Milan Stanković *Editor*

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Foreword

Nature is a major source of our current medicines, and many (semi)synthetic medicines have been developed from natural products. These compounds have been found by studying traditional medicines and by screening extracts from various organisms, particularly plants and microorganisms. In fact, about 25% of all western drugs are from plants. Microorganisms are particularly the source of antibiotics and antitumor medicines. In the meantime, for most ailments, good medicines are available; however, in the field of antibiotics and parasitic diseases, the resistance of microbes and parasites against available medicine is causing a major problem. New drugs are urgently needed, drugs that particularly should be available for the poor in developing countries at a reasonable price. As it concerns drugs cure a patient, the use is only for short periods. Consequently, the market is small if compared to, for example, an antihypertension drug that a patient takes every day for many years. Therefore, it is difficult to earn back the high costs for developing a new drug. The big pharma is thus not investing effort in developing such drugs, but if good leads are available, they may develop these further, as we can learn from the Nobel Prize of 2015. That prize shows the interest from the medical field for nature as a source of medicine, either by bioprospecting or by studying traditional medicines.

There is thus a unique opportunity for governmental and academic research institutes to look for novel leads from Nature for infectious and parasitic diseases. Particularly in Asia, with a well- documented traditional use of medicinal plants to treat patients, there is host of projects aiming at evidence-based traditional medicines and new leads for drug development. With the enormous biodiversity, with plants only having a rather small number of species (estimations run from 250,000 to 350,000) if compared with the largest group that of the insects (some 30 million species), there are ample materials to choose from. Concerning plants, I wrote in the year 2000 that only about 15% were studied phytochemically and 6% for one or more biological activities. At that time, the Dictionary of Natural Products had 139,000 entries. At present, the Dictionary of Natural Products claims to have now more than 300,000 entries (September 2019), which means an average increase of about 8000 compounds per year. That is quite a lot, but when we assume that each biological species can make one unique compound, there should be 10–100 million natural products, that means compared with the number of known ones, there must be many more compounds waiting to be discovered. Obviously, we will not be out of work for the coming years. But at the same time, it is clear that we must make choices for the goals of our research. The choices are made on the basis of previously reported results and the needs of the society for novel medicines and other sustainable products. Also, we will have to choose what approach to use, for example, to focus on evidence-based traditional uses or at random screening of extracts, which plant species or genera should be prioritized, and what type of activity does one focus on. Many questions and few answers, only a thorough study of the literature can help us make choices.

Publications like the present book are very helpful in making our choices, as one can learn about what type of activity is already shown and if that is of sufficient interest to warrant further studies. What kind of compounds may one expect, are they novel, and do they look like good candidates for further development from hit to lead, or is it already clear from other studies that the compounds are not promising. The editor have done an excellent job in compiling all the information and making this book a good navigator for further studies, as all aspects of the plants from the genus *Teucrium* are dealt with in this book. It will be an excellent guide for making new discoveries.

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Robert Verpoorte

Preface

Species of the genus *Teucrium* (Lamiaceae) are widespread in different climates as well as on different habitat types especially in the Mediterranean region. With more than 300 species, *Teucrium* is one of the largest genera of the Lamiaceae family. Some species have been used in folk medicine since Antiquity—the name *Teucrium* refers to the Achaean hero Teucros (Teucer), a great archer during the Trojan War. Species of this genus are commonly known as germanders. The species of genus *Teucrium* are mainly perennial herbaceous plants, shrubs, or subshrubs, while only a small number are annual species.

Known medicinal species belonging to the genus *Teucrium* are used as sources of natural therapy and beneficial bioactive compounds. Also, some *Teucrium* species are useful as spices, for the preparation of teas and bitter drinks. The species of the genus *Teucrium* are very rich in a variety of secondary metabolites with very significant biological activities. For medicinal purposes, these species are used in the treatment of digestive and respiratory disorders, abscesses, gout, and conjunctivitis as well as in the stimulation of fat and cellulite decomposition; they also possess anti-inflammatory, antioxidative, anticancer, antimicrobial, antidiabetic, and anthelminthic properties. However, their most significant therapeutic effect is the elimination of some digestive and respiratory problems.

Teucrium species are an interesting object of research in the numerous scientific projects from various aspects of science, with multiple applications. Numerous studies on the biological activity and phytochemical composition of plant extracts and essential oils, as well as biotechnological applications of *Teucrium* species, are based on ethnobotany as a primary source. All of these influenced the need for a book publication that will integrate such aspects. On that basis, the book *Teucrium Species: Biology and Applications* includes 15 systematically grouped chapters which highlight recent advances in exploring the unique features of *Teucrium* species. The chapters describe the systematics, morphology, ecology, biogeography, ethnobotany, and phytochemistry—secondary metabolites diversity; genotoxic, antioxidant, antibacterial, antifungal, antiviral, anticancer, anticholinesterase, and antidiabetic and anti-inflammatory activity of *Teucrium* secondary metabolites; as well as applications in biotechnology, food industry, and pharmacy, including current challenges and further perspectives. Excessive use of some medicinal plants of this genus can cause liver inflammation. This phenomenon and precaution are described in detail in a separate chapter in order to promote the safe use of certain species.

A book about *Teucrium* species will have several advantages as a literary material. First of all, this book is a comprehensive overview of the scientific information on the current achievements of research and their application of *Teucrium* species, as well as a rich source of the up-to-date bibliography of all scientific and practical fields related to *Teucrium* genus. The book will also provide the current list of the species of *Teucrium* genus, as well as color photographs of many species. Scientists in the field of biology, biotechnology, and pharmacy will have all the information and current literature in one place about *Teucrium* species from all aspects of science. The book would be initial literature review for beginners in the investigations of *Teucrium* species. Science enthusiasts and practitioners involved in medicinal plant applications will have the opportunity to get to know everything about the *Teucrium* species used in everyday life in Ethnomedicine for the treatment of various diseases and as the sources of additives as well as preservatives. The book can be used as a course literature at the university level. It is suitable for PhD students who focus on research on *Teucrium* species or other types of plant biological activity and applications.

Kragujevac, Serbia Milan Stanković

Acknowledgments

The book *Teucrium Species: Biology and Applications* includes 15 chapters written by 32 scientists in the field of Plant Biology and Ecology, Systematics, Morphology, Physiology, as well as Chemistry, Genetics, Microbiology, Molecular Biology, Pharmacognosy, Biotechnology, Food Sciences, Medicine and Pharmacy. Many of them are leading scientists in their field, my colleagues from the University, associates from foreign institutions, as well as laboratory colleagues from my research team working on the investigations of *Teucrium* species. I am highly grateful to all the chapter authors for their professional contributions and productive collaboration.

I am grateful to my colleague Prof. Dr. Teresa Navarro del Aguila (University of Malaga, Spain) for the expertise, effort, and valuable time devoted, as well as to other colleagues Prof. Dr. Trinidad Ruiz Tellez, Dr. Jose Blanco Salas (University of Extremadura, Spain) and Dr. Abhay Mishra (HNBG University, India) for friendly support.

I am thankful to the Springer's editorial team, especially to Mamta Kapila and Melanie van Overbeek, Joseph Daniel, and Anitta Camilya for valuable cooperation and guidance during initiating and editing process, as well as many other individuals acknowledged throughout the book.

I would like to specially thank Professor Emeritus Dr. Robert Verpoorte (Leiden University, The Netherlands) for supporting the book concept and idea in his Foreword to this book.

Book Editor
Prof. Dr. Milan Stanković

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About the Editor

Milan Stanković (1984) is an Associate Professor of Plant Science in the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Republic of Serbia, and Head of Department of Biology and Ecology. He earned his BSc in Biology from the Faculty of Science and Mathematics at the University of Niš, in 2007. Dr. Stanković began his scientific and teaching career as an Assistant in the Department of Biology and Ecology, Faculty of Science, University of Kragujevac (2008). He acquired PhD degree in Plant Science (2012) from the same University with the thesis "Biological activity of secondary metabolites of *Teucrium* L. species from Serbian flora." He completed Postdoctoral Research at the Université François-Rabelais de Tours, France. In the Faculty of Science, he was appointed as an Assistant Professor (2013) as well as Associate Professor (2019) and taught several BSc, MSc, and PhD courses on Plant Science as well as supervised thesis and dissertations in this field. His current research is focused on the biology, ecology, and applications of *Teucrium* species. Dr. Stanković is (co-)author of over 300 references including articles in peer-reviewed journals, edited books, book chapters, conference papers, meeting abstracts, etc. He is an editor, editorial board member, and reviewer of several scientific journals. Currently, he works as an Associate Editor of *Plants* (2012-) and *Rangeland Ecology and Management* (2015–). He was a member of the scientific and organizing committee of several conferences, congresses, and symposia in the fields of Biology and Plant Sciences. During his university career, he was a member of various scientific and professional associations such as the Society for Experimental Biology—Italy, Society for Medicinal Plants and Natural Product Research, Société Botanique de France, Organization for the Phyto-Taxonomic Investigation of the Mediterranean Area, and Serbian Biological Society. Currently he is a member of the State Commission for Expertise in the publication of school textbooks in Biology. In scientific collaboration, Dr. Stanković has published co-authored papers with colleagues from 23 various international universities from 17 countries. During his scientific career, he participated in the realization of over 30 scientific projects and studies.

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Chapter 1
Systematics and Biogeography of the Genus *Teucrium* (Lamiaceae)

Teresa Navarro

Abstract *Teucrium* L. is the second-largest genus of subfamily Ajugoideae with a subcosmopolitan distribution and 434 taxa (Govaerts RA, Paton A, Harvey Y, Navarro T, Del Rosario Garcia Pena M, World checklist of Lamiaceae. The Board of Trustees of the Royal Botanic Gardens Kew, Kew. www.kew.org/wcsp/, 2013). The Mediterranean region and surrounding floristic areas are the main center of diversity with around 90% of the total *Teucrium* species in the world. We describe the systematic of *Teucrium* following Bentham (Labiatarum genera et species. Ridgeway Sons, London, 1833), Boissier (Flora Orientalis IV. H. Georg, Geneva/Basel, 1879) and Kästner (Übersicht zur systematischen gliederung der gattung *Teucrium* L. Biocosme Mésogéen 6:63–78, 1989) with the recognition of the independent status of the section *Montana* Lazaro Ibiza (Navarro, *Teucrium* L. In: Castroviejo S et al (eds) Flora Iberica, VerbenaceaeLabiataeCallitrichaceae, vol 7. Real Jardín Botánico, CSIC, Madrid, pp 30–166, 2010). 341 herbarium specimens of 97 *Teucrium* taxa from throughout the world were studied, three taxa of its phylogenetic related genera *Spartothamnella* Briq., *Oncinocalyx* F. Muell., and *Teucridium* F. Hook, and two taxa of its segregated genera *Rubiteucris* Kudô and *Leucosceptrum* Smith. Based on the species biogeographical distribution and the main discriminant systematic characters, almost five major biogeographic and taxonomic species groups can differentiate. The group of species with 2-lipped corolla and zygomorphic calyx (sections *Pycnobotrys* Benth., *Stachyobotrys* Benth., *Scorodonia* (Hill) Schreb. and *Teucriopsis* Benth.) which provide a clear example of the Eurasian pattern of radiation from C Asia and China. The group of species with 1-lipped corolla and subactinomorphic calyx (sections *Polium* (Mill.) Schreb., *Chamaedrys* (Mill.) Schreb., *Montana* Lazaro Ibiza, *Isotriodon* Boiss., *Scordium* (Mill.) Schreb., and subsection *Pumila*), which represent the Mediterranean pattern of radiation, with a high degree of recent diversification. The typical Mediterranean species with 1-lipped corolla and actinomorphic calyx, consistent with the bulk of species of section *Teucrium* (Kästner, Übersicht zur systematischen gliederung der gattung *Teucrium* L. Biocosme Mésogéen 6:63–78, 1989). The Australian and S America 1-lipped corolla species with actinomorphic calyx, (*Teucrium* subsection

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Cretica (pro part)) (Kästner, Übersicht zur systematischen gliederung der gattung *Teucrium* L. Biocosme Mésogéen 6:63–78, 1989). Finally, the species with unclear 1-lipped corolla and actinomorphic calyx (Australian and Cape Region species belong to section *Teucrium*). The section *Teucrium* is the most heterogeneous and distinctive group within *Teucrium* with three different geographical distribution in Australian, N America and Mexico *Teucrium* while as other Ajugoideae genera closed to *Teucrium* such as Schnabelia Hand.-Mazz. All the sections occurs in the Mediterranean area, NW Africa, N Sahara Desert (Morocco, Algeria, Tunisia and Libya), N Egypt to the Ethiopia mountains, SW Arabian mountains, the Sinai Peninsula and through the arid regions up through W Asia and Europe. Section *Teucriopsis* is exclusive of Canary and Madeira islands, section *Pycnobotrys* distributed only in SE Asia and section *Leucosceptrum* Smith., exclusive from India.

Keywords *Teucrium* · *Teucrium* sections · Systematics · Biogeography · *Spartothamnella* · *Oncinocalyx* · *Teucridium* · *Rubiteucris* · Lamiaceae

1.1 Introduction

Teucrium L. is a polymorphic and widespread genus of Lamiaceae family including about 434 taxa (Appendix I) currently recognised species (Navarro and El Oualidi 2000a; Govaerts et al. 2013). They are shrubs, subshrubs and perennial herbs (rarely annuals or biennials) (Appendix II) with a subcosmopolitan distribution occurring mainly in the Mediterranean region and in the temperate parts of Asia (King 1988; Hedge 1992; Navarro and El Oualidi 2000a; Harley et al. 2004; Navarro 2010). *Teucrium* has an unusual distribution in the Lamiaceae family, only 7% of its species are present in the southern hemisphere (Australia, New Zealand, Cape Region and Argentina), while 93% are present in the north (Hedge and Miller 1977; Navarro and El Oualidi 2000a). The Mediterranean region, without doubt represents the major area of distribution, since it is represented by more of the 90% of the total of species in the world (Navarro and El Oualidi 2000a; Blanca et al. 2017) being the only genus of the subfamily Teucrioideae in the area (Cantino et al. 1992).

Teucrium has been divided into several sections mainly based on calyx shape, inflorescence structure and plant habitat. Bentham (1833) made the most relevant taxonomic classification with additions of Boisser (1879), and the most recent is whose of Kästner (1989). *Teucrium* is a paraphyletic genus (Salmaki et al. 2016) based on the placement of *Spartothamnella*, *Oncinocalyx* and *Teucridium* within it. All the sections, following Bentham (1833), occurs in the Mediterranean area, NW Africa, N Sahara Desert (Morocco, Algeria, Tunisia and Libya), N Egypt to the Ethiopia mountains, SW Arabian mountains, the Sinai Peninsula and through the arid regions up through W Asia and Europe. *Teucridium* is exclusive of Canary and Madeira islands, section *Pycnobotrys* distributed only in SE Asia and section *Leucosceptrum* exclusive from India.

The most recent hypothesis on evolutionary relationships within *Teucrium* have been proposed based on phylogenetic and chromosomatic studies, Salmaki et al. (2016) and Massoud et al. (2018) respectively. These authors proposed two main hypotheses, one of them considering the *Teucrium* section *Teucrium* (*Teucrium* core clade), as an old lineage within the genus as well as the Australian and S African lineages. The second and most speculative, consider the probable origin of the genus was the Mediterranean region, likely with the first aneuploidy originated in the Old World and dispersed in the New World (Massoud et al. 2018).

Due to the size of the taxa under study, a sample of 341 specimens of 97 *Teucrium* taxa from throughout the world have been studied in addition to the previous biosystematic revisions (Navarro and El Oualid 2000a; Navarro 1995, 2010). Species was taken to represent the total taxonomic variation and geographical distribution of *Teucrium*. For geographical regions where no infrageneric divisions of *Teucrium* have hitherto been recognised, representative species were chosen from the regional Floras. The monotypic genera *Oncinocalyx* and *Teucridium* were both examined. In *Sparthotamnella*, *Leucosceptrum* and *Rubiteucris* one member from each genera was chosen (Appendix III). The studied specimens are housed at: (Spain) MA, MAF, BC, SEV, MGC, GDAC, VAL, ABH, JACA; (Portugal) LISU; (United Kingdom) KEW, EDI; (Australia) NE, CANB; (New Zealand) CHR; (Italy) CAG; (France) MPU (for herbarium abbreviations see Holmgren et al. 1990).

In this chapter, we will discuss the systematic and biogeography of *Teucrium* focusing in the relationships within *Teucrium* infrageneric taxa and between *Teucrium* and its close related phylogenetic genera *Spartothamnella*, *Oncinocalyx* and *Teucridium*.

1.2 Systematic of *Teucrium* Genus and Its Infrageneric Delimitation Taxa

Teucrium has a corolla usually 1-lipped or 2-lipped, 5-lobed (with the anterior lobe much larger than the others), concave, most rarely unequal 5-lobed in the upper half; lobes slightly spreading, 4 posterior ± similar, (anterior lobe larger than the others). *Spartothamnella*, *Oncinocalyx* and *Teucridium* are the closest taxonomically related genera to *Teucrium* (Harley et al. 2004) and forming a phylogenetic clade (Salmaki et al. 2016). *Spartothamnella* is an endemic Australian genus that contains three species: *Spartothamnella teucriiflora* (F. Muell.) Moldenke, *S. juncea* Briq. Engl. and Prantl and *S. puberula* (F. Muell.) Maiden and Betchei. The main morphological difference between *Spartothamnella* and *Teucrium* is that the former has drupaceous fruit. *Teucridium*, is an endemic New Zealand genus represented by *T. parvifolium* F. Hook, which differs from *Teucrium* mainly in ovary form, unlobed or lobed up to a quarter part of its length in *Teucridium* and usually lobed from a quarter to half its length in *Teucrium*. *Oncinocalyx,* also an endemic genus from E Australia (represented by *Oncinocalyx betchei* F. Muell), differs from *Teucrium* in the conspicuously hooked calyx lobes. The pollen morphology supports the phylogenetic relationships between these genera and *Teucrium* (Abu Asab and Cantino 1993), forming the *Teucriina* clade (Cantino et al. 1997), based on the operculate and verrucate pollen. The phylogenetic analysis based on DNA sequence data has confirmed the affinities between *Teucrium* (throughout *T. fruticans* L.), *Teucridium* and *Oncinocalyx* (Wagstaff and Olmstead 1997; Wagstaff et al. 1998; Lindquist and Albert 2002) and *Spartothamnella* (Steane et al. 2004). *Teucridium*, *Oncinocalyx*, *Spartothamnella* and *Teucrium fruticans* L. form a morphologically recognised species group. They are erect or straggly shrubs with many branches with entire leaves, often-axillary cymes, actinomorphic calyx, and fruit in a schizocarp of four big haired reticulate-ridged nutlets.

The delimitation of infrageneric taxa in *Teucrium* is difficult because of the great variation in most morphological characters in many of the species. Bentam's (1833) system is the most traditional classification based on floral features that divides the genus into nine sections: *Leucosceptrum*, *Teucropsis*, *Teucris*, *Pycnobotrys*, *Stachyobotrys*, *Scorodonia*, *Scordium*, *Chamaedrys* and *Polium*. Bentham noted that the most distinct section is *Teucris*, recognising three distinct species (*Teucrium fruticans*, *T. corymbosum* R. Br. and *T. pseudochamaepitys* L.) which show different ways in this section. *Scorodonia*, *Pycnobotrys*, *Stachyobotrys*, *Leucosceptrum* and *Teucropsis* would, when considered together, form a well characterised group based on the broad upper lip of the calyx, which is usually but not always accompanied by less reticulate achenia. As well, section *Teucropsis* is similar to section *Scorodonia* in calyx morphology. Boissier (1879) described two new sections, *Spinularia* from species included by Bentham in *Scordium* and *Isotriodon* for the Asian species unknown to Bentham. Briquet (1895-1897) retained Bentham's circumscription but segregated *Leucosceptrum*, at present included in the subfamily Lamioideae (Harley et al. 2004). Lazaro Ibiza (1896) described two new sections, *Montana* and *Pumila*, for the Iberian species segregated from section *Polium*, based on leaf morphology. Rouy (1909) created a new monospecific section; section *Botrys* based on *Teucrium botrys*, a species included by Bentham in *Scordium*. Kudô (1929) segregated *Kinostemon* based on *Teucrium* section *Pleurobotrys*. *Kinostemon* included three species: *Kinostemon pernyi* (Franch.) Kudô, *K. bidentatum* (Hemsl.) Kudô and *K. ornatum* (Hemsl.) Kudô. Kästner (1989) and Harley et al. (2004) retained all *Kinostemon* within *Teucrium*. Cohen (1956) described the subsection *Rotundifolia* under *Polium*, based on the *Teucrium rotundifolium* species group. Rivas Martinez (1974) treated *Pumilum* (*Pumila*) as a subsection under *Polium*. Valdés Bermejo and Sánchez-Crespo (1978) recognised three subsections under *Polium*; *Polium*, *Pumilum* and *Rotundifolia*. Puech (1978) described the subsection *Simplicipilosa* for a *Polium* species group with simple hairs, endemic to the Iberian Peninsula. The most commonly used infrasectional arrangement of *Polium* is the one proposed by Valdés Bermejo and Sánchez-Crespo (1978), with an addition made by Puech (1980). As a result, *Polium* is one of the largest (50% of the total species, following Gobaerts et al. 2013) and most complex groups within *Teucrium*. Finally, Kästner (1989) produced the first substantial revision of the genus based on corolla, inflorescence and leaf characters. However, the taxonomic sampling of section *Polium* was poor, and many critical species have not been investigated. Kästner included *Polium* as a subsection under *Chamaedrys*, *Spinularia* and *Botrys* under *Scordium*, *Pycnobotrys* under *Isotriodon* and *Stachyobotrys* under *Scorodonia*. This author suggested new subsections; *Cretica* under *Teucrium*, *Canadensia* under *Scorodonia* and *Marum* under *Chamaedrys* and retained *Rubiteucris* palmata as *Teucrium palmatum* in the section *Pycnobotrys*. However, pollen morphology (Abu-Asab and Cantino 1993) and phylogenetic research segregate this genus.

The infrageneric taxonomic relationships within *Teucrium* have been investigated by different authors, karyology by Valdés Bermejo and Sánchez-Crespo (1978), growth form (Kästner 1985), chemical analyses (Harbone et al. 1986) and nutlet morphology (Marin et al. 1994) show *Teucrium* as the most distinctive section. The results of the study on trichome morphology (Manzanares et al. 1983; Navarro and El Oualidi 2000b) concluded that sections *Teucrium* and *Polium* are two well-differentiated groups. Navarro and El Oualidi (1999) investigated floral features and concluded that *Teucrium* and *Scorodonia* are the most distinct sections within *Teucrium*. A molecular study based on ITS analyses (El Oualidi et al. 1999) shows that *Polium* can be differentiated from the rest of the sections. The results obtained from pollen morphology (Abu Asab and Cantino 1993; Diez et al. 1993; Navarro et al. 2004) support Bentham's delimitation and agree with the distinctiveness of the sections *Teucrium* and *Spinularia* and establishing new taxonomic relationships between *Chamaedrys* and *Montanum*. Juan et al. (2004) studied seed amino acids and showed that sections *Scordium*, *Botrys* and *Spinularia* are different.

1.3 Systematic and Biogeographic Relationships Within *Teucrium* Infrageneric Taxa and Close Related Genera

Taxonomic relationships are established between *Oncinocalyx*, *Teucridium*, *Oncinocalyx*, *Spartothamnella* and the Austral *Teucrium* (section *Teucrium*) based on the no clearly lipped corolla. These relationships have been proven with results of phylogenetic analysis (Wagstaff and Olmstead 1997; Wagstaff et al. 1998; Lindquist and Albert 2002; Steane et al. 2004; Salmaki et al. 2016). Among them, *Spartothamnella* is the most morphologically close genus to *Teucrium*, differing substantially only based on it drupaceous fruit.

The primitive forms of subfamily Ajugoideae, such as section *Teucrium*, may have appeared in Australia (Chengyih and Hsiwen 1982). Section *Teucrium* is a basal group within *Teucrium* (Marin et al. 1994; Navarro and El Oualidi 1999; Navarro et al. 2004). This section has widespread floral characters in the structurally simple states, including the actinomorphic calyx and unclearly lipped corollas (Austral species of section *Teucrium*) present in geologically ancient areas. *Teucrium* is also the most worldwide spread section found in regions with a Mediterranean climate. As a result of climatic change (Pliocene/Pleistocene), closed forests started to open up and arid areas were established in Australia (Mummenhoff et al. 1992; Cox and Moore 1993) and in the Mediterranean (Quezel 2000). Therefore, it is not surprising to find two different centres of diversification of this section in Australia and in the Mediterranean region.

Section *Teucrium* can be separated from the rest of the sections mainly based on the actinomorphic ventral calyx (Bentham 1833; Kästner 1989; Marin et al. 1994; Navarro and El Oualidi 1999, 2000a). Three distinct groups can be recognized on the basis of corolla conformation. The first includes the 1-lipped species with spurred corollas, an annulus of hairs in the base, adpressed simple hairs in the stems and big nutlets. These are the much branched divaricate evergreen shrubs, including *Teucrium fruticans*, *T. brevifolium* Schreb., *T. malenconianum* and *T. chardonianum* Maire and Wilczek which form the most typically Mediterranean *Teucrium* with convergent traits of woody plants that belong to pre-Mediterranean lineages (Herrera 1987, 1992; Specht 1988; Verdú 2000; Verdú et al. 2003). The second is composed of the Australian *Teucrium racemosum* group characterized by their longer pedicellate flowers, 1-lipped non- spurred corollas with an annulus of hairs in the middle part of the tube. This group includes *Teucrium racemosum* R.Br., *T. corymbosum* R.Br. and *T. cubense* Jacq., and possibly, *T. integrifolium* Benth., *T. depressum* Small and *T. laciniatum* Torr. The third and most distinctive group is composed of the S African species *T. africanum* Thunb, *T. capense* Thunb., and *T. fililobum* F. Muell. ex Benth. Section *Teucriopsis* is discriminate from the rest of the sections according to previous morphological studies (Bentham 1833; Kästner 1989; Marin et al. 1994; Navarro and El Oualidi 2000a).

Trichome morphology (Bini-Maleci and Servettaz 1991; Servettaz et al. 1992) support the systematic affinities between the sections *Chamaedrys* and *Isotriodon* (Kästner 1985, 1986), both distributed in open forest, shrublands and rocky slopes and fissures in arid and sub-humid regions, mainly in the E Mediterranean area. Nevertheless, species of *Isotriodon* section are weel differenciated to be herbaceous often with a broad upper lip to the calyx and fruit in a schizocarp of four smooth or slightly reticulate coherent nutlets. An annulus of hairs in the calyx tube, a derived character (Abu Asab and Cantino 1993), also unites the species of this section.

The independent status of section *Stachyobotrys* is supported by pollen morphology (Abu Asab and Cantino 1993; Navarro et al. 2004) by chemical studies (Harbone et al. 1986) and floral features (Kästner 1989; Navarro and El Oualidi 1999). The Irano–Turanian and Mediterranean species of this section (*Teucrium lamiifolium* D'Urv., *T. hircanicum* L., *T. bracteatum* Desf. and *T. collincola* Greuter and Burdet) have a gibbous calyx, which is absent from the American species (*Teucrium canadense* L., and *T. vesicarium* Mill.). The Moroccan subshrubs, *Teucrium collincola*, seem to be the most different as regards hair type, corolla conformation and reticulate nutlets (Navarro and El Oualidi 1999). The N American and Mexican *Teucrium canadense* differs from the Mediterranean species on the basis of its tubular calyx, curved hairs and slightly reticulate nutlets, while the temperate S American *Teucrium vesicarium* differs in its rare sacciform calyx and reticulate-rugose nutlets.

The Mediterranean species of section *Scorodonia* with 2-lipped (1/4) calyx with a broad upper lip, 2-lipped corolla with straight stamens, the two last characters being the autopomorphies relative to *Teucrium* (Abu Asab and Cantino 1993). The two Chinese species, *Teucrium pernyi* (*Kinostemon pernyi*) and *T. bidentatum* (*K. bidentatum*), both with zygomorphic calyx (1/2/2), broad upper lip and slightly 2-lipped corollas, the five SE Asian species; *Teucrium veronicoides* Maxim., *T.*

stoloniferum Roxb., *T. viscidum* Blume and *T. japonicum* Will., with slightly 2-lipped corollas and zygomorphic calyx (3/2). *T. plectranthoides* Gamble appears to be separate group inside this section based on its 2-lipped calyx. The two SE Asian species, *Teucrium quadrifarium* Buchanan-Hamilton ex D. Don and *T. argutum* R. Br., and the Iberian *T. salviastrum* Schreb., all with 2-lipped corollas, calyx 1/2/2 with broad upper lip, are considered by Bentham (1833) as species belonging to the section *Scorodonia*. This author noted the strong affinity between *Teucrium quadrifarium* and *T. argutum* and the doubtful position of *T. salviastrum*.

Section *Polium* subsection *Polium* and *Simplicipilosa* were clearly different from the rest of the sections because they are the only group with sessile flowers and leaves, short corolla tube with a tuft of hairs inside. Close affinities between them have been reported (Puech 1978; Navarro and Rosúa 1988; Navarro and El Oualidi 1999, 2000b).

Species of section *Montana* can be differenciated based on the calyx, stem hair type, erect in the *Teucrium rotundifolium* group, and curved in *T. montanum* group. Systematic relationships between sections *Montana* and *Chamaedrys* are shown based on nutlet ornamentation (Marin et al. 1994) and trichome morphology (Navarro and El Oualidi 1999). Affinities between sections *Teucrium*, *Chamaedrys* and *Montana* were shown based on nutlet morphology (Marin et al. 1994), and trichome morphology (Navarro and El Oualidi 2000b).

The relationships between the 1-lipped taxa with subactinomorphic calyx, as occurs in the sections *Chamaedrys*, *Montana* and *Pumila* was shown by Kästner (1989), El Oualidi et al. (1999), Navarro and El Oualidi (2000b), Navarro et al. (2004) and between sections *Scordium* and *Polium* (on the basis of pollen morphology) by Diez et al. (1993), Navarro et al. (2004) and seed amino acids (Juan et al. 2004).

1.4 Biogeography of the Infrageneric *Teucrium* Taxa

Section *Teucrium* is distributes in the Mediterranean region, Europe, Africa, Australia, Asia and America (Fig. 1.1). Most species are perennials or short-lived herbs found in the E Mediterranean. They are branched shrubs like *Teucrium fruticans* looking like the relict elements from the old tropical Mediterranean flora. Section *Teucriopsis* comprises the semi-sclerophyllous shrub: *Teucrium heterophyllum* L'Hér from Canary Islands, and two perennial herbs; *T. abutiloides* L'Hér and *T. betonicum* L'Hér from Madeira Islands. It is an endemic section from the Macaronesian region distributed in forest, open habitats and rocky slopes. Section *Stachyobotrys* is distributed in America, Australia, Mediterranean region and Asia. The species are the perennials or short-lived herbs and half-shrubs. This section is distributed mainly on shady walls, rocky slopes and sandy areas in the semi-arid and sub-humid climate regions. It is represented by only two species in the W Mediterranean area, *Teucrium bracteatum* Desf. (Morocco and Spain) and *T. collincola* Greuter & Burdet endemic from Morocco. Section *Scorodonia* is distributed

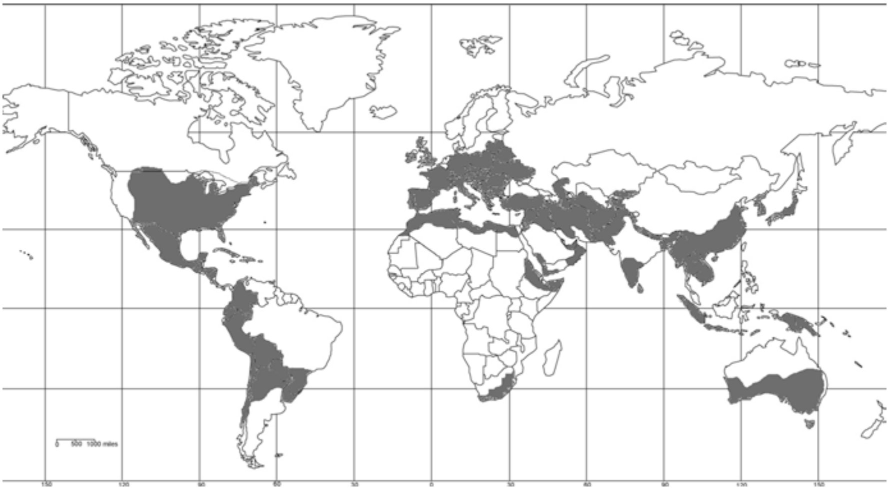

Fig. 1.1 Distribution map of *Teucrium* genus

in the Mediterranean, Europe and Asia but mainly in the humid climate of the W Mediterranean. This section includes half-shrubs and perennial herbs with asexual reproduction. Section *Scordium* is distributed in the Mediterranean, Europe and Asia. This section includes the stoloniferous erect and prostrate short-lived herbs. This is a widespread section in the meadows of the humid habitats. The most important species is the Saharo–Sindian *Teucrium scordium* L. Section *Scordium* subsection *Spinularia* is distributed in the Mediterranean, Europe and Africa and includes erect, prostrate and spinescent annual and short-lived herbs. This section is distributed mainly in the semi-arid and sub-humid regions of the W Mediterranean region (NW Africa). Section *Isotriodon* includes half-shrub species restricted to cliffs, rocky slopes and fissures in the Mediterranean semi-arid regions. Section *Chamaedrys* mainly occurring in the Mediterranean region, Europe, Africa and Asia. They are small half-shrubs and perennial herbs with asexual reproduction. The most important species is *Teucrium chamaedrys* L., distributed through the Mediterranean region. It is the only section of the genus found in the W Mediterrancan islands. Section *Polium* includes a difficult systematic group of vary diverse Mediterranean widespread species with adaptative radiations by polyploidy. The species of this section are common in exposed and disturbed areas of the Mediterranean area, N Africa (Morocco, Algeria and Tunisia). *Polium* is the most diversified section into the genus due its adaptative radiation in the Mediterranean region (Cohen 1956; Puech 1984; Navarro 1995, 2010; Navarro and El Oualidi 1999, 2000a, b; El Oualidi et al. 2002).

Section *Polium* comprises the subsection *Polium* that includes erect, prostrate, cushion-like, half-shrubs or scrubs and perennial herbs. The most important species are *T. polium* L. and *Teucrium capitatum* L., occur throughout the Mediterranean and Irano–Turanian regions. 70% of species are found in the W Mediterranean area

(SE Spain and N Morocco). Subsection *Simplicilpilosa* includes often endemic species from SE Spain, Ethiopia, and SE Arabian Peninsula and subsection *Pumila* is endemic to the Iberian Peninsula. Section *Montanum* is a diverse section throughout the Mediterranean area, NE Africa and E Mediterranean (Turkey, Greece and Cyprus), Ethiopia, E of the Arabia peninsula and W Asia mainly in from rocky slopes, fissures and cliffs.

1.5 Conclusions

Based on the biogeographical distribution of the species of *Teucrium* and the main discriminant systematic characters in the genus, such as corolla and calyx lobes structure (zygomorphy) as well as their indumentum type, almost five major biogeographic and taxonomic species groups can be differentiate. The group of the perennial herbs with 2-lipped corolla and zygomorphic calyx (sections *Pycnobotrys*, *Stachyobotrys*, *Scorodonia* and *Teucropsis*) which provide a clear example of the Eurasian pattern of radiation from C Asia and China. A group integrate for the subshrubs and half-shrubs with 1-lipped corolla and mainly subactinomorphic calyx (sections *Polium*, *Chamaedrys*, *Montana*, *Isotriodon*, *Pumila* and *Scordium*), that clearly represent the Mediterranean pattern of radiation, with a high degree of recent diversification (Hedge 1986). A third group composed by the typical Mediterranean shrubs with 1-lipped corolla and actinomorphic calyx, consistent with the bulk of species of section *Teucrium* subsection *Fruticantia* (pro part.) (Kästner 1989). A fourth group that includes the Australian and S America subshrubs and perennial herbs with 1-lipped corolla and actinomorphic calyx, which seem to be the most distinctive group (*Teucrium racemosum* group), which corresponds to the section *Teucrium* subsection *Cretica* (pro part) (Kästner 1989). This last group confirms the connection between Australasia and S American (through *Teucrium cubense*), the sole pattern of radiation in Lamiaceae in the southern hemisphere (Hedge 1992). Finally, the group formed of the perennial herbs and subshrubs with unclear 1-lipped corolla and actinomorphic calyx (Australian and Cape Region species belong to section *Teucrium*). The section *Teucrium* is the most heterogeneous and distinctive group within *Teucrium* with three different geographical distribution in Australian, N America and Mexico *Teucrium* while as other Ajugoideae genera closed to *Teucrium* such as *Schnabelia* and *Pseudocaryopteris*.

Teucrium genus is widespread in the Mediterranean, Macaronesian, Irano–Turanian and Saharo–Arabian regions with 434 taxa (Govaerts et al. 2013) in the world. The genus shows two centres of richness located in the E and W region of the Mediterranean Sea. This fact is exemplified by the geographical distribution of sections *Stachyobotrys*, *Isotriodon* and section *Polium* subsection *Simplicipilosa*. However, other sections, such as *Scordium*, have an homogeneous distribution in all the areas but are mainly represented by Saharo–Sindian species. The sections *Teucrium*, *Chamaedrys*, *Isotriodon* and *Stachyobotrys* mainly occur in the E Mediterranean and Irano–Turanian region. *Polium*, *Scorodonia* and *Spinularia* are predominantly in the W Mediterranean area. The E Mediterranean region, the S of the Arabian Peninsula and NE Africa are centres of floristic diversity of the section *Polium*. *Teucriopsis* is restricted to the Macaronesian region. Part of the floristic richness of *Teucrium* genus is the high number of endemic species in the Mediterranean region (63% of total species in the world). The main part of them (c. 50%) in the lberian Peninsula and Morocco and a high number (c. 18%) in Turkey and Greece.

Stachyobotrys (through *Teucrium canadense* and *T. inflatum*), *Pycnobotrys* (through *Teucrium vesicarium*) are the only taxonomic groups with amphiatlantic relationships, in which the basalmost members of these are largely Mediterranean and C Asian in origin. This pattern of distribution can be explained based on the Boreotropics hypothesis (Wolfe 1975). In this sense, *Teucrium* species have a distinct distribution in the Old and in the New World, among arid regions of S Africa, N America, N Africa and Asia (Mabberly 1997). All these sections can be considered as a remnant of widespread early Tertiary flora. Finally, section *Polium* is an example that reflects a rapid radiation and speciation with a high number of species in the Mediterranean region (Navarro and El Oualidi 2000a; El Oualidi et al. 2002).

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Appendices

Appendix I: List of Teucrium *Infrageneric Taxa in the World*

Teucrium L., taxa list of the accepted names from WCSP World Checklist of Selected Plant (WCSP) (familieshttp://wcsp.science.kew.org/qsearch.do). Govaerts RA, Paton A, Harvey Y, Navarro T, Del Rosario Garcia Pena M (2013) World Checklist of Lamiaceae. Kew. The Board of Trustees of the Royal Botanic Gardens, Kew.www.kew.org/wcsp/

***Teucrium* L., Sp. Pl.: 562 (1753).**

Teucrium abolhayatensis Ranjbar & Mahmoudi, Novon 25: 304 (2017).
Teucrium abutiloides L'Hér., Stirp. Nov.: 84 (1788).
Teucrium africanum Thunb., Prodr. Pl. Cap.: 95 (1800).
Teucrium afrum (Emb. & Maire) Pau & Font Quer in P.Font i Quer, Iter Marocc. 1927: n.° 510 (1928).

Teucrium afrum subsp. *afrum*.
Teucrium afrum subsp. *rhiphaeum* (Font Quer & Pau) Castrov. & Bayon, Anales Jard. Bot. Madrid 47: 513 (1989 publ. 1990).
Teucrium afrum subsp. *rubriflorum* (Font Quer & Pau) Castrov. & Bayon, Anales Jard. Bot. Madrid 47: 513 (1989 publ. 1990).

Teucrium aladagense Vural & H.Duman, Turkish J. Bot. 39: 319 (2015).
Teucrium albicaule Toelken, J. Adelaide Bot. Gard. 7: 296 (1985).
Teucrium albidum Munby, Bull. Soc. Bot. France 2: 286 (1855).
Teucrium alborubrum Hemsl., J. Linn. Soc., Bot. 26: 311 (1890).
Teucrium × alexeenkoanum Juz. in V.L.Komarov, Fl. URSS 20: 506 (1954).
Teucrium algarbiense (Cout.) Cout., Esboco Fl. Lenh. Portug., ed. 2: 262 (1936).
Teucrium alopecurus de Noé, Bull. Soc. Bot. France 2: 585 (1855).
Teucrium alpestre Sm. in J.Sibthorp & J.E.Smith, Fl. Graec. Prodr. 1: 395 (1809).
Teucrium × alvarezii Alcaraz, Sánchez-Gómez, De la Torre & S.Ríos, Datos Veg. Murcia: 144 (1991).
Teucrium alyssifolium Stapf, Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl. 50: 104 (1885).
Teucrium amplexicaule Benth., Labiat. Gen. Spec.: 687 (1835).
Teucrium andrusi Post, Bull. Herb. Boissier 5: 758 (1897).
Teucrium angustissimum Schreb., Pl. Verticill. Unilab. Gen. Sp.: 49 (1774).
Teucrium anlungense C.Y.Wu & S.Chow, Acta Phytotax. Sin. 10: 338 (1965).
Teucrium annandalei Mukerjee, Rec. Bot. Surv. India 14: 219 (1940).
Teucrium antiatlanticum (Maire) Sauvage & Vindt, Bull. Soc. Sci. Nat. Maroc 35: 286 (1956).
Teucrium antilibanoticum Mouterde, Saussurea 4: 25 (1973).
Teucrium antitauricum Ekim, Notes Roy. Bot. Gard. Edinburgh 38: 58 (1980).
Teucrium apollinis Maire & Weiller, Bull. Soc. Hist. Nat. Afrique N. 30: 86 (1939).
Teucrium aragonense Loscos & J.Pardo in H.M. Willkomm (ed.), Ser. Inconf. Pl. Aragon.: 85 (1863).
Teucrium arduinoi L., Mant. Pl. 1: 81 (1767).
Teucrium argutum R.Br., Prodr. Fl. Nov. Holland.: 504 (1810).
Teucrium aristatum Pérez Lara, Anales Soc. Esp. Hist. Nat. 18: 90 (1889).
Teucrium aroanium Orph. ex Boiss., Diagn. Pl. Orient., ser. 2, 4: 55 (1859).
Teucrium asiaticum L., Mant. Pl. 1: 80 (1767).
Teucrium atratum Pomel, Nouv. Mat. Fl. Atl.: 304 (1874).
Teucrium aureiforme Pomel, Nouv. Mat. Fl. Atl.: 113 (1874).
Teucrium aureocandidum Andr., Ind. Horti Bot. Univ. Budapest 5: 18 (1941).
Teucrium aureum Schreb., Pl. Verticill. Unilab. Gen. Sp.: 43 (1774).

Teucrium aureum subsp. *aureum*.
Teucrium aureum subsp. *turdetanum* Devesa & Valdés Berm., Anales Jard. Bot. Madrid 41: 88 (1984).

Teucrium* × *badiae Sennen, Bol. Soc. Aragonesa Ci. Nat. 11: 230 (1912).
Teucrium balearicum (Coss. ex Pau) Castrov. & Bayon, Anales Jard. Bot. Madrid 47: 508 (1989 publ. 1990).
Teucrium balfourii Vierh., Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl. 71: 436 (1907).
Teucrium balthazaris Sennen, Diagn. Nouv.: 95 (1936).
Teucrium baokangensis C.L.Xiang, Taxon 67: 390 (2018).
Teucrium barbarum Jahand. & Maire, Bull. Soc. Hist. Nat. Afrique N. 19: 85 (1928).
Teucrium barbeyanum Asch. & Taub. ex E.A.Durand & Barratte, Fl. Libyc. Prodr.: 191 (1910).
Teucrium* × *bergadense Sennen, Bol. Soc. Aragonesa Ci. Nat. 11: 214 (1912).
Teucrium betchei (F.Muell.) Kattari & Salmaki, Taxon 65: 818 (2016).
Teucrium betonicum L'Hér., Stirp. Nov.: 83 (1788).
Teucrium bicolor Sm. in A.Rees, Cycl. 35: n.° 25 (1817).
Teucrium bicoloreum Pau ex Vicioso, Bol. Soc. Esp. Hist. Nat. 16: 142 (1916).
Teucrium bidentatum Hemsl., J. Linn. Soc., Bot. 26: 312 (1890).
Teucrium bogoutdinovae Melnikov, Novosti Sist. Vyssh. Rast. 45: 81 (2014).
Teucrium botrys L., Sp. Pl.: 562 (1753).
Teucrium brachyandrum Puech, Naturalia Monspel., Sér. Bot. 21: 209 (1970 publ. 1971).
Teucrium bracteatum Desf., Fl. Atlant. 2: 7 (1798).
Teucrium brevifolium Schreb., Pl. Verticill. Unilab. Gen. Sp.: 27 (1774).
Teucrium* × *bubanii Sennen, Bol. Soc. Aragonesa Ci. Nat. 11: 229 (1912).
Teucrium bullatum Coss. & Balansa, Bull. Soc. Bot. France 20: 260 (1873).
Teucrium burmanicum Mukerjee, Notes Roy. Bot. Gard. Edinburgh 19: 306 (1938).
Teucrium buxifolium Schreb., Pl. Verticill. Unilab. Gen. Sp.: 42 (1774).
Teucrium campanulatum L., Sp. Pl.: 562 (1753).
Teucrium canadense L., Sp. Pl.: 564 (1753).

Teucrium canadense var. *canadense*.
Teucrium canadense var. *hypoleucum* Griseb., Cat. Pl. Cub.: 214 (1866).
Teucrium canadense var. *occidentale* (A.Gray) E.M.McClint. & Epling, Brittonia 5: 500 (1946).
Teucrium canadense var. *virginicum* (L.) Eaton, Man. Bot., ed. 5: 415 (1829).

Teucrium canum Fisch. & C.A.Mey., Index Seminum (LE, Petropolitanus) 1: 40 (1835).
Teucrium capitatum L., Sp. Pl.: 566 (1753).

Teucrium capitatum subsp. *capitatum*.
Teucrium capitatum subsp. *gracillimum* (Rouy) Valdés Berm., Acta Bot. Malac. 4: 40 (1978 publ. 1979).
Teucrium capitatum subsp. *majoricum* (Rouy) Nyman, Consp. Fl. Eur., Suppl. 2: 246 (1889).

Teucrium* × *carmelitanum Roselló, P.P.Ferrer, A.Guillén, Gómez Nav., Peris & E. Laguna, Flora Montiber. 55: 102 (2013).

Teucrium carolipaui Vicioso ex Pau, Bol. Soc. Ibér. Ci. Nat. 20: 185 (1921 publl. 1922).

Teucrium carolipaui subsp. *carolipaui*.

Teucrium carolipaui subsp. *fontqueri* (Sennen) Rivas Mart., Anales Inst. Bot. Cavanilles 31: 88 (1974).

Teucrium carthaginense Lange, Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn 1881: 97 (1882).

Teucrium* × *carvalhoae A.F.Carrillo, A.Hern., Coy, Güemes & Sánchez-Gómez, Acta Bot. Malac. 22: 221 (1997).

Teucrium caucasigenum Melnikov, Novosti Sist. Vyssh. Rast. 45: 72 (2014).

Teucrium cavanillesianum Font Quer & Jeronimo, Anales Jard. Bot. Madrid 6(2): 488 (1946).

Teucrium cavernarum P.H.Davis, Kew Bull. 6: 113 (1951).

Teucrium chamaedrys L., Sp. Pl.: 565 (1753).

Teucrium chamaedrys subsp. *albarracinii* (Pau) Rech.f., Bot. Arch. 42: 385 (1941).

Teucrium chamaedrys subsp. *algeriense* Rech.f., Bot. Arch. 42: 364 (1941).

Teucrium chamaedrys subsp. *chamaedrys*.

Teucrium chamaedrys subsp. *germanicum* (F.Herm.) Rech.f., Bot. Arch. 42: 379 (1941).

Teucrium chamaedrys subsp. *gracile* (Batt.) Rech.f., Bot. Arch. 42: 389 (1941).

Teucrium chamaedrys subsp. *lydium* O.Schwarz, Repert. Spec. Nov. Regni Veg. 36: 132 (1934).

Teucrium chamaedrys var. *multinodum* Bordz., Mat. Fl. Kavk. 4(3): 57 (1916).

Teucrium chamaedrys subsp. *nuchense* (K.Koch) Rech.f., Bot. Arch. 42: 370 (1941).

Teucrium chamaedrys subsp. *olympicum* Rech.f., Bot. Arch. 42: 368 (1941).

Teucrium chamaedrys subsp. *pectinatum* Rech.f., Bot. Arch. 42: 366 (1941).

Teucrium chamaedrys subsp. *pinnatifidum* (Sennen) Rech.f., Bot. Arch. 42: 383 (1941).

Teucrium chamaedrys subsp. *sinuatum* (Celak.) Rech.f., Bot. Arch. 42: 378 (1941).

Teucrium chamaedrys subsp. *syspirense* (K.Koch) Rech.f., Ann. Naturhist. Mus. Wien 51: 427 (1941).

Teucrium chamaedrys subsp. *tauricola* Rech.f., Bot. Arch. 42: 376 (1941).

Teucrium chamaedrys subsp. *trapezunticum* Rech.f., Bot. Arch. 42: 369 (1941).

Teucrium chardonianum Maire & Wilczek, Bull. Soc. Hist. Nat. Afrique N. 26: 130 (1935).
Teucrium charidemi Sandwith, Cavanillesia 3: 38 (1930).
Teucrium chasmophyticum Rech.f., Pl. Syst. Evol. 134: 287 (1980).
Teucrium chlorocephalum Celak., Bot. Centralbl. 14: 186 (1883).
Teucrium chlorostachyum Pau & Font Quer in P.Font i Quer, Iter Marocc. 1929: n.° 370 (1930).

Teucrium chlorostachyum subsp. *chlorostachyum*.
Teucrium chlorostachyum subsp. *melillense* (Sennen ex Maire) Ouqlidi, Mathez & T.Navarro, Fl. Medit. 7: 23 (1997).

Teucrium chowii Y.H.Tong & N.H.Xia, Phytotaxa 349: 99 (2018).
Teucrium chrysotrichum Lange, Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn 1881: 96 (1882).
Teucrium cincinnatum Maire, Bull. Soc. Sci. Nat. Maroc 13: 272 (1933 publ. 1934).
Teucrium clementiae Ryding, Edinburgh J. Bot. 55: 210 (1998).
Teucrium coahuilanum B.L.Turner, Lundellia 8: 7 (2005).
Teucrium × coeleste Schreb., Pl. Verticill. Unilab. Gen. Sp.: 49 (1774).
Teucrium compactum Clemente ex Lag., Gen. Sp. Pl.: 17 (1816).
Teucrium coniortodes Boiss. & Blanche in P.E.Boissier, Fl. Orient. 4: 818 (1879).
Teucrium × conquense M.B.Crespo & Mateo, Fl. Medit. 1: 197 (1991).

Teucrium × *conquense* nothosubsp. *conquense*.
Teucrium × *conquense* nothosubsp. *siyasense* A.F.Carrillo & Sánchez Gómez, Acta Bot. Malac. 21: 285 (1996).

Teucrium × contejeanii Giraudias, Bull. Soc. Études Sci. Angers 1888: 16 (1888).
Teucrium corymbiferum Desf., Fl. Atlant. 2: 8 (1798).
Teucrium corymbosum R.Br., Prodr. Fl. Nov. Holland.: 504 (1810).
Teucrium cossonii D.Wood, Bot. J. Linn. Soc. 65: 261 (1972).
Teucrium creticum L., Sp. Pl.: 563 (1753).
Teucrium cubense Jacq., Enum. Syst. Pl.: 25 (1760).
Teucrium cuneifolium Sm. in J.Sibthorp & J.E.Smith, Fl. Graec. Prodr. 1: 395 (1809).
Teucrium cyprium Boiss., Diagn. Pl. Orient. 5: 43 (1844).
Teucrium cyrenaicum (Maire & Weiller) Brullo & Furnari, Webbia 34: 167 (1979).
Teucrium davaeanum Coss., Bull. Soc. Bot. France 36: 102 (1889).
Teucrium dealianum Emb. & Maire, Bull. Soc. Sci. Nat. Maroc 15: 171 (1935).
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Teucrium similatum T.Navarro & Rosua, Candollea 45: 583 (1990).

Teucrium simplex Vaniot, Bull. Acad. Int. Géogr. Bot. 14: 186 (1904).

Teucrium sirnakense Özcan & Dirmenci, Turkish J. Bot. 39: 312 (2015).

Teucrium socinianum Boiss., Fl. Orient. 4: 818 (1879).

Teucrium socotranum Vierh., Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl. 71: 436 (1907).

Teucrium somalense Ryding, Edinburgh J. Bot. 55: 217 (1998).

Teucrium spinosum L., Sp. Pl.: 566 (1753).

Teucrium stachyophyllum P.H.Davis, Notes Roy. Bot. Gard. Edinburgh 21: 48 (1951).

Teucrium stocksianum Boiss., Diagn. Pl. Orient., ser. 2, 4: 58 (1859).

Teucrium stocksianum subsp. *incanum* (Hedge & Lamond) Rech.f., Fl. Iranica 150: 29 (1982).

Teucrium stocksianum subsp. *patulum* (Hedge & Lamond) Rech.f., Fl. Iranica 150: 29 (1982).

Teucrium stocksianum subsp. *stenophyllum* R.A.King, Notes Roy. Bot. Gard. Edinburgh 45: 32 (1988).

Teucrium stocksianum subsp. *stocksianum*.

Teucrium subspinosum Pourr. ex Willd., Enum. Pl.: 596 (1809).

Teucrium taiwanianum T.H.Hsieh & T.C.Huang, Taiwania 41: 86 (1996).

Teucrium tananicum Maire, Bull. Soc. Hist. Nat. Afrique N. 23: 210 (1932).

Teucrium teinense Kudô, J. Coll. Sci. Imp. Univ. Tokyo 43: 6 (1921).

Teucrium terciae (Sánchez-Gómez, M.A.Carrión & A.Hern.) Sánchez-Gómez, M.A.Carrión & A.Hern., Anales Biol., Fac. Biol., Univ. Murcia 25: 112 (2003).

Teucrium teresianum Blanca, Cueto & J.Fuentes, Nordic J. Bot. 35: 14 (2017 publ. 2016).
Teucrium teucriiflorum (F.Muell.) Kattari & Salmaki, Taxon 65: 818 (2016).
Teucrium thieleanum B.J.Conn, Telopea 11: 135 (2006).
Teucrium thymifolium Schreb., Pl. Verticill. Unilab. Gen. Sp.: 50 (1774).
Teucrium thymoides Pomel, Nouv. Mat. Fl. Atl.: 112 (1874).
Teucrium townsendii Vasey & Rose, Proc. U. S. Natl. Mus. 12: 146 (1890).
Teucrium trifidum Retz., Observ. Bot. 1: 21 (1779).
Teucrium tsinlingense C.Y.Wu & S.Chow, Acta Phytotax. Sin. 10: 334 (1965).

Teucrium tsinlingense var. *porphyreum* C.Y.Wu & S.Chow, Acta Phytotax. Sin. 10: 335 (1965).
Teucrium tsinlingense var. *tsinlingense*.

Teucrium × turianum M.B.Crespo, P.P.Ferrer, Roselló, M.A.Alonso, Juan & E. Laguna, Flora Montiber. 58: 70 (2014).
Teucrium turredanum Losa & Rivas Goday, Anales Inst. Bot. Cavanilles 25: 204 (1968).
Teucrium ussuriense Kom., Izv. Bot. Sada Akad. Nauk S.S.S.R. 30: 208 (1931 publ. 1932).
Teucrium veronicoides Maxim., Bull. Acad. Imp. Sci. Saint-Pétersbourg, sér. 3, 23: 388 (1877).
Teucrium vesicarium Mill., Gard. Dict. ed. 8: n.° 17 (1768).
Teucrium vincentinum Rouy, Naturaliste 4(3): 20 (1882).
Teucrium viscidum Blume, Bijdr. Fl. Ned. Ind.: 827 (1826).

Teucrium viscidum var. *leiocalyx* C.Y.Wu & S.Chow, Acta Phytotax. Sin. 10: 332 (1965).
Teucrium viscidum var. *longibracteatum* C.Y.Wu & S.Chow, Acta Phytotax. Sin. 10: 332 (1965).
Teucrium viscidum var. *macrostephanum* C.Y.Wu & S.Chow, Acta Phytotax. Sin. 10: 333 (1965).
Teucrium viscidum var. *miquelianum* (Maxim.) H.Hara, Bot. Mag. (Tokyo) 51: 145 (1937).
Teucrium viscidum var. *nepetoides* (H.Lév.) C.Y.Wu & S.Chow, Acta Phytotax. Sin. 10: 331 (1965).
Teucrium viscidum var. *viscidum*.

Teucrium wattii Prain, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 59: 317 (1890 publ. 1891).
Teucrium webbianum Boiss., Elench. Pl. Nov.: 78 (1838).
Teucrium werneri Emb., Bull. Soc. Sci. Nat. Maroc 15: 185 (1935).
Teucrium wightii Hook.f., Fl. Brit. India 4: 701 (1885).
Teucrium yemense Deflers, Voy. Yemen: 190 (1889).
Teucrium zaianum Emb. & Maire, Pl. Marocc. Nov. 2: 7 (1929).
Teucrium zanonii Pamp., Nuovo Giorn. Bot. Ital., n.s., 24: 153 (1917).

Appendix II: Photographs of Selected Teucrium *Species on Natural Habitats*


T. aureum Schreb.


T. carolipaui Vicioso ex Pau


T. carthaginense Lange


T. chrysotrichum Lange


T. compactum Clemente ex Lag.


T. dunense Sennen


T. freynii E. Rev. ex Willk.


T. gnaphalodes L'Hér.

T. haenseleri Boiss.

T. subspinosum Pourr. ex Willd.

T. intricatum Lange

T. moleromesae Sánchez-Gómez, T. Navarro, et al.

Teucrium pumilum Loefl. ex L.

Teucrium rotundifolium Schreb.

T. resupinatum Desf., Fl.

T. thymifolium Schreb.

T. barbarum Jahand. & Maire

T. bicoloreum Pau ex Vicioso

T. capitatum L.

T. eriocephalum Willk.

T. haenseleri Boiss.

T. hieronymi Sennen

T. pseudochamaepitys L.

T. similatum T. Navarro & Rosua

T. turredanum Losa & R. G.

Appendix III: List of Herbarium Specimens of Teucrium *L.,* Oncinocalyx *F. Muell.,* Sparthotamnella *Briq.,* Teucridium *F. Hook,* Rubiteucris *palmata Kudô and* Leucosceptrum canum *Sm., with Localities and Voucher. The Infrageneric Classification Follows Navarro (2010)*

Taxa of *Oncinocalyx* F. Muell., *Sparthotamnella* Briq., *Teucridium* F. Hook, *Rubiteucris palmata* Kudô and *Leucosceptrum canum* Sm.

Oncinocalyx betchei. AUSTRALIA: New South Wales, Gunnedak, vi-1909, Boorman, KEW; Curlevis, M. Martha, xi-1969, Johnson & Briggs, 2991, KEW; Deriah, Barra trail, ii-1986, Mackay 806, NE 051374; Tamworth, xii-1985, Hosking, NE 047136; Deriah, Myrtle, x-1916, Mackay, NE 043201A.

Spartothamnella teucriiflora. AUSTRALIA: Alice, vii-1962, Nelson 351, KEW; Uluru, v-1988, Lazarides & Palmer 45, KEW; Buró Plain, vii-1962, Nelson 351, KEW; Uluru, iv-1988, Lazarides & Palmer 045, KEW.

Teucridium parviflorum. NEW ZEALAND: Rangitikei, Taihapa, Mataroa, iii-1991, Ogle, CHR 471440; Whakatane, CHR 335270. Hangaroa, Tiniroto, xii-1967, Druce 179777, CHR 179777; Taihape, ii-1979, Druce, CHR 286453; Kawhatau, Taihape, CHR 286453; Ikawetea, 1-1975, Druce, CHR 275092; Gwavas, Hawkés bay, CHR 282979; Manonui, CHR 23800; Tawhaiti, i-1920, CHR 297158; Gwavas Bush, i-1976, Druce, CHR 282979; Manuni, iii-1949, Wood, CHR23800.

Rubiteucris palmata. NEPAL: Marsyandi Valley, x-1978, Davis, KEW; Sihkim, 1867, H.Hookerianum 1867, KEW.

Leucosceptrum canum. NEPAL: Sinduwa, 1963, Hara et al. 6300990, KEW; Kyapra, ix-1989, keke 342, KEW; Katmandú, ii-1966, Schilling 736, KEW; Sadon, i-1958, Mc Kee 6073, KEW.

Section *Teucrium*

T. fruticans. MOROCCO: Targuist, vi-1993, Jury 11301, MA 577056; Xauen, iv-1926, Font Quer, MA 99122; Igrouka, MA 595799; Targuist, MA 577056.

T. campanulatum. SPAIN: Zaragoza, Bujaraloz, BCF 32764. ITALIA: Sicilia, Catania, vi-2000, Güemes, VAL 119660.

T. aristatum. FRANCE: Arles, vi-2004, Yavercovski, MGC 63560. SPAIN: Sevilla, Carmona, v-1967, Mayor & Ladero, MA 278365; Huelva, Hinojos, v-2002, Talavera, MGC 51882.

T. creticum. CYPRUS: Larnaca, iv-1979, McClintock 2967, EDI 178048; Lefkara, vii-1940, Davis 1917, EDI 178047; Larnaca, Cape Kiti, EDI 2967; Larnaka, MA 495430; Montes Troodos, MA 418783; Larnaca, Cape Kiti, iv-1974, EDI 178048; Lefkara, vii-1940, EDI 178047.

T. pruinosum. TURKEY: Yesilhisar to Urgüp, vii-1980, Nydegger 15485, VAL 129347.

T. pseudoscorodonia. MOROCCO: Tizzi Iffni, MA 9839; Melilla, MA 98937; Nador, MA 562366.

T. parviflorum. Iran (Persia); Kurdistan, Hoseynabad, Sanandaj, v-1971, Rechinger, MA 417834.
T. brevifolium. CRETA: Akroteri, iv-1884, Reverchon 205, MA 9907, MA 99072.
T. malenconianum. MOROCCO: High Atlas, Imilchil, vii-1997, Jury 17728, MA 615566; Gorges du Dades, vi-1982, FC 6966, MA 633607.
T. pseudochamaepitys. SPAIN: Madrid, Aranjuez, MA 436850; Aranjuez, vi-1958, Guinea, MA 436850; Madrid, Algodor, v-1925, Caballero & Gonzalez, MA 436863.

Section *Teucrium* (species with no clearly lipped corolla)

T. africanum. S AFRICA: Grahamstown, xii-1980, Germishuizen 7212, KEW; Fort Beaufort, xii-1902, Brink 713, KEW; Riversdale, xi-1970, Taylor7833, KEW.
T. capense. S AFRICA: Grahanstown, x-1970, Brink 154, KEW; Transnaal, 1894, Transvaalensis 5780, KEW; Transvaal, iii-1958, Vauden 514, KEW; Rustenburg, 1904, Nation, KEW; Wolmaranstad, xii-1973, Hanekon 2185, KEW; Pretoria, Plaas Rooibos, 1977, Liebenberg 8804, KEW.
T. fililobum. AUSTRALIA: x-1909, H. Morrison, KEW; Norseman, xi-1962, Aplin 1851, KEW; Norseman, x-1904, Campbell, KEW.

Section *Teucrium* (*T. racemosum* group)

T. corymbosum. AUSTRALIA: Flinders, Partacoona, ix-1969, Kuchel 2827, MA 456302; Victoria, Mueller, MA 256743; Partacuona, MA 456302; Partacoona, ix-1969, Kuchel 2827, MA 456302.
T. cubense. MEXICO: Mexico, Tampico, i-1910, Palmer 13, KEW; San Luis Potosi, xi-1975, Peterson 471, KEW; Ejido Palmas, Martinez 1204, KEW; Castaños, 1936, Wynd, KEW. ARGENTINA: Corrientes, Mercedes, ix-1997, Ferruci & al. 1291, MA 622439. CUBA: La Habana, iv-1991, Herrera, MA 256749.
T. racemosum. AUSTRALIA: Borung, iii-1903, Reader, EDI 178056; v-1802, EDI 178055; Murray, xi-1968, Cole 131, MA 4563303; Melbourne, Borung, EDI 178058; Bryant creek Reserve, Morgan, ii-1968, Cole 131, MA 456303; Victoria, You-yango, MA 256850; Victoria, iii-1903, EDI 178050.

Section *Teucriopsis* Benth.

T. heterophyllum. SPAIN: Tenerife, Malpais, ii-1946, Sventenius, VAL 138501; Tenerife, Anaga, MGC 8272.
T. betonicum. PORTUGAL: Madeira, Encominada, MA 655341; S. Jorge valley, MA 522318; Mirodoiro do Porto, MA 27852; Madeira, San Jorge, vi-1966, Sjögren, 1178, MA 522318; Madeira, Encomiada, vi-2000, Velayos 9745, MA 655341.

Section *Montana* Lazaro Ibiza

T. pyrenaicum. SPAIN: Huesca, vii-1976, Cabezudo, MGC 37970; Cantabria, 1983, Casaseca, MA 256848; Huesca, Boltaña, vii-1987, Villar et al., MA 515728.

T. rotundifolium. MOROCCO: Alto Atlas, Oukaimeden, 1999, Güemes, VAL 41211. SPAIN: Valencia, Villalonga, 1980, Mateo, VAL 800654. Alicante, Benissa, 1998, Marin Camos, VAL 105784; Jaén, Los ladrones, 1977, Muñoz-Garmendia, MA 395212; Murcia, La Molata, 1923, Cuatrecasas, MA 97822.

T. musimonum. MOROCCO: Alto Atlas, Tizi-n-Ait Hamed, Güemes 1554, VAL 41104.

T. hifacense. SPAIN: Alicante, Pego, vi-1997, Signes & Soler 7130, MA 590236; Alicante, Altea, vi-1962, Rigual, MA 374228; Alicante, Benisa, iv-1993, Soler, MA 548713.

T. montanum. GREECE: Olympos, 1983, Aguilella, VAL 7311. SPAIN: Navarra, Lokiz, vii-1989, Alejandre, MA 485511; Aramendia, vii-1987, Urrutia, MA 477842; Lokiz, vii-1987, Uribe-Echevarria, MA 489242.

T. thymifolium. SPAIN: Cuenca, v-1975, G. López, MA 433643; Cuenca, Hoz de Tragaviros, vii-1978, G. López, MA 433630.

T. buxifolium. SPAIN: Alicante, Villena, vii-1955, Rigual, MA 375540; Albacete, rio Segura, v-1987, Izuzguiza 654A, MA 434376; Alicante, Serrella, vi-1984, Mateo & Figuerola, MA 434317. Alicante, Pego, 1997, Signer, MA 590256; Alicante, Altea, Rigual, MA 374228.

T. rivasii. SPAIN: Alicante, Monford del Cid, 1997, Serra, ABH 33976; Alicante, Novelda, Rigual, MA 375675; Alicante, Raspeig, v-1962, Rigual, MA 373637; Alicante, Monforte, v-1954, Rigual, MA 375546.

T. rivas-martinezii. SPAIN: Albacete, Yeste, MGC 45032.

T. compactum. SPAIN: Almería, Nijar, vi-1986, T. Navarro, MGC 39598.

T. grosii. MOROCCO: Alhucemas, 1999, Cabezudo, MGC 28014; Alhucemas, v-1989, Diaz et al., BC 164369.

T. alopecurus. TUNICIA: Gabes, Djebel Tebaga, 1992, Molero & Vicens, VAL 95/5441.

T ducellieri. MOROCCO: Midelt, iv-133, Wilczek, MA 97959; MA 97960; Haute Moulouya, iv-1925, Jahandiez 110, MA 9796; Midelt, 1933, Wilczek, MA 97959.

T. alpestre. CRETA: Nomos, v-2002, Vitek 02104, MA 710216.

Section *Chamaedrys* (Mill.) Schreb.

T. chamaedrys. SPAIN: León, Beberino, vii-1988, Luceño 2553, MA 489290; Soria, Cameros, viii-1991, Alejandre, MA 534072; Segovia, Fuentidueña, MA 317831. GREECE: Olimpos, viii-1983, Aguililla, VAL 41896. MOROCCO: Aknoul, vi-1995, Boratynski & Romo, 8787/5, BC 165411.

T. lucidum. FRANCE: Castellan, vii-1966, Berger, MA 581566; Toudon, vii-1994, Alziaz, MA 564241; Basses Alpes, vii-1886, Reverchon, MA 986335(2); Basses Alpes, vii-1886, Annot, MA 98635.

T. flavum. SPAIN: Valencia, Oliva, VAL 51220; Alicante, Fleix, VAL 48140. Valencia, Oliva, xi-1984, Mateo, VAL 51220; Alicante, Gallinera, Mateo, VAL 83820; Alicante, Gallinera, v-1979, Mansanet & Mateo, MA 462875. ITALIA: Siracusa, Sortino, VAL 119809.

T. fragile. SPAIN: Málaga, Alcaucín, MGC 57153; Granada, Almuñecar, MGC 57152; Granada, Sierra Tejeda, MGC 57151; Málaga, Sierra Tejeda, MGC 57150.

T. intricatum. SPAIN: Almería, Vicar, MA 542131; Felix, MA 501580.

T. pugionifolium. SPAIN: Valencia, Salvacañete, iv-1988, Mateo & Crespo 060, VAL 72107.

T. webbianum. SPAIN: Albacete, Sierra Ayna, vii-1971, Rivas Godoy & Borja, MA 558023; Ciudad Real, vii-1934, Alcaraz, MA 98676; Granada, Sierra Nevada, vii-1976, Castroviejo & Valdes Bermejo 778, MA 435011.; Jaén, Sierra de Mágina, vi-1926, Cuatrecasas, BC 47385; Jaén, Albanchez, vi-1925, Cuatrecasas, BC 47383.

Section *Chamaedrys* Subsection *Marum* Kästner

T. subspinosum. SPAIN: Menorca, Cap de Favaritx, MA 435671. Menorca, Binincola, vi-1980; Valdes-Bermejo 5611, MAF 118439; Binincola, Menorca, vi-1980, Castroviejo & al., MA 377857. Menorca, Ciutadella, JACA 1253; Menorca, Mercadal, JACA 444392; Cabrera, Canal Llarg, MA 98859; Cabrera, Coll Roig, MA 383095; ITALIA: Cerdeña, Cagliari, Limbara, CAG; Cerdeña. Isla S. Prieto, Porto Banda, CAG (Herbarium Horti Botanici Caralitani).

T. balearicum. SPAIN: Mallorca, Galatzó, MA 98854; Mallorca, Valldemosa, MA 98682; Mallorca, Soller, MA98856; Menorca, Monte Toro, MA 435647; Mallorca, Formentor, iv-1934, Rivas Goday, MAF 83869; Mallorca, Soller, vi-1879, Crespi, MAF 33134; Cabrera, Coll Roig, v-1948, Palau Ferrer, MAF 33135; Mallorca, Galatzo, vii-1934, Gros, MAF 33133.

T. marum. ITALIA: Livorno, VAL 025593; Corcega, Calvi, La Revellata, VAL 25594; Corse, Bavella, vii-1929, Litardiere, MAF 60255; Corse, Calvi, vi-1989, Lambinon 89145, MAF 145468; Livorno, vi-1988, Moggi & al. 14427, MAF 145467.

T. divaricatum. SIRIA: Liban, Ain-Halesoun, vi-1934, Bertschinger, MA 470817. PALESTINA: Jerusalem, Meyers 4025, MA 98679. GREECE: Athenes, MPU; Karpathos, Rhodas, Samos, MPU. LIBANO: Libano, Vallée de Maydouché, MPU.

Section *Polium* (Mill.) Schreb.

T. aureum. SPAIN: Tarragona, Montsant, vi-1954, MA 98498; Barcelona, Montserrat, vii-1914, Caballero, MA 96328; Querol, vi-1999, Castroviejo et al.,15065, MA 628840. ITALIA: Sicilia, Ragusa, vi-2000, Güemes 3398, VAL 119567. FRANCE: Aude, vii-1944, Rechinger 458, MA 648449.

T. capitatum subsp. ***capitatum***. SPAIN: Jaén, Jodar, x-1925, Cuatrecasas, BC 47262; Benidorm, v-1993, Verdú, ABH 11809; Alicante, Benitaxell, v-1992, Barber, ABH 9746. MOROCCO: Quebdana, vii-1939, Sennen, BC 138165.

T. homotrichum. SPAIN: Alicante, Sierra Mariola, 1949, Borja, MA 186190; Santa Pola, Muñoz Garmendia, MA 375840; Valencia, Pinet, 1975, Mateo, MA 484323; Valencia, Játiva, 1943, Bellot, MA 383032; Alicante, Serralla, 1981, Solanas, MA 557663.

T. dunense. SPAIN: Mallorca, El Arenal, vi-1919, Bianor, MA 98059; Valencia, Albufera, vi-1913, Sennen 7408, MA 98065; Mallorca, Font Santa, vi-1998, C. Navarro et al. 2115, MA 618607; Mallorca, El Arenal, vi-1919, Bianor & Sennen, MA 467631; Mallorca, Soller, 1919, Bianor, MA 98059; Mallorca, El Arenal, MA 98061.

T. polium. GREECE: Parnasos, viii-1983, Aguililla, VAL 07305. YUGOSLAVIA: Skopje, viii-1983, Aguililla, VAL 07312. ISRAEL: Nahal Qetalar, vi-1989, Heller, VAL 25590. ITALIA: Toscana, Moggi, VAL 04511. SPAIN: Lleida, Noguera, vii-1992, Aedo 2385, MA 511612; Huesca, Sopeira, vii-1992, Aedo 2343, MA 514397.

T. flavovirens. MOROCCO: Alto Atlas, Ait-Youly Bou Thrarar, Güemes 1511, VAL 41076; Ouarzazate, v-1985, Blanché et al. 1985, BC 165403; Ksar es Souk, vi-1985, Blanché et al., 1985, BC 165518.

T. murcicum. SPAIN: Alicante, San Miguel de Salinas, 1996, Crespo, ABH 33189; Alicante, Rabate, 1996, Crespo, ABH 33190; Mallorca, Sant Esteve, vi-1985, Benedi, MA 501870.

T. algarbiense. PORTUGAL: Tavira, Santa Maria, v-1969, Correia, Instituto Superior Agronomia de Lisboa; Margen del rio Chança, LISU 32527; Tavira, v-1907, Mendez, LISU 32524; Tavira, Mendez, LISU 32529.

T. angustissimum. SPAIN: Valencia, Requena, VAL 99910.

T. gnaphalodes. SPAIN: Madrid, Arajuez, iv-1950, Rivas Goday, MA 256782; Jaén, Sierra de Cabra, vi-1925, Cuatrecasas, BC 47364.

T. aragonense. SPAIN: Teruel, Valdealgorfa, v-1984, VAL 64593; Zaragoza, Caspe, VAL 129337; Zaragoza, v-1973, Castroviejo & Valdés Bermejo, MA 434365; Zaragoza, Mequinenza, v-1976, Segura Zubizarreta 3379, MA 256695.

T. carthaginenese. SPAIN: Murcia, Cartagena, iv-1998, Aran, 89-18-35, BC 631743; Murcia, Portman, iii-2000, Fuertes, BC 75145.

T. capitatum subsp. ***majoricum***. SPAIN: Ibiza, San Rafael, v-1918, Gros, MA 98221; Mallorca, Cala Fuent, xi-1947, Palau Ferrer, MA 98223; Mallorca, Bellver, vi-1955, Palau Ferrer 763, MA 167651; Mallorca, Burguesa, vi-1988, Morales et al. 1719, MA 617911.

T. similatum. SPAIN: Almería, Sierra de Filabres, vii-1989, T. Navarro, MGC 44958; Cádiz, Grazalema, vi-1984, Aparicio et al, BC 806325; Cádiz, Sierra del Pinar, vii-1984, Martin & Silvestre, BC 806328.

T. cossonii. SPAIN: Ibiza, LLadalt, v-1920, Font Quer, MA 98451; Mallorca, Puig Mayor, vi-1980, Castroviejo et al. 1935, MA 377938; Ibiza, Santa Agnes, vi-1979, FC 2905, MA 256744; Mallorca, Soller, MA 98081.

T. rouyanum. FRANCE: Aveyron, Tourmemire, vi-1906, VAL 141047.

T. mideltense. MOROCCO: Alto Atlas, Ighil M Goun, vii-1997, Güemes, VAL 41068; Oukaimeden, 1999, Güemes, VAL 41211.

Section *Polium* Subsection *Simplicipilosa* S. Puech

T. lanigerum. SPAIN: Almeria, Garrucha, vii-1950, Jerónimo, BC 146340; Almería, San José, vi-1983, T. Navarro, GDAC 23078; Almería, Mojacar, ii-1985, T. Navarro & Rosúa, GDAC 23080.
T. charidemi. SPAIN: Almería, Cabo de Gata, vii-1950, Losa, BC 145725; Almería, Cabo de Gata, vii-1948, Jerónimo, BC 146339.
T. chrysotrichium. SPAIN: Málaga, Coín, v-1994, Navas et al., MGC 39572; Málaga, Alhaurin Perez Gómez, MGC 15748.
T. haenseleri. SPAIN: Toledo, Orgaz, vii-1993, Aran, VAL 93346; Ciudad Real, Sierra Madrona, vi-1988, Sánchez Mata, VAL 77640; Málaga, Cerro Muriano, vii-1922, Gros, BC 802646.

Section *Polium* Subsection *Pumila* (Lazaro Ibiza) Rivas-Mart.

T. pumilum. SPAIN: Madrid, Chinchón, vi-1988, Aran, VAL 75540; Madrid, Arganda, vii-1984, VAL 47191; Arganda, vii-1981, Aguilella, VAL 72120.
T. carolipaui. SPAIN: Almería, Los Gallardos, vii-1983, Zubizarreta, VAL 11718; Alicante, La Nuncia, Mateo, VAL 46625; Villajoyosa, vi-1935, Martinez, VAL 72887.
T. libanitis. SPAIN: Murcia, Jumilla, viii-1959, Borja, MA 179404.
T. turredanum. SPAIN: Almería, Mojacar, ii-1985, Rosúa & T. Navarro, GDAC 22776; Almería, rio de Aguas, xii-1983, T. Navarro & Guirado, GDAC 22778; Turre, vii-1963, Rivas Goday, MAF 102670.

Section *Scordium* (Mill.) Rchb.

T. scordium. SPAIN: Huelva, Doñana, vi-1977, Castroviejo et al. 2256, MA 434926; Cádiz, Algodonales, viii-1978, Silvestre, MA 284365; Málaga, Cartama, vii-1888, Reverchon, MA 98796; Ciudad Real, vii-1980, Castroviejo et al. 1976, MA 256874.

Section *Scorodonia* (Hill) Schreb.

T. canadense. USA: Florida, St. Andrew bay, vi-1959, Dress & Read 7661, EDI 178059; Indiana, Riverside, viii-1958, Irvine 706, EDI 178060; Nebraska, Lancaster, vii-1988, Nieto-Feliner 2058GN, MA 472409; Chicago, Bennett, vii-1956, MA 173585. CANADA: Oke Pointe aux Anglais, vii-1941, Louis-Maie, MA 256731.

T. asiaticum. SPAIN: Mallorca, Soller, viii-1988, Orell, MA 497747; Mallorca, Valdemosa, ix-1946, Palau Ferrer, MA 98970; Mallorca, Masenella, vi-1980, Castroviejo et al. 5770EV, MA 25670.

T. scorodonia. SPAIN: Lugo, MA 503364; A Coruña, MA 551009; Ciudada Real, Valdeazogues, vi-1981, G. López, MA 256884; Asturias, Cudillero, viii-1996, Campón et al., MA 594089; Orense, Cassaio, viii-1992, Castroviejo 12125, MA 512523. PORTUGAL: Guarda, Sierra de Estrella, vii-1986, Bayón & Vogt, MA 489737.

T. massiliense. ITALIA: Cerdeña, vi-2003, Castroviejo 17097, MA 708252; Corse, Col de Sevi, vii-1982, Langhe 189, MA 281915; Corse, Galeria, vi-1978, Lambinon 78568, MA 256809; Col de Sevi, Cristinacce, MA 86927. Sicilia, Mesina, vi-2000, Güemes, VAL 119591.

T. siculum. ITALIA: Moni di Castellamare, Campania, VAL 141045; Campania Montidi, vi-1962, Pellanda, VAL 141045.

T. oxylepis. SPAIN: Almería, Sierra de Gádor, vii-1921, Gros, BC 46867; Almería, Sierra de Gador, MA 284360; MA 179418- MA 620040.

Section *Stachyobotrys* Benth.

T. collincola. MOROCCO: Sidi-Ifni, vi-1934, Caballero, MA 98997; Grand Atlas, Ouzoud, iv-1927, Jahandiez 315, MA 98998; Gran Atlas, Tizi'n Test, vi-1988, Romo, BC 165520; Agadir, v-1985, Blaché et al., BC 165525.

T. vesicarium. MEXICO: Tenozique, vi-1939, Matuda 3512, KEW; Quintana Roo, v-1962, Davidse et al. 20233, KEW.

T. bracteatum. SPAIN: Málaga, Casarabonela, v-1997, Cabezudo, MGC 36263; Málaga, Sierra Prieta, vii-1993, Cabezudo, MGC 36236. MOROCCO: Targuist, MA 98984; Targuist, vi-1927, Font Quer 508, BC 47409.

T. lamiifolium. TURKEY: Zonguldak; Karadere, vii-1962, Davis & al. 37681, EDI 178052. Estanbul, Balekesis, vii-1971, Baytoh 20780, EDI 178051. BULGARIA: Strandza, MA 256800.

T. hyrcanicum. ARMENIA: Zanahegunk, vii-1959, Yab, EDI 178053; Davis 20821, EDI 178054. PERSIA: sine loc., x-1783, Cavanilles, MA 256798.

Section *Pycnobotrys* Benth.

T. argutum. AUSTRALIA: Haeukesbrery, v-1802, EDI 178057. Queensland, Toowoumba, 1879, Warburg 18709, EDI 178058.

T. quadrifarium. BHUTAN: Wangdi, ix-1988, Wood 6640, EDI 178064. NEPAL: Godavari, ix-1974, Stainton 7188, EDI 178065.

T. salviastrum. PORTUGAL: Beira Alta, Serra da Estrela, vii-1983, Bayón & al. 8703, MA 387878; Serra da Estela, vi-1949, Fernandez & Sousa, MA 278698;

Sierra da Estela, vii-52, Beliz et al. 1287, MA 278700; Sierra de la Estrella, viii-1916, Sennen 2937, MA 98958.

T. pernyi. CHINA: Kiangsi, viii-1923, Steward 4718, KEW; Wangshan, 1973, Chow 45, KEW; Sushan, Kuling, viii-1923, KEW; Anhewi, Wangshan, 1973, KEW.

T. bidentatum. CHINA: Xi, 1974, Cheng & Hwa 1032, KEW; Sichuan, iii-1996, Zheng-yu 15317, KEW; Szechian, vii-1928, Kuan Hsien 2360, KEW; Omei Hsien, 1928, Fang 3158, KEW.

T. stoloniferum. CHINA: Shensi, viii-1932, Hao 4208, KEW; Banton, 1957, Sampson, KEW; Canton, vi-1884, KEW.

T. veronicoides. JAPAN: Hondo, ix-1947, Furuse 188818, KEW; Hokkaido, viii-1952, Furuse 25381, KEW.

T. plectranthoides. INDIA: Sengalheri, ii-1913, Hooper & Ramaswami 38609, KEW.

T. japonicum. JAPAN: Uragu, vii-1879, Bisser 253, EDI 178061.

T. viscidum. THAILANDIA: Petchabun, Phu Minao, EDI 178063; Phu Miang, x-1967, Shimizu & al. 11774, EDI 178062. JAPAN: Honsu, Tochu, EDI 178063; Ryukyu, Sonal, 1973, Furuse 3268, KEW; Tochigi, Aso-Gun, 1963, Furuse 44456 KEW; Honshu, x-1957, Togasi 1640, EDI 178063. CHINA: Yang, Shan, Linchow, vii-1932, Tsui 790, KEW. JAVA: Tahoha, ii-1894, Hoorders 14953B, KEW. NEW GUINEA PAPUA: Tufu, vii-1963, Darbyshire 1140, KEW. SUMATRA: 1938, Schiffer 2487, KEW.

Section *Scordium* subsection *Spinularla* (Boiss.) Kästner

T. decipiens Coss. & Balanza. MOROCCO: Rabat, Oued Akrech, VAL 11715.

T. spinosum. SPAIN: Cordoba, Montoso, 1981, Borja, MA 18178; Málaga, Coín, vi-1919, Gros, BC 124967; Sevilla, Alcalá de Guadaira, v-1981, Fernández & al. 168.81, BC 640242; Coin, MA 98823. MOROCCO: Kenitra, vii-1989, Garcia et al. 198/89, BC 165436.

T. resupinatum. MOROCCO: Zaggora, vi-1996, Lambinon & Sande 96806, MA 625762; Fés, iv-1987, Bayon et al., MA 486419; Tetuan, vi-1982, FC 6711, MA 393886; El Araix, vii-1930, Font Quer 546, BC 98044; Moyen Sebou, Zaggota, 1990, Lambinon, MA 625762; Kenitra, vi-1989, Podlech 46857, MA 472343. SPAIN: Cádiz, Alcalá, vi-1925, Font Quer, BC 805411.

T. botrys. SPAIN: Málaga, Torrox, MGC 53795; Jaén, Almaden, vii-1925, Cuatrecasas, BC 46940; Cádiz, Grazalema, vi-1984, Aparcio et al. BC 806331.

Section *Isotriodon* Boiss.

T. montbretii. TURKEY: Hatay; Antakya, vii-1984, Hartvig & al. 23561, EDI 178049-178050.

T. halacsyanum. GREECE: Aetolia, xi-1964, Rechinger 25334, EDI 178046; Aetolia, v-1964, Rechinger, MA 418724; Aetolia, Finike, vii-1984, Davis, EDI 178050.

T. tananicum. MOROCCO: Ida-ou-Tanan, v-1932, Maire-Iter Maroccanum xxii, MPU; Ida-ou-Tanan, SEV 165414.

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Chapter 2
Morphological Characteristics of *Teucrium* Species: Vegetative Morphology

Taner Özcan

Abstract In this chapter, vegetative morphological characteristics of *Teucrium* species have been described based on morphological and micromorphological studies. Morphological differentiation of *Teucrium* sections is also discussed. Most of the *Teucrium* species are perennial and also a small group of the genus is annual. The members of the genus *Teucrium* are shrubs, subshrubs, woody at base, rarely whole herbaceous. Several *Teucrium* species feature stolons or rhizomes and can spread vegetatively. Stem and leaf characters have been put forward in detail according to morphological investigations. The differentiation of leaf morphology is also described. Leaves of *Teucrium* species are opposite, petiolate, sessile or subsessile, simple, with entire or toothed margin, lobed or pinnatifid. The micromorphological characteristics of the *Teucrium* species are shown separately. Trichome micromorphology can be used as a discriminating character in the separation of species and subspecies. The trichome types are generally similar on adaxial and abaxial sides of the leaves, but lower sides are mostly denser. Subsessile peltate glandular trichomes are the most seen ones according to the literature and are occurred in almost all the species.

Keywords *Teucrium* · Sections · Vegetative morphology · Micromorphology

2.1 Introduction

Teucrium is a large and common genus of Lamiaceae family with about 400 species worldwide (Harley et al. 2004; Govaerts et al. 2013). Mediterranean is the major distribution area due to hosts about 195 taxa of the genus (Meusel et al. 1978; Navarro and El Oualidi 2000). *Teucrium* is a unique genus with its corolla structure. Species of this genus are characterized by the upper lip lackness of the corolla and this is a distinguishing character compared with the other members of Lamiaceae.

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Especially in some species, the stamens and stigma are greatly exserted from corolla (De Martino et al. 2010).

The members of the genus *Teucrium* are shrubs, subshrubs and mostly perennials, rarely annual or biennial. Leaves opposite, sessile or petiolate, simple, margines entire, toothed or lobed; inflorescences vary from simple to composites; calices vary from radial to bilabiate, tubular, campanulate or tubular-campanulate, 5-lobed, mostly 10 veins are also obvious, or sometimes the main veins are more obvious, calyx lobes differ from species to species, and all of them are same or sometimes different, the other 4 or different types of lobes, gibbouse or not at the base; corollas in very different colors, mostly short or almost equal to or longer than the calices, very rarely bilabiate and mostly one lipped (lower lip middle lobe), 5-lobed, the lower lobe is much larger than the others, usually inward; stamens 4, which of two short and two long filaments, exserted from corollas, obviously mostly longer than calices, filaments flat or curved, thecas separated; pistils with two carpels and four lobes, stigma lobes equal or almost equal, curved or not at the apex; nutlets obovate to almost orbicular, reticulated-wrinkled, with short eglandular and sessile glands or glabrous, endosperms usually available (Boissier 1879; Briquet 1895; McClintock and Epling 1946; Ekim 1982; Harley et al. 2004; Melnikov 2014; Özcan 2015).

Morphological variation can be seen in some *Teucrium* species according to the literature. Gaisberg (2000) divided *Teucrium heterophllyum* into three subspecies on the basis of their calyx lengths and the size of the branched, non-glandular trichomes on the calices and leaves. Trichome size, branched or unbranched trichomes, and the state of glandular or eglandular trichomes are used as very significant characters in many paper, too (Bentham 1833; Tutin et al. 1972; Kästner 1978; Manzanares et al. 1983; Antunes and Sevinate-Pinto 1991; Bini Maleci and Servettaz 1991; Servettaz et al. 1992; Navarro and El Oualidi 2000; Vural et al. 2015). *Teucrium orientale* L. (var. *glabrescens*, var. *orientale* and var. *puberulens*) is another species and its varieties can be easily distinguished by their trichome types and size on the adaxial and abaxial leaf surfaces. *Teucrium orientale* var. *orientale* has dense and elongated eglandular trichomes on the other hand *Teucrium orientale* var. *glabrescens* has sparse and shorter eglandular trichomes. All the three examined varieties have subsessile glandular trichomes (peltate) on the abaxial side of their leaves but only *Teucrium orientale* var. *orientale* has also adaxial side (Ecevit-Genc et al. 2015). Most of the *Teucrium* species have unbrunched trichomes on their leaves. *Teucrium polium* (Navarro and El Oualidi 2000; Lakušić et al. 2010; Eshratifar et al. 2011), *Teucrium capitatum* L. (Antunes et al. 2004) are two well known species with the branched trichomes on their leaves.

Most of the species of *Teucrium* grow mainly up on limestone cliffs, but some of endemics live on serpentine cliffs and some the other species live on basalt, volcanic tuff or marble. Section *Isotriodon* species grow only on rocky crevices and they are saxicolous plants, on the other hand most of the sect. *Scordium* members grow on wetlands. So, the species that live closest to the water are in this section (Stanković 2012).

The aim of this chapter is a general overview of the vegetative morphological characteristics of *Teucrium* species. The general characteristics of the life form, stem, leaf structure, as well as micromorphology will be presented. Comparative morphological differentiation of *Teucrium* sections will be discussed. Each subchapter will show accompanying micrographs, drawings and photographs of morphological details, as well as species on natural habitat.

2.2 Sectional Classification

The calyx shape and the inflorescence structure are used to identify the sections of *Teucrium* genus. The epidermal properties and trichome types of the nutlets show apparent differences among *Teucrium* sections. Kästner (1989) produced the first substantial revision of the sectional divisions by including corolla and vegetative characters. The detailed sectional classification was given in Table 2.1. The morphology of the leaf trichomes of *Teucrium* species with differentiation into thin and thick-walled trichomes provide valuable taxonomic distinguishing at the species level (Navarro and El Oualidi 2000; Eshratifar et al. 2011).

In addition to calyx shapes and trichome types, crenation of the leaves margins and attenuate leaves are very important for the classification of *Teucrium* sections. Leaves of the sect. *Teucrium* members entire or the crenation is very undetectable at first glance to deeply dissected, and their leaves are attenuate. This section is very interesting with crenation range among species. Leaves sessile or subsessile. Also, leaves are retrorse at the abaxial surface. Villous indumentum can be seen on different aerial parts of the plants. Some species of this section especially *Teucrium creticum* and *T. fruticans* are strongly fruticose, some of them are shrubs and the others are dwarf shrublets. The species are mostly rhizomatous. *Chamaedrys*, *Polium, Scordium*, *Stachyobotrys*, *Scorodonia*, *Spinularia* and *Isotriodon* sections have an obvious crenation on their leaf margins. Sect. *Chamaedrys* has obviously lobulate or dentate leaves, and the leaves are attenuate or cuneate at base and mostly subsessile or lower ones shortly petiolate. Leaves are glabrous to dense and sometimes retrorse-crispate hairy. This section members are generally small shrubs or suffrutescent. Especially *Teucrium chamaedrys* is rhizomatous and it can be found as heaps in the field trips. Stem lengths of the species are generally not as tall as the members of section *Teucrium*. Sect. *Scordium* members are obviously stoloniferous herbs and mostly live near or on wetlands. Leaves are toothed not crenated. Sect. *Polium* is distinguished with its terete and villous adpressed crispate hairy stems. Their leaves are usually tomentose and oblong to narrowly obovate or linear, obtuse, crenate to the base or middle, flat or revolute-margined. *Teucrium montanum* L. has not branched trichomes and dense indumenta on its aerial parts. All the species of the section *Polium* have globose heads consist of their flowers. *Isotriodon, Stachyobotrys* and *Scorodonia* sections are easily seperated with their calyx structure in the genus. With this properties they have some characteristic vegetative morphology. Section *Isotriodon* members are the most saxatile species of this genus. *Isotriodon* species are growing only the E. Mediterranean Region and Turkey has the most endemic species belonging to this section. This section members are

Table 2.1 Morphological differentiation of the *Teucrium* sections

Section	*Teucrium*	*Teucriopsis*	*Chamaedrys*	*Montanum*	*Polium*	*Scordium*	*Stachyobotrys*	*Scorodonia*	*Isotriodon*	*Spinularia*
Life form	Perennial	Perennial	Perennial	Perennial	Perennial	Perennial	Biennial or perennial	Perennial	Perennial	Annual
Stem	Shrubs, sub-shrubs or stoloniferous	Shrubs to 2–(3) m, short-hairy to tomentose	Dwarf shrubs, woody at base	Dwarf, woody and rotund at the base, erect to ascendance, sparsely hairy	Erect, woody at base, rotund to quadrangular, tomentose	Stoloniferous or erect, densely villous or velutinous	Shrubs, woody at base, hirsute or villous, with short eglandular trichomes to longer multicellular trichomes	Erect, conspicuously quadrangular	Dwarf, fragile, suffruticose, rotund to quadrangular	With spiny branches, hirsute
Leaf	Orbicular-ovate, oblanceolate, linear, obtuse, margin mostly entire to deeply divided, revolute at beneath, sparsely hairy	Ovate-lanceolate, obtuse, margin entire to bluntly crenate in the upper part, tomentose	Lobate or dentate-crenate	Lanceolate, elliptic to obovate, margin entire or unobvious dentate, revolute at beneath, sparsely glandular and eglandular hairy above, denser beneath	Sessile and simple, margin entire to deeply toothed-serrate, oblong-linear, spatulate, strongly revolute beneath, tomentose	Ovate to ovate-lanceolate, oblong, oblong-ovate, conspicuously toothed, crenate, subcordate at base, not petiolate to subpetiolate, villous-tomentose	Dentate-crenate, cuneate, truncate or cordate-subcordate at the base, short eglandular trichomes to long eglandular trichomes	Lanceolate to ovate, slightly rounded or blunt at the base, sessile or petiolate, tomentose	Ovate to ovate-lanceolate, slightly acute at apex, lower ones crenate, cuneate, subcordate to truncate at base, upper ones crenate to entire	Linear to elliptic-oblanceolate, obviously dentate, not rotund beneath, sparsely hairy above and denser beneath
Bract	Orbicular-ovate, Oblanceolate, linear, lanceolate	Leaf-like, obtuse	Leaf-like, dentate	Leaf-like, entire at margin	Bracts linear-spatulate	Leaf-like, rarely entire	Linear-subulate, shorter to longer than calyx	Ovate to ovate-lanceolate, sessile	Linear to oblanceolate, acute at apex, attenuate at base, pedicellate	Oblanceolate to ovate, entire or rarely crenated at the margin

obviously fragile and dwarf suffrutescent. Leaves are entire to dentate and subsessile to petiolate. Stems are mostly terete like sect. *Polium* and slender. Section *Stachyobotrys* members have the longest crenate-dentate leaves with the members of the section *Scorodonia*, and they can be around 80 mm length. Leaves have subsessile glandular trichomes but any capitate or clavate glandular trichomes could not be seen on abaxial or adaxial sides. Petioles of *Stachyobotrys* species are distinctive in *Teucrium* genus with around 35 mm length. Stems are villous or pubescent with 1–3 celled capitate glandular trichomes above. Petioles of section *Scorodonia* members are shorter than *Stachyobotrys* species, or subsessile. The colour of general view of the species are yellowish-greenish. Plants slightly rhizomatous like *Stachyobotrys* species, softly hirsute below, crisped-pubescent above. Retrorsing of leaves can also be seen in the sections *Polium*, *Chamaedrys*, and *Isotriodon* members (Tutin et al. 1972).

2.3 Vegetative Morphology

As mentioned before, most of the taxa belonging to different sections are perennial herbs from 20 cm to 2 m tall. Also, some members in the sect. *Stachyobotrys* are biennial, and some species from the sect. *Teucrium* are annual. On the other hand, the sect. *Spinularia* has totally annual species. When we exclude the members of *Spinularia*, *Teucrium* species are at least woody at base. But some members of the sect. *Teucrium* are woody (for instance *Teucrium creticum*, *T. fruticans* etc.). Section *Teucrium* is shrubby or subshrubby, *Teucriopsis* is shrubby to 2–(3) m, *Chamaedrys* is dwarf shrubby and woody at base, *Montanum* is dwarf, woody and terete at the base, *Polium* is woody at base, terete to quadrangular, *Scordium* is stoloniferous and rhizomatous, *Stachyobotrys* is shrubby and woody at base, *Scorodonia* is shrubby, *Isotriodon* is dwarf, fragile, suffruticose, rotund to quadrangular. So, the members of sections *Teucrium, Teucriopsis, Chamaedrys, Scordium, Stachyobotrys* and *Scorodonia* are mostly phanerophytes and the members of sections *Polium, Isotriodon* and *Spinularia* are mostly chamaephytes. The stems are frequently square in cross section, but this is not a strict characteristic of all *Teucrium* species, as well as whole family (Fig. 2.1). Several *Teucrium* species feature stolons or rhizomes and can spread vegetatively (Tutin et al. 1972).

Leaf morphology of *Teucrium* species vary considerably from species to species of plant, depending largely on their life form, although some species produce more than one type of leaf. The shape and size of the leaves are somewhat variable. Variability in shape and size, apart from the individual sections and species, is also observed on the same individual. The leaves of the upper and lower branches may be of different shapes and sizes, and the well lower and upper leaves of the same branch may vary to some extent, as well as leaves of the main stem and lateral branches. Leaves diameter can vary from 0.5 to 10 cm long and 0.5 to 5 cm across. The opposite arrangement of the leaves and branches on the stem is a feature of the whole family – leaf attachments are paired at each node and decussate. *Teucrium*

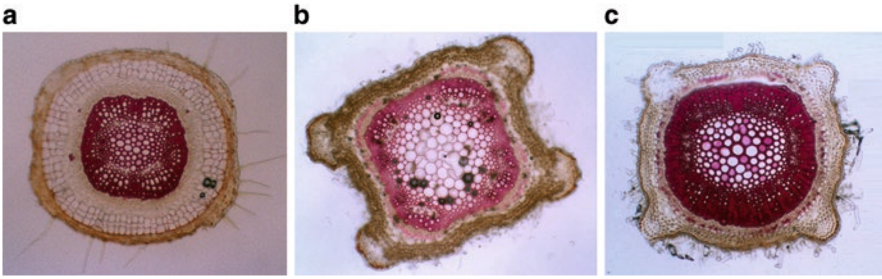

Fig. 2.1 Cross-section of *T. ekimii* (**a**), *T. sandrasicum* (**b**) and *T. divaricatum* (**c**) stem

species have a simple leaf blade, while some species have 1- to 2-pinatisect leaves. Leaf shape is characterized by most major types such as lanceolate, oblanceolate, ovate, obovate, triangular-ovate, ovate to orbicular, oblong, linear, linear-lanceolate, cuneate, cordate, truncate, eliptic, obtuse, acute, truncate, as well as different pinnatifid (Fig. 2.2). Leaf margins are very rarely entire, while the most common margin types are crenate, dentate, serrate and sinuate. The lamina is also characterized by different types of base and apex, while the surface may be glabrous, glaucous or pubescent with several degrees of hairiness, as well as differently expressed venation (Tutin et al. 1972).

Sect. *Teucrium* is characteristic in the genus with its leaf shapes. We can separate the section into four groups according to the leaf characters. For example, *Teucrium creticum* and *T. sandrasicum* have linear leaves with slightly toothed or entire margins; *Teucrium alyssifolium* and *T. pseudaroanium* have elliptic and entire leaves; *Teucrium multicaule* and *T. pestalozzae* have entire, bipartite and tripartite leaves on the same plants and *Teucrium orientale* and *T. parviflorum* have 2–3-pinnatipartite leaves. General characters of the leaves of the species belonging to section *Teucrium* are these followings: orbicular-ovate, oblanceolate, linear, obtuse, margin mostly entire to deeply divided, rotund at beneath, sparsely hairy. The leaves are mostly subsessile or rarely very short petiolate. Sect. *Teucriopsis* is characteristic with ovate-lanceolate and obtuse leaves, their margin entire to bluntly crenate in the upper part, and the leaves are obviously tomentose. Leaves of sect. *Chamaedrys* are oblong or obovate-oblong, cuneate at base, usually crenate-dentate or shortly lobulate, lobes entire or toothed. Obovate-oblong and dentate leaves are very significant to distinct the species belonging to this section. Leaves of sect. *Montanum* are lanceolate, elliptic to obovate, margin of the leaves are entire or unobvious dentate, rotund at beneath. On the other hand, the leaves of sect. *Polium* are sessile and simple, their margins are entire to deeply toothed-serrate, oblong-linear, spatulate, strongly rotund beneath, tomentose. Tomentose and spatulate leaves are distinguishing characters between *Polium* and *Montanum* sections. Sections *Scordium, Scorodonia* and *Stachyobotrys* have the longest leaves in the genus *Teucrium. Scordium* members have ovate to ovate-lanceolate, oblong, oblong-ovate leaves with conspicuously toothed, crenate, subcordate at base, subsessile to short petiolate, and villous or tomentose with thin or thick elongated trichomes. Leaves of sect. *Stachyobotrys* are more toothed-crenated than *Scordium* species, cuneate, truncate

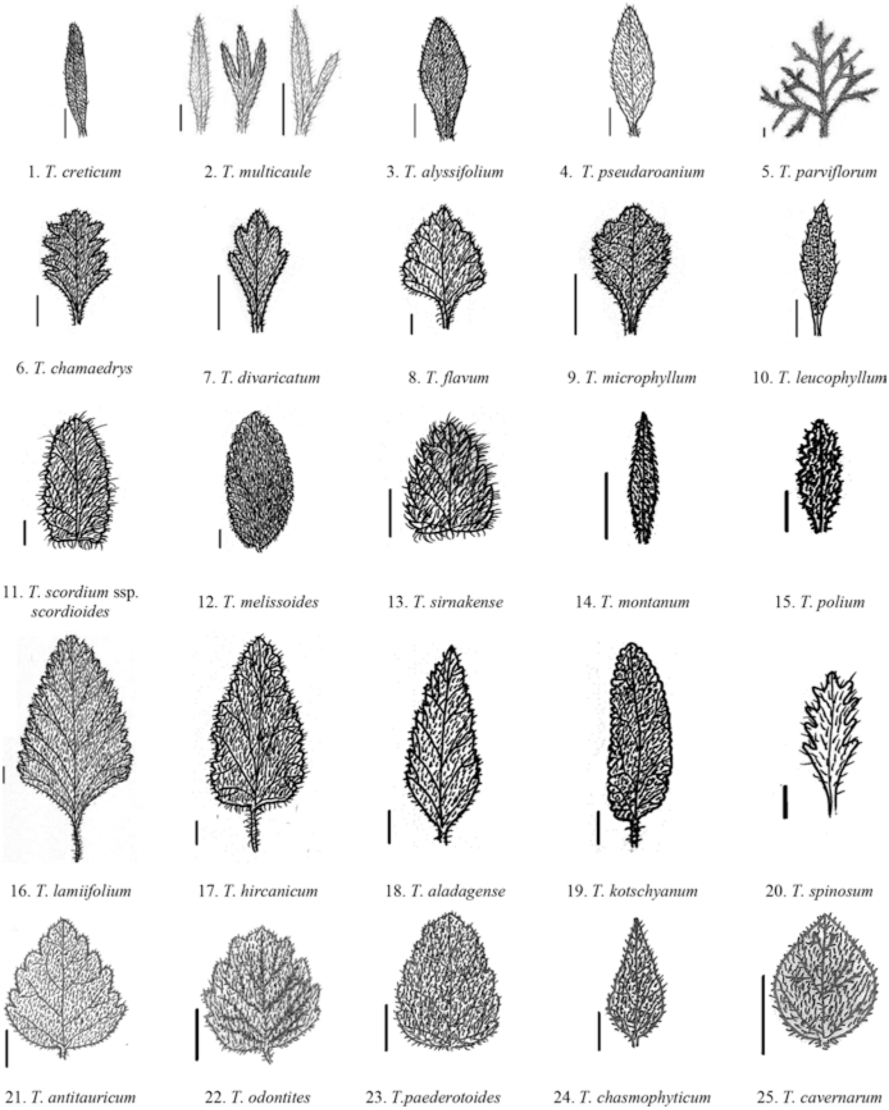

Fig. 2.2 Leaf morphology of selected *Teucrium* species 1–5: sect. *Teucrium*; 6–10: sect. *Chamaedrys*; 11–13: sect. *Scordium*; 14: sect. *Montanum*; 15: sect. *Polium*; 16–18: sect. *Stachyobotrys*; 19: sect. *Scorodonia*; 20: sect. *Spinularia*; 21–25 (scale bar 1 cm)

or cordate-subcordate at the base with short to elongated eglandular trichomes. We can see lanceolate to ovate leaves in the sect. *Scorodonia* and that means their leaves are narrower than the section *Stachyobotrys* and *Scordium* members, their leaves are slightly rounded or blunt at the base, sessile or petiolate and tomentose. The saxatile species of the genus (sect. *Isotriodon*) have ovate to ovate-lanceolate leaves, their leaves are slightly acute at apex, lower ones crenate, cuneate, subcordate to truncate at base, upper ones crenate to entire, glabrous or slightly hairy. And the annual *Spinularia* members have linear to elliptic-oblanceolate leaves with obviously toothed, their leaves are not rotund beneath. Sect. *Chamaedrys*, *Isotriodon* and *Stachyobotrys* are not branched or branched at base, the rest of the members have very branched stems from the middle or upper sides (Tutin et al. 1972).

2.4 Micromorphology

Trichome absence or presence and also their types have a significant role in the infrageneric classification of the genus *Teucrium*, and they are the most helpful characters to separate the species. There are many studies on *Teucrium* morphology and micromorphology (Antunes and Sevinato-Pinto 1991; Bini Maleci and Servettaz 1991; Servettaz et al. 1992; Marin et al. 1994; Navarro and El Oualidi 2000; Dönmez 2006; Parolly and Eren 2007; Jurišić Grubešic et al. 2007; Dinç et al. 2008, 2009, 2011a, b; Dönmez et al. 2010; Lakušić et al. 2010; Eshratifar et al. 2011; Kremer et al. 2012; Doğu et al. 2013; Özcan 2013; Ecevit-Genç et al. 2015, 2017, 2018; Özcan et al. 2015; Vural et al. 2015; Dinç and Doğu 2016).

El Oualidi and Puech (1993) provided the basic information for the members of *Polium* section based on the structure of the calyx teeth, the structure and degree of tessellation of the leaves from the bottom, and the internal and external indumentum of the corolla. Navarro and El Oualidi (2000) studied the trichome morphology of 56 Mediterranean species of *Teucrium* members. In their study, the indumenta of leaf, calyx, corolla, and nutlet were investigated. Twelve of the 25 trichome types were specific in this study. The indumentum structures on these parts are very distinctive for *Teucrium* sections. The trichome types are basically divided into four parts: simple capitate glandular, branched capitate glandular, peltate glandular and eglandular. El Beyrouthy et al. (2009) reported that corolla indumentum was very distinctive to separate sections. Eshratifar et al. (2011) studied the micromorphological characteristics of nutlets and leaves of some members of the genus *Teucrium*, and it was stated that although pubescence had obvious differences between different species, it was similar in the same section. In another study, the differences between glandular and eglandular trichomes of *Teucrium polium* leaves were examined in winter and summer months. In this study, it was revealed that the trichome structures, the volatile oils, and antioxidant activities changed periodically and it was clearly emphasized that the dimensions of this species differed in winter and summer months (Bosabalidis and Kofidis 2002). Gaisberg (2000) made a revision of *Teucrium heterophyllum* species, and as a result of this study, he discovered two new subspecies and presented morphological differences of subspecies of this species. Trichome morphology was very distinctive for the subspecies.

Lakušić et al. (2010) examined the adaptations of 5 *Teucrium* species due to their habitat differences. In this study, it was shown that indumentum was an important factor in adaptation to xeric environments. *Teucrium scordium* was investigated morpho-anatomically in this study, and according to their findings, glandular (peltate and capitate), and eglandular (unicellular or multicellular unbranched) trichomes were found on the leaves of this species. But Ecevit-Genç et al. (2018) did not observe any unicellular trichomes on the leaves of *Teucrium scordium* ssp. *scordium*. Jurišić Grubešić et al. (2007) investigated the trichomes of the leaves and stem of some *Teucrium* taxa from Italy. According to their findings, peltate, capitate glands and eglandular trichomes were found on *Teucrium montanum* leaves. Members of the sections *Polium* and *Montanum* are different from the other *Teucrium* sections with their inflorescences are arranged as a globose head. Also according to micromorphological studies, section *Polium* is also easily distinguished from all the other sections with the presence of branched eglandular trichomes on their leaves (*Teucrium polium*) (Ecevit-Genç et al. 2017).

Thin and thick-walled trichomes can be used to the delimitation of the species and could be regarded as a valid taxonomic character for the classification of *Teucrium* genus (Navarro and El Oualidi 2000; Eshratifar et al. 2011; Ecevit-Genç et al. 2015, 2017). Size, shape and density of glandular and eglandular trichomes are very significant for morphological differentiation of *Teucrium* species (Fig. 2.3).

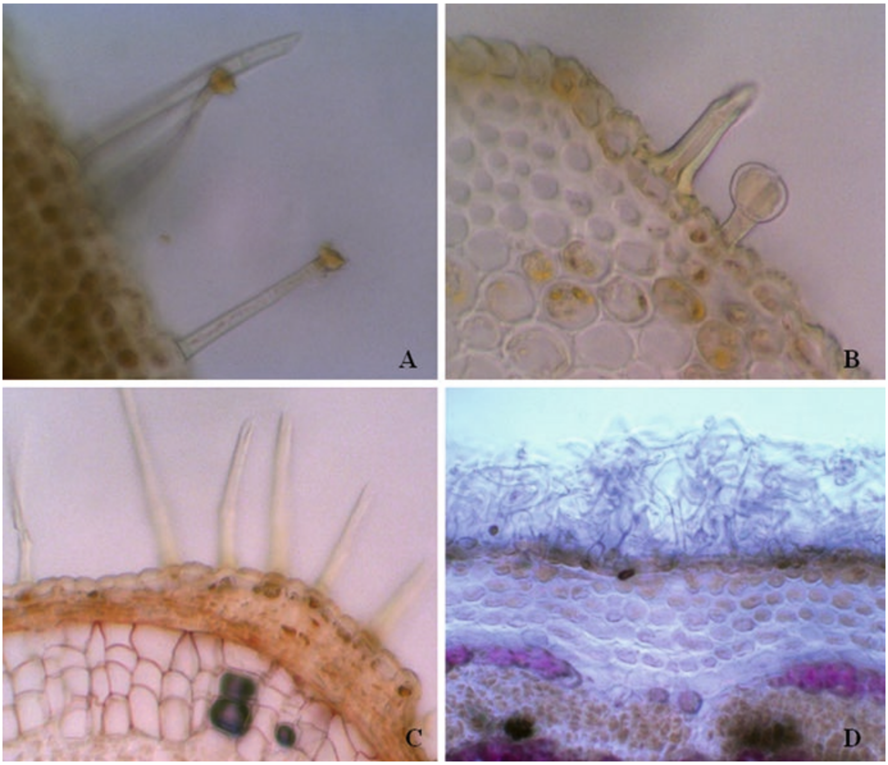

Fig. 2.3 General trichome types seen in transverse-sections of the stems: (**a**) *T. scordium* subsp. *scordioides* (sect. *Scordium*), (**b**) *T. chamaedrys* subsp. *chamaedrys* (sect. *Chamaedrys*), (**c**) *T. ekimii* and (**d**) *T. alyssifolium* (sect. *Teucrium*)

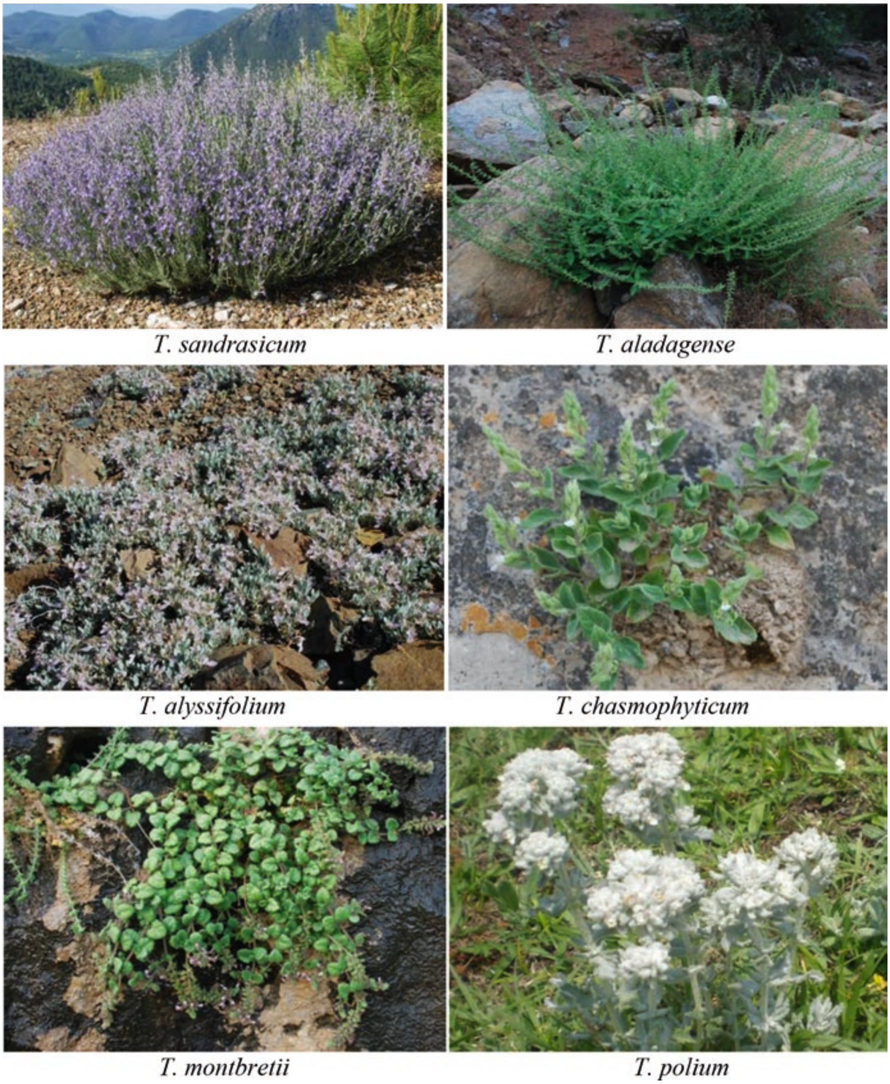

Fig. 2.4 General habitus of selected *Teucrium* species with different indumentum structure

The indument is also determined to some extent by the ecological conditions of the habitats. Figure 2.4 shows habitus of the six *Teucrium* species with different indumentum structure.

2.5 Conclusions

The genus *Teucrium* is one of the largest genera of Lamiaceae family. Certain morphological traits of this genus are characteristic of the whole family, but besides them, *Teucrium* species have many specificities regarding morphological differentiation. *Teucrium* species are perennial but a small group of species is annual. Most are shrubs, subshrubs, woody at base, rarely whole herbaceous plants. Some *Teucrium* species have stolons or rhizomes. Among the most common life forms are phanerophytes and chamephytes. The stems are frequently square in cross section. Leaves of *Teucrium* species are opposite, petiolate, sessile or subseeile, simple, with entire or toothed margin, lobed or pinnatifid. The lamina is also characterized by different types of base and apex, while the lamina surface may be glabrous, glaucous or pubescent with several degrees of hairiness, as well as differently expressed venation. The species of the genus *Teucrium* is characterized by a diverse indumentum structure composed of branched or unbranched eglandular as well as glandular trichomes. Trichome characteristics are a significant taxonomic character for the *Teucrium* genus. In addition to calyx shapes and trichome types, crenation of the leaves margins and attenuate leaves are very important for the classification of *Teucrium* sections.

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Chapter 3
Morphological Characteristics of *Teucrium* Species: Generative Morphology

Teresa Navarro

Abstract The generative morphology related with corolla and calyx construction, pollen, inflorescence type and nutlets are of great importance in *Teucrium* L. These characters are the basis for the classic systematic and recent classifications. *Teucrium* has a corolla usually 1-lipped or 2-lipped (bilabiate). *Spartothamnella* Briq., *Oncinocalyx* F. Muell., and *Teucridium* F. Hook, closely related to *Teucrium*, present a corolla 5-lobed in the upper half, lobes slighty spreading, 4 posterior ± similar, anterior lobe larger than the others and not concave. The calyx in these genera is actinomorphic. This is an exceptional corolla conformation in *Teucrium* and only found in these species. The main difference between *Spartothamnella* and *Teucrium* is that the former has drupaceous fruit and between *Teucrium* and *Teucridium* is the ovary form. *Oncinocalyx* differs from *Teucrium* in the conspicuously hooked calyx lobes. *Teucrium* Sections *Pycnobotrys* Benth., *Stachyobotrys* Benth., *Scorodonia* (Hill) Schreb., and *Teucriopsis* Benth., have weakly 2-lipped corolla and zygomorphic calyx. Species of section *Teucrium* have 1-lipped spurred corolla and actinomorphic calyx except for the Australian species with non-spurred corollas. *Teucrium* show a great diversity in floral phenotypic diversity and adaptive significance to pollination and dispersal. Functional redundancy is found in the gullet-shaped corollas with nectar guides and nototribic pollination of the sections *Chamaedrys* (Mill.) Kästner, *lsotriodon* Boiss., subsection *Scordium* (Mill.) Kästner, *Montana* Lazaro Ibiza and subsection *Pumila* (Lazaro Ibiza) Rivas Mart. *Teucrium* is the only genus within the sub family Ajugoideae with two derived pollen characteristics, granulate or verrucate sculpturing and operculate colpi and with a considerable variation in pollen sculpturing. Nototribic pollination of the gullet-shaped corollas with closed gullet mechanism (by a palate of hairs associated to a slight pouch in the corolla tube) are exclusive of the section *Teucrium.* The sternotribic pollination of the brush-shaped corollas closed by a tuft of hair in the entrance of the corolla tube (base of the anterior lobe), are specific of the ginodioecious species of section *Polium* (Mill.) Schreb. Resupinate flag-shaped corollas with sternotribic pollination

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is present in subsection *Spinularia* (Boiss.) Kästner and *Stachyobotrys*. The main dispersal modes in section *Chamaedrys, lsotriodon* and *Polium* is the semachory. Sections *Teucrium* and *Teucriopsis* are wind-dispersal by pogonospores, subsection *Spinularia*, section *Scorodonia* and *Teucrium betonicum* and *Teucrium abutiloides* L'Hér from section *Teucriopsis* are ballospores.

Keywords *Teucrium* · *Spartothamnella* · *Oncinocalyx* · *Teucridium* · Corolla and calyx construction · Inflorescence · Nutlets · Pollen · Pollination · Dispersal · Lamiaceae

3.1 Introduction

The “bilabiate blossom” characterizing the Lamiaceae is a floral construction for nototribic (dorsal) pollen deposition (Claßen-Bockhoff 2007). The floral construction is a syndrome of adaptive characters from generative structures needed for pollen transfer that have evolved under phylogenetic developmental and environmental constraints. The floral construction allows understanding of the ecological and morphogenetic constraints of flower evolution in Lamiaceae (Claßen-Bockhoff 2007) and it is particularly interesting in *Teucrium* because most of the species have one-lipped flowers except for the section *Pycnobotrys, Stachyobotrys, Scorodonia* and *Teucriopsis* with weakly bilabiate (two-lipped) flowers. The *Teucrium* phylogenetic related genera, such as *Spartothamnella* Briq., *Oncinocalyx* F. Muell., and *Teucridium* F. Hook, do not have one-lipped or two-lipped (bilabiate) flowers; they own flowers with corolla of five-lobed, four posterior ± similar, and anterior lobe larger.

Some studies have also investigated the variation of floral construction in different flower morphs related to the breeding system and the evolution of separate sexes in gynodioecius *Teucrium* species (Puech 1978; El Oualidi 1987; Alados et al. 1997; Rodríguez-Riaño and Dafni 2007) and *Teucridium* genus (Merrett 2005).

Teucrium and its related genera show a great phenotypic diversity and adaptive significance in the morphological generative characters related to breeding system, pollination and other functional generative traits such as dispersal. *Teucrium* is melittophilous and pollinated mainly by bees (van der Pijl 1972) and wasps (Petanidou 1996), it is a good example of a sympetal, tubular, hermaphrodite and gynodioecious, proterandrous flower. In particular, this flower type is interesting in *Teucrium*, because it has mainly one-lipped corollas with bilateral symmetry, slightly campanulate tube and the lower lip with five asymmetrical lobes; this corolla type also occurs in related genera of the Ajugoideae. The lower lip acts as a landing platform and provides an easier access to the nectar, but in some sections, a barrier in the tube may still act as a selective device. The variations in the corolla construction, size, lobes symmetry, specialized features of lower lip, resupination of the tube are the result of a selection by pollinators, which leads an adjustment in floral forms to efficient pollen transfer and fruit set. The most common *Teucrium* calyx is five-lobed and persistent, except when falls with the diaspores inside and plays a part in seed dispersal (van der Pjil 1972; Bouman and Meeuse 1992; Paton 1992; Navarro et al. 2009). The variation in the calyx symmetry, morphology of the tube and teeth while as functioning of the pedicel, express the adaptation to a certain kind of seed dispersal mechanisms and a protection of immature nutlets (Navarro and El Oualidi 1999). The sculpturing and size of the nutlets have an important role in dispersal mechanisms (Bouman and Meeuse 1992). Most of the species show a flowering peak at the end of spring but may also extend their flowering season into the summer and autumn (Navarro and Cabezudo 1995).

Teucrium pollen grains are trizonocolpate, isopolar and radiosymmetric. They are circular to circular-lobulate in polar view and sinuaperturate; in equatorial view they are elliptic and usually subprolate to prolate. Pollen surface is minutely perforated with granules, less often with verrucae and with a supractectal reticulum or with an intermediate sculpturing composed of short, discontinuous supratectal ridges (Navarro et al. 2004). *Teucrium* is the only genus within the sub family Ajugoideae in the Mediterranean region with two derived pollen characteristics, granulate or verrucate sculpturing and operculate colpi. Abu Asab and Cantino (1993) pointed to the considerable variation in pollen sculpturing within *Teucrium*, distinguishing three sculpturing types: the verrucate sculpturing (bearing supratectal wart-like projections), which is considered as a derived pollen character in Lamiaceae, the suprareticulate exine (supratectal ridges forming a reticulate pattern), a plesiomorphic condition within the subfamily Nepetoideae (Wagstaff 1992), and an intermediate sculpturing type transition from the verrucate to the suprareticulate composed of short discontinuous supratectal ridges. The first type is considered as the norm in *Teucrium*, while the last two are less common. Nabli (1970, 1976), El Oualidi (1987), Diez et al. (1993), Abu-Asab and Cantino (1993) and Navarro et al. (2004) described the pollen morphology of many species of section *Polium*. The pollen morphology supports the phylogenetic relationships between these genera and *Teucrium* (Abu Asab and Cantino 1993), forming the *Teucriina* clade (Cantino et al. 1997), based on the operculate and verrucate pollen.

The generative characters related with corolla and calyx construction, pollen, inflorescence type and nutlet morphology are of great importance in *Teucrium.* These characters are the basis for the classic and recent systematic classifications, the great useful for delimiting and structuring the most problematic taxonomic groups. In order to examine the floral phenotypic diversity, a sample of 341 specimens of 97 *Teucrium* taxa and its close related phylogenetic genera *Spartothamnella*, *Oncinocalyx* and *Teucridium* from throughout the world have been studied in addition to whose examined in previous works (Navarro and El Oualid 1999; Navarro 1995, 2010). This study based on the field, herbarium specimens and bibliographic references of the regional Floras. Flowers and nutlets were collected in the field or from authorized Herbarium specimens (see Appendix in Chap. 1). The main floral features, such as the inflorescence type, corolla size, corolla colour, flower sexuality and main flowering season were measures or observed. The flower form related to the pollination based on Faegry and van der Pjil (1979). The primary dispersal mode follows van der Pijl (1972), Zohary (1937) and Bouman and Meeuse (1992) and the diaspore type is described in accordance with Dansereau and Lems (1957) and Jenny (1995). The sectional delimitation of *Teucrium* is according to Bentham (1833), Boissier (1879) and Navarro (2010). The examined specimens are listed in the Appendix included in the Chap. 1.

The aim of this study is described the phenotypic diversity and adaptive significance of the *Teucrium* flower construction to carry out an overview of the diversity of the generative characters, especially floral features related to pollination and dispersal mechanisms, providing a synthesis of the predominant generative strategies and their infrageneric diversity.

3.2 Floral Phenotypic Diversity and Adaptive Functional Significance in *Teucrium* and Related Genera

Teucrium has a corolla usually 1-lipped or 2-lipped, 5-lobed (with the anterior lobe much larger than the others), concave, occasionally unequal 5-lobed in the upper half; lobes slightly spreading, 4 posterior ± similar (anterior lobe larger than the others). *Oncinocalyx betchei, Spartothamnella teucriiflora* and *Teucridium parvifolium* present a corolla 5-lobed in the upper half, lobes slighty spreading, 4 posterior ± similar, anterior lobe larger than the others and not concave, with an annulus of hairs at the middle part of insertion of stamens in the corolla tube (Table 3.1). The calyx in these genera is actinomorphic. This is an exceptional corolla conformation in *Teucrium* and only found in these species. The main difference between *Spartothamnella* and *Teucrium* is that the former has drupaceous fruit and between *Teucrium* and *Teucridium* is the ovary form, unlobed or lobed up to a quarter part of its length in *Teucridium* and usually lobed from a quarter to half its length in *Teucrium*. *Oncinocalyx* differs from *Teucrium* in the conspicuously hooked calyx lobes. Variation in corolla construction is show in Table 3.1 and corolla characters related with pollination in Table 3.2. A schematic drawing of the general conformation of the *Teucrium* flower is shown in Fig. 3.1.

Teucrium and its close related genera also differs from the other members of Lamiaceae based on non-gynobasic style. The ovary is bicarpellary, syncarpous, 4-lobed, 4-locular, with one ovule in each cell attached to an axile placenta, the style terminal, exserted, filiform, with 2 stigmatic lobes at the top. Fruit dry, schizocarpic, splitting into 4 mericarps (nutlets), enclosed in the persistent calyx, except for the drupaceous fruit in *Spartothamnella*.

The basic inflorescence is a raceme derived from a tyrse of verticillasters by floral reduction, originating contracted verticillasters, grouping into heads (Navarro and Cabezudo 1995). The infrageneric variation from axillary flowers or lax verticillasters (2–8 flowers) to simple dense terminal or branched raceme of contracted verticillasters is show in Table 3.1.

The calyx is tubular or campanulate, urceolate (gibbous) in plate or obconical, ventral, dorsiventral or dorsally pedicellated, 10-nerved, zygomorphic (bilabiate),

Table 3.1 Floral construction of the infrageneric taxa of *Teucrium* and its related genera *Oncinocalyx, Spartothamnella* and *Teucridium*

Teucrium infrageneric taxa	Broad upper calyx lip	Calyx construction	Corolla construction	Internal indumentum of corolla	Calyx pedicellum insertion	Corolla tube short apically broadly
Teucrium (Mediterranean area)	Absent	Actinomorphic Calyx	1-lipped, llp. not forming angle with the tube	Annulus of hairs at the base of the ct.	Ventral	Present
Teucrium (*T. racemosum* group)	Absent	Actinomorphic Calyx	1-lipped, llp. not forming angle with the tube	Annulus of hairs at the middle part of the ct.	Ventral	Present
Teucrium (Austral areas)	Absent	Actinomorphic Calyx	5-lobed,4 posterior ±similar, anterior lobe larger	Annulus of hairs at the middle part of the ct.	Ventral	Present
Teucriopsis	Absent	2-lipped 1/4	2-lipped 2/3, llp. emarginated or two lobed,	Without indumentum	Dorsi-ventral	Absent
Scorodonia	Present	2-lipped 1/4	2-lipped 2/3, ul. developed	Without indumentum	Dorsi-ventral	Absent
Pycnobotrys	Present	2-lipped(1/4)/ zygomorphic (1/2/2)/ Sub-actinomorphic (3/2)	Sligth 2-lipped (pseudo-2-lipped)	Without indumentum	Dorsi-ventral	Absent
Stachyobotrys	Present	Zygomorphic (1/2/2)	Sligth 2-lipped (pseudo-2-lipped)	Without indumentum	Dorsal	Absent
Isotriodon	Absent	Zygomorphic (1/2/2)	1-lipped, llp forming angle with the ct.	Hairs at the base of ml. forming two guides	Dorsi-ventral	Absent
Chamaedrys subsection *Chamaedrys*	Absent	Sub-actinomorphic (3/2)	1-lipped, llp forming angle with the ct.	Hairs at the base of ml. forming two guides	Dorsi-ventral	Absent
Chamaedrys subsection *Marum*	Absent	Sub-actinomorphic (3/2)	1-lipped, llp forming angle with the ct.	Hairs at the base of ml. forming two guides	Dorsi-ventral	Absent

(continued)

Table 3.1 (continued)

Teucrium infrageneric taxa	Broad upper calyx lip	Calyx construction	Corolla construction	Internal indumentum of corolla	Calyx pedicellum insertion	Corolla tube short apically broadly
Montana	Absent	Sub-actinomorphic (3/2)	1-lipped, llp forming angle with the tube,	Hairs at the base of ml. forming two guides	Dorsi-ventral	Absent
Polium subsection *Polium*	Absent	Sub-actinomorphic (3/2)	1-lipped, llp not forming angle with the ct.	Hairs at the base of the ml. forming a tuf	Dorsi-ventral	Present
Polium subsection *Pumila*	Absent	Sub-actinomorphic (3/2)	1-lipped, llp forming angle with the ct.	Hairs at the base of ml. forming two guides	Dorsi-ventral	Absent
Polium subsection *Simplicipilosa*	Absent	Sub-actinomorphic (3/2)	1-lipped, llp not forming angle with the ct.	Hairs at the base of the ml. forming a tuf	Dorsi-ventral	Present
Scordium	Absent	Sub-actinomorphic (3/2)	1-lipped, llp forming angle with the ct.	Hairs at the base of ml. forming two guides	Dorsi-ventral	Absent
Scordium subsection *Botrys*	Absent	2-lipped (1/4)	1-lipped, llp forming angle with the ct.	Hairs at the base of ml. forming two guides	Dorsal	Absent
Scordium subsection *Spinularia*	Absent	2-lipped (1/4)	1-lipped, llp forming angle with the ct.	Hairs at the base of ml. forming two guides/ without indumentum	Dorsal	Absent
Oncinocalyx genus	Absent	Actinomorphic Calyx	5-lobed,4 posterior ±similar, anterior lobe larger	Annulus of hairs at the middle part of the ct.	Ventral	Present
Spartothamnella genus	Absent	Actinomorphic Calyx	5-lobed,4 posterior ±similar, anterior lobe larger	Annulus of hairs at the middle part of the ct.	Ventral	Present

(continued)

Table 3.1 (continued)

Teucrium infrageneric taxa	Broad upper calyx lip	Calyx construction	Corolla construction	Internal indumentum of corolla	Calyx pedicellum insertion	Corolla tube short apically broadly
Teucridium genus	Absent	Actinomorphic Calyx	5-lobed,4 posterior ±similar, anterior lobe larger	Annulus of hairs at the middle part of the ct.	Ventral	Present

ct corolla tube, *ml* middle lobe, *llp* latero-posterior corolla lobes

sub-actinomorphic or actinomorphic, tube within glabrous or pilose at throat, the 5 teeth, equal or unequal, some times mucronate to hooked at end or cucullate (Table 3.1).

The infrageneric adaptive functional significance of floral features to pollination and dispersal is summarized below. Very detailed illustrations of the inflorescence type, corolla and clayx construction and nutlets morphology can be consulted in Navarro (2010).

The corolla of section *Teucrium* is one-lipped, gullet-shaped and slightly personate (with a slight pouch) and shortened tubuled, lilac or white with closed gullet mechanisms by a palate of hairs; the lateral and latero-posterior lobes are developed. Flowers are axillary in the leaves or in lax verticillasters (Fig. 3.2) and the flowering occurs in spring or multi-seasonal floration in winter-spring-summer. In this group, the most important factor for attracting pollinators is presumably the strong visual signal of their large flowers described by Shmida and Dafni (1989) as flowers engaged in a "discovery advertisement" (Fig. 3.2). The campanulate actinomorphic calyx does not have a closed mechanism to protect the immature seed. They are wind-dispersal species with a big hairy nutlet, ancestral character in the genus (Marin et al. 1994; Navarro and El Oualidi 2000b). The diaspore type is pogonospore. Section *Teucrium* is a basal group within *Teucrium* (Marin et al. 1994; Navarro and El Oualidi 2000a; Navarro et al. 2004; Salmaki et al. 2016). This section has widespread floral characters in the structurally simple states, including the actinomorphic calyx and unclearly lipped corollas (Austral species of section *Teucrium*) such as *Spartothamnella, Oncinocalyx* and *Teucridium* present in geologically ancient areas.

Section *Teucriopsis* comprise endemic species from Macaronesian region. The flowers are in verticillasters or in lax axillary cymes in the upper leaves. The corolla blue, orange or cream is weakly bilabiate, wide and shortly tubular; the calyx is campanulate, actinomorphic, some with an annulus of hairs inside; the nutlets are large and haired.

Species from section *Scorodonia* have one-lipped gullet-shaped corollas with long and narrow tube and a lower lip and latero-posterior lobes well-developed without nectar guides; the calyx is bilabiate with an annulus of hairs inside. Flowers

Table 3.2 Main inflorescence type, corolla size (mm), corolla colour, main flowering season and pollinators in the infrageneric taxa of *Teucrium* L

Teucrium infrageneric taxa	Inflorescence	Corolla size (mm) Length of tube and lower lip	Corolla colour	Main flowering season	Pollinators
Teucrium	Axillary flowers or lax verticillasters	(10)12.5–14.5(22)	Lilac/white	End winter-spring-summer (III–VI)	Large solitary bees
Teucriopsis	Axillary flowers or lax verticillasters	(7.5)9.5–11.5(13.5)	Blue/orange/white	Summer (V–VI)	Large solitary bees
Chamaedrys	Lax verticillasters/sub-spirally racemes	(9.5)11.5–12.5(14.5)	Purple/pink/yellow	Summer (V–VI)	Small solitary bees, flies
Montana	Sub-spirally raceme	(8)10.5–12.5(17)	Cream/white-pink/purple/yellow	Summer (V–VII)	Solitary bees, ants, flies
Scorodonia	Verticillasters	(5.5)7–8(11)	Cream/pink	End spring-summer (IV–VI)	Long-tongued butterflies, bees
Scordium	Axillary flowers/verticillasters (2–8) flowered	7.5–8.5	Cream/pink	Summer (V–VII)	Small solitary bees
Stachyobotrys	Dense Verticillasters like spike	7.5–9.5	Cream/cream-yellow	End spring-summer (IV–VI)	Small solitary bees, flies
Scordium subsection *Spinularia*	Verticillasters (2–4) flowered	(6.8)8.5–9(15.5)	Cream/cream-pink	Summer (V–VIII)	Solitary bees
Isotriodon	Sub-spirally racemes	(5.5)8–10(14.5)	Cream/pink-purple/yellow	Spring (IV–V)	Solitary bees, flies
Polium subsection *Polium*	Simple dense terminal or branched raceme	(3)3.5–4.5(6.5)	White/cream-pink/yellow	End spring-end summer (IV–VIII)	Small solitary bees, wasps, ants, flies
Polium subsection *Simplicipilosa*	Simple dense terminal or branched raceme	(3)3.5–4.5(6.5)	White/cream	Summer (VI–VII)	Small solitary bees, wasps, ants, flies

(continued)

Table 3.2 (continued)

Teucrium infrageneric taxa	Inflorescence	Corolla size (mm) Length of tube and lower lip	Corolla colour	Main flowering season	Pollinators
Polium subsection *Pumila*	Simple dense terminal or branched raceme	5.5–6	White/ purple	Summer (V–VII)	Solitary bees, long-tongued butterflies, ants

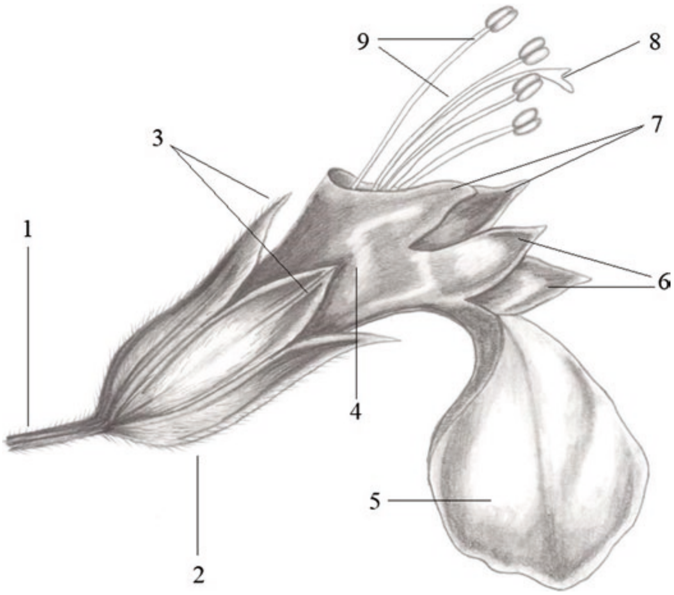

Fig. 3.1 Schematic drawing of the general conformation of the *Teucrium* flower. 1 – Flower pedicel; 2 – Calyx; 3 – Calyx lobes; 4 – Corolla tube; 5 – Anterior corolla lobe; 6 – Lateral corolla lobes; 7 – Latero-posterior corolla lobes; 8 – Style; 9 – Stamines

are disposed in verticillasters and the main flowering season is the summer Fig. 3.3. They are wind-ballistic or/and rain-ballistic species with glabrous and smooth nutlets. These features are in accordance with the ones described by Paton (1992) in *Scutellaria*.

Section *Isotriodon* includes species with infloreseence in a sub-spiral raceme; the calyx is tubular-campanulate, sub-actinomorphic, gibbous at the base, ±pedicellate with the upper tooth shorty ovate, the lower teeth narrower and acute; the corolla has long tube with well-developed lobes and two nectar guides of hairs on the lower lip; the nutlets have glandular hairs. In the species of section *Chamaedrys,* the flowers are placed in lax verticillasters or in sub-spirally lax racemes; the corolla has

Fig. 3.2 *T. fruticans* L. (section *Teucrium*)

Fig. 3.3 *T. oxylepis* Font Quer (section *Scorodonia*)

long latero-posterior lobes, acute and haired and the lower lip has two clear nectar guidelines of hairs; the calyx is tubular-campanulate and actinomorphic (Fig. 3.4) with an annulus of hairs inside and the nutlets are haired.

The greatest convergence of floral features is show in the species of section *Isotriodon*, the rocky species of section *Chamaedrys* and the rocky species of section *Montana* indicating a higher incidence of functional redundancy. The corolla is one-lipped, gullet-shaped and with regular tube (tube >5 mm), with two clear nectar guides in the lower lip, without closed gullet mechanisms and with well-developed

Fig. 3.4 *T. webbianum* Boiss. (section *Chamaedrys*)

Fig. 3.5 *T. charidemi* Sandwith (section *Polium* subsection *Simplicipilosa*)

and specialized latero-posterior lobes acting as guiding visitors. Flowers are purple, white or cream, rarely yellow, flowering mainly at the end spring and summer. They are wind dispersal species with campanulate, sub-actinomorphic calyx generally without annulus of hairs inside. They are also wind-ballistic species, or seed-dispersal inside the calyx. In this last case, the calyx is easily detached and generally dry and papery. In some species such as *Teucrium charidemi* Sandwith (Fig. 3.5), *T. compactum* Clemente ex Lag., and *T. yemense* Deflers, the inflated calyx ensure long distance dispersion by the wind, this type is found by Zohary (1937) and

Fig. 3.6 *T. scordium* L. (section *Scordium*)

Navarro et al. (2009) in desert species. This group of species shows a highly convergence exemplified by the lax inflorescence in sub-spirally simple racemes.

Species from the section *Scordium* have one-lipped, gullet-shaped corollas with nectar guides. Flowers are placed in verticillasters (2–8) flowered axillary in upper leaves; the calyx is tubular-campanulate, and sub-actinomorphic; the corolla has two nectar guides on lower lip and the nutlets are glandular haired (Fig. 3.6).

The species of section *Scordium* subsection *Spinularia* have corolla one-lipped, gullet and flag-shaped with a long and narrow tube, resupinate in some species. The strongly zygomorphic, gibbous calyx have an annulus of hairs inside. They are wind-ballistic species and possibly epizoochores, since most of the species show spinescent calyx teeth. The flowers (2–4) placed in axillary verticillasters in the upper leaves; the nutlets are small and glabrous. The species flowering mainly in spring and summer (Fig. 3.7).

In the section *Stachyobotrys,* the flowers are in dense verticillasters like spikes; the lateral lobes of the corolla are reduced or absent, in some cases, the tube is resupinate; the calyx is campanulate, bilabiate often-gibbous al the base with spinescent teeth, upper tooth weakly broad, 2 lower teeth lanceolate and the nutlets have glandular hairs. Some species from section *Stachyobotrys* such as *Teucrium collincola* Greuter & Burdet show a particular resupinate corolla without nectar guides and with a well-developed lower lip, the latero-posterior lobes are almost totally reduced and the lateral lobes are very short. The calyx is strongly zygomophic and campanulate. They are rain-ballistic or/and wind-ballistic species, some of them show the long mucronated calyx teeth, perhaps related to the epizoochory.

Species belonging to section *Polium* subsections *Polium* and *Simplicipilosa* are ginodioecious species (Fig. 3.8) with one-lipped brush-shaped corollas shortened tubuled with closed gullet mechanisms by a tuft of hairs to exclude inefficient

Fig. 3.7 *T. spinosum* L. (section *Scordium* subsection *Spinularia*)

Fig. 3.8 *T. teresianum* Blanca, Cueto & J. Fuentes (section *Montana*)

visitors and limit pollinator diversity. Flowers are compacted in the dense racemes forming the branched inflorescence (Figs. 3.8 and 3.9). Flowering mainly in spring, the ginodioecious system imposes limitations in pollination and rewards partitioning between females, generally pink and with nectar reward flowers and hermaphrodite flowers (white or cream). The calyx is sub-actinomorphic. They are wind-dispersal species and the calyx falls with the nutlet inside. Some species from arid regions have cucullate calyx teeth that close the calyx tube and protect the nutlets. In other species like *Teucrium charidemi* (Fig. 3.5) or *T. musimonum* Humbert, the dense vermiform hairs cover the calyx outside to facilitate the dispersion by the air that pushes them hovering over the ground.

Fig. 3.9 *T. lusitanicum* Schreb. (section *Polium*)

3.3 Corolla Construction and Pollination Adaptive Significance

Teucrium exhibit a great diversity in flower construction. Table 3.1 show the main infrageneric generative traits in *Teucrium* related to the pollination. *Teucrium* has with white, cream, pink and purple flowers. The orange and blue flowers are exclusive from section *Teucriopsis* and the lilac from section *Teucrium*. The most common flower construction related to pollination in *Teucrium* are the gullet-shaped corollas with nototribic pollen deposition; this type occurs in all sections of the genus except in section *Polium* subsection *Polium.* The flag-shaped corollas with sternotribic pollen deposition are rare and exclusive of the species with resupinate corollas from subsections *Spinularia* (Fig. 3.7) and section *Stachyobotrys*. In these cases, the gullet-shaped corollas are reversed and function like a flag-shaped and there may be a simple resupination through torsion of the corolla tube, and the lower lip only acts as an attraction and the lateral and latero-posterior lobes are reduced or absent (Fig. 3.7). The brush-shaped corollas with diffuse pollen deposition are exclusive of the ginodioecious flowers of the section *Polium.* The bilateral symmetric flowers such as those of the section *Teucrium* induce a more specific behavior to ensure that stigma and anthers are touched effectively (Fig. 3.2). Sternotribic and nototribic pollen deposition occur in this last section.

Teucrium species have corollas closed to ineffective insect visitors. We observed two closed gullet mechanisms related to pollination: (1) A barrier, which offers resistance and demands precision on the part of the pollinator. This barrier formed by a palate of hairs in the region of the common basal part of the latero-posterior lobes and stamine insertion, which is associated to a slight pouch (Navarro and El Oualidi 1999). When the insect poses on the lower lip, its force makes the lip bend down and the tube closed with an annulus of hair. This mechanism is specific of the species from the section *Teucrium.* (2) A barrier to exclude the inefficient visitors, formed by an annulus of hairs from the base of the stamine insertion to the basal part of the anterior lobe. This basal part is cover densely by hair forming a tuft, which also serves as a foothold for the pollinator visitors. This mechanism is exclusive of the unspecialized ginodioecious species of section *Polium* subsections *Polium* and *Simplicipilosa* and section *Montana* (Navarro and El Oualidi 1999) and *Teucridium* genus (Merrett 2005).

The gullet-shaped corollas without closed gullet mechanism show the guiding of visitors such as the nectar guides formed by two clear rows of hairs in the adaxial side of the lower lip extending to the entrance of the corolla tube. These guidings are common in the species of the sections *Chamaedrys, lsotriodon, Scordium, Montana* and *Polium*. The calyx does not play an important role in the pollination syndromes because it does not have a known semaphyll function, but has glandular hairs emitting scent adding to the attraction for visitors.

3.4 Calyx Construction, Nutlet Morphology and Dispersal Adaptive Significance

Table 3.3 show the main infrageneric generative traits in *Teucrium* related to the dispersal. The predominant primary dispersal mode in *Teucrium* is the semachory and ballautochory. Wind-dispersal or wind-ballistic species (semachores or ballospores) are found in *Teucrium heterophyllum* L'Her from section *Teucriopsis* and in the species of the sections *Teucrium, Chamaedrys, Scordium*, *Isotriodon* and *Polium*. Ballautochory, wind-ballistic or rain-ballistic species (ombrochory) found in the section *Scorodonia, Stachyobotrys*, subsection *Spinularia* and in *Teucrium abutiloides* and *T. betonicum* from the section *Teucriopsis.* Epizoochory is rare in the genus, found in some species with spinescent calyx from subsection *Spinularia*. Meteo-anemochory only found in the section *Polium.*

The sculpturing and size of the nutlets (diaspore) have an important role in dispersal mechanisms (Bouman and Meeuse, 1992). Pericarp surface of the nutlets in *Teucrium* are variously ornamented (Marin et al. 1994); smooth (section *Scorodonia*); haired (section *Teucrium*, *Teucriopsis* and *Chamaedrys*) (Fig. 3.10); with glands (sections *Chatnaedrys, Isotriodon* and *Montana*) and very reticulated (section *Polium* subsection *Polium*) to maximize the dispersion by the wind. The variation in the type of indument of the nutlets are described in Navarro and El Oualidi (2000b).

The main diaspore type present in *Teucrium* are the semaspores associated to the species with actinomorphic or sub-actinomorphic, pedicelled calyx. This dispersal mode characterized *Thymus* (Bouman and Meeuse 1992). Ballospores are associated to species with bilabiate or strongly zygomorphic calyx and pogonospores (diaspores largest than 2 mm) with the actinomorphic and campanulated calyx.

The calyx morphology vary in relation to the protection of the immature nutlets by two type of system: (1) an annulus of hairs inside the calyx tube, considering its presence as a derived state character (Abu-Asab and Cantino 1993) frequent in other

Table 3.3 Main dispersal mode, diaspore type and diaspore size (mm) in the infrageneric taxa of *Teucrium* L

Teucrium infrageneric taxa	Dispersal mode	Diaspore type	Diaspore size (mm) length × width
Teucrium	Semachory wind-dispersal	Pogonospore	(1.5)2 × 3(3.5)
Teucriopsis	Semachory wind-dispersal	Pogonospore	(1.5)1.8 × 2.5
Chamaedrys	Semachory wind-dispersal	Semaspore/ pogonospore	(0.7)1.5 × 1.8(2.5)
Montana	Ballautochory semachory wind dispersal	Semaspore/calyx with seed inside	(0.5)1.2 × 1.3(2.5)
Scorodonia	Ballautochory semachory wind-dispersal	Ballospore	(1)1.3 × 1.5(1.8)
Scordium	Ballautochory semachory wind-dispersal	Semaspore	(0.8)1 × 1.3
Stachyobotrys	Ballautochory epizoochory	Ballospore	(1)1.3 × 1.8
Scordium subsection *Spinularia*	Ballautochory epizoochory	Semaspore/ ballospore/ sclerospore	(0.1)1 × 1 × (1.5)
Isotriodon	Ballautochory/ semachory wind-dispersal	Semaspore	(0.7)1.3 × 1.5(2.5)
Polium subsection *Polium*	Meteo-anemochory wind-dispersal	Calyx with seed inside	(0.8)1.3 × 1.5(2.5)
Polium subsection *Simplicipilosa*	Meteo-anemochory wind-dispersal	Calyx with seed inside	(0.6)1.2 × 1.5(1.7)
Polium subsection *Pumila*	Meteo-anemochory	Calyx with seed inside	(0.6)0.8 × 1(1.5)

Lamiaceae genera such as *Marrubium, Origanum, Sideritis, Ocimum* and *Scutellaria*; (2) the mature cucullate teeth closing completely the calyx tube (El Oualidi and Puech 1993). The last is exclusive of some species of section *Polium* subsection *Polium* as *Teucrium lusitanicum* Schreb., or (3) a gibbous in the base of the calyx like *Teucrium botrys* L., covered inside by long hairs, making difficult the exit the nutlets, this mechanisms is exclusive of the subsection *Spinularia.*

The pedicel of the calyx and the upper tooth in strongly zygomorphic calyces plays a role in the dispersal mechanism of *Teucrium.* The pedicel is present in all *Teucrium* flowers except in the section *Polium* subsection *Polium* and some rocky and arid species from section *Montana*. The pedicel is not rigid and acts in the bal-listic mechanisms contracting itself to eject the mature nutlets (subsections

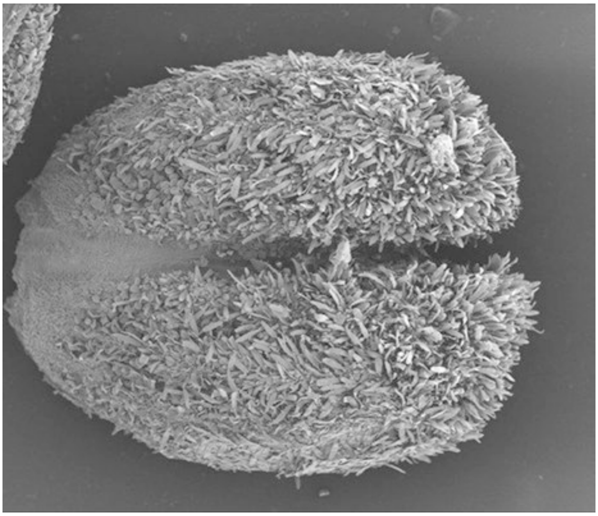

Fig. 3.10 Haired nutlet of *T. racemosum* R.Br. (section *Teucrium*)

Spinularia, section *Scorodonia,* some species from section *Stachyobotrys* and in *Teucrium abutiloides* and *T. betonicum* from section *Teucriopsis*). The broad and more or less horizontally upper lobe tooth of the calyx reinforces this ballistic action.

3.5 Conclusions

The most important discriminant generative characters within *Teucrium* are the corolla and calyx construction. Sections *Pycnobotrys, Stachyobotrys, Scorodonia* and *Teucriopsis* have species with wrakly 2-lipped corolla and zygomorphic calyx (2-lipped calyx that in the majority of cases present an upper broad lip). Sections *Polium, Chamaedrys, Montana, Isotriodon, Pumila* and *Scordium* have 1-lipped corolla and mainly sub-actinomorphic calyx. Section *Polium* is separate into two groups: species of the subsection *Simplicipilosa* with 1-lipped corolla, tube apically broading and a tuft of hairs inside, and species of subsection *Pumila,* with 1-lipped corollas and latero-posterior lobes forming an angle with the corolla tube, which has two guides of hairs at the base of the anterior corolla lobe. Section *Teucrium* is the most heterogeneous and distinctive group within *Teucrium.* Species of section *Teucrium* subsection *Fruticantia* (Kästner 1989) have 1-lipped corolla and actinomorphic calyx. Species of section *Teucrium* subsection *Cretica* (Kästner 1989) have 1-lipped spurred corolla with an annulus of hairs in the base and actinomorphic calyx and the Australian species of section *Teucrium* are characterized by their longer pedicellate flowers, 1-lipped non-spurred corollas with an annulus of hairs in the middle part of the tube.

Flower shapes in the genus range from weakly bilabiate corollas to one-lipped gullet shaped corollas with guiding visitors; gullet shaped slightly personate corollas closed by a palate of hairs and rare flag-shaped resupinate corollas with poorly developed lateral lobes and finally the brush-shaped corollas closed by a tuft of hairs at the base of the anterior lobe. All corolla forms exemplify the evolutionary potential of tubular one-lipped corollas. A personate corolla is universally present in all the species of section *Teucrium* and totally absent from other sections. This type occurs in species of *Anthirrhitteae, Gratioleae* and *Cheloneae* and considered as derived characters in *Scrophulariaceae* (Kampny 1995). Bilabiate corollas is the common type in Lamiaceae (Bouman and Meeuse 1992), weakly bilabiate corollas are only present in the species of the sections *Pycnobotrys, Stachyobotrys, Scorodonia* and *Teucriopsis.* The tuft of hairs at the entrance of the corolla tube is present in the species from section *Polium* subsection *Polium* but is absent in the rest of the sections. The species of this section are mainly ginodioecious with white or cream corollas and brush-shaped pollination such as the typical thymoideas of the Mediterranean maquis (Dafni and O'Toole 1994).

Seed dispersal inside the calyx is exclusive of the section *Polium*. This suggests that the above are characters, which may be of use in phylogenetic studies and an additional taxonomic use at infrageneric level. The pogonospores is the unique diaspore type in the section *Teucrium, Teucriopsis* and in some species of section *Chamaedrys.* The presence of the annulus of hairs on the inner side of the calyx in all species of the section *Scorodonia* and in some ones of the sections *Chamaedrys* and *Stachyobotrys,* suggests that these two characters express the intersectional taxonomic boundaries.

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Chapter 4
Ecology of *Teucrium* Species: Habitat Related Metal Content Dynamics

Dolja Pavlova, Irina Karadjova, Milan Stanković, and Nenad Zlatić

Abstract The chapter reviews the available data about the effect of habitat related metal content on *Teucrium chamaedrys* and *T. montanum* (Lamiaceae). The study was focused on element concentrations in plant and soil samples, both on metalliferous and non-metalliferous soils. Metal concentrations varied depending on species and habitat type. The levels of elements in plant tissues from non-metalliferous localities were always lower, compared to those from metalliferous (serpentine) ones. None of the species could not hyperaccumulate metals although the metal concentration in some of them exceeded the range, which is naturally found in plants. Depending on the nickel accumulation, both analyzed species are classified as excluders. The level of tolerance was related to the amount of metals and their bioavailability in the soil. The metal concentrations for the toxic elements were above the permissible limits for the toxic elements, in both species. The populations of the studied species demonstrated some adaptations to the serpentine habitats related to their secondary metabolites and its morphology, which is known as serpentinomorphoses. As a result of the heavy metal profiles of the soils, significantly higher values and differences in the quantity of secondary metabolites were recorded in plant populations growing on serpentines compared to non-serpentine ones. *Teucrium chamaedrys* and *T. montanum* populations on metalliferous habitats, possess morphological differences in contrast of populations on non-metalliferous habitats.

Keywords *Teucrium* · Ecology · Distribution · Metal content · Soil · *Teucrium chamaedrys* · *Teucrium montanum*

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Abbreviations

Al	Aluminium
B	Boron
BAF	Bioaccumulation factor
Ca	Calcium
Cd	Cadmium
CEC	Cation exchange capacity
Co	Cobalt
CO2	Carbon dioxide
Cr	Chromium
Cu	Copper
Fe	Iron
GR	Glutathione reductase
GSH	Reducted glutathione
H	Hydrogen
H_2O_2	Hydrogen peroxide
Hg	Mercury
K	Potassium
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
ROS	Reactive oxygen species
Zn	Zinc

4.1 Introduction

The genus *Teucrium* L. is the one of the largest in Lamiaceae family. It is widely distributed in Europe, Asia, Africa and Australia and have two centers of richness located in the east and western regions of the Mediterranean area. The genus *Teucrium* comprises approximately 300 taxa of herbs and shrubs grouped in 9 sections according to Bentham (1833) with additions proposed by Boissier (1879) (Navarro and El Oualidi 2000). The Mediterranean area represents the major area of distribution of the genus, since it comprises about 90% of the total species in the world (Navarro and El Oualidi 2000; Navarro 2010).

Teucrium chamaedrys L. (wall germander) is the most important species of sect. *Chamaedrys* (Mill.) Schreb., distributed throughout the Mediterranean, mainly in the Balkan peninsula and Turkey. The species *Teucrium montanum* L. (mountain germander) is a representative of sect. *Polium* (Mill.) Schreb., subsect. *Rotundifolia* Berm. & Sanchez Crespo, distributed in South and East Europe, the west Mediterranean area and in the mountains of the south Arabian peninsula (Navarro and El Oualidi 2000).

Reichinger (1941) considered *Teucrium chamaedrys* as the most variable taxon in the *Teucrium* genus and described 15 subspecies, most of them locally distributed. These subspecies were not initially included in Flora Europaea (Tutin et al. 1972) but, it was later added by Greuter et al. (1986), and some of them in local floras (Ekim 1982; Peev 1989). This perennial plant occurs in open forests, shrublands and rocky slopes in semi-arid, arid and sub-humid climates (Navarro and El Oualidi 2000). In the Balkan countries, the plant inhabits rocky limestone areas, dry mountain meadows and pastures, edges of sparse oak and pine forests up to 1000 m in Serbia and up to 1500 m in Bulgaria, demonstrating a high degree of morphological diversity (Diklić 1974; Peev 1989; Markova 1992). It was also reported for the serpentine territories in Bulgaria, Serbia, Albania, Turkey (Ekim 1982; Shallari et al. 1998; Pavlova et al. 2003; Pavlova 2010). *Teucrium montanum* L. is a perennial, shrub-like plant with half ligneous branches, up to 25 cm high and inhabits thermophilic calcareous rocks, dry mountain meadows and edges of forests in Europe and Anatoly (Diklić 1974; Zlatić et al. 2017).

A large number of known medicinal species, belonging to the genus *Teucrium*, are used in folk medicine and pharmacy, in food industry as spices and for bitter beverages. The most popular species in the flora of Europe and Asia (*Teucrium chamaedrys*, *T. montanum* and *T. polium*) have a history of traditional use in the Balkan countries for herbal teas and basic medicinal healing treatments (Ivancheva and Stancheva 2001; Obratov-Petković et al. 2006, 2008; Evstatieva et al. 2007; Redzić 2010). For pharmaceutical purposes aboveground plant parts (stems, leaves, flowers) are collected from natural populations and from cultivated plants (Nikolov 2006). The mentioned plants are included in the list of medicinal plants permitted for export, and annually hundreds of tons are exported (Evstatieva et al. 2007). They are used in the treatment of digestive and respiratory disorders, abscesses, gout, conjunctivitis, in the stimulation of fat and cellulite decomposition. These species possess anti-inflammatory, antioxidative, antimicrobial, antidiabetic, and antihelmintic effects (Harborne et al. 1986; Stanković et al. 2011a, 2012; Grubešić et al. 2012; Milošević-Djordjević et al. 2013; Vlase et al. 2014; Zdraveva et al. 2018).

According to Obratov-Petković et al. (2006), the distribution and the degree of presence of medicinal plants are directly correlated to the edaphic factors. Moreover, the quantity of active substances in plant tissues depends of many ecological factors that affect the vegetative plant organs (Lombini et al. 1998). The properties of soil generally depended on the combined effects of climate, biological activity, topography and the mineralogical composition of the parent rock and play a central role in the distribution and ecology of plant species and their associated biota (Jenny 1980; Whitea and Claxtonb 2004). Usually, extreme edaphic conditions, like limestone, gypsum, dolomite, granite, guano deposits, salt marshes, and even mine tailings, provide ideal settings for examining the role of the edaphic factor in the distribution, ecology and natural selection of plants (Kruckeberg 1969; Rajakaruna 2004; Rajakaruna and Boyd 2008).

Fig. 4.1 *T. chamaedrys* L. on serpentine (left) and calcareous habitat (right). (Photo D. Pavlova and M. Stanković)

Fig. 4.2 *T. montanum* L. on serpentine (left) and calcareous habitat (right). (Photo M. Stanković and N. Zlatić)

The species *Teucrium chamaedrys* (Fig. 4.1) and *T. montanum* (Fig. 4.2) demonstrate a wide ecological tolerance as they were found in open grasslands, oak woodlands, pastures, rocky calcareous and siliceous terrains, and on serpentine outcrops. They occurred either on soils that are naturally metalliferous (e.g. serpentine soils) or in territories which secondarily have been enriched with metals (industrial plants emitting metals, mine tailings, etc.). As the serpentines and their soils are characterized by toxic quantities of heavy metals (particularly Ni and Cr), low Ca/Mg ratio, drought, and wide temperature fluctuations (Kruckeberg 1984, 1992; Brooks 1987; Brady et al. 2005), they probably have the most extreme habitat conditions for these species. Most populations of *Teucrium chamaedrys* and *T. montanum* grow on nonmetalliferous soils in their range and are quite sensitive to metal toxicity. There is a strong selection favoring the metal-tolerant genotypes, when plants colonize metalliferous soils, causing a rapid increase in the average tolerance of the local populations, which leads to the establishment of metal-tolerant "races" or ecotypes (Antonovics et al. 1971; Pollard et al. 2002). It could be concluded that their adaptive mechanisms includes exclusion of metals (either by restricting them to the roots or through the absence of any uptake mechanisms), compartmentalization of metals in various organs, or toxicity tolerance as it was summarized by Kay et al. (2011) for plants growing on such soils. The level of tolerance is related to the amount of metals in the soil and their bioavailability. The metal bioavailability in the system soil-plant could be an indicator of potential risk for environment and human health, especially for medicinal plants.

This chapter will describe the influence of soil metal concentration on the therapeutically important medicinal plants – *Teucrium chamaedrys* and *T. montanum* (Lamiaceae) throughout their range in the Mediterranean region. Additionally, this chapter will describe the components of edaphic factors and their role, through metal concentrations in different types of soils. Related to soil analysis, metal accumulation by *Teucrium chamaedry*s and *T. montanum* will be presented as well as plant-soil correlations with bioaccumulation factor of selected plant species. Furthermore, this chapter will include the morphological characteristics of presented species related to the substrate and the quantity of secondary metabolites as one of the mechanisms of species adaptation to the metalliferous soil.

4.2 Soils and Their Role in the Distribution of *Teucrium* Species

4.2.1 Components of the Edaphic Factor and Its Role

The edaphic factor as a combination of numerous physical and chemical properties of the substrate significantly determines plant species distribution, diversity and composition. Very significant soil factor is combination and concentration of mineral nutrients available in the soil. Bioavailability is the proportion of total metals or metal species that are available for incorporation into biota (bioaccumulation). Among the chemical elements two classes are considered essential for plants: macronutrients and micronutrients. Macronutrients like K and Mg are required in high quantities as they form the basis of crucial cellular components like proteins and nucleic acids. Micronutrients like Zn, Mn, Cu, Fe, Ni etc. often required as cofactors for enzyme activity are needed in small amounts and, as expected, in excess they become toxic. The highly toxic elements like Cd, Pb, Hg usually can not be distinguished by the nutrient uptake system and enter into the plant causing reduced uptake of the essential nutrients and significantly reduced plant growth and quality. Plant uptake of trace elements is dependent on (1) movement of elements from the soil to the plant root, (2) elements crossing the membrane of epidermal cells of the root, (3) transport of elements from the epidermal cells to the xylem, in which a solution of elements is transported from roots to shoots, and (4) possible mobilization, from leaves to storage tissues used as food (seeds, tubers, and fruits).

Many factors can affect the efficiency of chemical elements biouptake mainly from the soil through the plant roots. Among these are factors connected with specific plant biouptake mechanism and root system. Micro- and macronutrient homeostasis in plants is realized by various strategies for mobilization and uptake which differ for different plants species. Metal ions reach plant tissue through ion exchange, passive diffusion or through a membrane transporters localized in the root. Various defense mechanism has been developed by plants against toxic elements: extracellular immobilization near root region (using root exudates or mycorrhizal association), intracellular detoxification by the formation of complexes between metal ions and peptides – phytochelatins, or complexes with organic acids or aminoacids. On the other hand, metals tolerant for some plants are associated with increased synthesis of phytochelatins proline or antioxidant enzymes.

In addition to the factors connected with specific plant biouptake mechanism and root system, very important are factors connected with soil properties, edaphic factors and chemical elements bioavailability. Acid soils have contributed to a great extent to the solubilization of metals. It is clear that with increasing pH the solubility of Fe, Cr, Ni and Co in serpentine soils decreases. The order of increasing solubility is Fe, Cr, Ni, Co (Brooks 1987). According to Blake and Goulding (2002) Pb is not mobilized until pH < 4.5. Zinc is readily soluble relative to the other metals in soils and available in acid light mineral soils. It is considered that the Zn fraction associated with Fe and Mn oxides is likely to be the most available to plants. The greatest amounts of adsorbed Cu have always been found for Fe and Mn oxides, amorphous Fe and Al hydroxides, and clays (Kabata-Pendias 2011). The solubilization of Cd and its transformation into an available ionic form, which is preferentially absorbed by plant roots depend on soil pH and other soil factors (Sharma and Dubey 2006). In acid soils the mobility and availability of Cd is much higher than in non-calcareous, neutral and slightly alkaline soils. Acidification to pH 4 mobilizes 60–90% of the total soil Cd, but it is adsorbed on ion exchange surfaces and complexed with soil organic matter (Obratov-Petković et al. 2006). The rapid increase in the Cd concentration at the 0.5 mg kg^{-1} level (in straw) is considered to be related to a break of the physiological barrier controlling a metabolic absorption of this metal (Kabata-Pendias 2011).

In most cases above separation of factors is quite operational as far as plant can to some extend regulate chemical elements biouptake as a respond to the changes in the soil properties and chemical elements bioavailability. The rhizosphere is a zone at the root-soil interface controlled by plant root and root released metabolites or exudates where additionally mutually interacting physical, chemical and biological processes take place. The health of both the plant and the soil associated is dependent on this rhizosphere region and its biochemical reactions.

Evidently, equilibria between different chemical elements in soil solid phase (mineral and organic/soil solution/plants rhizosphere) is extremely complex depending on various biotic and abiotic factors. Even more, reliable determination of bioavailable concentrations of nutrients and evaluation of their behavior is almost impossible. Although various operationally defined analytical procedures have been proposed and applied, the results obtained for chemical elements, their distribution between soil fractions and their chemical species are far from real situation in a complex soil-plants system. From such point of view the investigations on soils with unusual features (extreme pH, nutrient imbalances, limited depth, etc.) accepted as a strong selective force shaping plant evolution are very important lightening in a specific manner soil-plant connection and plant response to soil edaphic factors.

Together, these mechanisms allow plants to maximize their nutrient acquisition abilities while protecting against the accumulation of excess nutrients, which can be toxic to the plant. It is clear that the ability of plants to utilize such mechanisms exerts significant influence on plant community structure, soil ecology, ecosystem health, and biodiversity.

4.2.2 Metal Content in Serpentine Soil

Serpentine soil (formed after serpentine rocks (ultramafic rock consists of magnesium-iron silicate minerals, such as olivine and pyroxene) weathering under different climatic conditions) is a naturally occurring model system ideal for the study of the physiological responses of plants to edaphic factors. Serpentine soils are characterized with (i) low Ca content combined with elevated concentrations of Mg, which creates an unusually low Ca:Mg ratio; (ii) low concentrations of several of the macronutrients essential for plant growth, especially N, P, and K; (iii) high concentrations of toxic elements like Ni, Cr, Co and less toxic Mn. The parent material from which serpentine soil originates is highly variable leading to variations within them with respect to the absolute concentrations of Ca and Mg and the presence and concentration of toxic elements. Within serpentine ecosystems, additional abiotic stresses are also often present, including drought, low nutrient cycling rates, and shallow soil depth. This suite of abiotic factors and the sparse vegetation observed on serpentine soils has been described as the "serpentine syndrome" (Jenny 1980). It is a physiologically challenging environment for most plant species driving the development of unique physiological adaptations and high levels of endemism (Brooks 1987; Kruckeberg 1992).

Various hypotheses (reviewed by Brady et al. 2005; Kazakou et al. 2008), including tolerance to low Ca:Mg ratio, avoidance of Mg toxicity, an increased requirement for Mg, and tolerance/exclusion or hyperaccumulation of toxic elements have been discussed for plants that do grow on serpentine soil. Tolerances and adaptations range from those at the cellular level to those apparent to the naked eye. High concentrations of Ni are tolerated in some serpentine plants by exclusion, reduced transfer of Ni from root to shoot, or hyperaccumulation. Other plants adapt to a low Ca environment, by selectively up taking this nutrient rather than Mg.

Although physical-chemical features of serpentine soils where *Teucrium chamaedrys* and *T. montanum* are distributed vary considerably from site to site (Pavlova 2009; Pavlova and Karadjova 2013; Obratov-Petković et al. 2008; Branković et al. 2017; Zlatić et al. 2017) and within a site (Pavlova and Karadjova 2013), species populations are often found in open, steep landscapes with shallow and rocky soils with reduced moisture. Sparse plant cover on serpentines promotes elevated soil temperature and erosion (Kruckeberg 1992). Each of these factors poses an additional stress to plant life.

4.2.3 Metal Content in Calcareous Soil

Calcareous parent materials are the most widespread type of sedimentary rock in the world. They are made of calcium carbonate and originally divided into chemical, over gammon and rocks of organogenic origin. Usually, they contain primers of other metals such as Mn, Fe, Mg, clays, organic matter, sand, etc. Soils formed on the calcareous geological substrate contain calcium carbonate in free form (Ewald 2003). Calcareous soils are characterized with (i) high Ca content; (ii) high concentrations of several macronutrients essential for plant growth, especially K, P, and N; (iii) low concentrations of toxic elements like Co, Cr, and Ni, which is opposite to serpentine soils (Zlatić et al. 2017). This type of soil is characteristic for dry and semi-arid areas, as well as humid and moderately humid regions, especially in those areas where the parent material is rich in $CaCO_3$. At the chemical level, the presence of $CaCO_3$ determines the alkaline reaction in calcareous soils and affects the availability of certain metals, such as N, P, K, Mg, Zn, Cu, and Fe, to plants. The carbonates presented in the soil contribute to a alkaline pH value, ranging between 7.5 and 8.5. In addition to the chemical influence of $CaCO_3$ in calcareous soils, it also affects the physical characteristics of the soil (Lambers et al. 2008).

Plant species that inhabits limestone habitats are not exposed to the negative impact of heavy metals in the substrate. Plants that inhabit such habitats are adapted to other ecological factors by specific physiological mechanisms (Zlatić et al. 2017). These species are exposed to different stress conditions in opposed to the serpentine ones. The alkaline pH value of the calcareous soil, the deficiency of water in the substrate, the high temperature and erosion, are factors that plants needs to be adapt (Lambers et al. 2008).

4.3 Plant Life on Selected Edaphic Conditions – *Teucrium chamaedrys*

4.3.1 Metal Accumulation by **Teucrium chamaedrys**

The physiological response of plants to the stress provided by the elevated metal concentrations in the soil can be related to the different ways to cope with metals in the soil and to their genotypic response. Plants tolerate elevated metal concentrations on serpentine soils either by a constitutive trait present in all members of the species growing either on serpentine or non-serpentine substrate or by an adaptive mechanism present only in tolerant ecotypes (Antonovics et al. 1971; Kazakou et al.

2008). *Teucrium chamaedrys* absorbs a number of elements from soil both essential (Cu, Zn, Fe, Ni, Mn) and non-essential (Cr, Co, Pb) for its biological functions. The availability of metals in a soil-plant system depends on a number of factors which include pH of the soil, soil organic matter content, cationic exchange capacity as well as plant species, stage of development, and others (Farago 1994; Kassim and Rahim 2014). The species is widely distributed without preferences to any type of rock and should be considered as a "bodenvag" species, which are those widely distributed (Fig. 4.1) in serpentine and non-serpentine habitats (Kruckeberg 1992). The significant ecological differences between populations are related to the differences in edaphic conditions of the serpentine sites from the non-serpentine sites. According to Obratov-Petković et al. (2008) there are also differences in the heavy metal uptake between the individuals of the same species, which is first of all the consequence of climatic conditions and moisture regime.

The mechanism by which a serpentine-tolerant plant copes with elevated levels of Mg and relatively insufficient quantities of Ca in the soil is considered its most defining character (Brooks 1987; Brady et al. 2005; Kazakou et al. 2008). The ratios Ca:Mg are generally always above 1 in plants. However, serpentine plants are better able to maintain a greater than 1 ratio despite minimal levels of Ca found in the soils. They either have very efficient Ca uptake systems or ability to exclude Mg despite high concentrations in the soil. The low Ca:Mg ratio in serpentine soils has elicited a wide range of adaptive responses based on either ion exclusion at the root/soil interface, selective translocation of Ca from root to shoot, sequestration of Mg in the vacuole, or internal mechanisms of tolerance (Kay et al. 2011). Calcium concentrations found in tissues of *Teucrium chamaedrys* are between 2369 and 6345 mg kg^{-1} in serpentine plant populations in Bulgaria (Pavlova 2009; Pavlova and Karadjova 2012) and between 324 and 4872.2 mg kg^{-1} in plant populations from Serbia (Obratov-Petković et al. 2008; Branković et al. 2017; Zlatić et al. 2017). Similar to previous findings (Karataglis et al. 1982; Brooks 1987; Roberts and Proctor 1992; Kay et al. 2011; Pavlova and Karadjova 2013, Branković et al. 2017), while the amounts of Ca in the serpentine soils were small, the amounts of Ca taken up by the plant were higher. Serpentine species populations are much more tolerant to low Ca levels and elevated concentrations of Mg in the soil. Calcium concentrations in *Teucrium chamaedrys* populations are in all cases lower than in Ni hyperaccumulating plants distributed in the same areas such as *Alyssum* and *Thlaspi* species. The remarkable Ca uptake ability is considered an important feature in Ni-hyperaccumulator physiology (Broadhurst et al. 2004; Chaney et al. 2007).

Calcium is one of the elements contributing to the inhibition of the heavy-metal toxicity, and it is possible that plant takes up Ca to compensate the toxic action of different toxic metals (Brooks 1987; Lombini et al. 1998; Brady et al. 2005). Studying populations of *Buxus sempervirens* L. (Common Box) in Greece, Karataglis et al. (1982) suggested that the plant developed a mechanism to permit an excess soil Ca to be taken by plant.

Several species adapted to serpentine environments have higher external and internal Mg requirements than their non-serpentine relatives and variety of responses shown suggests that the physiological basis for tolerating low Ca:Mg may involve more than one mechanism in a given species (Brady et al. 2005; Kay et al. 2011). Normally, the high Mg of serpentine soils is reflected in high Mg concentrations in plant tissues, but this result might also indicate an unusual Mg and low Ca requirement (Proctor and Woodell 1975). The mean Mg concentrations for *Teucrium chamaedrys* in the range 0.13–0.35% and 0.11–0.19%, respectively for serpentine and non-serpentine sites, were reported by Pavlova and Karadjova (2012). The mean Mg concentrations for the species from Serbia were almost the same for serpentine and non-serpentine (calcareous) populations as reported by Zlatić et al. (2017).

Brooks and Yang (1984) found the concentration of Mg in plant tissue to be inversely proportional to the concentrations of other nutrients: B, Fe, Co, Mn, P, and Na. Their findings showed that these relationships were caused by high Mg levels in the soil; high Mg concentration is probably the major cause of infertility of serpentine soils and hence of the development of specialized serpentine floras adapted to this unfavorable edaphic condition (Kazakou et al. 2008). These data clearly suggest that the uptake of Mg comes at a cost to the plant as the uptake of other elemental nutrients is forfeited. More complex is this interaction in Ni-hyperaccumulator plants because Ni is also involved. Some publications demonstrated that in the presence of Ni internal Ca and Mg concentrations counteract Ni toxicity or in any case enhance Ni tolerance (Gabbrielli and Pandolfini 1984), or decrease Ca uptake and increase uptake of Mg (Kazakou et al. 2008). It is likely, therefore, that the Ca/Mg quotient of the soil solution has a strong influence on Ni absorption, translocation and accumulation.

The accumulation of Ni, Cr, and Co in the above-ground plant parts for all serpentine populations of *Teucrium chamaedrys* is higher than in non-serpentine ones which was proved by a number of studies (Shellari et al. 1998; Jurišić et al. 2001; Obratov-Petković et al. 2008; Pavlova 2009; Pavlova and Karadjova 2012, 2013; Branković et al. 2017; Zlatić et al. 2017). Metal concentrations also vary at population level in relation to soil properties, mainly soil metal availability. This species demonstrates, as many other, specific strategies of adaptation to elevated levels of toxic metals in soil probably result of mineral element imbalance characteristic of serpentine soils (Ater et al. 2000) or due to inter-population variation in parts of the uptake and translocation processes (Adamidis et al. 2014). Nickel concentrations measured in *Teucrium chamaedrys* serpentine populations in Bulgaria (Pavlova 2009; Pavlova and Karadjova 2012, 2013) are lower than the data of Shallari et al. (1998) for Albanian serpentine populations, and higher from data provided for this medicinal plant from Serbia (Obratov-Petković et al. 2008). It was shown that different Ni concentrations in serpentine populations correlate with the bioavailable fraction of trace elements in the soils (Pavlova and Karadjova 2013; Zlatić et al. 2017). The concentrations of Ni even higher for all serpentine populations of *Teucrium chamaedrys* were not exceptional. The amounts of Ni in *Teucrium chamaedrys* were low compared to the data for other plants growing on serpentines (Karataglis et al. 1982; Vergnano Gambi et al. 1982; Konstantinou and Babalonas 1996; Bani et al. 2013; Sawidis et al. 2014), but higher than levels normally considered to be toxic to most plants (Kabata-Pendias and Pendias 1984). The concentrations of Ni in plants from some of the investigated sites were higher than 60 mg kg^{-1} considered to be the threshold of physiological evidence of toxicity in plants of serpentine habitats (Kazakou et al. 2008, 2010). Concentrations of Ni above 100 mg kg^{-1} were documented for some serpentine populations of *Teucrium chamaedrys* in Bulgaria and Serbia (Pavlova and Karadjova 2012; Branković et al. 2017; Zlatić et al. 2017). Values from 145.7 mg kg^{-1} Ni found in *Teucrium chamaedrys* growing on serpentine in Goč Mt. (Serbia) give reason this species to be considered as accumulating plant of Ni (Branković et al. 2017). Despite of high Ni values documented they are rarely found in species populations and can be considered exception for the species. Only a few species appear to show extreme variations in metal uptake, even when confined to metalliferous soils. This behavior may be a reflection of widely varying metal availability caused by variations in pH or other soil properties (Reeves 2017). The first record for Ni accumulation above 1000 mg kg^{-1}, threshold for Ni hyperaccumulation, reported for the species of *Teucrium* were those of Hossain (2007) after Kassim and Rahim (2014) who studied metal accumulation in *Teucrium polium* populations in Iran. The values 8140 mg kg^{-1} in leaves and 2300 mg kg^{-1} in stem are reported for serpentine populations of the species. Later on, Ni concentrations between 9678 and 14.110 mg kg^{-1} in above ground parts of *Teucrium polium* grown in serpentine soils in Turkey were reported by Yaman (2014). The same species is included in the list of accumulating species that belong to the Italian flora (Bazan and Galizia 2018), altogether with Pb accumulator *Teucrium flavum* subsp. *glaucum* and Hg accumulator *Teucrium scorodonia*.

The populations of *Teucrium chamaerdrys* growing on non-serpentine soil types also differ in their ability to accumulate Ni and in most cases reported values are below 1 mg kg^{-1} (Obratov-Petković et al. 2008; Pavlova 2009; Pavlova and Karadjova 2012). An exception is the data provided for a calcareous site from Kopaonik Mt. (Zlatić et al. 2017) where a value of 9.65 mg kg^{-1} was measured. In all cases Ni concentrations were below the levels considered threshold for Ni toxicity.

The Ellenberg's indicator values used to describe the ecological relationship amongst accumulating plants, climate and soil conditions demonstrate that most of the phytoextractors naturally grow in neutral to basic soils (Bazan and Galizia 2018). The experiments with two *Alyssum* species (including *A. murale*) showed that increasing soil pH through liming increased Ni uptake when plants are grown on Ni-rich non-serpentine soils. However, liming an serpentine soil decreased Ni uptake (Kukier et al. 2004).

The phytotoxic Ni concentrations range widely among native species populations and cultivars of *Teucrium chamaedrys* growing on different substrates (Jurišić et al. 2001; Bazan and Galizia 2018). The higher concentrations were found in cultivated plants, while in *Teucrium montanum* the highest quantities of Ni and other metal ions (Cr and Cu) were found in wild species populations. This indicates that abilities of handling metal cations are not specific for serpentine populations, but can also be found in non-serpentine populations (Vicić et al. 2013a). Hence, Ni toxicity did not appear to be a universal feature of serpentine soils, but was dependent on other variables, including Mg concentration in particular; the influence of Ni sometimes even varied within a site (Kazakou et al. 2008).

In *Teucrium chamaedrys* from serpentine sites Ni is taken up in higher quantities than Cr, and the Ni/Cr ratio in plant tissues is always much higher than in the soil. Most data from Bulgarian and Serbian serpentines demonstrated low Cr concentrations in populations of *Teucrium chamaedrys* (Obratov-Petković et al. 2008; Pavlova 2009; Pavlova and Karadjova 2012). These results corroborate the data of Brooks (1987) that serpentine plants contain only trace quantities of Cr. The phytoextraction of Cr from serpentine plant populations in Goč Mt. (Serbia) was above the threshold of 50 mg kg^{-1} and according to Branković et al. (2017) it may be considered as accumulation. However, even on serpentine soils, Cr concentrations > 50 mg kg^{-1} are so uncommon that this may be used as an indicator of soil contamination (Reeves 2006). Low mobility of this metal in the soil also serves to possible dust contamination. Several native plants (from different families) from areas of serpentine or chromite deposits were found to accumulate Cr as much as 0.3% or 3.4% (Kabata-Pendias 2011).

Although, most soils contain significant amounts of Cr, its availability to plants is highly limited and controlled mainly by the soluble Cr contents of the soils. Cr bioaccessibility is a function of soil type and retention time and not easily translocated within plants, thus it is concentrated mainly in roots (Kabata-Pendias 2011). Data provided for some *Teucrium* species (Pavlova and Alexandrov 2003; Vicić et al. 2013a) confirmed this conclusion. There is a great difference in the accumulation of Cr by shoots and roots of various plants and in vegetable crops the ratio shoot/root varies widely from 0.005 to 0.027 (Kabata-Pendias 2011).

Besides the elevated concentrations for Ni and Cr measured for *Teucrium chamaedrys* from serpentine soils such high concentrations were reported also for Fe (Obratov-Petković et al. 2008; Pavlova and Karadjova 2012, 2013; Branković et al. 2017; Zlatić et al. 2017). The quantity of Fe in the tissues of the species growing on serpentines varied in wide range from extremely high unusual concentrations (1781.9 mg kg^{-1}) to almost typical contents for the species from Serbia (Obratov-Petković et al. 2008; Branković et al. 2017, Zlatić et al. 2017) and in range 538–2740 mg kg^{-1} for the species from Bulgaria (Pavlova and Karadjova 2012). The same high concentrations were also reported for different species growing on metalliferous soils (Cornara et al. 2007; Dudić et al. 2007; Golubović and Blagojević 2013; Franco et al. 2013, Sawidis et al. 2014). Values > 1000 mg kg^{-1} often indicate contamination by serpentine soil or dust, not easily removed by simple washing procedures (Reeves et al. 1999). However, higher Fe concentration seems to be characteristic for the serpentine flora of the Balkans and more attention should be given on this phenomenon (Babalonas et al. 1984). Such behavior of some serpentine plants might be explained with their ability to acidify the rhizosphere and to increase the solubility of ferric ions and their reduction to even more soluble ferrous ion. However, where Fe is easily soluble, plants may take up a very large amount of Fe and this is clearly shown by vegetation grown in soils derived from serpentine and excessive Fe uptake can produce toxic effects in plants. The variation among plants in their ability to absorb Fe is not always consistent and is affected by abiotic factors – soil, climate, and biotic conditions like the stages of plant growth and the ability to accumulate Fe (Kabata-Pendias and Pendias 1984; Kabata-Pendias 2011). In most cases *Teucrium chamaedrys* growing on serpentines demonstrates abnormal levels of Fe (> 200 mg kg^{-1}) in tissues according to the Element Concentration Cadastre in Ecosystems presented at the 25th General Assembly of the International Union of Biological Sciences (Lieth and Markert 1988). The accumulation of the metal in plant organs is different depending on the species, for example Sawidis et al. (2014) mentioned that Fe in the root was more in tree, shrub and cultivated plant samples but in non-cultivated and aquatic plants the leaves showed the highest Fe values. Also, Fe concentrations in roots of *Teucrium montanum* and *T. polium* were higher than in the leaves, stems and flowers (Pavlova and Alexandrov 2003; Kassim and Rahim 2014).

The concentrations of Co in *Teucrium chamaedrys* were lower than Cr in samples suggesting that the plant adapted to serpentine soil did not accumulate Co (Wallace et al. 1982). The concentrations for Co seldom reached 10 mg kg^{-1} in plants on normal soils and even on serpentine soil this level is not often exceeded. Co concentrations in *Teucrium chamaedrys* were in range 0.9–9.4 mg kg^{-1} for serpentine populations and 0.07–0.4 mg kg^{-1} in non-serpentine populations in Bulgaria (Pavlova and Karadjova 2012). Data from serpentines in Serbia (Branković et al. 2017) also fall in this range.

The concentrations of essential elements Mn, Cu and Zn measured in *Teucrium chamaedrys* from Bulgaria and Serbia (Obratov-Petković et al. 2008; Pavlova 2009; Pavlova and Karadjova 2012, 2013; Branković et al. 2017; Zlatić et al. 2017) were within the ranges considered normal for plants both from serpentine and non-serpentine sites (Kabata-Pendias and Pendias 1984; Reeves 2006). Usually, serpentine soils are not rich in Cu, Zn, and Pb, and typical concentrations of these elements in *Teucrium chamaedrys* tissues are below the limits for toxicity. However, results for Zn and Pb concentrations were higher in species populations from Bulgaria in comparison with the data from Serbia (Obratov-Petković et al. 2008; Branković et al. 2017) and from Albania (Shallari et al. 1998) result of possible contamination from recent polymetal mining activities and aerosol deposition due to heavy road traffic near some sites. Also, the quantity of Zn in plants changes with the growing season and often shows an increase throughout the season (Antonovics et al. 1971).

Cadmium is also not part of the "serpentine syndrome" and in most serpentine soils demonstrates relatively lower levels close to typical ranges for unpolluted regions. The concentrations of this element in tissues of *Teucrium chamaedrys* reported from some serpentine sites in Bulgaria were between 0.2 and 1.43 mg kg^{-1} (Pavlova and Karadjova 2012), while in Serbia Cd was below the toxic levels (Branković et al. 2017). The degree to which plants are able to take up Cd depends on its concentration in the soil and its bioavailability modulated by the presence of organic matter, pH, redox potential, temperature and concentration of other elements (Di Toppi and Gabbrielli 1999). Cd and Pb as well, are considered to be effectively absorbed by the root and leaf systems showing a great difference in the ability of various species to accumulate them (Kabata-Pendias and Pendias 1984). In general, Cd concentration in plant tissues is in the order:

root > stem > leaves > grains, although possible absorption directly from the atmosphere can change it (Zhang et al. 2006). Cadmium is variable within species populations of *Teucrium chamaedrys* and the variability of Cd accumulation within populations can be of genetic origin or due to local environmental factors (Reeves 2006). The absorption and accumulation of Cd in plants is also dependent on the growth stage and metabolic activity (Zhang et al. 2006). Based on the difference in absorption, transport and accumulation of heavy metals, *Teucrium chamaedrys* can be classified as an excluder.

Over the diverse range of sites, sampling times of the species and climatic conditions involved in the field collections, it is not appropriate to try to draw conclusions about the detailed interactions of the major and trace elements.

4.3.2 Plant-Soil Correlations

Correlations between elements in the aerial plant parts and elements in the rhizosphere serpentine soils were calculated, taking into account all studied soils and plants (*Teucrium chamaedrys*, *Teucrium polium* and *T. montanum*) as average values for Bulgaria (Pavlova and Karadjova 2012, 2013). Positive correlation coefficients were found for Ni, Cr, Fe, Co, and Cu concentrations in plants with Ca in the soil. Significant negative correlations were found between Mg in plants and Mg in soil confirming plant ability to exclude Mg from soil. However it should be also emphasized that the correlations between measured elemental concentrations in plant populations and their rhizosphere soils are species specific (Pavlova and Karadjova 2013). Highly significant correlations at level $p < 0.01$ were observed between Fe, Ni, Cr, and Co in *Teucrium chamaedrys* samples and Fe in the soils for Bulgarian serpentine sites. However, it should be mention that all these correlation patterns are for total element content in soils, which most probably will be changed if their bioavailable fractions have been considered.

4.3.3 Bioaccumulation Factors

The uptake of heavy metals depends on their concentration in the soil solution and the rate of the transfer from the solid phase into soil solution for replenishment of the heavy metals taken up by the plant roots (Kashem and Singh 2002). The bioaccumulation factor (BAF) calculated as a ratio between the concentrations of elements in plants and in the respective soils provides information or potential bioavailability modes of absorption and accumulation of specific elements in plant tissues. For most cases in *Teucrium chamaedrys* BAF has a value below 1 (Pavlova and Karadjova 2013; Zlatić et al. 2017). BAF values higher than 1 were reported for K both from serpentine and calcareous populations of *Teucrium montanum* and *T. chamaedrys* by Zlatić et al. (2017), whereas BAF values higher than 1 were calculated for Ca from serpentine populations of the same species. The bioaccumulation trends in the species are the following: Zn > Cu > Cd > Pb > Ni > Co > Mn > Cr > Fe. It is worth mentioning that the bioaccumulation trend follows the bioavailability of trace elements. Higher BAFs could be expected for elements like Zn, Cu, Ni, and Pb. Extractable fraction for these elements is reported to be between 2% and 8%. BAFs for elements like Fe and Cr with extractable fraction below 0.05% are much lower (Pavlova and Karadjova 2013). Almost the same is the bioaccumulation trend for the species shown by Zlatić et al. (2017) and this fact confirms the specific adaptation strategy of the species to the metals. Zinc and Cu are the metals with the highest biouptake abilities, while Cr and Fe have the lowest. Zn is regarded highly mobile from some authors, while others consider Zn to have intermediate mobility. Soluble forms of Zn are readily available to plants and uptake of Zn was reported to be linear with concentration in soils and in nutrient solution (Kabata-Pendias 2011). The mechanism of Cu uptake is different from the uptake of Zn (Antonovics et al. 1971). In the above ground plant parts of *Teucrium chamaedrys,* both from serpentine and non-serpentine soils, Cu uptake stays low and almost constant at low levels of soil Cu. The plant reaction to Cu in the soil suggests that the mechanism of exclusion is available. Plant Cu concentrations are controlled within a remarkably narrow range and extractable Cu was about 2% (Pavlova and Karadjova 2013). The bioavailability of Pb in *Teucrium chamaedrys* above-ground plant parts determined through extractable fraction in 0.43 M acetic acid was about 3%, higher compared to extractable Cu (Pavlova and Karadjova 2013). It is considered that plants are able to take up Pb from soils to a limited extent even under contaminated soil conditions (Kabata-Pendias 2011).

Ni is considered to be a major contributor to the serpentine factor because of the very low availability of Cr and the relatively low abundance of Co compared to Ni (Brooks 1987). The uptake of Ni in *Teucrium chamaedrys* populations was demonstrated to be variable and extractable Ni was about 5% (Pavlova and Karadjova 2013). Nickel is usually easily extracted from soils by plants and its contents of plants are functions of Ni forms in soils. Both plant and pedological factors affect these processes and the most pronounced factor is soil pH (Kabata-Pendias 2011). Although only 1% of the total Ni in soils is available to plants, this far exceeds the percentage of available Cr (Brooks 1987). The low solubility of Cr is reflected in the very slight uptake of this element by plants. In the case of *Teucrium chamaedrys* Cr demonstrates very low extractable fraction, below 0.05%, similarly to Fe. According to Brooks (1987) Fe in serpentine soils is very insoluble and typically only about 0.01% of the total Fe is extracted. It is possible that *Teucrium chamaedrys* strictly selects elements and the absorbed amount and this mechanism enables survival. Thus, in addition to the internal factors of control of mineral content in plant such as genetic specificity, the properties of the root system and leaves, ontogenetic development of the species, external factors should also be considered as very important (Rašić et al. 2006). It is known that the availability of some heavy metals decreases with rising pH of the soil, organic matter and clay content (Mengel and Kirkby 1987; Obratov-Petković et al. 2006; Kabata-Pendias 2011). Among the external factors soil acidity has a strong influence on ion mobility and their uptake.

Weak acidic to weak alkaline conditions of the rhizosphere soil favor strong binding of toxic element in the soil, on one hand, and optimal bioavailability of nutrients as they are essential elements (Rašić et al. 2006).

4.3.4 Species Adaptations to Metalliferous Soils: Quantity of Secondary Metabolites

Secondary metabolites play a major role in the environmental adaptation of plants. The concentrations of various secondary plant products are strongly dependent on the growing conditions and their accumulation occurs in plants subjected to stresses including various elicitors or signal molecules (Nasim and Dhir 2010; Akula and Ravishankar 2011). Heavy metals, as well as other abiotic stress factors (temperature, salinity, water, radiation, etc.), can influence biosynthetic pathways of secondary metabolites (Mahajan and Tuteja 2005) and suppress or stimulate their production. The impact of heavy metals on plant secondary metabolite production is in relation to their concentration and exposure time (Berni et al. 2018). The plant secondary metabolites play numerous roles in plant protection under different biotic and abiotic influences (Ibrahim et al. 2017). Drought resistance is attributed to flavonoids or other phenolic compounds which may also help plants to tolerate soils that are rich in toxic metals (Treutter 2006). It is confirmed that due to the presence of heavy metals certain plants increase the synthesis of phenolics (Michalak 2006; Zlatić et al. 2017; Zdraveva et al. 2018).

As a result of the specific heavy metal profiles of the soils, differences in the quantity of secondary metabolites in *Teucrium chamaedris* were found. In all serpentine plant populations significantly higher values of secondary metabolites were reported compared to non-serpentine ones (Stanković et al. 2010; Zlatić et al. 2017; Zdraveva et al. 2018). The serpentine populations of *Teucrium chamaedrys* are enriched in phenolic compounds similarly to other medicinal plants growing on serpentine substrates (Stanković et al. 2011a, b, 2015; Veličković et al. 2014) and they could be considered as appropriate sources of natural antioxidants. The total amount of phenolic compounds and flavonoids both in extracts and plant material (above-ground parts) of *Teucrium chamaedrys* populations from serpentines were higher compared to non-serpentine (calcareous) populations according to Zlatić et al. (2017). The data presented for Bulgaria (Zdraveva et al. 2018) also confirmed higher values of these compounds in serpentine populations. The content of phenols and flavonoids in plant extracts of *Teucrium chamaedrys* depends on the type of extracts and polarity of solvents used for extractions (Stanković et al. 2010).

The concentrations of phenols and flavonoids vary in the plant body of the species. Stanković et al. (2010) showed the highest concentrations of phenols and flavonoids in leaf extracts in water, acetone and petroleum ether, smaller in flower extracts, and lowest in stem extracts. The large number of glandular hairs, rich in phenolic components, is considered as a reason for higher concentrations of phenols found in some stem extracts of *Teucrium chamaedrys* var. *glanduliferum* from Serbia (Stanković et al. 2010). The concentrations of phenols and flavonoids in different plant parts compared to those of the whole plant are also different.

The phenolic compounds contribute to the antioxidant potential of plants by neutralizing free radicals and preventing decomposition of hydroperoxides into free radicals (Jain et al. 2014). The high concentration of phenolics, as well as the properties of these molecules and the positive correlation found between the concentration of phenolic compounds and antioxidant activity of different plant extracts of *Teucrium chamaedrys*, indicated that these compounds contributed to the strong antioxidant activity (Stanković et al. 2010). The highest antiradical effect has been also detected in purified methanol *Teucrium chamaedrys* leaf and root extracts from Italy (Pacifico et al. 2009) but the methanol extracts collected from serpentine *Teucrium chamaedrys* populations possessed stronger antioxidant capacity. According to Stanković et al. (2010) both methanol and acetone extracts of *Teucrium chamaedrys* have high concentration of total phenols and flavonoids, which is in correlation with the intense antioxidant activity of these extracts and have a crucial role in the adaptation of this plant to the adverse effects of heavy metals.

The plant extracts of *Teucrium chamaedrys* populations demonstrated variation in the concentrations of tannins as well (Zdraveva et al. 2018). The tannin content was higher in serpentine samples and it was comparable to data for Croatia reported by Grubešić et al. (2012). The amounts of total polyphenols and tannins was found to vary also among the *Teucrium* species (Grubešić et al. 2012; Maleš et al. 2015) and their quantity was higher in native than in cultivated samples in coherence with the environmental factors (temperature, humidity, light intensity, water, minerals and CO_2). The content of total polyphenols and tannins in the samples of *Teucrium chamaedrys* and other studied species reported by Maleš et al. (2015) is significantly lower in comparison to data presented by Grubešić et al. (2012) because of the different method used for quantitative analysis. However, polyphenols represent most abundant and widely distributed antioxidants that have beneficial health promoting effects and confirm that *Teucrium* species are good source of polyphenols (Maleš et al. 2015).

Exposure to heavy metals leads to accumulation of harmful reactive oxygen species (ROS). Reactive oxygen species (ROS), generated during heavy metal stress, may cause lipid peroxidation that stimulates formation of highly active signaling compounds capable of triggering production of bioactive compounds (secondary metabolites) that enhance the medicinal value of the plant (Nasim and Dhir 2010; Berni et al. 2018).

Phenolic compounds and other secondary metabolites have numerous pharmacological properties and thus any environmental condition that affects either the quantity or composition of phytochemical compounds may potentially influence the efficacy of the medicinal plant product. There is limited information on specific physiological responses of medicinal plants to heavy metals in soils and the resulting changes in the efficacy of the plant (Ibrahim et al. 2017).

4.3.5 Species Adaptations to Metalliferous Soils: Morphology

Plants growing on metalliferous soils often possess morphologies distinct from their relatives growing on normal soils. Soil nutrients, moisture stress, photoperiodism, temperature effects, photosynthesis and respiration, all have elicited racial differentiation in a variety of seed plants (Kruckeberg 1969). Intraspecific variation leading to local adaptation (i.e. ecotypic differentiation) has been frequently cited (Štepankova 1996, 1997; Rajaharuna and Bohm 1999; O'Dell and Rajakaruna 2011) for species found on and off serpentine soils. The specific serpentinomorphoses are described and summarized by many authors (Pichi-Sermolli 1948; Krause 1958; Ritter-Studnička 1968). The morphological characteristics of serpentine plants are three main groups: xeromorphic foliage, reduction in stature, and increase in root system (Kruckeberg 1984). Several of these traits have been shown to be genetically determined (Westerbergh 1994).

Although *Teucrium chamaedrys* is widely distributed in serpentine and non-serpentine habitats it has high abundance and potential preference for alkaline soils (Obratov-Petković et al. 2006). The growth habit of *Teucrium chamaedrys* differs between serpentine and non-serpentine sites as it was documented for many species (Brooks 1987; Vergnano Gambi 1992; Westerbergh and Saura 1992; Mayer and Soltis 1994; Štepankova 1996, 1997; Westerbergh and Rune 1996; Bratteler et al. 2006; Wright et al. 2006; Boyd et al. 2009; Salmeroŕn-Saŕnchez et al. 2018). The plants are typically adapted to dry soils and are of smaller stature than non-serpentine relatives. *Teucrium chamaedrys* populations are dense and well developed in mesophilous habitats on calcareous and siliceous terrains compared to serpentine populations. The serpentine populations are characterized by their reduced size – lower stems, smaller leaves, and shorter internodes compared to the non-serpentine populations (Pavlova 2009). The variation is higher for the vegetative features and not clearly expressed for the generative ones. The positive correlations within the floral and vegetative trait groups do not indicate functional relationships among traits. Confirming Conner and Sterling (1996) it was concluded that correlations between both groups of traits were due at least in part to overall size relationship and reflect common developmental pathways.

Studying ecobiology of *Teucrium chamaedrys* in some regions of Serbia Obratov-Petković et al. (2006) noted that populations of *Teucrium chamaedrys* growing on serpentines were less compact than those growing on limestone; the plants growing on serpentine were smaller than the same species on limestone; the leaves of plants on serpentine were smaller, covered with more hairs and many of them were grey-green in comparison with the plants on limestone. The leaf indumentum has adaptive value for species because the hairs allow to gain a higher rate of carbon under arid condition than the leaf acquire without hairs to avoid potentially lethal condition of soil and loss of water which allows the plant to extend its growth for a longer period into the drought (Azmat et al. 2009). Important characteristic for the individuals growing on serpentines is denser indumentum and longer simple or glandular hairs covering the stems, leaves, and bracts which is related to the dry habitat conditions provided by the serpentine. For most serpentine populations of *Teucrium chamaedrys* are reported 3–5 celled glandular hairs densely covered plant parts – leaves, stem and bracts (Pavlova 2009). Such kind of hairs is not mentioned by Bini-Maleci and Servettaz (1991), Navarro and El Oualidi (2000) or Grubešić et al. (2007) for this species and can be considered specific for the serpentine populations. Because hairs can play an important role in various aspects of plant physiology and ecology in *Teucrium*, further investigations are needed to demonstrate the ecotypic differentiation in *Teucrium chamaedrys* found on serpentine and non-serpentine soils (Pavlova 2009).

4.4 Plant Life on Selected Edaphic Conditions – *Teucrium montanum*

4.4.1 Metal Accumulation by Teucrium montanum

Plant species are exposed to different environmental factors. Among them, the influence of geological substrate and soil was considered as the most important. The effects of the edaphic factor originate from the abiotic and biotic components of the soil as a dynamic system. The process of pedogenesis is a complex of physical and chemical process determined by the type of geological substrate, climatic conditions, living organisms, etc. Edaphic factors in plants affect the appearance of various morpho-anatomical and ecophysiological properties in the function of adaptation. Differentiated groups of species was adapted to a wider range of edaphic conditions variation, as well as groups of species specialized for a substrate making special edaphic ecotypes. Due to the development of complex adaptation mechanisms to a certain type of substrate, the distribution of some ecological groups was completely determined by the type of substrate, while for some groups, there is a certain degree of tolerance in terms of variation of edaphic conditions.

Plant species, that are widespread on habitats with a certain type of substrate, developed the ability to tolerate habitats that are characterized by completely different edaphic conditions. Soil formed on the calcareous geological substrate contained calcium carbonate in free form, which determined the alkaline reaction and limited availability of certain elements to plants, such as N, P, K, Mg, Zn, Cu, and Fe. The soil formed on the serpentine geological substrate represents a special substrate, with extreme physical and chemical characteristics. The disturbed mineral regime, the disturbed Ca:Mg ratio, the lack of essential nutrients Ca, P, N, K, increased concentrations of heavy metals, such as Mn, Ni, Cr and the variation in pH from acidic to strong alkaline, are one of the most significant characteristics of serpentine soils (Zlatić et al. 2017).

Teucrium montanum L. (Lamiaceae) is a facultative serpentinophyte (Fig. 4.2) and it is an ideal model organism for comparative analysis of ecological differentiation relative to the substrate such as serpentine and calcareous substrate (Fig. 4.3).

Fig. 4.3 Serpentine (left) and calcareous (right) habitat of *T. montanum* L. (Photo M. Stanković)

Teucrium montanum absorbs a large number of elements from soil, essential (Cu, Fe, Mn, Ni, Zn) and non-essential (Cr, Co, Pb) for its biological functions. Many authors state that the most stressful component of the plant was disturbed Ca:Mg ratio, that affects the establishment of viable populations of *Teucrium montanum* species on serpentine habitats. The Ca:Mg ratio is generally in values above 1 in plants, while the plants present on the serpentine could have values higher than 1, regardless of the minimum concentration of Ca in the soil. It has been confirmed that Ca deficiency had an influence on root elongation and shoots growth (Marschner 1995). Since Ca and Mg are available to the plants through the roots, plants need a special mechanisms to maintain the adequate Ca:Mg ratio in the species that grow on serpentine. These mechanisms allow selective Ca and Mg exclusion, despite high concentrations in the soil, as well as the translocation of Ca and Mg from the root into the above-ground plant parts (Vicić et al. 2013b).

In the study of *Teucrium montanum* populations on the serpentine localities in Serbia and Bulgaria, similar amounts of accumulated Ca and Mg was found in the above-ground plant parts. Plant populations sampled from serpentine soil accumulated higher quantities of Ca than the population sampled from calcareous soil (Pavlova and Karadjova 2012). Juranović-Cindrić et al. (2013) described that the population of *Teucrium montanum* accumulated between 30 and 40 times more Mg than Ca, which indicated that the species process a high ability to absorb Mg in habitats where the concentration of Mg is lower than the concentration of Ca.

Calcium concentrations found in tissues of *Teucrium montanum* were between 1356.5 and 2237.7 mg kg^{-1} in serpentine plant populations from Serbia (Zlatić et al. 2017), and between 2980 and 4798 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results in presented research indicated that the amount of Ca, in soil samples and in samples of plants on the calcareous substrate, is higher in relation to the quantity in samples from the serpentine substrate. The increased amount of Ca in the soil affects the amount of Ca in the plants (Zlatić et al. 2017). Calcium is involved in 0.1–2.0% of dry matter of plants. The normal concentrations of this element in the above-ground parts of the plants were 5 g kg^{-1} (Shallari et al. 1998). The content of Ca in plants growing on serpentine soil is lower than 0.8% (Kataeva et al. 2004). Concentration of calcium was higher in the above-ground parts of plants compared to the substrate (serpentine or calcareous), although the serpentine species are better adapted to low Ca concentrations. The plants, which have normal growth, the Ca:Mg ratio is higher than 1. The serpentine species are adapted to low Ca concentrations relative to Mg, since Mg prevents the absorption of Ca (Brooks 1987). Calcium is an essential macronutrient which participates in the construction of the cell wall, provides normal transport through membrane proteins and acts as a cofactor for many enzymes (Marschner 1995). Differences in concentration of Ca in soil and plant material from serpentine habitats could be due to the presence of organic matter and the effects of various ecological factors presented on a specific type of ecosystem (Zlatić et al. 2017).

The amount of Mg in plant samples of *Teucrium montanum* was in the range from 1476.6 to 3651.4 mg kg^{-1} (Zlatić et al. 2017), and in the range from 1932 to 4626 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results of the study indicated that the amount of Mg in the plant samples on the serpentine substrate (Goč and Kamenica) was higher than in the samples from the calcareous substrate (Kopaonik and Durmitor) (Zlatić et al. 2017). Increased concentrations of Mg in serpentine plants population indicated their ability to adapt and survive under stressful conditions. The differences in quantity between Mg and Ca affects the appearance of undeveloped vegetation on serpentine soils. Plants could grow successfully, if the ratio of the total amount of Ca:Mg in the soil is at least in ratio 1:1 (Brooks 1987). Magnesium is macronutrient that plants used for their growth and development in large quantities (Karley and White 2009). Also, this element is located at the center of the chlorophyll molecule and had a significant function for many enzymes and chelating nucleotide formations (Shaul 2002). Lack of Mg in plants could affects the increase in the activity of antioxidant mechanisms in order to neutralize harmful effects (Cakmak and Kirkby 2008). The obtained results of the comparative analysis showed that the samples of soil and plant material from the serpentine geological substrate had a higher concentration of Mg from the calcareous substrate. Certain amounts of Mg are required by plant nutrition, and studies showed that plants could metabolically control the absorption of Mg from the soil. The differences in the concentration of Mg between serpentine localities could be attributed to the different influence of pedological processes and ecological factors that were dominate on the investigated habitats (Zlatić et al. 2017).

The amount of K in plant samples of *Teucrium montanum* was in the range from 6221.4 to 12338.6 mg kg^{-1} (Zlatić et al. 2017). The results of this study indicated that the amount of K in soil and plant samples from calcareous substrate, was higher in relation to the quantity in samples from the serpentine substrate. The increased amount of K in the soil influenced the amount of K in the plants (Zlatić et al. 2017). Potassium is one of the main nutrient, which affect the growth and development of plants. It is the most important element in plant nutrition of all alkali elements found in the soil (K, Ca, Mg, and Na) (Haby et al. 1990). Due to the disturbed water regime, plants require higher concentrations of K for adaptation to stress caused by photo-oxidative damage. Potassium had an important role in the protection of plants, which are presented in arid habitats (Sen Gupta et al. 1989).

The accumulation of Ni, Cr, and Co in the above-ground plant parts for all serpentine populations of *Teucrium montanum* were higher than in non-serpentine ones (Pavlova and Karadjova 2012; Branković et al. 2017; Zlatić et al. 2017). Element concentrations vary between the populations in relation to soil properties. Nickel quantity in plant samples for *Teucrium montanum* was in the range from 9.8 to 108.6 mg kg^{-1} (Zlatić et al. 2017). The results of this study indicated that the amount of Ni, in soil samples and in samples of plants on the serpentine substrate, was higher than the quantity in samples from the calcareous substrate, as well as that the increased amount of Ni in the soil influenced the amount of Ni in the plants. In the plant species from serpentine, the absorption of certain nutrients had been changed because of stress induced by the presence of Ni. Certain concentrations of this element in plant tissue inhibited the process of photosynthesis and transpiration. In natural conditions, the toxicity of Ni is in conjunction with serpentine soils which possess great amounts of this metal (Kabata-Pendias 2011). Nickel is very mobile in the soil and showed the ability to cross from one soil layer to another. Fe and Mg oxides could accumulate large amounts of Ni. After this accumulation, Ni is available to plants. Influence on the bioavailability of Ni in the soil depended of the presence of organic matter, clay fractions and pH values (Kastori 1993). It has been showed that the soils formed on the serpentine substrate had a higher concentration of Ni than those on limestone (Zlatić et al. 2017). The composition of the geological substrate plays an important role in the presence of Ni in the soil. The difference between localities with the same geological substrate could be attributed to the various environmental factors, that affect the certain habitat, as well as the chemical processes in the upper layers of the soil.

The amount of Cr in plant samples of *Teucrium montanum* was in the range from 4.15 to 45.3 mg kg^{-1} (Zlatić et al. 2017) and between 0.3 and 28 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results of presented research indicated that the amount of Cr, in soil samples and in samples of plants on the serpentine substrate, was higher than the amount in samples from the calcareous substrate on the territory of Bulgaria and Serbia. The increased quantity of Cr in the soil affects the quantity of Cr in plants (Zlatić et al. 2017). Chromium content in plants is affected by the amount of soluble Cr in the soil. In the mentioned studies, there is no data based on the important role of Cr in plant metabolism. However, there are data which described the positive influence of Cr on plant growth. The most accessible for plants is Cr^{6+}, which in certain soils is unstable and whose distribution depends on the characteristics of the soil, its texture, and pH value. In some plant species from the serpentine region, the Cr concentration varies from 0.3% to 3.4% (Kabata-Pendias 2011).

The amount of Fe in the plant samples of *Teucrium montanum* was in the range from 351.9 to 2673.1 mg kg^{-1} (Zlatić et al. 2017) and between 148.5 and 1490 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). In the presented studies the amount of Fe, in soil samples and in samples of plants on the serpentine substrate, was higher than the quantity in samples from the calcareous substrate. Iron is a reactive element and it is similar like Co and Ni. Studies have confirmed that the reactivity of Fe depends on the pH value and the oxidative degree of the Fe compound (Kabata-Pendias 2011). The distribution of the Fe compound in the soil can be useful in determination the type and characteristics of the soil (Zonn 1982). The concentration of Fe in the soil solution at usual pH value varies between 30 and 550μg l^{-1}. In very acidic soils, it could be over concentration of 2000μg l^{-1}. Therefore, in calcareous soil types, with pH from 7 to 7.8, concentration of Fe ranged from 100 to 200μg l^{-1}. In sandy substrates, whose pH varies from 2.5 to 4.5, concentration of Fe ranged between 1000 and 2223μg l^{-1}. The solubility level of Fe is minimal in alkaline soils. Acidic soils are rich in soluble inorganic Fe in relation to soil from neutral and limestone substrates. Reduced quantities of Fe are characteristic for dry areas (Kabata-Pendias 2011). Although the Fe compounds in the soil are mobile, there is a tendency for the formation of mobile organic compounds and chelates. These compounds caused the distribution of Fe in the soil layers, as well as the transition of ion Fe from one layer to another that plants absorb through the root system. The quantity of Fe, that plants could absorb, depends on the changing conditions of the soil and climate, and the stage of plant development. However, when Fe is soluble, plants may absorb a higher amount (Kabata-Pendias 2011). This was clearly demonstrated in plant samples from serpentine habitats where the Fe concentration in the tested grasses were between 2127 and 3580 mg kg^{-1} (Johnston and Proctor 1977). Fe oxides bind to Mn compounds and this is the reason of why Fe is more represented in the serpentine substrate (Kabata-Pendias 2011). Certain amounts of Fe are required by plants in the nutrition. Plants regulate the concentration of the same in their tissues through metabolism. The concentration of Fe affects the composition of the parent material, pedogenetic processes, the presence of organic matter and other factors. The difference in the concentration of Fe between similar sites could be explained through different environmental factors, which are dominated in the specific habitat.

The concentrations of essential elements, Mn, Cu and Zn measured in *Teucrium montanum* from Serbia and Bulgaria (Pavlova and Karadjova 2012; Zlatić et al. 2017) were within the ranges considered normal for plants from serpentine and non-serpentine sites (Kabata-Pendias 2011). Usually, serpentine soils are not rich in Cu, Zn, and Pb. The amount of Mn in the plant samples of *Teucrium montanum* was in the range from 22.16 to 78.3 mg kg^{-1} (Zlatić et al. 2017) and between 16 and 99 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results of the study indicated that the amount of Mn, in soil samples and in samples of plants on the serpentine substrate, was higher than the quantity in samples from the calcareous substrate. By reducing the amount of Mn in the soil, the amount of Mn increases in the above-ground parts of the plants. High levels of Mn are observed in soils that are present on serpentine soil, in soil rich in Fe and organic matter, as well as in arid and semi-arid regions of the earth (Kabata-Pendias 2011). Certain quantities of Mn are required in plant nutrition, and studies showed that plants could metabolically control the absorption of Mn from the soil (Skinner et al. 2005). Numerous studies showed the relationship between the amount of Mn and Fe represented in the soil. Manganese was significantly more present in areas where Fe oxides are present (Kabata-Pendias 2011).

The amount of Cu in plant samples of *Teucrium montanum* was in the range from 1.68 to 8.35 mg kg^{-1} (Zlatić et al. 2017) and between 5.7 and 15.4 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The quantity of Cu, in the soil samples and in the samples of plants on the serpentine substrate (Goč and Kamenica), was higher than the quantity in the samples from the calcareous substrate (Kopaonik and Durmitor). With reducing the amount of Cu in the soil, the amount of Cu in plants grows. Plants sampled on serpentine soil had a higher concentration of Cu than those on limestone. However, minor deviations were observed, which is in accordance with the above data on the bioavailability of Cu (Zlatić et al. 2017). The concentration of Cu was influenced by the presence of organic matter, pedogenetic processes, and the composition of the parent material.

According to the results presented by the study from Zlatić et al. (2017), the quantity of Zn in the above-ground plant parts of the species *Teucrium montanum* parts varied from 14.79 to 39.75 mg kg^{-1} in populations from Serbia, and between 29 and 70 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results indicated that the quantity of Zn, in soil samples and in samples of plants from the calcareous substrate, was higher than the quantity in samples from the serpentine substrate, and that the increased amount of Zn in the soil influenced the amount of Zn in the plants. The composition of the parent material, the structure of the soil, the presence of organic matter, the ecological factors, the pH value are one of the factors that influenced the amount of Zn in the soil. The concentration of Zn in plant tissues varies between 15 and 150 mg kg^{-1}, while maximum values were up to 300 mg kg^{-1} (Brunetti et al. 2009). In the acidic environment, Zn was very soluble (Kastori 1993). In previous studies, it has been found that the lack of Zn disrupted the absorption of Fe (Graham et al. 1987). Tolerant plant species had ability to reduce the effect of increased concentrations of Zn. Mechanisms of defense are reflected in the adaptation of metabolic reactions in the plant organism. Plants accumulate Zn in some parts of the cell and tissue (Kabata-Pendias 2011).

The amount of Pb in plant samples of *Teucrium montanum* was between the detection limits to 6.02 mg kg^{-1} (Zlatić et al. 2017), and between 0.6 and 21.7 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results of the authors suggested that the amount of Pb in the soil samples from the calcareous substrate was higher than the quantity in the samples from the serpentine substrate, while the opposite results were showed in the plant samples. Lead is the least mobile among heavy metals in the soil, although its sorption was lower than Zn and Cu (Vega et al. 2007). The Pb concentrations in soils formed on the calcareous substrate are always higher than those formed on the serpentine (Kabata-Pendias 2011). Lead behaves like Ca, therefore, it had less in plants on calcareous soil. The obtained results were in accordance with numerous studies related to the absorption of Pb by plants present on the serpentine substrate (Reeves et al. 2007; Kabata-Pendias 2011).

Based on the results presented in the studies conducted by Pavlova and Karadjova (2012) and Zlatić et al. (2017), it can be concluded that *Teucrium montanum*, which accumulate a higher amounts of heavy metals (Ni, Fe, Mn, Cu), is not a hyperaccumulator of these elements. The investigated species possess physiological mechanisms that allow adaptation of various stress conditions, caused by increased concentrations of heavy metals in the soil, as well as specific conditions of the habitat on the serpentine substrate.

4.4.2 Plant-Soil Correlation

Correlations between elements in the above-plant parts of *Teucrium montanum* and elements in the serpentine soils from the territory of Serbia were calculated as average values (Zlatić et al. 2017). Significant positive correlations at level $p < 0.01$ were observed between Ca and Zn in *Teucrium montanum* samples and Ca in the soils for Serbian serpentine sites. Negative correlation coefficients were found for Ni and Mg plant samples and Ca in the serpentine soils. Also, the synergistic effect between elements Fe, Ni, Cr, Mn in serpentine soils were found. It has been established that the contents of Mn and Cu in the soil and those of Mn and Fe in the plants correlate. The studies confirmed the antagonistic and synergistic effects of Mn in the process of absorption of Pb and Cd; antagonistic effects of Mn in the process of N, Na and K absorption and the substantial impact of Zn on the low absorption of Mn (Kabata-Pendias 2011). Other scientists confirmed the existence of correlations between certain metals (Yan et al. 2012; Gonneau et al. 2014).

4.4.3 Bioaccumulation Factors

Concentration and the absorption of elements in plant tissues depended on the concentration in the substrate and the ability of the plant to absorb certain amounts of the same. Absorption of certain macro- and microelements is regulated by special mechanisms in plants. Bioaccumulation factor (BAF) is the ratio between the total content of metals in the plants and the content of metals in the soils, and it is used to determine the total amount of metal that the plant accumulated from the soil through the root system into organs (Pandy and Tripathi 2010). The BAF results for *Teucrium montanum* on calcareous and serpentine habitats in most cases had values below 1 (Zlatić et al. 2017). BAF values higher than 1 were observed for K from calcareous and serpentine populations of *Teucrium montanum* while values higher than 1 for Ca were observed for populations of the species of serpentine habitat (Zlatić et al. 2017). The bioaccumulation trends in the species are as follow: Zn > C u > Cr > Mg > Mn > Ni > Pb > Fe.

Factors affecting the mobility, accessibility, and dynamics of soil elements for plants were the pH value, the content of organic matter and clay in the soil, the mechanical composition and humidity of the soil, the content and the presence of hydrated Al and Fe oxides. Elements, such as Zn and Cd, are very mobile in the soil and could be easily accumulated by plants. Elements such as Cu, Mn, Ni, are moderately mobile in the soil and could be rapidly absorbed by plants, while Pb is relatively strongly related to soil particulates and could not be transported to the above-ground parts of plants (Kabata-Pendias 2011).

Zinc is a very mobile in most types of soil. Zinc has an essential metabolic function in the plants, and in the soil solution, it is in the form of free ions or ionic complexes. Plants absorb Zn mainly from the soil in the form of divalent cation (Zn^{2+}). It has been showed that plants absorb Zn in the form of hydrated zinc (Zn^{2+}), in the form of ionic complexes and Zn organic chelates (Kabata-Pendias 2011). The copper is quite immobile in the soil and its mobility was influenced by the organic matter of the soil, the dissolved organic matter, the pH and the content of Cu in the soil. The bioavailability of the soluble Cu forms depended on the molecular weight of the Cu complex, and on their amounts in the soil. Copper is an essential element for plants. Soil pH value play an important role in the availability of Cu and its toxicity to plants. The plants can absorb small quantities of Cu, mainly in the form of Cu^{2+} ions and chelates. Copper concentration is influenced by its concentration and the presence of other ions in soil such as heavy metals, like Zn, Mn, Fe (Kastori 1993). Chromium is a crucial element for plants, which stimulate the growth and development of some plant species. Higher concentrations of Cr showed a toxic effect on plants. The effect of Cr in plants depends on its concentration in soil, but also on the chemical and physical properties of the soil (Kastori 1993). Plants could absorb Mg in the form of Mg^{+2} from the soil solution. The availability of Mg is affected by the parent material, climatic factors, as well as the accumulation capacity of the soil. In the conditions of the acidic soil reaction, the availability of Mg was reduced by its competence with Al or Mn, while the presence of carbonates and increased concentrations of Ca, K and Na could reduce the absorption of Mg in alkaline conditions (Sigel and Sigel 1990). Manganese could be present in soils in several forms (Kabata-Pendias 2011). The plants absorb Mn in acidic soil where it is in the form of Mn^{2+} ions. Absorption of Mn is metabolically controlled while higher concentrations of this element depend on the level of Mn concentration in the soil. The accumulation of Ni had a significant influence on the content of other elements in the substrate, while it had no effect on the absorption and metabolism of Fe (Kastori 1993). Dynamics of Pb in the soil is influenced by numerous factors, like pH value and the presence of organic matter. Lead is mainly occurred in the soil, in the form of Pb^{2+}. Plants poorly can absorb and move inorganic forms of Pb into the above-ground parts, while the adsorption and transport of organic forms are relatively strong. Lead have very low bioavailability, so the roots have a great ability to accumulate Pb, which is one aspect of protecting the above-ground parts of plants. The content of easily soluble Fe fractions is limited compared to the total content in the soil. Iron is a slow-moving element in the soil and showed the tendency to form organic complexes and chelates. Iron ions are not absorbed directly from the plant, but from the complexes with organic compounds. The high level of Fe compound, its precipitation on carbonates and the competence of other cations with Fe^{2+}, are responsible for the low absorption of Fe (Kabata-Pendias 2011).

4.4.4 Species Adaptations to Metalliferous Soils: Quantity of Secondary Metabolites

Secondary metabolites are compounds that plays a role in the interaction of plants with the environment and have an influence on plant adaptation on different abiotic and biotic stresses (Kliebenstein and Osbourn 2012). Biosynthetic pathways of secondary metabolites are to some extent influenced by environmental factors such as the presence of heavy metals in the substrate, altitude, water regime, radiation, etc. (Endt et al. 2002; Politycka and Adamska 2003). The quantity of secondary metabolites is different in plant parts and depends on the development phase, season and several ecological factors (Filippini et al. 2010). Secondary metabolites participate in the process of adaptation of plant organisms to the ecological environmental conditions, and their quantity varies in plant organs depending on the abiotic and biotic factors (Ramakrishna and Ravishankar 2011). The presence of heavy metals in the substrate caused increased synthesis of secondary metabolites. It has been determined experimentally that certain plants increase the synthesis of phenolic compounds due to the presence of heavy metals. Due to the increase in the number of phenolic compounds, the activity of enzymes involved in their biosynthesis is also enhanced (Michalak 2006).

Phenolic compounds represent a large group of secondary metabolites, which have one or more aromatic rings with one or more hydroxyl groups in the structure. Within the group of phenolic compounds, two subgroups are distinguished: flavonoids and phenolic acids (Quideau et al. 2011). Flavonoids are present in all plant organs, while their amount varies according to the ecological and genetic factors (Stanković et al. 2010, 2012). The role of phenolic acids in plants is various and it reflected in the process of absorption of necessary materials from the substrate and the adaptation of the plant to abiotic and biotic stresses (Mandal et al. 2010). The particularly important role of phenolic compounds is the antioxidant activity. Phenolic compounds act as an reducing agents, donors of hydrogen, and have the properties of metal healing. The antioxidant activity of these compounds is primarily the result of their role as a donor of hydrogen, after which there are less reactive phenoxyl radicals (Quideau et al. 2011).

Zlatić et al. (2017) indicated that the total amount of phenolic compounds in plant extracts of *Teucrium montanum* was in the range from 143.42 to 190.2 mg GA g^{-1} of extract, while the quantity of flavonoids ranges from 46.5 to 54.19 mg Ru g^{-1} of the extract. For *Teucrium montanum*, the concentration of phenolic compounds and flavonoids was higher in extracts from sample originated from serpentine sites than in samples from the calcareous substrate. Also, authors indicated that the total amount of phenolic compounds in plant extracts of *Teucrium montanum* was in the range from 37.11 to 66.52 mg GA g^{-1} of the extract, while the quantity of flavonoids was in range from 15.38 to 20.9 mg Ru g^{-1} of the extract. The concentration of phenolic compounds as well as flavonoids of *Teucrium montanum* was higher in extracts from samples originated from the habitat with serpentine geological substrate compared to samples from the limestone substrate (Zlatić et al. 2017).

There are differences in the quantitative composition of secondary metabolites of plants that were sampled on calcareous and serpentine substrates (Pavlova 2009). The presence of heavy metals in the soil caused increase of secondary metabolites synthesis. It had been determined that certain plants enhance the synthesis of phenolic compounds due to the presence of heavy metals (Michalak 2006; Stanković et al. 2011b).

The increase of phenolic compounds concentration in the plant extracts from *Teucrium montanum* occurred in the response of plants to stress conditions, caused by heavy metals (Lavid et al. 2001). The total amount of phenolic compounds was higher due to the increased concentration of heavy metals (Hamid et al. 2010). The antioxidant system of *Aeluropus littoralis*, under the influence of stress caused by heavy metals, goes through biochemical changes, which are reflected in increase the concentration of phenolic compounds involved in chelation (Rastgoo and Almzadeh 2011). Rusak et al. (2005) indicated that flavonoids have multiple protective functions, such as antioxidant activity. Phenols and flavonoids can be oxidized by peroxidase and react in H_2O_2 phenolic systems, which regulate the input of heavy metals in plants (Michalak 2006). Some heavy metals can cause a decrease in volume of reducted glutathione (GSH) and insufficient activity of antioxidant enzymes, especially glutathione reductase (GR) (Schützendübel and Polle 2002). Based on this knowledge, alternative antioxidants, such as flavonoids, could be produced by plants. Flavonoids build complexes with heavy metals, making them the main systems in the adaptation of plants to stress conditions caused by increasing the concentrations of heavy metals (Michalak 2006; Korkina 2007).

The qualitative and quantitative analysis of the composition of *Teucrium montanum* secondary metabolites confirmed the presence of gentisic acid which is the most represented phenolic acid in the extracts. Also, the phenolic acids, that are present in lower concentrations, are chlorogenic, coumaric, syringic, gallic, vanillin, caffeic, and ferulic acid (Tumbas et al. 2004; Čanadanović-Brunet et al. 2006). A qualitative analysis of the essential oil showed that the main components of the volatile compounds are: β-cadenine, β-selinene, α-calacorene, β-caryophyllene, β-pinene and germacrene (Vuković et al. 2008; Bežić et al. 2011).

The phenolics contribute to the antioxidant potential of plants by neutralization of free radicals (Stanković et al. 2010). Populations of plants sampled from the serpentine localities are differentiated by the increased quantity of total phenolic compounds, as well as intense antioxidant activity in relation to plant populations sampled from calcareous localities. Higher concentration of total phenolic compounds are in correlation with the intense antioxidant activity of *Teucrium montanum* extracts and have a crucial role in the adaptation of this plant to habitats with the higher concentration of heavy metals. Differences in the concentration of secondary metabolites and expressed antioxidant activity could be attributed to a different type of habitat as well as the abiotic factors on which the populations are represented. There are differences in the quantity of phenolic compounds and antioxidant activity among plant populations from serpentine soil. This difference was induced by a different amount of metal at a specific locality, a difference in thermal and water regime, as well as the altitude at which the locality is located (Zlatić et al. 2017).

4.4.5 Species Adaptations to Metalliferous Soils: Morphology

Serpentine soils represent a special substrate for the development of flora and vegetation (Brooks 1987). Plants, which are present on the serpentine soil, possesses numerous structural and functional adaptations, which are different from the plants represented on other types of soil. Serpentine plants are characterized by "serpentine syndrome", which is a result of the disturbed relationship between Mg and Ca, heavy metal toxicity and other pedological physicochemical characteristics (Kruckeberg 1984; Brooks 1987). Additionally, to the low water potential of the soil, the plants on the serpentine have great tolerance to the increase of concentration of heavy metals from the substrate (Proctor 1999). Numerous studies have showed that populations represented in different serpentine soils have similar characteristics (Proctor and Woodell 1975; Kruckeberg 1984; Proctor and Nagy 1992). Facultative serpentinophytes showed a "serpentine syndrome" such as a complex of morpho-functional adaptations that differentiate the plant populations present on serpentine soils. These adaptations include smooth-surface leaves with waxy coatings, plants with high developed roots, plagiotropism and nanism (Kruckeberg 1984; Proctor and Woodel 1975; Brady et al. 2005).

The habitats of *Teucrium montanum* are calcareous rocks, pine forests on calcareous soil, pastures on serpentine substrate. The species occupy different type of habitats from 30 to 2000 m above sea level. Species is represented in the communities of deciduous, mixed and coniferous forests, in the communities of serpentine rocky and continental calcareous pastures, gorge and canyons, in the communities of the submediterranean region, the communities of the xerophilous type, and the slopes of the mountainous and mountain regions. In the territory of Serbia, the species *Teucrium montanum* is the most represented on the calcareous and serpentine substrate, rocky meadows and dry pastures around the pine forests (Stanković and Zlatić 2019).

Previous morpho-anatomical studies of *Teucrium montanum* from different habitats indicated that species possess phenotypic plasticity manifested through more or less xeromorphic properties. Studies of *Teucrium montanum* from calcareous and serpentine localities showed physiological and ecological differences between populations, presented on the serpentine and calcareous substrate, with specific features in the morpho-anatomical structure of serpentine plants designated as serpentinomorphoses (Stevanović and Stevanović 1985). Morpho-anatomic differences were observed in plants from different populations as a response to the adaptive abilities of the species to certain environmental conditions. In plant populations from calcareous habitats, xeromorphic characteristics were observed, which are in accordance with the general habitat conditions determined by the hygrothermal soil regime.

Teucrium montanum on these habitats is compact and densely branched bushes. The leaves are small, covered with hairs and moderately curved to the abaxial side. At the cross-section, the leaves are thicker with a distinct cuticle compared to the serpentine population. They possess a large number of vascular bundles and a more developed mesophyll. On the serpentine habitats, *Teucrium montanum* is represented in the bushy habitus form with low density. On the shoots, there are fewer leaves than on the plants from calcareous habitats. Leaves are smaller and ticker in relation to leaves from calcareous populations, while the edges of the leaves are strongly curved to the abaxial side. At the cross-section, the leaves are thin with a well-developed cuticle. Cells of mesophyll are smaller with poorly expressed intercellular space. In plants with serpentine habitats, xeromorphic characteristics are strongly expressed in connection with the serpentine substrate and the effect of physiological drought (Stevanović and Stevanović 1985).

4.5 Conclusions

This chapter is a review of the ecological, physical, chemical, morphological and geographical distribution of two selected species of the genus *Teucrium*. *Teucrium chamaedrys* and *T. montanum* demonstrate a wide ecological tolerance as they could be found in different habitats, like rocky calcareous and serpentine terrains, open grasslands, pastures, and pine forests. Both species showed the ability to absorb a large number of elements from soil both essential (Cu, Fe, Ni, Mn, Zn) and non-essential (Co, Cr, Pb) for its biological functions. Plant populations from serpentine habitats possess a higher content of heavy metals, like Mn, Ni, Cr, than calcareous one. Among the selected species *Teucrium montanum* can accumulate more metals (Cr, Fe, Ni) compared to *Teucrium chamaedrys*. Based on the difference between the ability of absorption and accumulation of heavy metals, described plant species could not be classified as metal-hyperaccumulating plants. *Teucrium chamaedrys* and *T. montanum* are classified as excluders, containing relatively low metal concentrations in their aerial parts, despite the high element concentrations in the soil. The presence of heavy metals and other environmental conditions from serpentine possess significantly higher values of secondary metabolites in plant populations, compared to non-serpentine ones. The significant morpho-anatomic differences between populations are related to the differences in edaphic conditions of the serpentine and non-serpentine sites. The serpentine populations are characterized by their reduced size – lower stems, smaller leaves, and shorter internodes, compared to the non-serpentine populations. Selected plant species collected from naturally metalliferous (serpentine) soils should be avoided because of the increased concentration of heavy metals in plant tissues. Traditional medicinal products, which contains heavy metals in concentrations hazardous to human health. Usage of these plants should be limited. Plants collected from serpentine soils require strict control. Medicinal herbs should be gathered and cultivated only from non-metalliferous sites.

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Chapter 5
Ethnobotanical Features of *Teucrium* Species

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Abstract Species of the *Teucrium* genus have been used in ethnopharmacology for centuries, helping to treat many pathophysiological conditions, such as diabetes, gastrointestinal disorders, rheumatism, inflammations, and tuberculosis. They can also be used as a diuretic, antipyretic, tonic, diaphoretic, analgesic and antihyperlipidemic. This study provides a comprehensive analysis of the traditional use of *Teucrium* species based on research conducted up to now in different parts of the world. With this aim, 72 ethnobotanical studies were analyzed and a total of 20 *Teucrium* species were identified for this analysis. Nineteen of these species are used in human ethnomedicine, 3 in veterinary ethnomedicine and 4 for other purposes. *Teucrium chamaedrys*, *T. polium* and *T. montanum* are mentioned in the largest number of studies and have the widest range of uses. The aerial parts of the plant are those that are most frequently used, with infusions (77.8%) and decoctions (48.6%) the most common methods of preparation. The use of *Teucrium* species for treating abdominal problems is mentioned in 56 of the studies and in 21 studies for treating disorders heart and blood vessels. Treating respiratory problems is referenced in 17 studies and problems in the functioning of the endocrine glands in 16 studies. The medicinal properties of *Teucrium* species can be ascribed to their chemical composition, specifically essential oils, phenolic acids, flavonoids, and other secondary metabolites. However, the long-term use of some of the preparations can have negative side effects, such as hepatotoxicity or gradual deterioration in neuromuscular coordination.

Keywords Ethnobotanical research · *Teucrium* species · *T. chamaedrys* · *T. polium* · *T. montanum*

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5.1 Introduction

Medicinal plants and products made from them have been used in ethnomedicine all across the world since time immemorial. Numerous plant species are central to beliefs, methods and institutions for diagnosing and treating diseases and preventing them. Hence, botanical folk knowledge plays a crucial role in the ethnomedicine of every civilisation (Nedelcheva and Draganov 2014). Indeed, the most useful starting point for developing new pharmaceuticals is provided by the array of botanical sources, particularly metabolites and their biological activities, in addition to the various practices developed by ethnic groups over the centuries in terms of the preparation and application of herbal remedies (Jarić et al. 2007). According to estimations provided by Uritu et al. (2018), up to 70,000 plant species are used for ethnomedicine worldwide, while according to data from the World Health Organization (WHO), approximately 80% of the world's population still relies mainly on plant-based drugs (Bahmani et al. 2014). The reasons for the wide use of medicinal plants in ethnomedicine across the world include their relative ease of availability and the fact that they are quite a cost effective alternative when it comes to daily health care and self-medication; in light of this, many people, particularly those in poorer rural areas or when economic crisis hits, depend on them as home remedies for health problems (Leonti 2011). Their therapeutic value is based primarily on the link between the chemical structure of the active substances they contain and their pharmacodynamic effects on the body (Rafieian-Kopaei 2012; Jarić et al. 2014, 2015a).

Medicinal and aromatic plants come from a whole variety of plant families that often have common active ingredients (due to their biosynthetic pathways being similar). One, highly significant example is the plant family Labiatae (Lamiaceae), which includes many species that contain essential oils (Máthé 2015; Jarić et al. 2015b) and, as such, have biological and medical applications. Their high concentrations of biologically active substances mean they contribute significantly to both traditional and modern medicine (phytotherapy). This family includes aromatic herbs such as thyme, mint, oregano, basil, sage, savory, rosemary, self-heal, hyssop, and lemon balm, while other species have more limited uses (Bekut et al. 2018).

This chapter provides a comprehensive analysis of the traditional uses of *Teucrium* species from the ethnobotanical and ethnomedicine points of view, based on ethnobotanical research undertaken in different parts of the world. With this aim, 72 ethnobotanical studies were analysed and 20 species of the *Teucrium* genus were identified for the purposes of this analysis.

5.2 Botanical Characterization and Distribution of *Teucrium* Species

Teucrium L. (germander) is a large, polymorphic, cosmopolitan genus, belonging to the family Lamiaceae, within the subfamily Ajugoideae. This family has a cosmopolitan distribution with about 236 genera and contains between 6900 (Heywood et al. 2007) and 7200 species (Harley et al. 2004), although the World Checklist lists 7534 (https://wcsp.science.kew.org/qsearch.do). The genus *Teucrium* comprises more than 300 species, which are widely found in Europe, North Africa, and temperate parts of Asia, but 96% are distributed in the Mediterranean region (Tutin et al. 1972; Meusel et al. 1978; Navarro and El Oualidi 2000). About 195 taxa are present at the level of species and subspecies, 83 of which are on the Pyrenean peninsula, 72 in the northwestern part of Northern Africa (Algeria and Morocco), 61 in Asia Minor, 41 on the Balkan Peninsula, and 23 on the Apennine Peninsula (Greuter et al. 1986). In terms of European flora, the genus *Teucrium* has been divided into seven sections with 49 species (Tutin et al. 1972), while a few are spread throughout South America, mountainous tropical Northeast and South Africa, and Australia (Wielgorskaya 1995).

Species of the genus *Teucrium* are mostly perennial herbs, shrubs or subshrubs, and more rarely, annual herbaceous plants (e.g. *T. botrys*) (Tutin et al. 1972). Research into micromorphological characteristics, especially trichomes and pollen, nutlet morphology and vegetative anatomy has shown that these have taxonomic value for sectional and interspecific classification in this genus (Oybak-Dönmez and Inceoğlu 1988; Navarro and El Oualidi 2000; Jurišić Grubešić et al. 2007; Dinç et al. 2008, 2009; Eshratifar et al. 2011; Lakušić and Lakušić 2014).

5.3 Ethnobotanical and Ethnomedicinal Research into *Teucrium* Species

5.3.1 A Historical Review of the Use of Teucrium *Species in Ethnomedicine*

For over two millennia, *Teucrium* species have been used as medicinal herbs, with many still used in folk medicine today (Milošević-Djordjević et al. 2013). Used to treat coughs and asthma since ancient Greek times (Menichini et al. 2009), there are many other records of their use in ethnomedicine. Pharmaceutical manuscripts from Persia, dating from the period between the ninth and eighteenth centuries AD, note the use of the aerial part of *T. montanum* as a decoction to treat headaches (Zarshenas et al. 2013). Moreover, in medieval written documents, i.e. in lists of *materia medica* used by the Jewish community of Medieval (eleventh to fourteenth centuries) Cairo, found at the Taylor–Schechter Genizah collection, Cambridge, the species

Teucrium capitatum L. is mentioned twice, with its leaves and stems used in the treatment of eye diseases, stomach ailments and colic (Lev 2007). Similarly, the botanical *materia medica* of the *Iatrosophikon* – A collection of prescriptions from a monastery in Cyprus, which dates back to the Ottoman period (1571–1878), includes *Teucrium micropodioides* Rouy as an exotic taxon originating from the New World, used to treat catarrh and the common cold (Lardos 2006). In their work on the use of medicinal herbs for the treatment of rheumatic disorders in the sixteenth and seventeenth centuries, Adams et al. (2009) stated that important European herbals (Fuchs 1543; Bock 1577; Matthiolus 1590; Tabernaemontanus 1687) mention the ethnomedicinal use of *Teucrium chamaedrys* L. Namely, its aerial parts were boiled in wine and this concoction was taken for 60 days, 3 h before meals on an empty stomach. Any patient seeking to use this as a remedy for gout of the feet or hip pains also had to avoid sour and salty foods.

5.3.2 The Use of **Teucrium** *Species in Human Ethnomedicine*

Teucrium species are generally aromatic, ornamental plants and also a valuable source of pollen, while many are used in folk medicine and pharmacy. Numerous phytochemical and pharmacological studies have confirmed their medicinal and biological properties, such as hypoglycemic (Gharaibeh et al. 1988; Baluchnejadmojarad et al. 2005), hypolipidemic (Rasekh et al. 2001), hepatoprotective (Shtukmaster et al. 2010), antipyretic (Autore et al. 1984), anti-inflammatory and antiulcerogenic (Sghaier et al. 2011a), anticarcinogenic (Sghaier et al. 2011b; Stanković et al. 2011a), and antimicrobial activities (Vuković et al. 2007). These species are very rich in phenolic compounds with very strong biological activity (Yin et al. 2009; Hasani-Ranjbar et al. 2010).

Knowledge on the use of *Teucrium* species, which are used by locals in various geographical regions, has been well documented in numerous ethnobotanical studies. In this chapter, 72 ethnobotanical studies from across the world were analysed and 20 species of the genus *Teucrium* were identified for their medicinal properties. Nineteen species are used in human ethnomedicine (Table 5.1) and 3 in veterinary ethnomedicine (Table 5.2), while 4 are also used for other purposes (Table 5.3). It should be noted that some of them have multiple uses, while *Teucrium scorodonia* is only used in veterinary medicine.

Our analysis of these studies revealed that the people of Turkey, Iran, Bosnia and Herzegovina, Italy, Pakistan, Serbia and Palestine know most about *Teucrium* species and use them most frequently (Fig. 5.1). It was also determined that the species *Teucrium chamaedrys* was the most frequently mentioned of all the species of the genus *Teucrium*. Details on its use were found in 34 (47.2%) ethnobotanical studies; 13 (18.15%) of these were conducted in different parts of Turkey, 5 (7%) in Italy and the same number in Bosnia and Herzegovina. The species *Teucrium polium* is used in 23 regions of the world (31.9%), but is most familiar to and most frequently used by the people of Iran (6 studies, 26.1%) and Turkey (5 studies, 21.8%).

Table 5.1 The use of *Teucrium* species in traditional medicine

Teucrium species	Locality	Part used	Forms of preparation and method of administration	Traditional uses	References
T. arduini	Bosnia and Herzegovina	Aerial part	**I:** Infusion	Stomach diseases	Redžić (2007)
T. arduini	Bosnia and Herzegovina (Central, Southern and Western)	Aerial part	**I:** Infusion	Gastrointestinal ailments	Šarić-Kundalić et al. (2010a)
T. arduini	Bosnia and Herzegovina (Eastern, Northern and Northeastern)	Aerial part	**I:** Infusion	Diarrhoea	Šarić-Kundalić et al. (2011)
T. capitatum	Palestine (West Bank)	Leaf	**E:** Decoction (about 50 g leaves are boiled in 100 ml water and the affected area is bathed with this decoction once a day)	Psoriasis	Shawahna and Jaradat (2017)
T. capitatum subsp. *capitatum*	Lebanon (Mount Hermon)	Aerial part	**I:** Infusion	Diabetes, insomnia and neurological disorders, abdominal cramps	Baydoun et al. (2015)
T. chamaedrys	Turkey (Nigde, Aladaglar)	Aerial part	**I:** Infusion	Abdominal pain, haemorrhoids, diabetes, a painkiller, stomach pains	Özdemir and Alpınar (2015)
T. chamaedrys	Turkey (Afyonkarahisar, Inner-West Anatolia)	Leaf, flower	**I:** Infusion	A painkiller, stomach problems and haemorrhoids	Ari et al. (2015)
T. chamaedrys	Turkey (East Anatolia)	Herb/aerial part	**I:** Infusion, decoction	Toothache, kidney pains, a stomachic, indigestion, heart disease	Altundag and Ozturk (2011)
T. chamaedrys	Turkey (Edremit Gulf, Balıkesir Province)	Flowering branches	**I:** Infusion (drink one cupful twice a day for a week)	Abdominal pains, kidney stones	Polat and Satil (2012)
T. chamaedrys	Turkey (Bozyazı district of Mersin)	Aerial part	**I:** Decoction (gargle 1 glassful and spit, once a day for 3–5 days)	Mouth sores	Sargin (2015)

(continued)

Table 5.1 (continued)

Teucrium species	Locality	Part used	Forms of preparation and method of administration	Traditional uses	References
T. chamaedrys	Turkey (Hatay Province, Antakya)	Aerial part	**I:** Decoction	An antidiabetic	Güzel et al. (2015)
T. chamaedrys	Turkey (Central Anatolia)	Herb/aerial part	**I:** Infusion (as tea)	Stomachaches	Sezik et al. (2001)
T. chamaedrys	Italy (Rotonda, Pollino National Park)	Flowering tops	**I:** Decoction	A febrifuge	Di Sanzo et al. (2013)
T. chamaedrys	Italy (Mundimitar/ Montemitro)	Aerial part	**I:** Decoction	An antimalarial (in the past), hypertension	di Tizio et al. (2012)
T. chamaedrys	Italy (Dolomiti Lucane)	Aerial part	**I:** Decoction	An antimalarial	Pieroni et al. (2004)
T. chamaedrys	Italy (North-western Ligurian Alps)	Leaf	**I:** Decoction	A hypotensive, abdominal pain	Cornara et al. (2014)
T. chamaedrys	Italy (Lucania region)	Aerial part	–	An antimalarial	Pieroni and Quave (2005)
T. chamaedrys	Serbia (Kosovo, Albanian Alps)	Aerial part	**I:** Infusion	An antihaemorrhoidal	Mustafa et al. (2012)
		Whole plant		An antidiabetic	
T. chamaedrys	Serbia (Kopaonik)	Aerial part	**I:** Infusion (tea)	Gastrointestinal ailments	Jarić et al. (2007)
T. chamaedrys	Serbia (Suva planina)	Aerial part	**I:** Infusion (tea)	A digestive, stomach problems	Jarić et al. (2015a)
T. chamaedrys	Serbia (Zlatibor)	Leaf	**I:** Infusion	Digestive complaints, diarrhoea	Šavikin et al. (2013)
T. chamaedrys	Bosnia and Herzegovina (Central, Southern and Western)	Aerial part	**I:** Infusion (tea)	Spasms (a mixture of *Agrimonia* sp., *Frangula* sp., *Melissa* sp., *Mentha* sp., and *T. montanum*), diarrhoea (a mixture of *Agrimonia* sp., *Matricaria* sp., *Rosa* sp., and *Tilia* sp.)	Šarić-Kundalić et al. (2010a)

T. chamaedrys	Bosnia and Herzegovina (Eastern, Northern and North-eastern)	Aerial part	**I:** Infusion (tea)	Anaemia, digestive ailments	Šarić-Kundalić et al. (2011)
T. chamaedrys	Bosnia and Herzegovina (Pristine Village of Prokoško Lake on Mt. Vranica)	Aerial part	**I:** Infusion (tea)	Spasms	Šarić-Kundalić et al. (2010b)
T. chamaedrys	Bosnia and Herzegovina (Mt. Javor)	Aerial part	**I:** Infusion	Digestive complaints, diarrhoea	Savić et al. (2019)
T. chamaedrys	Bosnia and Herzegovina	Aerial part	**I:** Fresh juice, infusion	Diarrhoea	Redžić (2007)
T. chamaedrys	Spain (Balearic Islands, Eastern Mallorca, Mediterranean Sea)	Flowering tops	**I:** Drops	Earache	Carrió and Vallès (2012)
T. chamaedrys	Macedonia (Southeastern)	Leaf	**E:** Compress from fresh leaves on the neck area	An aphrodisiac (anti-aphrodisiac), 'anti sex crusader'	Nedelcheva et al. (2017)
		Aerial part	**I:** Infusion (tea)	Childlessness	
T. chamaedrys	Greece (Epirus, Zagori)	–	–	A haemostatic, haemorrhoids, a tonic and stimulant, rheumatism and arthritis	Vokou et al. (1993)
T. chamaedrys	Albania (Theth, a village in the Northern Albanian Alps)	Aerial part	**I:** Infusion *Aerial part dried and sold to the city markets.*	Stomachaches, fevers	Pieroni (2008)
T. chamaedrys	Israel (the Golan Heights and the West Bank region)	Leaf	**I:** Decoction (a standard decoction is prepared from 50 g leaves and stems and taken orally, 100 cc, three times a day)	Stomach and intestinal pain and inflammation, lack of appetite, jaundice	Said et al. (2002)
T. chamaedrys	Iran (North Khorasan Province)	Aerial part	–	An anti-inflammatory, an aperient, an astringent, a carminative, a diaphoretic, a digestive, a diuretic, a stimulant	Mashayekhan et al. (2015)

(continued)

Table 5.1 (continued)

Teucrium species	Locality	Part used	Forms of preparation and method of administration	Traditional uses	References
T. chamaedrys	Palestine (West Bank)	–	–	Digestive disorders	Ali-Shtayeh et al. (2000)
T. chamaedrys subsp. *chamaedrys*	Turkey (Honaz Mountain National Park, Middle Aegean Region of Turkey)	Aerial part	**I:** Decoction (use 2–3 cupfuls daily to relieve pain)	An analgesic	Kargıoğlu et al. (2010)
T. chamaedrys subsp. *chamaedrys*	Turkey (Ulukışla)	Aerial part	**E:** Decoction (bath; take a bath; take a sitz bath twice a day)	Haemorrhoids, itching	Paksoy et al. (2016)
T. chamaedrys subsp. *lydium*	Turkey (Bayramiç, Çanakkale)	Aerial part	**I:** Infusion	Eczema	Bulut and Tuzlacı (2015)
T. chamaedrys subsp. *sinuatum*	Turkey (Hakkari- Geçitli)	Aerial part	**I:** Decoction, infusion (drink one glassful of the tea from the plant twice a day)	Gastric pain, poisoning, rheumatism	Kaval et al. (2014)
T. chamaedrys subsp. *sinuatum*	Turkey (Bingöl, Solhan)	Aerial part	**I:** Infusion (drink one teacupful after meals)	Insomnia, colds and influenza, a sedative	Polat et al. (2013)
T. chamaedrys subsp. *sinuatum*	Turkey (Sivrice, Elazığ)	Aerial part	**E:** Infusion (compress)	An antispasmodic, haemorrhoids	Cakilcioglu and Turkoglu (2010)
T. chardonianum	Western Sahara (Moroccan occupied territories)	Flowering tops (dried and triturated)	**E:** Infusion	Mixed with water and applied to hair to perfume it and stimulate hair growth	Volpato et al. (2012)
T. cubense	Mexico (Nuevo León, Bustamante)	Whole plant	**I/E**: Infusion (as a tea to drink and for bathing)	Fevers	Estrada-Castillón et al. (2018)
		Leaf	**I:** Infusion (as a tea to be drunk for several weeks)	Kidney disorders	

T. divaricatum subsp. *canescens*	Cyprus	Aerial part (in flower)	**I/E:** Infusion (taken orally but also together with inhalation of the steam)	A stomachic, fevers and common colds	Arnold (1985)
			E: Infusion	Wound healing (cicatrisant)	
T. divaricatum subsp. *divaricatum*	Turkey (Mugla, Marmaris)	Aerial part	**I:** Infusion	Coughs, a tonic for the eyes, stomachaches, urinary diseases	Gürdal and Kültür (2013)
T. fruticans	Morocco (middle region of Oum *Rbai*)	Aerial part	**I:** Infusion	Influenza (in the treatment of respiratory diseases)	Fatiha et al. (2017)
T. fruticans	Spain	–	–	Cardiovascular problems, mental/nervous disorders	Gonzalez-Tejero et al. (2008)
T. lusitanicum	Spain (Granada province)	Aerial part	**I:** Decoction	Fevers	Benítez et al. (2010)
T. micropodioides	Cyprus (Paphos and Larnaca)	–	–	A digestive, mental/nervous disorders	Gonzalez-Tejero et al. (2008)
T. montanum	Bosnia and Herzegovina (Mt. Javor)	Aerial part	**I:** Infusion	Digestive complaints	Savić et al. (2019)
T. montanum	Bosnia and Herzegovina (Eastern, Northern and North-eastern)	Aerial part	**I:** –	Gastrointestinal ailments, digestive ailments, rheumatism, arthritis, hangovers, biliary tract purification, lung cancer	Šarić-Kundalić et al. (2011)
T. montanum	Bosnia and Herzegovina (Pristine Village of Prokoško Lake on Mt. Vranica)	Aerial part	**I:** Infusion/tea	Spasms, blood purification	Šarić-Kundalić et al. (2010b)
			E: Balm	Rheumatism	
T. montanum	Bosnia and Herzegovina (Lukomir)	–	**I:** –	Diabetes	Ferrier et al. (2014)
T. montanum	Bosnia and Herzegovina	Aerial part	**I:** Infusion	Liver and stomach diseases	Redžić (2007)

(continued)

Table 5.1 (continued)

Teucrium species	Locality	Part used	Forms of preparation and method of administration	Traditional uses	References
T. montanum	Serbia (Zlatibor)	Aerial part	**I:** Infusion	Digestive complaints	Šavikin et al. (2013)
T. montanum	Serbia (Mt. Suva planina)	Aerial part	**I:** Infusion	Disorders of the abdominal organs, 'male' headaches, a tonic (tea), improving appetite, an antipyretic ('bitter tea' – blend: mountain germander, wall germander, yarrow, sage, pennyroyal and oregano)	Jarić et al. (2015a)
			E: Infusion: 'bitter tea'	Tuberculosis – a bath soak: add yellow chamomile (*Anthemis tinctoria* L.) to the 'bitter tea' or set the plants mentioned above alight for the patient to inhale the 'smoke' **a combination of internal and external use is recommended*	
T. montanum	Serbia (Rtanj)	Aerial part	**I:** Infusion	Strengthening the immune system	Zlatković et al. (2014)
T. montanum	Croatia (Dubrovnik coast)	Leaf, flower	**I:** Infusion/tea, brandy	–	Dolina and Łuczaj (2014)
T. montanum	Montenegro (Mt. Prokletije)	Aerial part	**I:** Infusion/tea	Cirrhosis	Menković et al. (2011)
T. montanum	Iran = Persia (pharmaceutical manuscripts from ninth to eighteenth century AD)	Aerial part	**I:** Decoction	Headaches	Zarshenas et al. (2013)
T. oliverianum	Saudi Arabia (Al-Rass province)	Whole plant	–	Diabetes	El-Ghazali et al. (2010)
T. orientale	Iran (Hormozgan province)	Leaf, flower	**I:** Decoction	Hoarseness	Safa et al. (2013)

T. orientale subsp. *orientale*	Lebanon (Mount Hermon)	Aerial part	**I:** Infusion	Fevers	Baydoun et al. (2015)
			E: Infusion	Wounds and skin injuries	
T. parviflorum	Turkey (East Anatolia)	Aerial part	**I:** Decoction	An antihemorrhoidal	Altundag and Ozturk (2011)
T. polium	Turkey (East Anatolia)	Aerial part	**I:** Fresh, decoction	Stomach problems, diarrhoea, an antihemorrhoidal, internal diseases, diabetes, an analgesic, an anti-inflammatory, an oedema, stomachaches, a digestive, an orexigenic, a carminative, tuberculosis, abdominal pain	Altundag and Ozturk (2011)
			E: Direct application	Sunstroke, a haemostatic	
T. polium	Turkey (Midyat)	Aerial part	**I:** Infusion, decoction	Stomachaches	Akgul et al. (2018)
T. polium	Turkey (Honaz Mountain National Park, Middle Aegean Region of Turkey)	Aerial part	**I:** Decoction (use 2–3 cupfuls daily to relieve abdominal pain)	Stomach diseases	Kargıoğlu et al. (2010)
			I: Decoction (use 2–3 cupfuls daily)	Rheumatic diseases, alleviating spasms and cramps	
T. polium	Turkey (Manisa, Turgutlu)	Aerial part	**I:** Infusion (one glassful of the infusion after meals)	Haemorrhoids	Bulut and Tuzlaci (2013)
			I: Crushed with honey (eaten before breakfast)	Haemorrhoids	
			I: Decoction	Diabetes	
T. polium	Turkey (Bayramiç, Çanakkale)	Aerial part	**I:** Infusion (before breakfast)	Haemorrhoids, eczema	Bulut and Tuzlacı (2015)
T. polium	Iran (Turkmen Sahra)	Aerial part	**I:** Decoction	Digestive problems, a stomachic, liver disorders	Ghorbani (2005)
T. polium	Iran (Mt. Hezar, Southeastern Iran)	Aerial part	**I:** Hydrodistillation	Stomachaches, a carminative	Rajaei and Mohamadi (2012)

(continued)

Table 5.1 (continued)

Teucrium species	Locality	Part used	Forms of preparation and method of administration	Traditional uses	References
T. polium	Iran (Arjan, Parishan protected area in Fars Province)	Leaf, flower	**I:** Infusion	Regulating blood lipids and sugars, diabetes, a scent	Dolatkhahi et al. (2014)
T. polium	Iran (Kohghiluyeh va Boyer Ahmad province)	Aerial part	**I:** Decoction, infusion, cooked aerial part	Menstruation disorders, toothache, body and joint pains, abortions, gynaecological infections, a carminative	Mosaddegh et al. (2012)
T. polium	Iran (Hormozgan province)	Flower, leaf, seed (fresh)	**I:** Decoction	Stomachaches, abdominal pain, flatulence, diarrhoea, regulating blood pressure, menstruation in women who have just given birth, measles, eye pain, headaches	Safa et al. (2013)
			E: Powder	Scorpion stings, snake bites, wound healing	
T. polium	Iran (Kurd tribe, Dehloran and Abdanan districts, Ilam province)	Leaf, flower	**I:** –	An antiseptic for gastric problems, a breath freshener	Ghasemi Pirbalouti et al. (2013)
T. polium	Albania (Albanians of Lepushe, Northern Albanian Alps)	Aerial part	**I:** Decoction	A digestive	Pieroni et al. (2005)
T. polium	Albania	–	**I:** –	A digestive	Gonzalez-Tejero et al. (2008)
T. polium	Jordan (the Ajloun Heights region)	Aerial part	**I:** Infusion	An antispasmodic, flatulence, an antidiabetic, kidney stones	Aburjai et al. (2007)
T. polium	Jordan (Al-Mafraq region)	Leaf	**I:** Fresh, soaked, cooled and taken orally whenever needed	An anti-inflammatory for the stomach and intestines	Al-Quran (2014)
T. polium	Morocco (Seksaoua Region)	Aerial part	**I:** Decoction	Abdominal pain	Sbai-Jouilil et al. (2017)

T. polium	Israel (the Golan Heights and the West Bank region)	Leaf	**I:** Decoction (a standard decoction is prepared from 50 g plant material and taken orally, 100 cc, three times a day)	Kidney and liver diseases, diabetes, stomach and intestinal pain and inflammation	Said et al. (2002)
T. polium	Algeria	–	**I:** –	A digestive	Gonzalez-Tejero et al. (2008)
T. polium	Bosnia and Herzegovina	Aerial part	**I:** Infusion	Stomach diseases	Redžić (2007)
T. polium	Palestine	Leaf, aerial part	**I:** Decoction	Diabetes	Ali-Shtayeh et al. (2012)
T. polium	Palestine	Leaf	**I:** –	Local treatment of stomach and intestinal inflammation, an antispasmodic, an anthelmintic, smallpox	Jaradat (2005)
T. polium	Libya (Wadi Alkuf, Al-Jabal Al-Akhder)	–	–	Diabetes, gastritis, thyroiditis, anaemia, common colds, hypertension, kidney stones, arthritis, herpes, hair loss, rheumatism, scabies	El-Mokasabi (2014)
T. pruinosum	Lebanon (Mount Hermon)	Whole plant	**I:** Infusion	Gastrointestinal disorders	Baydoun et al. (2015)
			E: Infusion	Wounds, fevers	
			I: Steam	Colds	
T. royleanum	Pakistan (Dir Lower, Talash Valley)	Aerial part	**I:** Decoction	Fevers, an antiseptic and stimulant, a vermifuge (anthelmintic)	Khan et al. (2018a)
T. scordium	Bosnia and Herzegovina	Aerial part	**I:** Infusion	Diarrhoea	Redžić (2007)
T. scordium	Bosnia and Herzegovina (Central, Southern and Western)	Aerial part	**I:** Infusion	Gastrointestinal ailments	Šarić-Kundalić et al. (2010a)
T. scordium	Bosnia and Herzegovina (Eastern, Northern and Northeastern)	Aerial part	**I:** –	Diarrhoea	Šarić-Kundalić et al. (2011)

(continued)

Table 5.1 (continued)

Teucrium species	Locality	Part used	Forms of preparation and method of administration	Traditional uses	References
T. stocksianum	Pakistan (Kurram agency, lower Kurram)	Leaf, flower	**I:** Infusion	A blood purifier, an antipyretic, malaria, weight loss/obesity, an antidiabetic	Hussain et al. (2018)
T. stocksianum	Pakistan (Khyber Pakhtunkhwa, Lower Dir District, Tehsil Khall)	Leaf	**I:** Decoction	Diabetes	Irfan et al. (2018)
T. stocksianum	Pakistan (Bahadur Khel, Karak District, Khyber Pakhtunkhwa)	Seeds, leaf	**I/E:** Powder	Arthritis, coughs, a blood purifier, asthma, pneumonia, jaundice, diarrhoea	Khan et al. (2018b)
T. stocksianum	Pakistan (Madyan Valley in the Swat district)	Leaf	–	An expectorant	Ahmad et al. (2013)
T. stocksianum	Iran (Hormozgan province)	Leaf	**I:** Decoction, powder (when fresh)	Stomachaches, abdominal pain, flatulence, toxicity, emesis, stomach acidification, regulating blood pressure, lowering lipids, aiding the recovery of women who have recently given birth	Safa et al. (2013)
T. trifidum	South Africa (Southeastern Karoo)	Aerial part	**I:** Infusion	Colds, back pain, bladder problems in women, influenza	Van Wyk et al. (2008)

Table 5.2 The use of *Teucrium* species in veterinary medicine

Teucrium species	Locality	Part used	Forms of preparation and method of administration	Traditional uses	References
T. chamaedrys	Macedonia (Southeastern)	Aerial part	**E:** Compress with honey	For foot-and-mouth disease in cloven-hoofed animals	Nedelcheva et al. (2017)
T. scorodonia	Canada (British Columbia)	–	**I:** Tincture	Mastitis in cattle (cows)/Woodsage (*T. scorodonia*) tincture is infused into the udder	Lans et al. (2007)
T. trifidum	South Africa (Southeastern Karoo)	Aerial part	**I:** Infusion	Fever in sheep and cattle	Van Wyk et al. (2008)

Table 5.3 Uses of *Teucrium* species for other purposes

Teucrium species	Locality	Part used	Forms of preparation and method of administration	Traditional uses	References
T. chamaedrys	Italy (Campania, National Park of Cilento and Vallo di Diano)	Aerial part	**I:** *T. chamaedrys* with the aerial part of *Urtica dioica* L., *Gallium verum* L. and *Veronica chamaedrys* L. is chopped and put in white vinegar. This mixture is used in the preparation of cheese.	For human nutrition	Di Novella et al. (2013)
T. chamaedrys	Italy (Rotonda, Pollino National Park)	Whole plant	**I:** Infusion	As an appetizer	Di Sanzo et al. (2013)
T. polium	Egypt	–	**I:** –	For human nutrition	Gonzalez-Tejero et al. (2008)
T. cubense	Mexico (Nuevo León, Bustamante)	Whole plant	**E:** Rub the whole body with the dry plant while praying	Fright	Estrada-Castillón et al. (2018)
			Ornamental	Planted in private gardens	
T. chardonianum	Western Sahara (Moroccan occupied territories)	Flowering tops (dried and triturated)	– (In its dry state)	Burnt inside a tent to perfume the air	Volpato et al. (2012)

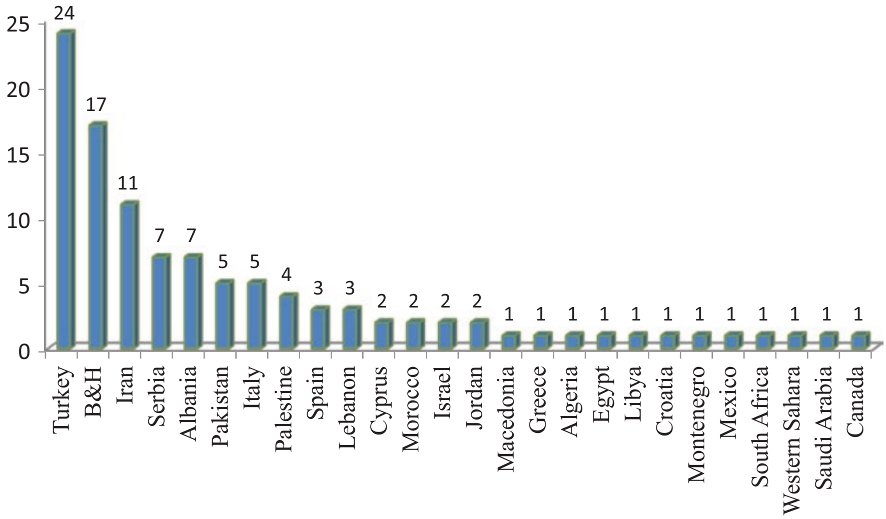

Fig. 5.1 The number of analysed studies in the different countries where *Teucrium* species are used

Furthermore, the species *Teucrium montanum* also features significantly in ethnomedicine, as is confirmed by 11 (15.3%) ethnobotanical studies. It is used most in the mountain regions of Bosnia and Herzegovina (5 studies, 45.4%) and Serbia (4 studies, 27.3%). In addition, the use of *Teucrium stocksianum* was recorded in 5 (6.94%) ethnobotanical studies conducted in Pakistan and Iran (Fig. 5.2).

Research showed that infusions (77.8%) and decoctions (48.6%) were the dominant methods for preparing and using *Teucrium* species; however, these plants were also found to be used in the form of fresh juice, drops, brandy, tincture, balm or by applying the fresh aerial parts wrapped either whole or chopped and mixed with honey in a gauze (Table 5.1). According to the available data, their internal use was the dominant form (85%), while their use externally (for rheumatism, haemorrhoids, psoriasis, wound healing, and sunstroke, as an antidote to the snake bites and scorpion stings, and as a haemostatic) was significantly lower (12%). Combined internal/external use was mentioned in 3% of the studies analysed.

Teucrium species were used most often to treat various gastrointestinal disorders (56 of the analysed studies), followed by problems related to the functioning of the heart (e.g. hypertension) and blood vessels (e.g. haemorrhoids) (21 studies), and respiratory tract problems (colds, lung cancer, tuberculosis, asthma, pneumonia, influenza, etc., 17 studies). Their use in the treatment of problems related to endocrine function disorders (diabetes, thyroiditis) was mentioned in 16 ethnobotanical studies, while 14 studies noted their being used to treat problems connected to muscle and skeletal disorders and skin diseases (Fig. 5.3). In addition, *Teucrium* species were also used to treat problems concerning the excretory organs (7 studies), fevers (7 studies), as a stimulant and tonic (6 studies), to treat inflammations (5 studies), gynaecological ailments (4 studies), malaria (4 studies), mental disorders (3) and

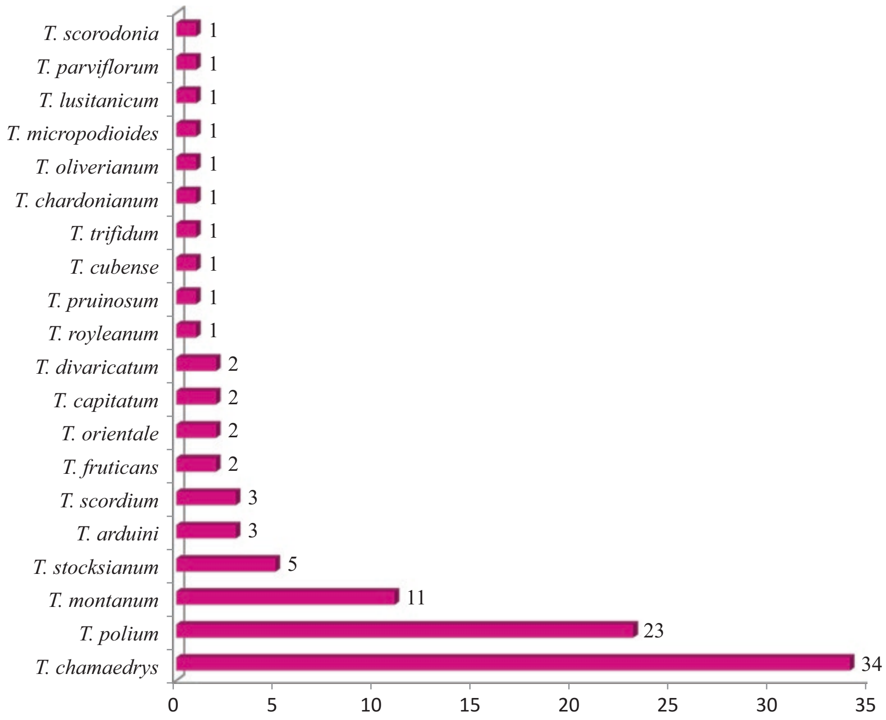

Fig. 5.2 The total number of ethnobotanical studies in which *Teucrium* species are cited

headaches (3 studies). Twenty-four studies noted health problems and diseases that were only mentioned once or twice (Other, Fig. 5.3).

5.3.3 *The Use of* Teucrium *Species in Veterinary Ethnomedicine*

Our research established that three *Teucrium* species, mentioned in three ethnobotanical studies, are used in veterinary ethnomedicine (Table 5.2). Namely, in Southeastern Macedonia, Nedelcheva et al. (2017) recorded the use of *Teucrium chamaedrys* in the treatment of foot-and-mouth disease in cloven-hoofed animals. The South African endemic species *Teucrium trifidum* (Codd 1977) is used to treat fevers in sheep and cattle (Van Wyk et al. 2008). In the British Columbia region (Canada), tincture of *Teucrium scorodonia* is used to treat mastitis in cattle, particularly cows (Lans et al. 2007). In line with this, laboratory research by Djilas et al. (2006) found that ethyl acetate, chloroform and n-butanol extracts of *Teucrium montanum* exhibit a wide range of inhibitory activities against Gram (+) and Gram (−) bacteria, representing mid-level validity for mastitis.

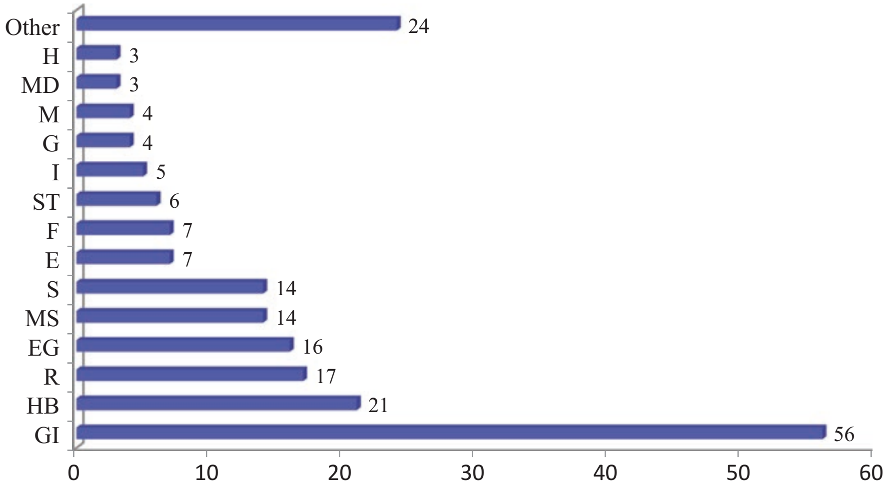

Fig. 5.3 The frequency of the use of *Teucrium* species in human therapy based on the analysed studies: **GI** – gastrointestinal disorders; **HB** – heart and blood vessels; **R** – respiratory diseases; **EG** – diseases of the endocrine glands; **MS** – muscular and skeletal problems; **S** – skin diseases; **E** – excretory organ problems; **F** – fever; **ST** – stimulant and tonic; **I** – inflammation; **G** – gynaecological ailments; **M** – malaria; **MD** – mental disorders; **H** – headaches; **Other –** toothache, earache, poisoning, lack of appetite, an aphrodisiac, oedema, sunstroke, weight loss, an analgesic, etc

5.3.4 The Use of Teucrium *Species for Other Purposes*

Besides the traditional uses for treating a variety of health problems and complaints in people and animals, *Teucrium* species are also used for other purposes. Namely, they are important alimentary plants with some used to prepare flavoured wines, herbal teas, bitters and liqueurs, while people use infusions of leaves and flowers to flavour beer in some regions (Maccioni et al. 2007). Furthermore, in studies by Saroglou et al. (2007), Ulubelen et al. (2000) and Özkan et al. (2007), this genus was found to be important in the food industry with many species serving as natural preservatives due to their antimicrobial, antioxidant and antifungal properties. Hence, in ethnobotanical research conducted in two national parks in Italy, *Teucrium chamaedrys* is mentioned as being used in cheese preparation (Di Novella et al. 2013) and as an appetizer (Di Sanzo et al. 2013) (Table 5.3). *Teucrium polium* is also used in Egypt in human nutrition (Gonzalez-Tejero et al. 2008). Estrada-Castillón et al. (2018) mentioned *Teucrium* cubense as an ornamental plant in Mexico and its use in rituals, while *Teucrium chardonianum* is used as an air freshener in the Western Sahara region (Volpato et al. 2012).

5.4 The Medicinal Properties of *Teucrium* Species

As with other Lamiaceae, the aerial organs of *Teucrium* species are covered by an indumentum of glandular and non-glandular trichomes in which essential oils are secreted. The essential oil content in these species is high and sesquiterpenes predominate in it (Cavalerio et al. 2002; Kucuk et al. 2006; Hachicha et al. 2007; Saroglou et al. 2007). In addition to sesquiterpenes, the essential oil of *Teucrium* species is a great source of neoclerodane diterpenes, in fact one of the richest, with over 220 diterpenes described so far (Piozzi et al. 2005).

The yield of essential oil in *Teucrium* species varies from species to species, but ranges from 0.5% to 1.5%, while the proportion of the main chemical constituents (primarily monoterpene/sesquiterpene hydrocarbons and oxygenated sesquiterpenes) also varies dramatically (Kovačević et al. 2001; Saroglou et al. 2007). Some of the differences in the chemical composition of the essential oil are believed to be linked to different subspecies and/or the plants' geographical origin and a range of environmental factors (Menichini et al. 2009).

Used widely due to their antioxidant and antidiabetic properties, the essential oils and volatile constituents extracted from *Teucrium* species also have a part to play in the prevention and treatment of a whole variety of human disorders, ailments and diseases, including cancer, cardiovascular problems, such as atherosclerosis and thrombosis, and bacterial and viral infections (Leyel 1984; Bruneton 1995; Edris 2007). They are also of interest ecologically, being used as antifeedants, inhibiting attack by different species of insects, due to the properties of their components, especially the diterpenes (Piozzi et al. 2005).

The chemical composition of the essential oil largely determines the medicinal properties of a plant and hence its uses. In this chapter, analysis of 72 ethnobotanical studies showed that the following species were used most, and as such, were the most popular among the human population: *Teucrium chamaedrys, T. polium* and *T. montanum*.

5.4.1 Teucrium chamaedrys *(Wall Germander)*

A perennial herbaceous plant with a half-ligneous and shrub-like low stem, *Teucrium chamaedrys* (section *Chamaedrys*) grows up to 30 cm high. It is found in rocky limestone areas, dry mountain meadows and pastures, and on the edges of sparse oak and pine forests, up to 1000 m above sea level in Central Europe, the Mediterranean region and Western Asia. Flowering and harvesting takes place between July and September, while fruiting occurs from August to September (Tutin et al. 1972; Diklić 1974; Fig. 5.4). *Teucrium chamaedrys* has a very wide range of uses in traditional medicine, particularly in its centres of distribution, as is confirmed by 34 of the analysed ethnobotanical studies. It is mainly the aerial parts that are used, most frequently when the plant is flowering, as then it has the greatest

Fig. 5.4 *Teucrium chamaedrys* L. (Photo P. Lazarević)

concentrations of active substances. It is prepared in the form of an infusion, decoction, and fresh juice, while it is usually used internally, and more rarely externally (as a compress).

It is used as a painkiller and to treat stomach pains, gastric pains, indigestion, digestive complaints, haemorrhoids, toothache, kidney pains, kidney stones, heart disease, hypertension, mouth sores, poisoning, rheumatism, insomnia, colds and flu, itching, eczema, malaria, diarrhoea, spasms, anaemia, earache, childlessness, rheumatism and arthritis. Furthermore, it is used as a stomachic, antidiabetic, analgesic, sedative, antispasmodic, febrifuge, aphrodisiac, haemostatic, tonic, and stimulant, as well as an appetizer (Table 5.1). In addition, it is used in veterinary ethnomedicine for foot-and-mouth disease in cloven-hoofed animals (Table 5.2). Nencini et al. (2014) stated that it is used as Portland Powder in traditional medicine in England for the treatment of rheumatism and gout.

The chemistry of *Teucrium chamaedris* has been thoroughly researched due to its proven in vitro antioxidant activity (Kadifkova-Panovska et al. 2005) and also as there have been several cases of hepatotoxicity arising from the use of this species (Kouzi et al. 1994; Stickel et al. 2000; Perez Alvarez et al. 2001). Namely, it is very rich in phenolic compounds, exhibiting very strong biological activity and antioxidative effects (Ozgen et al. 2006; Gursoy and Tepe 2009; Stanković et al. 2010). In a study by Prescott et al. (2011), teucrioside was identified as the main active ingredient of the plant, which was shown in preclinical research to be effective in inhibiting calcineurin, meaning it could aid in the reduction of inflammatory states.

5.4.2 **Teucrium polium** ***(Felty Germander)***

Teucrium polium (section *Polium*) is a perennial, aromatic plant, 20–50 cm high, appearing from June to August, and found abundantly in Southwestern Asia, Europe (the Mediterranean region), and North Africa (Diklić 1974; Djabou et al. 2012; Fig. 5.5). It grows on well-drained land, hillsides, sands, in stony mountains, and in sunny regions with a semiarid and arid bio-climate. The aerial parts of this plant are characterised by a pleasant aroma and a bitter taste (Barceloux 2008).

Numerous ethnobotanical studies (23) have highlighted the major ethnomedicinal importance of this species, mainly in the treatment of abdominal pain (digestive problems, stomach and intestinal pain and inflammation, and diarrhoea), kidney and liver diseases, kidney stones, diabetes, haemorrhoids, oedema, rheumatism, tuberculosis, and sunstroke. Moreover, it is used as an analgesic, an anti-inflammatory, an orexigenic, a carminative, a haemostatic, a digestive, an antispasmodic, and in human nutrition (Table 5.1). The medicinal properties of *Teucrium polium* for treating most of these health problems have been confirmed by laboratory research, which justifies its use in traditional medicine. Likewise, numerous in vivo and in vitro studies have confirmed the various biological activities of this species. These include anti-inflammatory and antirheumatic (Tariq et al. 1989), hypoglycemic (Kasabri et al. 2011), antipyretic and antibacterial (Autore et al. 1984), antioxidant and antimicrobial (Ilhami et al. 2003; Balmekki et al. 2013), hypolipidemic (Rasekh et al. 2001), and antihypertensive properties (Suleiman et al. 1988), as well as its benefits for treating stomach disorders (Malakov and Papanov 1983; Aqel et al. 1990), improving mental performance (Perry et al. 1996), and aiding weight loss (Gharaibeh et al. 1988).

Research by Kadifkova-Panovska et al. (2005) showed that different *Teucrium polium* extracts exhibit significant free radical scavenging activity, hydroxyl radical

Fig. 5.5 *Teucrium polium* L. (Photo M. Stanković)

scavenging, and antioxidant activity in vitro. These antioxidative properties of *Teucrium polium* are the result of the presence of flavonoids (rutin, apigenin, apigenin-4, 7-dimethylether, cirsimaritin, cirsiliol, luteolin, etc.) (Sharififar et al. 2009), which has been confirmed by in vitro and in vivo studies (Djeridane et al. 2006; Hasani et al. 2007). In his research, Rajabalian (2008) demonstrated that the methanol extract of *Teucrium polium* could potentially be used as an effective and safe chemo-sensitizer agent in cancer chemotherapy. In addition, laboratory research confirmed that the ethanol extract also exhibited potent antibacterial activity against Gram (+) and Gram (−) microorganisms, while the aqueous extract of *Teucrium polium* inhibits the growth of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* (Autore et al. 1984; Rojas et al. 1992; Aggelis et al. 1998; Essawi and Srour 2000; Darabpour et al. 2010). Due to the presence of sterols and flavonoids, *Teucrium polium* exhibits anti-inflammatory properties (Tariq et al. 1989), while its analgesic properties are the result of the presence of essential oils (Abdollahi et al. 2003). The antispasmodic activity of *Teucrium polium* can be put down to the high level of sesquiterpene alcohols (Kamel and Sandra 1994). In experimental conditions, it was established that the aqueous extract of the aerial parts of this species brought about a significant reduction in serum triglycerides and cholesterol in hyperlipidemic rats (Rasekh et al. 2001). Moreover, Movahedi et al. (2014) demonstrated that a decoction of *Teucrium polium* can protect liver cells against hepatocellular carcinoma in carcinogenesis-induced animal models. In one study, burn wounds healed more quickly through the topical application of *Teucrium polium* extract (Ansari et al. 2013). Similarly, the anti-inflammatory effects of this plant were demonstrated through it inhibiting carrageenan-induced inflammation in an animal study and its methanol and ethanol extracts have also been shown to be effective on veterinary pathogens (Darabpour et al. 2010).

5.4.3 **Teucrium montanum** ***(Mountain Germander)***

The species *Teucrium montanum* (section *Polium*) is a perennial, shrub-like plant that is native to the Mediterranean region of Europe and North Africa, and to the Middle East (Tutin et al. 1972; Diklić 1974; Lakušić and Lakušić 2014; Fig. 5.6). It has half-ligneous branches, grows up to 25 cm high and can be found on thermophilic limestone and serpentine rocks, in dry mountain meadows and on the edges of forests. It flowers between June and September.

According to the ethnomedicinal data available, *Teucrium montanum* is used for digestive disorders, biliary tract purification, liver (cirrhosis) and stomach diseases, diabetes, spasms and blood purification, lung cancer, tuberculosis, rheumatism, arthritis, and headaches (in some regions, for “male” headaches), to improve appetite, as an antipyretic, to strengthen the immune system, as a hangover cure, and as a tonic (tea) (Table 5.1). The dominant method of preparation is an infusion, while in some areas it is used in the form of a decoction or balm. In Bosnia and Herzegovina and Serbia, *Teucrium montanum* is one of the most popular plants in traditional

Fig. 5.6 *Teucrium montanum* L. (Photo M. Stanković)

medicine and according to local people's beliefs "heals every disease" (Redžić 2010; Jarić et al. 2015a).

Phytochemical studies have shown that *Teucrium montanum* is very rich in phenolic compounds, exhibiting very strong biological activity and antioxidative effects (Čanadanović-Brunet et al. 2006; Stanković et al. 2011b), which justifies its use in traditional medicine. Furthermore, data promoting the use of *Teucrium montanum* in ethnomedicine confirms the free-radical scavenging activity of flavonoids and phenolic acids in extracts made from different solvents (Djilas et al. 2006).

5.5 Potentially Toxic Effects of *Teucrium* Species

The use of *Teucrium* species in traditional medicine spanning several centuries points unequivocally to its medicinal properties. However, there have been instances when the long-term use of preparations made from these plants can have unwanted side effects. An illustrative example of the toxic effects of one species of the genus *Teucrium* was reported by a 33-year-old woman who drank *Teucrium chamaedrys* tea every day for 2 weeks. It was found that she had symptoms of acute icteric hepatitis and all other causes of acute hepatitis were ruled out. When she stopped drinking the tea, the patient made a clinical recovery and her serum bilirubin, aminotransferase and alkaline phosphatase levels returned to normal within 9 weeks (Ural et al. 2011). This case indicates that *Teucrium chamaedrys* can cause acute icteric hepatitis, which can clinically mimic acute viral hepatitis. Similar hepatotoxicity was observed during the use of preparations made from other species of the *Teucrium* genus (Chitturi and Farrell 2008; Kotsiou and Tesseromatis 2017) with most patients presenting with very high liver aminotransferases or developing jaundice. Hepatitis and intrahepatic cholestatic liver disease were the etiology of liver damage in these cases. *Teucrium* species are rich in neoclerodane diterpenoids, which may be the cause of hepatotoxicity (Sundaresan et al. 2006). Therefore, those suffering from hepatic abnormalities should be careful when using *Teucrium* species (Perez Alvarez et al. 2001). Likewise, taking high doses of *Teucrium* species or using them over a longer period of time might cause the gradual deterioration in neuromuscular coordination, as is supported by histopathological and biochemical evidence (Tanira et al. 1996, 1997).

5.6 Conclusions

A comprehensive analysis of the traditional use of *Teucrium* species from the ethnobotanical and ethnomedicinal points of view took in 72 ethnobotanical studies from different parts of the world. It revealed a wide range of uses for different species of this genus, but for the purposes of this chapter the traditional use of 20 species of the genus *Teucrium* has been presented. Nineteen of these species are used in ethnomedicine and 3 in veterinary ethnomedicine, while 4 are used for other purposes. It has been established that these species are most frequently used traditionally in the treatment of abdominal problems, followed by problems related to the functioning of the heart, blood vessels, and endocrine glands, muscular and skeletal problems, and skin diseases. Those species which stand out for their wide range of uses are *Teucrium chamaedrys, T. polium* and *T. montanum*.

The medicinal properties of *Teucrium* species and their favourable impact on numerous ailments is undeniable, as has been confirmed by many studies. On the one hand, this justifies their use in ethnomedicine; on the other hand, it leads to the need for caution, particularly in terms of dosage and treatment duration and the overall health of the patient must be taken into consideration. Namely, histopathological and biochemical studies have revealed that when *Teucrium* species are taken in high doses or over a long period of time, they might cause progressive deterioration in neuromuscular coordination and hepatic damage, which may or may not be irreversible. Therefore, it is necessary to undertake further research aimed at isolating and characterizing the constituents of *Teucrium* species so as to obtain suitable drugs.

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Chapter 6
Phenolic Compounds Diversity of *Teucrium* Species

Vladimir Mihailović, Jelena S. Katanić Stanković, and Nevena Mihailović

Abstract A main phytochemical property of *Teucrium* species, apart from essential oils production, is the presence of phenolic compounds in their composition. The aim of this chapter was to review the main publications on the genus *Teucrium*, focusing on studies of phenolic compounds identification. A total of 78 different phenolic compounds have been reported in *Teucrium* species, such as phenolic acids, phenylethanoids, and flavonoids. Current literature describes the chemical richness of flavonoid metabolites present in *Teucrium* genus, making these compounds the most diverse group of phenolics identified in *Teucrium* plants and chemotaxonomically relevant for species belonging to this genus. The information presented in this chapter also describes all the known phenolic acids and phenylethanoids of *Teucrium* species as reported in the literature. Also, available data about the quantification of different phenolic compounds in these plants will be described and compared. Based on the published results, biosynthetic pathways for every class of phenolic compounds present in these species will also be provided. In addition, the methods for extraction of phenolic compounds from *Teucrium* species, as reported in the literature, will be compared and discussed. Taking into account that the genus *Teucrium* includes more than 300 species and that phenolic composition has been investigated for only 48 of them so far, this chapter aims to survey current literature, improve the knowledge and provide sufficient data for future research of phenolic compounds of *Teucrium* genus.

Keywords Phenolic acids · Phenylethanoids · Flavonoids · Biosynthesis of phenolic compounds

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Abbreviations

CHI	Chalcone isomerase
CHS	Chalcone synthase
DAHP	3-deoxy-D-arabino-heptulosonic acid 7-phosphate
DOPA	3,4-dihydroxyphenylalanine
EPSP	5-enolpyruvylshikimic acid 3-phosphate
F3H	Flavanone 3′-hydroxylase
FHT	Flavanone 3-hydroxylase
FLS	Flavonol synthase
FNS	Flavone synthase
GAE	Gallic acid equivalents
HPLC-DAD	High-performance liquid chromatography with diode array detector
HPLC-MS	High-performance liquid chromatography with mass spectrometry
HPLC-PDA	High-performance liquid chromatography with photodiode array detector
LC/DAD/ESI-MS^n	Liquid chromatography with diode array detector coupled to an electrospray ionization mass detector
PABA	*p*-aminobenzoic acid
TPC	Total phenolic content
UPLC–MS/MS	Ultra performance liquid chromatography-tandem mass spectrometry

6.1 Introduction

Phenolic compounds represent a widespread group of plant phytochemicals having a wide range of structures and functions (Rispail et al. 2005). They are considered to be the most important and numerous groups of secondary plant metabolites, which is ubiquitous in the plant kingdom. The term phenols include a very large and diverse group of chemical compounds which have one or more hydroxyl groups directly attached to the aromatic ring. Polyphenols are compounds with more than one phenolic hydroxyl group in its structure, attached to one or more benzene rings (Vermerris and Nicholson 2006).

Since polyphenols include a large group of compounds, with over 8000 characterized structures, there are several ways of their classification. These compounds are generally classified according to the number of phenolic rings they contain in their structure (phenolic acids, stilbenes, flavonoids, lignans, and tannins). The most accepted classification by Harborne and Simonds (1964) is based on the number of carbon atoms in the molecule. According to this classification phenolic compounds are classified to: simple phenols (C_6), phenolic acids – derivatives of benzoic acid (C_6-C_1), acetophenones and phenylacetic acids (C_6-C_2), cinnamic acids (C_6-C_3), coumarins (C_6-C_3), flavonoids (C_6-C_3-C_6), biflavonoids (C_{30}), benzophenones (C_6-C_1-C_6), xanthones and stilbenes (C_6-C_2-C_6), quinones (C_6, C_{10}, C_{14}), tannins, lignans, and lignins (polymers). According to another type of classification, phenolic compounds are divided into simple phenols, phenylpropanoids, flavonoids, tannins, and quinones (Vermerris and Nicholson 2006).

Simple phenols generally include substituted phenols monomeric components (C_6) and acids that make up some plant tissues, such as lignins. Phenolic acids, classified as simple phenols, are divided into two subgroups: hydroxybenzoic acids and hydroxyacetic acids. Phenolic acids are rarely found free in plant species, and usually are present in the form of esters with various compounds (glucose, organic acids, flavonoids, terpenes, etc.). Well-known representatives of phenolic acids are gallic acid, salicylic acid, vanillic acid, protocatechuic acid, etc. (Brielmann et al. 2006).

The phenylpropanoids represent phenolic compounds consisting of three-carbon side chain attached to a phenol basis (C_6-C_3 skeleton). This class of phenolic compounds includes the hydroxycoumarins, phenylpropenes, and lignans. The most common phenylpropenes are hydroxycinnamates, including the caffeic, *p*-coumaric, ferulic, and sinapic acid. These are found in plants as conjugates with tartaric acid or quinic acid. Chlorogenic acids, principally 3-*O*-, 4-*O*- and 5-*O*-caffeoylquinic acids, found in high amount in coffee beans together with caffeic acid, are the main hydroxycinnamates of dietary significance (Crozier et al. 2009).

Flavonoids are the most extensive group of phenolic compounds found in plants. They represent a family of polyphenols consisting of 15 carbon atoms (Fig. 6.1) that

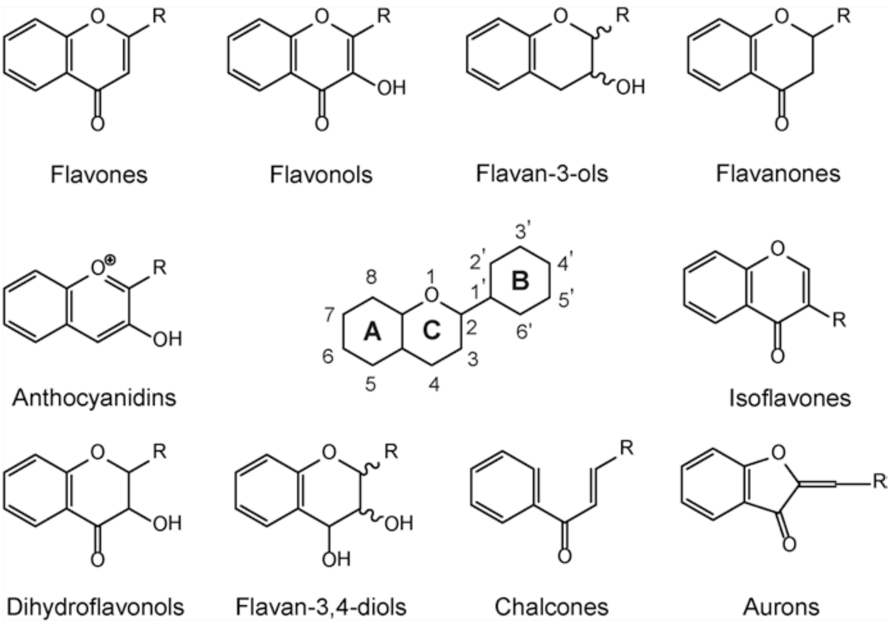


Fig. 6.1 Classes of structurally different flavonoids (R = C_6H_5)

are chemically defined to have a basic structure of two aromatic rings (A and B) linked via three carbon atoms that usually form an oxygenated heterocycle (C ring). This structure is most commonly described as C_6-C_3-C_6 (Ignat et al. 2011). The C ring is the key factor to define the different groups of flavonoids by providing different chemical structures containing various functional groups (hydroxy, methoxy, and glycosidic groups) and forming polymers (Fraga and Oteiza 2011). Flavonoids are usually divided into 6 main subclasses: flavonols, flavones, flavanols (flavan-3-ol), flavanones, anthocyanidins, and isoflavones (Fig. 6.1). The less represented subclasses include dihydroflavonols, flavan-3,4-diols, chalcones, dihydrochalcones, and aurons (Ignat et al. 2011).

Phenolic compounds are synthesized during the normal plant growth, but also as a response to various ecological conditions, such as, among others, stress and UV radiation (Naczk and Shahidi 2004). The synthesis of phenolic compounds in plants is performed by the biosynthetic pathway of the shikimic acid and is associated with the metabolism of carbohydrates (Isabelle et al. 2010; Steffen et al. 2003). Distribution and accumulation of phenolic compounds within the plant are not uniform but rather determined by the solubility of phenolic compounds. In that sense, water-soluble phenols are located in cellular vacuoles and other organelles, while lignans are concentrated mainly in the subepidermal and epidermal layers as the basic structural elements of the cell wall of plants, contributing to its mechanical resistance and defense against microorganisms (Naczk and Shahidi 2004). In addition, the presence of phenolic compounds in certain parts of the plant is conditioned by the stage of growth and development of the plant (Haminiuk et al. 2012).

Phenolic compounds include signaling molecules, pigments, and aromas that can attract or repel, as well as compounds that can protect plants against insects, fungus, bacteria, and viruses. The most important and most studied role of phenolic compounds of plant origin, which has an impact on human health, is their antioxidant activity. Oxidative stress can cause a range of degenerative diseases in humans, such as cancer, multiple sclerosis, autoimmune diseases, Parkinson's disease, etc. Antioxidants present in plants, e.g., phenolic acids, flavonoids, anthocyanins, and tannins, are often associated with positive effects of plants on human health (Haminiuk et al. 2012; Theriault et al. 2006).

The aim of this chapter is a comprehensive and comparative review of the phenolic compounds which are, in addition to volatile terpenoids, the most characteristic for the genus *Teucrium*. It will also review available data about the quantification of the phenolic compounds in these plants and biosynthetic pathways for every class of phenolic compounds present in these species. On the evidence so far, the methods for extraction of phenolic compounds from *Teucrium* species will be compared and discussed.

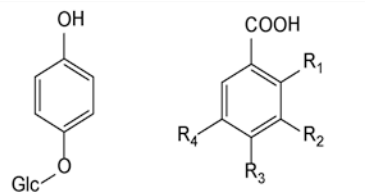
6.2 Phenolic Compounds of *Teucrium* Species

Species belonging to the genus *Teucrium* have extensively been the subject of phytochemical studies. These plants biosynthesize various compounds, predominantly phenolics, iridoid glycosides, and volatile terpenoids, found in the essential oils (De Marino et al. 2012; Frezza et al. 2019). Through phytochemical studies of *Teucrium* species, three groups of phenolic compounds, hydroxycinnamic acid derivatives, phenylethanoid glycosides, and flavonoid glycosides have been reported as the most abundant phenolics in these species. Previous phytochemical studies identified phenylethanoids as the major group of polyphenols in some *Teucrium* species (Lin et al. 2009; Mitreski et al. 2014), while specific flavonoids were reported as chemotaxonomic markers for the classification of the various species of this genus (Mitreski et al. 2014; Harborne et al. 1986). Numerous reports have been published on total phenolic content of species from the genus *Teucrium*. The results showed high percentage of total phenolics in these plants ranging from 7 to 93 mg GAE/g dry herb (Mitreski et al. 2014; Šamec et al. 2010; Zlatić et al. 2017), while total phenolic contents of examined *Teucrium* extracts were in the range from 53 to 255 mg GAE/g dry extract (Ben Sghaier et al. 2011; Khled-Khoudja et al. 2014a, b; Vlase et al. 2014; Belarbi et al. 2017; Nastić et al. 2018a, b; Farahmandfar et al. 2019) depending on the growing location, examined plant part or applied extraction procedures. This chapter will focus on major groups of phenolic compounds in the genus *Teucrium* – phenolic acids, phenylethanoids, and flavonoids.

6.2.1 Phenolic Acids

The genus *Teucrium* is characterized by the presence of diverse groups of phenolic compounds. Phenolic acids represent significant, but not so extensive group of compounds and they can be associated in the two classes: derivatives of benzoic acid (Fig. 6.2) and derivatives of cinnamic acid (Fig. 6.3) (Manach et al. 2004).

A comprehensive review of literature data on the phytochemical composition of plant species from genus *Teucrium* revealed the presence of several simple phenolic acids, derivatives of benzoic acid, in a few tested species (Table 6.1). The



(1) β-Arbutin

		R_1	R_2	R_3	R_4
(2)	Salicylic acid	OH	H	H	H
(3)	4-Hydroxybenzoic acid	H	H	OH	H
(4)	Protocatechuic acid	H	OH	OH	H
(5)	Gentisic acid	OH	H	H	OH
(6)	Gallic acid	H	OH	OH	OH
(7)	Vanillic acid	H	OCH_3	OH	H
(8)	Syringic acid	H	OCH_3	OH	OCH_3

Fig. 6.2 Benzoic acid derivatives identified in *Teucrium* species

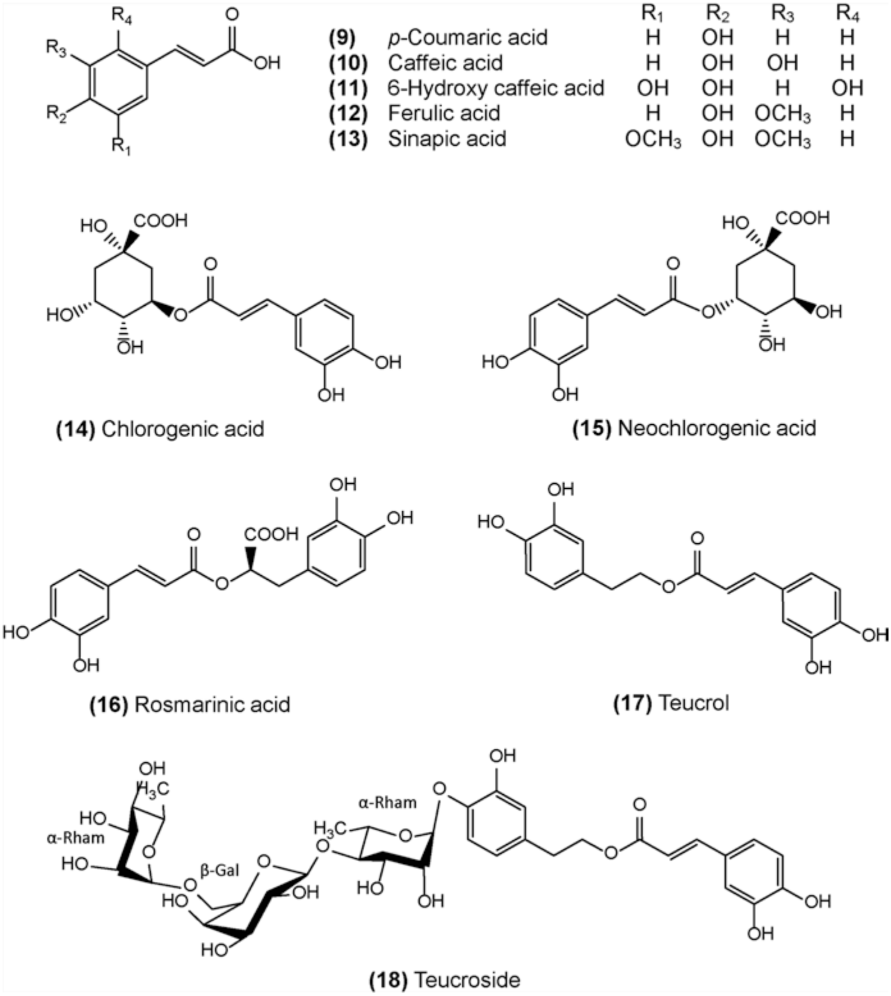


Fig. 6.3 Hydroxycinnamic acids identified in *Teucrium* species

phytochemical composition of extracts or infusions made from *Teucrium* leaves or flowers was most frequently estimated using HPLC-DAD, HPLC-PDA, HPLC-MS, and UPLC–MS/MS analyses. Several scientific reports showed the presence of a total of eight compounds (Fig. 6.2) that can be classified as simple phenolic acids with the core structure of benzoic acid.

One of the phenolic acids derivatives (Fig. 6.2), *β*-arbutin (**1**), was identified in ethanolic extract of *Teucrium chamaedrys* aerial parts, collected in Italy. This glycosylated hydroquinone was defined by Frezza et al. (2018) as a new compound for the whole genus since it is a quite uncommon metabolite for the whole Lamiaceae family. Salicylic (**2**) and 4-hydroxybenzoic acids (**3**) were found only in *Teucrium arduini* flower and leaf extracts so far, while protocatechuic (**4**) and gentisic acids

Table 6.1 Benzoic acid derivatives identified in *Teucrium* species

No.	Compounds reported	*Teucrium* species	References
1.	*β*-Arbutin	*T. chamaedrys*	Frezza et al. (2018)
2.	Salicylic acid	*T. arduini*	Šamec et al. (2010)
3.	4-Hydroxybenzoic acid	*T. arduini*	Šamec et al. (2010)
4.	Protocatechuic acid	*T. chamaedrys*	Nastić et al. (2018a)
		T. montanum	Tumbas et al. (2004) and Nastić et al. (2018b)
		T. arduini	Šamec et al. (2010)
5.	Gentisic acid	*T. pseudoscorodonia*	Vlase et al. (2014)
		T. montanum	Tumbas et al. (2004)
		T. arduini	Šamec et al. (2010)
6.	Gallic acid	*T. polium*	Milošević-Djordjević et al. (2018)
		T. chamaedrys	Nastić et al. (2018a)
		T. montanum	Tumbas et al. (2004) and Nastić et al. (2018b)
7.	Vanillic acid	*T. polium, T. scordium*	Milošević-Djordjević et al. (2018)
		T. arduini	Šamec et al. (2010)
		T. chamaedrys	Nastić et al. (2018a)
		T. montanum	Tumbas et al. (2004) and Nastić et al. (2018b)
8.	Syringic acid	*T. pseudoscorodonia*	Belarbi et al. (2017)
		T. montanum	Tumbas et al. (2004)
		T. arduini	Šamec et al. (2010)

(**5**) were present, besides *Teucrium arduini,* also in *Teucrium montanum*. Moreover, protocatechuic acid was also identified in *Teucrium chamaedrys*, whilst gentisic acid was detected in *Teucrium pseudoscorodonia*. Gallic acid (**6**), as one of the most abundant phenolic acids in plant kingdom, was found in several *Teucrium* species, such as *Teucrium polium*, *T. chamaedrys*, and *T. montanum* in high quantities, particularly in subcritical water extracts of *T. chamaedrys* and *T. montanum* (217 and 345 mg/100 g dry extract, respectively). Generally, in eight plant species where benzoic acids were analyzed, the presence of vanillic acid (**7**) was observed in five of them (in *T. arduini*, *T. scordium, T. polium*, *T. chamaedrys*, and *T. montanum*). Literature sources revealed the presence of one more benzoic acid derivative, syringic acid (**8**) in *T. pseudoscorodonia*, *T. arduini*, and *T. montanum* extracts.

Besides simple phenolic acids, ten representatives of hydroxycinnamic acids or phenylpropanoids and their derivatives (Fig. 6.3) were identified in a notably higher number of *Teucrium* plant species, even 13 of them (Table 6.2). The hydroxycinnamic acids are significantly more represented in plants than the hydroxybenzoic acids and they are not often found in free form, but mostly as glycosylated derivatives or esters of shikimic, quinic, or tartaric acids (Manach et al. 2004). The presence of main secondary metabolites (Fig. 6.3) in plants obtained in the process of

Table 6.2 Hydroxycinnamic acids identified in *Teucrium* species

No.	Compounds reported	*Teucrium* species	References
9.	*p*-Coumaric acid	*T. chamaedrys*	Vlase et al. (2014) and Stanković (2012)
		T. montanum	Tumbas et al. (2004) and Stanković (2012)
		T. arduini	Šamec et al. (2010) and Stanković (2012)
		T. pseudoscorodonia	Belarbi et al. (2017)
		T. polium, T. scordium	Milošević-Djordjević et al. (2018) and Stanković (2012)
		T. botrys	Stanković (2012)
10.	Caffeic acid	*T. polium*	Proestos et al. (2006), Tepe et al. (2011), Milošević-Djordjević et al. (2018) and Stanković (2012)
		T. pseudoscorodonia	Belarbi et al. (2017)
		T. hyrcanicum	Oganesyan (2005)
		T. arduini	Šamec et al. (2010) and Stanković (2012)
		T. chamaedrys	Lin et al. (2009), Nastić et al. (2018a), Mitreski et al. (2014) and Stanković (2012)
		T. montanum	Tumbas et al. (2004), Nastić et al. (2018b), Mitreski et al. (2014) and Stanković (2012)
		T. scordium	Mitreski et al. (2014) and Stanković (2012)
		T. botrys	Stanković (2012)
11.	6-Hydroxy caffeic acid	*T. chamaedrys, T. flavum, T. fruticans, T. scorodonia*	Pedersen (2000)
12.	Ferulic acid	*T. polium*	Proestos et al. (2006) and Stanković (2012)
		T. pseudoscorodonia	Belarbi et al. (2017)
		T. chamaedrys	Nastić et al. (2018a) and Stanković (2012)
		T. arduini	Šamec et al. (2010), Kremer et al. (2013) and Stanković (2012)
		T. montanum	Tumbas et al. (2004), Nastić et al. (2018b) and Stanković (2012)
		T. scordium, T. botrys	Stanković (2012)
13.	Sinapic acid	*T. pseudoscorodonia*	Belarbi et al. (2017)
		T. scordium	Milošević-Djordjević et al. (2018)
		T. chamaedrys	Nastić et al. (2018a)
		T. arduini	Šamec et al. (2010)

(continued)

Table 6.2 (continued)

No.	Compounds reported	*Teucrium* species	References
14.	Chlorogenic acid	*T. canadense*	Lin et al. (2009)
		T. flavum, T. fruticans, T. marum	Pedersen (2000)
		T. polium	Milošević-Djordjević et al. (2018) and Stanković (2012)
		T. chamaedrys	Vlase et al. (2014), Nastić et al. (2018a) and Stanković (2012)
		T. arduini, T. montanum, T. botrys, T. scordium	Stanković (2012)
15.	Neochlorogenic acid	*T. chamaedrys*	Lin et al. (2009) and Mitreski et al. (2014)
		T. polium, T. montanum, T. scordium	Mitreski et al. (2014)
16.	Rosmarinic acid	*T. scorodonia*	Pedersen (2000)
		T. chamaedrys	Vlase et al. (2014) and Stanković (2012)
		T. arduini	Kremer et al. (2013) and Stanković (2012)
		T. pseudoscorodonia	Belarbi et al. (2017)
		T. montanum, T. polium, T. scordium, T. botrys	Stanković (2012)
17.	Teucrol	*T. pilosum* (syn. *T. japonicum* var. *pilosum*)	El-Mousallamy et al. (2000)
18.	Teucroside	*T. pilosum* (syn. *T. japonicum* var. *pilosum*)	El-Mousallamy et al. (2000)

phenylpropanoid biosynthesis, *p*-coumaric (**9**), caffeic (**10**), and ferulic (**12**) acids were confirmed in *T. chamaedrys*, *T. montanum*, *T. arduini*, *T. pseudoscorodonia*, *T. polium*, *T. scordium*, and *T. botrys* by different research groups. Chlorogenic acid (**14**) was identified in ten species while sinapic acid (**13**) was present in four *Teucrium* species. *p*-Coumaric acid was greatly present in *T. arduini* from Croatia, especially in flower infusions, ranging from 3.4 to 10.8 μg/g of dry weight (Šamec et al. 2010). On the other hand, much higher content of *p*-coumaric acid was found in the *Teucrium* extracts, particularly in *T. montanum* (1794 μg/g dry extract) (Tumbas et al. 2004).

Some of *Teucrium* species were able to synthesize rosmarinic acid (**16**), another very important ester of caffeic acid and 3, 4-dihydroxyphenyllactic acid. Moreover, El-Mousallamy et al. (2000) reported the presence of two derivatives of rosmarinic acid, a decarboxyrosmarinic acid or teucrol (**17**) and its' 4′-*O*-triglycoside teucroside (**18**) in *T. pilosum*.

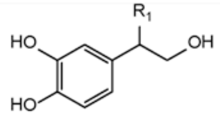
6.2.2 Phenylethanoids and Phenylethanoid Glycosides

Besides phenolic acids, including phenylpropanoids, another very important class of phenolic secondary metabolites identified in *Teucrium* spp. are phenylethanoids and their glycosides. Phenylethanoid glycosides are secondary metabolites widely distributed in plants and they often appear together with iridoids in several plant families. Phenylethanoid glycosides are characterized by a hydroxyphenylethyl moiety (C_6-C_2 structure) to which a sugar molecule, mainly β-glucopyranose, is linked through glycosidic bond formation (Fu et al. 2008). Additional monosaccharides (i.e. rhamnose, apiose, galactose, xylose, etc.) and phenylpropanoid (C_6-C_3) residues are usually attached to the glucose center of the molecule (Alipieva et al. 2014). The synonyms caffeic acid glycoside esters or caffeoyl phenylethanoid glycosides are frequently in use instead of phenylpropanoid glycosides, because the most common phenylpropanoid residue in these molecules is caffeic acid (Luca et al. 2019). Verbascoside (**21**), also known as acteoside, the most common phenylethanoid glycoside in plants, has been identified in more than 200 plants (Schaluer et al. 2004). According to the previous studies, phenylethanoid glycosides have been the subject of many scientific researches in the past few decades. They possess numerous biological activities and pharmacological properties of some of them have been confirmed in certain clinical trials (Jimenez and Riguera 1994).

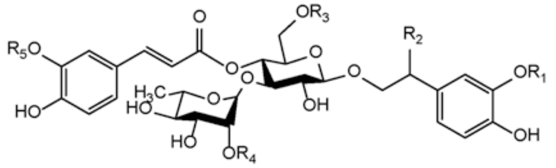
The phenylethanoids and phenylethanoid glycosides reported from the *Teucrium* genus are shown in Fig. 6.4. A total of 16 different phenylethanoids and phenylethanoid glycosides, listed in Table 6.3, have been recorded from 13 species belonging to *Teucrium* genus. The most common phenylethanoid glycoside reported from 12 species was verbascoside (**21**). Other most abundant phenylethanoid glycosides of *Teucrium* species are forsythoside B (**28**) reported in 4 species, poliumoside (**29**), and samioside (**31**) reported in 3 species listed in Table 6.3. Other phenylethanoids identified in *Teucrium* species were found only in one or two species.

All phenylethanoid glycosides identified in *Teucrium* genus contain caffeic acid as phenylpropanoid residue in these molecules, except leucoseptoside A (**23**) found in *T. polium*, and alyssonoside (**24**) found in *T. polium* (Mitreski et al. 2014) and *T. chamaedrys* (Mitreski et al. 2014; Frezza et al. 2018) which contain ferulic acid residue. Considering the number of monosaccharide moieties in identified phenylethanoids, phenylethanoid triglycosides are the most distributed class of these compounds in *Teucrium* genus. Among 16 reported phenylethanoids, 5 phenylethanoid diglycosides, verbascoside (**21**), 6-hydroxyacteoside (**22**), leucoseptoside A (**23**), isoverbascoside (**33**), and forsythoside A (**34**) were found in *Teucrium* species. Only two phenylethanoid aglycons, tyrosol (**1**) and 3,4-dihydroxy-β-phenylethanol (**2**), were found in species from this genus.

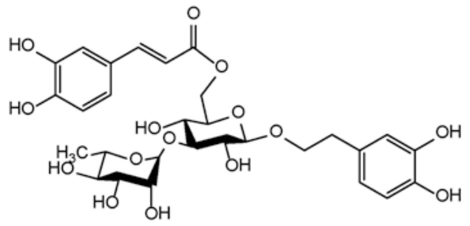
T. polium, *T. montanum*, and *T. chamaedrys* are the most studied plant species from *Teucrium* genus with the highest number of identified phenylethanoids. A total of 11 distinct phenylethanoid glycosides were reported in *T. polium* species, while 7 and 5 different phenylethanoids were identified in *T. montanum* and *T. chamaedrys*, respectively. 3,4-Dihydroxy-β-phenylethanol (**2**) was found only in



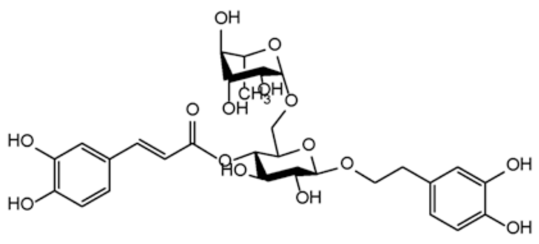
(19) Tyrosol R_1 = H
(20) 3,4-Dihydroxy-β-phenylethanol R_1 = OH



		R_1	R_2	R_3	R_4	R_5
(21)	Acteoside (Verbascoside)	H	H	H	H	H
(22)	6-Hydroxyacteoside	H	OH	H	H	H
(23)	Leucoseptoside A	H	H	H	H	CH_3
(24)	Alyssonoside	H	H	Apiose	H	CH_3
(25)	Caerulescenoside	H	H	H	Glucose	H
(26)	Echinacoside	H	H	Glucose	H	H
(27)	Castanoside A	CH_3	H	Glucose	H	H
(28)	Forsythoside B	H	H	Apiose	H	H
(29)	Poliumoside	H	H	Rhamnose	H	H
(30)	Poliumoside B	H	H	Rhamnose	Apiose	H
(31)	Samioside	H	H	H	Apiose	H
(32)	Teucreoside	H	H	H	Xylose	H



(33) Isoverbascoside



(34) Forsythoside A

Fig. 6.4 Phenylethanoids and phenylethanoid glycosides identified in *Teucrium* species

Table 6.3 Phenylethanoids and phenylethanoid glycosides identified in *Teucrium* species

No.	Compounds reported	*Teucrium* species	References
19.	Tyrosol	*T. polium*	Proestos et al. (2006)
20.	3,4-Dihydroxy-*β*-phenylethanol	*T. hyrcanicum*	Oganesyan (2005)
21.	Acteoside (Verbascoside)	*T. capitatum, T. flavum, T. fruticans, T. marum, T. scorodonia*	Pedersen (2000)
		T. polium	Pedersen (2000), Tepe et al. (2011) and Mitreski et al. (2014)
		T. chamaedrys	Frezza et al. (2018), Pedersen (2000), Mitreski et al. (2014) and Lin et al. (2009)
		T. montanum, T. scordium	Mitreski et al. (2014)
		T. polium var. *gnaphalodes*	Boghrati et al. (2016)
		T. canadense	Lin et al. (2009)
		T. belion (syn. *T. dunense*)	Andary et al. (1985)
22.	6-Hydroxyacteoside	*T. scorodonia*	Pedersen (2000)
23.	Leucoseptoside A	*T. polium*	Mitreski et al. (2014)
24.	Alyssonoside	*T. polium*	Mitreski et al. (2014)
		T. chamaedrys	Frezza et al. (2018) and Mitreski et al. (2014)
25.	Caerulescenoside	*T. montanum*	Mitreski et al. (2014)
26.	Echinacoside	*T. polium, T. montanum*	Mitreski et al. (2014)
		T. belion (syn. *T. dunense*)	Andary et al. (1985)
27.	Castanoside A	*T. montanum*	Mitreski et al. (2014)
28.	Forsythoside B	*T. chamaedrys*	Frezza et al. (2018) and Mitreski et al. (2014)
		T. polium , T. montanum, T. scordium	Mitreski et al. (2014)
29.	Poliumoside	*T. polium*	De Marino et al. (2012) and Venditti et al. (2017)
		T. polium var. *gnaphalodes*	Boghrati et al. (2016)
		T. chamaedrys	Mitreski et al. (2014)
		T. belion (syn. *T. dunense*)	Andary et al. (1985)
30.	Poliumoside B	*T. polium*	De Marino et al. (2012) and Mitreski et al. (2014)
31.	Samioside	*T. chamaedrys*	Frezza et al. (2018)
		T. polium, T. montanum	Mitreski et al. (2014)
32.	Teucreoside	*T. polium*	Tepe et al. (2011)
		T. chamaedrys	Lin et al. (2009) and Mitreski et al. (2014)
33.	Isoverbascoside	*T. canadense*	Lin et al. (2009)
34.	Forsythoside A	*T. polium, T. scordium*	Mitreski et al. (2014)
		T. belion (syn. *T. dunense*)	Andary et al. (1985)

T. hyrcanicum, while verbascoside (**21**) was the only identified phenylethanoid glycoside in *T. capitatum, T. flavum, T. fruticans,* and *T. marum*.

Only a few studies reported phenylethanoids contents in *Teucrium* species. According to the results of Mitreski et al. (2014), the verbascoside concentrations were 6.7 for *T. scordium*, 4.5 for *T. chamaedrys*, 2.8 for *T. polium,* and 2.0 mg/g dry herb for *T. montanum*. The same study showed that forsythoside B (**28**) was the most abundant phenylethanoid glycoside in these species, except *T. polium*, in the concentration range of 39.0 mg/g dry herb for *T. chamaedrys* to 10.2 mg/g dry herb for *T. montanum*, while samioside (**31**) was the dominant phenylethanoid glycoside for *Teucrium polium* in the concentration of 11.5 mg/g dry herb (Mitreski et al. 2014). Lin et al. (2009) showed that different *T. canadense* samples contain verbascoside in amounts from 0.23% to 9.66% of dry plant weight (**21**).

6.2.3 Flavonoids

Flavonoids represent the largest group of phenolic compounds found in *Teucrium* species, and totally 44 compounds belonging to this subclass of phenolics were identified in plants from this genus (Table 6.4). Four subclasses of flavonoids were detected in *Teucrium* species: flavones, flavonols, flavone-3-ols, and flavanones. Flavones are the major subclass of flavonoids recorded in these plants, represented by apigenin and luteolin derivatives (Figs. 6.5 and 6.6). As can be seen in Table 6.4, a total of 34 flavones have been reported from a total of 45 *Teucrium* species so far. Luteolin (**49**), identified in 42 *Teucrium* species, is the most common flavone within this genus. Also, luteolin 7-*O*-glucoside (**50**) and luteolin 7-*O*-rutinoside (**52**) are widely distributed flavone glycosides in these species found in 35 and 38 *Teucrium* plants, respectively (Table 6.4). On the other hand, apigenin (**35**) was identified only in 5 species, while its glycoside derivatives, such as apigenin 7-*O*-glucoside (**36**) and apigenin 7-*O*-rutinoside (**38**), were found in more than 20 different *Teucrium* plants. According to the studies dealing with flavonoids analysis of this genus, salvigenin (**43**), cirsimaritin (**44**) (Fig. 6.5), cirsiliol (**61**), and cirsilineol (**62**) (Fig. 6.6) were also reported as common flavone aglycones. Some of *Teucrium* species are able to synthesize flavone *C*-glycosides; vicenin-2 (**48**), as a common flavone *C*-glycoside found in these species, was observed in thirty of them. Harborne et al. (1986) reported the presence of two flavone *C*-glycosides, isoscutellarein 7-allosylglucoside (**37**) and hypolaetin 7-allosylglucoside (Fig. 6.5) in *T. chamaedrys* and *T. webbianum*.

As shown in Table 6.4, the most abundant flavonols reported from *Teucrium* species are quercetin glycosides, isoquercitrin (**69**) and rutin (**70**) (Fig. 6.7) which were identified in 13 and 14 *Teucrium* plants, respectively. The presence of quercetin (**68**) and myricetin (**75**) was observed in 6 *Teucrium* species. Flavan-3-ols (Fig. 6.7), catechin (**76**) and epicatechin (**77**), were reported by Nastić et al. (2018b) only for *T. chamaedrys* and *T. montanum*, while Milošević-Djordjević et al. (2018) confirmed the presence of catechin in *T. polium* and *T. scordium*. Flavanone glycoside

Table 6.4 Flavonoids identified in *Teucrium* species

No.	Compounds reported	*Teucrium* species	References
Flavones and flavone glycosides			
35.	Apigenin	*T. polium*	Sharififar et al. (2009), Milošević-Djordjević et al. (2018), Mitreski et al. (2014), Harborne et al. (1986), Carreiras et al. (1989) and Venditti et al. (2017)
		T. chamaedrys, T. montanum, T. scordium	Mitreski et al. (2014)
		T. pilosum (syn. *T. japonicum* var. *pilosum*)	Hawas et al. (2008)
36.	Apigenin 7-*O*-glucoside	*T. arduini, T. scorodonia, T. massiliense, T. asiaticum, T. spinosum, T. resupinatum, T. botrys, T. webbianum, T. divaricatum, T. villosum, T. flavum, T. intrincatum, T. fragile, T. marum, T. subspinosum, T. pyrenaicum, T. rotundifolium, T. buxifolium, T. freynii, T. thymifolium, T. cossoni, T. libanitis, T. cartaginensis, T. pumilum, T. carolipaui, T. capitatum, T. pii-fontii, T. gnaphalodes, T. eriocephalum, T. charidemi*	Harborne et al. (1986)
		T. polium	Kawashty et al. (1999) and Mitreski et al. (2014)
		T. chamaedrys, T. scordium	Harborne et al. (1986) and Mitreski et al. (2014)
		T. leucocladum	Kawashty et al. (1999)
37.	Apigenin 7-*O*-glucuronide	*T. polium*	Tepe et al. (2011)
		T. chamaedrys, T. canadense	Lin et al. (2009)
38.	Apigenin 7-*O*-rutinoside (Isorhoifolin)	*T. massiliense, T. asiaticum, T. resupinatum, T. webbianum, T. divaricatum, T. villosum, T. intrincatum, T. fragile, T. marum, T. subspinosum, T. buxifolium, T. freynii, T. cossoni, T. libanitis, T. cartaginensis, T. pumilum, T. carolipaui, T. pii-fontii*	Harborne et al. (1986)
		T. chamaedrys	Harborne et al. (1986) and Mitreski et al. (2014)
		T. scordium	Mitreski et al. (2014)
		T. polium	Venditti et al. (2017)
		T. polium var. *gnaphalodes*	Boghrati et al. (2016)

39.	Apigenin 5-galloylglucoside	*T. polium, T. leucocladum*	Kawashty et al. (1999)
40.	Apigenin *p*-coumaroylglucoside	*T. polium*	Mitreski et al. (2014)
41.	4′,7-Dimethoxy apigenin	*T. polium*	Sharififar et al. (2009) and Verykokidou-Vitsaropoulou and Vajias (1986)
42.	7-Methyl scutellarein	*T. hyrcanicum*	Oganesyan (2005)
43.	Salvigenin	*T. massiliense, T. divaricatum, T. villosum, T. subspinosum, T. pyrenaicum, T. rotundifolium, T. buxifolium, T. thymifolium, T. libanitis, T. cartaginensis, T. aragonense, T. pumilum, T. carolipaui, T. aureum, T. capitatum, T. pii-fontii, T. gnaphalodes, T. eriocephalum, T. haenseleri*	Harborne et al. (1986)
		T. polium	Rizk et al. (1986)
		T. pilosum (syn. *T. japonicum* var. *pilosum*)	Hawas et al. (2008)
44.	Cirsimaritin	*T. brevifolium, T. pseudochamaepitys, T. arduini, T. scorodonia, T. massiliense, T. asiaticum, T. spinosum, T. webbianum, T. divaricatum, T. villosum, T. intrincatum, T. fragile, T. marum, T. subspinosum, T. compactum, T. pyrenaicum, T. rotundifolium, T. buxifolium, T. freynii, T. thymifolium, T. cossoni, T. libanitis, T. cartaginensis, T. aragonense, T. pumilum, T. carolipaui, T. aureum, T. capitatum, T. pii-fontii, T. gnaphalodes, T. eriocephalum, T. haenseleri, T. charidemi*	Harborne et al. (1986)
		T. polium	Verykokidou-Vitsaropoulou and Vajias (1986), Mitreski et al. (2014), Harborne et al. (1986) and Venditti et al. (2017)
		T. chamaedrys, T. montanum	Harborne et al. (1986) and Mitreski et al. (2014)
		T. pilosum (syn. *T. japonicum* var. *pilosum*)	Hawas et al. (2008)
45.	Cirsimaritin 4′-glucoside	*T. arduini*	Harborne et al. (1986)
46.	5-hydroxy-6,7,3′,4′-tetramethoxyflavon	*T. fruticans, T. brevifolium, T. pseudochamaepitys, T. arduini, T. salviastrum, T. scordium, T. divaricatum, T. villosum, T. flavum, T. fragile, T. marum, T. subspinosum, T. compactum*	Harborne et al. (1986)

(continued)

Table 6.4 (continued)

No.	Compounds reported	*Teucrium* species	References
47.	3′,6-Dimethoxy apigenin	*T. polium*	Sharififar et al. (2009)
48.	Vicenin-2	*T. polium*	Harborne et al. (1986) and Kawashty et al. (1999)
		T. leucocladum	Kawashty et al. (1999)
		T. fruticans, T. brevifolium, T. pseudochamaepitys, T. arduini, T. massiliense, T. asiaticum, T. chamaedrys, T. webbianum, T. divaricatum, T. villosum, T. intrincatum, T. fragile, T. compactum, T. pyrenaicum, T. buxifolium, T. freynii, T. montanum, T. cossoni, T. libanitis, T. cartaginensis, T. aragonense, T. pumilum, T. carolipaui, T. aureum, T. capitatum, T. pii-fontii, T. haenseleri, T. charidemi	Harborne et al. (1986)
		T. pilosum (syn. *T. japonicum* var. *pilosum*)	Hawas et al. (2008)
49.	Luteolin	*T. fruticans, T. brevifolium, T. pseudochamaepitys, T. arduini, T. scorodonia, T. massiliense, T. salviastrum, T. asiaticum, T. scordium, T. spinosum, T. resupinatum, T. botrys, T. divaricatum, T. villosum, T. flavum, T. intrincatum, T. fragile, T. marum, T. subspinosum, T. pyrenaicum, T. rotundifolium, T. buxifolium, T. freynii, T. thymifolium, T. cossoni, T. libanitis, T. cartaginensis, T. aragonense, T. pumilum, T. carolipaui, T. aureum, T. capitatum, T. pii-fontii, T. gnaphalodes, T. eriocephalum, T. haenseleri, T. charidemi*	Harborne et al. (1986)
		T. polium	Harborne et al. (1986), Proestos et al. (2006), Tepe et al. (2011), Milošević-Djordjević et al. (2018) and Mitreski et al. (2014)
		T. chamaedrys	Harborne et al. (1986), Lin et al. (2009) and Vlase et al. (2014)
		T. hyrcanicum	Oganesyan (2005)
		T. montanum	Harborne et al. (1986) and Mitreski et al. (2014)
		T. pilosum (syn. *T. japonicum* var. *pilosum*)	Hawas et al. (2008)

(continued)

50.	Luteolin 7-*O*-glucoside	*T. arduini, T. scorodonia, T. massiliense, T. salviastrum, T. asiaticum, T. spinosum, T. resupinatum, T. botrys, T. divaricatum, T. villosum, T. flavum, T. intrincatum, T. fragile, T. marum, T. subspinosum, T. pyrenaicum, T. rotundifolium, T. buxifolium, T. freynii, T. thymifolium, T. cossoni, T. libanitis, T. cartaginensis, T. pumilum, T. carolipaui, T. aureum, T. capitatum, T. gnaphalodes, T. eriocephalum, T. haenseleri, T. charidemi*	Harborne et al. (1986)
		T. polium	Harborne et al. (1986), Tepe et al. (2011), De Marino et al. (2012), Mitreski et al. (2014) and Kawashty et al. (1999)
		T. chamaedrys	Harborne et al. (1986), Lin et al. (2009) and Mitreski et al. (2014)
		T. leucocladum	Kawashty et al. (1999)
		T. montanum, T. scordium	Harborne et al. (1986) and Mitreski et al. (2014)
51.	Luteolin 4′-*O*-glucoside	*T. polium*	De Marino et al. (2012)
52.	Luteolin 7-*O*-rutinoside	*T. fruticans, T. brevifolium, T. pseudochamaepitys, T. arduini, T. scorodonia, T. massiliense, T. salviastrum, T. asiaticum, T. spinosum, T. resupinatum, T. botrys, T. divaricatum, T. villosum, T. intrincatum, T. fragile, T. marum, T. subspinosum, T. pyrenaicum, T. rotundifolium, T. buxifolium, T. freynii, T. thymifolium, T. cossoni, T. libanitis, T. cartaginensis, T. aragonense, T. pumilum, T. carolipaui, T. aureum, T. capitatum, T. gnaphalodes, T. eriocephalum, T. haenseleri, T. charidemi*	Harborne et al. (1986)
		T. polium	Harborne et al. (1986), Tepe et al. (2011), De Marino et al. (2012) and Mitreski et al. (2014)
		T. chamaedrys	Harborne et al. (1986), Lin et al. (2009) and Mitreski et al. (2014)
		T. montanum, T. scordium	Harborne et al. (1986) and Mitreski et al. (2014)

(continued)

Table 6.4 (continued)

No.	Compounds reported	*Teucrium* species	References
53.	Luteolin 7-*O*-neohesperidoside	*T. polium*	De Marino et al. (2012)
54.	Luteolin 7-sambubioside	*T. scordium, T. spinosum, T. resupinatum, T. botrys, T. chamaedrys, T. flavum, T. compactum, T. rotundifolium, T. polium, T. pii-fontii*	Harborne et al. (1986)
55.	Diosmetin	*T. polium*	Tepe et al. (2011), Mitreski et al. (2014) and Venditti et al. (2017)
		T. chamaedrys	Lin et al. (2009)
		T. scordium	Mitreski et al. (2014)
		T. pilosum (syn. *T. japonicum* var. *pilosum*)	Hawas et al. (2008)
56.	Diosmetin 7-*O*-rutinoside	*T. spinosum, T. resupinatum, T. gnaphalodes, T. eriocephalum, T. haenseleri, T. charidemi*	Harborne et al. (1986)
		T. polium	Harborne et al. (1986) and Mitreski et al. (2014)
		T. montanum, T. scordium	Mitreski et al. (2014)
		T. chamaedrys	Lin et al. (2009) and Mitreski et al. (2014)
57.	Diosmetin *p*-coumaroylglucoside	*T. polium*	Mitreski et al. (2014)
58.	6-Hydroxyluteolin	*T. polium*	Harborne et al. (1986)
59.	6-*OH*-Luteolin 7-glucoside	*T. arduini, T. scorodonia, T. massiliense, T. asiaticum, T. flavum, T. rotundifolium, T. buxifolium, T. thymifolium, T. libanitis, T. cartaginensis, T. aragonense, T. pumilum, T. carolipaui, T. polium, T. aureum, T. capitatum, T. gnaphalodes,T. eriocephalum*	Harborne et al. (1986)
60.	6-*OH*-Luteolin 7-rhamnoside	*T. scorodonia, T. massiliense, T. buxifolium, T. thymifolium, T. libanitis, T. cartaginensis, T. aragonense, T. carolipaui, T. gnaphalodes,T. eriocephalum, T. haenseleri, T. charidemi*	Harborne et al. (1986)

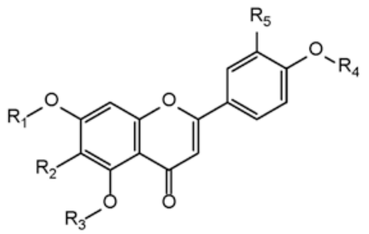
61.	Cirsiliol	*T. fruticans, T. brevifolium, T. pseudochamaepitys, T. arduini, T. scorodonia, T. massiliense, T. salviastrum, T. asiaticum, T. spinosum, T. botrys, T. webbianum, T. divaricatum, T. villosum, T. flavum, T. fragile, T. marum, T. subspinosum, T. compactum, T. pyrenaicum, T. rotundifolium, T. buxifolium, T. freynii, T. thymifolium, T. cossoni, T. libanitis, T. cartaginensis, T. aragonense, T. pumilum, T. carolipaui, T. aureum, T. capitatum, T. pii-fontii, T. gnaphalodes, T. eriocephalum, T. haenseleri*	Harborne et al. (1986)
		T. polium	Rizk et al. (1986), Verykokidou-Vitsaropoulou and Vajias (1986), Harborne et al. (1986), Carreiras et al. (1989) and Mitreski et al. (2014)
		T. chamaedrys, T. montanum, T. scordium	Harborne et al. (1986) and Mitreski et al. (2014)
62.	Cirsilineol	*T. brevifolium, T. pseudochamaepitys, T. massiliense, T. scordium, T. resupinatum, T. divaricatum, T. villosum, T. marum, T. subspinosum, T. compactum, T. pyrenaicum, T. rotundifolium, T. buxifolium, T. freynii, T. montanum, T. thymifolium, T. libanitis, T. cartaginensis, T. aragonense, T. pumilum, T. carolipaui, T. aureum, T. capitatum, T. pii-fontii, T. gnaphalodes, T. eriocephalum, T. haenseleri, T. charidemi*	Harborne et al. (1986)
		T. polium	Harborne et al. (1986) and Venditti et al. (2017)
63.	Pedalitin	*T. hyrcanicum*	Oganesyan (2005)
64.	Eupatorin	*T. polium*	Verykokidou-Vitsaropoulou and Vajias (1986)
65.	Desmethoxycentaureidin	*T. pilosum* (syn. *T. japonicum* var. *pilosum*)	Hawas et al. (2008)
66.	6-Methoxyluteolin-7,3′,4′-trimethylether	*T. pilosum* (syn. *T. japonicum* var. *pilosum*)	Hawas et al. (2008)
67.	Isoscutellarein 7-allosylglucoside	*T. chamaedrys, T. webbianum*	Harborne et al. (1986)

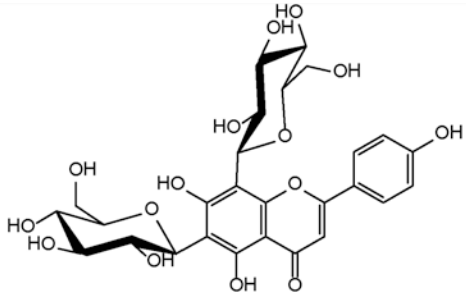
(continued)

Table 6.4 (continued)

No.	Compounds reported	*Teucrium* species	References
68.	Hypolaetin 7-allosylglucoside	*T. chamaedrys, T. webbianum*	Harborne et al. (1986)
Flavonols and flavonol glycosides			
69.	Quercetin	*T. polium*	Stanković (2012) and Milošević-Djordjević et al. (2018)
		T. arduini	Stanković (2012) and Kremer et al. (2013)
		T. chamaedrys, T. montanum, T. botrys, T. scordium subsp. *scordioides*	Stanković (2012)
70.	Isoquercitrin	*T. chamaedrys*	Vlase et al. (2014)
		T. fruticans, T. brevifolium, T. pseudochamaepitys, T. botrys, T. compactum, T. montanum, T. aragonense, T. polium, T. aureum, T. capitatum, T. pii-fontii, T. haenseleri	Harborne et al. (1986)
71.	Rutin	*T. chamaedrys*	Stanković (2012), Vlase et al. (2014) and Zdraveva et al. (2018)
		T. pseudoscorodonia	Belarbi et al. (2017)
		T. montanum	Harborne et al. (1986), Stanković (2012) and Nastić et al. (2018b)
		T. scordium	Milošević-Djordjević et al. (2018)
		T. polium	Sharififar et al. (2009), Stanković (2012) and Milošević-Djordjević et al. (2018)
		T. arduini, T. scordium subsp. *scordioides*	Stanković (2012)
		T. botrys	Harborne et al. (1986) and Stanković (2012)
		T. fruticans, T. brevifolium, T. pseudochamaepitys, T. compactum, T. aragonense, T. haenseleri	Harborne et al. (1986)
72.	Quercitrin	*T. chamaedrys*	Vlase et al. (2014) and Mitreski et al. (2014)
		T. montanum	Mitreski et al. (2014)
73.	Isorhanmetin 3-glucoside	*T. fruticans, T. brevifolium, T. pseudochamaepitys, T. compactum, T. aragonense*	Harborne et al. (1986)

74.	Isorhamnetin 3-rutinoside	*T. fruticans, T. brevifolium, T. pseudochamaepitys, T. compactum, T. aragonense*	Harborne et al. (1986)
75.	Myricetin	*T. polium*	Stanković (2012) and Milošević-Djordjević et al. (2018)
		T. chamaedrys, T. arduini, T. montanum, T. botrys, T. scordium subsp. *scordioides*	Stanković (2012)
Flavan-3-ols			
76.	Catechin	*T. polium, T. scordium*	Milošević-Djordjević et al. (2018)
		T. chamaedrys	Nastić et al. (2018a)
		T. montanum	Nastić et al. (2018b)
77.	Epicatechin	*T. chamaedrys*	Nastić et al. (2018a)
		T. montanum	Nastić et al. (2018b)
Flavanone glycoside			
78.	Naringin	*T. montanum*	Nastić et al. (2018b)





(48) Vicenin-2

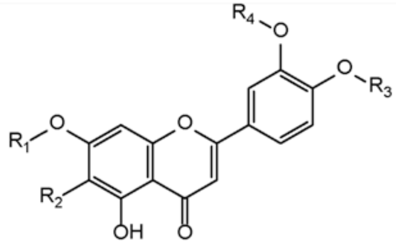
		R1	R2	R3	R4	R5
(35)	Apigenin	H	H	H	H	H
(36)	Apigenin 7-*O*-glucoside	Glucose	H	H	H	H
(37)	Apigenin 7-*O*-glucuronide	Glucuronic acid	H	H	H	H
(38)	Apigenin 7-*O*-rutinoside (Isorhoifolin)	Rutinose	H	H	H	H
(39)	Apigenin 5-galloylglucoside	H	H	Glucose-galloyl	H	
(40)	Apigenin *p*-coumaroyl glucoside	Glucose-*p*-coumaroyl	H	H	H	
(41)	4',7-Dimethoxy apigenin	CH_3	H	H	CH_3	H
(42)	7-Methyl scutellarein	CH_3	OH	H	H	H
(43)	Salvigenin	CH_3	OCH_3	H	CH_3	H
(44)	Cirsimaritin	CH_3	OCH_3	H	H	H
(45)	Cirsimaritin 4'-glucoside	CH_3	OCH_3	H	Glucose	H
(46)	5-Hydroxy-6,7,3',4'-tetramethoxyflavon	CH_3	OCH_3	H	CH_3	OCH_3
(47)	3',6-Dimethoxy apigenin	H	OCH_3	H	H	OCH_3

Fig. 6.5 Flavones (apigenin and its derivatives) identified in *Teucrium* species

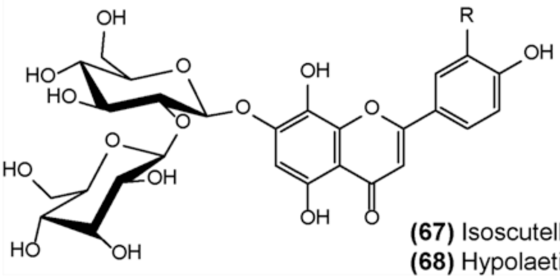
(Fig. 6.7), naringin (**78**), was currently reported (Nastić et al. 2018b) only for *T. montanum.*

T. polium, *T. chamaedrys*, and *T. montanum* are the most studied plants from *Teucrium* genus with the highest number of identified flavonoids. A total of 30 distinct flavonoids have been recorded in *T. polium* so far, while 22 and 17 flavonoids have been reported in *T. chamaedrys* and *T. montanum*, respectively. Apigenin 5-galloylglucoside (**39**), apigenin *p*-coumaroylglucoside (**40**), 4′,7-dimethoxy apigenin (**41**), 3′,6-dimethoxy apigenin (**47**), luteolin 4′-*O*-glucoside (**51**), luteolin 7-*O*-neohesperidoside (**53**), diosmetin *p*-coumaroylglucoside (**57**), 6-hydroxyluteolin (**58**), and eupatorin (**64**) were found only in *Teucrium polium*. 7-Methyl scutellarein (**42**) and pedalitin (**63**) were reported only in *T. hyrcanicum*, while cirsimaritin 4′-glucoside (**45**) was identified only in *T. arduini* so far.

Although there are numerous literature data about flavonoids composition of *Teucrium* plants, publications on their quantitative analysis are relatively rare. Mitreski et al. (2014) showed that flavonoid glycosides represent about 7% of total



		R_1	R_2	R_3	R_4
(49)	Luteolin	H	H	H	H
(50)	Luteolin 7-*O*-glucoside	Glucose	H	H	H
(51)	Luteolin 4’-*O*-glucoside	H	H	Glucose	H
(52)	Luteolin 7-*O*-rutinoside	Rutinose	H	H	H
(53)	Luteolin 7-*O*-neohesperidoside	Neohesperidose	H	H	H
(54)	Luteolin 7-sambubioside	Sambubiose	H	H	H
(55)	Diosmetin	H	H	CH_3	H
(56)	Diosmetin 7-*O*-rutinoside	Rutinose	H	CH_3	H
(57)	Diosmetin *p*-coumaroylglucoside	Glucose-*p*-coumaroyl	H	CH_3	H
(58)	6-Hydroxyluteolin	H	OH	H	H
(59)	6-*OH*-Luteolin 7-glucoside	Glucose	OH	H	H
(60)	6-*OH*-Luteolin 7-rhamnoside	Rhamnose	OH	H	H
(61)	Cirsiliol	CH_3	OCH_3	H	H
(62)	Cirsilineol	CH_3	OCH_3	H	CH_3
(63)	Pedalitin	CH_3	OH	H	H
(64)	Eupatorin	CH_3	OCH_3	CH_3	H
(65)	Desmethoxycentaureidin	H	OCH_3	CH_3	H
(66)	6-Methoxyluteolin-7,3′,4′-trimethylether	CH_3	OCH_3	CH_3	CH_3



(67) Isoscutellarein 7-allosylglucoside R = H
(68) Hypolaetin 7-allosylglucoside R = OH

Fig. 6.6 Flavones (luteolin and its derivatives) identified in *Teucrium* species

phenolic content detected in *T. montanum* and *T. chamaedrys* and about 30% in *T. polium* and *T. scordium*. In the same study, rutin, luteolin, and apigenin 7-*O*-glycosides were found in the highest concentration among all identified flavonoids in *T. polium, T. chamaedrys, T. montanum*, and *T. scordium* extracts examined using LC/DAD/ESI-MS^n. According to the results of Proestos et al. (2006), *T. polium* contained 0.48 mg of quercetin in 100 g of dry plant. Vlase et al. (2014) quantified

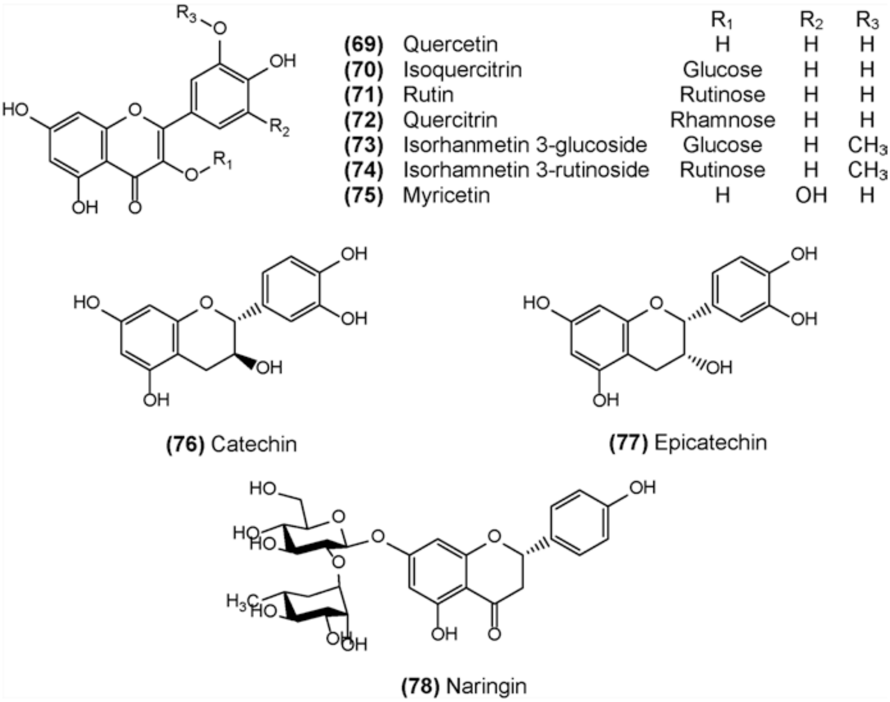


Fig. 6.7 Flavonols, flavan-3-ols and flavanones identified in *Teucrium* species

isoquercitrin, rutin, quercitrin, and luteolin in *T. chamaedrys* extract in the concentrations of 524.8, 85.42, 18.52, and 20.42 μg/g plant material, respectively, while catechin and epicatechin in subcritical water extract of *T. chamaedrys* were found in amounts of 73.7 and 54.2 mg/100 g extract, respectively (Nastić et al. 2018a). Subcritical water extraction of *T. montanum* showed that this extract contained epicatechin, rutin, and naringin in the concentrations of 120, 125, and 996 mg/100 g dry extract, respectively (Nastić et al. 2018b). The results of qualitative and quantitative analyses of flavonoids in *Teucrium* plants suggest that flavonoids represent the most diverse group of phenolic compounds present in these plant species, while their concentrations were lower compared to phenylethanoid glycosides.

6.3 Biosynthesis of Plant Phenolic Compounds

The shikimate pathway serves for the biosynthesis of the aromatic amino acids tryptophan, phenylalanine, and tyrosine. This pathway is common to plants and microorganisms, but not to animals, and because of that, the aromatic amino acids are placed in a group of the essential amino acids for humans (Dewick 2009; Vermerris and Nicholson 2006). The shikimate pathway got its name after shikimic acid, as the main intermediate compound in this pathway. Shikimic acid was named after shikimino-ki or shikimi, the Japanese name for the fruits of star anise (*Illicum anisatum* L., Illicaceae), from which it was first isolated in 1885, many years before its role in plant metabolism was elucidated (Dewick 2009; Samuelsson and Bohlin 2015). Precursors for the formation of aromatic amino acids are phosphoenolpyruvate, from the glycolytic pathway, and erythrose 4-phosphate, from the pentose phosphate cycle (Fig. 6.8). Briefly, these two compounds condense to form firstly 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP), catalyzed by DAHP synthase. Thereafter, the elimination of phosphate from DAHP and an intramolecular cyclisation occur to form 3-dehydroquinic acid, which can be reduced to quinic acid. The side reactions on 3-dehydroquinic acid can lead to the formation of some simple phenolic acids of C_6-C_1 skeleton type (protocatechuic and gallic acids). The removal of water in 3-dehydroquinic acid leads to 3-dehydroshikimic acid, from which shikimate is formed by the action of shikimate dehydrogenase. Shikimic acid is then converted with a series of enzymatic reactions to 5-enolpyruvylshikimic acid 3-phosphate (EPSP), which is transformed by chorismate synthase to chorismic acid. Chorismic acid itself represents a key precursor and branch point for two biosynthetic pathways, one is biosynthesis of tryptophan (L-Trp) and another is biosynthesis of phenylalanine (L-Phe) and tyrosine (L-Tyr) via formation of prephenic acid. The pathways to the formation of aromatic amino acids L-Phe and L-Tyr may vary in different organisms, where usually more than one route may be present in a particular species (Dewick 2009). Besides, chorismate is the starting point for biosynthesis of many other simple phenolic compounds (C_6-C_1), e.g., 4-hydroxybenzoic acid, *p*-aminobenzoic acid (PABA), and salicylic acid.

The synthesized aromatic amino acids are the precursors of the important class of phenolic compounds, the phenylpropanoids (C_6-C_3), as well as several other classes of phenolic compounds (flavonoids, monolignols, hydroxycinnamic acids, sinapoyl esters, coumarins, and stilbenes). After deamination of L-Phe and L-Tyr in plants and some microorganisms, cinnamic acid and 4-coumaric acid are formed, respectively. Thereupon, a sequence of hydroxylation and methylation reactions lead to the formation of metabolites called hydroxycinnamic acids (caffeic, ferulic, 5-hydroxyferulic, and sinapic acids, Fig. 6.8). *p*-Coumaric acid (4-coumaric acid) can be enzymatically converted to *p*-coumaroyl Coenzyme A which ends the general phenylpropanoid pathway, as further reactions lead to the biosynthesis of specific classes of compounds (Vermerris and Nicholson 2006). The mutual interaction of some hydroxycinnamic acids may lead to the formation of many other plant metabolites. For example, in the reaction of caffeoyl-CoA and quinic acid, chlorogenic acid is formed. Also, rosmarinic acid can be synthesized from L-Tyr and 4-coumaroyl-CoA via a range of enzymatic reactions. Gallic acid, besides direct synthesis in shikimate pathway, can be formed via a wide range of metabolites, such as phenylalanine, cinnamic, *p*-coumaric, caffeic, and protocatechuic acids (Dewick 2009; Vermerris and Nicholson 2006).

Starting molecule in flavonoids biosynthetic pathway, as the most abundant group of phenolic compounds in *Teucrium* species, is 4-hydroxycinnamoyl-CoA (*p*-coumaroyl-CoA) which is extended with three molecules of malonyl-CoA (Fig. 6.9). The resulting product of this reaction, catalyzed by the enzyme chalcone

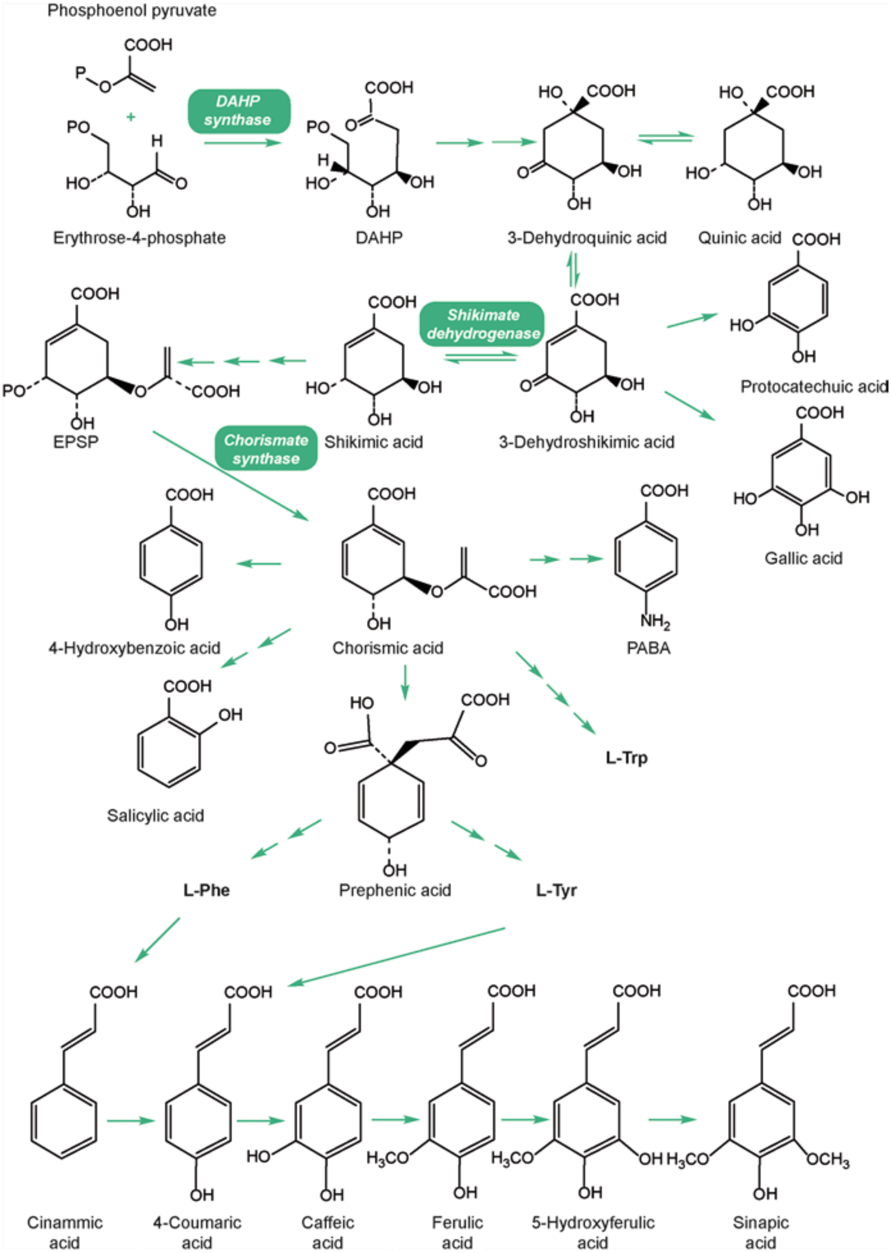


Fig. 6.8 Biosynthesis of shikimic acid with routes of aromatic amino acids (L-Trp, L-Phe, and L-Tyr) and phenylpropanoids synthesis

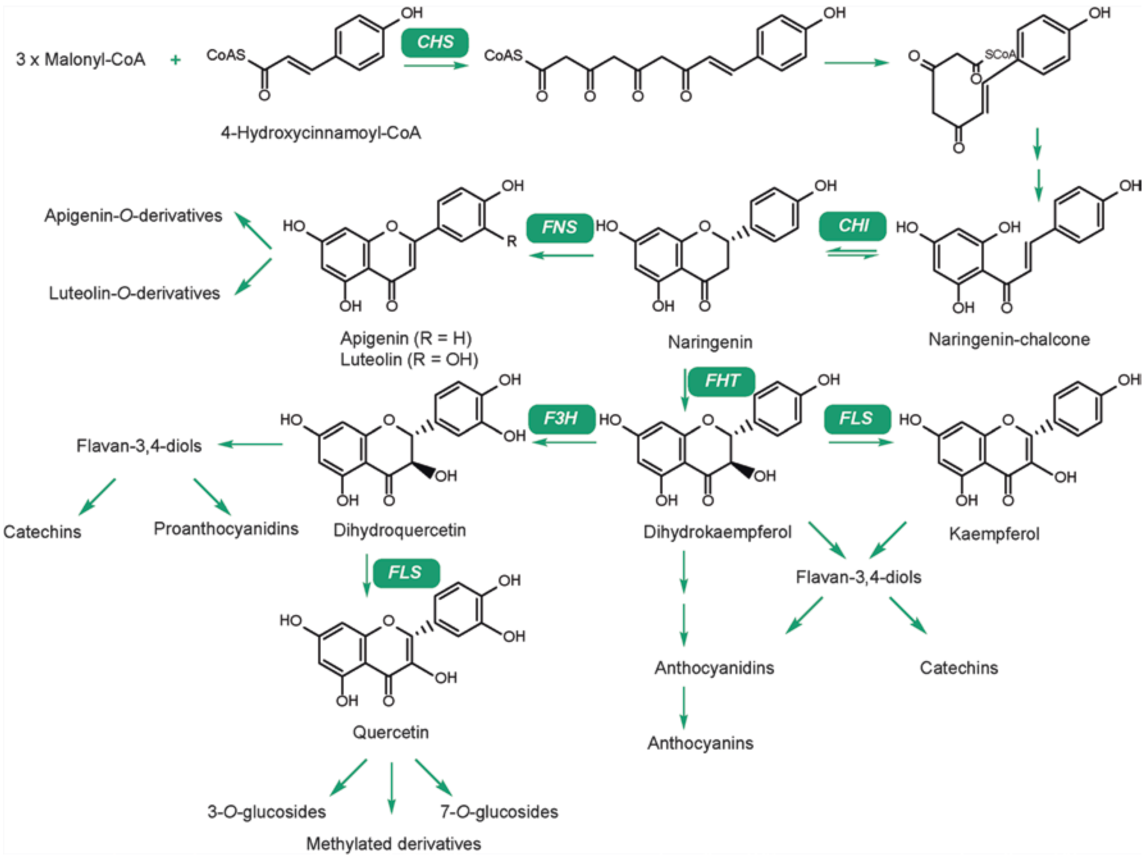


Fig. 6.9 Biosynthetic pathways for production of flavonoids. The enzymes: *CHS* chalcone synthase, *CHI* chalcone isomerase, *FNS* flavone synthase, *FHT* flavanone 3-hydroxylase, *F3H* flavanone 3′-hydroxylase, *FLS* flavonol synthase

synthase (CHS), is initially in a polyketide form, but it can be folded to generate aromatic rings. The key compound for further synthesis of flavonoids is naringenin-chalcone (4,2′,4′,6′ tetrahydroxychalcone) which is transformed by chalcone isomerase (CHI) via closing the pyrane ring to generate a flavanone naringenin. Naringenin-chalcone and chalcones generally are considered as precursors and can undergo a number of reactions to form different classes of polyphenolic metabolites, such as aurones (Dewick 1998, 2009).

Naringenin is subsequently converted by flavanone 3-hydroxylase (FHT) to the dihydrokaempferol (dihydroflavanonol). Besides that, the action of flavone synthase (FNS) on naringenin leads to the formation of flavones, apigenin (**35**) or luteolin (**49**), from which a wide range of metabolites identified in *Teucrium* species can be derived. Dihydrokaempferol can be converted by flavonol synthase (FLS) to the flavonol, kaempferol, but on the other hand, action of flavonoid 3′-hydroxylase (F3H) generates another dihydroflavonol, dihydroquercetin. This compound can be converted by FLS to its corresponding flavonol, quercetin (**69**), and thereafter to many quercetin derivatives. Kaempferol can also be enzymatically hydroxylated to quercetin (Dewick 1998). The most common form of flavonoids in plants is glycosylated for storage or transport, where the most often sugar attached to an OH group in the flavonoid is glucose (but also galactose, xylose, arabinose or rhamnose can be found) (Samuelsson and Bohlin 2015). Extension of the formed molecule with another sugar molecule takes place by addition to the first one, such as naringin (**78**, naringenin-7-hesperidoside), a compound that contains two sugar molecules, rhamnose and glucose. But, there is a possibility that two sugars can be attached to the core of the flavonoid structure, like in vicenin-2 (**48**).

As shown in Fig. 6.9, flavanones, particularly naringenin, can give rise to many different forms of their basic skeleton (C_6-C_3-C_6), leading to the formation of diverse groups of secondary metabolites in plants, e.g., flavones, flavonols, flavan-3,4-diols, catechins, anthocyanidins, proanthocyanidins, and anthocyanins.

One of the most studied biosynthetic pathways of phenylethanoid glycosides, another very important class of phenolic secondary metabolites identified in *Teucrium* spp., is a synthesis of acteoside (**21**, verbascoside) in plants, but it still remains not elucidated in detail. Tentative mechanism of biosynthesis, proposed by Alipieva et al. (2014) and Saimaru and Orihara (2010), is shown in Fig. 6.10. The acteoside molecule is comprised of phenylethanoid (C_6-C_2) and caffeic acid (C_6-C_3) moieties linked *via* α-rhamnopyranosyl-β-glucopyranose to form the disaccharide caffeoyl ester. It has been suggested that the biosynthesis of acteoside begins with the generation of aromatic acids, phenylalanine and tyrosine, in the pathway of shikimic acid. Thereafter, from L-Tyr can be synthesized the hydroxytyrosol moiety in acetoside, and caffeic acid moiety can be derived from L-Phe in phenylpropanoid pathway. Monohydroxyl compounds synthesized in this process, such as tyramine, tyrosol, and salidroside, can be effectively incorporated into acteoside, as well as dopamine and DOPA (Alipieva et al. 2014).

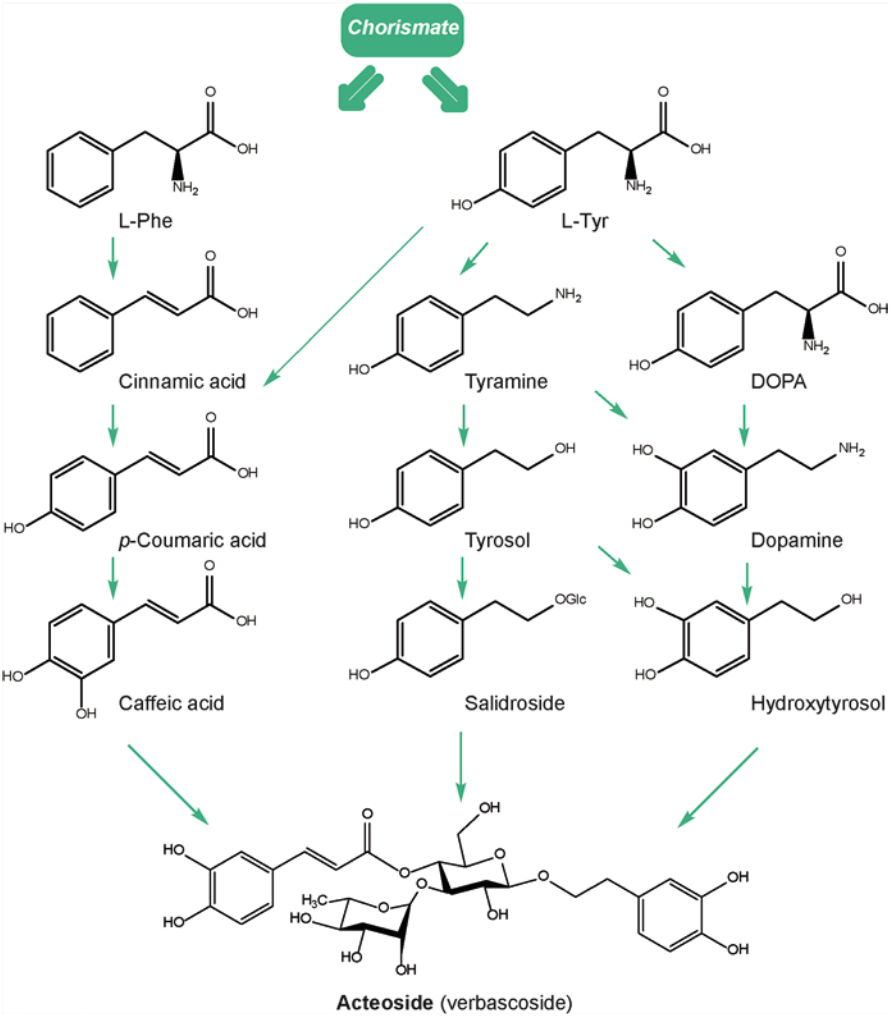


Fig. 6.10 Biosynthetic pathway of acteoside (tentative, adopted and modified from Alipieva et al. (2014) and Saimaru and Orihara (2010))

6.4 Extraction of Phenolic Compounds from *Teucrium* Species

There are a number of extraction methods that can be applied for the extraction of phenolic compounds from plant matrices, and generally can be divided into traditional and modern techniques. Traditional methods include maceration, maceration with mixing, and Soxhlet extraction. These methods are time-consuming, often giving low extraction yields, and also not suitable from the environmental protection point of view due to the large volumes of used organic solvents. Nowadays, modern techniques for the extraction of bioactive compounds, such as ultrasonic extraction, microwave extraction, sub- and supercritical extraction, solid-phase microextraction, and accelerated solvent extraction, are increasingly used (Khoddami et al. 2013). Each of these techniques has its advantages and disadvantages, and the main goal of the chosen method is to achieve the maximum extraction yield of compounds of interest and to avoid their chemical modification (Ćujić et al. 2016).

A number of studies have shown that chosen extraction method can alter the total phenolic content (TPC) and antioxidant activity (Chan et al. 2007; Sikora et al. 2008; Yeh et al. 2014). The choice of the solvent for extraction is one of the main factors that have an influence on the phenolic compounds content. Phenolic compounds present in plant matrices have different characteristics and polarities, so they may not be soluble in a particular solvent (Turkmen et al. 2006). Many studies have shown that aqueous mixtures containing methanol, ethanol, acetone, and ethyl acetate are the most suitable solvents for the extraction of phenolic compounds in plant material. Ethanol is an appropriate solvent for polyphenol extraction. Methanol has been found to be more efficient for extraction of lower molecular weight phenolic compounds, while an application of aqueous acetone is appropriate for higher molecular weight flavonols extraction (Dai and Mumper 2010).

A wide range of extraction methods were applied for the extraction of phenolic compounds from different species of *Teucrium* genus, including traditional methods, such as maceration or Soxhlet's extraction, or modern techniques, such as ultrasonic-assisted and subcritical extraction. Farahmandfar et al. (2019) applied maceration and ultrasonic-assisted extraction with various solvents in their study, in order to analyze TPC in the aerial part of *T. polium*. The highest extraction yield was obtained by ultrasonic-assisted extraction using ethanol:water (50:50) solvent mixture. This is in agreement with the determined TPC, which was the highest (65.74 mg GAE/g extract) by applying the above-mentioned extraction technique and solvent. Considering the classic, maceration methods, this solvent mixture (ethanol:water = 50:50) also proved to be the most appropriate for TPC extraction from *T. polium* aerial part (60.50 mg GAE/g extract). No difference was found between ethanol:water (50:50) maceration and ethanol ultrasonic-assisted extraction, while ethanol maceration had intermediate content of TPC (55.29 mg GAE/g extract). Water maceration and water ultrasonic extraction showed the lowest values for TPC (47.42 and 49.60 mg GAE/g extract, respectively), which may be attributed to the presence of more nonphenol compounds (polar) in water extracts than in other extracts. Ben Sghaier et al. (2011) compared extraction yields and total phenolics, flavonoids, and tannins content in *T. ramosissimum* water (boiling), methanol and chloroform (Soxhlet's extraction) plant extracts. The aqueous extract showed the highest extraction yield (21.6%), followed by methanol extract (16.78%) and chloroform extract (6.53%). The results also showed that aqueous extract was more enriched in total phenolic compounds than the methanol and chloroform extract with TPC of 121.66 μg GAE/mg extract. However, the results of total flavonoid and tannin contents showed to be reversed considering aqueous and methanol *T. ramosissimum* extracts; methanol extract was significantly more enriched in flavonoid and tannin compounds. The content of flavonoids and tannins in *T. ramosissimum* chloroform extract was negligible compared to methanol and aqueous extracts. Stanković et al. (2010) examined the influence of water and different organic solvents (methanol, acetone, ethyl acetate, and petroleum ether) on extraction yields and TPC in the whole plant and plant parts of *T. chamaerdys* using Soxhlet extraction. The results showed that the highest extraction yield was obtained using polar solvents, such as water and methanol. TPC in the examined extracts ranged from 15.98 to 208.17 mg GAE/g extract, and the highest TPC was found in leaf methanol extract, followed by methanol extract of the whole plant (169.50 mg GAE/g extract), while all ethyl acetate and petroleum ether extracts contained considerably lower amounts of phenolic compounds. Phenolic content of *T. chamaerdys* was determined by applying different extraction techniques and solvents in a number of studies. Considering the obtained results, it can be concluded that the highest TPC (243.65 mg GAE/g plant material) was determined in *T. chamaerdys* ethanol extract (70% EtOH at 60 °C) (Vlase et al. 2014). Also, a high amount of total phenols (176.74 mg GAE/g dry extract) in *T. chamaerdys* was obtained by applying environmentally-friendly extraction technique, such as subcritical water extraction on the temperature of 160 °C for 30 min. The application of such methods eliminates the use of organic solvents, contributing to safety, quality, and applicability of plant extracts (Nastić et al. 2018a). Nastić et al. (2018b) also applied a subcritical water technique for the phenolic compounds extraction of *T. montanum* species and examined the influence of extraction pressure and temperature on TPC. Extracts obtained at a temperature of 160 °C and 10 bar showed the highest TPC. Under optimal extraction conditions, the extraction yield of *T. montanum* was 42.63%, while TPC was 174.61 mg GAE/g dry extract.

According to literature data, it can be concluded that modern extraction techniques, such as ultrasonic-assisted extraction, using aqueous organic solvents (methanol and ethanol) and subcritical water extraction are the most effective methods for the extraction of phenolic compounds in plant material for the most of *Teucrium* species.

6.5 Conclusions

Combining the known chemical data about the phenolic composition of genus *Teucrium*, 78 different phenolic compounds have been reported from 48 species. A total of 44 different flavonoids, including flavonoid aglycons, and their *O*-glycoside and *C*-glycoside forms, have been identified in plants from this genus so far, representing the largest class of phenolic compounds of *Teucrium* species. Flavones represent the most abundant subclass of flavonoids in this genus with a total of 34 compounds reported so far. Luteolin is the most common flavone identified in 42 *Teucrium* species. Among flavone derivatives, luteolin and apigenin glycosides are also frequently found in these species, suggesting that these compounds may be chemotaxonomic markers for genus *Teucrium*.

Teucrium genus is also characterized by the presence of phenylethanoids and derivatives of benzoic and hydroxycinnamic acids. We have recorded a total of 16 different phenylethanoids and 15 phenolic acids (7 of them belonging to the group of hydroxybenzoic acids and 8 to the hydroxycinnamic acids group). The hydroxybenzoic acids have been rarely investigated in *Teucrium* species and their presence was confirmed only in 6 species, while hydroxycinnamic acids and phenylethanoids have been reported in 13 species. The most common phenylethanoid is verbascoside, found in 12 investigated plants from *Teucrium* genus. According to the quantitative analysis of phenolic compounds in *Teucrium* plants, phenylethanoids are present in higher concentration compared with flavonoids and phenolic acids.

The genus *Teucrium* includes more than 300 species, but an analysis of phenolic compounds was reported only for 48 of them so far. Taking into account the limited number of investigated *Teucrium* species, the diversity of phenolic compounds in these species is probably significantly higher. Considering the number of unexplored *Teucrium* species and biological importance of phenolic compounds identified in plants from this genus, many possibilities for the further investigation of the chemical composition and application of neglected plants of the *Teucrium* genus are opened.

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Chapter 7
Essential Oils Diversity of *Teucrium* Species

Laura De Martino, Raffaele Coppola, Vincenzo De Feo, Lucia Caputo, Florinda Fratianni, and Filomena Nazzaro

Abstract The Mediterranean flora is characterized by an abundance of aromatic plants. These plants make a significant contribution to the Mediterranean ecological communities both in terms of species numbers and biomass. In this area, *Teucrium* is a polymorphic and cosmopolitan genus of perennial plants, the largest of the Lamiaceae family, which comprises more than 300 species, of which almost 50 are native to Europe. The members of this genus are commonly known as germanders: they are bitter, astringent and antirheumatic herbs, utilized as antispasmodic agents for gastric ulcer and intestinal inflammation, as diuretic, antiseptic, antipyretic and antihelmintic agents. Moreover, they were also used in food preparation. *Teucrium* species are rich in essential oils and the presence of various biologically active monoterpenoid and sesquiterpenoid with several biological activities has been reported. This review summarizes the chemical features of the essential oils of *Teucrium* species reported in literature: it provides an overview on their compositions with particular reference to the more abundant components and to the reported biological activities. *Teucrium polium*, *T. chamaedrys*, *T. flavum* and *T. capitatum* are the most studied taxa concerning the volatile oil composition. Moreover, it is possible to distinguish different types of essential oils, characterized by the prevalence of a specific class of compounds: in particular, we recognized volatile oils rich in caryophyllene and caryophyllene derivatives, α- and β-pinene, germacrene B and D, α-, γ-, δ-cadinene and/or cadinol, limonene, and bisabolol/bisabolene. This richness in chemodiversity confers to the essential oils from *Teucrium* species distinctive biological properties and applications, often confirming the traditional and folk uses of this genus. This review testifies the high great variability of *Teucrium* essential oils and shows that the possible factors responsible for the chemical polymor-

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phism of essential oils and consequently for their bioactivity need further investigation.

Keywords *Teucrium* · Essential oil · Caryophyllene · α-pinene · β-pinene · Germacrene D · Limonene · Bisabolol · Biological activity

Abbreviations

AChE	Acetylcholinesterase
AD	Alzheimer's disease
BChE	Butyrylcholinesterase
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EOs	Essential oils
ICAM	Intercellular adhesion molecule
MIC	Minimal inhibitory concentration
MRSA	Methicillin-resistant *S. aureus*
NO	Nitric oxide
TNF-α	Tumor necrosis factor-α
VCAM	Vascular cell adhesion molecule

7.1 Introduction

The use of herbal alternative medicinal therapeutics has increased the interest of herbalists and pharmacologists over the past decade. Historically, plants have provided excellent sources of inspiration for new effective medications and, as herbal medicines, they contribute since ancient time to the human health and well-being. Although natural plants medications for over 50 years became less common in evolved countries, however, they are still used in the developing countries and poor section populations. Essential oils (EOs) and extracts from plants, herbs, and spices have long been used for culinary and medicinal purposes throughout the world (Bakari et al. 2015). In particular, essential oils are extracted from aromatic plants, generally found in temperate to warm locations such as the Mediterranean and tropical countries, where they represent an important part of the traditional pharmacopeias. EOs are secondary metabolites, deriving from a specific biosynthetic pathway (Fig. 7.1), characterized by a strong odor. They are composed principally by terpenoids, especially monoterpenes (C_{10}), and sesquiterpenes (C_{15}), although diterpenes (C_{20}) may also be present along with a variety of other molecules (De Martino et al. 2015). EOs are liquid, volatile, limpid, colored, and soluble in lipids and in organic solvents with a density lower than water. They can be present in all plant organs and generally are stored in secretory cells, cavities, canals, epidermal cells, or glandular

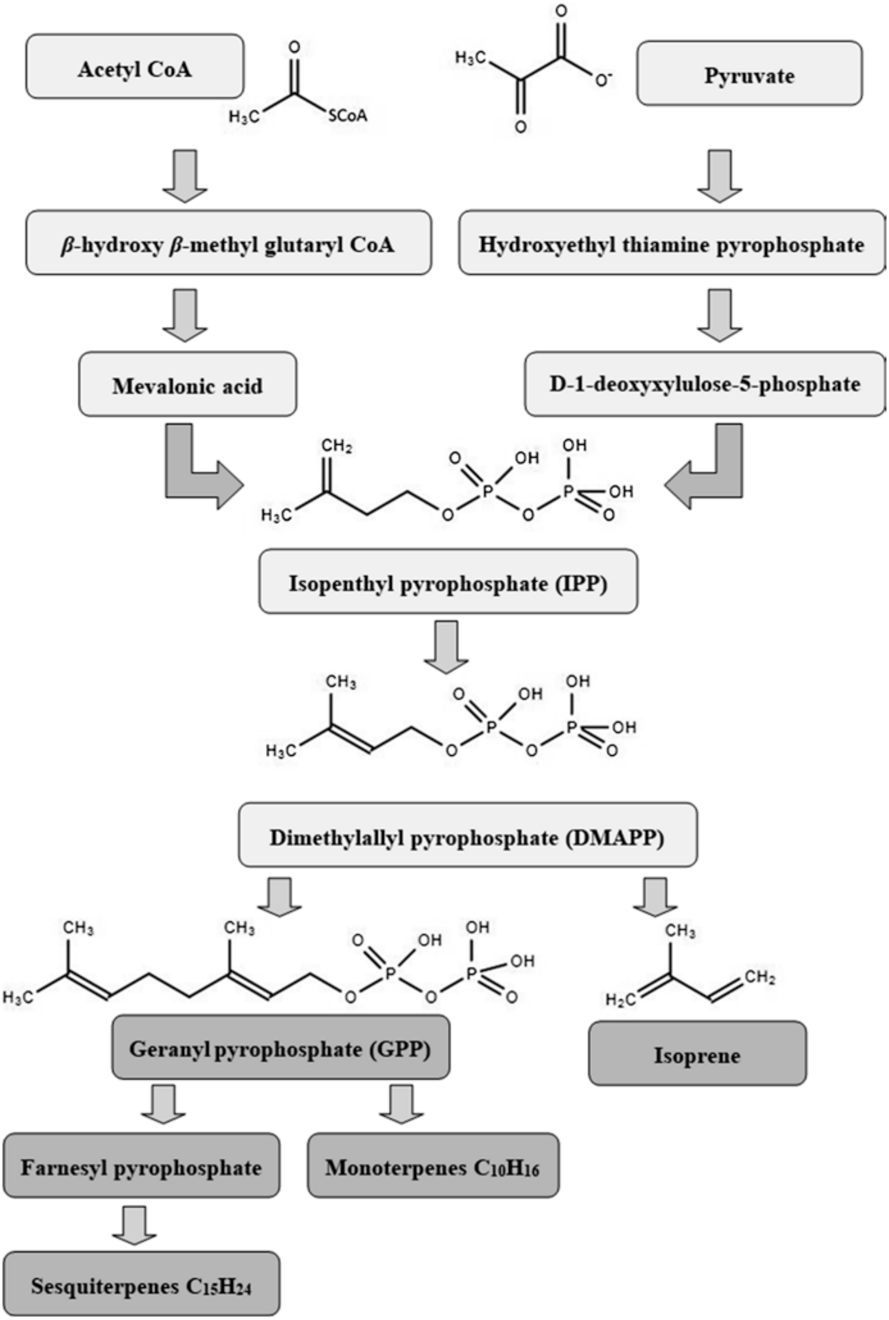

Fig. 7.1 Biosynthetic pathway of essential oils

trichomes. Known for their bactericidal, virucidal, fungicidal, and medicinal properties and for their fragrance, EOs are used also in the preservation of foods, in perfumery and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic, and locally anaesthetic remedies (Bakkali et al. 2008).

The Mediterranean flora is characterized by an abundance of aromatic plants. Such plants make a significant contribution to the Mediterranean ecological communities both in terms of species numbers and biomass (Vokou 1992): apart from their medical properties, plants of the genera *Thymus* (Adzet et al. 1977), *Salvia* (Ulubelen et al. 2002), *Teucrium* (Stankovic et al. 2010) and others, represent a source of substances that might benefit the food industry or agriculture or be used for the human health and well-being (De Martino et al. 2015). The multiple roles of the essential oils and their main components make them natural substances of great importance in several fields such as physiological function of growth, ecological function, development (Wink 2003), resistance against diseases and insects (Gershenzon and Dudareva 2007). They also possess antimicrobial, antiviral, antimycotic, antioxigenic, antiparasitic and insecticidal properties (Bishop 1995; Lamiri et al. 2001; Juglal et al. 2002; Moon et al. 2006; Michaelakis et al. 2007).

This chapter is focused on the essential oils extracted from different species of *Teucrium* genus; specifically, this review has the aims of: (1) describing the chemical composition and the high variability of *Teucrium* essential oils; (2) highlighting the existence of chemical variability these *Teucrium* essential oils oils, differentiated according to the dominant components; (3) making a compendium of the biological activities of the essential oils; (4) testifying the great variability of *Teucrium* essential oils, as influenced by several endogenous and exogenous factors. The chapter would a comprehensive resource on these aspects of the plant.

7.2 The Genus *Teucrium*

Teucrium is a polymorphic and cosmopolitan genus of perennial plants, the largest of the Lamiaceae family in the Mediterranean area, which comprises more than 300 species (Djabou et al. 2012), of which almost 50 are known in Europe (Menichini et al. 2009). In fact, it is a genus of temperate regions, with relatively few representatives in the tropics and the greatest concentration of species in the Mediterranean basin and Central Europe. Some of these species are annual herbs, others are shrubs or subshrubs (Djabou et al. 2011).

The name *Teucrium* derives from Teucer, a son of Talomon, king of Salamis, who was the first to use these plants for curative purposes. In fact, *Teucrium* species have been known for a long time for their medicinal properties, and they are commonly used as diuretic, antiseptic, antipyretic and antispasmodic agents for the treatment of digestive and pulmonary disorders (Lo Presti et al. 2010; Djabou et al. 2011). Members of this genus are commonly known as germanders. Most of these species are bitter, astringent and antirheumatic herbs. Moreover, *Teucrium* species were also alimentary plants and are currently used in the preparation of flavoured wines,

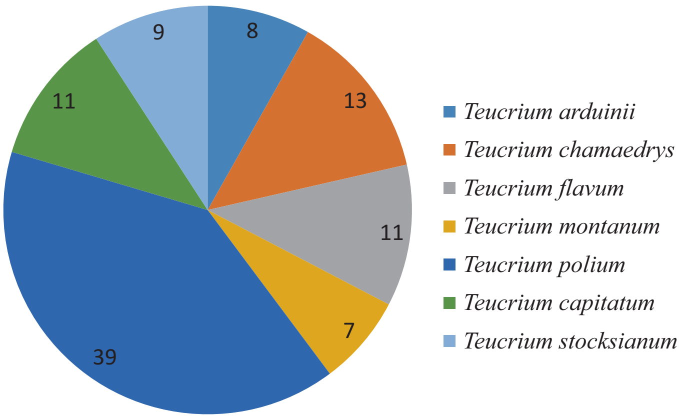

Fig. 7.2 The most studied *Teucrium* species

herbal teas, bitters and liqueurs, as well as aerial part infusions are used for flavoring beer (Maccioni et al. 2007). Some species (notably *Teucrium* fruticans L.) are valued as ornamental plants (Djabou et al. 2011).

From a taxonomical point of view, the genus *Teucrium* has been divided into nine sections identifiable through the calyx shape and the inflorescence structure (Navarro and El Oualidi 2000).

An unusual feature of *Teucrium* genus, if compared with other members of Lamiaceae, is that its flowers completely lack the upper lip of the corolla. Moreover, as in other Lamiaceae, the aerial organs of *Teucrium* species are covered by an indumentum of glandular and non-glandular trichomes (De Martino et al. 2010). *Teucrium* species are rich in essential oils and various active monoterpenoid/sesquiterpenoid have been reported in them, possessing several biological activities. This genus has drawn attention of phytochemists and pharmacognosists and the scientific literature reports a consirable number of papers: in Fig. 7.2, *Teucrium* taxa with more bibliographic reference concerning essential oil composition, are reported.

Above all, *Teucrium polium* is the species with the highest number of references: its common names are polium, pouliot de montagne, germandée tomenteuse (French); germander, mountain germander, felty germander, cat thyme, hulwort (English); camendrio di montagna, polio, polio primo, timo bianco (Italian); poleigamander, berggamander (German) (Jaradat 2015). This species grows wildly in sunny regions of semiarid and arid in bioclimates, and is capable to grow also on hillsides, sands and in the stony mountains. It is a 10–35 cm high shrub, with a white or yellow corolla contained in a globular small inflorescence. The leaves are white, tomentose on both sides; the fruits are brown and all the plant aerial parts have aromatic pleasant odor and bitter taste (Barceloux 2008). In the traditional herbal medicine, decoctions of *Teucrium polium* have been used for treatment of inflammations, gastrointestinal tract disorders, rheumatism, and diabetes. A tea from the plant is used in traditional Iranian medicine for treating many diseases such as indigestion, abdominal pain, Type 2 diabetes and common cold (Bahramikia and Yazdanparast

2012); the same formulation is used in the Palestinian folk medicine for the treatment of gastrointestinal inflammations, as well as an antispasmodic and an anthelmintic and for the treatment of claustrophobia (Jaradat 2005).

Teucrium chamaedrys L. is widely distributed in Azerbaijan, Golestan, Khorasan, Lurestan, Mazandaran and Yazd provinces of Iran (Zargari 1993): the plant has been used medicinally since ancient Greek times, with similar applications to other *Teucrium* species; in Iranian herbal medicine, it is used as a diuretic, antiseptic, antipyretic and antihelmintic agent (Djabou et al. 2011). *Teucrium flavum* L. is a 60 cm evergreen perennial shrub, with triangular-ovate leaves and yellow flowers, which appear from May to August (Jeanmonod and Gamisans 2007; Lo Presti et al. 2010). The plant can be found in the cracks of lime rocks up to 1000 m (Lo Presti et al. 2010). Previously classified in *Chamaedrys* (Miller) Schreber section, successively, on the basis of investigation of flowers and inflorescences, *Teucrium flavum* was included in section *Polium* Schreber (Lakusic et al. 2006). *Teucrium capitatum* L. [syn. *T. polium* L. ssp. *capitatum* (L.) Arcang.] is widespread diffused in the dry and stony places of Greece and almost in all Mediterranean countries: it is a perennial, prostrate species with white to bright pink flowers, crenate leaves and ramified indumentum (De Martino et al. 2010). This plant is used in folk medicine to treat diabetes and intestinal troubles (Boulila et al. 2008). The Iranian *Teucrium stocksianum* Boiss. is a polymorphous perennial plant distributed just in southern regions of Iran, 10–35 cm high and with a very pleasant aromatic odor (Bakhtiari and Asgarpanah 2015). The purple globular inflorescences appears from April to May; the fruits are dark brown with a latticed surface. This perennial aromatic herb is extensively exploited as a medicinal plant and is used in folk medicine to treat diarrhea, cough, jaundice and abdominal pain (Rahim et al. 2012). *Teucrium arduini* L. (Arduini's germander) is an endemic Illyric Balcan species, distributed in the mountains along the Adriatic coast of Croatia, Bosnia and Herzegovina, Montenegro, Serbia and Northern Albania. It is a 10–40 cm dwarf shrub, semi-woody, branchy, erect or ascending; it grows on calcareous rocky slopes up to 1600 m a.s.l. Whitish flowers form simple, very dense, up to 16 cm long inflorescences (De Martino et al. 2010). *Teucrium montanum* L. is a grass crop which has long been used both as an herbal medicine and a nourishing food. It is a widely used as a diuretic, stomachic, analgesic and antispasmodic agent. Moreover, it possess antibacterial, antifungal, anti-inflammatory and antioxidative activities (Vukovic et al. 2008).

7.3 Essential Oils of *Teucrium* Species

In literature, the essential oils of 93 different species and or subspecies are reported in more than 200 papers. It is possible to distinguish different types of essential oils or essential oils characterized by a prevalence of a specific class of compounds. The majority of *Teucrium* essential oils can be divided on the basis of predominant components: caryophyllene and caryophyllene derivatives (Table 7.1), α- and β-pinene

Table 7.1 *Teucrium* taxa with predominance of caryophyllenes

Teucrium species	Percentage (%)	References
T. alyssifolium	16.87	Semiz et al. (2016)
T. apollinis	22.4	Elabbara et al. (2014)
T. arduinii	32.9	Kremer et al. (2012)
	35.2	Kremer et al. (2013)
	13.7–50.7	Kremer et al. (2015)
	34.5	Dunkic et al. (2011)
	17.7	De Martino et al. (2010)
	24.5	Kovacevic et al. (2001)
T. botrys	20.4	Kovacevic et al. (2001)
T. capitatum	15.1	De Martino et al. (2010)
T. cavernarum	47.0	Kaya et al. (2013)
T. chamaedrys	33.9	Djabou et al. (2013a)
	7.1–52.0	Bezic et al. (2011)
	27.0–29.4	Muselli et al. (2009)
	26.9	Kovacevic et al. (2001)
T. chamaedrys ssp. *syspirense*	28.0	Kaya et al. (2009)
	23.0	Sajjadi and Shookohinia (2010)
T. chamaedrys ssp. *trapezunticum*	25.0	Kaya et al. (2009)
T. creticum	24.6	Valentini et al. (1997)
T. divaricatum	36.2	Formisano et al. (2010)
T. flavum	32.5	Hammami et al. (2015b)
	22.0	Formisano et al. (2012)
	23.1	Bezic et al. (2011)
	20.1	Menichini et al. (2009)
	30.7	Baher and Mirza (2003)
T. flavum ssp. *hellenicum*	22.2	Bellomaria et al. (1998)
T. fruticans (fruiting tops)	22.0	Flamini et al. (2001)
T. marum	1.3–39.4	Djabou et al. (2013b)
	15.1	Djabou et al. (2013a)
	27.4	Ricci et al. (2005)
T. montbretii ssp. *heliotropiifolium*	17.0	De Martino et al. (2010)
T. multicaule	16.5	Polat et al. (2010)
T. orientale var. *orientale*	42.8	Javidnia and Miri (2003)
	29.3	Kucukbay et al. (2011)
T. orientale var. *lydium*	19.7	Kucuk et al. (2006)
T. orientale var. *puberulens*	38.0	Kucukbay et al. (2011)
	21.7	Kucuk et al. (2006)
T. parviflorum	27.4	Bagci et al. (2011)
T. pestalozzae	27.6	Baser et al. (1997)
T. pilosum	21.52	Chen et al. (2010)

(continued)

Table 7.1 (continued)

Teucrium species	Percentage (%)	References
T. polium	29.0	Raei et al. (2014)
	7.1–52.0	Bezic et al. (2011)
	100.0	Al-Qudah et al. (2011)
T. quadrifarium	25.0	Tandon and Mittal (2018)
	38.3	Mohan et al. (2010)
T. scordium	31.4	Morteza-Semnani et al. (2007)
T. scorodonia ssp. *scorodonia*	20.5	Djabou et al. (2012)
	26.2–41.4	Makowczynska et al. (2016)
T. scorodonia ssp. *baeticum*	39.7	Djabou et al. (2012)
T. stocksianum	37.0	Shah et al. (2015)
T. turredanum	15.6–32.6	Blazquez et al. (2003)
T. yemense	23.4–31.3	Awadh Ali et al. (2017)

(Table 7.2), germacrene B and D (Table 7.3), α-, γ-, δ-cadinene and or cadinol (Table 7.4), limonene (Table 7.5), bisabolol/bisabolene (Table 7.6) (Fig. 7.3).

Thirty *Teucrium* taxa have been reported for an essential oil rich in caryophyllene and caryophyllene derivatives (Table 7.1). Thirteen of these taxa are reported only for this characteristic chemical characteristic: *Teucrium alyssifolium* Stapf, *T. apollinis* Maire & Weiller, *T. botrys* L., *T. cavernarum* P. H. Davis, *T. chamaedrys* ssp. *syspirense* (C. Koch) Rech. F., *T. chamaedrys* ssp. *trapezunticum* Rech. F., *T. creticum* L., *T. marum* L., *T. multicaule* Montbret & Aucher ex Benth., *T. pestalozzae* Boiss., *T. scorodonia* ssp. *baeticum* (Boiss. & Reut.) Tutin and *T. turredanum* Losa & Rivas Goday. Moreover, *Teucrium flavum* L. ssp. *hellenicum* Reich. F. essential oil showed almost identical amounts of caryophyllene (22.2%) and germacrene D (22.3%).

Seventeen taxa possess essential oils with a predominance of α- and β-pinene (Table 7.2). For nine of these species [*Teucrium algarbiense* (Cout.) Cout., *T. gnaphalodes* L'Hér., *T. libanitis* Schreb., *T. lusitanicum* Schreb., *T. melissoides* Boiss. et Hausskn. ex Boiss., *T. polium* L. ssp. *gabesianum, T. polium* L. ssp. *valentinum* and *T. stocksianum*], the literature reports only this chemical feature. Seventeen taxa possess essential oils with a predominance of germacrenes (Table 7.3). In literature, for the species *Teucrium antiatlanticum* (Maire) Sauvage & Vindt, *T. bidentatum* Hemsl., *T. labiosum* C. Y. Wu & S. Chow, *T. paederotoides* Boiss. and *T. zanonii* Pamp., only essential oils with germacrene predominance have been reported. Sixteen *Teucrium* taxa have been reported for essential oils with a predominance of cadinenes (Table 7.4). For *Teucrium carolipaui* Vicioso ex Pau, *T. maghrebinum* Greuter & Burdet, *T. persicum* Boiss. and *T. polium* ssp. *aurasiacum* (Maire) Greuter & Burdet only essential oils with cadinenes predominance have been reported. Five taxa (Table 7.5) have been reported for their limonene-rich essential oil. Among these taxa, *Teucrium flavum* L. ssp. *glaucum* (Jord. & Fourr.) Runninger, *T. polium* L. ssp. *geyrii* Maire and *T. polium* L. ssp. *aurasiacum* are known only for this chemical characteristic. For five taxa (Table 7.6) an essential oil rich in bisabolol/

Table 7.2 *Teucrium* taxa with predominance of pinenes

Teucrium species	Percentage (%)	References
T. algarbiense	18.5	Cavaleiro et al. (2004)
T. capitatum	18.0	Antunes et al. (2004)
	24.1	Djabou et al. (2013a)
	36.0	Cozzani et al. (2005)
	36.1	Mitic et al. (2012)
T. flavum	29.0	Stanic et al. (1993)
	36.2–39.6	Djabou et al. (2011)
	40.0	Djabou et al. (2013a)
T. fruticans (flowering phase)	21.0	Flamini et al. (2001)
T. gnaphalodes	27.5	Perez-Alonso et al. (1993)
T. haenseleri	42.0–54.0	Gaspar et al. (1997)
T. libanitis	9.9–21.2	Blazquez et al. (2003)
T. lusitanicum	3.3–20.4	Cavaleiro et al. (2004)
T. melissoides	44.1	Ahmadi et al. (2002)
T. polium	29.7	Bakari et al. (2015)
	23.3	Keykavousi et al. (2016)
	29.4–36.1	Lograda et al. (2014)
	19.61	Moghtader (2009)
	8.9	Nikpour et al. (2018)
	25.8	Purnavab et al. (2015)
	28.3	Sabzeghabaie and Asgarpanah (2016)
	42.8	Rowshan and Najafian (2012)
	14.43	Stanciu et al. (2006)
	19.8	Kovacevic et al. (2001)
	30.0	Cakir et al. (1998)
	11.94	Sarer and Konuklugil (1987)
T. polium ssp. *gabesianum*	49.29	Ben Othman et al. (2017)
T. polium ssp. *valentinum*	27.5	Perez-Alonso et al. (1993)
T. sandrasicum	55.67	Ali et al. (2013)
T. scordium	27.7	Kovacevic et al. (2001)
T. stocksianum	50.76	Mojab et al. (2003)
T. stocksianum ssp. *gabrielae*	36.0	Sonboli et al. (2013)
T. zanonii	14.13	Abdelshafeek et al. (2010)

bisabolene is reported. *Teucrium gracile* Barbey & Fors.-Major is known only for this characteristic. Oct-1-en-3-ol is reported as the main constituent of the essential oils of *Teucrium abutiloides* L'Hér. and *T. betonicum* L'Hér. in which the amonuts are 20% and 24%, respectively (Barroso et al. 1993). The EO of *Teucrium lepicephalum* Pau contains sabinene in percentage between 47.54% and 57.43% (Perez et al. 2000), *Teucrium leucocladum* Boiss. has an essential oil with a predominance of patchouli alcol (31.24%) (El-Shazly and Hussein 2004). The EO of *Teucrium* orientale ssp. *glabrescens* (Hausskn. ex Bornm.) Rech.f. shows a predominance of β-(26.9%) and α-cubebene (9.0%) (Oezek et al. 2012).

Table 7.3 *Teucrium* taxa with predominance of germacrenes

Teucrium species	Percentage (%)	References
T. antiatlanticum	13.2	Kartah et al. (2015)
T. antitauricum	28.2	Baser et al. (1999)
T. arduinii	17.0	Vukovic et al. (2011)
	57.8	Blazevic et al. (1992)
T. bidentatum	32.55	Chen et al. (2010)
T. chamaedrys	32.1	Bagci et al. (2010)
	16.7	Kucuk et al. (2006)
	16.5	Morteza-Semnani et al. (2005)
T. flavum ssp. *hellenicum*	22.3	Bellomaria et al. (1998)
T. fruticans (fruiting tops)	24.0	Flamini et al. (2001)
T. labiosum	34.08	Chen et al. (2010)
T. montanum	31.0	Radulovic et al. (2012)
	17.2	Bezic et al. (2011)
	15.0	Kovacevic et al. (2001)
T. orientale var. *orientale*	24.6	Oezek et al. (2012)
T. orientale var. *puberulens*	33.4	Oezek et al. (2012)
T. paederotoides	20.8	Kaya et al. (2013)
T. polium	12.5–13.8	Bendif et al. (2018)
	8.7–19.5	Lianopoulou et al. (2014)
	18.26	Mahmoudi and Nosratpour (2013)
	25.81	Belmekki et al. (2013)
	31.0	Radulovic et al. (2012)
T. polium ssp. *capitatum*	31.8	Mitic et al. (2012)
T. quadrifarium	8.8	Liu et al. (2016)
T. royleanum	28.9	Mohan et al. (2010)
T. sandrasicum	27.9	Baser et al. (1997)

Teucrium pseudochamaepitys L. has been reported for its essential oil with a predominance of hexadecanoic acid (26.1%) (Hammami et al. 2015a); the EO of *Teucrium scorodonia* ssp. *baeticum* presents N-formylmorpholine (25.07%) and morpholine, 4-acetyl- (17.61%) as the main components (Tahoum et al. 2017); *Teucrium salviastrum* Schreb. essential oil has in prevalence *β*-farnesene (26.1–29.3%) followed by E-caryophyllene (19.1–24.1%) (Cavaleiro et al. 2002). For *Teucrium scordium* L. ssp. *scordioides* (Schreb.) Arcang. essential oil, menthofuran (11.9%) is reported as the main component (Radulovic et al. 2012). *Teucrium massiliense* L. showed 6-methyl-3-heptyl acetate as the main compound with percentage between 19.1% (Djabou et al. 2013a) and 23.83% (Djabou et al. 2010). However, the same plant gave an essential oil rich in 3,7-dimethyloctan-2-one (15.2%) and bu-2-methylbutyrate (12.1%). Some papers reports no quantitative data on essential oil compositions of *Teucrium africanum* Thunb. (Ruiters et al. 2016), *T. brevifolium* Schreb. (Menichini et al. 2009), *T. pruinosum* Boiss. (Jaradat et al. 2018), *T. sauvagei* Le Houér. (Salah et al. 2006) and *T. trifidum* Retz. (Ruiters

Table 7.4 *Teucrium* taxa with predominance of cadinenes

Teucrium species	Percentage (%)	References
T. alopecurus	13.4	Hachicha et al. (2007a)
T. atratum	45.8	Kabouche et al. (2006)
T. capitatum	31.6	Antunes et al. (2004)
	46.2	Khani and Heydarian (2014)
	18.3	Kerbouche et al. (2015)
T. carolipaui	21.5	Pala-Paul et al. (2001)
	13.12–29.32	Perez et al. (2000)
T. divaricatum	27. 5–33.9	Tzakou et al. (1997)
T. heterophyllum	26.0–32.0	Barroso et al. (1996)
T. hircanicum	13.4	Rahimi et al. (2018)
T. maghrebinum	22.2	Formisano et al. (2009)
	21.0	De Martino et al. (2010)
T. montanum	17.2	Vukovic et al. (2007)
	17.2	Vukovic et al. (2008)
T. persicum	12.9	Javidnia et al. (2007)
	23.2	Masoudi et al. (2009)
	18.9	Miri et al. (2012)
T. polium	13.6	Vokou and Bessiere (1985)
	13.82	Guetat and Al-Ghamdi (2014)
T. polium ssp. *aurasiacum*	46.8	Kabouche et al. (2007)
T. ramosissimum	23.7–42.5	Ghazouani et al. (2016)
	37.33	Ghazouani et al. (2017)
	29.9	Hachicha et al. (2007b)
T. stocksianum	24.4–28.4	Al-Yousuf et al. (2002)
	12.7	Hisham et al. (2006a)
	12.9	Shah et al. (2012)
	37.6	Bakhtiari and Asgarpanah (2015)
T. yemense	34.9	Awadh Ali et al. (2008)

Table 7.5 *Teucrium* taxa with predominance of limonene

Teucrium species	Percentage (%)	References
T. flavum ssp. *glaucum*	21.1–31.8	Djabou et al. (2011)
	27.4	Djabou et al. (2013b)
T. lusitanicum ssp. *aureiformis*	15.9	Velasco-Negueruela and Perez-Alonso (1990)
T. mascatense	17.2	Oraimi et al. (2012)
T. polium	37.70	Vahdani et al. (2011)
T. polium ssp. *geyrii*	11.18	Roukia et al. (2013)
T. polium ssp. *aurasiacum*	34.72	Bencheikh et al. (2015)

Table 7.6 *Teucrium* taxa with predominance of bisabolol/bisabolene

Teucrium species	Percentage (%)	References
T. alopecurus	16.16	Guesmi et al. (2017)
T. flavum	35.0	Kovacevic et al. (2001)
	31.5–48.2	Lo Presti et al. (2010)
T. gracile	1.9–30.7	Arnold et al. (1998)
T. heterophyllum	20.8	Velasco-Negueruela et al. (1999)
T. polium	32.3	Sadeghi et al. (2014)

et al. 2016). For other taxa, only the classes of main components are reported: *Teucrium polium* L. var. *album* and *T. polium* var. *pilosum* (L.) Arcang. (Kamel and Sandra 1994) as well as *T. viscidum* Blume (Wei et al. 2010).

Teucrium polium essential oil is mainly represented by a pinenes type: in fact, in 12 different papers α- and/or β-pinene are reported as the most abundant components; in five papers, germacrene D and/or germacrene B are reported as the main components, while caryophyllene and derivatives predominate in other four oils. Probably, the origin of the plants can influence the essential oil composition. Yugoslavian (Kovacevic et al. 2001) and Iranian germander (Eikani et al. 1999) were mainly represented by germacrene D rich essential oil (11.9% and 23.6–13.2% respectively), while the major components of a Turkish EO were β- (18%), α-pinene (12%) and β-caryophyllene (18%) (Cakir et al. 1998). On the other hand, Jordanian germander EOs contain 8-cedren-13-ol (24.8%), β-caryophllene (8.7%), sabinene (5.2%) and germacrene D (6.8%) (Aburjai et al. 2006). The phytochemical screening of the Saudi Arabia *Teucrium polium* volatile oil identified the presence of ten isoprenoid compounds, including alcohols as linalool, cedrenol, cedrol, guaiol and terpine4-ol and the hydrocarbons, β-pinene, γ-, δ-cadinene, limonene and α-phellandrene (Hassan et al. 1979).

Thirteen studies on the EOs of *Teucrium chamaedrys* reported the presence of caryophyllene and germacrene as principal components. Moreover, Zhou et al. (2004) indicated the presence of the diterpenoid teucrin A in the oil. *Teucrium flavum* essential oils have been subject of some studies. The volatile oils appeared qualitatively quite similar and only quantitative differences occurred in the relative amounts of components. These differences were presumably due to environmental conditions and they might be explained by ambiguous taxonomic determination of the taxa studied (Djabou et al. 2011). The essential oils of *Teucrium capitatum* is variously characterized: in fact, a part from the relative aboundance of pinenes, some literature data report the predominance of oxygenated compound, as cadinol (Table 7.4), carvacrol (Menichini et al. 2009) and endo borneol (El Amri et al. 2017). Probably, the reported differences also depend on the part of the plant studied, its stage of growth as well as the nature of the soil and of the environment. In any case, a chemical polymorphism is present in this plant (El Amri et al. 2017). Ten chemotypes are reported for *Teucrium arduini*, based on the essential oil profile and molecular analyses: one with prevalence of caryophyllenes and the others with predominance of germacrene, although such a basis requires further clarification

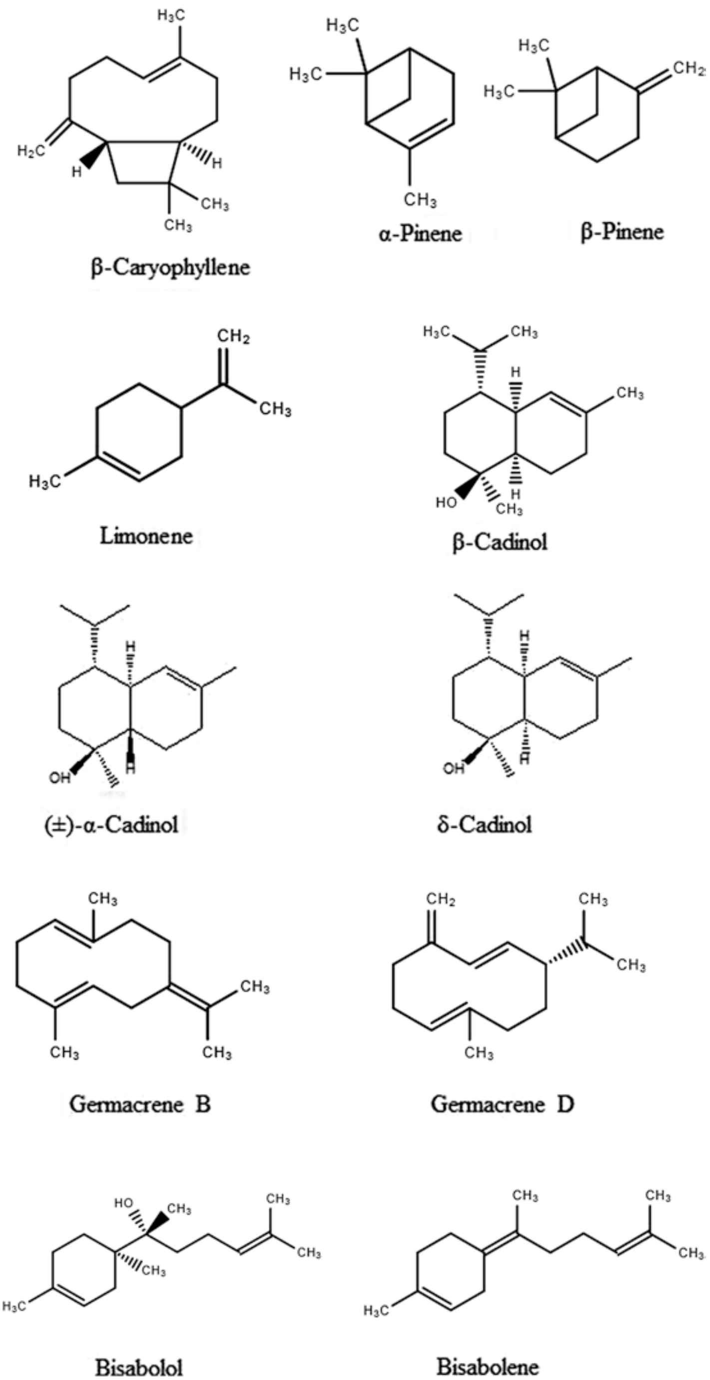

Fig. 7.3 The main components of *Teucrium* essential oils

(Kremer et al. 2015). *Teucrium montanum* EOs showed the presence of sesquiterpene hydrocarbons as characteristic compounds and monoterpenes as minor constituents, with an high prevalence of germacrene and cadinene (Vukovic et al. 2007, 2008; Bezic et al. 2011; Radulovic et al. 2012). The essential oils of *Teucrium stocksianum* are mainly constituted by cadinene and/or cadinol (Al-Yousuf et al. 2002; Hisham et al. 2006a; Shah et al. 2012). Similar results were obtained also by Bakhtiari and Asgarpanah (2015), who found α-cadinol (37.6%), α-pinene (9.7%) and caryophyllene oxide (4.9%) as the main components of the fruits.

On the basis of the available literature, the most part of *Teucrium* essential oils are characterized by the presence of sesquiterpenes. In this feature, caryophyllenes constitute the most common principal class of compounds in combination with germacrenes and, in minor extent, with cadinenes. In some taxa, caryophyllenes are combined with pinenes. Only in few taxa monoterpenes predominate, with pinenes and limonenes as main classes. A generalization about the chemical expression of *Teucrium* essential oils need certainly of more studies: there is no homogeinity in chemical characterization of EOs and some species (for example *Teucrium polium, T. capitatum, T. chamaedrys* and *T. flavum*) have been more extensively studied. These *Teucrium* accessions showed a high degree of variability in essential oil composition: in fact, *Teucrium polium* appear the most variable species, with 6 different types of essential oils. Also *Teucrium flavum* and *T. capitatum* show essential oils with 5 and 4 different types, respectively. The high degree of chemical variability, a part on intrinsic and extrinsic factors linked to plant and environment, appears in part attributable to different methodological approaches used in the studies.

7.4 Biological Activity of the Genus *Teucrium*

Generally, most, if not all, countries of the Mediterranean area have a consolidated experience in the utilization and in the study *Teucrium*. At present, due to the concerns about the harmful consequences and side effects showed by many drugs, an in-depth study on the biological properties of natural substances is considered with increasingly strong interest. Thus, research concerning also *Teucrium* species is not an exception. Many biological properties of the genus *Teucrium* are well known and exploited in folk medicine. Such properties are ascribable both to the hydroalcholic extracts and to the essential oils giving rise from most of the plant.

Based on the study of the chemical composition of several EOs derived from *Teucrium*, it is now established that the biological properties, with a positive effect on health, are essentially due to the presence of some molecules, such as sesquiterpenes, of which the essential oil from many species of *Teucrium* is rich (Bezic et al. 2011). The main effects due to the *Teucrium* EOs can be listed as below indicated. Naturally, not all species of this genus exhibit the same properties, which depend also on the genetic factors, as well as on the environmental conditions of growth, climate, and geography, as therefore demonstrated for many other species of plants. Furthermore, for the same organ and the same plant, the extraction methods, the area of origin as well as the operating conditions can affect the chemical composition of the oil or extract, subsequently their biological activities. In general, however, *Teucrium* species are reported for their antibacterial, antioxidant, antidiabetic, antielmintic, antirheumatic, and anticancer effects (Miri et al. 2012; Mahmoudi and Nosratpour 2013; Guesmi et al. 2018).

7.4.1 Antibacterial Activity

The antimicrobial activity has been evaluated for different species of *Teucrium.* The essential oils of three African endemic species, *Teucrium africanum, T. kraussii* Codd and *T. trifidum* have been assayed to determine their minimal inhibitory concentration (MIC) against *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* (Ruiters et al. 2016); *Teucrium africanum* showed antibacterial activity against *Escherichia coli* and *Streptococcus pyogenes*. The essential oil from croatian *Teucrium arduini* showed antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Microsporum gypseum* and *Aspergillus brasiliensis*, with MIC values ranging from 6.25 to 37.50 mg/ml. Fungal species were susceptible with MIC values from 7.81 to 25.00 mg/ml (Kremer et al. 2012). The same species was tested by Vukovic et al. (2011) against *Klebsiella pneumonia*e with a MIC of 6.25 μl/ml. Moreover, with a MIC value of 25 μl/ml, the oil showed a good antibacterial activity against *Staphylococcus aureus* (ATCC 25923) and *S. aureus* (FSB 30), as well as against *Enterobacter cloacae* (FSB 22) and *Proteus mirabilis* (FSB 34). *Teucrium polium* ssp. *aurasiacum* was included in a comparative study of antimicrobial activity of five Lamiaceae essential oils from Algeria (Kabouche et al. 2005). Three populations of *Teucrium polium* from the same region of eastern Algeria were evaluated to determine the potential antibacterial activity: the essential oil of the Beni Aziz population has a high antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and the yeast *Saccharomyces cerevisiae*. The population of Boutaleb showed significant activity against *Bacillus cereus* and no action against *Staphylococcus aureus* and the yeast *Saccharomyces cerevisiae* (Lograda et al. 2014).

Belmekki et al. (2013) and Fertout-Mouri et al. (2017) studied the antibacterial activity of the essential oil of *Teucrium polium* from Western Algeria: the oil showed moderate inhibitory effects on *Bacillus cereus, Enterococcus faecalis*, *Escherichia coli* and *Staphylococcus aureus*. This latter recorded the highest sensitivity level (100%); *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* exhibits a resistance (33.33%) towards the tested oil. Moreover, Sevindik et al. (2016) highlighted that the antimicrobial effects of essential oil from *Teucrium polium* growing in North Anatolian region were stronger against *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA), whereas *Escherichia coli* Q157:H7 showed the smallest inhibition zone (11 mm). Considering the alarming spread of antibiotic resistance to many bacterial pathogens, interest in natural plant products as alternative antibacterial agents has increased: thus, Raei et al. (2014) demonstrated that *Teucrium polium* essential oil may have a potential for use against multidrug resistant organisms such as clinical isolates of *Klebsiella pneumoniae*. An Iranian *Teucrium polium* essential oil was active against *Streptococcus pneumoniae* and *Staphylococcus aureus* (Vahdani et al. 2011).

The essential oils of *Teucrium polium* ssp. *gabesianum* (Ben Othman et al. 2017) and *Teucrium polium* L. ssp. *aurasianum* (Bencheikh et al. 2015) were assayed for a possible antimicrobial activity. The essential oil of the first subspecies was most effective against *Proteus mirabilis*, *Staphylococcus aureus* and *Citrobacter freundei* and against some fungi as *Microsporum canis*, *Scopulariopsis brevicaulis* and *Trichophyton rubrum*; the oil of the second one was active against *Staphylococcus aureus*. The EOs of *Teucrium chamaedrys* L. ssp. *chamaedrys*, *T. orientale* L. var. *puberulens*, and *T. chamaedrys* L. ssp. *lydium* were tested for their antimicrobial activity, showing moderate antibacterial activity against Gram-positive and Gram-negative bacteria, but no antifungal activity against two yeast-like fungi (Kucuk et al. 2006). Food spoilage is an enormous economic problem worldwide: the research is focus on discovery of new antimicrobial agents and biological additives from plants. Mahmoudi et al. (2015) investigated the growth and survival of *Salmonella typhimurium* and *Lactobacillus casei* at a 7-day interval during storage (28 days) by addition of *Teucrium polium*, that showed the best growth inhibition of *Salmonella typhimurium* at different doses tested. *Teucrium polium* essential oil was evaluated also against some phytobacteria: the oil did not show antibacterial activity against *Pseudomonas aeruginosa* and *Pectobacterium carotovorum*; instead it showed antibacterial activity against *Pantoea agglomerans*, *Brenneria nigrifluens*, *Streptomyces scabies* and *Ralstonia solanacearum* (Purnavab et al. 2015).

The antibacterial activity of Corsican *Teucrium marum*, *T. massiliense*, *T. chamaedrys*, *T. scorodonia* L. ssp. *scorodonia*, *T. capitatum*, *T. flavum* ssp. *glaucum* and *T. flavum* L. ssp. *flavum* was evaluated against the foodborne pathogens, *Campylobacter jejuni* and *Listeria innocua* and four toxi-infectious bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter aerogenes* wild type (CIP 60.86) and the multi-drug resistant bacterium *E. aerogenes* (EAEP289) (Djabou et al. 2013a). Almost all *Teucrium* essential oils (with exception of *Teucrium scorodonia* ssp. *scorodonia* and *T. chamaedrys*) inhibited the bacterial growth: *Campylobacter jejuni* was found to be extremely sensitive to all the Corsican *Teucrium* oils. suggesting that Corsican *Teucrium* essential oils have the potential to be used as food preservatives and to prevent the growth of nosocomial bacteria (Djabou et al. 2013a). The essential oil of *Teucrium capitatum* growing in Morocco has been evaluated for its potential antimicrobial activity against the bacterial strain *Staphylococcus aureus*, two yeasts (*Candida albicans* and *C. glabrata*), the mold *Aspergillus niger*, and 5 dermatophytes: *Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. mentagrophytes* and *Epidermophyton floccosum*. The results showed a large antimicrobial activity of this essential oil (El Amri et al. 2017). *Teucrium marum* essential oil was reported by Ricci et al. (2005) for its antimicrobial activity against four phytopathogenic fungi: *Rhyzoctonia solani* resulted the most sensitive microorganism.

The essential oil of *Teucrium hircanicum* L. exhibited a dose-dependent antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Aspergillus niger* but it did not showed antimicrobial activity against *Escherichia coli* and *Candida albicans* (Morteza-Semnani et al. 2011). The essential oil of *Teucrium leucocladum* was tested for its bacteriostatic and antifungal activities, showing broad and potent activity against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* (El-Shazly and Hussein 2004). The antimicrobial activity of the essential oil of *Teucrium mascatense* Boiss. from Oman was evaluated against a panel of 17 bacterial and six fungal strains by the disk diffusion method: the oil inhibited the growth of all test organisms at various levels as reported by Hisham et al. (2006b). Vukovic et al. (2007, 2008) studied the antibacterial and antifungal activities of the essential oil of *Teucrium montanum* against several bacterial and fungal strains: the highest activities were obtained specifically against *Klebsiella pneumoniae*, *Bacillus subtilis*, *B. mycoides* and *Enterobacter cloacae*.

Thoppil et al. (2001) studied the antimicrobial activity of the essential oil of *Teucrium plectranthoides* Gamble against seven bacteria and eight fungi using the filter paper disk diffusion method. The growth of almost all microorganisms was inhibited suggesting the possible use of this essential oil as a source of potential microbicides. With the same method, the antimicrobial activities of the essential oil from *Teucrium polium* L. ssp. *geyrii* was evaluated (Roukia et al. 2013) against *Staphylococcus aureus* ATCC 27923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Candida albicans*, showing mainly an efficient fungicidal activity. *Teucrium stocksianum* essential oil exhibited profound antibacterial potential against both Gram-positive and Gram-negative bacterial strains as reported by Shah et al. (2016) and Hisham et al. (2006a). Finally, two different populations of *Teucrium yemense* Deflers were evaluated for their antimicrobial activity: one of these essential oils showed antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Aspergillus niger* and *Botrytis cinerea* (Awadh Ali et al. 2017).

7.4.2 Cytotoxic Effects

Anticancer effects have been attributed to different species of *Teucrium*. The hydrophobic and hydrophilic fractions of the essential oil of *Teucrium alopecurus* Noë, for instance, were studied in the context of cancer prevention and therapy. In particular, the molecular mechanism involved was elucidated, with the aim to determine the effectiveness of such fractions against cancer, through their capability to trigger apoptosis and suppress the tumorigenesis, using as a model human colon cancer cells. The EO acted clearly as free radical scavenger and inhibited the growth and proliferation of several cancer cells, in a time and concentration dependent way. The EO was also capable to induce the apoptotic process, by acting on caspase-3, -8 and -9, as well as on poly-adenosine diphosphate ribose polymerase. In addition, it was able to suppress the gene expression that is involved in the different steps (from survival to proliferation, from the invasion to angiogenesis until metastatic events occurring in human colon cancer cells), without inducing toxicity. Phenolics and sesquiterpenes (in particular δ-limonene, α-bisabol, humulene, thymol and (+)-epi-bicyclosesquiphellandrene) might be also capable to alleviate the inflammatory process (Guesmi et al. 2018). In vitro, through the Ames test, the EO of *Teucrium ramosissimum* Desf. showed antimutagenic effects against sodium azide, aflatoxin B1, benzo[α]pyrene, and 4-nitro-o-phenylenediamine by the bacterial reverse mutation assay in *Salmonella typhimurium* TA98, TA100, and TA1535, with and without exogenous metabolic activation (Sghaier et al. 2010). The EO of *Teucrium pruinosum* from Palestine exhibited in vitro cytotoxic activity against HeLa cervical adenocarcinoma cells. This EO showed also potential antioxidant activity and COX-1 and COX-2 inhibitory effects, with IC_{50} values of 0.25 μg/ml and 0.5 μg/ml, respectively, demonstrating also potential anti-inflammatory properties (Jaradat et al. 2018).

The essential oil of *Teucrium alopecurus* demonstrated the capability to exert in vitro anti-inflammatory and anti-cancer activity by a combination of several actions, such as the block of IκBα kinase, downregulation of NF-κB activation, enhancement of apoptosis and destruction of NF-κB-regulated gene expression (Guesmi et al. 2017). Moreover, terpenes and an hydrolate from this EO oil triggered apoptotic events dependent on caspases activation and PARP cleavage in human colon cancer cells through decreased protein expressions (Guesmi et al. 2018). The essential oil of *Teucrium yemense* demonstrated positive effects against HT-29 human colorectal adenocarcinoma cell lines, with an IC_{50} of 43.7 μg/ml. Coherent with this, this oil was active against both MCF-7 and MDA-MB-231 human breast adenocarcinoma cells (Awadh Ali et al. 2017).

7.4.3 Anti-cholinesterase Activity

Alzheimer's disease (AD) represents actually the fifth leading age-related neurological disorders in the world. AD is characterized by several symptoms, such as cognitive dysfunction, memory loss, psychological changes, and disability, which increase day by day, due to an oxidative stress-induced scarceness in cholinergic neurotransmission, the accumulation of amyloid plaques, and neurofibrillary tangles in the brain (Reitz 2012; Ayaz et al. 2015). In this context, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitor compounds, such as donepezil, galanthamine, rivastigmine, tacrine, etc., represent, at present, the majority of drugs approved for clinical use in the symptomatic AD relief. Nevertheless, EOs target several pathways and thus provide better healing.

Members of the Lamiaceae family and their phytochemicals are known for pharmacological and some clinical effects relevant to dementia. Among Lamiaceae plants, especially *Salvia*, *Rosmarinus* and *Melissa* species, have neuroprotective properties known for many years, besides some other Lamiaceae plants, such as *Teucrium polium*. These plants have been used since ancient times in Turkey for memory enhancement (Perry and Howes 2011; Tuncer 1978). The EOs of some species of *Teucrium* showed an anticholinesterase activity, thus a putative capability to bind to the acetylcholinesterase enzyme, inhibiting its activity. For this reason, as cholinesterase could lead to an increase in the acetylcholine that acts at the nervous level with consequent bradycardia, hypotension, bronchoconstriction and prolonged muscle contraction, the EO with anticholinesterase activity could be used in the pharmacological field (treatment of Alzheimer's disease, or as antidotes for anticholinergic poisoning). *Teucrium polium* EO, extracted from plants collected in Algeria, exhibit such property, in particular against butyrylcholinesterase, resulting also more effective than *Thymus algeriensis* Boiss. & Reut. (Bendjabeur et al. 2018).

7.4.4 Antinociceptive Activity

Pain is a sensorial modality that applies nature from a protective point of view. However, it often can cause discomfort. At present, opiates and non-steroidal anti-inflammatory drugs are the most diffused analgesic drugs used in the current pharmacology to mitigate the pain: however, they cannot be so often used, due to their side effects. Thus, in last decades, the search for new analgesic compounds was one of the main aims for scientists and pharmaceutical companies (Mattison et al. 1998). Therefore, it is well known that herbs and medicinal plants represent a significant font of substances capable to exert potential therapeutic effects (Farnsworth 1989; Blumenthal 2000). Thus, the research and study of plant species traditionally used to mitigate or eliminate the pain could be seen as a logical research strategy, in search for new analgesic drugs (Rang et al. 1998). The genus *Teucrium* can supply, both in the form of aqueous extract (Zendehdel et al. 2011) and in form of essential oils, products with several interesting properties. The EO of *Teucrium polium* is the most known and studied as analgesic. The anti-visceral pain properties discovered for this EO might be comparable to those exhibited by hyoscine and indomethacin. This suggests a good place for it in antispasmodic therapies in humans. The presence of flavonoids and sterols might be also responsible for the anti-inflammatory activity of this plant (Abdollahi et al. 2003). The antispasmodic activity exhibited by *Teucrium polium* is widely demonstrated through the study of plants collected in different countries of the Mediterranean/Middle East. *Teucrium polium* collected in South Arabia, for instance, exhibited a powerful antispasmodic activity and the presence of β-pinene, limonene, α-phellandrene, γ- and δ-cadinenes, and of some alcohols such as linalool, terpinen-4-ol, cedrol, cedrenol and guaiol, which could contribute, further than the alleviation of the pain, also to the other well-known properties provided by the species (Hassan et al. 1979).

Few *Teucrium* EOs were studied for their anti-nociceptive activity. The essential oil of *Teucrium stocksianum* from Pakistan, showed outstanding anti-nociceptive activity. It was observed that, in an animal model, the decrease of writhes occurred using from 20 to 80 mg/kg (b.w), with the maximum of writhes inhibition noted at a concentration of 80 mg/kg (b.w). This oil contains mainly α-pinene, myrcene, limonene, linalool and caryophyllene oxide, all of them with antinociceptive effects.

The high potency of the EO of *Teucrium stocksianum,* as compared to diclofenac used as positive control, might be due to the synergistic effect of some of its components (Shah et al. 2012).

7.4.5 Anti-inflammatory Activity

Free radical species and nitric oxide (NO) or its derivatives represent key denominators in carcinogenesis, further to have biological significance in viral and bacterial infections. Treatment with chemical carcinogens, such as carbon tetrachloride and heterocyclic amines, also generated superoxide. Furthermore, most experimental solid tumours have elevated levels of iNOS in the tumoral tissue, and NO thus generated facilitates vascular permeability, which quickens nutritional supply to the tumor tissue and hence supports the rapid tumour growth (Maeda and Akaike 1998). Inflammation can be considered as a normal protective response induced by tissue injury or infection and functions against microorganisms and non-self cells, when these invade the body, and as response to remove dead or damaged host cells (Stevenson and Hurst 2007; Miguel 2010). In the inflammatory response, we find an increase of permeability of endothelial lining cells and influxes of blood leukocytes into the interstitium, oxidative burst, with release of cytokines [interleukins and tumor necrosis factor-α (TNF-α)]. At the same time, it occurs also an induction of the activity of several enzymes (in particular oxygenases, nitric oxide synthases, peroxidases) and of the arachidonic acid metabolism. In addition, the expression of cellular adhesion molecules, such as intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) can be observed (Gomes et al. 2008). Further than the capability of some essential oils to scavenge free radicals, there is also evidence that some of them possess anti-inflammatory activity. Under this aspect, several essential oils, including those obtained from some species of *Teucrium,* can show beneficial effect by inhibiting the production of inflammatory mediators in macrophages.

The incubation of cell such as RAW 264.7 cells with essential oils obtained from *Teucrium* spp. can positively cause a significant inhibitory effect on the LPS-induced nitrite production, with IC_{50} values lower than those of the positive control, indomethacin. Among the essential oils recovered by the *Teucrium* spp., the oil with the most effective inhibitory action against LPS-induced NO production could be that obtained from *Teucrium brevifolium,* which, as reported by Menichini et al. (2009), gave an IC_{50} value of 7.1 μg/ml, followed by *Teucrium montbretii* ssp. *heliotropiifolium* (Berbey) P. H. Davis and *T. capitatum,* which IC_{50} values resulted of 16.5 and 29.4 μg/ml, respectively. *T. flavum* EO shows a weaker activity (IC_{50} = 41.4 μg/ml). Such activities are noteworthy if compared with those of other essential oils. For instance, the essential oil from the *Farfugium japonicum* (L.) Kitam. (Asteraceae) produced a 25% of inhibition of NO production at a concentration of 100 μg/ml (Kim et al. 2008); on the other hand, the EO from *Cinnamomum osmophloeum*

Kaneh. (Lauraceae) can inhibit by 100% the NO production when used at a concentration of 50 μg/ml (Tung et al. 2008).

A survey of literature reveals that some *Teucrium* EOs, such as that of *Teucrium buxifolium* Schreb.*,* have potent anti-inflammatory activity against both the acute and the delayed phases of inflammation (Fernández Puntero et al. 1997). Some essential oils, containing major constituents similar to those in the oil of *Teucrium brevifolium,* have shown anti-inflammatory activity. For example, the EO of *Garcinia brasiliensis* Mart. (Clusiaceae)*,* which contains spathulenol (8.7%) as a major constituent (Martins et al. 2008), exhibited anti-inflammatory activity, in the rat-paw oedema model induced by carrageenan. The highest anti-inflammatory activities exhibited by *Teucrium capitatum* and *T. montbretii* ssp. *heliotropiifolium* could be probably due to their major components, caryophyllene and carvacrol, which showed anti-inflammatory activity in previous studies (Sosa et al. 2005; Passos et al. 2007).

7.4.6 Antioxidant and Hypoglycemic Activities

Generally, the species of *Teucrium* exhibit a good antioxidant activity, which can be a support for other biological properties. Several *in vitro* studies demonstrated the antioxidant potentiality of *Teucrium polium* with a lot of the assays usually performed in this context, starting from the DPPH test to metal chelating activity until the evaluation of the reducing power (Abdollahi et al. 2003). *Teucrium polium* EO even demonstrated the same potency of tocopherol (Couladis et al. 2003).

Hypoglycemic activity has been reported – in addition for the flavonoids – also for the volatile oils of different species of *Teucrium*. Traditionally, especially in the Mediterranean countries, some *Teucrium* species, such as *Teucrium polium* and *T. mascatense* are used for their hypoglycemic activities by the native inhabitants and recommended by the herbalists (Afifi et al. 2005; Rehman et al. 2016). However, the EO from *Teucrium polium* ssp. *polium* Briq., and its major terpene components, showed strong antioxidant, anti-inflammatory and antimicrobial activities (Kerbouche et al. 2015).

7.4.7 Allelopathic and Biocide Effects

Allelopathic effects gives rise from the release, by the plant, of certain compounds, known as allelochemicals, which are biosynthesized in the plant as secondary metabolites (essenatial oils, polyphenols, coumarins, terpenoids, etc.) and may be present in all tissues including pollens (Whittaker and Feeny 1971). The isolation and identification of EOs and/or components with allelopathic, anthelminthic and in general biocide activity is of great interest, due to the increasing request, in food, health and agriculture, of substances/compounds of natural origin that can be used with safety, are no toxic and have a great spectrum of action without induce resistance (Ahmad et al. 2011; De Martino et al. 2015).

The EO of *Teucrium royleanum* Wall. ex Benth. has been evaluated as allelopathic agent (Ahmad et al. 2011). De Martino et al. (2010) studied the chemical composition and the phytotoxic effect of four *Teucrium* EOs. All oils, although rich in sesquiterpenes (50.0–61.9%) differed for composition, being caryophyllene and caryophyllene oxide the main components of *Teucrium arduini*, germacrene D, δ-cadinene and γ-cadinene predominated in *Teucrium maghrebinum*. Carvacrol and caryophyllene were abundant in *Teucrium capitatum*, while carvacrol, caryophyllene oxide and caryophyllene were the most main components in *Teucrium montbretii* ssp. *heliotropiifolium*. The germination of radish and garden cress was less sensitive to the four essential oils. The radicle elongation, above all of radish, was significantly inhibited by all oils, in particular by that of *Teucrium arduini*. Among the main components of the oils, monoterpenes resulted the more active compounds. Some of the sesquiterpenes isolated by the essential oil of *Teucrium ramosissimum*, in particular homalomenol C, 4β-hydroxy-11,12,13-trinor-5-eudesmen-1,7-dione, oxo-T-cadinol and 1β,4β,6β-trihydroxyeudesmane exhibit a significant *in vitro* antiplasmodial activity against *Plasmodium falciparum,* with IC_{50} values ranging from 1.2 to 5.0 μg/ml, without any cytotoxic effect upon the human diploid lung cell line MRC-5 (Sathiyamoorthy et al. 1999; Henchiri et al. 2009).

7.5 Conclusions

This review testifies the great variability of the essential oils produced by different species of *Teucrium*, as influenced by several endogenous and exogenous factors and by the possible presence of chemotypes. The essential oils constitute the basis for most of the uses of *Teucrium* species in traditional medicine and alimentation.

Teucrium polium, *T. chamaedrys*, *T. flavum* and *T. capitatum* are the more studied taxa concerning the essential oil composition. The literature data permit to categorize the essential oils in some groups, following the prevalence of some components, even if the majority of the essential oils contain a prevalence of sesquiterpenic compounds. Caryophyllene and caryophyllene derivatives, α- and β-pinene, germacrene B and D, α-, γ-, δ-cadinene and or cadinol, limonene, bisabolol/bisabolene have been identified as the marker components in *Teucrium* essential oils. However, *Teucrium* volatile oils are charaterized by a high degree of chemical polymorphism and often the same species possessed essential oils with very different chemical profiles. This inhomogeneity could reflect differences in genetic structure, environmental effects, and/or a combination of both factors. Moreover, in most cases, also the seasonal influence or climatic factors, such as altitude, annual rainfall, winter cold stress, summer precipitation, summer drought stress, evapotranspiration and humidity could contribute to explain the complex essential oil variability.

These differences constitute an asset both in terms of biodiversity and in terms of practical use of *Teucrium* species, primarily for their biological properties. In fact, the available literature reports data about antimicrobial, antioxidant, antinflammatory, cytotoxic, nociceptive and allelopathic activity of the essential oils, often confirming the traditional uses of *Teucrium* species.

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Chapter 8
Secondary Metabolites of *Teucrium* Species with Toxic Effects

Maria-Eleni Grafakou, Christina Barda, and Helen Skaltsa

Abstract The genus *Teucrium* L. has a long-term use in folk medicine, however there are some implications for human health related to the consumption of germander-containing products. Numerous cases of hepatotoxicity have been reported, mostly in Europe, where the plants were used as herbal products for weight loss and still are used in folklore medicine to treat hypercholesteraemia and diabetes. During the last decades several cases of germander toxicity including chronic hepatitis as well as acute cytolytic hepatitis and a case of death have been reported. The most well known toxic *Teucrium* species are *Teucrium chamaedrys*, *T. polium* and *T. capitatum*, the use of which as herbal medicines and dietary supplements should be strictly controlled. The neo-clerodane diterpenoids occurring in *Teucrium* species have been the subject of intensive studies, since they have been correlated to the toxic effects of the genus. Studies in mice showed that the hepatotoxicity of the genus is being induced by this type of diterpenes, which are transformed into their reactive metabolites by P450. Teucrin A and teuchamaedrin A have been subjected to numerous studies in order to reveal the mechanism of action. This chapter aims to summarize the diterpenes isolated from *Teucrium* spp., as well as the hepatotoxicity of some of them.

Keywords *Teucrium* · Toxicity · Hepatotoxicity · Diterpenes

Abbreviations

CEFS	Council of Europe Committee of Experts on Flavouring Substances
FDA	Food and Drug Administration
NFG	Nerve growth factor
PP	Pyrophosphate
UV	Ultraviolet

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8.1 Introduction

Natural products are structurally diverse compounds, deriving from natural sources, mainly plants, in the form of primary and secondary metabolites (Dewick 1999). Secondary metabolites differ from primary metabolites in having a districted distribution throughout the plant kingdom (Anulika et al. 2016). Plants and other living organisms have uniquely evoluted to biosynthesize secondary metabolites, compounds which unlike primary metabolites are generally not essential for growth, development or reproduction of the organism (Theis and Lerdau 2003). However, secondary metabolites play significant roles in defence mechanisms against insects, pretadors and microorganisms, as attractants for pollinators and seed-dispersing animals, allelopathic agents in natural habitats, barriers to abiotic stressors such as UV, salty or drought environmental conditions (Wink 2009; Theis and Lerdau 2003; Pichersky and Gang 2000). Because of structure similarity to humans metabolites, secondary metabolites from plants exert biological activities of a wide range, thus plants have been used for a variety of internal and external ailments since ancient times (Dias et al. 2012). Many secondary metabolites, however, act as toxins, which is explained by their major role in defence mechanisms (Wink 2009). In western civilization, there is an increasing use of herbal medicines, because of their supposed safety in contrast to chemical drugs, however, using herbal remedies may pose health hazards (De Smet 2002). Plant derived products should not be assumed safe; safety is evaluated either in the case of herbal medicinal products or isolated compounds. There are even reports from poisoning associated with widely used medicinal plants, which are usually due to misidentification of the plants or incorrectly preparation and administration (Nasri Hamid Shirzad 2013; Karimi et al. 2015).

The genus *Teucrium* L. (germander) of the Lamiaceae family, comprises about 300 species, distributed worldwide, mainly in the Mediterranean area (Tutin et al. 1972; Greuter et al. 1986). Members of the genus have a long term use in traditional medicine and the first records of the medicinal use of *Teucrium* date back to Greek mythology. Probably the name *Teucrium* comes from Teukros (Teucer), the best Achaean archer in the Trojan War (son of Telamon, the king of the island Salamina); his step-brother Aias healed using this plant the wounds of Teukros caused by Hector (Carnoy 1959). Later on, Aias was killed by Hector. At his return to Salamina, Teukros was kicked out by his father, because he did not defend his brothers' death. The bitter taste and the toxicity of the plant make allusion to the Teukros' misfortune. According to Dioscorides, the plant as an oxycrate beverage, or combined with figs, helps the spleen; it has also anticonvulsant, antitussive, diuretic, abortive and emmenagogue activities. Also, the poultices embedded in germander decoctions are antidotes against venomous bites; combined with honey it could be used to treat old ulcers and with oil in ocular discomforts (Berendes 1902; Wellmann 1958); The medicinal uses of the genus include the treatment of digestive and respiratory disorders, tuberculosis, jaundice, rheumatics, diabetes, kidney diseases, skin inflammations and for weight loss (Stanković et al. 2011; Pacifico et al. 2012; Stanković and Zlatić 2019).

Teucrium species despite being popular in folk medicine, have been also associated to potential toxicity, especially hepatotoxicity which has been attributed to their content of furano-*neo*-clerodane diterpenes (Ulubelen et al. 2000). As a result, the aim of the present study is to summarize the secondary metabolites of the genus *Teucrium* with toxic effects. A selection of the relevant data was made through a search using the keyword "Teucrium" and "toxicity", "hepatotoxicity", "diterpenes" in PubMed, Scopus, Google Scholar and Science Direct databases. Only papers in English language are included.

8.2 Diterpenes from the Genus *Teucrium*

Phytochemical investigations have revealed a plethora of bioactive secondary metabolites produced by members of the genus *Teucrium*, including monoterpenes, iridoids, sesquiterpenes, diterpenes, sterols, saponins, polyphenolic compounds, flavonoids, tannins, coumarins, glycosides, alkaloids, and essential oils (Djabou et al. 1974; Piozzi et al. 1998; Hao et al. 2013; Fatima 2016) However, the most abundant compounds are diterpenes, especially *neo*-clerodane type diterpenes; so far, more than 230 compounds with this skeleton have been isolated from the aerial part of the taxa (Table 8.1; Piozzi et al. 1998, 2005), while this group of secondary metabolites has been described as chemotaxonomic markers of the genus *Teucrium* (Ulubulen et al. 2000). Moreover, some rearranged *neo*-clerodane diterpenes, as well as some abietane type diterpenes have been isolated, mainly from the roots of *Teucrium* taxa (Ulubulen et al. 2000). *Neo*-clerodanes have been also isolated from other plant sources; in addition to the Lamiaceae family, those diterpenes have been reported from plants of Asteraceae, Salicaceae and Menispermaceae families (Li et al. 2016). This group of compounds have captured the attention of the scientific community, as they can exhibit a wide range of pharmacological activities, such as antitumor, NGF-potentiating, antibiotic, anti-ulcer, hypoglycaemic, hypolipidaemic, antiplatelet; however, insect antifeedant activity and opioid receptor agonist effects have been assumed to be the most significant activities (Li et al. 2016).

8.2.1 Chemistry of Diterpenes in Teucrium *spp*

The following section summarizes the diterpenes isolated from *Teucrium* spp. Our study aims to outline the up-to-date investigated *Teucrium* species, characterized by the presence of diterpenes (Table 8.1).

Table 8.1 Literature Survey of ditepenes from *Teucrium* spp.

Teucrium species	Isolated diterpenes	References
T. abutiloides	12-epiteupolin II, montanin C, teubutilin A, teubutilin B	Torre et al. (1990)
T. alpestre	3-acetylteumicropin, teumicropin, teupyrenone, deacetylteupyrenon	Piozzi et al. (1997)
T. asiaticum	Auropolin, teuftin	Camps et al. (1987)
	Auropolin, teucrasiatin	Rodríguez et al. (1996)
	Auropolin, teucrasiolide, teucrasiatin	Rodríguez et al. (1997)
T. barbeyanum	Teucrin A, teucrin F, teucrin G	Bruno et al. (1985)
T. betonicum	19-acetylgnaphalin (teucrin H_3), 6*β*-hydroxyteuscordin, 6*α*-hydroxyteuscordin, teucrin E, teucrin H_2, teucvin, teubetonin	Gaspar et al. (1995)
T. bicolor	Montanin C, 12-epi-teucvin, teucvin, teucrin H2, teupolin I, 12-epi- teupolin I, (12R)-epi- teuscordon	Labbe et al. (1989)
T. botrys	19-deacetylteuscorodol, 6*β*-hydroxyteuscordin, montanin D, teubotrin, teucvidin, teuchamaedrin C	Torre et al. (1986)
T. brevifolium	Teubrevin C, teubrevin D, teubrevin E, teubrevin F, teubrevin G, teubrevin H, teubrevins I	Rodríguez et al. (1995)
	Teubrevin A, teubrevin B	Rodríguez et al. (1994)
T. buxifolium	19-acetylteulepicin, 19-acetylgnaphalin	Savona et al. (1986)
T. canadense	18-acetylmontanin D, 12-epiteupolin II, isoteuflin, teucvidin, teuflin, teucvin, teuscorodal, (12R)-teupolin I	Bruno et al. (1989)
T. capitatum	19-acetylgnaphalin, lolin, teucapitatin	Marquez et al. (1981)
T. chamaedrys	2(*S*)-15,16-epoxy-19-hydroxy-*neo*-cleroda-13(16),14-dien-18,6*α*:20,12-diolide	Bedir et al. (2003)
	Dihydroteugin, syspirensin A, teuchamaedryn D, teucrin A, teucroxide	Elmastas et al. (2016)
	Chamaedryoside A, chamaedryoside B, chamaedryoside C	Fiorentino et al. (2009)
	Teucrin F, teucrin G	Bruno et al. (1985)
	Teucrin A	Kouzi et al. (1994)
	Dihydroteugin, 6*α*-hydroxyteuscordin, teuchamaedrin C	Papanov and Malakov (1985)
T. chartaginense subsp. *homotrichum*	19-acetylgnaphalin, eriocephalin	Bruno et al. (1985)
T. cosoonii	Teucossiin A, teucossiin B, montanin H	Alcazar et al. (1992)

(continued)

Table 8.1 (continued)

Teucrium species	Isolated diterpenes	References
T. cubense	Eugarzasadone	Dominguez et al. (1974)
T. cuneifolium	Deacetylteupyrenone	Piozzi et al. (1997)
T. divaricatum subsp. *canescens*	2-deoxychamaedroxide, 6β-hydroxy-teuscordin, montanin D, dihydroteugin, teuflin, teucrin H_2, teuflidin, teucrin A, teucrin F, teucrin G	Bruno et al. (1987)
T. divaricatum subsp. *divaricatum* syn. *T. divaricatum*	Teucrin A	Rodríguez et al. (1996)
T. divaricatum subsp. *villosum*	Hydroxyteuscordin, dihydroteugin, montanin D, teuflin, teuscordinone, teuflidin, teucrin A, 6β- teugin	Piozzi et al. (1997)
	Villosin A, villosin B, villosin C, imbricatolic acid, dehydroabietic acid, teuvincenone B, teucrin A	Ulubelen et al. (1994)
T. flavum subsp. *glaucum*	Teuflin, teuflavin, teutlavoside	Savona et al. (1984)
T. flavum subsp. *hellenicum*	Teucvidin, 12-epiteucvidin, teuflin	Piozzi et al. (1997)
T. fruticans	Fruticolone, isofruticolone, 8β-hydroxyfruticolone, 6-acetylteucjaponin B, 7β-hydroxyfruticolone, 11-hydroxyfruticolone, deacetylfruticolone, 6-acetyl-10-hydroxyteucjaponin B	Coll and Tandrón (2004)
	Difuranofruticol, deoxyfruticolone, 10-hydroxyteucjaponin B, 7,8-didehydrofruticolone	Coll and Tandrón (2005)
	Teufruintins A–G, fruticolone, isofruticolone, 6α-hydroxyfruticolone, 6-acetyl-10-hydroxyteucjaponin B, 6-acetyl-teucjaponin B, 8β-hydroxyfruticolone, teucretol, 11-hydroxyfruticolone	Lv et al. (2015)
	Fruticolide, fruticolone, isofruticolone, 8β-hydroxyfruticolone	Bruno et al. (1991)
	Teuvincenone F, teuvincenone G, teuvincenone E	Sexmero Cuadrado et al. (1992)
T. gnaphalode	Gnaphalin, 19-acetylgnaphalin	Savona et al. (1979)
T. gracile	Teugracilin A, 3-*O*-deacetylteugracilin A, teugracilin B, teugracilin C, teumicropodin	Bruno et al. (1991)
	19- acetylteulepicin, teugracilin D, teugracilin E	Bruno et al. (1992a)
T. hyrcanicum	Teucrin H_1, teucrin H_2, teucrin H_3, teucrin H_4	Gács-Baitz et al. (1978)
T. intricatum	Teucvin	Bruno et al. (1985)
T. japonicum	Teuponin	Zhi-da et al. (1991)

(continued)

Table 8.1 (continued)

Teucrium species	Isolated diterpenes	References
T. lamiifolium	12-epiteupolin II, teuscordinon, teuflin, montanin C, 19-acetylgnaphalin	Boneva et al. (1988)
	Teulamioside, montanin E, teuspinin	Malakov et al. (1993)
T. lanigerum	Teuvincenones A teuvincenones B, teuvincenones C, teuvincenones D, teuvincenone J	Rodríguez et al. (2009)
	2*O*-deacetyleriocephalin, isoeriocephalin, eriocephalin, auropolin	Fernandez-Gadea et al. (1984)
T. lepicephalum	Teulepicin, 19-acetylteulepicin, teulepioephin	Savona et al. (1986)
T. lucidum	Teucvidin, teuflin	Bruno et al. (1985)
T. luteum subsp. *flavovirens*	3*β*-hydroxyteucroxylepin and teuluteumin A, teuluteumin B	Castro et al. (2010)
T. maghrebinum	12-epi-teucjaponin A, 12-epi-montanin D, 12-epi-montanin B, teucjaponin A, montanin D, 19-deacetylteuscorodol, teusalvin C, montanin B	Bruno et al. (2000)
T. marum	Teumarin B	Bianco et al. (2004)
T. massiliense	Deacetylajugarin-II, teumassilin, 6,19-diacetylteumassilin, montanin C, teucjaponin A	Savona et al. (1984)
	Teumassilenins A-D, teumassin, teumassilin, 6,19-diacetylteumassilin, teumarin, deacetylajugarin II, montanin C, teucjaponin A	Fontana et al. (1998)
T. micropodioldes	3-acetylteumicropin, deacetylteupyrenone, 3-deacetyl-20-epi-teulanigin teumicropodin, teumicropin	Torre et al. (1988)
T. montanum	Montanin A, montanin B	Malakov et al. (1992)
T. montanum subsp. *montanum*	19-acetylgnaphalin, montanin B, D, E, teubotrin (teulamifin B), montanin H	Malakov et al. (1992)
T. montbretii subsp. *libanoticum*	3*α*-hydroxyteubutilin A, 12-epi-montanin G, 20-epi-3,20-di-*O*-deacetylteupyreinidin, 6-ketoteuscordin, teuscordinon, 6*α*-hydroxyteuscordin, montanin D, 3,20-di-*O*-deacetylteupyreinidin, montanin G, 3-*O*-deacetylteugracilin A	Bruno et al. (2002)
T. montbretii subsp. *montbretii*	2-deoxychamaedroxide, teuflin, 6-keto-teuscordin, montanin C, 6-acetylteucjaponin B, teucrin H_2, 6-hydroxyteuscordin, 2*β*-hydroxyteuscordinon, montanin D, teugin	Bruno et al. (1992b)
T. montbretii subsp. *heliotropiifolium*	Teucrin H, 6-*β*-hydroxyteuscordin, montanin D, teugin	Alcazar et al. (1992)
T. montanum subsp. *pannonicum*	Auropolin, montanin H	Kisiel et al. (1995)
T. oliverianum	Teucrolin E, teucrolin F, teucrolin G	Al-Yahya et al. (2002)

(continued)

Table 8.1 (continued)

Teucrium species	Isolated diterpenes	References
T. orientale	6-deacetyl-teucrolivin A, 8*β*-hydroxy-teucrolivin B, teucrolivin A, teucrolivin B, teucrolivin C, teucrolivin H	Bruno et al. (2004)
T. oxylepis subsp. *marianum* syn. *Teucrium oxylepis*	Teucroxylepin, 12-*O*-acetylteugnaphalodin, gnaphalin, 19-acetylgnaphalin, teuscorodonin, montanin D, teucrin H_4, 19-deacetylteuscorodo, teubotrin, teucroxide, teukotschyn, isoteucrin H	Sexmero Cuadrado et al. (1991)
T. polium	Teulolin A, teulolin B	Bedir et al. (1999)
	12,16-Epoxy-6,11,14-trihydroxy-17(15->16)-abeo-3*α*,18-cyclo-5,8,11,13,15-abietapentaen-7-one, 12,16-Epoxy-6,11,14,17-tetrahydroxy-17(15->16)-abeo-3*α*,18-cyclo-5,8,11,13,15-abietapentaen-7-one, 12,16-Epoxy-6,11,14-trihydroxy-17(15->16)-abeo-5,8,11,13,15-abietapentaen-7-one, 12,16-Epoxy-6,11,14,17-tetrahydroxy-17(15->16)-abeo-5,8,11,13,15-abietapentaen-7-one, 12,16-Epoxy-6,11,14,15-tetrahydroxy-17(15->16)-abeo-3*α*,18-cyclo-5,8,11,13-abietatetraen-7-one, 12,16-Epoxy-6,11,14,15-tetrahydroxy-17(15->16)-abeo-5,8,11,13-abietatetraen-7-one, vilocin C, teuvincenone A, teuvincenone B, teuvincenone C, teuvincenone D	Fiorentino et al. (2010)
	Teupolins VI–XII	Fiorentino et al. (2011)
	20-*O*-acetyl-teucrasiatin, 20-*O*-acetyl-teucrasiatin	Venditti et al. (2017)
T. polium var. *polium*	Teupolin III	Malakov et al. (1982)
	Teupolin IV, teupolin V	Malakov and Papanov (1983)
T. polium subsp. *aurasianum*	Teumicropodine, 3-deacetylteumicropodine, 3,20-bis-deacetylteupyreinidine, 6,20-bis-deacetylteupyreinidine, 3,6,20-tri-deacetylteupyreinidine	Ladjel et al. (1994)
T. poliun subsp. *belion*	19-acetylgnaphalin, teucrin A	Camps et al. (1987)
T. polium subsp. *capitatum*	19-acetylgnaphalin, auropolin	Camps et al. (1987)
	7-deacetycapitatin, picropolinol, 2-*O*-epi-isoeriocephalin, picropolin, picropolinone, 19-acetylgnaphalin, teucjaponin B	Fernández et al. (1986a,)
T. polium subsp. *exansum*	Picrolinone, 19-acetylteulepicin, 3-*O*-deacetylteugracilin A	Alcazar et al. (1992)
	Ferruginol, teuvincenones, teuvincenones B, teuvincenones H, teuvincenones I	Sexmero Cuadrado et al. (1992)
T. polium subsp. *polium*	Auropolin, capitatin, 20-epi-auropolin	Bruno et al. (2003)

(continued)

Table 8.1 (continued)

Teucrium species	Isolated diterpenes	References
T. polium subsp. *vincentinum*	Teuvincentin A, teuvincentin B, teuvincentins C, 19-acetylgnaphalin, eriocephalin, isoeriocephalin, 3-deacetyl-20-epi-teulanigin	Carreiras et al. (1990)
	Euvincenone A, euvincenone B, euvincenone C, euvincenone D	Torre et al. (1990)
T. pyrenaicum	Teupyrin A, teupyrin B, teutlin, teucrin H_2, 6α-hydroxyteuscordin	Fernández et al. (b)
T. tomentosum	Euctomin, along with 6-*O*-acetyl-teucmicropodin, teugracilin A, 6α-hydroxyteuscordin, 6α-acetoxyteuscordin	Aravind et al. (2010)
	Teuctosin, teuflin, teucrin H_2, 6β-hydroxyteuscordin, 6β-acetylteuscordin, montanin D	Kumari et al. (2003)
	3β-acetoxy-4α,18:15,16-diepoxy-6β, 12-dihydroxyneocleroda-13(16),14-dien-19,20-olide	Kumari et al. (2003)
T. racemosum	Teuracemin, eutrifidin, 4α,18- epoxytafricanin A, 20-oxo-teufiavin	Rodríguez et al. (1995)
T. rivas-martinezii	19-acetylgnaphalin, 19-acetylteulepicin	Rodríguez et al. (1996)
T. royleanum	Royleanumin	Ahmad et al. (2014)
T. salviastrum	Teusalvin A, teusalvin B, teusalvin C, teusalvin D, teusalvin E, teusalvin F, teucvidin, teucroxide	Torre et al. (1986)
T. sandrasicum	Sandrasin A, 6-deacetylsandrasin A, sandrasin B	Topcu et al. (1996)
T. scordium	Teucrin F, teucrin G, 6α-hydroxyteuscordin	Bruno et al. (1985)
	Teucrin E, 6α-hydroxyteuscordin, 6β-hydroxyteuscordin, teuscordinone, teugin, dihydroteugin, teucjaponin B acetate, teucroxide, 2,3-Dehydroteucrin E, 2β-6α-Dihydroxyteuscordin, 2β-Hydroxyteuscordinone, 6,20 Bisdeacetylteupyreinidin, 6-deacetylteupyreinidin	Jakupovic et al. (1985)
	Teuscordinon	Papanov et al. (1981)
	2-keto-19-hydroxyteuscordin, teucrin E, teucrin H_4	Papanov and Malakov (1985)
T. scorodonia subsp. *euganeum* syn. *T. siculum*	Teuilin	Bruno et al. (1985)
T. scorodonia	Teuflin, teuscorolide, teuscorodin, teupolin I, teuscorodal, teuscorodol, teuscorodonin, 2α-hydroxyteuscorolide	Bruno et al. (1985)

(continued)

Table 8.1 (continued)

Teucrium species	Isolated diterpenes	References
T. viscidum	Teuvisone, biteuvisones A, biteuvisones B, 2α,11,12-trihydroxy-7β,20-epoxy-8,11,13-abietatriene	Gao et al. (2015)
	19-acetyl-teuspinin, 6α-acetoxyteuscordin, deoxychamaedroxide, isoteuflin, kinalborin C, teucvisin A, teucvisin B, teucvisin C, teucvisins D, teucvisin E, teucvin, teucvidin, teuflin, teuspinin, 2- teuscordal	Lv et al. (2014)
T. viscidum var. *miquelianum*	Teucvin, teuflin (6-epiteucvin), teucvidin	Node et al. (1981)
T. yemense	Fatimanol A, fatimanol B, fatimanol C, fatimanol D, fatimanol E, fatimanone, teulepicephin	Nur-E-Alam et al. (b)
	3-*O*-deacetylteugracilin, teugracilin B	Nur-E-Alam et al. (2017a)
	6β-*O*-acetyl-3β-hydroxyteucroxylepin, teucryemin, 19-*O*-acetylteucryemin, teucryeminone	Sattar et al. (1995)

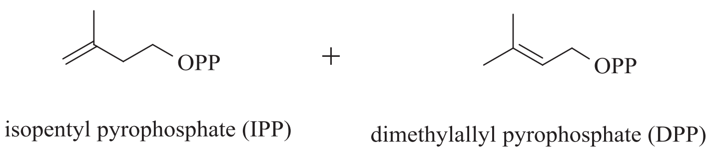


Fig. 8.1 Building blocks of terpenoids

8.2.2 Biosynthesis of Diterpenes

Terpenes or isoprenoids are a large and structural diverse class of secondary metabolites formed through the mevalonate pathway or the deoxyxylulose phosphate pathway. Biosynthetically, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, derived from C5 isoprene units commonly joined in a head to tail orientation and they are classified according to the number of isoprene units. The active building blocks biochemically are the diphosphate esters dimethylallyl diphosphate and isopentenyl diphosphate. The precursor of diterpenes (C20) is the geranylgeranyl diphosphate (Figs. 8.1 and 8.2) and it is further modified by cyclization reactions, involving numerous of parallel steps to yield the mentioned natural compounds (Table 8.1). Their large structural diversity is attributed to their different biosynthetic pathways, and for this reason the diterpenes are classified by their biosynthesis (Bruneton 1993). The biosynthetic pathway of cyclic diterpenes comprises two different types of cyclization A and B. In the first case, its characteristic conversion into cebrenes or casbanes. From the cebrene, a group of macrocyclic diterpenes is initiated. The casbane, leads to the formation of skeletons such as tigliane, daphnane, etc. The second type (B) of cyclization is characteristic of the upper terpenes. This point of biosynthesis is critical because it can lead either to the normal order (n-) or to the enantiomer type (*ent*-) of labdane skeleton. N-labdane derivatives are believed to be derived from the labdadienyl PP, while the antipodes

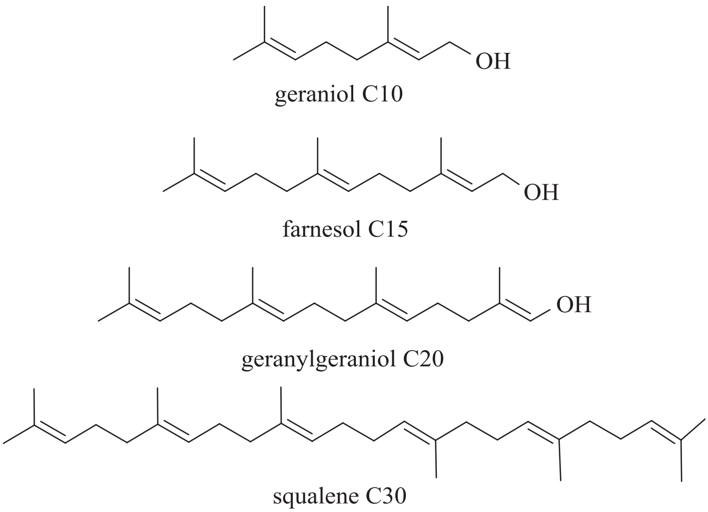


Fig. 8.2 Precursors of terpenes

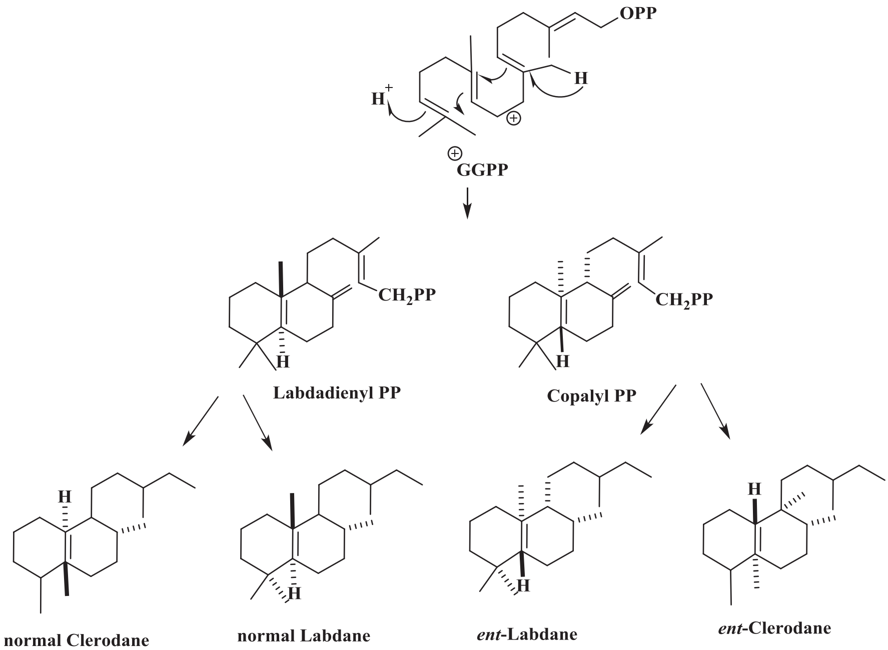


Fig. 8.3 Biosynthetic pathway of clerodane and labdane diterpenoids

from its enantiomer, copalyl diphosphate. Through the above, we can understand the basic principles in the biosynthesis of labdanes and clerodanes which appear to be closely related (Fig. 8.3) (Merritt and Ley 1992; Mac Milan and Beale 1999).

8.3 Hepatotoxicity Related to *Teucrium* spp.

In modern civilization, the use of herbal remedies is becoming an increasingly popular form of alternative medicine because of their assumed safety, but a survey on the literature reveals the concerns related to their health risks. One such health hazard, is hepatotoxicity; liver injury has been associated with herbal medicines and especially with terpenoid-containing dietary supplements (Chitturi and Farrell 2008; Hasani-Ranjbar et al. 2010). Wild germander (*Teucrium chamaedrys*) was one from the first herbal products to be related with cases of acute liver failure, attributed to its content of diterpene derivatives.

In 1986, an advertisement promoted germander tea bags and capsules for weight loss and mild diarrhoea in France, while germander was also consumed as a constituent of aromatized wines and liquors (Stickel and Shouval 2015). The use of high amounts of germander, previously regarded as safe, led to an epidemic of cytolytic hepatitis (De Berardinis et al. 2000; Stickel et al. 2000). Numerous cases of hepatic injury were reported (Larrey et al. 1992; De Berardinis et al. 2000), mainly acute cytolytic hepatitis, as well as some reports of chronic hepatitis. Most patients recovered after discontinuation of the herbal remedy except for those with cirrhosis, and a case of death, because of fulminant hepatic necrosis (Mostefa-Kara et al. 1992). The daily intake was ranging between 600 and 1620 mg of germander daily (Larrey et al. 1992), and the developed hepatitis (2 months after permanent administration) was described by jaundice and increased amino-transferase levels and serum bilirubin levels, but in some cases of chronic course of liver disease also fibrosis and cirrhosis have been described. More women consumed germander products, in an attempt of losing weight, which reflects the higher levels of toxicity in this gender (Stickel et al. 2000).

As a result of these indices, all herbal preparations of *Teucrium chamaedrys* were banned in France in 1992, and no further cases were recorded in this country. In Italy 1996, the sale of germander products was also prohibited, while more *Teucrium* spp. (*Teucrium marum*, *T. montanum*, *T. polium*, *T. scordium* and *T. scorodonia*) are included in "Plants that contain substances that are definitely toxic to humans" (Carratù et al. 2010). Similar prohibitions had been applied also in 1997 in Belgium for products containing both *Teucrium chamaedrys* and *T. polium* (Gori et al. 2011). FDA permits *Teucrium chamaedrys* in alcoholic beverages only, which is in accordance with the recommendation of the CEFS that the use of germander products including teucrin A should not extended beyond flavoured beverages (European Commission 2003).

Despite those restrictions, two further cases were reported in Canada in 1996 (Laliberté and Villeneuve 1996) and in more recent years, more cases were reported, such as two in Spain in 2001 (Pérez Alvarez et al. 2001), and four more in Italy, in 2011 and 2014 (Gori et al. 2011; Nencini et al. 2014).

Moreover, hepatotoxicity has also been related with other members of the genus *Teucrium.* In 1995 the first case of liver transplantation for severe acute liver injury after treatment for 10 days with *Teucrium polium* for hypercholesterolaemia was reported in Greece (Mattéi et al. 1995). Regarding *Teucrium polium* (golden germander) more cases have been reported; acute cholestatic hepatitis has been reported in two cases in Greece, after the use of the plant as hypoglycaemic or hypolipidaemic agent (Polymeros et al. 2002; Mazokopakis et al. 2004). Furthermore, the use of *Teucrium polium* products for the same reasons led to three other cases of hepatitis in Greece (Starakis et al. 2006; Savvidou et al. 2007).

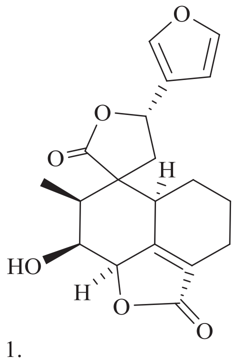
Teucrium capitatum is also related to hepatotoxicity. Dourakis et al. (2002) reported a patient with acute icteric hepatocellular necrosis in Greece, who used *Teucrium capitatum* because of hypercholesterolaemia and hyperglycaemia. Moreover, *Teucrium viscidum* has been linked to hepatotoxicity. Poon et al. (2008) reported a case of acute hepatotoxicty from a patient in China who used a preparation of *Teucrium viscidum* for low back pain.

8.3.1 Mechanism of Action

At the time of the first records of hepatotoxicity related to *Teucrium* spp. and the resulting restrictions to the use of germander containing products, studies relevant to the phytochemical profiles of the *Teucrium* plants have not been conducted yet, thus the chemical constituents which were responsible for the hepatotoxicity remained unknown (Stickel et al. 2000). The next step was the evaluation of wild germander hepatotoxicity in animal studies, both in vivo and in vitro (Kouzi et al. 1994; Loeper et al. 1994; Lekehal et al. 1996; Fau et al. 1997). Except from *Teucrium chamaedris*, also *Teucrium polium* has been evaluated in animal models. More specifically, studies in diabetic rats proved the hypoglycaemic properties of *Teucrium polium*, but also revealed hepatotoxic effects (Zal et al. 2001; Shahraki et al. 2007). In addition, Mehdinia et al. (2013) reported decreased body weight and changes in liver parameters in experiments with petroleum ether extracts of *Teucrium polium* in mice. Regarding *Teucrium stocknaum,* a toxicological investigation in rats did not revealed significant hepatotoxicity rather than neurotoxicity (Tanira et al. 1996)

Regarding the mechanism of action, studies in mice revealed that the hepatotoxic constituents of germander are the furano-*neo*-clerodane diterpenes, which are transformed into their reactive metabolites by P450, especially CYP3A (Kouzi et al. 1994; Loeper et al. 1994). Kouzi et al. (1994) showed that the exodiation of the furan ring of those diterpenes through the CYP3A-metabolism is required for the hepatotoxicity of teucrin A, a major diterpene from the aerial parts of *Teucrium chamaedrys*. Further studies in isolated rat hepatocytes showed that teucrin A and teuchamaedrin.

A (Fig. 8.4) are being metabolized in electrophilic derivatives, which in turn cause apoptosis through decreasing of cellular thiols and [Ca^+]-involving mechanisms (Lekehal et al. 1996; Fau et al. 1997). Covalent modification of proteins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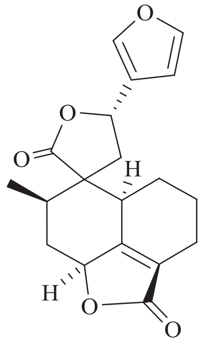
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Fig. 8.4 Stuctures teucrin A (1) and teuchamaedrin A (2)

(mitochondrial, endoplasmatic, cytosolic, peroxisomal and secreted proteins) has been linked to the hepatotoxicity of the reactive metabolites of teucrin A (Druckova et al. 2007). Furthermore, these reactive metabolites may cause hepatotoxicity through an immunoallergic reaction, as anti-microsomal epoxide hydrolase autoantibodies have been found in the sera of patients who have been long term using germander products (De Berardinis et al. 2000; Larrey and Faure 2011).

8.3.2 Hepatoprotective Activity, Cell Cultures and Possible Protection from Total Polyphenolics

A survey on the literature reveals some controversial studies. Panovska et al. (2007) evaluated the hepatoprotective activity of ethyl acetate extract of *Teucrium polium* against carbon tetrachloride induced liver damage in rats, and reported significant liver regeneration after 7 days injection of *Teucrium polium* extract. These results are in accordance with some similars studies (Al-Kubaisy 2013; Rahmouni et al. 2019). Forouzandeh et al. (2013) further evaluated the hepatoprotective activity of this plant, and reported significant inhibition of acetaminophen-induced hepatotoxicity in mice. Similar results were reported for another member of the genus, *Teucrium polium-geyrii*, attributed to its phenolic content (Baali et al. 2016). Moreover, a safety data review (De Vincenzi et al. 2003), reports that teucrin A from *Teucrium chamaedrys* showed no mutagenic effects and no hepatotoxicity, as the changes in body weights of the rats in comparison to the absence of other morphological findings can be regarded as an adaptative metabolic effect.

Teucrium chamaedrys contains substances that are both hepatoprotective and hepatotoxic, saponines and *neo*-clerodane diterpenoids, respectively (Stickel et al. 2000). A study revealed that cell cultures from leaf explants of *Teucrium chamaedrys* (both solid and liquid) continue to biosynthesize phenylethanoid glycosides, especially teucrioside, but not *neo*-clerodanes (Antognoni et al. 2012). More recently, two cases of acute hepatotoxicity induced by the use of *Teucrium chamaedrys* were evaluated in relation to the diterpene concentration and differences in the preparation of the traditional family decoction, which had been used without toxic effects. The study showed the patients used a decoction, in which the extraction time was much higher, resulting in high levels of teucrin A, low antioxidant activity and total polyphenolics content in comparison to the traditional family preparation (Nencini et al. 2014).

8.4 Conclusions

The current review reveals that *Teucrium* genus contain several toxic species, the most well known are *Teucrium chamaedrys*, *T. polium* and *T. capitatum*, responsible for several cases of hepatotoxicity in S. Europe, where the plants were used as herbal products for weight loss and still are used in folklore medicine to treat hypercholesteraemia and diabetes. The use of these plants as herbal medicines and dietary supplements should be strictly controlled.

The plants have been reported to contain a plethora of secondary metabolites, belonging to terpenes, phenolics, alkaloids and essential oils. The *neo*-clerodane diterpenoids occurring in *Teucrium* species have been the subject of intensive studies, since they have been correlated to the toxic effects of the genus.

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Chapter 9
Genotoxic Activity of Secondary Metabolites of *Teucrium* Species

Darko Grujičić, Dragoslav Marinković, and Olivera Milošević-Djordjević

Abstract *Teucrium* species have been used in traditional medicine for the treatment of different diseases and for this reason, it is very important to know their effect on the human genome. In this chapter are presented literature data of genotoxic potential of secondary metabolites (phenolic acids and flavonoids) and different extracts from *Teucrium* species using different valid methods for the detection of genotoxicity. The presented results of investigating the genotoxic effect of methanolic extracts from *Teucrium chamaedrys, T. montanum, T. scordium, T. arduini* and *T. flavum*, methanolic, ethanolic, and aqueous extracts from *Teucrium polium*, as well as methanolic, ethanolic and chloroform extracts from *Teucrium ramosissimum* using micronucleus assay in human peripheral blood lymphocytes (PBLs), Comet assay in PBLs, Ames test/*Salmonella typhimurium*. It is known that the biological activities of the herbal extracts associated with its phytochemical composition. Phytochemical analyses of examined extracts of *Teucrium* species showed that they were rich in polyphenolic compounds and could be considered responsible for antimutagenic properties of the plants. Presented results have shown that the investigated of the *Teucrium* species possess the protective properties against antineoplastic drug-induced genetic damage in a different type of cells. There are two possible genoprotective effects, antimutagenic/antioxidative (*Teucrium polium* and *T. ramosissimum*) and proapoptotic (*Teucrium chamaedrys, T. montanum, T. scordium, T. arduini* and *T. flavum*). The investigated *Teucrium* species showed a genotoxic effect in high concentrations, while in combination with known mutagenic in the same concentrations had a protective effect. Presented investigations from dif-

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ferent genotoxicity studies have showed that secondary metabolites have a genoprotective effect on different types of cells (PBLs, mice bone marrow cells, PCEs; Chinese hamster ovary cells, CHO; bacteria *Salmonella typhimurium*). Moreover, present results clearly showed that tested extracts *Teucrium* species had a protective effect on the genotoxicity of different agents (mitomycin C, MMC; N-methyl-N-nitro-N-nitrosoguanidine, MNNG; benzo[a] pyrene, B[a]P; aflatoxin B1, AFB1; hydrogen peroxide, H_2O_2) in a different type of cells in vitro and, therefore, they can be a potential source of new pharmaceutics that will have a significant protective effect on induced genetic damage by chemotherapeutics in healthy cells.

Keywords Genotoxic activity · Secondary metabolites · *Teucrium* species · Methanolic extracts · Flavonoids · Phenolic acids

Abbreviations

BN	Binucleated Cells
CA	Chromosome aberrations
CBMN	Cytokinesis Block Micronucleus Assay
CHO cells	Chinese hamster ovary cells
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1- picrylhydrazyl radical
EMS	Ethyl methanesulfonate
H_2O_2	Hydrogen peroxide
HPLC	High-Performance Liquid Chromatography
MMC	Mitomycin C
MN	Micronuclei
NDI	Nuclear Division Index
PBLs	Peripheral blood lymphocytes
PCEs	Polychromatic erythrocytes
SD	Standard deviation

9.1 Introduction

Plants have been used for centuries in the treatment of many human diseases. According to data from the World Health Organization (WHO), about 70–80% of the world's population in developing countries relies on plants for their primary health care (Chan 2003). Medicinal plants can be considered the main source of new chemical substances with potential therapeutic effects (Blumenthal 2000; Celik 2012). It is known that the specific parts of the herb (aerial parts, root, leaves, fruit, flowers, and seeds) are already being used in form of suitable preparations (tablets, teas, extracts, creams or tinctures) in therapeutic purposes (Saad et al. 2017).

Teucrium L. (Lamiaceae) is a large, polymorphic, and cosmopolitan genus of perennial plants, the largest member of the family Lamiaceae distributed in Europe, North Africa, and the temperate parts of Asia, but mainly in the Mediterranean area, including more than 300 species (Tutin et al. 1972). In the flora of Europe, the genus *Teucrium* has been divided into seven sections with 49 species.

The preservation of the genetic integrity of human populations is very important, and any disturbance can either induce or contribute to, the onset of disease. It is known that are the genotoxic agents involved in the pathogenesis of several chronic degenerative diseases, including neurodegenerative and cardiovascular disorders, diabetes, chronic inflammation, cancer and aging (Lopez-Romero et al. 2018). Genotoxic agents present in the environment and occupational are numerous, and they are divided into physical, chemical and biological, and according to their origin, they can be natural and synthetical. The mutagenic effect they produce can be on somatic or germ cells are showed in Fig. 9.1.

Precisely for these reasons that a relatively new scientific discipline, genotoxicology, has been created, the primary goal of which is the detection of genotoxic agents, which ability to induce genetic damage regardless of the level of life organization, from bacteria to human cells (Izquierdo-Vega et al. 2017). Over the last 30 years, different methodologies, strategies and approaches have been developed to asses chemicals that could demonstrate genotoxic and/or carcinogenic effects, in vitro or in vivo, on somatic or germ cells (Sponchiado et al. 2016; Lopez-Romero et al. 2018). Accordingly, at what level the changes in genetic material are detected,

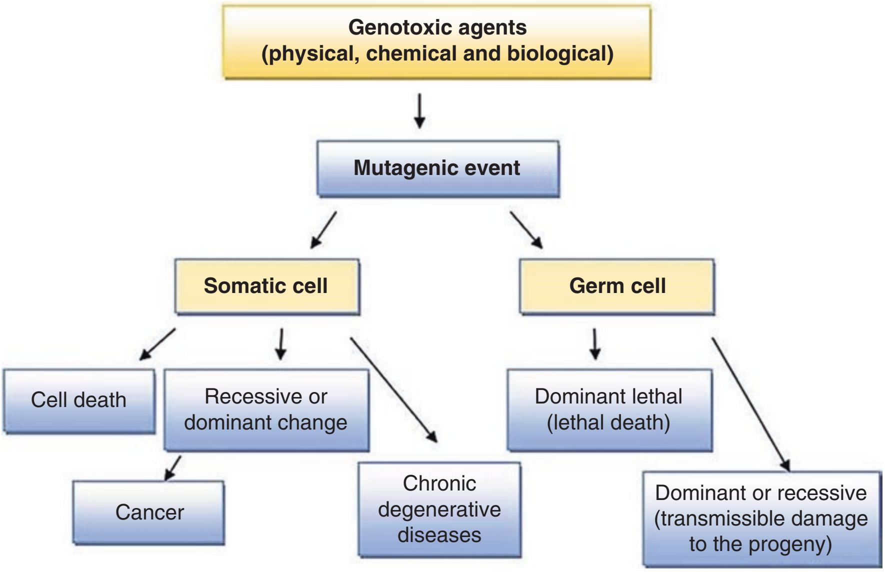


Fig. 9.1 Effect of genotoxic agents on mutagenic event in somatic and germ cells

all tests are divided into (1) tests detect changes in the level of DNA molecules (sister chromatid exchanges, SCE; single cell gel electrophoresis SCGE, or Comet assay); (2) gene mutation detection tests (Ames test, bacteria *Salmonella typhimurium*; SLRL test; *Drosophila melanogaster*); and (3) tests for detection chromosomal damage (chromosomal aberrations, CA; micronucleus test, MN).

The CA, SCEs, and MN assay in PBLs, as well as Comet assay, are used as the most useful assays to detect the potential genotoxicity of different agents (Joksić 1990; Celik 2012). They have been considered to be markers of early biological effects of carcinogen exposure (Liou et al. 2002). Numerous studies are showed that different drugs presented genotoxic effect in human periphelal blood lymphocytes (PBLs) in vivo (Milošević-Djordjević et al. 2003; Dimitrijević et al. 2006; Grujičić et al. 2007, 2008) and in vitro (Grujičić et al. 2009; Milošević-Djordjević et al. 2010a, b); as well as environmental contamination (Milošević-Djordjević et al. 2004, 2005, 2007). Traditionally, short term tests (STTs) for evaluating the genotoxic potential of hazardous chemicals were introduced and modified decades ago. STTs include the Ames test, in vivo cytogenetics tests, and the MN assay (Ren et al. 2017).

Each test has its advantages and disadvantages, but, overall, it is sensitive methods that are rapid, simple, and able to evaluate the genotoxic effect, provide an opportunity for assessment antigenotoxic effect in the same cells. The compounds that reduce the DNA damage caused by genotoxic agents called antigenotoxic agents (De Flora and Ferguson 2005; Ferguson et al. 2005). Numerous studies have shown the antigenotoxic potential of different plants (Behravan et al. 2011; Andrade et al. 2016; Lopez-Romero et al. 2018), mushroom (Tubić et al. 2019; Kosanić et al. 2019), and lichens (Turkez et al. 2012; Grujičić et al. 2014; Kosanić et al. 2016).

The detection of novel bioactive phytocompounds in counteracting mutagenic and carcinogenic effects is now gaining credence. In recent decades, numerous studies have been carried out in the last decades in order to identify compounds that might protect humans against DNA damage and its consequences. There are continual efforts all over the world to explore the rich biodiversity of edible (fruits, vegetables) as well as medicinal plants and other edible non-toxic plants in pursuit of the most effective phyto-antimutagens (Bhattacharya 2011; Lopez-Romero et al. 2018). In general, the antimutagens have been classified as desmutagens and bio-antimutagens. According to Kada and Shimoi (1987) antimutagenic agents are divided into two large groups. The first group is desmutagens, agents that can inactivate the mutagenic agents extensively before they reach the DNA and prevent the formation of premutation lesions. The second group belongs to bio-antimutagens, who reduce the effect of mutagens by acting inside the cell and participating in the suppression of mutations after DNA damage. Prevent treatment of premutation lesion in mutations, modulating replication and repair of DNA molecules (Fig. 9.2).

Intensive research over the last few decades has shown the beneficial protective effects of medicinal plant extracts on different cell cultures both in vitro (Ljubuncic et al. 2006; Leskovac et al. 2007; Milošević-Djordjević et al. 2013, 2018) and

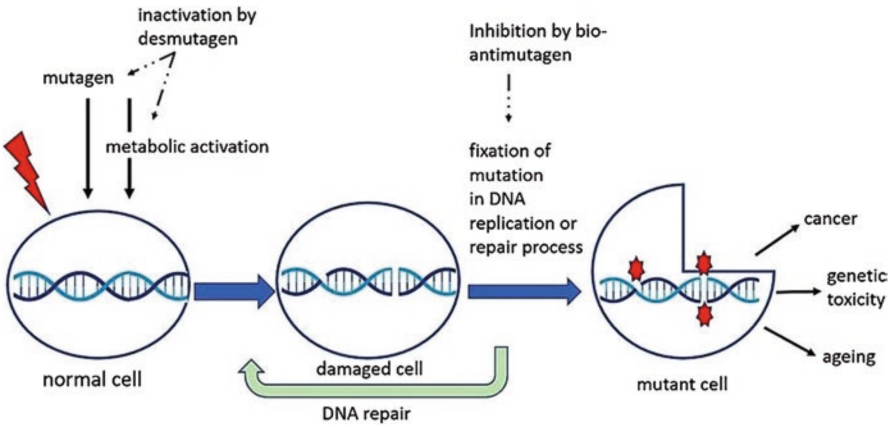


Fig. 9.2 Schematic representation showing the process of antimutagenesis

in vivo conditions (Damasceno et al. 2016; Rahmouni et al. 2017). Numerous studies were showed that species of the *Teucrium* genus have significant biological effects such as antimicrobial, antioxidant, cytotoxic and anti-cancerogenic properties. In Table 9.1 presented some of the biological effects and application in the traditional medicine of *Teucrium* species.

Genotoxicity assessment is an indispensable component in the safety assessment, aiming to prevent certain substances from affecting human health. Concerning the plant use, especially medicinal, long historical use of many practices of traditional medicine has indicated the safety and efficacy of traditional medicine. On the other side, some of studies have revealed that some plants frequently used in traditional medicine are potentially genotoxic (Marques et al. 2003; Ananthi et al. 2010; Mello-Reis et al. 2011; Regner et al. 2011; Shin et al. 2011). Therefore, it is extremely important to assess genotoxicity of plant extracts or secondary metabolites in order to verification their mutagenic potential since medicinal plants are widely used in traditional medicine and as a resource for the development of new drugs (Mello-Reis et al. 2011). However, to provide its efficacy more scientific research is needed. Moreover, in order to evaluate the genotoxicity risk of medicinal plants, it is necessary to review the literature and, as well, explore the genotoxicity assays most used and, then, compare these to the current legal requirements (Šavikin et al. 2013; Harutyunyan et al. 2019).

In relation to the described biological effects, the genotoxic properties of *Teucrium* species have been slightly studied. Accordingly, the purpose of this chapter is to collect available data on previous genotoxicity studies of *Teucrium* species. This chapter unifies scientific knowledge currently and to answer some questions concerning the genotoxic and antimutagenic properties of the investigated *Teucrium* species. In this chapter, we are showing the genotoxic and antimutagenic effect of different extracts of so far tested species *Teucrium* species.

Table 9.1 Biological effects and application in the traditional medicine of analysed *Teucrium* species

Teucrium species	Application in traditional medicine	References	Biological effects	References
T. chamaedrys	Gastrointestinal disorders: diarrhea, metabolic disorders, diuretic	Redžić (2007) and Jarić (2007)	Anti-inflammatory Antirheumatoid Antioxidative	Redžić (2007) and Jarić (2007)
T. montanum	Gastrointestinal disorders	Redžić (2007) and Jarić (2007)	Anti-inflammatory Antibacterial Antioxidative	Redžić (2007) and Jarić (2007)
T. polium	Gastrointestinal disorders, inflammation, eczema, urinary tract inflammation, diabetes, rheumatic disease mental performance	Everest and Ozturk (2005), Abu-Irmaileh and Afifi (2003), Said et al. (2002) and Perry et al. (1996)	Anti-inflammatory Antirheumatic Antimicrobial Antihypertensive Hypolipidemic Hypoglicemic Antioxidative	Balmekki et al. (2013), Kasabri et al. (2011), Kadifkova-Panovska et al. (2005), Rasekh et al. (2001), Tariq et al. (1989) and Suleiman et al. (1988)
T. scordium	Gastrointestinal disorders: diarrhea, fever, hemorrhoids, and intestinal parasites, bronchial ailments	Morteza-Semnani et al. (2007)	Anti-inflammatory	Morteza-Semnani et al. (2007)
T. arduini	Gastrointestinal disorders	Redžić (2007) and Jarić (2007)	Antimicrobial Antiproliferative Antiphytoviral Antioxidative	Kremer et al. (2013), Stanković et al. (2012), Dunkić et al. (2011), Šamec et al. (2010) and Vuković et al. (2010)
T. flavum	Heal skin wounds gastrointestinal disorders	Dall'Acqua et al. (2008), Bellomaria et al. (1998) and Calatayud et al. (1998)	Anti-inflammatory Antidiabetic Hypotensive Antioxidant	Dall'Acqua et al. (2008), Bellomaria et al. (1998), Calatayud et al. (1998) and Barrachina et al. (1995)

9.2 Evaluation of Genotoxic and Antimutagenic Activities of Medicinal Plants

9.2.1 PBLs as a Model System in Genetic Toxicology

Studies on cultured cells have shown that the PBLs is an extremely sensitive indicator in vitro and in vivo induced chromosome structural changes (Lerda et al. 2005; Milošević-Djordjević et al. 2013; Turkez et al. 2015). The PBLs are the best material for determination of cytogenetic effects (Eroglu et al. 2011; Grujičić et al. 2016; Milošević-Djordjević et al. 2018). PBLs have proved to be exceptional markers of exposure to a variety of mutagenic agents, since they represent long living cells able to accumulate various types of cytogenetic damages for years. Via the blood circulation lymphocytes are distributed through the entire organism. Thus, the chromosomal aberrations are not just the indicators of the state of the lymphocytes in the circulation but can also be induced in any part of the organism (Lou et al. 2007; Holland et al. 2011: Fortin et al. 2015).

9.2.2 MN Assay in Genetic Toxicology

For screening chromosomal damage in PBLs, MN assay is the most frequently used assay (Fenech 2000; Fenech and Bonassi 2011). Otherwise, Countryman and Heddle (1976) are the time were the first who detected MN in these cells investigating chromosome aberrations. MN test is one of the valid tests used as a quick check for detection of acute disorders caused by chemical substances in cells that have undergone cell division. Following the frequency of MN gives us insight into the level of chromosomal aberrations in mutagen-exposed cells (Speit et al. 2012; Kirsch-Volders et al. 2014; Ghosh and Godderis 2016). MN originates mainly from either acentric chromosome fragments or whole chromosomes. Acentric chromosome fragments originate from unrepaired DNA strand breaks or misrepaired DNA strand breaks. These acentric fragments often occur in conjunction with forming dicentric chromosomes. MN may also arise from dicentric chromosome fragments during anaphase. Malsegregation of whole chromosomes may be induced if the chemical either causes centromere/kinetochore malfunction or impairs the mitotic spindle or centrosome (Fenech et al. 2011; IAEA 2011; Kirsch-Volders et al. 2011; Luzhna et al. 2013; Fortin et al. 2015; Guo et al. 2019).

MNs are formed spontaneously or are induced, as a cellular response to the effects of genotoxic agent. For years, the presence of MN in PBLs has been used as biological dosimetry at exposure to ionizing radiation (Joksić 1990; Vral et al. 2011; Ryu et al. 2014, 2016), as well as to chemical genotoxic agents in working and living environment (Huang et al. 2009). Several studies have shown that air pollution can contribute to the increase of MN frequency in adults, as well as in children (Neri et al. 2006; Milošević-Djordjević et al. 2007; Hallare et al. 2008; Feretti et al. 2014).

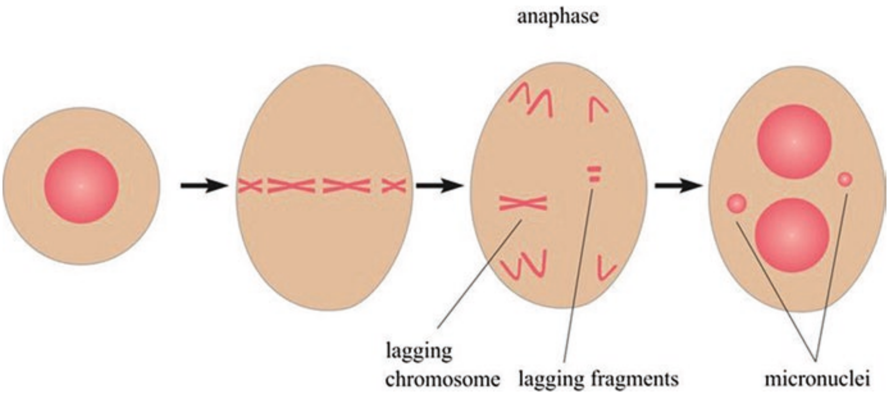


Fig. 9.3 Mechanisms of micronuclei formation during cell division

The correlation between exposure to various inorganic chemicals, among which heavy metals were the most prominent, and the MN frequency has also been determined, mostly in workplace settings (Bonassi et al. 2005). Research has shown that MN frequency can also be influenced by dietary, genetic, and some lifestyle factors (Bonassi et al. 2011; Fenech and Bonassi 2011; Kadioglu et al. 2012). High MN frequency in PBLs indicates a high level of CAs, and, therefore, MN is an important biomarker for the risk assessment and prediction of cardiovascular disease (Andreassi and Botto 2003), and different cancers (Bonassi et al. 2007; Milošević-Djordjević et al. 2010a, b, 2011a, b; Vrndić et al. 2013; Stošić et al. 2014; Pardini et al. 2017). MN is a small additional nucleus which is located near the main nucleus in the cytoplasm (Fig. 9.3). Its diameter is usually between 1/16 and 1/3 of the diameter of the main nucleus. They are mostly oval or round, but they can also be pyramidal, hemispheric, elliptical, cylindrical or very rarely irregular. MN adopts the same texture as the main nucleus. Generally, there is only one MN in a cell, but occasionally there can be two. Some cells can contain triple or more MN, but very rarely (Fenech et al. 2003; Fenech 2007).

One of the most commonly used cytogenetic assays in the genotoxic assessment of different agents is the cytokinesis-block micronucleus (CBMN) assay in cultivated human PBLs and is simultaneously used as a method in human biomonitoring studies (Decordier et al. 2009; Speit et al. 2011). Fenech and Morley have developed the CBMN assay in human lymphocytes (Fenech and Morley 1985). It is one of the most commonly used methods for measuring structural and numerical chromosomal changes in human cells in vitro and in vivo (Fenech 2007; Fenech et al. 2011; OECD Library 2010; Nersesyan et al. 2016; Gajski et al. 2018). The advantage of CBMN assay is its speed and simplicity, as well as the possibility of MN frequency analysis in binucleated (BN) cells. Using this test, thousands of lymphoblasts can be analyzed on a single-sample basis, which provides a more objective viewpoint as far as the damage to the genetic material is concerned. CBMN assay ensures simultaneous information on chromosomal breakage, chromosomal rearrangement, and gene amplification. MN test has a great advantage over other cytogenetic tests because it offers tools for a damage assessment at the level of function and mitotic spindle integrity (Mateuca et al. 2006).

The analysis of CBMN assay is based on the cultivation of PBLs and it allows the identification of cells that have undergone only one division in culture. These results were achieved with cytochalasin B. It was added 44 h after the beginning of incubation, before the first mitosis, to block the division of cytoplasmic cytokinesis without inhibiting nuclear division. This is the mechanism of the formation of BN cells. Cytochalasin B may help to establish the difference among mononuclear cells (those that did not divide in vivo) or those that have completed one or more nuclear divisions. Generally, MN has been used as a biomarker of genome instability, chromosomal damages and cancer risk, integrating acquired mutations and genetic susceptibility towards mutations (Holland et al. 2008; Iarmarcovai et al. 2008; Vrndić et al. 2013). In recent years, the CBMN assay has evolved into a "cytome" assay (CBMN Cyt) of chromosomal instability in which more nuclear anomalies and many more cells in the sample can be included in the analysis and categorized to provide a more comprehensive view of DNA damage, cytostasis, and cell death. The key components of the CBMN Cyt assay are nuclear buds (NBUDs), nucleoplasmic bridges (NPBs) and apoptotic and necrotic cells (Fenech 2006, 2007).

In addition to chromosomal aberrations, the second cytogenetic parameter that can be detected by the CBMN assay is the nuclear division index (NDI), as a significant indicator proliferative status of the cells. NDI is calculated according to the formula proposed by Eastmond and Tucker (1989) as follows: NDI = ((1 × M1) + (2 × M2) + (3 × M3) + (4 × M4))/N, where M1 to M4 represents the number of cells with one to four nuclei and N is the total number of cells scored (Fig. 9.4). The NDI is the parameter that shows the number of cell cycles through which the treated cells have passed in culture (Surralles et al. 1995; Fenech 2000). Having determined NDI value we can also establish the cytostatic and cytotoxic effect in the lymphocytes that can be the level of mutagenic response (Fenech 2007). The numerous authors proposed NDI as a screening marker for different cancers (El-Zein et al. 2011; Ionescu et al. 2011) because is NDI marker of cell proliferation in cultures and is considered a measure of general toxicity (Speit 2013; Ionescu et al. 2015). Cells with greater chromosomal damage will either die before cell division or may be less able to enter in this phase (Santos-Mello et al. 2004; Minozzo et al. 2004). Therefore,

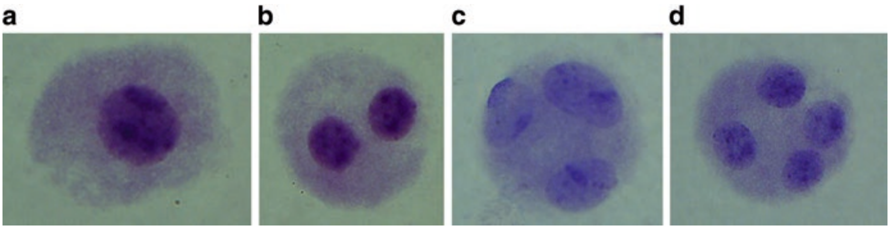


Fig. 9.4 Representative image of cells for calculating NDI: (**a**) mononucleated cell; (**b**) binucleated cell; (**c**) trinucleated cell; (**d**) tetranucleated cell

more a cell will accumulate genetic alterations, less it will be able to divide, and NDI will be lower (Ionescu et al. 2015).

CBMN assay is widely used to determine the mutagenic effect of both physical (Das and Karuppasamy 2009) and chemical agents (OECD Library 2010; Kirsch-Volders et al. 2014). According to a number of authors, this method is used as an alternative to fixed metaphase analysis. It is particularly suitable for population monitoring studies, when fast and accurate information, whether a substance is genotoxic or not, is wanted. For that reason, there has been a growing interest in the use of the MN assay to detect genotoxic agents. The latest research showed that the MN assay as a test is most frequently used in the evaluation of genotoxic risks of medicinal plants (Sponchiado et al. 2016).

9.3 Phytochemical Characteristics of the Examined *Teucrium* Species

Phenolic compounds are products of secondary plant metabolism and occur during normal development of plants, but also in stress conditions, for example during UV radiation, infection, after injury, etc. In the past research of secondary plant metabolites, more than 8000 phenolic compounds have been identified (Bravo 1998; Ferguson 2001; Ghasemzadeh and Ghasemzadeh 2011; Kenndey 2014). These compounds are present in all plant organs, leaves, flowers, tree, root, fruits and seeds of plants (Stanković 2012). There are different classifications of phenolic compounds with more than 15 classes different in chemical structure: from simple molecules to high molecular weight polymers. The most common polyphenolic compounds are divided into phenolic acids, flavonoids, stilbene, and lignans.

Phenolic acids are hydroxybenzoic acid and hydroxycinnamic acid derivatives. The most prominent benzoic acid derivatives are gallic acid, protocatechuic acid, vanillic acid, and the most commonly derivatives of cinnamic acid are the *p*-coumaric, caffeic and ferulic acid. Flavonoids are a class of polyphenol secondary metabolites. They are presented broadly in plants and diets and have various bioactive effects (antiviral, anti-inflammatory, cardioprotective, antidiabetic, antiaging, etc.). Moreover, flavonoids, shown to have anticancer activity notably. This was confirmed through numerous in vitro and in vivo studies (Havsteen 2002; Esmaeili et al. 2014; Hammami et al. 2015; Veeramuthu et al. 2017).

Flavonoids are frequently found as glycosylated or esterified forms, consisting of C6—C3—C6 rings, namely rings A and B linked by three-carbon-ring C. Due to their poor bioavailability, however, information about associations between structure and biological fate is limited. This review, therefore, attempts to bring some order into relationships between structure, activity as well as pharmacokinetics of bioactive flavonoids (Wang et al. 2018). Flavonoids are a group of natural compounds with variable phenolic structures and are found in plants. In 1930 a new substance was isolated from oranges. At that time, it was believed to be a member of a new class of vitamins and was designated as vitamin P (Batra and Sharma 2013). Later it became clear that this substance was a flavonoid (rutin) and till now more than 4000 varieties of flavonoids have been identified (Middleton 1998; Kumar and Pandey 2013). They can be divided into a variety of classes such as flavones (apigenin, luteolin, and chrysin), flavonols (quercetin, kaempferol, and myricetin), flavanones (naringenin, and hesperetin), and others (Veeramuthu et al. 2017).

The extracts of the investigated *Teucrium* species had a very rich content of secondary metabolites such as phenolic compounds (Stanković 2012; Milošević-Djordjević et al. 2018). The most important polyphenolic compounds in plants are phenolic acid and flavonoids (Petti and Scully 2009; Khoddami et al., 2013). They play an important role in reducing the risk of cardiovascular disease, as antioxidants, and exhibited a wide variety of other biological activities, including hepatoprotective and antibacterial activities (Tiwari 2001). Due to their importance in plants and human health, it would be useful to know the concentration of the polyphenolic compounds and biological activities that could indicate their potentials as therapeutic agents (Benedec et al. 2013).

Results of qualitative analysis of methanolic extracts of examined *Teucrium* species are presented in Table 9.2. The analyzes were performed by High-performance liquid chromatography – HPLC (Stanković 2012; Milošević-Djordjević et al. 2018; Grujičić et al. 2020). Presented results showed that the most prevalent phenolic acids were chlorogenic acid (for *Teucrium chamaedrys* and *T. polium*), caffeic acid (for *Teucrium arduini* and *T. flavum*) and rosmarinic acid (for *Teucrium montanum*).

Table 9.2 The of polyphenolic contents of methanolic extract from analyzed *Teucrium* species

Teucrium species	Phenolic acids ↑ ↓		Flavonoids ↑ ↓		References
T. chamaedrys	Chlorogenic acid	Caffeic acid *p*-coumaric acid	Rutin	Myricetin Kaemferol	Stanković (2012)
T. montanum	Rosmarinic acid	Caffeic acid *p*-coumaric acid	Rutin	Kaempferol Myricetin	
T. polium	Chlorogenic acid *p*-coumaric acid	Caffeic acid Vanillic acid	Catechin Apigenin	Luteolin Quercetin	Milošević-Djordjević et al. (2018)
T. scordium	Vanillic acid *p*-coumaric acid	Sinapic acid	Catechin	Rutin	
T. arduini	Caffeic acid Ferulic acid	Chlorogenic acid Vanillic acid	Quercetin	Epicatechin Rutin	Grujičić et al. (2020)
T. flavum	Caffeic acid *p*-coumaric acid	Vanilic acid Chlorogenic acid	Quercetin	Epicatechin Catechin	

P-coumaric acid was present in *Teucrium polium*, *T. scordium* and *T. flavum* extracts, as a second most abundant phenolic acid. Analyzing the content of flavonoids, present results showed that *Teucrium chamedrys* and *T. montanum* extracts contained the highest content of rutin, while the lowest contents were measured for myricetin and kaempferol. The extracts of *Teucrium polium* and *T. scordium* had the highest content of catechin and apigenin, while the lowest contents were measured for luteolin and rutin. Both extracts of *Teucrium arduini* and *T. flavum* contained the highest content of quercetin, and the lowest contents were measured for epicatechin. The second most abundant flavonoid in the extract of *Teucrium polium* was apigenin.

9.4 Genotoxic Activity of *Teucrium* Extracts in PBLs

The results of the genotoxic activity of methanolic extracts of examined *Teucrium* species on PBLs of healthy donors are shown in Table 9.3 (Milošević-Djordjević et al. 2013, 2018; Grujičić et al. 2020). The results indicated that the extract of *Teucrium chamaedrys* did not show genotoxic effect in all tested concentrations, while both extracts of *Teucrium montanum* and *T. polium* only in the highest concentration (1000 μg/ml) significantly induced chromosomal damage. On the other hand, *Teucrium scordium* showed genotoxic effect in the two highest tested concentrations (500 and 1000 μg/ml). Also, the high concentrations (250–1000 μg/ml) extracts of *Teucrium arduini* and *T. flavum* have a genotoxic effect. In relation to cellular kinetics, present results have show that, in separately treatment PBLs, only extract of *Teucrium scordium* significantly decreased NDI values in two highest tested concentrations (500 and 1000 μg/ml), while others plant extracts did not change NDI values in comparation to negative control (untreated) cells.

All plant extracts, except *Teucrium chameaedrys*, in a separately treatment, with an increased concentration, showed a genotoxic effect. *Teucrium arduini* and *T. flavum* showed a genotoxic effect at concentrations from 250 to 1000 μg/ml, while at the lowest test concentration of extracts (125 μg/ml) none of the six analyzed plants showed a genotoxic effect. None of the plants influence on cells kinetics, except *Teucrium scordium*, which in higher concentrations (500 and 1000 μg/ml) significantly decreased NDI. These results may be explained by high levels of flavonoids contained in higher concentrations of the extract which can possibly contribute to the pro-mutagenic effect, as pro-oxidants in generating free radicals that damage DNA. Stopper et al. (2005), Silva et al. (2000) and Boos and Stopper (2000) showed that flavonoids may cause genetic damage in a variety of prokaryotic and eukaryotic systems. Also, numerous studies provide evidence for dual biological activities of flavonoids, which are showed that in high concentrations can act both protective and mutagenic (Bhattachar 2011; Kunwar and Priyadarsini 2011; Baldim et al. 2017). The possible mechanism has shown in Table 9.4.

The anti-mutagenic/pro-mutagenic and antioxidant/pro-oxidant activities largely depend on the concentration used (Labieniec et al. 2003; Labieniec and Gabryelak 2003; Stopper et al. 2005), as well as the physiological parameters and

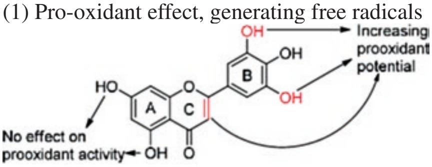
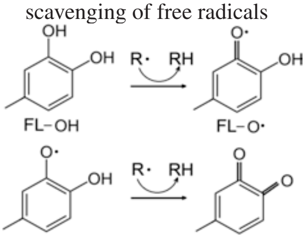
Table 9.3 The results of genotoxic activities of different methanolic extracts concentrations from *Teucrium* species in vitro

Teucrium species	Concentration (μg/ml)	MN in comparison to negative controls	NDI in comparison to negative controls	Effect	References
T. chamaedrys	125	No sig. difference	No sig. difference	No genotoxic	Milošević-Djordjević et al. (2013)
	250				
	500				
	1000				
T. montanum	125	No sig. difference	No sig. difference	No genotoxic	
	250				
	500				
	1000	Sig. ↑ MN*		Genotoxic	
T. polium	125	No sig. difference in MN	No sig. difference	No genotoxic	Milošević-Djordjević et al. (2018)
	250				
	500				
	1000	Sig. ↑ MN*		Genotoxic	
T. scordium	125	No sig. difference	No sig. difference	No genotoxic	
	250				
	500	Sig. ↑ MN*	Sig. ↓ NDI*	Genotoxic	
	1000				
T. arduini	125	No sig. difference	No sig. difference	No genotoxic	Grujičić et al. (2020)
	250	Sig. ↑ MN*		Genotoxic	
	500				
	1000				
T. flavum	125	No sig. difference	No sig. difference	No genotoxic	
	250	Sig. ↑ MN*		Genotoxic	
	500				
	1000				

*significant difference in comparison to negative controls; *sig.* significant

physiological conditions in the body (Skibola and Smith 2000; Noel et al. 2006). The genotoxic damage caused by flavonoids at higher concentrations may be due to DNA intercalation and inhibition of DNA topoisomerase II and inhibition of key enzymes involved in hormone metabolism (Skibola and Smith 2000; Sharma 2013). Some phenolic acids which otherwise as well-known antioxidants are, possible can be pro-oxidants, depending on concentration, and thus being a significant cause of DNA damage. Accordingly, Erdem et al. (2012) demonstrated that a high dose of the vanillic acid itself had genotoxic effects on DNA in PBLs in vitro, and when used at an appropriately low dose this acid could prevent oxidative DNA damage. Similar results were obtained by Bhat et al. (2007) that caffeic acid induced prooxidant DNA breakage in PBLs.

Table 9.4 The possible mechanism of the protective/mutagenic effect of high concentrations of flavonoides in vitro

Mutagenic effect	Protective effect
(1) Pro-oxidant effect, generating free radicals  Structure–prooxidant activity relationship in flavonoids (Eghbaliferiz and Iranshahi 2016) (2) DNA interaction and inhibition of DNA topoisomerase II, enzyme who play importance role in the mitotic chromosome segregation after DNA replication (Skibola and Smith 2000)	(1) Genoprotective: (a) Inhibition of genotoxic effects- antioxidant effect and scavenging of free radicals  Scavenging of ROS (R*) by flavonoids (Fl-OH) (Gupta et al. 2010) (b) proapoptotic effect for cells with collected chromosome damage (Milošević-Djordjević et al. 2013, 2018)
(3) Inhibition of key enzymes involved in hormone metabolisms, and alter the activity other key metabolizing enzymes (Sharma 2013)	(2) Modulation of signal transduction pathways (3) Induce antioxidant enzymes and detoxifying enzymes (Bahramikia and Yazdanparast 2012)
(4) Unrepaired or misrepaired oxidative DNA strand breaks and mutation (Skibola and Smith 2000)	(4) As blocking agents thus preventing the metabolic activation of promutagens (Daradka and Alshibly 2012)

Of all flavonoids, perhaps the greatest scientific interest was caused by quercetin. It is known that the prooxidant property of quercetin is important as it is related to the generation of mutagenic quinone-type metabolites. Mutagenic property of quercetin has been demonstrated in bacteria (Czeczot et al. 1990) as well as in mammalian cell culture (Silva et al. 2000), including human DNA (Duthie et al. 1997). Mazumdar et al. (2011) showed that quercetin induced MN and CAs in polychromatic erythrocytes in bone marrow cells in vivo. The findings same authors suggest that quercetin may result in genomic instability in the tested dose range and significant reduction in MMC induced genotoxicity in the highest dose tested. However, numerous studies have shown that the genotoxicity of one compound present in the extract can be alleviated by the other compound from extract when they are found together since the extracts act as a mixture of different compounds (De Marino et al. 2014; Andrade et al. 2016).

9.5 Protective Effect of Plant Extracts Against Mitomycin C (MMC) Induced Chromosome Damage in PBLs

To determine the protective effects of plant extracts against MMC-induced chromosomal damages in PBLs, the cell cultures were treated with the tested concentrations of extracts in combination with MMC (mitomycin C). MMC is a natural antitumor antibiotic drug used in clinical chemotherapy regimens in various carcinomas (Verweij and Pinedo 1990; Sontakke and Fulzele 2009). However, MMC induces chromosomal aberrations as a non-cell-cycle-specific agent, therefore showing strong genotoxic effects in different cell lines (Ho and Schärer 2010; Vasquez 2010). During metabolism, the MMC can generate reactive oxygen species (ROS) such as superoxide radical anions, hydrogen peroxide and hydroxyl radicals (Albertini et al. 2003; Ortega-Gutierrez et al. 2009). ROS induce numerous lesions in DNA including deletions, base modifications and single and double-strand breakages (Cadet et al. 1994; Tiwari 2001).

It is known that free radicals involve the MN formation (Vijayalaxmi et al. 1999) and MMC generates MN-induced genotoxic damage in animal models (Hayashi et al. 1992; Grisolia 2002). Thus, for example, MMC induced genotoxic effect which can be detected by different methods such as CA, SCEs (Krishnaja and Sharma 2008) and MN frequency (Fauth et al. 2000) in the cultures of PBLs. In the studies from Milošević-Djordjević et al. (2013, 2018) it was observed that MMC induced micronuclei in PBLs in comparison to untreated PBLs. These chromosomal damages were, to some extent, the result of the activity of free radicals formed during the metabolism of MMC. Similar results have been obtained in other studies that have shown that MMC caused genotoxicity in PBLs (Sontakke and Fulzele 2009; Erdem et al. 2012; Turkez et al. 2012, 2015). Therefore, MMC is often used as a positive control in genotoxicology studies (Siddique et al. 2008; Santovito et al. 2011; Akyil et al. 2015; Turkez et al. 2015).

The effects of methanolic extracts of *Teucrium* species on MN frequency and NDI in cultured PBLs treated with MMC showed in Table 9.5 (Milošević-Djordjević et al. 2013, 2018; Grujičić et al. 2020). All the tested concentrations of extracts of *Teucrium chamaedrys, T. polium, T. arduini* and *T. scordium* (except on the lowest, 125 µg/ml) significantly reduced MMC-induced MN in PBLs in a dose-dependent manner. The extract of *Teucrium montanum* significantly reduced MMC-induced MN frequency only in lowest concentration, at 125 µg/ml, however, with increasing concentration protective effect of extract decreased. The extract *Teucrium flavum* in lower concentrations (125–500 µg/ml) evident no significance effect on decrease MMC-induced MN frequency, while at the highest concentration (at 1000 µg/ml) it significantly reduces MN frequency, and expressed protective effect on PBLs. Cell proliferation was evaluated by NDI, which indicates the average number of the cell cycle in culture. Many authors showed that significant decrease of NDI in the cultures of PBLs treated with various agents in vitro was associated with the activation of apoptosis mechanism (Hess et al. 1999; Abou-Eisha et al. 2004). Present results showed that high concentrations of extracts *Teucrium chamaedrys*, *T. scordium,*

Table 9.5 Genotoxic/antigenotoxic effects different concentrations of methanolic extracts from *Teucrium* species on PBLs in vitro

Teucrium species	Concentrations extract (μg/ml) + MMC (0.5 μg/ml)	MN in comparison to positive controls	NDI in comparison to positive controls	Effect against MMC	References
T. chamaedrys	125 + MMC	Sig. ↓ MN*	No sig. difference	Protective	Milošević-Djordjević et al. (2013)
	250 + MMC				
	500 + MMC		Sig. ↓ NDI*		
	1000 + MMC				
T. montanum	125 + MMC	Sig. ↓ MN*	Significant ↓ NDI*	Protective	
	250 + MMC	No sig. difference			
	500 + MMC				
	1000 + MMC				
T. polium	125 + MMC	Sig. ↓ MN*	No sig. difference	Protective	Milošević-Djordjević et al. (2018)
	250 + MMC				
	500 + MMC				
	1000 + MMC				
T. scordium	125 + MMC	No sig. difference	No sig. difference	Protective	
	250 + MMC	Sig. ↓ MN*			
	500 + MMC				
	1000 + MMC		Sig. ↓ NDI*		
T. arduini	125 + MMC	Sig. ↓ MN*	No sig. difference	Protective	Grujičić et al. (2020)
	250 + MMC				
	500 + MMC				
	1000 + MMC		Sig. ↓ NDI*		
T. flavum	125 + MMC	No sig. difference	No sig. difference	Protective	
	250 + MMC				
	500 + MMC				
	1000 + MMC	Sig. ↓ MN*	Sig. ↓ NDI*		

*significant difference in comparison to positive controls; *sig.* significant

T. arduini and *T. flavum*, in combined treatment with MMC, a significant decrease of NDI in PBLs. The extract of *Teucrium montanum* significant reduced NDI in all tested concentrations, while extract of *Teucrium polium* non reduced NDI not even in one tested concentration.

Researches Milošević-Djordjević et al. (2013, 2018) and Grujičić et al. (2020) showed that the methanolic extracts of the genus *Teucrium* clearly indicate a protective effect against MMC-induced MN. Presented results showed that methanolic extracts of *Teucrium chamaedrys*, *T. polium* and *T. arduini* in all concentrations, reduced MMC-induced genetic damage, reducing the frequency of MN in PBLs. The most of all tested plants (*Teucrium chamaedrys*, *T. scordium*, *T arduini* and *T. flavum*) are at high concentrations affecting on cellular proliferation because NDI reduced, can be explained as a proapoptotic effect these plant extracts. It is known that a significant decrease in NDI indicates that cells with the greater chromosomal damage did not survive in culture and were entered a process of apoptosis and that they were as such eliminated (Abou-Eisha et al. 2004; Minozzo et al. 2004; Milošević-Djordjević et al. 2011a). The methanolic extract of *Teucrium montanum* is it significantly reduced the MMC-induced MN frequency at the lowest tested concentration but is in all concentrations reduces NDI in MMC treated PBLs, which also indicates its proapoptotic effect. The methanolic extract of *Teucrium polium* significantly reduced MN in all concentrations but without the reduction of NDI, which indicating the antimutagenic effect this extract. All the concentrations of *Teucrium scordium* (except the lowest one) significantly decreased the genotoxic effect of MMC. However, the strongest protective activity was observed in the highest tested concentrations.

This plant extract reduced significantly NDI at the highest concentration. The reduced proliferation of cells indicates and the proapoptotic mechanism of the action of the plant extract and the introduction of cells with significant genetic damage in apoptosis. The similar effect was showed the methanolic extract of *Teucrium flavum* which is reduced MN frequency in PBLs only in the highest concentration, and reduced significantly NDI only at the highest concentration, indicating a proapoptotic effect. Therefore, we think that MN is a possible mechanism for introduction to cell death – apoptosis. Similar results were obtained from Hess et al. (1999) and Abou-Eisha et al. (2004) who studied NDI values in cultured PBLs treated with various agents. In Fig. 9.5 were presented BN cells after MMC treatment PBLs, while in Fig. 9.6 were presented BN cells after combined treatment plant extracts and MMC. The antimutagenic effect detected in tested plants may be attributed to actions of secondary metabolites who presence in the extract. It is known that secondary metabolites to have antioxidant properties are known to have inhibitory effects on the genotoxic actions of several known mutagens (Ferguson 2001; Matkowski and Wołniak 2005; Bhattachar 2011). The active ingredients, secondary metabolites of the extract have a role of collecting free radicals, thus alleviating the mutagenic effect of MMC. Many polyphenols in plant extracts have ideal structure

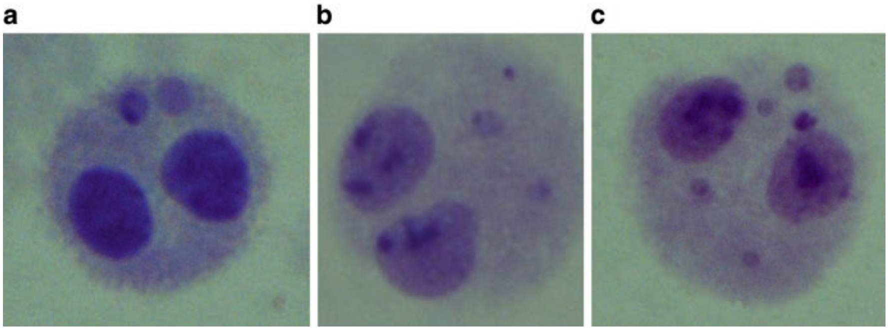


Fig. 9.5 The binucleated (BN) cells after MMC treatment: (**a**) BN with 2 MN; (**b**) BN with 3MN; (**c**) BN with 5MN

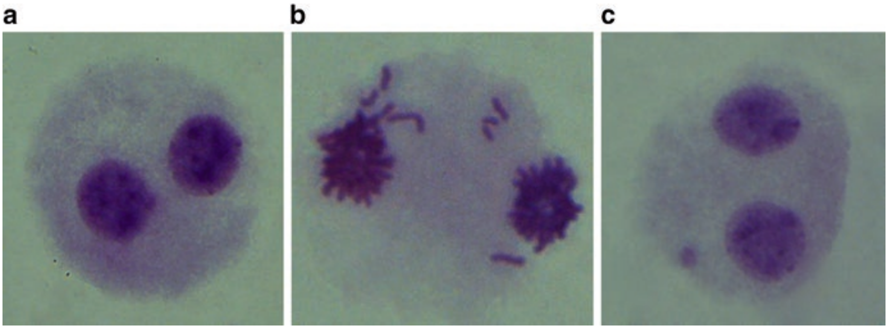


Fig. 9.6 The binucleated (BN) cells after treatment plant extracts of *Teucrium* species against MMC-induced MN (**a**) without MN (normal BN cell), (**b**) BN cell with MNs in development; (**c**) BN cell with an MN

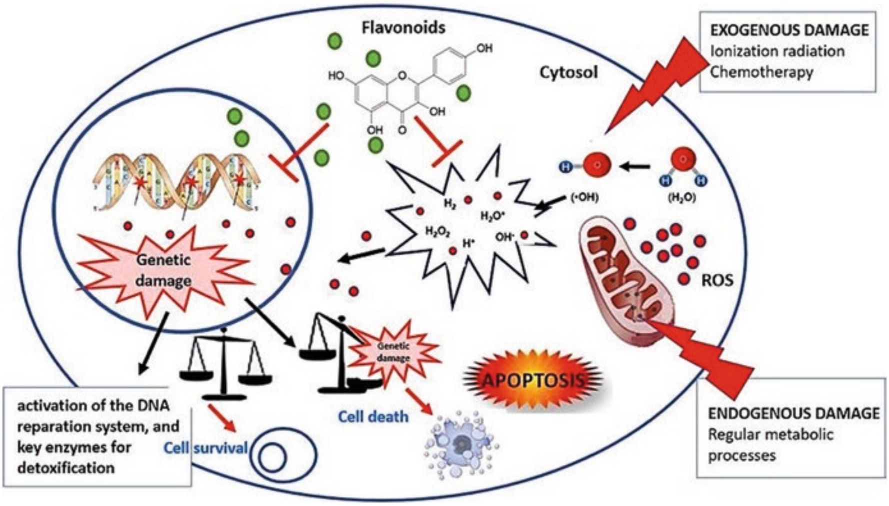


Fig. 9.7 Protective effect of plant secondary metabolites in human cell

and redox properties for free radical scavenging and can chelate transition metal ions thus preventing the formation of ROS (Montoro et al. 2004).

On the other hand, numerous studies have demonstrated that plant-derived natural compounds exhibit protective activities against genotoxicity caused by oxidative stress (Glei and Poll-Zobel 2006; Plazar et al. 2008; Behravan et al. 2011; Mazumdar et al. 2011). The primarily, the antimutagenic effect can be attributed to flavonoids (Calomme et al. 1996), tannins (Baratto et al. 2003) and total polyphenols (Ben Ammar et al. 2008), which cause by radical scavenging effects (Edenharder and Grunhage 2003) (Fig. 9.7).

The phenolic compounds and many flavonoids were reported to have the capacity to scavenge mutagens or free radicals and the ability to neutralize reactive oxygen species (Rice-Evans et al. 1997; Saxena et al. 2012). Flavonoids and phenolic acids are known to be beneficial to human health and disease prevention thanks to their strong antioxidant activity, capacity to scavenge free radicals. Numerous studies demonstrated that plant extracts possess significant antioxidative properties (Sghaier et al. 2011), as well as that, exist a positive correlation between antioxidative and antimutagenic properties of tested plant extracts. Also, exist a positive correlation between total polyphenolic content, total flavonoid content, antimutagenic, antigenotoxic, and antioxidant activities (Ben Sghaier et al. 2011a, b).

9.6 Protective Effect of Phenolic Acids

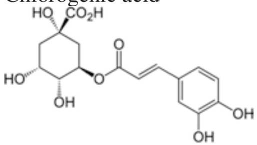
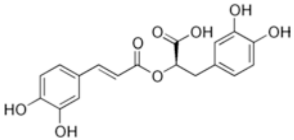
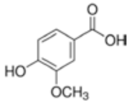
The protective effects of the methanolic extracts of *Teucrium* species can be explained by the presence of phytochemical compounds. Phytochemical analysis showed that the extracts were rich in polyphenolics. From phenolic acids presence chlorogenic, rosmarinic, vanillic, caffeic and ferulic acid, and of flavonoids: rutin, catechin, apigenin, quercetin. In Table 9.6 presence review of some literature data of the genoprotective effect of phenolic acids, using application different genotoxicology tests and on different cell types.

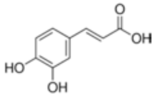
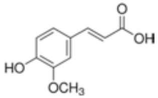
The results Cinkilic et al. (2013) showed that chlorogenic and quinic acids had a protective effect in vitro radiation-induced DNA damage in PBLs from two healthy human donors, using the comet assay. The comet assay is a well-known test system used to analyzed DNA damaging agents in vitro and in vivo (Singh et al. 1988). Chlorogenic acid is an important plant polyphenol that is widely distributed in the leaves and fruits of dicotyledonous plants, such as coffee beans. In the investigations from Milošević-Djordjević et al. (2013, 2018) chlorogenic acid most presented in *Teucrium chamaedrys* and *T. polium* methanolic extracts.

Most phytochemicals act by inhibiting the binding of the free radicals to DNA (Lahouel and Fillastre 2004) although the antioxidant properties of chlorogenic acid are primarily attributed to its capacity to donate hydrogen atoms of the phenolic ring to free radicals (Farah et al. 2008). In a few studies, antioxidant properties of chlorogenic acid were shown using superoxide anion and 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging method (Chen and Ho 1997; Sato et al. 2011). Chlorogenic and quinic acids have a vicinal hydroxyl group on an aromatic residue, which might be the cause of antimutagenic, anticarcinogenic and antioxidant activities in vitro, allowing the compound to scavenge ROS (Sato et al. 2011). Consequently, the decrease in the DNA damage in human lymphocytes might be attributed to the proposed antioxidant mechanisms of quinic and chlorogenic acid.

Furtado et al. (2008) showed that treatments with different concentrations of rosmarinic acid combined with doxorubicin a significantly reduced the frequency of micronuclei compared to Swiss mice treated with doxorubicin only. Although the mechanism the protective effect of rosmarinic acid are not completely understood, the antioxidant activity of rosmarinic acid might explain its effect against doxorubicin mutagenicity. In the next research, authors Furtado et al. (2010) demonstrated

Table 9.6 Literature data of protective effect of phenolic acids using different tests and cell types

The phenolyc acid	Effect	Genotoxicology test	Model system	References
Chlorogenic acid 	Radioprotective, antimutagenic (decreased the DNA damage induced by X-ray irradiation)	Comet assay	PBLs	Cinkilic et al. (2013)
Rosmarinic acid 	Antimutagenic, protective effect chemotherapeutic agent doxorubicin	MN test	PCEs of Swiss mice	Furtado et al. (2008)
	Antimutagenic, protective effect chemotherapeutic agent doxorubicin	MN assay, Comet test	V79 cells (CHO cells)	Furtado et al. (2010)
	Protective effect against ethanol-induced DNA damage in mice	MN assay, Comet test	Mice bone marrow	De Oliveira et al. (2012)
Vanilic acid 	Antimutagenic, (decreased DNA damage induced H_2O_2)	CBMN assay, Comet test	PBLs	Taner et al. (2016)
	Chemoprotective, antimutagenic against MMC induced genomic damage in human lymphocytes at low dose	CBMN assay, Comet test	PBLs	Erdem et al. (2012)
	Antimutagenic	Ames test	*Salmonella typhimurium*	Shaughnesy et al. (2001)
	Chemoprotective, antimutagenic against MMC	MN test	Mice bone marrow cells	Inouye et al. (1988)
	Reduced DNA damage induced EMS and H_2O_2	CA	V79 cells (CHO cells)	Tamai et al. (1992)

The phenolyc acid	Effect	Genotoxicology test	Model system	References
Caffeic acid 	Radioprotective, antimutagenic against radiation induced DNA damage	CBMN assay, CA, Comet test	PBLs	Devipriya et al. (2008a)
Ferulic acid 	Prevention of radiation-induced MN and dicentric aberrations in human lymphocytes	CA, CBMN assay	PBLs	Prasad et al. (2006)

H_2O_2 hydrogen peroxide, *EMS* ethyl methanesulfonate, *PBLs* peripheral blood lymphocytes, *PCEs* polychromatic erythrocytes, *CA* chromosome aberrations, *CHO cells* Chinese hamster ovary cells that rosmarinic acid showed the same, antimutagenic effect in V79 cells. For the evaluate they were used the MN assay and comet test. The results of De Oliveira et al. (2012) showed that rosmarinic acid decreases the levels of DNA damage induced by ethanol probably due to it exerts antioxidant activity.

Numerous in vitro and in vivo studies have shown that phenolic acids exhibit powerful effects on the biological response by scavenging free radical. Vanillic acid found in vanilla is natural plant phenolic acid which are secondary aromatic plant products that are presumed to possess many physiological and pharmacological functions. According to Milošević-Djordjević et al. (2018) vanillic acid is the most presented in *Teucrium scordium* methanolic extract. Vanillic acid is an oxidized form of vanillin and exhibits more free radical scavenging activity then vanillin (Sasaki et al. 1990). Taner et al. (2016) showed that vanillic acid indicated antioxidant activity, as well as did not have cytotoxic and genotoxic effects alone at the tested concentrations in compared with the controls. Some author demonstrated that vanillic acid decreased DNA damage induced by hydrogen peroxide in PBLs. Vanillic acid shows an antigenotoxic effect on MMC-induced genetic damage in healthy PBLs measured by the CBMN and alkaline comet assays. Vanilic acid could prevent oxidative damage to DNA and chromosomes when used at an appropriately low dose. Despite the antioxidant and antigenotoxic capabilities of vanillic acid, care should be taken with regard to its concentration in the daily diet (Erdem et al. 2012). Earlier studies also confirmed the protective role of vanillic acid against MMC, EMS and hydrogen peroxide in different animal models (Inouye et al. 1988; Tamai et al. 1992).

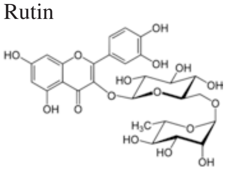
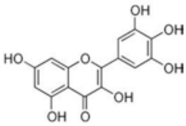
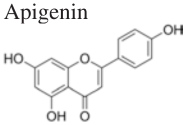
Caffeic acid (3,4-dihydroxicinnamic acid) is a naturally occurring polyphenol widely distributed in plant-derived materials such as fructus, vegetables, tea, wine, olive oil, coffee bean (Challis and Bartlett 1975). Of all polyphenolic acids, coffee acid was present in the largest amount in *Teucrium flavum* and *T. arduini* methanolic extracts (Grujičić et al. 2020). The investigations (Prasad et al. 2006; Devipriya et al. 2008a) were showed that this polyphenol compound has protective properties in PBLs against radiation-induced genetic damage (MN, dicentric aberration, comet attributes).

9.7 Protective Effect of Flavonoides

In recent years, there has been a growing academic and industrial interest in the health benefits of flavonoids (Chebil et al. 2007; Resende et al. 2012; Kozłowska and Szostak-Węgierek 2014). In Table 9.7 gives an overview of some literature data of the genoprotective effect of flavonoids, using application different genotoxicology tests (CBMN assay, CA, Comet test, Ames test) on different cell types (PBLs, polychromatic erythrocytes- PCEs, V79 cell, *Salmonella typhimurium*).

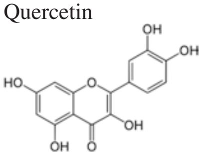
Rutin is most abundant flavonoid in *Teucrium chamaedrys* and *T. montanum* methanolic extracts (Stanković 2012), while quercetin mostly abundant in *Teucrium arduini* and *T. flavum* methanolic extracts. According to our knowledge, there are

Table 9.7 Literature data of protective effect of secondary metabolites, flavonoides using different tests and cell types

The flavonoides	Effect	Genotoxicology test	Model system	References
Rutin 	Radioprotective, antimutagenic against radiation induced damage	CBMN assay	PBLs	Patil et al. (2017)
	Radioprotective, antimutagenic against radiation induced damage	Comet assay	PBLs	
	Antimutagenic, (decreased DNA damage induced H_2O_2)	CA	PBLs	Boligon et al. (2012)
	Chemoprotective, antimutagenic against MMC	Comet assay	PBLs	Undeğer et al. (2004)
Catehin 	Antigenotoxic potential catechin hydrate against cadmium toxicity	DNA fragmentation assay	PBLs	Alshatwi et al. (2014)
Apigenin 	Chemoprotective, antimutagenic against ethinylestradiol	CA, SCEs	PBLs	Siddique et al. (2010)
	Antimutagenic, (decreased DNA damage induced H_2O_2)	CBMN assay SCE	PBLs	Siddique and Afzal (2009a)
	Chemoprotective, antimutagenic against MMC	MN test	Mice bone marrow cells	Siddique and Afzal (2009b)
	Chemoprotective, antimutagenic against MMC and cyclophosphamide (anti-cancerous drug)	CA, SCE	Mice bone marrow cells	Siddique et al. (2008)
	Radioprotective, except in highest concentration	CBMN assay	PBLs	Rithidech et al. (2005)

(continued)

Table 9.7 (continued)

The flavonoides	Effect	Genotoxicology test	Model system	References
Quercetin 	Radioprotective, antimutagenic	CBMN assay	PBLs	Patil et al. (2017)
	Radioprotective, antimutagenic against radiation induced DNA damage	Comet assay	PBLs	
	Chemoprotective, antimutagenic against MMC	MN test	Mice bone marrow cells	Mazumdar et al. (2011)
	Chemoprotective, antimutagenic against MMC and idarubicin induced DNA strand breaks	DNA strand breakage assay	pBR322 plasmid DNA	Çelik and Arinç (2010)
	Radioprotective, antimutagenic against radiation induced DNA damage	CBMN assay Comet test	PBLs *in vitro*	Devipriya et al. (2008b)
	Chemoprotective, antimutagenic, against t-butylhydroperoxide	Ames	*Salmonella typhimurium* TA102	Geetha et al. (2005)
	Chemoprotective, antimutagenic against MMC	Comet assay	PBLs	Undeğer et al. (2004)

CA chromosomal aberrations, *SCEs* sister chromatid exchange

few studies which determined the antigenotoxic and radioprotective effects of rutin and quercetin on PBLs (Undeğer et al. 2004; Boligon et al. 2012). Study Patil et al. (2017) demonstrates the protective effect of rutin and quercetin against radiation-induced DNA damage in PBLs. The authors explained that these results may be partly attributed to scavenging of radiation-induced free radicals and also by the inhibition of radiation-induced oxidative stress.

Methanolic extract of *Teucrium polium* and *T. scordium* are characterized by a high presence of flavonoids catechin (Milošević-Djordjević et al. 2018). Catechin is a polyphenolic flavonoid that has been isolated from a variety of natural source, including tea leaves, grape seeds, and the wood and bark of trees such as acacia and mahogany. Catechin is a more potent antioxidant then ascorbate or tocopherol in certain in vitro assays of lipid peroxidation (Alshatwi 2010). Catechins (flavan-3-ol) are typical natural phenols. Catechin hydrate, also known as taxofilin (Makena et al. 2009), a type of flavonoid that can be found mostly in the Siberian larch (*Larix sibirica*) and silymar in extract from milk thistle seeds (Hoffman 2003). Some studies indicate the antigenotoxic potential of catechin hydrate against cadmium toxicity in PBLs (Alshatwi et al. 2014).

The second most abundant flavonoid in the extract of *Teucrium polium* is apigenin. Some studies have shown that apigenin is potent in reducing the genotoxic damage inducing by MMC, thereby reducing the chances of developing secondary tumors during the therapy (Siddique et al. 2008). Similarly, Siddique and Afzal (2009a, b) indicated a protective role of apigenin against the genotoxicity of H_2O_2 and MMC both on PBLs and mouse bone marrow cells. Also, Siddique et al. (2010) showed a clear reduction in genotoxic damage in PBLs induced by ethinylestradiol observed with increasing doses of apigenin, suggesting a protective role for apigenin during ethynylestradiol therapy. On the other hand, apigenin had protective effects on radiation-induced chromosome damage in human lymphocytes (Rithidech et al. 2005; Sharma 2013). Quercetin, a flavonol group of plant flavonoid, has generated immense interest because of its potential antioxidant, antiproliferative, chemoprotective, anti-inflammatory and gene expression modulating properties (Mazumdar et al. 2011). Quercetin as the most common flavonoid is found in the methanolic extracts of *Teucrium arduini* and *T. flavum* (Grujičić et al. 2020).

The findings Mazumdar et al. (2011) suggest that quercetin may result in genomic instability in the tested dose range and a significant reduction in MMC-induced genotoxicity in the highest dose tested. Pre-treatment with quercetin significantly reduced the frequency of MMC-induced MN as well as CA. These effects of quercetin should be taken into account when assessing the potential use of quercetin as a therapeutic agent. The same authors point out that further studies are required to elucidate the possible interaction of quercetin with DNA as well as with other DNA damaging agents like MMC in vivo. On the other hand, Çelik and Arinç (2010) concluded that quercetin is highly effective in reducing the DNA damage caused by the antitumor agents, idarubicin, and MMC, following bioactivation by P450 reductase. Devipriya et al. (2008b) showed that quercetin significantly decreased radiation-induced genetic damage and biochemical changes in PBLs. The important mechanisms offered by quercetin in protecting lymphocytes from radiation is free radical scavenging property, DNA binding potential, increasing the antioxidant status and antiperoxidative potential.

Although some studies have shown that quercetin in Ames test is regarded as mutagenic (Gaspar et al. 1993), later in vitro studies indicate that quercetin is protective against genotoxicants and regarded as antimutagenic, as well as that in vitro free radical scavenging activity of quercetin correlated well with the antimutagenic activity (Geetha et al. 2005). In 1999, the International Agency for Research on Cancer (IARC) concluded that quercetin is not classified carcinogenic to humans. In the U.S. and Europe, supplements of quercetin are commercially available, and beneficial effects of quercetin supplements were reported in clinical trials (Okamoto 2005).

9.8 Protective Effect of Secondary Metabolites of *Teucrium* Species

However, although numerous studies have confirmed the antimutagenic effect of phenolic components of secondary metabolites from herbal extracts, it should be noted that the extracts are a mixture of different compounds. Since a plant extract is the mixture of natural compounds, their different biological activities (antioxidant, antimicrobial, antigenotoxic and anticancer) are not only the result of different activities of individual components and may also be the result of their interactions, possibly producing different effects on the overall activity of extracts. Therefore, numerous authors suggested that the activity of the extracts may be the result of synergistic effects of several compounds (Romero-Jimenez et al. 2005; Daradka and Alshibly 2012; Kosanić et al. 2016; Milošević-Djordjević et al. 2013, 2018). On the other hand, it is well documented that often a mixture of plant polyphenols present in nature, by their synergistic collaboration acts better than its individual components separately (Rice-Evans et al. 1997; Havsteen 2002). As well it is quite possible that uptake of complex plant-derived mixture modulates the genotoxicity of one flavonoid by the antigenotoxic capacity of another flavonoid and vice-versa (Di Virgilio et al. 2004).

Table 9.8 gives an overview of some literature data of genoprotective effect of different *Teucrium* species extracts using application different genotoxicology tests and on different cell types. According to our knowledge, there are very few scientific studies who investigated of genotoxic properties of the extracts genus *Teucrium*. Except of the investigate by Milošević-Djordjević et al. (2018), genotoxic effect of *Teucrium polium* extract, also, has been investigated by Khader et al. (2007, 2010). The extract of *Teucrium polium*, had protective, antimutagenic effect against MMC-induced genetic damage, and this extract had no effect on proliferation PBLs in vitro. These results are consistent with previous results from Milošević-Djordjević et al. (2013) which showed that both methanolic extracts of *Teucrium chamaedrys* and *T. montanum* administered alone in PBLs in vitro, did not significantly affect the NDI in the concentrations tested.

Numerous studies have shown that extracts of *Teucrium polium* have significant effects on cell proliferation in different cell types. Khader et al. (2007) demonstrated that aqueous extract of *Teucrium polium* significantly reduced the mitotic indices in primary rat hepatocyte cultures after simultaneous treatment with mutagen *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG), indicating that the extract enhances MNNG-mediated cytotoxicity. In a later study, Khader et al. (2010) showed that the ethanolic extract of *Teucrium polium* had the antimutagenic effect on primary rat hepatocyte cultures before, during and after the treatment with MNNG. Also, investigations demonstrated that the extract of *Teucrium polium* potentiates the cytotoxic and apoptotic effects of anticancer drugs (vincristine, vinblastine, and doxorubicin) against a panel of cancerous cell lines (Rajabalian 2008).

Similar results were obtained on another plant species as a *Teucrium ramosissimum*. Sghaier et al. (2016) showed that methanolic extract of *Teucrium*

Table 9.8 Protective effect of different extracts of *Teucrium* species

Teucrium species	Extract	Protective effects	Test/model system	References
T. chamaedrys	Methanolic	Proapoptotic against MMC	MN assay/PBLs in vitro	Milošević-Djordjević et al. (2013)
T. montanum	Methanolic	Proapoptotic against MMC	MN assay/PBLs in vitro	
T. polium	Methanolic	Antimutagenic against MMC (free radical scavenging activities)	MN assay/PBLs in vitro	Milošević-Djordjević et al. (2018)
	Aqueous	Reduced the mitotic index	Primary rat hepatocyte in vitro	Khader et al. (2007)
	Ethanolic	Antimutagenic (free radical scavenging activities), during and after treatment with MNNG	Primary rat hepatocyte in vitro	Khader et al. (2010)
T. scordium	Methanolic	Proapoptotic (at highest concentration)	MN assay/PBLs in vitro after treatment with MMC	Milošević-Djordjević et al. (2018)
T. arduini	Methanolic	Proapoptotic	MN assay/PBLs in vitro after treatment with MMC	Grujičić et al. (2020)
T. flavum	Methanolic	Proapoptotic	MN assay/PBLs in vitro after treatment with MMC	Grujičić et al. (2020)
T. ramosissimum	Methanolic	Antimutagenic, against hydrogen peroxide (H_2O_2)	Comet assay/human lymphocytes	Sghaier et al. (2016)
	Methanolic, ethanolic, chloroform	Antimutagenic, against AFB1, nitrofurantoin	Ames test/*Salmonella typhimurium*	Ben Sghaier et al. (2011a)
	Methanolic ethanolic chloroform	Antimutagenic, against AFB1, nitrofurantoin	SOS chromotest/*Escherichia coli* PQ37	Ben Sghaier et al. (2011b)
	Total flavonoids, ethyl acetate, petroleum ether	Antimutagenic, against SA, AFB1, B[a]P, NOPD	Ames test/*Salmonella typhimurium*	Sghaier et al. (2011)
	Essential oil	Antimutagenic, against SA, AFB1, B[a]P, NOPD	Ames test/*Salmonella typhimurium*	Ben Sghaier et al. (2010)

MNNG mutagen *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, *SA* sodium azide, *AFB1* aflatoxin B 1, *B[a]P* benzo[a] pyrene, *NOPD* 4-nitro-*o*-phenylenediamine *ramosissimum* had antigenotoxic properties against genotoxicity induced by the direct-acting mutagen, hydrogen peroxide (H_2O_2), using comet assay. The same authors have opinions that tested *Teucrium ramosissimum* extract possess potent antioxidant, antiproliferative and antigenotoxic activities, which could be derived from compounds such as flavonoids and polyphenols. The studies Ben Sghaier et al. (2011a, b) has demonstrated that *Teucrium ramosissimum* extracts (methanolic, ethanolic, and chloroform) possess antimutagenic activity against all the tested genotoxicants (aflatoxin B1, benzo[a]pyrene, NOPD -4-nitro-o-phenylenediamine and sodium azide) in the *Salmonella* assay systems and SOS chromotest in the *Escherichia coli*. Antimutagenic activity of the tested extracts may be ascribed to flavonoids, tannins and total polyphenols (Calomme et al. 1996; Baratto et al. 2003; Ben Ammar et al. 2008), at least partly to their antioxidative properties but we cannot exclude other additionally mechanisms. *Teucrium ramosissimum* extracts could give rise to antimicrobial, anti-inflammatory agents and could be promising candidates for further studies designed to obtain more evidence on their components with potential chemopreventive activity. The results Sghaier et al. (2011) demonstrated that total oligomers flavonoids (TOF), ethyl acetate (EA) and petroleum ether (PE) extracts from aerial parts of *Teucrium ramosissimum* was assessed using Ames *Salmonella* tester strains TA98, TA100 and TA1535 with and without metabolic activation (S9). In addition, all extracts showed important free radical scavenging activity toward the radical's DPPH and ABTS except the PE extract. Same authors suspected an eventual correlation between antiradical and antimutagenic effects of *Teucrium ramosissimum* extracts. Antioxidant potential expressed by the different extracts may provide a common mechanism for inhibiting the genotoxicity of both direct and indirect tested mutagens. In fact, phenolic compounds are ubiquitous in fruit, vegetables, and nuts, several of which have been reported to be inhibitors of chemical carcinogenesis (Fiala et al. 1985; Stich 1991). The study Ben Sghaier et al. (2010) showed that *Teucrium ramosissimum* essential oil possesses antimutagenic effects against SA, AFB1, B[a]P, and NOPD. Finally, the antimutagenic and antioxidant properties of the essential oil from *Teucrium ramosissimum* and its constituents could make it a promising candidate for future applications in chemoprevention and therapy. Antigenotoxic plant extracts can counter or prevent the adverse effects caused by DNA-damaging chemicals. Indeed, it has been shown that plant-derived polyphenolic compounds can exert an antigenotoxic activity (Zhao et al. 1999; Bhatia et al. 2001).

The previous researches (Milošević-Djordjević et al. 2013, 2018; Grujičić et al. 2020) have shown that investigated *Teucrium* species possess the protective properties against MMC-induced genetic damage in PBLs. In our opinion, two genoprotective effects are possible: (1) antimutagenic, antioxidant effect, and (2) proapoptotic effect. The type of genoprotective effect depends on the influence of the extract on cell kinetics in the culture of PBLs. We think that the extracts do not affect cellular kinetics (NDI is not changed), but they reduce MMC-induced MN frequency. Those extracts have an antimutagenic, antioxidant effect that manifest as free radical scavenging activities. In this way, the plant extract relieves the harmful mutagenic effect of antitumor drug on healthy human cells (PBLs). If the extract influence on PBLs is such that NDI is decreased and that the cell kinetics slows down, with, of course, additionally reduction of MMC-induced MN frequency, this extract shows a genoprotective, proapoptotic effect. This means that apoptotic mechanisms are activated for cells that have suffered significant damage to the genetic material and such cells are eliminated.

Presented observations indicate that these genoprotective properties can belong to antioxidative type, as in methanolic extracts of *Teucrium polium* and *T. ramosissimum*, or proapoptotic type, as noted in all other tested extracts *Teucrium* species (*Teucrium chamaedrys, T. montanum, T. scordium, T. arduini* and *T. flavum*). That means that except *Teucrium polium* and *T. ramosissimum*, all the others tested extracts have proapoptotic properties that can be responsible for their protective effects against MMC-induced genetic damage. These finds are in agreement with reports of other authors who pointed out that antimutagenic properties of plant extracts depend on the phytochemical composition and the content of secondary metabolites. The different genoprotective effects of tested *Teucrium* extracts on MMC-induced mutagenicity can be explained with differences in the phytochemical content of the polyphenols present. Phytochemical analyses of methanolic extract of *Teucrium* species showed that the extracts were rich in polyphenolic compounds (Stanković 2012), and could be considered responsible for antimutagenic properties of the plant. In support of this, the fact is that many studies on the different cell lines (normal and cancer) suggest that polyphenols, separately and in combination with chemotherapeutic agents induced apoptosis (Rajabalian 2008; Ćurčić et al. 2012). It is an important mechanism for the maintenance of cellular homeostasis in both in chemoprevention and chemotherapy of cancer. Studies on cultured cells have shown that the human PBLs is a sensitive indicator both in vitro and in vivo induced chromosome damage (Lerda et al. 2005). In fact, the MN assay provides a measure of both chromosome breakage and chromosome loss or non-disjunction in clastogenic and aneugenic events, respectively (Karaman et al. 2009). An increase of the frequency of chromosomal aberrations in PBLs is in associated with an increased overall risk of cancer (Bonassi et al. 2000; Natarajan 2002; Rossner et al. 2005; Boffetta et al. 2007).

The antimutagenic activity of compounds derived from plants may be due to a variety of mechanisms, such as inhibition of genotoxic effect, signal transduction modulation, antioxidant activity, and scavenging of free radicals (Yen and Chen 1995; Marnewick et al. 2000; Fragoso et al. 2008). The recent studies have suggested the use of medicinal plants as antimutagenic agents in the prevention of genotoxic effects of different chemotherapeutic agents (Verschaeve et al. 2004; Sibanda and Okoh 2007; Kumar et al. 2012; Vlastos et al. 2013). Since the use of chemotherapeutic drugs can cause the emergence of secondary carcinoma (Vlastos et al. 2013), which results from genetic damage in healthy cells, the reduction of the risk of this phenomenon is of great importance (Unal et al. 2013). Natural antioxidant compounds are important agents for potential use in chemoprevention and/or during or after cancer chemotherapy, showed extensive studies, both in vitro and in vivo (Çelik and Arinç 2010; Veeramuthu et al. 2017; Zyad et al. 2018).

The recent research has shown the dual activity of flavonoids presenting both antioxidative and prooxidant activities revealed that the existence of a balance between these two features could be important to the development of adequate therapeutic strategies (Baldim et al. 2017). Additionally, presented results have shown that it is necessary to be careful when using these extracts because plants except *Teucrium chamaedrys* have shown a genotoxic effect in high concentrations, while they in combination with MMC in the same concentrations, all plants, had a protective effect.

9.9 Conclusions

An overview of the literature data suggests that *Teucrium* extracts have a significant protective effect against various genotoxic agents (MMC, AFB1, B[a]P, NOPD), alleviating their effect, either as potent antioxidants or introducing cells into the apoptosis process. Presented results showed that secondary metabolites, as well as extracts from *Teucrium* species, have a genoprotective effect on different types of cells. The different genoprotective effects of tested *Teucrium* extracts can be explained with differences in the phytochemical content of the polyphenols present. Generally, presented results clearly showed that tested extracts *Teucrium* species, as well as secondary metabolites, had a protective effect on the genotoxicity of the different agents in different type cells in vitro and, therefore, they can be a potential source of new pharmaceutics that will have a significant protective effect on induced genetic damage by chemotherapeutics in healthy cells.

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Chapter 10
Antioxidant Activity of Secondary Metabolites of *Teucrium* Species

Milan Stanković

Abstract *Teucrium* species are characterized by valuable secondary metabolites content and long traditional ethnopharmacological use. Bearing in mind that the therapeutic benefit of medicinal plants is often based on their antioxidant activity, this chapter is a comprehensive review of the antioxidant activity of medicinal and potentially medicinal species of the genus *Teucrium*, as well as the secondary metabolites content and diversity including methods for antioxidant activity determination. Analysis of the current literature indicates that antioxidant activity has been tested for over 30 species of this genus using different methodological approaches. *Tucrium polium* is the most tested species followed by *Teucrium chamaedrys* and *Tucrium montanum*. Plant extracts are often used for antioxidant activity analysis followed by essential oils and isolated chemical compounds. The most commonly used methods include DPPH – 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, Ferric Reducing Antioxidant Power (FRAP), *β*-carotene/linoleic acid assay, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging capacity (ABTS) etc. *Teucrium* species is characterized by the presence of monoterpenes and sesquiterpenes, as well as flavonoids, phenylethanoids and phenolic acids. The significant correlation of the antioxidant activity and phenolic content was determined in many studies. The antioxidant potential of some *Teucrium* species has been confirmed by in vivo testing. Variability during vegetative period, habitat-related variability as well as variability between plant parts were determined for the antioxidant activity and content of the active compounds of some *Teucrium* species. Numerous studies confirm that species of the genus *Teucrium* possess secondary metabolites with significant antioxidant effects.

Keywords *Teucrium* species · Antioxidant activity · Plant extracts · Phenolics · Flavonoids · Essential oil · Antioxidant assays

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Abbreviations

ABTS	2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging capacity
BDE	Bond dissociation energy
BR	Briggs-Rauscher oscillating reaction
CUPRAC	Cupric Reducing Antioxidant Capacity assay
DPPH	2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity
EPR	Electron Paramagnetic Resonance radicals detection
FRAP	Ferric Reducing Antioxidant Power
HAPX	Hemoglobin Ascorbate Peroxidase activity inhibition
HAT	Hydrogen atom transfer
HRSA	Hydroxyl Radical Scavenging Activity
IP	Ionization potential
NBT/riboflavine	Nitroblue Tetrazolium riboflavine assay
ORAC	Oxygen Radical Absorbance Capacity
OSI	Oxidative Stability Index
RP	Reducing Power
SET	Single electron transfer
SOD	Superoxide Dismutase Activity assay
SRSA	Superoxide Radical Scavenging Activity
TAC	Total Antioxidant Capacity
TAP	Total Antioxidant Power
TBA	Thiobarbituric Acid Assay
TEAC	Trolox Equivalent Antioxidant Capacity
X/XOD	Xanthine/Xanthine Oxidase assay
XOA	Xanthine Oxidase Activity assay

10.1 Introduction

Genus *Teucrium* (germander) is a member of the Lamiaceae family. It includes more than 300 species and together with genera *Salvia*, *Stachys*, *Plectranthus*, and *Hyptis* makes the group of the most numerous genera of this plant family. The species of genus *Teucrium* are mainly perennial herbaceous plants, shrubs or subshrubs, while only a small number are annual. The species of this genus are present in moderate climate regions, mostly in Mediterranean and upper Asia. In Europe the most species are found in the south, southwest and southeast areas, thus making this continent characterised by the greatest number of species of this genus. In Asia, the greatest number of species are found in the southwest part, while on the territory of Africa it is present in the far north areas, and only a small number is in the far south areas. In America the areal of genus *Teucrium* covers the south areas of North

America and the southwest areas of South America. In Australia, these species are found in the southern parts as well as on some surrounding islands (Stanković 2012).

The species of genus *Teucrium* are important plant group for research in biology and ecology, as well as in the domains of applied disciplines such as biotechnology, pharmacy and food and drink industries. One of significant aspects of for development of the application of *Teucrium* species is their ethno botanic use which dates thousands of years ago. The species of genus *Teucrium* are very popular in traditional medicine from the very beginnings of plant use in the treatment of diseases. The species of this genus were used in the treatments of tuberculosis, scurvy, jaundice, rheumatism and diseases of digestive tract. Therapeutic products, prepared from the parts of these plants above the ground are characterised by bitter taste, whereby their positive effect on digestive tract is found. High contents of aromatic compounds with antioxidant and antimicrobial activity contributes to their effect in treatment of many infectious diseases. Due to their bitter aromatic compounds, the species of genus *Teucrium* are used in cookery as spices and additives in preparation of soft and alcoholic drinks. Because of the presence of the several toxic neoclerodane diterpenes application of some *Teucrium* species should be controlled and careful (Keršek 2006; Starakis et al. 2006; Savvidou et al. 2007; Stanković 2012).

Due to characteristic qualitative and quantitative contents of secondary metabolites in the group of flavonoids, phenolic acids and mono and sesquiterpenes, the species of *Teucrium* genus are significant sources of antioxidant, antimicrobial and anticancer natural components, and bearers of many therapeutic properties. For that reason, plant extracts, essential oils and isolated components are extensively analysed for their biological activity. The literature data show that plant extracts, essential oils and isolated secondary metabolites of the genus *Teucrium* are largely analysed due to their antioxidant activity. Detailed review of literature showed that the research results of the antioxidant activity of more than 30 species were published in more than a 80 scientific papers. The greatest number of data for the antioxidant activity of the species of this genus are found for *Teucrium polium* (felty germander), *Teucrium chamaedrys* (wall germander) and *Teucrium montanum* (mountain germander). The chapter shows the review of literature about antioxidant activity of secondary metabolites of the *Teucrium* species. In introductory parts general information on secondary metabolites as bearers of antioxidant role is presented together with the mechanisms of their effect. It is followed by the table of plant species of genus *Teucrium,* analysed for antioxidant activity of their extracts, essential oils and isolated components, together with the methodological approach to the investigation.

10.2 Plant Secondary Metabolites

Besides primary, secondary metabolism also occurs in plant cells, and its products, i.e. secondary metabolites are not of essential importance for the plant, but are most often the result of plant adaptation to environmental conditions. The term "secondary" was first used by A Kossel in 1891; it designated metabolic pathways that resulted in products that were not essential, but were involved in the reaction of a plant organism to environmental factors (Edreva et al. 2008). These substances play a role in ecological interaction of plants; they show regulatory effect and protect plants from the attack of predators, parasites and other negative factors, and also play a role in the processes of pollination and seed distribution by animals attracting. Their role in protective response to unfavourable abiotic influences (radiation, temperature changes, disturbances in water regime etc.) is significant. They appeared during evolution of plants; mutually, they differ in chemical structure and quantity in various groups of taxon, thus they can be characteristic of only one species or taxon of higher rank. Quantitative and qualitative composition of secondary metabolites changes during a vegetation period, season or a day, depending on the state of a plant, the presence or absence of stress etc. (Oh et al. 2009).

Metabolic pathways of secondary biomolecules are extremely complex. The enzymes that are included in metabolic pathways of secondary metabolites are mainly under the influence of a great number of ecological factors, while the pathways of regulation of these enzymes are a significant domain of scientific research today, since many products of secondary metabolism in plants are extremely important, especially for pharmaceutical and food industry (Zwenger and Basu 2008; Quideau et al. 2011). According to their chemical composition, plant secondary metabolites are divided into two groups. The first group includes molecules without nitrogen atoms – phenolic compounds and terpenoids. The second group includes secondary metabolites with nitrogen atoms in their molecules, i.e. alkaloids (Wink 2004; Roberts 2007). Besides a great number of biological effects in plants, secondary metabolites that are isolated from plants show strong biological effects. Plant secondary metabolites are characterised by wide use in pharmaceutical industry, medicine, agricultural and food production. A wide spectrum of their application is related to structural properties of their molecules, which shows their antioxidant, antimicrobial and anticancer activity, as well as many therapeutic effects (Quideau et al. 2011). Due to varied chemical structure, secondary metabolites produce effects at all levels of organisation in biological systems, which is the reason of their intensive application. Their pharmacological effects are very important, thus they are used in therapy and prevention of many pathological states (Fraga 2010).

10.2.1 Biological Effects of Plant Secondary Metabolites

In addition to their significant biological role in the interactions of plants in an ecosystem, secondary metabolites that are isolated from a plant organism show biological activity in both in vitro and in vivo conditions. The mechanism of their activity in the given biological system involves mainly stimulation or inhibition of enzymatic activity and other metabolic processes where their therapeutic benefit is expressed (Korkina 2007). Plants have mechanisms to defend themselves from occurrence of oxidative stress, as well as mechanisms to reduce negative impact of oxidative stress. Antioxidant activity of phenolic compounds is based on their chemical structure, which allows them to be donors of hydrogen atoms and electrons. In addition to the stated processes, there are a few significant mechanisms of antioxidant activity of phenolic compounds and other secondary metabolites in plant cells and tissues (Blokhina et al. 2003; Michalak 2006). Besides important and necessary biological effects in the process of the plant response to negative impact of environmental factors, i.e. stress conditions, isolated plant secondary metabolites possess different biological effects (Quideau et al. 2011). The biological activity of plant secondary metabolites is based on their capability to react with numerous regulatory molecules and other cellular and subcellular structures, thus having positive or negative impact on a great number of metabolic processes. The effect of secondary metabolites is most often manifested through the control of enzyme reactions, control of hormone activity, gene expression, cellular transport, cellular division, immune response and many other physiological processes. Acting by stimulatory or inhibitory mechanisms, the secondary metabolites show antioxidant, antimicrobial, antiproliferative, apoptotic, anti-inflammatory, antihypertensive and many other activities (Briskin 2000).

10.2.2 Antioxidant Activity of Plant Secondary Metabolites

Free radicals are molecules, ions or atoms of carbon, oxygen, nitrogen or sulphur with unpaired electrons that make them very reactive. Free radicals occur during a great number of metabolic processes in an organism under the influence of negative effects of physical and chemical environmental factors. They most often occur during reactions such as homolytic cleavage within a molecule, oxidoreduction reactions, thermolysis, radiolysis, transmission of one electron from transient ion of metal to organic molecule, the effect of ozone, nitrogen (IV) – oxide and singlet oxygen as well as during numerous enzymatic processes in the organism (Sen et al. 2010). The most common and most reactive free radical and non-radical forms are oxygen reactive forms that can appear as superoxide anion radicals, hydroxyl radicals, hydroperoxyl radicals, singlet oxygen, carbon dioxide and carbon monoxide radicals (Halliwel and Whiteman 2004). The mechanism of negative effect of free radical forms in biological systems is based on their reaction with biomolecules such as nucleic acids, proteins, lipids and enzymes, thus causing their damage and inhibiting their primary biological role in cells. The increased production of free radicals in a human organism intensifies its ageing process and causes a great number of pathological conditions such as neurodegenerative changes, cancer genesis, cardiovascular disorders and inflammatory processes (Valko et al. 2006).

Harmful effects induced by free radicals are prevented by the actions of antioxidant substances which inhibit their activity. Antioxidants are substances which partly inhibit or completely prevent oxidation of substrates exposed to the oxidation process. The mechanism of effect of antioxidant substances is based on their role of a donor of electron or hydrogen atom, i.e. their scavenging ability, then on their capability to chelate metal ions (Fe^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}), thus reducing their redox potential, destruction of hydro peroxide of lipid molecules which create non-radical forms etc. (Gupta and Sharma 2006). Antioxidant capacity of phenolic compounds depends on the number and position of hydroxyl groups. In addition, significant physical and chemical characteristics are bond dissociation energy (BDE) and ionization potential (IP). One of leading mechanisms of antioxidant activity of phenolic compounds is the role of a donor of hydrogen atom (HAT – hydrogen atom transfer) and the role of a donor of electron (SET – single electron transfer) to the molecule exposed to oxidation process (Quideau et al. 2011).

10.3 Antioxidant Activity of Secondary Metabolites from *Teucrium* Species

Numerous studies confirm that species of the genus *Teucrium* are an important plant group with a variety of secondary metabolites with antioxidant effects. A review of the literature revealed that over 30 species of this genus have been studied for the antioxidant activity using different model systems. *Teucrium* species with traditional application have been analyzed in many studies of antioxidant activity. Based on the literature review presented in Table 10.1, it was found over 80 references about antioxidant activity of the *Teucrium* species, as well as results about 34 tested *Teucrium* species such as *Teucrium alyssifolium*, *T. arduini*, *T. barbeyanum*, *T. botrys*, *T. cavernarum*, *T. chamaedrys*, *T. creticum*, *T. flavum*, *T. fruticans*, *T. hyrcanicum*, *T. marum*, *T. massiliense*, *T. montanum*, *T. montbretii*, *T. muscatense*, *T. orientale*, *T. oxylepis*, *T. parviflorum*, *T. persicum*, *T. pilosum*, *T. polium*, *T. pruinosum*, *T. pseudochamaepitys*, *T. pseudoscorodonia*, *T. ramosissimum*, *T. royleanum*, *T. salviastrum*, *T. sandrasicum*, *T. sauvagei*, *T. scordium*, *T. siculum*, *T. stocksianum*, *T. subspinosum,* and *T. yemense*. Review of current literature data about the antioxidant activity analysis of *Teucrium* species including sample types as well as methods for antioxidant activity determination were presented in Table 10.1.

The results for the antioxidant activity and amount of phenolic compounds and flavonoids of leaf, flower and stem extracts obtained by water extraction, as well as the composition of the essential oil, were determined for *Teucrium alyssifolium* (Semiz et al. 2016). The water extract of the leaves of this plant showed the highest antioxidant activity and content of phenolic compounds. The main components of the essential oil were trans-*β*-caryophyllene, ar-curcumene and bisabolene. The antioxidant activity of *Teucrium arduini* has been investigated using various methods. Leaf and flower infusions of *Teucrium arduini* were analyzed in order to determine phenolic content and antioxidant activity. Antioxidant activity was evaluated using the FRAP, DPPH and ABTS radical scavenging assays. The authors found significant antioxidant activity as well as a positive correlation between antioxidant activity and the amount of phenolic compounds (Šamec et al. 2010). Screening of

Table 10.1 Antioxidant activity of *Teucrium* species – review of analyzed species, sample types as well as methods for antioxidant activity determination

Teucrium species	Sample type – extract/essential oil	Method used	References
T. alyssifolium	Leaf, flower and stem water extract	DPPH	Semiz et al. (2016)
T. arduini	Infusions	FRAP, DPPH, ABTS	Šamec et al. (2010)
	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH, FRAP, BR	Stanković (2012)
	Leaf, flower, stem ethanolic extract	DPPH, Fe^{2+} chelating activity, β-carotene/linoleic acid assay	Kremer et al. (2013)
	Ethanolic extracts	DPPH, TAC	Vladimir-Knežević et al. (2014)
T. barbeyanum	Petroleum ether, dichloromethane, methanolic, chloroform, ethyl acetate, butanolic and water extract	DPPH, ABTS, TEAC, FRAP	Alwahsh et al. (2015)
T. botrys	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH, FRAP, BR	Stanković (2012)
	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH	Stanković et al. (2014)
T. cavernarum	Methanolic extract	DPPH, ABTS, TEAC	Goger et al. (2019)
T. chamaedrys	Diethyl ether, ethyl acetate and n-butanolic extract	DPPH, HRSA, β-carotene/linoleic acid assay	Kadifkova Panovska et al. (2005)
	Water extract	DPPH, RP, TBA	Ozgen et al. (2006)
	Isolated chemical compounds	DPPH, TBA	Pacifico et al. (2009)
	Methanolic extract	DPPH, β-carotene/linoleic acid assay	Gursoy and Tepe (2009)
	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH	Stanković et al. (2010a)
	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH, FRAP, BR	Stanković (2012)
	Ethanolic extracts	DPPH, TAC	Vladimir-Knežević et al. (2014)
	Ethanolic extract	DPPH, TEAC, HAPX, EPR	Vlase et al. (2014)
	Methanolic-water extract	DPPH	El Guiche et al. (2015)
	Methanolic, ethanolic, acetone and ethyl acetate extract	DPPH	Stanković and Zlatić (2017)
	Methanolic extract and water infusion	DPPH	Zlatić et al. (2017)
	Methanolic extract	DPPH	Faiku et al. (2019)

(continued)

Table 10.1 (continued)

Teucrium species	Sample type – extract/essential oil	Method used	References
T. creticum	Ethanolic extract	DPPH, β-carotene/linoleic acid assay	Husein et al. (2014)
T. flavum	Essential oil	DPPH	Hammami et al. (2015b)
	Ethanolic extract	DPPH, SOD	Acquaviva et al. (2017)
	Methanolic, ethanolic, acetone and ethyl acetate extract	DPPH	Stanković and Zlatić (2017)
T. fruticans	Ethanolic extract	DPPH, SOD	Acquaviva et al. (2017)
	Methanolic, ethanolic, acetone and ethyl acetate extract	DPPH	Stanković and Zlatić (2017)
	Methanolic extract	DPPH, BR	Stanković et al. (2017)
T. hyrcanicum	Methanolic extract	DPPH, FRAP	Golfakhrabadi et al. (2015)
T. marum	Essential oil	DPPH, XOA, 5-lipoxygenase test	Ricci et al. (2005)
	Dichlormethane, acetone and ethanolic extract	DPPH, ABTS, β-carotene/linoleic acid assay	Poli et al. (2007)
T. massiliense	Essential oil	DPPH, Inhibition of lipid peroxide formation aasay	Giamperi et al. (2008)
T. montanum	Diethyl ether, ethyl acetate and n-butanolic extract	DPPH, HRSA, β-carotene/linoleic acid assay	Kadifkova Panovska et al. (2005)
	Petroleum ether, chloroform, ethyl acetate, n-butanolic and water extract	DPPH	Djilas et al. (2006)
	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH	Stanković et al. (2011a)
	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH, FRAP, BR	Stanković (2012)
	Ethanolic extracts	DPPH, TAC	Vladimir-Knežević et al. (2014)
	Methanolic extract and water infusion	DPPH	Zlatić et al. (2017)
	Water extract	DPPH, FRAP	Nastić et al. (2018)
T. montbretii	Methanolic extract	DPPH, Phosphomolybdenum assay	Ozkan et al. (2007)
T. muscatense	n-hexane, chloroform, ethanolic, n-buthanolic and water extract	DPPH, ABTS, TBA	Ur Rehman et al. (2017)

Teucrium species	Sample type – extract/essential oil	Method used	References
T. orientale	Petroleum ether, chloroform, acetone and methanolic extract	DPPH, Thiocyanate method	Cakir et al. (2006)
	Essential oil and methanolic extract	DPPH, β-carotene-linoleic acid assay	Amiri (2010)
T. oxylepis	Methanolic extract	DPPH, BR	Stanković et al. (2017)
T. parviflorum	Ethanolic and water extract	ABTS, DPPH, RP, SRSA, H_2O_2 scavenging capacity, Metal chelating activity	Turkoglu et al. (2010)
T. persicum	Essential oil	DPPH, FRAP, RP	Monsef-Esfahani et al. (2010)
T. pilosum	Isolated chemical compounds	DPPH, α-glucosidase inhibitory activity assay	Mou et al. (2009)
T. polium	Diethyl ether, ethyl acetate and n-butanolic extract	DPPH, HRSA, β-carotene/linoleic acid assay	Kadifkova Panovska et al. (2005)
	Water extract	β-carotene/linoleic acid assay, X/XOD, HRSA	Ljubunčić et al. (2006)
	Ethanolic extract	DPPH, TAP, TBA (*in vivo*)	Hasani et al. (2007)
	Methanolic extract	ABTS	Tarawneh et al. (2010)
	Methanolic, acetone and ethyl acetate extract	DPPH, FRAP, BR	Stanković et al. (2011b)
	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH, FRAP, BR	Stanković (2012)
	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH	Stanković et al. (2012)
	Methanolic extract	DPPH, FRAP	Belmekki and Bendimerad (2012)
	Methanolic, chloroform and ethyl acetate extract	XOA, SRSA, β-carotene/linoleic acid assay, Fe^{2+} chelating activity, DPPH, FRAP	Boumerfeg et al. (2012)
	Crude extracts and isolated chemical compounds	DPPH, RP, XOA, β-carotene/linoleic acid assay	De Marino et al. (2012)
	Isolated chemical compounds	DPPH, ABTS, ORAC	D'Abrosca et al. (2013)

(continued)

Table 10.1 (continued)

Teucrium species	Sample type – extract/essential oil	Method used	References
	Methanolic extract	DPPH, ABTS	Chedia et al. (2013)
	Essential oil	DPPH, β-carotene/linoleic acid assay	Mahmoudi and Nosratpour (2013)
	Ethanolic extract	DPPH, β-carotene/linoleic acid assay	Husein et al. (2014)
	Ethanolic extracts	DPPH, TAC	Vladimir-Knežević et al. (2014)
	Methanolic extract	TAC, DPPH, FRAP	Bilto et al. (2015)
	Essential oil and acetone extract	DPPH, RP, β-carotene/linoleic acid assay	Bakari et al. (2015)
	Essential oil and ethanolic extract	DPPH, RP	Kerbouche et al. (2015)
	Methanolic extract	DPPH, FRAP	Dridi et al. (2016)
	Methanolic extract	DPPH, BR	Stanković et al. (2017)
	Essential oil	DPPH, β-carotene/linoleic acid assay	Sayyad and Farahmandfar (2017)
	Water-ethanolic extract	DPPH	Fazeli-Nasab et al. (2017)
	n-hexane, dichloromethane, chloroform, ethyl acetate extract and methanolic fractions	ABTS	Dehghan and Sadani (2018)
	Essential oil, ethanolic extract	β-carotene/linoleic acid assay, FRAP, CUPRAC, ABTS, DPPH, SRSA	Bendjabeur et al. (2018)
	Methanolic, ethanolic, ethyl acetate, chloroform, hexane and water extract	DPPH, ABTS, RP	Ait Chaouche et al. (2018)
	Methanolic extract	DPPH, FRAP *(in vivo)*	Krache et al. (2018)
	Water and ethanolic extract	DPPH, β-carotene/linoleic acid assay, OSI	Farahmandfar et al. (2019)
	Methanolic, ethanolic, water and ethyl acetate extract	DPPH, FRAP, TAC	El Atki et al. (2019a)
	Essential oil	DPPH, FRAP, TAC	El Atki et al. (2019b)

Teucrium species	Sample type – extract/essential oil	Method used	References
T. pruinosum	Essential oil	DPPH	Jaradat et al. (2018)
T. pseudochamaepitys	Essential oil	DPPH	Hammami et al. (2015a)
T. pseudoscorodonia	Butanolic fraction of leaves, crude methanolic extract and ethyl acetate fraction	TAC, DPPH, FRAP, β-carotene/linoleic acid assay	Belarbi et al. (2017)
T. ramosissimum	Essential oil	X/XOD, NBT/riboflavine, DPPH	Ben Sghaier et al. (2010)
	Crude extracts	NBT/riboflavine, X/XOD	Ben Sghaier et al. (2011a)
	Crude extracts	NBT/riboflavine, DPPH, ABTS	Ben Sghaier et al. (2011b)
	Methanolic extract	CUPRAC, RP, FRAP	Ben Sghaier et al. (2016)
T. royleanum	Essential oil	DPPH	Saroglou et al. (2007)
T. salviastrum	Dichloromethane, ethanolic and water-ethanol extract; essential oil	DPPH	Cabral et al. (2010)
T. sandrasicum	Essential oil	TEAC	Ali et al. (2013)
	Water-ethanolic and water-methanolic extract	DPPH, ABTS, RP, β-carotene/linoleic acid, Phosphomolybdenum assays	Kaska et al. (2019)
T. sauvagei	Methanolic extract	DPPH, ABTS	Salah et al. (2006)
T. scordium	Water, methanolic, acetone, ethyl acetate and petroleum ether extract	DPPH, FRAP, BR	Stanković (2012)
T. siculum	Ethanolic extract	DPPH, SOD	Acquaviva et al. (2017)
T. stocksianum	Ecetone, butanolic, chloroform, ethyl acetate, ethanolic, methanolic, n-hexane and petroleum ether extract	DPPH	Rahim et al. (2013)
	Methanolic extract	DPPH	Shah et al. (2014)
	Water extract	DPPH	Shah and Shah (2015)
	Methanolic and water extract	DPPH	Uddin et al. (2016)

(continued)

Table 10.1 (continued)

Teucrium species	Sample type – extract/essential oil	Method used	References
T. subspinosum	Dichlormethane, acetone and ethanolic extract	DPPH, ABTS, β-carotene/ linoleic acid assay	Poli et al. (2007)
T. yemense	Essential oil	DPPH	Ali et al. (2017)

ABTS 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging capacity, *BR* Briggs-Rauscher oscillating reaction, *CUPRAC* Cupric Reducing Antioxidant Capacity assay, *DPPH* 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, *EPR* Electron Paramagnetic Resonance radicals detection, *FRAP* Ferric Reducing Antioxidant Power, *HAPX* Hemoglobin Ascorbate Peroxidase activity inhibition, *HRSA* Hydroxyl Radical Scavenging Activity, *NBT/riboflavine* Nitroblue Tetrazolium riboflavine assay, *ORAC* Oxygen Radical Absorbance Capacity, *OSI* Oxidative Stability Index, *RP* Reducing Power, *SOD* Superoxide Dismutase Activity assay, *SRSA* Superoxide Radical Scavenging Activity, *TAC* Total Antioxidant Capacity, *TAP* Total Antioxidant Power, *TBA* Thiobarbituric Acid Assay, *TEAC* Trolox Equivalent Antioxidant Capacity, *X/XOD* Xanthine/Xanthine Oxidase assay, *XOA* Xanthine Oxidase Activity assay antioxidant activity by analyzing extracts of different polarity from plant organs and whole herb using DPPH, FRAP and BR methods showed significant antioxidant activity of leaf methanolic extract (Stanković 2012). Antioxidant activity of *Teucrium arduini* was also investigated in a study of Kremer et al. (2013). The content of quercetin, ferulic and rosmarinic acid, as well as the antioxidant activity of ethanol leaf, flower and stem extracts using DPPH, Fe^{2+} chelating activity and *β*-carotene/linoleic acid assay were determined in this study. Further, the antioxidant activity of the ethanol extract of *Teucrium arduini* was examined by DPPH and TAC method (Vladimir-Knežević et al. 2014).

In a detailed study of the antioxidant activity of different extracts from *Teucrium barbeyanum*, ethyl acetate and buthanol extract showed significant antioxidant activity as well as highest totalphenolic content. In this study, 5-hydroxy-3,6,7,4′-tetramethoxyflavone, salvigenin, 5-hydroxy-6,7,3′,4′-tetramethoxyflavone, chrysosplenetin, cirsilineol, cirsimaritin, cirsiliol, apigenin, luteolin, methyl caffeate and 4-hydroxybenzoic acid were identified as significant chemical constituents (Alwahsh et al. 2015). The antioxidant activity of extracts of different polarity from *Teucrium* botrys were investigated by application of DPPH, FRAP and BR methods. The results showed that polar extracts of this species possess the highest antioxidant activity but moderate compared to other *Teucrium* species (Stanković 2012; Stanković et al. 2014). In the further studies, antioxidant activity and phenolic content of methanolic extract was determined for *Teucrium cavernarum*. In this experiment, DPPH, ABTS, TEAC assays were used for analysis. In qualitative study of secondary metabolites, forsythoside A, forsythoside B, luteolin-7-gucoside, cirsimaritin and cirsiliol were determined as the major compounds (Goger et al. 2019).

The antioxidant activity of *Teucrium chamaedrys* was determined for extracts of different polarity, infusions, as well as for the isolated chemical components. A significant level of antioxidant activity was determined in the study of diethyl ether, ethyl acetate and n-butanolic extract by DPPH, HRSA, *β*-carotene/linoleic acid assay (Kadifkova Panovska et al. 2005), as well as of water extract of *Teucrium chamaedrys* by DPPH, RP and TBA methods (Ozgen et al. 2006). In a study of the antioxidant activity of the isolated chemical components of *Teucrium chamaedrys*, significant activity was determined for phenylethanoid and iridoid glycosides (Pacifico et al. 2009). Remarkable antioxidant activity of this species was determined for polar fractions using DPPH, *β*-carotene/linoleic acid assays (Gursoy and Tepe 2009). In the comparative analysis of extracts with different polarity such as water, methanolic, acetone, ethyl acetate and petroleum ether extract, significant antioxidant activity correlated with the amount of phenolic compounds was determined. In these comparative studies, the variability of the amount of phenolic compounds and antioxidant activity were determined for the extracts from different plant parts of *Teucrium chamaedrys* such as leaf, flower and stem extracts, as well as for *Teucrium chamaedrys* samples at different stages of development, such as before flowering, during flowering, and after flowering. The results showed that leaf extracts obtained with polar solvents and extracts from plants during flowering period possess the highest amount of phenolic compounds and antioxidant activity (Stanković et al. 2010a; Stanković 2012; Stanković and Zlatić 2017). Further studies analyzed the antioxidant activity and phytochemical composition of ethanolic extracts (Vladimir-Knežević et al. 2014; Vlase et al. 2014). In the tested ethanolic extracts, the presence of isoquercitrin, rutin, luteolin, quercitrin, *p*-coumaric, rosmarinic, chlorogenic and gentisic acid was determined. The antioxidant activity of ethanol extracts was confirmed by the DPPH and TAC methods (Vladimir-Knežević et al. 2014) as well as DPPH, TEAC, HAPX, EPR (Vlase et al. 2014). Antioxidant activity assay was conducted for methanolic-water (El Guiche et al. 2015) and methanolic (Faiku et al. 2019) extracts of *Teucrium chamaedrys* by DPPH method.

Within the group of the analyzed medicinal plant species, antioxidant activity was determined for *Teucrium creticum* and *Teucrium polium* using DPPH and *β*-carotene-linoleic acid assay. In addition, the amount of total phenolic compounds and flavonoids was determined, as well as a strong correlation between the amount of these compounds and free radical scavenging activity and total antioxidant activity (Husein et al. 2014). Various studies of biological activity have examined the antioxidant activity of *Teucrium flavum*. Hammami et al. 2015b identified *β*-caryophyllene and *α*-humulene as the most abundant components of essential oil and its moderate antioxidant activity. The antioxidant activity of the ethanolic extract was evaluated by application of DPPH and SOD-like methods (Acquaviva et al. 2017) as well as of the methanolic, ethanolic, acetone and ethyl acetate extract by DPPH method (Stanković and Zlatić 2017). The results showed that the extracts of this species have a significant amount of secondary metabolites with antioxidant activity. Also, *Teucrium* fruticans extracts were tested to evaluate the content of phenolic compounds as well as antioxidant and other biological activities (Acquaviva et al. 2017; Stanković and Zlatić 2017; Stanković et al. 2017). By testing methanolic, ethanolic, acetone and ethyl acetate extracts using DPPH, SOD-like and BR methods, the antioxidant activity of *Teucrium* fruticans extracts was determined. Also, methanolic extract from *Teucrium hyrcanicum* showed antioxidant activity and significant amount of flavonoids. In this study, antioxidant activity was determined using the DPPH and FRAP method (Golfakhrabadi et al. 2015).

The antioxidant activity of *Teucrium marum* has been studied by testing essential oil (Ricci et al. 2005) and extracts (Poli et al. 2007). Qualitative analysis of the essential oil revealed that the isocaryophyllene, *β*-bisabolene, *β*-sesquiphellandrene, *α*-santalene and *α*-caryophyllene were the most components. The antioxidant activity of the essential oil was evaluated using the DPPH test, 5-lipoxygenase test and XOA assay. Dichlormethane, acetone and ethanolic extract of *Teucrium marum* were analyzed by application of DPPH, ABTS, *β*-carotene/linoleic acid assay. A significant level of antioxidant activity was determined for polar *Teucrium marum* extracts. In the analysis of the essential oil chemical composition from *Teucrium massiliense*, within 35 identified compounds, 3,7-dimethyloctan-2-one, butyl 2-methylbutyrate, linalool, linalyl acetate, zingiberene, *γ*-cadinene are the most represented. In this study, the antioxidant activity of the essential oil was determined using the DPPH and lipid peroxidation methods (Giamperi et al. 2008).

The antioxidant activity of secondary metabolites of *Teucrium montanum* has been investigated in several studies. The antioxidant activity of the diethyl ether, ethyl acetate and n-butanolic extract of this species was determined by the DPPH, HRSA, *β*-carotene/linoleic acid assays. The authors also found that the presence and quantitative composition of flavonoids were associated with high values for antioxidant activity (Kadifkova Panovska et al. 2005). Significant antioxidant activity of *Teucrium montanum* was confirmed by testing petroleum ether, chloroform, ethyl acetate, n-butanolic and water extract using DPPH method (Djilas et al. 2006). In a comparative study of the antioxidant activity of *Teucrium montanum*, water, methanolic, acetone, ethyl acetate and petroleum ether extract of the whole plant, flowers, leaves and stems were investigated using DPPH, FRAP, BR methods. In addition, antioxidant activity of the extracts obtained from *Teucrium montanum* sampled during different phenophases, such as before flowering, during flowering and after flowering were evaluated. The results of the studies indicate that the quantity of secondary metabolites as well as the antioxidant activity of the extracts depend on the plant part and the phenological phase of sample collection (Stanković et al. 2011a; Stanković 2012). Also, the variability in the amount and antioxidant activity of secondary metabolites in relation to the type of geological substrate on the habitat was examined for *Teucrium montanum*. The results of this comparative study showed that plants from harshest habitats possess a higher amount of secondary metabolites with possible adaptive significance, as well as notable antioxidant activity (Stanković et al. 2010b; Zlatić et al. 2017). Further studies analyzed the antioxidant activity of water (Vladimir-Knežević et al. 2014) and ethanolic (Nastić et al. 2018) extract of *Teucrium montanum*. The results of the phytochemical study of these extracts confirmed the presence of naringin, gallic, chlorogenic, caffeic and ferulic acid in the investigated extracts.

The total amount of phenolic compounds, flavonoids and flavonols was determined for the methanolic extract of *Teucrium montbretii*. In addition to these methods, antioxidant activity has been determined by use DPPH and phosphomolybdenum assay (Ozkan et al. 2007). In the phytochemical and pharmacological study of *Teucrium muscatense* n-hexane, chloroform, ethanolic, n-buthanolic and water extract was examined. Antioxidant activity was determined by DPPH, ABTS, TBA assays (Ur Rehman et al. 2017). GC/MS analysis of the essential oil showed higher percentage of linalool, linalyl acetate, and *β*-eudesmol. The antioxidant activity of *Teucrium* orientale was analyzed in two studies. In the first study, Chakir et al. (2006) analyzed the petroleum ether, chloroform, acetone and methanolic extract of this species using the DPPH and thiocyanate method. In this study, the authors found higher antioxidant activity as well as the amount of phenolic compounds for acetone extract during the flowering phase. In the second study, the essential oil and methanolic extract were tested using DPPH and *β*-carotene-linoleic acid assay (Amiri 2010). Forty compounds have been found in the essential oil of this species. The major constituents were linalool, caryophyllene oxide, 1,8-cineol, *β*-pinene, 3-octanol, *β*-caryophyllene and germacrene-D. High antioxidant activity was found for polar fractions of *Teucrium* orientale extracts.

In screening of phytochemical composition and biological activity of *Teucrium oxylepis*, methanolic extract was tested for the content of phenolic compounds as well as for the antioxidant activity by DPPH and BR methods (Stanković et al.

2017). Ethanolic and water extracts from leaves and flowers of *Teucrium parviflorum* were tested for antioxidant activity. Different methods were used for this study such as ABTS, DPPH, RP, SRSA, H_2O_2 scavenging capacity, metal chelating activity (Turkoglu et al. 2010.). For the essential oil of *Teucrium persicum* seasonal variation in chemical content and antioxidant activity has been studied (Monsef-Esfahani et al. 2010). Essential oil were analyzed during pre-flowering, flowering and post-flowering phenological phase. Analysis of the chemical profile of the isolated oils revealed the presence of sesquiterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes. They contained α-terpinyl acetate, α-cadinene, 1,4-cadinadiene, linalool and cadinol. For the analyzed oils, good antioxidant activity was measured by DPPF, FRAP and RP method. In the conducted research of *Teucrium pilosum* the authors are isolated chemical constituents and analyzed the antioxidant activity. The antioxidant activity was tested by the DPPH and alpha-glucosidase inhibitory activity assay for glyceryl tristearate, 2,5-dioxolanone, fernenol, stigmasta-5,22-dien-3Pol, 24-nor cholesta-5,22 (E)-dien-3beta-ol, ca-spinasterol, (3,4-dihydroxyphenyl) acrylate, 3,4-dihydroxy phenyl acrylic acid (Mou et al. 2009).

The antioxidant activity of *Teucrium polium* has been analyzed in a large number of studies. This species is one of the most tested medicinal plant from the *Teucrium* genus. In the conducted studies, different extracts, isolated components and essential oils were examined. In addition to investigating antioxidant activity, the quantitative composition of secondary metabolites was examined (Table 10.1). Significant antioxidant activity of *Teucrium polium* extracts have been reported in numerous studies. In the study of the antioxidant activity of *Teucrium polium*, extracts obtained using solvents of different polarity were investigated, such as acetone, chloroform, diethyl ether, ethanolic, ethyl acetate, hexane, methanolic, n-butanolic, petroleum ether, water and water-ethanolic extracts. Antioxidant activity has been determined using almost all known methods for antioxidant activity determination – DPPH, FRAP, β-carotene/linoleic acid assay, ABTS, BR, X/XOD, XOA, HRSA, TAP, TAC, TBA, SRSA, Fe^{2+} chelating activity, RP and OSI method (Kadifkova Panovska et al. 2005; Ljubunčić et al. 2006; Hasani et al. 2007; Tarawneh et al. 2010; Stanković et al. 2011b, 2012, 2017; Stanković 2012; Belmekki and Bendimerad 2012; Boumerfeg et al. 2012; Chedia et al. 2013; Husein et al. 2014; Vladimir-Knežević et al. 2014; Bilto et al. 2015; Dridi et al. 2016; Fazeli-Nasab et al. 2017; Ait Chaouche et al. 2018; Dehghan and Sadani 2018; Krache et al. 2018; Farahmandfar et al. 2019; El Atki et al. 2019a). Additionally, antioxidant activity of *Teucrium polium* has also been identified for isolated components of plant extracts such as flavones (D'Abrosca et al. 2013) as well as phenyletanoid and iridoid glycosides (De Marino et al. 2012). The composition and antioxidant activity of *Teucrium polium* essential oil have been analyzed in several studies. The results of these chemical analyzes indicate that spathulenol, β-pinene, β-myrcene, germacrene B, germacrene D, bicyclogermacrene, *t*-cadinol and linalool were the predominant components in *Teucrium polium* essential oil. The results also show significant antioxidant activity of the essential oil determined using various methods such as DPPH, β-carotene/linoleic acid assay, RP, FRAP, TAC and CUPRAC (Mahmoudi and Nosratpour 2013; Bakari et al. 2015; Kerbouche et al. 2015; Sayyad and

Farahmandfar 2017; Bendjabeur et al. 2018; El Atki et al. 2019b). In a comparative study of antioxidant activity, whole plant extracts and extracts from different plant parts such as leaves, flowers and stem were analyzed. The results of these tests show that *Teucrium polium* leaf extract contain the highest amount of phenolic compounds and show the significant antioxidant activity (Stanković 2012; Stanković et al. 2012). In some studies of the antioxidant activity and chemical composition of *Teucrium polium* extracts, the presence of various phenolic compounds such as luteolin, apigenin, myricetin, quercetin, kaempferol, resveratrol, coumaric, rosmarinic, chlorogenic, ferulic and caffeic acid (Kadifkova Panovska et al. 2005; Stanković 2012; Vladimir-Knežević et al. 2014).

In a study by Jaradat et al. (2018) the chemical composition of the essential oil and the biological activity were studied for *Teucrium pruinosum*. The authors found that agarospirol and caryophyllene were the most represented components of the essential oil. For the isolated essential oil, antioxidant activity was determined using the DPPH method in addition to other methods of biological activity. Also, the essential oil of *Teucrium pseudochamepitys* was analyzed in next experiment (Hammami et al. 2015a). Of 31 identifed compounds hexadecanoic acid was most abundant component followed by caryophyllene oxide, myristicin and α-cubebene. Belarbi et al. (2017) studied antioxidant activity as well as the amount of active secondary metabolites of different extracts from *Teucrium pseudoscorodonia.* For this experiment, the authors analyzed extracts and fractions of different polarity. Total phenolic, flavonoid, and condensed tannin contents followed by antioxidant assays such as TAC, DPPH, FRAP, β-carotene/linoleic acid assay were determined. The authors also showed a correlation of antioxidant activity with total phenolic and flavonoid content.

Essential oil and various extracts from *Teucrium ramosissimum* have been examined in several studies using different enzymatic and non-enzymatic methods such as X/XOD assay, NBT/riboflavine, ABTS, DPPH, CUPRAC, RP and FRAP assays. The result of conducted studies has demonstrated that *Teucrium ramosissimum* extracts possess potent antioxidant activity which could be derived from compounds such as polyphenols (Ben Sghaier et al. 2010, 2011a, b, 2016). The chemical composition of the essential oil and the antioxidant activity have also been investigated for *Teucrium royleanum* (Saroglou et al. 2007). For the essential oil of this species, sesquiterpene hydrocarbons are the most represented group of chemical compounds among which β-santalene and cis-α-bisabolene were the predominant compounds. The antioxidant activity was investigated using the DPPH method. By same method, antioxidant activity of dichloromethane, ethanolic and water-ethanol extract from *Teucrium salviastrum* was determined, as well as total phenolic content (Cabral et al. 2010). The antioxidant activity of the *Teucrium sandrasicum* was investigated for essential oil by TEAC method (Ali et al. 2013), as well as for water-ethanolic and water-methanolic extract using different methods such as DPPH, ABTS, RP, β-carotene/linoleic acid and phosphomolybdenum metal chelating assays (Kaska et al. 2019). In addition to testing for antioxidant activity, the composition of the essential oil was analyzed. The main components of the *Teucrium sandrasicum* essential oil were detected as α-pinene, β-pinene, germacrene-D and sabinene. For the analyzed extracts, the presence of certain phenolic acids and flavonoids was examined. Also, the antioxidant activity of essential oil and the methanolic leaf extracts from *Teucrium sauvagei* was investigated by DPPH and ABTS method. Results of the analysis of the chemical composition of the essential oil show that β-eudesmol, T-cadinol, α-thujene, γ-cadinene, and sabinene were the most represented chemical components (Salah et al. 2006).

In order to analyze the antioxidant activity of *Teucrium scordium*, aqueous, water, methanolic, acetone, ethyl acetate and petroleum ether extract were tested using DPPH, FRAP and BR methods. The results showed that the methanolic extract possess the most significant level of antioxidant activity compared to the extracts obtained using other solvents (Stanković 2012). Infloresence ethanolic extract from *Teucrium siculum* was tested for antioxidant activity by DPPH and SOD-like methods. Based on phenolic content determined as well as the antioxidant activity, authors indicate important biological potential of this *Teucrium* species (Acquaviva et al. 2017). Also, *Teucrium stocksianum* extracts have been tested in various studies using the DPPH method. Acetone, butanolic, chloroform, ethyl acetate, ethanolic, water, methanolic, n-hexane and petroleum ether extract were tested in four studies (Rahim et al. 2013; Shah et al. 2014; Shah and Shah 2015; Uddin et al. 2016). Studies have shown that *Teucrium stocksianum* is characterized by significant antioxidant potential, especially extracts obtained by polar solvents. In a study of *Teucrium subspinosum,* in dichlormethane, acetone and ethanolic extract, the presence of verbascoside and arabinosyl-verbascoside has been determined. These compounds are associated with a significant level of antioxidant activity determined by DPPH, ABTS, β-carotene/linoleic acid assays (Poli et al. 2007). For *Teucrium yemense* essential oil Ali et al. (2017) identifed remarkable radical inhibition potential by DPPH method, as well as α-pinene, (E)-caryophyllene, α-humulene, δ-cadinene, caryophyllene oxide, α-cadinol and shyobunol as the major compounds.

Analysis of the presented papers on the study of the antioxidant activity of *Teucrium* species indicates that *Tucrium polium* (over 30 papers) is the most tested species followed by *Teucrium chamaedrys* (over ten papers), and *T. montanum* (over five papers), while less than five papers were found for the other analyzed *Teucrium* species (Table 10.1). The antioxidant activity of the extracts obtained by solvents of different polarity is the most common way of testing in relation to the antioxidant activity of the essential oils and isolated chemical components. The most commonly used methods include DPPH – 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, Ferric Reducing Antioxidant Power (FRAP), β-carotene/linoleic acid assay, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging capacity (ABTS) etc. In the conducted studies of the antioxidant activity of *Teucrium* species, the quantitative and qualitative composition of secondary metabolites have been analyzed in many studies. The most frequently analyzed is the amount of total phenolic compounds and flavonoids, as well as the chemical composition of essential oils (Ricci et al. 2005; Saroglou et al. 2007; Giamperi et al. 2008; Amiri 2010; Ben Sghaier et al. 2010; Cabral et al. 2010; Monsef-Esfahani et al. 2010; Mahmoudi and Nosratpour 2013; Bakari et al. 2015; Hammami et al. 2015a, b; Kerbouche et al. 2015; Ali et al. 2017; Sayyad and Farahmandfar 2017;

Bendjabeur et al. 2018; Jaradat et al. 2018; El Atki et al. 2019b) and plant extracts (Kadifkova Panovska et al. 2005; Poli et al. 2007; Stanković 2012; Kremer et al. 2013; Vladimir-Knežević et al. 2014; Vlase et al. 2014; Alwahsh et al. 2015; Goger et al. 2019; Nastić et al. 2018). Essential oil of *Teucrium* species is characterized by the presence of monoterpenes (limonene, α- and β-pinene) and sesquiterpenes (caryophyllene and its derivates, germacrene B and D, α-, γ-, δ-cadinene, cadinol, bisabolol), while phenolics of this genus is characterized by the presence of flavonoids (luteolin and apigenin), phenylethanoids (verbascoside) and phenolic acids (hydroxybenzoic and hydroxycinnamic acids). The significant correlation between the antioxidant activity and content of phenolic compounds, as well as the antioxidant potential of the presented chemical compounds in many individual studies (Quideau et al. 2011) indicates that species of the genus *Teucrium* are a source of secondary metabolites with significant antioxidant potential.

10.3.1 Correlation Between Phenolic Content and Antioxidant Activity

A significant correlation between antioxidant activities and phenolic content of *Teucrium* species was found for *Teucrium arduini* (Stanković 2012), *T. barbeyanum* (Alwahsh et al. 2015), *T. botrys* (Stanković 2012), *T. chamaedrys* (Ozgen et al. 2006; Gursoy and Tepe 2009; Stanković et al. 2010a; Stanković 2012; Zlatić et al. 2017), *T. montanum* (Stanković et al. 2011a; Stanković 2012; Zlatić et al. 2017), *T. orientale* (Amiri 2010), *T. polium* (Stanković 2012; Stanković et al. 2012; Vladimir-Knežević et al. 2014; Bakari et al. 2015; Dehghan and Sadani 2018; Krache et al. 2018; El Atki et al. 2019a) and *T. scordium* (Stanković 2012).

When examining the results of the numerous studies in various fields of science, the correlation coefficient is used as a measure for the total ratio of statistical relationships. The correlation coefficient is a parameter of summarizing the relationship between two variables in a single number. The correlation coefficient has values between −1 and + 1 and is it marked with the symbol (r). When the correlation values are close to 0, it is understood that there is no significant correlation between the variables being compared, or it is very small. When the correlation values are close to +1 it means that there is a positive relationship between the two variables. This further implies that as the value of the first variable increases, the values of the second variable increase, which is why they are in a positive correlation. When the correlation values are −1, this indicates a negative relationship between the two variables, which means that as the values of the first variable increase, the values of the second variable decrease (Schober et al. 2018).

In biological research, the correlation coefficient is used as one of the numerous statistical methods. When comparing the results of the total amount of phenolic compounds in the plant extracts and their antioxidant activity, the determination of the correlation coefficient is an essential part. Phenols are very important plant constituents because of their scavenging ability on free radicals due to the presence of hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant activity (Tosun et al. 2009). Numerous researchers have shown that there is a highly significant correlation between phenolics and antioxidant activity which supports the hypothesis that phenolic compounds directly contribute to the total antioxidant capacity of plant species (Tosun et al. 2009; Moein and Moein 2010; Stanković et al. 2010a; Alwahsh et al. 2015; Zlatić et al. 2017). Stanković et al. (2010a) reported a significant correlation ($r = 0.701$) between total phenolic compounds and antioxidant activity of different plant extracts of *Teucrium chamaedrys*. Bakari et al. (2015) noted a strong positive correlation between total phenols and IC_{50} of DPPH (coefficient of determination $R^2 = 0.999$) for the acetone extract of species *Teucrium polium*. Also, for the same species is determined a significant correlation between antioxidant activities and contents of polyphenols in methanol, ethanol, water, ethyl acetate, and petroleum ether extracts (Stanković et al. 2012). El Atki et al. (2019a) studied a correlation between the results of antioxidant tests and the contents of total phenolics of two *Teucrium polium* subspecies. They reported a significant and negative correlation between DPPH and total phenols ($r^2 = -0.78$). For the ferric reducing power test, the IC_{50} values were significantly and negatively correlated with total phenols ($r^2 = -0.81$). Zlatić et al. (2017) showed a significant positive correlation between total phenols and antioxidant activity in extract $r = 0.900$ ($p < 0.05$) and plant material $r = 0.957$ ($p < 0.01$) for *Teucrium chamaedrys* and *T. montanum* species sampled on limestone and serpentinite geological substrates. For six species and one subspecies of the genus *Teucrium* examined by Stanković (2012), a correlation factor ($r = 0.911$) was determined between the total phenol content and the antioxidant activity of DPPH by the 140 tested extracts. The previous research has shown that species belonging to the genus *Teucrium* can be used as good antioxidants because of the strong correlation between total phenol content and antioxidant activity.

The presented values for the correlation between total phenol content and antioxidant activity (DPPH) had different values. Since a lower IC_{50} value indicates higher antioxidant activity, it can be observed that with increasing phenol content, activity against DPPH radicals increases, i.e. the IC_{50} value decreases. The linear correlation is therefore negative and strong. Some results of correlation analysis indicate that antiradical activity is not necessarily correlated with the content of phenolic compounds. When considering the antioxidant potential of the sample, it is necessary to analyze content of all present active components. Some studies suggest that it is not always possible to correlate total phenolics and antioxidant capacity. This could be explained by the presence of different active compounds in the plant and the structural forms included in the nature of the phenolic groups. Phenolics include complex compounds with one or more hydroxyl groups (-OH), with phenylpropanoid side chains and with additional aromatic/heterocyclic ring structures, which differentiates the antioxidant activity of the tested extracts. Antioxidant activity is not simply reflective of total phenolic substances in a complex mixture. Antioxidant activity depends on the number of molecules of certain compounds and their structural forms that have potential radical scavenging activity. This indicates that phenolic compounds are partial, if not completely, responsible for the antioxidant activity observed in the tested species of the genus *Teucrium* (Stanković 2012).

10.3.2 Methods Used for Determining the Antioxidant Activities of **Teucrium** *Species*

For determination of the antioxidant activity of plant extracts, essential oils as well as isolated chemical compounds from *Teucrium* species, about 30 analytical methods have been used (Table 10.1). The most commonly applied assays include DPPH – 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity followed by Ferric Reducing Antioxidant Power (FRAP), *β*-carotene/linoleic acid assay and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging capacity (ABTS), as well as moderately applied methods such as: Total Antioxidant Capacity (TAC), Reducing Power (RP), Briggs-Rauscher oscillating reaction (BR), Hydroxyl Radical Scavenging Activity (HRSA), Trolox Equivalent Antioxidant Capacity (TEAC), Thiobarbituric Acid Assay (TBA), Nitroblue Tetrazolium riboflavine assay (NBT/riboflavine), Superoxide Dismutase Activity assay (SOD), Superoxide Radical Scavenging Activity (SRSA), Xanthine/Xanthine Oxidase assay (X/XOD), XOA – Xanthine Oxidase Activity assay (XOA), Fe^{2+} chelating activity, Cupric Reducing Antioxidant Capacity assay (CUPRAC), Phosphomolybdenum assay, Electron Paramagnetic Resonance radicals detection (EPR), Hemoglobin Ascorbate Peroxidase activity inhibition (HAPX), Oxygen Radical Absorbance Capacity (ORAC), Oxidative Stability Index (OSI), Total Antioxidant Power (TAP), 5-lipoxygenase test, *α*-glucosidase inhibitory activity assay, H_2O_2 scavenging capacity, Thiocyanate method and Metal chelating activity and Inhibition of lipid peroxide formation aasay.

Each listed method will always have advantages and disadvantages, which need to be taken into account in terms of complexity, sample preparation, required reagents and equipment, the chemical mechanism, the quantification method, values presentation and its relevance in biological systems. Scientific evidence indicates that DPPH is the most used assay for the analysis of the antioxidant activity (Table 10.1). The method was described by Brand-Williams et al. (1995) and adopted with suitable modifications for different samples of natural and synthetic origin. The DPPH method is based on the ability of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical to react with different hydrogen donors as well as absorbance reduction of the radical anion DPPH• by antioxidants. Based on the chemical structure, DPPH is very stable organic nitrogen free radical. In dry form, the DPPH is a dark-purple colored powder with absorbance peak at 515–528 nm. After dissolution, dark-purple color loses its intensity, depending on the concentration. In the reduction reaction by antioxidant, the color of the solution changes to yellow, based on the concentration of the reduced molecules in the reaction mixture.

Fig. 10.1 Structure of DPPH and its reduction by an antioxidant

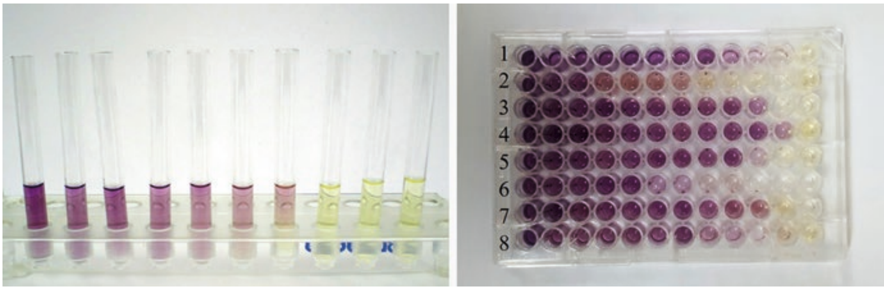

Fig. 10.2 Samples prepared for DPPH assay after reaction with reagent – sample series with increasing concentration gradient of the one plant extract for spectrophotometric absorbance reading and eight plant extract series for microplate absorbance reading

Antioxidant compounds as donors of the hydrogen atom reduce the stable 1,1-diphenyl-2-picrylhydrazine radical by transforming it into 1,1-diphenyl-2-(2,4,6-trinitrophenyl)-hydrazine (Fig. 10.1).

During this process, the purple color of the initial solution derived from the stable DPPH radical is changed to the yellow due to the presence of a newly formed 1,1-diphenyl-2-(2,4,6-trinitrophenyl)-hydrazine which resulting in a decrease in absorbance at 517 nm. This property allows visual and spectrophotometric monitoring of the reaction, i.e., the quantity of radicals at the beginning can be determined by changing of the absorbance at 517 nm or by the ECP signal of the DPPH radical. In an experimental study of the antioxidant activity of plant extracts, the intensity of color change from violet to yellow is in correlation with the antioxidative potential of the plant extract (Fig. 10.2), which can be monitored by spectrophotometric measurement. Based on this, the DPPH method is widely used to measure the ability of different substances to act as free radical scavengers or hydrogen donors, as well as to evaluate antioxidant activity of plant compounds as plant extracts or isolated metabolites (Stanković et al. 2019).

Plant extracts obtained by various solvents have been found to be the most common form of samples for testing the antioxidant activity of *Teucrium* species followed by essential oils as well as isolated chemical components. In studies of the antioxidant activity of *Teucrium* species methanolic, ethanolic, acetone, n-butanolic, chloroform, ethyl acetate, n-hexane, petroleum ether, dichlormethane, diethyl ether and water extracts were tested (Table 10.1). Essential oils of *Teucrium* species are characterized by significant chemical composition and biological activity. In previous studies, the antioxidant activity and chemical composition of essential oils have been investigated for *Teucrium flavum* (Hammami et al. 2015b), *T. marum* (Ricci et al. 2005), *T. massiliense* (Giamperi et al. 2008), *T. orientale* (Amiri 2010), *T. persicum* (Monsef-Esfahani et al. 2010), *T. polium* (Mahmoudi and Nosratpour 2013; Bakari et al. 2015; Kerbouche et al. 2015; Sayyad and Farahmandfar 2017; Bendjabeur et al. 2018; El Atki et al. 2019b), *T. pruinosum* (Jaradat et al. 2018), *T. pseudochamaepitys* (Hammami et al. 2015a), *T. ramosissimum* (Ben Sghaier et al. 2010), *T. royleanum* (Saroglou et al. 2007), *T. salviastrum* (Cabral et al. 2010) and *T. yemense* (Ali et al. 2017). In addition to investigating the chemical composition and biological activity of plant extracts and essential oils, some of the more detailed studies have assaying isolated chemical components. The antioxidant activity of isolated chemical constituents for the species of the genus *Teucrium* was determined for phenylethanoid and iridoid glycosides from *Teucrium chamaedrys* (Pacifico et al. 2009), glyceryl tristearate, 2,5-dioxolanone, fernenol, stigmasta-5,22-dien-3Pol, 24-nor cholesta-5,22 (E)-dien-3beta-ol, ca-spinasterol, (3,4-dihydroxyphenyl) acrylate, 3,4-dihydroxy phenyl acrylic acid from *Teucrium pilosum* (Mou et al. 2009) and flavones (D'Abrosca et al. 2013) as well as phenyletanoid and iridoid glycosides (De Marino et al. 2012) from *Teucrium polium*.

10.3.3 Antioxidant Activity of Traditionally Used Teucrium *Species*

Some species of the genus *Teucrium* are known medicinal plants with long traditional use (Fig. 10.3). Teas and other herbal remedies are used to treat certain diseases and disorders of the digestive and respiratory organs as well as skin disorders and diabetes (Stanković 2012). Investigations of biological activity as well as the phytochemical composition of many plants are based on their previous traditional use. In the studies of the *Teucrium* species conducted so far, many medicinal species of this genus has been the subject of different biological activities. The antioxidant activity of extracts, essential oils, and isolated chemical components has been tested for many medicinal species of the genus *Teucrium*, such as *T. chamaedrys* (Kadifkova Panovska et al. 2005; Ozgen et al. 2006; Pacifico et al. 2009; Gursoy and Tepe 2009; Stanković et al. 2010a; Stanković 2012; Vladimir-Knežević et al. 2014; Vlase et al. 2014; El Guiche et al. 2015; Stanković and Zlatić 2017; Zlatić et al. 2017; Faiku et al. 2019), *T. creticum* (Husein et al. 2014), *T. flavum* (Hammami et al. 2015b; Acquaviva et al. 2017; Stanković and Zlatić 2017), *T. massiliense* (Giamperi et al.

Fig. 10.3 Selected species of the genus *Teucrium* with traditional ethnomedicinal use. (Photo M. Stanković)

2008), *T. montanum* (Kadifkova Panovska et al. 2005; Djilas et al. 2006; Stanković et al. 2011a; Stanković 2012; Vladimir-Knežević et al. 2014; Zlatić et al. 2017; Nastić et al. 2018), *T. arduini* (Šamec et al. 2010; Stanković 2012; Kremer et al. 2013; Vladimir-Knežević et al. 2014), *T. polium* (Kadifkova Panovska et al. 2005; Ljubunčić et al. 2006; Hasani et al. 2007; Tarawneh et al. 2010; Stanković et al. 2011b, 2012, 2017; Stanković 2012; Belmekki and Bendimerad 2012; Boumerfeg et al. 2012; De Marino et al. 2012; D'Abrosca et al. 2013; Chedia et al. 2013;

Mahmoudi and Nosratpour 2013; Husein et al. 2014; Vladimir-Knežević et al. 2014; Bilto et al. 2015; Bakari et al. 2015; Kerbouche et al. 2015; Dridi et al. 2016; Sayyad and Farahmandfar 2017; Fazeli-Nasab et al. 2017; Dehghan and Sadani 2018; Bendjabeur et al. 2018; Ait Chaouche et al. 2018; Krache et al. 2018; Farahmandfar et al. 2019; El Atki et al. 2019a, b), *T. pruinosum* (Jaradat et al. 2018), *T. pseudochamaepitys* (Hammami et al. 2015a), *T. ramosissimum* (Ben Sghaier et al. 2010, 2011a, b, 2016), *T. sandrasicum* (Ali et al. 2013; Kaska et al. 2019), *T. stocksianum* (Rahim et al. 2013; Shah et al. 2014; Shah and Shah 2015; Uddin et al. 2016) *T. scordium* (Stanković 2012) and *T. yemense* (Ali et al. 2017). The results of the above studies of antioxidant activity of *Teucrium* medicinal species, as well as the amounts and diversity of their secondary metabolites as bioactive compounds justify their previous traditional use in the treatment of many diseases and health disorders.

10.3.4 In Vivo Study of the Antioxidant Activity of **Teucrium** *Species*

For extracts, essential oils, as well as isolated chemical components of *Teucrium* species, antioxidant activity was investigated using a model system under in vitro conditions. A review of the literature indicates that some experiments for the determination of antioxidant activity have been carried out using an in vivo mode system, whereby selected laboratory animals have been treated with bioactive substances of these plant species. Hasani et al. (2007) investigated the in vivo antioxidant potential of ethanolic extract from *Teucrium polim* and compared the obtained results with α-tocopherol as a reference antioxidant for comparison. The ethanol extract was administered to laboratory Wistar rats by intragastric intubation at various concentrations. Antioxidant activity was measured by three methods (DPPH, TAP and TBA) in serum. Results obtained by used assays indicate that *Teucrium polium* effectively inhibits oxidative stress in vivo. An in vivo study of antioxidant activity was also conducted by Krache et al. (2018) for methanolic extract of *Teucrium polium*. Adults of laboratory mice were treated with methanol extract of *Teucrium polium*, after which the blood parameters of antioxidant activity were measured using several methods. Significant antioxidant potential of *T. polium* has been demonstrated using these in vivo model systems.

10.3.5 Variability of the Antioxidant Activity of **Teucrium** *Species*

Variability during vegetative period, habitat-related variability as well as variability between plant parts were determined for the antioxidant activity and content of the active compounds of some *Teucrium* species. Dynamics of the phenolic content and antioxidant activity for *Teucrium* orientale (Cakir et al. 2006), *Teucrium chamaedrys* and *Teucrium montanum* (Stanković 2012), as well as essential oil composition and antioxidant activity of *Teucrium persicum* (Monsef-Esfahani et al. 2010) during several successive phases such as before flowering, during flowering and after flowering has been investigated. The results of these studies showed that plants during the flowering phase contain a significant amount of bioactive compounds and exhibits the strongest antioxidant activity. Habitat-related variability has been studied for *Teucrium* species in several comparative studies. Variability in the amount and composition of secondary metabolites as well as antioxidant activity in relation to the type of geological substrate, soil mineral composition, habitat type altitude, latitude etc., were investigated for *Teucrium chamaedrys* (Stanković et al. 2011c; Stanković 2012, 2018; Zlatić et al. 2017; Faiku et al. 2019), *T. montanum* (Stanković 2011, 2012; Zlatić et al. 2017), *T. polium* (Stanković 2012, 2013) and *T. scordium* (Stanković 2012). The results of the studies indicate significant ecological variability of the investigated parameters as well as their adaptive importance to the specific habitats. Ecological conditions on the habitats with a specific geological substrate such as serpentinite, halophytic habitats as well as high altitude habitats all affect the phenolic content and diversity and antioxidant activity of the investigated *Teucrium* species. The plant part variability determination is based on a comparative analysis of the antioxidant activity of extracts obtained from different plant parts such as inflorescences, flowers, leaves, stems etc. The variability of the phenolic content and antioxidant activity in relation to plant part of *Teucrium* species was investigated for *Teucrium arduini* (Šamec et al. 2010; Stanković 2012), *T. botrys* (Stanković 2012; Stanković et al. 2014), *T. chamaedrys* (Stanković et al. 2010a; Stanković 2012), *T. flavum*, *T. fruticans* and *T. siculum* (Acquaviva et al. 2017), *T. montanum* (Stanković et al. 2010b, 2011a; Stanković 2012), *T. polium* (Ljubunčić et al. 2006; Stanković 2012; Stanković et al. 2012) and *T. scordium* (Stanković 2012). The obtained results showed that the leaves and flower parts are the richest in active compounds and that they exhibit significant antioxidant activity compared to the other plant parts.

10.4 Conclusions

Based on the long traditional application and the significant content of secondary metabolites, the antioxidant activity of *Teucrium* species has been analyzed in numerous studies using various methodological approaches. It was found over 80 references about antioxidant activity of *Teucrium* species, as well as results for 34 tested *Teucrium* species. *Tucrium polium* (over 30 papers) is the most tested species followed by *Teucrium chamaedrys* (over ten papers), and *T. montanum* (over five papers), while less than five papers were found for the other analyzed *Teucrium* species.

For determination of the antioxidant activity of *Teucrium* species, about 30 analytical methods have been used. The most commonly applied methodological approaches include DPPH – 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity followed by Ferric Reducing Antioxidant Power (FRAP), *β*-carotene/linoleic acid assay, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging capacity (ABTS) etc. Plant extracts are most commonly used in this process followed by essential oils as well as isolated chemical compounds. Antioxidant activity and chemical composition of essential oils have been investigated for *Teucrium flavum*, *T. marum*, *T. massiliense*, *T. orientale*, *T. persicum*, *T. polium*, *T. pruinosum*, *T. pseudochamaepitys*, *T. ramosissimum*, *T. royleanum*, *T. salviastrum, T. yemense* etc. The antioxidant activity of isolated chemical constituents for the species of the genus *Teucrium* was determined for phenylethanoid and iridoid glycosides from *Teucrium chamaedrys*, glyceryl tristearate, 2,5-dioxolanone, fernenol, stigmasta-5,22-dien-3Pol, 24-nor cholesta-5,22 (E)-dien-3beta-ol, ca-spinasterol, (3,4-dihydroxyphenyl) acrylate, 3,4-dihydroxy phenyl acrylic acid from *Teucrium pilosum* and flavones as well as phenyletanoid and iridoid glycosides from *Teucrium polium*.

In addition to the antioxidant activity, the amount of total phenolic compounds and flavonoids, as well as the chemical composition of essential oils were frequently analyzed. Essential oil of *Teucrium* species is characterized by the presence of monoterpenes (limonene, *α*- and *β*-pinene) and sesquiterpenes (caryophyllene and its derivates, germacrene B and D, *α*-, *γ*-, *δ*-cadinene, cadinol, bisabolol), while phenolics of this genus is characterized by the presence of flavonoids (luteolin and apigenin), phenylethanoids (verbascoside) and phenolic acids (hydroxybenzoic and hydroxycinnamic acids). The significant correlation between the antioxidant activity and the content of phenolic compounds were determined in the study of many *Teucrium* species such as *Teucrium arduini*, *T. barbeyanum*, *T. botrys*, *T. chamaedrys*, *T. montanum*, *T. orientale*, *T. polium, T. scordium* etc.

Some medicinal species of the genus *Teucrium* such as *T. chamaedrys, T. creticum, T. flavum, T. massiliense, T. montanum, T. arduini*, *T. polium, T. pruinosum, T. pseudochamaepitys, T. ramosissimum, T. sandrasicum, T. stocksianum, T. scordium* and *T. yemense* are characterized by long-term traditional use. The results of testing the antioxidant activity of these species as well as the diversity of their secondary metabolites justify their traditional application in many diseases of the digestive and respiratory systems based on alterations of the antioxidant defense system and indicates their use as natural antioxidant agents. In addition to numerous in vitro experiments conducted, the antioxidant activity of the secondary metabolites of *Teucrium polium* has been confirmed in vivo. Interpopulation and phenological variability including variability during vegetative period, habitat-related variability as well as variability between plant parts were determined for the antioxidant activity and content of the active compounds of some *Teucrium* species. Data found in the current literature on the antioxidant activity of species of the genus *Teucrium* indicate that these species are characterized by secondary metabolites with significant antioxidant potential.

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Chapter 11
Antiviral Activity of Secondary Metabolites of *Teucrium* Species

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Abstract Unefficiency of chemical treatments and recent plant protection that is focused on natural products, have pointed the importance of finding natural substances that can help in a control of plant virus diseases. Current knowledge about the antiviral effects of essential oils, although limited, indicates the potential of these secondary metabolites to control or at least reduce the spread of viral infection. Essential oils isolated form *Teucrium* species showed antiviral activity in tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV) infected plants. The aim of this chapter is to present findings dealing with antiphytoviral potential of selected species of genus *Teucrium* and to improve the knowledge about possible application of essential oils for the prevention of diseases caused by plant viruses. Essential oil of *Teucrium arduini* applied on the leaves of local host plants, significantly reduced the number of lesions on both TMV and CMV infected plants. Aside from *Teucrium arduini*, four additional *Teucrium* species (*T. montanum, T. polium, T. chamaedrys* and *T. flavum*) are also source of bioactive molecules with antiphytoviral activity against CMV infection. This promising biological activity of *Teucrium* species opens new area of research that could help in a control of virus diseases of plants.

Keywords *Teucrium* · Essential oil · Antiphytoviral activity · CMV · TMV · β-caryophyllene · Caryophyllene oxide

Abbreviations

CMV	Cucumber mosaic virus
EO	Essential oil
TMV	Tobacco mosaic virus

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11.1 Introduction

The aromatic plants produce a diverse array of organic compounds, which are known as secondary metabolites. Secondary metabolites are often referred as specialized metabolites since it is often impossible to distinguish primary and secondary metabolic reactions. Essential oils are secondary metabolites that are important for life and survival plants for numerous reasons, such as physiological function of growth, ecological function and development, and resistance against diseases and insects (Wink 2003; Gershenzon and Dudareva 2007). Xerophyte plants produce essential oils in response to the specific conditions of life, such as hypersensitivity reaction to the temperature cross flow and water stress (Kremer et al. 2012). Numerous aromatic species are rich in strongly-bioactive phenolic compounds. Since ancient times, aromatic plants have been used as herbal formulations in diverse forms, like tinctures, teas, powders and poultices, for their growing interest as alternative therapies for the prevention of various diseases (Bhuwan et al. 2015). Biological effects of essential oils are often attributed to prooxidant effects at the cellular level. Thus, they are widely used for their bactericidal, virucidal, fungicidal, antiparasitic, and insecticidal properties in pharmaceutical, sanitary, cosmetic, agricultural, and food industries (Bakkali et al. 2008; Poma et al. 2018). In addition, many of them show a valuable potential as anticancer therapeutic agents (Russo et al. 2015).

The antimicrobial activity of the essential oils constituents has already been described by many authors (Nostro et al. 2007; Ćavar et al. 2008; Vazirian et al. 2019; Sojić et al. 2019). Clinical observations reported antibacterial, antifungal, and antiviral activities of *Melaleuca alternifolia* tea tree oil whereas in vitro experiments ascribed most of biological properties to complex interaction among different components (Brun et al. 2019). Drevinskas et al. (2019) described novel chromatographic data segmentation method which demonstrates the capability of finding what volatile substances are responsible for antiviral and cytotoxic effects in the medicinal plant extracts. Their findings suggested that β-cis-caryophyllene, linalool, and eucalyptol possess antiviral activity, while thujones do not, and α-thujone, β-thujone, cis-*p*-menthan-3-one, and estragole show cytotoxic effects. Antiviral activity of essential oils highlight their potential use in fresh produce sanitation (Battistini et al. 2019). Described antiviral activity of different plant extracts demonstrate that secondary plant metabolites could be new natural antiviral agents. For example, *Helichrysum arenarium* and *H. armenium* showed notable antiviral activity against both Herpes simplex virus Type-1 and Parainfluenza-3 virus (Kutluk et al. 2018), *Osmunda regalis* essential oil showed antiviral activity against Coxsackievirus B4 (Bouazzi et al. 2018), *Teucrium chamaedrys* extracts against herpes simplex virus type 2 (Todorov et al. 2015), essential oil of *Salvia fruticosa* has a strong activity against *Herpes simplex* virus (Sivropoulou et al. 1997) and the activity of savory's essential oils against HIV has been documented (Yamasaki et al. 1998).

Significant variations of essential oil components have been observed between different species, but also within populations or within individual species, with implications for plant–pathogen interactions. The activity of essential oils in plant defense can be direct, through toxicity, or some volatile organic compounds act as specific cues to attract parasitoids (Giamakis et al. 2001; Padovan et al. 2013). Many individual compounds act as antifungal, antibacterial or allelopathic agents, or for priming of systemic defences in both the host and neighbouring plants (Zulak and Bohlmann 2010; Sharifi-Rad et al. 2017). Although various substances of natural and synthetic origin have been assessed for their antiphytoviral activity, only limited number of scientific publications have revealed the antiphytoviral activity of essential oils. In this sense, essential oil of *Melaleuca alternifolia* (Maiden & Betche) Cheel reduced lesion number in TMV-infected plants (Bishop 1995), while *Plectranthus tenuiflorus* essential oil inhibited infection with Tobacco Necrosis Virus (TNV) (Othman and Shoman 2004). Essential oil of *Satureja montana* L. ssp. *variegata* (Host) P.W. Ball as well as its dominant phenol compounds thymol and carvacrol applied simultaneously with the infecting virus, reduced the number of local lesions on both tobacco mosaic virus and cucumber mosaic virus infected plants (Dunkić et al. 2010). The essential oils of *Eryngium* species reduced the number of viral lesions in plants infected with satellite associated cucumber mosaic virus (Dunkić et al. 2013). Plants treated with essential oil of *Helichrysum italicum* showed delay in the development and strong reduction of local lesions in the early stage of infection with tobacco mosaic virus (Bezić et al. 2016). Essential oil from *Micromeria graeca* caused delay in the development and reduction of the number of lesions in the plants infected with satellite associated cucumber mosaic virus (Vuko et al. 2012).

Results dealing with antiphytoviral activity indicate the role of these plant metabolites in response to viral infection. Qualitative differences in essential oil composition of different plant species do not exhibit a uniform response that would explain the mechanism of antiphytoviral activity. Overall conclusion based on our previous results is that sesquiterpene-rich oils can reduce viral infection in local host plants (Dunkić et al. 2010, 2011; Bezić et al. 2011; Vuko et al. 2012). It is known that synthesis of certain *Arabidopsis thaliana* sesquiterpenes, including *β*-caryophyllene, is induced by the phytohormones gibberelline and jasmonate. Thus, that the production of sesquiterpenes is obviously integrated within the gibereline and jasmonate signalling pathways (Hong et al. 2012).

Many authors described the different biological activities and medical properties of *Teucrium* species as a traditional plants (Khazaei et al. 2018; Vujanović et al. 2019). GC-MS analysis showed sesquiterpenes as abundant components in the oil composition of selected *Teucrium* species, indicating its possible antiphytoviral activity (Dunkić et al. 2011). Thus, we focused on this promising biological activity of *Teucrium* that is unsufficiently explored and consequently opens new area of research that could help in a control of diseases caused by plant viruses. The aim of this chapter is to present findings dealing with antiphytoviral potential of selected species of genus *Teucrium* and to improve the knowledge about possible application of essential oils as antiphytoviral agents. Findings presented in this chapter indicate that sesquiterpene-rich essential oils of *Teucrium* species are potent inhibitors of viral infection and natural substances with possible role in the control of plant virus diseases.

11.2 Tobacco Mosaic Virus and Cucumber Mosaic Virus as a Worldwide Occurring Disease Agents

Tobacco mosaic virus (TMV) is a positive-sense single stranded RNA virus belonging to genus Tobamovirus, family Virgaviridae. TMV causes mottling and discoloration of leaves of an infected plants, especially tobacco and other members of the Solanaceae. It was the first infectious agent to be recognised as being distinct from bacteria and thus the first to bear the designation "virus". It can infect over 350 different plant species. TMV can multiply only inside a living cell but it can survive in a dormant state in dead tissue. Opposite to TMV, most other viruses die when the plant tissue dies. TMV is one of the most stable viruses that retains ability to infect plants for years after the infected plant part died. Infection by TMV causes serious losses on many crops including ornamental plants, tomatoes, peppers and many others. Although the virus almost never kills plants, infection lowers the quality and quantity of the crop. As opposed to fungicidal chemicals used to control fungal diseases, there are no efficient chemical treatments that protect plant parts from virus infection. Additionally, there are no known chemical treatments used under field conditions that eliminate viral infections of plants. Therefore, plants infected by viruses remain so. Thus, control of TMV is primarily focused on reducing and eliminating sources of the virus as the most important practice in controlling tobacco mosaic virus infection.

Cucumber mosaic virus (CMV) is the type member of the genus *Cucumovirus* in the family *Bromoviridae*. This plant pathogenic virus has a worldwide distribution and the widest host range among plant viruses infecting about 1000 plant species. As a worldwide occurring disease agent, it has been reported in many crops, for example in tomatoes, peppers, cucurbits and cucumbers inducing diverse symptoms (Galliteli 2000). As described above, chemicals can not cure a plant of this virus, or of any other virus infection. CMV is a small virus with icosahedral shape and a tripartite genome and two subgenomic RNAs (Shi et al. 1997; Kwon and Chung 2000). Some strains of CMV encapsidate satellite RNA (satRNA) that is completely dependent on the CMV for its replication and spread. Accompanied by their helper virus, different variants of satRNA can induce severe symptoms on infected plants (Garciá-Arenal and Palukaitis 1999).

Recent plant protection that is focused on natural products emphasizes the importance of finding natural substances that can help in a control of plant virus diseases. Current knowledge about the antiviral effects of essential oils, although limited, indicates the potential of these secondary metabolites to control or at least reduce the spread of viral infections. Accordingly, we present bioactivity of selected *Teucrium* species that showed inhibition in development of local lesions in tobacco mosaic virus and cucumber mosaic virus infected plants.

11.3 Antiphytoviral Activity of *Teucrium arduini*

Family Lamiaceae includes about 3500 species spread all over the world, especially in the Mediterranean area. Plants in the family Lamiaceae are a very important source of essential oils and other biologically active molecules. Genus *Teucrium* (Lamiaceae), includes 300 species widespread all around the world, chiefly in the Northern Hemisphere. In the flora of Europe the genus *Teucrium* includes 49 species, of which 13 are widespread in the Croatian flora (Trinajstić 2008; Nikolić 2011). *Teucrium arduini* is Mediterranean endemic plant with restricted range in the Western Balkans, distributed along the Adriatic Coast from the Istra Peninsula in Croatia in the north to Albania in the south (Tutin et al. 1972; Šilić 1990). Representatives of *Teucrium* genus have been used for more than 2000 years as medicinal herbs (Ulubelen et al. 2000; Bedir et al. 2003). Previous chemical research on *Teucrium* species revealed the presence of volatile oils, tannins, terpenoids (mostly diterpenoids), flavonoids and steroid compounds (Šamec et al. 2010). *Teucrium* species were found to possess a wide range of biological activities such as antioxidant, antimicrobial, antifungal, larvicidal, antispasmodic, antinociceptive, antiinflammatory, antiulcer, hypoglycemic, antiacetylcholinesterase and hepatoprotective activities (Vuković et al. 2007; Menichini et al. 2009), some of which are attributed to the presence of the essential oils. Despite a wide spectrum of biological activities of essential oils and secondary metabolites in general, there is only little information available about the effects of these compounds on viruses or viral infection in plant systems. Thus, all results showing antiphytoviral potential of secondary plant metabolites are valuable resource for further research and possible development of a new natural antiviral compounds. Antiphytoviral activity of *Teucrium arduini* essential oil against Tobacco mosaic virus (TMV) and Cucumber mosaic virus (CMV) infection indicate that plants of this genus could be used as a natural source of bioactive molecules for control of plant virus diseases.

Our results showed that essential oil of *Teucrium arduini* is characterized by a high concentration of sesquiterpene hydrocarbons (43.8%) with β-caryophyllene (19.9%) being the major compound, followed by oxygenated sesquiterpenes (19.6%) with caryophyllene-oxide (14.6%) as the main fraction. Essential oil, β-caryophyllene and caryophyllene oxide applied as a spray solution on the leaves of local host plants prior to virus inoculation, significantly reduced the number of local lesions. Treatment with essential oil reduced lesion number on both TMV and CMV infected plants. The percentage of inhibition was 25.7% on plants infected with TMV, and 21.9% on CMV-infected plants (Table 11.1) (Dunkić et al. 2011).

The main oil components, sesquiterpene β-caryophyllene and oxigenated sesquiterpene caryophyllene oxide, applied individually to plants prior to virus inoculation, showed significant antiviral activity against CMV, but weak activity against TMV. β-caryophyllene and caryophyllene oxide inhibited lesions on the leaves of CMV-infected plants with percentage 30.8% and 36.9%, respectively (Table 11.2). Antiviral efficiency of both components was increased in relation to the essential oil. These sesquiterpene-essential oil components are probably responsible for

Table 11.1 *Teucrium arduini* essential oil, *β*-caryophyllene and caryophyllene oxide activity on TMV and CMV infection (Dunkić et al. 2011)

	TMV			CMV		
	Mean of L.L ± SEM (treated)	Mean of L.L ± SEM (control)	% of inhibition	Mean of L.L ± SEM (treated)	Mean of L.L ± SEM (control)	% of inhibition
T. arduini essential oil	26 ± 1.3*	35 ± 1.3*	25.7	25 ± 1.1*	32 ± 0.9*	21.9
β-caryophyllene	52.6 ± 1.8	56.9 ± 2.1	7.6	13.7 ± 1.6*	19.8 ± 2.1*	30.8
Caryophyllene-oxide	53.5 ± 2.1	56.9 ± 2.1	5.9	12.5 ± 1.4*	19.8 ± 2.1*	36.9

Mean of L.L the mean number of local lesions, *SEM* Standard Error Mean. * Significance reduction in disease compared with control ($p \leq 0.05$)

Table 11.2 Antiviral activity of *Teucrium flavum*, *T. chamaedris*, *T. polium* and *T. montanum* essential oils on cucumber mosaic virus (Bezić et al. 2011)

Species	Mean of L.L ± SEM	% of inhibition
Control plants	7.0 ± 0.5	/
T. polium	4.1 ± 0.3*	41.4
T. flavum	5.4 ± 0.3*	22.9
T. montanum	3.9 ± 0.4*	44.3
T. chamaedrys	5.2 ± 0.4*	25.7

Mean of L.L the mean number of local lesions, *SEM* Standard Error Mean. * Significance reduction in disease compared with control ($p \leq 0.05$)

antiviral activity of *Teucrium arduini* essential oil against CMV infection. Inhibition of TMV infection could be explained with antiviral activity of other oil components or their synergistic effect. This is supported by the fact that different extracts of many plants were tested for antiviral activity and it was explained due to their content of diterpenoids and essential oils (Romero et al. 1989; Bishop 1995).

Teucrium arduini essential oil and dominant components of the oil reduce infection of plants with CMV and TMV by inducing resistance response in the host plants. Main oil components *β*-caryophyllene and caryophyllene oxide are responsible for described activity, at least in the reduction of CMV infection. The possibility of other mechanisms as well as antiviral activity of other essential oil compounds, remains as a questions for further research. Answering to above questions will improve our understanding of antiviral activity of *Teucrium arduini* essential oil.

11.4 Antiphytoviral Activity of *Teucrium montanum, T. polium, T. chamaedrys* and *T. flavum*

Evaluation of antiphytoviral effects of the volatiles of additional four *Teucrium* species on the development of local lesions in CMV infected plants indicated that, aside from *Teucrium arduini*, other *Teucrium* species are also source of bioactive molecules with antiphytoviral activity. A comparison of the mean number of lesions on the essential oil-treated local host plants with the corresponding control plants showed activity of *Teucrium* essential oils in reduction of CMV infection. *Teucrium montanum* essential oil showed the strongest antiviral effect with percentage of inhibition 44.3%, followed by *T. polium* with 41.4%, *T. chamaedrys* with 25.7% and *T. flavum* with 22.9% (Table 11.2) (Bezić et al. 2011). The chemical composition of essential oils of above *Teucrium* species is characterised with sesqiuiterpenes *β*-caryophyllene and germacrene D, that are represented in relatively high percentages. Since we above discussed about antiphytoviral potential of sesquiterpene-rich oils, activity of these four *Teucrium* species against CMV infection confirms that these aromatic species are valuable source of bioactive molecules. With the exception of *Teucrium montanum*, the percentage of *β*-caryophyllene in the essential oil correlates with its antiviral activity. Essential oil of *Teucrium montanum* showed the strongest reduction of local lesions, despite the fact that *β*-caryophyllene is not the most abundant component in the *Teucrium montanum* essential oil. The most abundant component in the oil of *Teucrium montanum* is germacrene D, followed by *β*-pinene, *β*-caryophyllene and limonene. These components or their synergistic effect could be responsible for strongest antiphytoviral effect of *Teucrium montanum* essential oil compared to other three oils. *β*-caryophyllene is the main component in the essential oil composition of *Teucrium polium, T. chamaedrys* and *T. flavum* and the percentage of *β*-caryophyllene in the essential oil correlates with antiviral activity of these oils. Comparing the percentages of inhibition, essential oils of these four *Teucrium* species showed stronger activity against CMV infection than above described *Teucrium arduini*. In addition, *Teucrium montanum* and *T. polium* essential oil showed significantly stronger antiviral activity against CMV than *Satureja montana* (Dunkić et al. 2010). Our conclusion is that sesquiterpene-rich essential oils of *Teucrium* species are potent inhibitors of CMV infection and natural substances with possible role in the control of plant virus diseases.

11.5 Conclusions

Although no single answer is expected to explain the antiphytoviral activity of essential oils, further gathering of information will improve the existing knowledge about antiphytoviral activity of secondary plant metabolites as well as our understanding of the role of essential oils in plant-virus interactions. In this sense, species of the genus *Teucrium* are worth exploring, since several *Teucrium* species showed potent activity against CMV and TMV infection. Antiviral activity of the *Teucrium* essential as well as the antiviral activity of the main oil components, indicated the role of β-caryophyllene and caryophyllene oxide in the activity of the essential oil in the plant defence against viruses. Other components of essential oil, such as germacrene D as abundant compound or additional, lower represented components, could also be responsible and sinergistically contribute to effectiveness of essential oil in reducing plant virus infection. Understanding the defence response mechanisms at the molecular level will improve our understanding of the plant defence responses and enhance our knowledge about the antiphytovial potential of essential oils and their use in the prevention of viral plant diseases. The above presents several directions for future research that should aim to clarify the mechanism of antiviral activity of essential oils.

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Chapter 12
Antibacterial and Antifungal Activity of Secondary Metabolites of *Teucrium* Species

Olgica Stefanović

Abstract *Teucrium* species are known for their medicinal properties and have exhibited different biological activities including broad-spectrum antimicrobial activity. Considering that *Teucrium* species produce various bioactive compounds (phenolic acids, flavonoids, saponines, alkaloids, monoterpenes, neo-clerodane diterpenes, sesquiterpenes, essential oils) they could be an important source of new antimicrobial compounds. The expanding of knowledge on the antimicrobial plant compounds has opened wide opportunities for their application in medicine, pharmacy, and food industry. Accordingly, the aim of this chapter was to collect and summarize the results of antimicrobial (antibacterial and antifungal) studies. The results for 44 *Teucrium* species were processed including both the activity of different types of plant extracts and essential oils. Antimicrobial properties were based on in vitro determination of zones of growth inhibition and minimal inhibitory concentrations, using diffusion and dilution method. *Teucrium* species established broad-spectrum antimicrobial activity. Generally, the species were more active against Gram-positive than Gram-negative bacteria and fungi. The essential oils were more potent than plant extracts. The following *Teucrium* species exhibited a promising antimicrobial activity: plant extracts of *Teucrium flavum*, *T. fruticans*, *T. siculum*, *T. yemense*, *T. sokotranum*, *T. persicum* and *T. scordium*, especially against Gram-positive bacteria, and essential oils from *Teucrium orientale*, *T. africanum*, *T. ramosissimum*, *T. mascatence*, *T. yemense*, *T. massiliense* and *T. scordonia*. *Teucrium polium* was one of the most tested *Teucrium* species and has exhibited pronounced activity. The activity was observed against important pathogenic bacteria (*Staphylococcus* sp., *Bacillus* sp., *Enterococcus* sp., *Streptococcus* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella* sp.) and fungi (*Candida* sp., *Trychophyton* sp.). In the last 20 years, the significant database of antimicrobial properties of *Teucrium* species is formed. However, the mechanisms of activity are still poorly explored. Only with exact knowledge of these mecha-

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nisms, it will be possible to develop a new generation of standardized, effective biopreparations. Future studies on bioavailability, pharmacodynamics, and mechanisms of action will contribute to the development of new *Teucrium* antimicrobial agents.

Keywords *Teucrium* · Plant extracts · Essential oils · Bacteria · Fungi · Antibacterial effects · Antifungal effects

Abbreviations

AcOH	Acetone
BuOH	Buthanol
CFU	Colony forming unit
$CHCl_3$	Chloroform
conc.	Concentration
DIZ	Diameter of inhibition zone
DMC	Dihlormethane
EtAc	Ethyl acetate
EtOH	Ethanol
G^-	Gram-negative bacteria
G^+	Gram-positive bacteria
Hex.	Hexane
MDR	Multi-drug resistant
MeOH	Methanol
MIC	Minimal inhibitory concentrations
MRSA	Methicillin-resistant *Staphylococcus aureus*
RPMI	Roswell Park Memorial Institute
TLC	Thin layer chromatography

12.1 Introduction

Various medicinal plants were, for a long time, used for infectious diseases healing. Medicinal plants synthesized different volatile and non-volatile compounds which exhibit inhibitory effects on growth and division of pathogenic microorganisms. Essential oils, balsams, resins of many aromatic plants (exp. *Salvia officinalis*, *Matricaria chamomilla*, *Eucalyptus globulus*, *Syzygium aromaticum*, *Myrtus communis*) are used as remedies for oral hygiene, tooth decay and gingivitis, wound healing. The essential oils of *Thymus vulgaris*, *T. serphyllum*, *Origanum vulgare*, *Ocimum basilicum* content thymol and carvacrol, compounds which interrupt the growth of microorganisms. *Melaleuca alternifolia* essential oil is a common therapeutic agent to treat acne and other skin infectious problems. The well-known aromatic plants with the antimicrobial property are *Allium sativum*, *A. cepa* and other species of *Allium* genus which by hydrolysis of glycosides realized volatile sulfur-containing bioactive compounds. Antimicrobial activity possesses and numerous nonvolatile plant's compounds efficient for wound healing, infection, and inflammation of mucous membrane of the digestive tract, digestive disorders, and diarrhea. Proanthocyanidins from *Vaccinium macrocarpon* are used to treat urinary tract infections. Moreover, the plant- and spice-based antimicrobial compounds derived from *Cinnamomum* sp., *Syzygium aromaticum*, *Sinapsis alba*, *Laurus nobilis*, *Carum carvi*, *Coriandrum sativum*, *Cuminum cyminum*, *Origanum vulgare*, *Rosmarinus officinalis* and *Salvia officinalis* are used as natural preservatives for food preservation and food safety (Stefanović et al. 2009, 2012; Tajkarimi et al. 2010; Saleem et al. 2010; Ličina et al. 2013; Antolak and Kregiel 2017; Ionescu 2018).

The healing properties of species from genus *Teucrium* (family Lamiaceae) are well-known. In traditional medicine, the most common uses of these plants are as a diuretic, diaphoretic, tonic, analgesic, antipyretic, antirheumatic and antiseptic agent. In the Serbian herbal medicine, *Teucrium* species (*Teucrium chamaedrys*, *T. montanum*, *T. polium* and *T. scordium*) are used as a tonic, antirheumatic, digestion stimulant as well as an antiseptic agent (Sarić 1989). In recent years, numerous studies on *Teucrium* species bioactivities including antispasmodic, anti-inflammatory, antihypertensive, hypoglycemic, anticancer, antimicrobial and antioxidant, were reported. The expanding of knowledge on the antimicrobial plant compounds has opened wide opportunities for their application in both the medical and cosmetic purposes, and, also, in the food industry for storing and preserving food products. Accordingly, the goal of this chapter was an up-to-date literature review on antimicrobial activity of *Teucrium* species summarizing the results and selecting the most potent species for further potential application.

12.2 Bioactive Plant Compounds

Medicinal and prophylactic properties of medicinal plants are attributed to the synthesized bioactive organic compounds, the products of secondary metabolism of plants. The one of widely accepted classification of secondary metabolites is into three large classes: phenolic compounds (8000 types), terpenes (25,000 types), and alkaloids (12,000 types) (Antolak and Kregiel 2017).

The phenolic compounds are one of the largest groups of secondary metabolites that have exhibited antimicrobial activity. Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavonoids, tannins, and coumarins. Phenols are a class of chemical compounds consisting of a hydroxyl functional group (-OH) attached to an aromatic phenolic group. The site(s) and the number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity (Cowan 1999). Quinones have aromatic rings with two ketone substitutions. Quinones have the potential to form an irreversible complex with nucleophilic amino acids in proteins (Cowan 1999). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. They are classified according to their biosynthetic origin into the following classes: flavones, flavonols, flavanones, flavanols, anthocyanidins, isoflavones (Cowan 1999; Cushnie and Lamb 2011). Hydrolyzable and condensed tannins are found in almost every plant part: bark, wood, leaves, fruits, and roots. Hydrolyzable tannins are polyesters of gallic acid (or its derivatives), and the sugar. The condensed tannins are formed by the coupling of flavan-3-ol or flavan-3,4-diol monomers. Coumarins are benzo-α-pyrones and could be categorized as simple coumarins and cyclic coumarins (furanocoumarins and pyranocoumarins) (Ojala 2001).

Terpenes are organic compounds built up from isoprene subunits. According to the number of isoprene subunits, there are monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), tetraterpenes (C40) and polyterpenes. Monoterpenes, diterpenes, and sesquiterpenes are the primary constituents of the essential oils (Kovačević 2004).

Alkaloids, one of the earliest isolated bioactive compounds from plants, are divided into the three classes: true alkaloids – heterocyclic nitrogen compounds derived from amino acids, protoalkaloidis – derived from amino acids, but not contain nitrogen in a heterocyclic ring and pseudoalkaloids – not derived from amino acids (Kovačević 2004).

12.3 Mechanisms of Antimicrobial Activity of Plant Compounds

The antimicrobial efficiency of plant compounds depends on several factors: (i) characteristics of target microorganism (the type, genus, species, strain), (ii) characteristics of plant material (botanical source, composition of the bioactive compounds as well as time of harvesting, stage of development or method of extraction) and (iii) chemical properties (hydrophilicity, lipophilicity, concentration, pH value).

The antibacterial activity occurs through several mechanisms: (i) disruption of membrane structure and function (including the efflux system), (ii) interruption of DNA/RNA synthesis and function, (iii) interference with intermediary metabolism, (iv) induction of coagulation of cytoplasmic constituents (Cowan 1999; Kovačević 2004; Buzzini et al. 2008; Cushnie and Lamb 2011; Daglia 2012; Radulović et al. 2013; Coppo and Marchese 2014). The mechanisms of antibacterial action for the particular class of bioactive compounds are summarized in Table 12.1.

In addition, plant compounds are being able to suppress bacterial toxin production by reducing the expression of major virulence genes. Selected plant extracts inhibit the production of cholera toxin by *Vibrio cholerae*, reduce the production of *Staphylococcus aureus* α-hemolysin, enterotoxins and toxic shock syndrome toxin

Table 12.1 Mechanisms of antibacterial activity of plant compounds

Class of plant compounds	Mechanism of action
Phenols	Changing the permeability of cell membrane and causing the leakage of cellular content, interfere with membrane proteins resulting in structure disrupting
Flavonoids	Cell membrane disruption, inhibition of nucleic acid synthesis (caused by topoisomerase inhibition), inhibition of energy metabolism (caused by NADH-cytochrome c reductase or ATP synthase inhibition), disturbance of cell wall and cell membrane synthesis
Quinones	Formation of irreversible complex with surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes
Proanthocyanidins	Destabilization of cell membrane, inhibition of extracellular microbial enzymes, direct actions on microbial metabolism, deprivation of the substrates required for microbial growth
Gallotannins	Inactivation of membrane-bound proteins, strong affinity for iron
Coumarins	Decrease cell respiration
Essential oils	Cell membrane disruption
Alkaloids	Intercalate with DNA, inhibition of enzymes (esterase, DNA- polymerase, RNA-polymerase) or cell respiration

1, reduce the production of vero toxin and inactivate Shiga toxins (Upadhyay et al. 2014). Moreover, it was observed that plant extracts and essential oils inhibit bacterial biofilm formation, motility, attachment, and cell communication (Huber et al. 2003; Borges et al. 2015; Stefanović et al. 2015; Muruzović et al. 2016; Silva et al. 2016). Biofilm is defined as a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface (Hall-Stoodley et al. 2004). The ability to form biofilm is recognized as an additional virulence factor which contributes to the long-lasting persistence of bacterium. In biofilms, bacteria show great tolerance toward antibacterial agents and host immune defenses what makes difficulties in control and eradication of bacterial infections (Høiby et al. 2010).

Studies investigating the mechanism of antifungal activity showed that plant compounds (essential oils, plant extracts) demonstrate several negative effects on fungal cell: (i) cause changes in cell membrane function or structure and, also, in the membranes of organelles (ii) inhibit key enzymes in normal physiological metabolism (respiration and energy production), (iii) inhibit the synthesis of the main polymers of the fungal cell wall, (iv) induce differential expression of critical genes including those involved in the cellular drug response, oxidation-reduction processes, pathogenesis, and the cellular starvation response (Carson et al. 2006; Li et al. 2013, 2014, 2016).

12.4 Extraction of Plant Compounds

Medicinal plants may have been specifically cultivated or collected from the wild. After harvesting, plant material should be cleaned, sorted, and then subjected to extraction. Fresh or dried plant material can be used. Extraction and isolation of the bioactive compounds from the plant tissues can be performed by using different procedures and methods (solvent extraction, distillation, supercritical fluid extraction). The appropriated extraction procedure used depends on the physical and chemical properties of the plant material, texture, water content in plant material, as well as the nature and kind of the compound to be isolated.

Solvent extraction is a process of separation of active compounds from plant material using different solvents. During extraction, solvents diffuse into the plant tissues and solubilise compounds with similar polarity. After the extraction process has finished, solvents have been evaporated, so that an extract is a concentrated mixture of plant active compounds. Successful extraction is largely dependent on the type of solvent used in the extraction procedure. The desirable properties of extracting solvents are: selectivity, large extraction capacity, no interference with plant components, no toxicity, and high evaporation level. The most often tested extracts are: water extract as a sample of extract that primarily used in traditional medicine and extracts from organic solvents such as methanol, ethanol as well as ethyl acetate, acetone, chloroform, dichlormethane (Ncube et al. 2008).

Distillation is a process of separation of volatile active compounds from plant material by physical process of evaporation, treating plant material with steam or boiling. After cooling and condensation of vaporized compounds, the water distillate is formed. Due to essential oils are insoluble in the water and because of specific gravity, essential oils are separated at the surface or top of the water distillate and further collected. Essential oils are extracted by steam distillation or hydrodistillation (Tongnuanchan and Benjakul 2014).

Supercritical fluid extraction is a diffusion-based process of separation of plant active compounds using supercritical fluids as the extracting solvent. Supercritical fluids are formed by heating of gases above their critical temperatures or by compressing of liquids above their critical pressure. Carbon dioxide is the most used supercritical fluid. Supercritical fluids have dissolving properties that depend on their density and can be controlled with temperature and pressure. In relation to solvent extraction, supercritical fluid extraction has some advantages such as low extraction temperature, minimal degradation of the active substances, selectively dissolving of compounds, and faster extraction (Janaćković et al. 2017).

12.5 Antimicrobial Susceptibility Methods

Antimicrobial (antibacterial and antifungal) activity of plant extracts, essential oils, and pure compounds might be analysed using several methods: diffusion, dilution and bioautography method (Fig. 12.1). Diffusion and dilution method are frequently

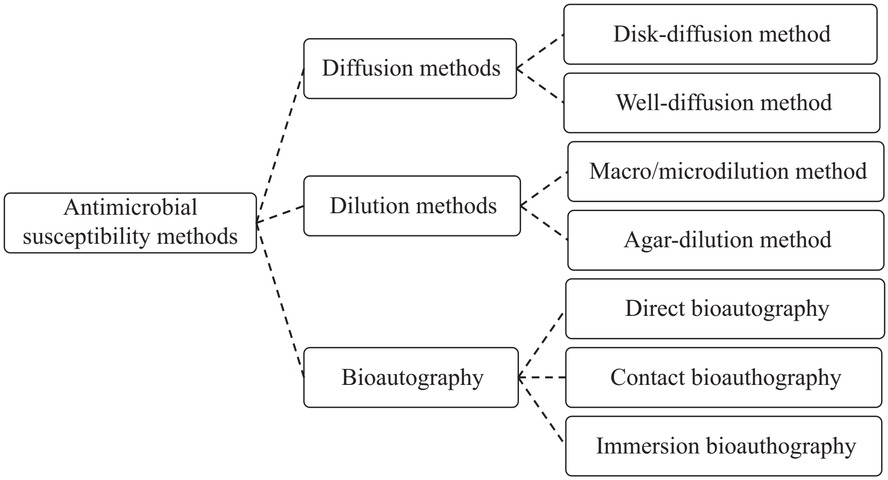

Fig. 12.1 Classification of antimicrobial susceptibility methods

used. These methods are standard antimicrobial susceptibility testing methods used for antibiotics/antifungal drugs recommended by the Clinical and Laboratory Standards Institute, USA.

Diffusion method is a qualitative test which, according to a size of a diameter of the zone of growth inhibition, shows the intensity of antimicrobial effect of the tested compound and allows classification of microorganisms as susceptible or resistant. The tested compound has applied on disk or into a hole in agar medium inoculated with a microorganism to be tested. During incubation, the compound diffuses into the medium inhibiting the growth of a microorganism. The reference medium for testing is Mueller-Hinton agar for bacteria and Mueller-Hinton agar supplemented with 2% glucose for fungi. The density of inoculum should be equivalent to 0.5 McFarland standard (1–2 × 10^8 CFU/ml). The results are recorded as zones of growth inhibition and when the diameter of the inhibition zone is wider, the antimicrobial activity of the compounds is higher (Fig. 12.2). The rate of diffusion of the tested compounds through the agar is dependent on solubility properties and the molecular weight of the tested compound. Hence, this method is not suitable to natural antimicrobial compounds that are scarcely soluble or insoluble in water and thus their hydrophobic nature prevents uniform diffusion through the agar medium (Klancnik et al. 2010).

Dilution (macrodilution and microdilution) method is more appropriate when investigating the activity of plant products. It can be carried out by using test tubes (macrodilution) or 96-wells microtiter plates (microdilution). The serial twofold dilutions of a tested compound are prepared in a liquid medium, Mueller-Hinton broth for bacteria and RPMI 1640 broth for fungi. The density of final inoculum is 5 × 10^5 CFU/ml for bacteria and 5 × 10^2 – 2.5 × 10^3 cell/ml for yeasts. This method is quantitative, the activity of a compound or a plant extract is determined as minimal inhibitory concentration (MIC). MIC is defined as the lowest concentration of

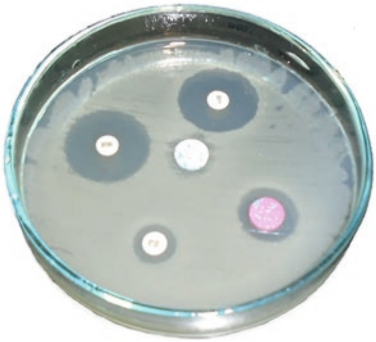

Fig. 12.2 Diffusion method – the zones of growth inhibition around disks indicate the activity of tested compound

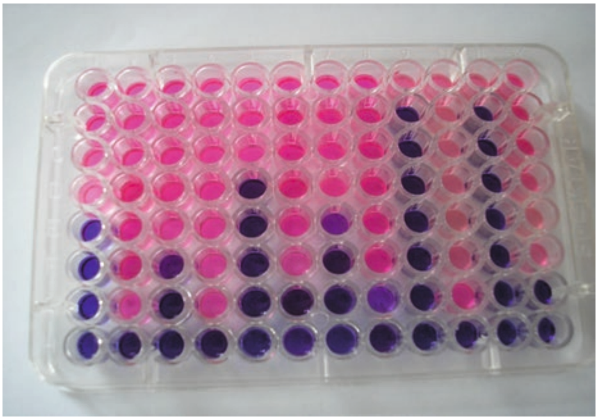

Fig. 12.3 Microdilution method – the change of colour of indicator (resazurine) from blue to pink indicates the growth of bacteria

tested compound able to inhibit the visible growth of microorganisms. Microbial growth (growth of bacteria and yeasts) could be assessed either visually by grading turbidity or spectrophotometrically by measuring optical density. Moreover, easy MIC detection and increased credibility of this method allow using of growth indicators which changing the colour indicate the growth of microorganisms (Cos et al. 2006; Fig. 12.3).

Once more method used for determination of MIC is agar dilution method. In this method, dilutions of a plant extract or a compound are prepared by adding into a precise volume of molten agar medium which then poured into sterile petri plates. Each agar plate containing a different concentration of tested compound. MIC is recorded as the lowest concentration of the compound that completely inhibits the growth, no colony observed.

Bioautography rely on fractionalisation of the extracts on its different components simplifies the identification of compounds with antimicrobial potency. Bioautography may be direct, when microorganisms grow directly on a TLC plate, then contact, when the active compound is transferred from a TLC plate to inoculated agar, and immersion when the inoculated agar medium is spilled over a TLC plate. Clear (white) zones on the TLC plate indicate antimicrobial activity of the extracts and their components (Rahalison et al. 1991).

12.6 Antimicrobial Activity of *Teucrium* Species

Antibacterial and antifungal activity of *Teucrium* species has been extensively investigated. In the following sections, the results of the literature search of databases Scopus, PubMed, Google Scholar for period 1999–2019 (the last accessed was on April 1, 2019) were presented. According to published data, 16% of species of the genus *Teucrium* (total of 44 species) were screened for antimicrobial testing. The genus *Teucrium* includes 268 species. The species from different regions (Serbia, Croatia, Italy, Romania, Turkey, Pakistan, Jordan Iran, Tunisia, Algeria, Mexico) were investigated, so the results were summarised and compared.

Evidently, bioactive compounds from *Teucrium* species were frequently isolated in a form of plant extracts. Plant extracts were prepared from fresh or dried plant material using conventional extraction methods (Soxhlet extraction, maceration, decoction) and most recent supercritical fluid extraction. The most commonly used extracting solvents were methanol, ethanol, and water. The more detailed information on plant parts used or extraction procedure and solvents used are presented in Table 12.2.

The essential oils were isolated by hydrodistillation or steam distillation using a Clevenger-type apparatus. A total of 20 *Teucrium* species essential oils were analysed and screened for antimicrobial activity (Table 12.3). For the most part, antimicrobial activity was determined using diffusion or dilution method. However, activity has also been demonstrated using time-kill assays. Antimicrobial activity studies were carried out on numerous bacterial species (Gram-positive and Gram-negative strains; sensitive and resistant, pathogens and opportunistic pathogens, clinical isolates and standard strains) and fungal species (yeasts, dermatophytes, and filamentous fungi, human and phytopathogens, clinical isolates and standard strains).

Teucrium species exhibited broad-spectrum antimicrobial activity (Fig. 12.4). Mostly, antimicrobial properties of *Teucrium* species were based on their activity against planktonic bacteria (bacteria in suspensions). In most environments, bacteria form biofilms, surface-attached communities. Many chronic infections (urinary tract infections, middle-ear infections, tooth decay and paradontosis, prostheses-associated infection) or problems with contamination in food processing industry are being connected with biofilms (Costerton et al. 1999; Wood et al. 2011). So, in recent years, the investigation of new active plant-derived antibiofilm agents is

Table 12.2 List of different plant extracts, plant part used, tested microorganisms, and results of *Teucrium* species antimicrobial activity

Teucrium species (part used)	Type of extract (extraction method)	Tested microorganisms	DIZ (tested concentration) range, MIC range		References
			Antibacterial activity	Antifungal activity	
T. polium (flowers) Iran	Ethanol extract (Maceration)	489 isolates of *Staphylococcus aureus*	DIZ (30 mg/ml) 10–11 mm	Not tested	Mansouri (1999)
T. polium (aerial parts) Turkey	Ethanol extract (Soxhlet)	*Listeria monocytogenes*, *L. ivanovii*, *L. innocua*, *L. murrayi*	DIZ (133 mg/ml) 10–11 mm MIC 12.5–50μg/ml	Not tested	Altanlar et al. (2006)
T. polium (aerial parts) Jordan	Ethanol extract (Maceration)	*S. aureus*, *Pseudomonas aeruginosa*, *Candida albicans*	DIZ (80 ppm) 14–15 mm MIC 80 ppm	Not active	Khalil et al. (2009)
T. polium (aerial parts) Jordan	Methanol extract (Soxhlet)	*Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 426, *Enterobacter cloaceae* ATCC 29004, *S. aureus* ATCC 25923	DIZ (10 mg/ml) 11–26 mm MIC 1.2–2.4 mg/ml	Not tested	Tarawneh et al. (2010)
T. polium (aerial parts) Iran	Ethanol, methanol extracts (Maceration)	*Brucella melitensis*	DIZ_{EtOH} (100–400μg/ml) 9–12 mm DIZ_{MeOH} (100–400μg/ml) 8–11 mm	Not tested	Motamedi et al. (2010)
T. polium (aerial parts) Iran	Ethanol, methanol extracts (Maceration)	*Bacillus pumilis*, *B. anthracis*, *B. licheniformis*, *B. cereus*, *S. aureus*, *S. epidermidis*, *E. coli*, *Yersinia enterocolitica*, *S. typhy*, *P. mirabilis*, *Bordetella bronchispetica*, *Streptococcus pyogenes*	DIZ_{EtOH} (400 mg/ml) 9–18 mm DIZ_{MeOH} (600 mg/ml) 9–26 mm	Not tested	Darabpour et al. (2010)

T. polium (aerial parts) Egypt	Ethanol, ethyl acetate, chloroform, n-hexane extracts (Maceration)	*Trichophyton rubrum*, *T. tonsurans*, *C. albicans*, *Chrysosporium tropicum*, *Paecilomyces lilacinus*, *P. variotii*, *Scopulariopsis bervicaulis*	Not tested	MIC_{EtOH} 75– > 150 mg/ml MIC_{EtAc} 75– > 150 mg/ml MIC_{CHCl3} 25–125 mg/ml $MIC_{Hex.}$ 25– > 150 mg/ml	Hashem (2011)
T. polium (aerial parts) Turkey	Water extract (Maceration)	*Klebsiella pneumoniae*, *Haemophilus influenzae* ATCC 49766, *P. aeruginosa* ATCC 10145, *Acinetobacter baumannii*, *Streptococcus pneumonia* ATCC 19615, *S. pyogenes* ATCC 13615, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Mycobacterium tuberculosis*, *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, *C. krusei*, *C. tropicalis*	MIC 64µg/ml (G^+ bacteria) MIC 16–32µg/ml (G^- bacteria)	MIC 8–64µg/ml	Deliorman Orhan et al. (2012)
T. polium (aerial parts) Serbia	Methanol, acetone, ethyl acetate extracts (Maceration)	*S. aureus* ATCC 25923, *S. aureus*, *E. coli* ATCC 25922, *E. coli*, *P. aeruginosa*, *C. albicans* ATCC 10231, *C. albicans*, *A. niger*	MIC_{MeOH} 0.3–20 mg/ml MIC_{AcOH} 0.3–20 mg/ml MIC_{EtAC} 0.3, 10 mg/ml (*S. aureus* ATCC 25923, *P. aeruginosa*)	MIC_{MeOH} > 20 mg/ml MIC_{AcOH} > 20 mg/ml MIC_{EtAC} > 10 mg/ml	Stanković et al. (2012)
T. polium (aerial parts) Iran	Ethanol extract (Maceration)	*S. typhi*, *P. aeruginosa*, *B. cereus*, *L. monocytogenes*, *S. aureus*	DIZ (50 mg/ml) 6–21 mm MIC 6.25–50 mg/ml	Not tested	Mirzaei et al. (2013)
T. polium (aerial parts) Palestine	Ethanol extract (Maceration)	*S. aureus* ATCC 29213, *B. subtilis* ATCC 6633, *M. luteus* ATCC 10240, *E. coli* ATCC 10536, *P. aeruginosa*, *K. pneumoniae*, *C. albicans* ATCC 10231, *A. niger* ATCC 16404	DIZ (25 mg/ml) 8.7–18.3 mm	DIZ (25 mg/ml) 19.7 mm (*C. albicans*)	Qabaha (2013)

(continued)

Table 12.2 (continued)

Teucrium species (part used)	Type of extract (extraction method)	Tested microorganisms	DIZ (tested concentration) range, MIC range		References
			Antibacterial activity	Antifungal activity	
T. polium (leaves) Algeria	Methanol extract (Maceration)	*E. coli* ATCC 25922, *S. aureus* ATCC 6538	DIZ (2 mg/ml) 14–15 mm MIC 13 mg/ml (*S. aureus*) MIC 13 mg/ml (*E. coli*)	Not tested	Khaled-Khodja et al. (2014)
T. polium (aerial parts) Algeria	Methanol extract (Maceration)	*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. typhimurium* ATCC 10428, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *K. pneumoniae* ATCC 700603, *B. subtilis* ATCC 7033	DIZ (600 mg/ml) 11.5–26 mm MIC 3.125–50 mg/ml	Not tested	Dridi et al. (2016)
T. polium subsp. *gabesianum* (aerial parts) Tunisia	Ethanol, water extracts (Maceration)	*E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *C. freundeii*, *P. mirabilis*, *C. albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *T. soudanense*, *Microsporum canis*, *A. fumigatus*, *Scopulariopsis brevicaulis*	MIC_{EtOH} 0.156–0.625 mg/ml MIC_{H2O} 0.156–0.625 mg/ml	MIC_{EtOH} 0.625– > 1 mg/ml MIC_{H2O} > 1 mg/ml	Ben Othman et al. (2017)
T. polium (aerial parts) Pakistan	Ethanol, ethyl acetate, dichloromethane acetone, hexane extracts (Maceration)	*E. coli* ATCC 15224, *B. subtilis* ATCC 6663, *S. aureus* ATCC 29213, *S. flexneri* ATCC 14028, *A. flavus* ATCC 32611, *A. niger*, *C. albicans* ATCC 2091, *T. longifusus*	EtOH (1 mg/ml) 37–73% of inhibition EtAc (1 mg/ml) 24–56% DCM (1 mg/ml) 25–37% AcOH (1 mg/ml) 30–31% Hex. (1 mg/ml) 10–35%	EtOH (1 mg/ml) 40–70% of inhibition EtAc (1 mg/ml) 10–65% DCM (1 mg/ml) 27–58% AcOH (1 mg/ml) 15–25% Hex. (1 mg/ml) 25–30%	Ali et al. (2018)

T. polium (leaves, flowers, stem) Turkey	Ethanol, acetone, ether extracts (Maceration)	*B. subtilis* NRRL 558, *B. cereus* NRRL 3711, *S. aureus* NRRL 767, *S. epidermidis* NRRL 4377, *S. lutea* NRRL 4370, *E. coli* NRRL 3008, *E. aerogenes* NRRL 3567, *S. typhimurium* NRRL 4420, *Shigella* sp., *C. albicans* NRRL 27077, *Pichia membranifaciens* NRRL 2026, *S. cerevisiae* NRRL 2034, *Shizosaccharomyces pombe* NRRL 12796, *Zygosaccharomyces rouxii* NRRL 229	DIZ (1 mg/ml) 8–14 mm (G^+ bacteria) DIZ (1 mg/ml) 8–9 mm (G^- bacteria)	DIZ (1 mg/ml) 8–18 mm	Kunduhoglu et al. (2011)
T. chamaedrys subsp. *chamaedrys* (flowers) Turkey			DIZ (1 mg/ml) 8–22 mm (G^+ bacteria) DIZ (1 mg/ml) 8–13 mm (G^- bacteria)	DIZ (1 mg/ml) 8 mm	
T. chamaedrys Turkey	Polar and non-polar fractions of methanol extract (Soxhlet)	*S. pneumoniae*, *B. cereus*, *Acinetobacter lwoffii* ATCC 19002, *E. coli*, *K. pneumoniae*, *Clostridium perfringens*, *C. albicans*, *C. krusei* ATCC 6258	MIC_{polar} > 72 mg/ml $MIC_{non-polar}$ 9– > 72 mg/ml	MIC_{polar} > 72 mg/ml $MIC_{non-polar}$ 18–36 mg/ml	Gursoy and Tepe (2009)
T. chamaedrys (aerial parts) Turkey	Water, ethanol, ethyl acetate, hexane extracts (Maceration)	*B. cereus*, *S. aureus* ATCC 6538P, *E. coli* O157:H7, *K. pneumoniae*, *C. albicans* ATCC 10239, *S. aureus* ATCC 49444, *L. monocytogenes* ATCC 13076, *E. coli* ATCC 25922, *S. typhymurium* ATCC 14028, *C. albicans* ATCC 10231	MIC_{H2O} 12.5–150 mg/ml MIC_{EtOH} 400 mg/ml (G^+ bacteria) MIC_{EtAC} 3.12–12.5 mg/ml $MIC_{Hex.}$ – not active MIC (*S. aureus*) 3.12 mg/ml	MIC_{EtAC} 400 mg/ml	Ceyhan et al. (2012)
T. chamaedrys (aerial parts) Serbia	Methanol, acetone, ethyl acetate extracts (Maceration)	*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* ATCC 10231, *C. albicans*, *A. niger*	MIC_{MeOH} 0.078–5 mg/ml MIC_{AcOH} 5– > 20 mg/ml MIC_{EtAC} 0.6– > 10 mg/ml	MIC_{MeOH} > 20 mg/ml MIC_{AcOH} 10 mg/ml (*A. niger*) MIC_{EtAC} 10 mg/ml (*A. niger*)	Stanković et al. (2012)

(continued)

Table 12.2 (continued)

Teucrium species (part used)	Type of extract (extraction method)	Tested microorganisms	DIZ (tested concentration) range, MIC range		References
			Antibacterial activity	Antifungal activity	
T. chamaedrys (aerial parts) Romania	Ethanol extract (Maceration)	*S. aureus* ATCC 49444, *L. monocytogenes* ATCC 13076, *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, *C. albicans* ATCC 10231	DIZ 11–20 mm	DIZ 22 mm	Vlase et al. (2014)
T. chamaedrys (aerial parts) Serbia	Water extract (Supercritical water extraction)	*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *P. vulgaris* ATCC 13315, *P. mirabilis* ATCC 14153, *K. pneumoniae* ATCC 13883, *C. albicans* ATCC 10231, *A. niger* ATCC 16404	MIC 78.125–312.5μg/ml	MIC 78.125–156.25μg/ml	Nastić et al. (2018)
T. montanum (aerial parts) Serbia	Petroleum ether, chloroform, ethyl acetate, n-butanol (Maceration)	*P. aeruginosa*, *E. coli*, *S. aureus*, *S. lutea*, *Bacillus* sp.	DIZ_{Ether} (10 mg/ml) 0 mm, MIC_{Ether} 10– > 10 mg/ml DIZ_{CHCl3} (10 mg/ml) 15–18 mm, MIC_{CHCl3} 5–10 mg/ml DIZ_{EtAC} (10 mg/ml) 16–19.8 mm, MIC_{EtAC} 1– > 10 mg/ml $DIZ_{Butanol}$ (10 mg/ml) 16–20 mm, $MIC_{Butanol}$ 1– > 10 mg/ml	Not tested	Djilas et al. (2006)

T. montanum (aerial parts) Serbia	Methanol, acetone, ethyl acetate extracts (Maceration)	*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* ATCC 10231, *C. albicans*, *A. niger*	MIC_{MeOH} 0.15–5 mg/ml MIC_{AcOH} 0.15–20 mg/ml MIC_{EtAC} 0.3, 10 mg/ml (*S. aureus* ATCC, *P. aeruginosa*)	MIC_{MeOH} > 20 mg/ml MIC_{AcOH} 20 mg/ml (*A. niger*) MIC_{EtAC} > 20 mg/ml	Stanković et al. (2012)
T. arduini (leaves, flowers) Croatia	Infusions	*B. subtilis* NCTC 8236, *S. aureus* ATCC 6538, *E. coli* ATCC 10535, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 10231, *A. niger* ATCC 16404	MIC_{leaves} 25–50 mg/ml (*B. subtilis*) MIC_{leaves} 1.56–4.16 mg/ml (*S. aureus*) $MIC_{flowers}$ 16.66 mg/ml (*S. aureus*)	Not active	Šamec et al. (2010)
T. arduini (aerial parts) Serbia	Methanol, acetone, ethyl acetate extracts (Maceration)	*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* ATCC 10231, *C. albicans*, *A. niger*	MIC_{MeOH} 0.15–10 mg/ml MIC_{AcOH} 0.6– > 20 mg/ml MIC_{EtAC} 0.6– > 20 mg/ml	MIC_{MeOH} > 20 mg/ml MIC_{AcOH} > 20 mg/ml MIC_{EtAC} > 20 mg/ml	Stanković et al. (2012)
T. arduini (leaves, flowers, stem) Croatia	Ethanol extract (Ultrasonication)	*S. aureus* ATCC 6538, *E. faecalis* ATCC 21212, *E. coli* ATCC 10535, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 10231, *A. brasiliensis* ATCC 16404, *Microsporum gypseum*	MIC_{leaves} 2–4 mg/ml $MIC_{flowers}$ 2– > 4 mg/ml MIC_{stem} > 4 mg/ml	MIC_{leaves} 2– > 4 mg/ml $MIC_{flowers}$ 1– > 4 mg/ml MIC_{stem} 2– > 4 mg/ml	Kremer et al. (2013)

(continued)

Table 12.2 (continued)

Teucrium species (part used)	Type of extract (extraction method)	Tested microorganisms	DIZ (tested concentration) range, MIC range		References
			Antibacterial activity	Antifungal activity	
T. scordium subsp. *scordioides* (aerial parts) Serbia	Methanol, dichlormetan, cyclohexane extracts (Maceration)	*S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *M. luteus* ATCC 10240, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6633BB, *B. cereus* ATCC 11778, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* NCIMB 9111, *C. albicans* ATCC 10259	DIZ_{MeOH} (100 mg/ml) 6.5 mm (*P. aeruginosa*) DIZ_{DCM} (100 mg/ml) 6–11 mm (*P. aeruginosa, B. subtilis*) $DIZ_{Hex.}$ (100 mg/ml) 6.5–11 mm	Not active	Kundaković et al. (2011)
T. scordium subsp. *scordium* Serbia	Methanol, acetone, ethyl acetate extracts (Maceration)	*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* ATCC 10231, *C. albicans*, *A. niger*	MIC_{MeOH} 0.3–20 mg/ml MIC_{AcOH} 0.6– > 20 mg/ml MIC_{EtAC} 0.6, 10 mg/ml (*S. aureus* ATCC, *P. aeruginosa*)	MIC_{MeOH} > 20 mg/ml MIC_{AcOH} > 20 mg/ml MIC_{EtAC} 5–10 mg/ml	Stanković et al. (2012)
T. scordium subsp. *scordioides* (aerial parts) Serbia			MIC_{MeOH} 0.3–20 mg/ml MIC_{AcOH} 0.6 mg/ml (*S. aureus* ATCC) MIC_{EtAC} 0.6, 10 mg/ml (*S. aureus* ATCC, *P. aeruginosa*)	MIC_{MeOH} > 20 mg/ml MIC_{AcOH} > 20 mg/ml MIC_{EtAC} > 10 mg/ml	
T. stocksianum (whole plant) Pakistan	Methanol extract and sub-fractions: n-hexane, chloroform, ethyl acetate	*E. coli*, *S. aureus*, *S. typhi*, *S. flexneri*, *B. subtilis*, *A. niger*, *A. flavus*, *A. fumigatus*, *Fusarium solani*	DIZ_{MeOH} (1 mg/ml) 12–24 mm $DIZ_{Hex.}$ (1 mg/ml) 12–26 mm DIZ_{CHCl3} (1 mg/ml) 14–26 mm DIZ_{EtAC} (1 mg/ml) 21–26 mm	MIC_{MeOH} 2–3 mg/ml $MIC_{Hex.}$ 2.5–4 mg/ml MIC_{CHCl3} 2.5–3 mg/ml MIC_{EtAC} 2–3 mg/ml	Shah et al. (2015a, b)

T. africanum (leaves, flowers, stem) South Africa	Water, methanol, dichloromethane – methanol extracts (Maceration)	*B. cereus* ATCC 11778, *E. coli* ATCC 8739, *K. pneumoniae* ATCC 13883, *Moraxella catarrhalis* ATCC 23246, *P. aeruginosa* ATCC 27858, *S. aureus* ATCC 25923, *S. pyogenes* ATCC 8668	MIC 1– > 8 mg/ml (G^+ bacteria) MIC 0.125– > 8 mg/ml (G^- bacteria)	Not tested	Ruiters et al. (2016)
T. kraussii (leaves, flowers, stem) South Africa			MIC 0.8–6.6 mg/ml (G^+ bacteria) MIC 1–8 mg/ml (G^- bacteria)	Not tested	
T. trifidum (leaves, flowers, stem) South Africa			MIC 1– > 8 mg/ml (G^+ bacteria) MIC 1– > 8 mg/ml (G^- bacteria)	Not tested	
T. montbretii subsp. *pamphylicum* (aerial parts) Turkey	Methanol extract (Soxhlet)	*E. coli*, *S. typhi*, *Yersinia enterocolitica*, *S. aureus, S. pneumoniae*, *E. aerogenes*, *L. monocytogenes*, *Lactobacillus reuteri*, *L. acidophilus, Micrococcus luteus*	DIZ (10% extract) 13.5–22.5 mm	Not tested	Özkan et al. (2007)
T. flavum (flowers) Italy	Ethanol (80%) extract (Maceration)	32 clinical isolates and 8 standard strains of bacteria	MIC 1024–16,384μg/ml (G^+ bacteria) MIC 8192– > 16,384μg/ml (G^- bacteria)	Not tested	Acquaviva et al. (2018)
T. fruticans (flowers) Italy			MIC 256–8192μg/ml (G^+ bacteria) MIC 2048–8192μg/ml (G^- bacteria)	Not tested	
T. siculum (flowers) Italy			MIC 256–8192μg/ml (G^+ bacteria) MIC 1024–8192μg/ml (G^- bacteria)	Not tested	

(continued)

Table 12.2 (continued)

Teucrium species (part used)	Type of extract (extraction method)	Tested microorganisms	DIZ (tested concentration) range, MIC range		References
			Antibacterial activity	Antifungal activity	
T. oliverianum (aerial parts) Saudi Arabia	Methanol (80%) extract (Maceration)	*K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, *Serratia marcescens*, *B. cereus*, *M. luteus*, *M. roseus*, *S. aureus*, *A. flavus*, *A. ochraceus*, *C. albicans*, *F. moniliforme*	DIZ (10 mg/ml) 9 mm (*B. cereus*, *K. pneumoniae*)	Not active	Shahat et al. (2017)
T. orientale var. *glabrescens* Turkey	Water extract (Maceration)	*E. coli* ATCC 25922, *Y. pseudotuberculosis* ATCC 911, *P. aeruginosa* ATCC 43288, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *B. cereus* 709 Roma; *M. smegmatis* ATCC 607, *C. albicans* ATCC 60193, *S. cerevisiae*	DIZ 6–16 mm	Not active	Yildirmiş et al. (2017)
T. cubense (aerial parts) Mexico	Water extract (Maceration)	*S. aureus*, *S. haemolyticus*, *E. faecalis*, *P. aeruginosa*, *E. coli*, *Acinetobacter lwoffii*, *A. baumanii*, *Burkholderia cepacin*, *C. albicans*, *C. tropicalis*, *T. belgeii*	DIZ (375µg/disk) 22 mm (*A. lwoffii*) DIZ (750µg/disk) 10 mm (*P. aeruginosa*)	DIZ (750µg/disk) 12 mm (*C. albicans*)	Jacobo-Salcedo et al. (2011)
T. yemense (aerial parts) Yemen	Methanol, water extracts (Maceration)	*S. aureus* ATCC 6538, *B. subtilis* ATCC 6059, *M. flavus*, *E. coli* ATCC 11229, *P. aeruginosa* ATCC 27853, *C. maltose*, multiresistant strains – *S. epidermidis*, *S. haemolyticus*, *S. aureus*	DIZ_{MeOH} (4 mg/ml) 9–26 mm MIC_{MeOH} 250–500µg/ml DIZ_{H2O} (4 mg/ml) 10–16 mm $MIC_{H2O} > 1000$µg/ml	Not active	Mothana et al. (2009a, b)
T. sokotranum (aerial parts) (Soqotra Island) Yemen			DIZ_{MeOH} (4 mg/ml) 10–22 mm MIC_{MeOH} 500–1000µg/ml DIZ_{H2O} (4 mg/ml) 10–22 mm MIC_{H2O} 250–1000µg/ml	Not active	

T. persicum (aerial parts) Iran	Methanol extract (Maceration)	*E. coli* PTCC 1338, *B. subtilis* PTCC 1023, *S. aureus* PTCC 1112, *S. epidermis* PTCC 1114, *P. aeruginosa* PTCC 1074, *S. typhi* PTCC 1693, *K. pneumonia* PTCC 1031, *A. niger* PTCC 5010, C. *albicans* PTCC 5027	MIC 0.5–2 mg/ml	MIC 2 mg/ml	Javidnia et al. (2009)
T. leucocladum (aerial parts) Egypt	n-Hexane-ether extract (Maceration)	*E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *C. albicans*	DIZ (1 mg/ml) 16–19 mm	DIZ (1 mg/ml) 15 mm	El-Shazly and Hussein (2004)
T. botrys (aerial parts) Serbia	Methanol, acetone, ethyl acetate extracts (Maceration)	*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* ATCC 10231, *C. albicans*, *A. niger*	MIC_{MeOH} 1.25 mg/ml (*S. aureus* ATCC) MIC_{AcOH} 2.5 mg/ml (*S. aureus* ATCC) MIC_{EtAC} > 10 mg/ml	MIC_{MeOH} > 20 mg/ml MIC_{AcOH} 10 mg/ml (*A. niger*) MIC_{EtAC} 5–10 mg/ml	Stanković et al. (2012)
T. bicolor (aerial parts) Mexico	Methanol extract (Maceration)	*C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *M. tuberculosis*	MIC 125μg/ml (*M. tuberculosis*)	MIC 31.2–125μg/ml	Garza et al. (2017)
T. sauvagei (leaves) Tunisia	Methanol extract (Maceration)	Dermatophytes	Not tested	Active against dermatophytes	Salah et al. (2006)
T. royleanum (whole plant) Pakistan	Methanol extract and sub-fractions: n-butanol, chloroform, ethyl acetate (Maceration)	*E. coli*, *B. subtilis*, *K. pneumoniae*, *S. flexneri*, *S. aureus*, *S. typhi*, *P. aeruginosa*, *T. longifusus*, *C. albicans*, *C. glaberata*, *A. flavus*, *M. canis*, *F. solani*	DIZ_{MeOH} (3 mg/ml) 9–16 mm DIZ_{BuOH} (3 mg/ml) 5–6 mm DIZ_{CHCl3} (3 mg/ml) 4–24 mm DIZ_{EtAC} (3 mg/ml) 14–25 mm	MeOH (24 mg/ml) 40–50% of inhibition $CHCl_3$ (24 mg/ml) 53–87% EtAc (24 mg/ml) 40–71% BuOH (24 mg/ml) 70% (*T. longifusus*)	Ahmad et al. (2008)

Table 12.3 List of major oils' compounds, tested microorganisms, and results of *Teucrium* species essential oils antimicrobial activity

Teucrium species	Major oil's compounds	Tested microorganisms	DIZ (tested concentration) range, MIC range		References
			Antibacterial activity	Antifungal activity	
T. polium Iran	Limonene (37.70%), 2,4 di-tetra-butylphenol (10.81%) p-cymene (8.20%)	*Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Escherichia coli*	DIZ (10μl) 7–17 mm MIC 5–50μl	Not tested	Vahdani et al. (2011)
T. polium Algeria	Germacrene, bicyclogermacrene, β-pinene, carvacrol	*S. aureus* ATCC 25923, *Enterococcus feacalis* ATCC 29212, *Bacillus cereus* ATCC 11778, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus stolonifer*	DIZ (15μl) 9–16 mm MIC 3–5μl/ml	2–10μl/ml – 6.58–25.9% of inhibition	Belmekki et al. (2013)
T. polium subsp. *capitatum* Italy (Corsica Island)	α-pinene (24.71%), α-thujene (8.1%), terpinen-4-ol (6.6%), limenone (5.2%)	*S. aureus*, *S. epidermidis*, *Listeria innocua*, *Campylobacter jejuni*, *Enterobacter aerogenes*, *E. aerogenes* (MDR)	DIZ (15μl) 10–44 mm MIC 0.2–12.5 mg/ml	Not tested	Djabou et al. (2013)
T. polium Iran	β-caryophyllene (29%), farnesene (13%), β-pinene (11%), germacrene D (6.5%), α-pinene (5.5%)	15 clinical isolates of *K. pneumoniae*	DIZ 14–28.5 mm MIC 0.62–1.25 mg/ml	Not tested	Raei et al. (2014)
T. polium Iran	α-pinene (25.79%), myrcene (12.50%)	*P. aeruginosa*, *Pantoea agglomerans*, *Brenneria nigrifluens*, *Rhizobium radiobacter*, *Rhizobium vitis*, *Streptomyces scabies*, *Ralstonia solanacearum*, *Xanthomonas campestris*, *Pectobacterium cartovorum*	Active against – *R. solanacearum*, *P. agglomerans*, *B. nigrifluens*, *S. scabies*	Not tested	Purnavab et al. (2015)

T. polium subsp. *capitatum* Algeria	t-cadinol (18.3%), germacrene D (15.3%), *β*-pinene (10.5%)	*S. aureus* ATCC 6538, *B. subtilis* ATCC 9372, *P. aeruginosa* ATCC 9027, *K. pneumoniae* ATCC 4352, *Candida albicans* ATCC 24433	DIZ (20µl) 14.7–35.3 mm	DIZ (20µl) 13.7 mm	Kerbouche et al. (2015)
T. polium Morocco	Not analysed	*S. aureus* ATCC 29213, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 10231, *A. brasiliensis* ATCC 16404	DIZ (10µl) 12 mm (*S. aureus*) MIC 1.3µl/ml (*S. aureus*)	DIZ (80µl) 10.33 mm (*C. albicans*)	Boukhira et al. (2016)
T. polium Turkey	(Z)-*β*-farnesene (15.49%), *β*-phellandrene (10.77%), *α*-farnesene (10.71%)	*S. aureus* (MRSA), *S. aureus* ATCC 6538, *P. aeruginosa*, *E. coli* O157:H7, *B. cereus*	DIZ (5µl) 11–15 mm	Not tested	Sevindik et al. (2016)
T. polium subsp. *gabesianum* Tunisia	*β*-pinene (35.9%), *α*-pinene (13.32%), *α*-thujene (8.46%)	*E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *Citrobacter freundeii*, *Proteus mirabilis C. albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *T. soudanense*, *Microsporum canis*, *A. fumigatus*, *Scopulariopsis brevicaulis*	MIC 0.078–0.156 mg/ml	MIC 0.062– > 1 mg/ml	Ben Othman et al. (2017)
T. marum subsp. *marum* Italy (Sardinia Island)	Isocaryophyllene (20.24%), *β*-bisabolene (14.73%), *β*-sesquiphellandrene (11.27%), *α*-santalene (10.97%), dolichodial (9.38%), *α*-caryophyllene (7.18%)	*F. oxysporum*, *Botrytis cinerea*, *Rhyzoctonia solani*, *Alternaria solani*	Not tested	MIC 250–3800µg/ml	Ricci et al. (2005)
T. marum Italy (Corsica Island)	Caryophyllene oxide (9.8%), (E)-*α*-bergamotene (8.2%), *β*-bisabolene (7.5%)	*S. aureus*, *S. epidermidis*, *L. innocua*, *C. jejuni*, *E. aerogenes*, *E. aerogenes* (MDR)	DIZ (15µl) 8–40 mm MIC 0.4–1 mg/ml	Not tested	Djabou et al. (2013)

(continued)

Table 12.3 (continued)

Teucrium species	Major oil's compounds	Tested microorganisms	DIZ (tested concentration) range, MIC range		References
			Antibacterial activity	Antifungal activity	
T. arduini Montenegro	Germacrene D (16.98%), *β*-caryophyllene (14.98%), *β*-burbonene (5.59%), *α*-amorphene (4.68%), linalool (7.05%), *α*-terpinolene (5.25%), 1-octene-3-ol (4.69%)	*B. subtilis*, *E. cloaceae*, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *K. pneumoniae*, *M. lysodeikticus* ATCC 4698, *P. mirabilis*, *S. aureus* ATCC 25923, *S. aureus*, *C. albicans* ATCC 10259	MIC 6.25–50μl/ml	MIC 50μl/ml	Vuković et al. (2011)
T. arduini Croatia	*β*-caryophyllene (32.9%), germacrene D (16.4%), borneol (5.4%)	*S. aureus* ATCC 6538, *E. faecalis* ATCC 21212, *E. coli* ATCC 10535, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 10231, *M. gypseum, A. brasiliensis*	MIC 6.25–37.5 mg/ml	MIC 7.81–25 mg/ml	Kremer et al. (2012)
T. chamaedrys Italy (Corsica Island)	(E)-*α*-caryophyllene (33.9%), germacrene D (18.5%), *α*-humulene (7.5%), *δ*-cadinene (4.6%)	*S. aureus*, *S. epidermidis*, *L. innocua*, *C. jejuni*, *E. aerogenes*, *E. aerogenes* (MDR)	DIZ (15μl) 6–22 mm MIC 1– > 50 mg/ml	Not tested	Djabou et al. (2013)
T. chamaedrys subsp. *chamaedrys* Turkey	Germacrene D (16.7%), *α*-pinene (15.8%), *β*-caryophyllene (11.8%), *β*-pinene (8.9%), *β*-myrcene (4.1%)	*E. coli* ATCC 35218, *K. pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Serratia marcescens* ATCC 13880, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *C. albicans* ATCC 60193, *C. tropicalis* ATCC 13803	DIZ (1000μg/ml) 5–15 mm	Not active	Küçük et al. (2006)
T. chamaedrys subsp. *lydium* Turkey	*β*-caryophyllene (19.7%), *α*-pinene (12.5%), germacrene D (9.3%), *β*-pinene (6.6%), caryophyllene oxide (6.1%)		DIZ (1000μg/ml) 5–15 mm	Not active	
T. orientale var. *puberulens* Turkey	*β*-caryophyllene (21.7%), 2-methyl cumarone (20.0%), germacrene D (10.6%), *α*-humulene (4.8%), *δ*-cadinene (4.1%)		DIZ (1000μg/ml) 5–15 mm	Not active	

T. orientale var. *orientale* Turkey	*β*-caryophyllene (15.3–19.0%), germacrene D (14.2–12.8%), caryophyllene oxide (14.0–19.0%)	*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *C. albicans*, *C. tropicalis*	MIC 100–400μg/ml	MIC 25–50μg/ml	Kucukbay et al. (2011)
Teucrium orientale var. *puberulens* Turkey	*β*-caryophyllene (15.3–19.0%), germacrene D (14.2–12.8%), caryophyllene oxide (14.0–19.0%)		MIC 50–400μg/ml	MIC 12.5–25μg/ml	
T. massiliense Italy (Corsica Island)	6-methyl-3-heptyl acetate (19.1%), 3-octanyl acetate (7.0%), pulegone (6.9%), germacrene D (6.1%)	*S. aureus*, *S. epidermidis*, *L. innocua*, *C. jejuni*, *E. aerogenes*, *E. aerogenes* (MDR)	DIZ (15μl) 6–41 mm MIC 0.8–6 mg/ml	Not tested	Djabou et al. (2013)
T. scorodonia subsp. *scorodonia* Italy (Corsica Island)	(E)-*α*-caryophyllene (21.1%), germacrene B (8.3%), *α*-humulene (6.9%), germacrene D (6.7%), *α*-cubebene (6.2%)		DIZ (15μl) 6–26 mm MIC 0.2– > 50 mg/ml	Not tested	
T. flavum subsp. *glaucum* Italy (Corsica Island)	Limenone (27.4%), *α*-pinene (12.2%), (Z)-*α*-ocimene (6.0%), (E) phytol (4.5%)		DIZ (15 μl) 16–39 mm MIC 0.2–1.5 mg/ml	Not tested	
T. africanum South Africa	*β*-cubebene (20.5%), *α*-cubebene (29.3%)	*B. cereus* ATCC 11778, *E. coli* ATCC 8739, *K. pneumoniae* ATCC 13883, *Moraxella catarrhalis* ATCC 23246, *P. aeruginosa* ATCC 27858, *S. aureus* ATCC 25923, *S. pyogenes* ATCC 8668	MIC 0.16– > 8 mg/ml	Not tested	Ruiters et al. (2016)
T. kraussii South Africa	Not analysed		MIC 4 mg/ml (*S. pyogenes*)	Not tested	
T. trifidum South Africa	*β*-cubebene (31.1%), *α*-cubebene (11.4%), *β*-caryophyllene (7.7%)		MIC 2– > 8 mg/ml	Not tested	
T. leucocladum Egypt	Patchouli alcohol (31.24%), *β*-pinene (12.66%), *α*-pinene (10.99%), *α*-cadinol (l9.27%), viridiflorol (5.36%), myrcene (5.35%)	*E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *C. albicans*	DIZ (20 mg/ml) 12–30 mm	DIZ (20 mg/ml) 26 mm	El-Shazly et al. (2004)

(continued)

Table 12.3 (continued)

Teucrium species	Major oil's compounds	Tested microorganisms	DIZ (tested concentration) range, MIC range: Antibacterial activity	DIZ (tested concentration) range, MIC range: Antifungal activity	References
T. stocksianum subsp. *stocksianum* Oman	*α*-cadinol (7.6%), *β*-selinene (6.4%), trans-verbenol (5.9%), caryophyllene oxide (5.7%), *α*-phellandren-8-ol (5.0%), verbenone (5.0%), *δ*-cadinene (5.1%)	*S. aureus* NCTC 6571, *E. coli* NCTC 10418, *P. aeruginosa* NCTC 10662, *S. aureus*, *S. albus*, *S. epidermidis*, *S. mitis*, *S. sanguis*, *M. luteus*, *B. subtilis*, *B. cereus*, *E. coli*, *E. aerogenes*, *K. pneumoniae*, *S. typhy*, *P. vulgaris*, *P. aeruginosa*, *C. albicans*, *Sacharomyces cerevisiae*, *R. stolonifer*, *Penicillium notatum*, *F. oxysporum*	DIZ (2 mg/disk) 9–18.5 mm MIC 4.5–5 mg/ml (G^+ bacteria) MIC 6.5–11 mg/ml (G^- bacteria)	DIZ (2 mg/disk) 3.5–6 mm	Hisham et al. (2006a, b)
T. mascatense Oman	Linalool (27.8%), linalyl acetate (12.6%), *β*-eudesmol (10.1%), *α*-bergamotene (5.0%)		DIZ (0.2 mg/disk) 3.5–17.5 mm MIC 1.5–2.5 mg/ml (G^+ bacteria) MIC 6–8.5 mg/ml (G^- bacteria)	DIZ (1.6 mg/disk) 3.5–6.5 mm	
T. hyrcanicum Iran	(E)-*β*-farnesene (34.1%)	*B. subtilis*, *S. aureus*, *S. typhi*, *P. aeruginosa*, *E. coli*, *A. niger*, *C. albicans*	Active against *B. subtilis*, *S. aureus*, *S. typhi*, *P. aeruginosa*	Active against *A. niger*	Morteza-Semnani et al. (2011)
T. ramosissimum Tunisia	*β*-eudesmol (44.52%), caryophyllene oxide (9.36%), *α*-thujene (5.51%), sabinene (4.71%), *t*-cadinol (3.9%)	*S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *S. enteridis* ATCC 13076, *S. typhymurium* NRRL B- 4420	MIC 0.24–0.36 mg/ml	Not tested	Ben Sghaier et al. (2007)
T. yemense Yemen	*α*-pinene (6.6%), (E)-caryophyllene (19.1%) *α*-humulene (6.4%), *δ*-cadinene (6.5%), caryophyllene oxide (4.3%), *α*-cadinol (9.5%), shyobunol (4.6%)	*E. coli* ATCC 10536, *P. aeruginosa* ATCC 25619, *S. aureus* ATCC 29737, *B. cereus*, *A. niger* ATCC 16888, *B. cinerea* ATCC 126943, *C. albicans* ATCC 90028	MIC 156–1250µg/ml	MIC 313–1250µg/ml	Ali et al. (2017)

T. sauvagei Tunisia	*β*-eudesmol, *t*-cadinol, *α*-thujene, gamma-cadinene, sabinene	Dermatophytes	Not tested	Active against dermatophytes	Salah et al. (2006)
T. divaricatum subsp. *villosum* Lebanon	(E)-caryophyllene (30.1%), caryophyllene oxide (6.1%)	G^+ bacteria, G^- bacteria	Active against G^+ bacteria	Not tested	Farmisano et al. (2010)
T. plectranthoides India	Not analysed	*B. megaterium*, *B. subtilis*, *E. coli*, *S. aureus, P. aeruginosa, P. vulgaris*, *A. niger, Xanthomonas campestris*, *A. parasiticus*, *Rhizopus oryzae*, *Rhizoctonia oryzae-sativae*, *Colletotrichum musae*, *F. solani*, *C. albicans*, *Alternaria brassicicola*	DIZ 20–38 mm	DIZ 16–27 mm	Thoppil et al. (2001)

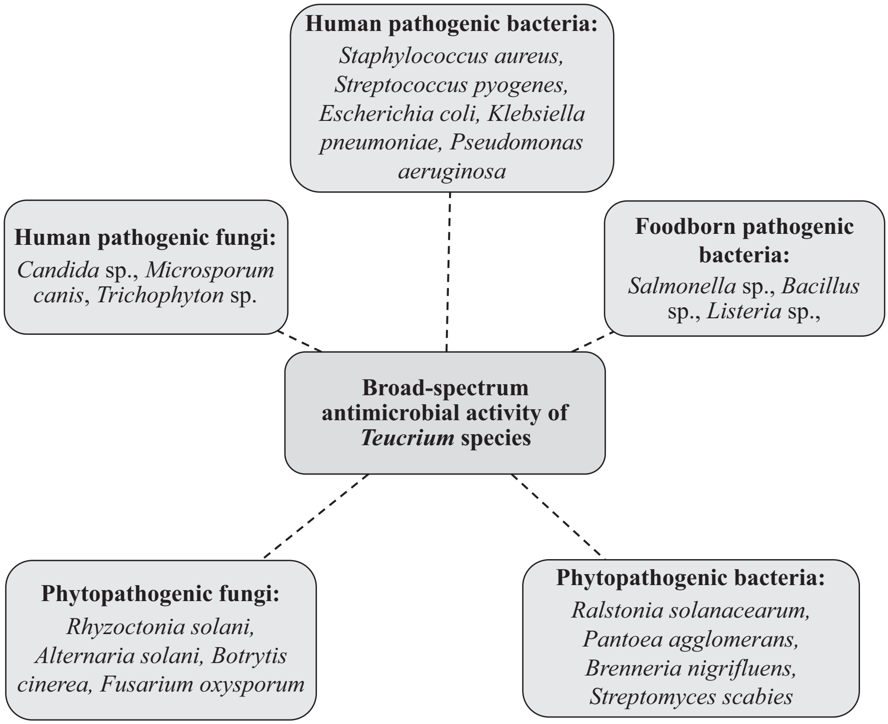

Fig. 12.4 The most susceptible bacteria and fungi to *Teucrium* species

necessary. So far, only, Elmasri et al. (2014, 2015) have evaluated antibiofilm activity of *Teucrium* species. The authors have noticed that several sesquiterpenes and flavonoids from *Teucrium polium* were effective in prevention of *Staphylococcus aureus* biofilm formation.

12.6.1 *Antimicrobial Activity of* Teucrium *Species Plant Extracts*

Generally, three levels of investigation of antimicrobial activity of plant extracts are established: (i) testing of crude extract, (ii) testing of sub-fractions, and (iii) testing of pure compounds. According to the literature search, crude extracts of *Teucrium* species were frequently researched. Several studies were focused on testing of sub-fractions of extracts, but no studies on testing of isolated pure compounds. Also, the mechanism of antimicrobial action of extracts from *Teucrium* species has not been determined yet.

The MIC values are used as cut-off points for classification of the antimicrobial potential of plant extracts. The one of that classification was defined by Tamokou et al. (2017), as follows: MIC below 100µg/ml – highly active, 100 ≤ MIC ≤ 512µg/ml – significantly active, 512 < MIC ≤ 2048µg/ml – moderately active, MIC > 2048µg/ml – low active, MIC > 10 mg/ml – not active. The stated cut-off points were applied for interpretation of antimicrobial activity of extracts from *Teucrium* species. Summary results of antimicrobial activity are presented in Table 12.2. In the following paragraphs, the most significant results were discussed.

Teucrium polium was one of the most tested *Teucrium* species and plant materials, collected from different regions, have been screened for antimicrobial activity. Extracts from *Teucrium polium* originated from Tunisia and Turkey showed the most promising antimicrobial activity in relation to commonly used Gram-positive bacteria (*Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis*, *B. cereus*, *Micrococcus luteus*, *Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi, Enterobacter cloaceae*) and fungi (*Candida albicans*, *Aspergillus niger*, *A. fumigatus*, *Trychophyton rubrum*). The MIC values of ethanol and water extract from Tunisian *Teucrium polium* were 0.156–0.625 mg/ml against bacteria and 0.625 mg/ml against fungal species *Microsporum canis* (Ben Othman et al. 2017). Moreover, Turkish *Teucrium polium* extract, in a form of decoction, showed better activity with MIC range of 16–64µg/ml for bacteria and 8–64µg/ml for fungi (Deliroman Orhan et al. 2012). The methanol extract from Jordanian plant showed moderate antibacterial activity, the best activity was noticed against *Escherichia coli* (MIC of 1.2 mg/ml) (Tarawneh et al. 2010). In addition, the ethanol extract of plant material from the same region were active on *Staphylococcus aureus* and *Pseudomonas aeruginosa* at MIC of 80 ppm (Khalil et al. 2009). On the other side, organic solvent extracts, tested in other studies, exhibited low antimicrobial activity or they were not active, MIC values were 3.125–50 mg/ml for bacteria and up to > 150 mg/ml for fungi (Hashem 2011; Stanković et al. 2012; Mirzei et al. 2013; Khaled-Khodja et al. 2014; Dridi et al. 2016). Using time-kill assay, the ethanol extract at concentration of 0.3 mg/ml has no effect on the growth of *Escherichia coli* O157 NCTC 1290 (Mashreghi and Niknia 2012). In the study of Mansouri (1999), *Teucrium polium* flower ethanol extract had very low antistaphylococcal activity. Against 489 isolates of *Staphylococcus aureus*, at concentration of 30 mg/ml, it showed activity against 0.61% of tested isolates. The antilisterial activity of ethanol extract was noticed, the inhibitory zones of 10–11 mm (at conc. 133 mg/ml) and MIC values 12.5–50µg/ml were detected (Altanlar et al. 2006). Against pathogenic bacteria, *Brucella melitensis*, the extracts of *Teucrium polium* showed moderate activity compared to other medicinal plants tested in the same study (Motamedi et al. 2010). Furthermore, the synergistic activity of *Teucrium polium* extract and antibiotics was investigated. The methanol extract in combination with antibiotics (amoxicillin, chloramphenicol, neomycin, doxycycline, clarithromycin, cephalexin, and nalidixic acid) enhanced the activity of antibiotics against the resistant *Escherichia coli* strain (Darwish and Aburjai 2010).

Studies on *Teucrium chamaedrys* extracts indicated their similar antimicrobial activity even that plant material was collected in the different regions of the world.

In general, low to moderate antimicrobial activity was noticed and activity was higher against Gram-positive bacteria then Gram-negative bacteria and fungi (Table 12.2). In the recent paper (Nastić et al. 2018), the authors have reported the activity of *Teucrium chamaedrys* extract obtained by supercritical water extraction. If we compare the results with the results of the study (Stanković et al. 2012) where plant material was extracted by maceration, the higher activity of supercritical water extract was obtained. The plant materials were from the same region (Serbia). In *Teucrium chamaedrys* supercritical water extract several phenolic compounds were identified: vanillin acid, caffeic acid, epicatechin, ferulic acid, sinapic acid, gallic acid, protocatechuic acid, catechin, and chlorogenic acid. In addition, in ethanol extract of *Teucrium chamaedrys* from Romania the following phenolic compounds, luteolin, p-coumaric acid, isoquercitrin, rutin, quercitrin, were isolated (Vlase et al. 2014).

Teucrium montanum is one of the most popular medicinal plants in Serbia. Two independent research groups investigated the antimicrobial activity of this plant and they observed that the extracts (methanol, acetone, ethyl acetate, butanol) were active against *Staphylococcus aureus* strains (MIC of 0.15–1 mg/ml) (Djilas et al. 2006; Stanković et al. 2012). In addition, the methanol, acetone, ethyl acetate extracts were not active against tested fungi (Stanković et al. 2012).

Teucrium arduini is an endemic Illyric Balkan species distributed in Croatia, Bosnia and Herzegovina, Montenegro, Serbia and northern Albania. Šamec et al. (2010) have conducted a detailed study including six different localities in Croatia. The leaf and flower infusions were tested. The lowest MIC values were found against *Staphylococcus aureus* ATCC 6538 (MIC of 1.56 and 4.16 mg/ml), depending on the location from which plant material (leaves) was collected. *Teucrium arduini* flower and leaf infusions did not exhibit activity against Gram-negative bacterial species, or against fungal species tested in this study. Except for water extract in form of infusion, antimicrobial activity of organic solvent extracts from different location (Croatia and Serbia) were tested and promising activity against Gram-positive bacteria (MIC of 0.15–2 mg/ml) was noticed (Stanković et al. 2012; Kremer et al. 2013). In Croatian samples of *Teucrium arduini* several phenolic compounds were identified: protocatechuic acid, 4-hydroxybenzoic acid, salicylic acid, gentisic acid, ferulic acid, vanillic acid, caffeic acid, syringic acid, sinapic acid, 4-coumaric acid, rosmarinic acid and quercetin (Kremer et al. 2013).

Two subspecies of *Teucrium scordium* (*T. scordium* subsp. *scordioides* and *T. scordium* subsp. *scordium*) have been subjected to antimicrobial testing of two research groups from Serbia. The plant material was collected from different localities and organic solvent extracts were prepared. The promising activity was noticed against *Staphylococcus aureus* ATCC 25922 (MIC of 0.3–0.6 mg/ml) (Kundaković et al. 2011; Stanković et al. 2012).

Numerous studies of antimicrobial activity of different *Teucrium* endemic species, rare species or species characteristic for a particular region were, also, performed. According to detected MIC values, extracts of *Teucrium flavum*, *T. fruticans*, *T. siculum*, *T. yemense*, *T. sokotranum* and *T. persicum* exhibited pronounced activity against tested Gram-positive bacteria, MIC range was 250–2000μg/ml

(Table 12.2). *Teucrium bicolor* methanol extract was active against *Mycobacterium tuberculosis* (MIC of 125µg/ml) and *Candida* species (MIC of 31.2–125µg/ml) (Garza et al. 2017). Three endemic species (*Teucrium africanum*, *T. kraussii* and *T. trifidum*) from southern Africa were active against some important pathogens; *Teucrium africanum* against *Escherichia coli* with MIC value of 0.13 mg/ml. *Teucrium kraussii* against *Streptococcus pyogenes* with MIC value of 0.8 mg/ml and *Teucrium trifidum* showed activity against *Pseudomonas aeruginosa* with MIC value of 0.5 mg/ml (Ruiters et al. 2016). Oppositely, extracts of *Teucrium oliverianum*, *T. orientale*, *T. royleanum* and *T. botrys* showed low activity (Table 12.2).

12.6.2 *Antimicrobial Activity of* Teucrium *Species Essential Oils*

Essential oils are recognized as the most active products of plant metabolism. They present the mixture of bioactive compounds including monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, esters, and alcohols). Generally, essential oils have strong antimicrobial properties and could be used as food preservatives or therapeutic antimicrobial agents. The level of *Teucrium* essential oils bioactivity was determined according to cut-off points, it was estimated that MIC values lower than 2 mg/ml indicate significant activity of essential oils (Van Vuuren and Viljoen 2006). Hence, most of the essential oils from *Teucrium* species exhibited significant activity against Gram-positive bacteria (*Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp.) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Salmonella* sp.) and fungi (*Candida* sp.). Essential oils were isolated from *Teucrium* plants originated from different regions, especially from the Mediterranean basin. It was obvious that the chemical composition and the amounts of main constituents (mono- and sesquiterpene hydrocarbons and oxygenated sesquiterpenes) differed notably and showing intraspecific variations in the chemical composition of the essential oils according to environment and/or genetic parameters. The essential oils' main compounds as well as detailed results of antimicrobial activity were summarized in Table 12.3. In the following sections, the most significant results were discussed.

Antibacterial activity of *Teucrium polium* essential oil was investigated in several studies. The oil in the quantity of 5–20µl inhibit the growth of *Staphylococcus aureus* and *Bacillus subtilis* (Vahdani et al. 2011; Belmekki et al. 2013; Djabou et al. 2013; Kerbouche et al. 2015; Boukhira et al. 2016; Sevindik et al. 2016). Moreover, *Teucrium polium* essential oil extracted from plant material collected in North Anatolia (Turkey) showed promising activity against *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (Sevindik et al. 2016). In addition, essential oil of *Teucrium polium* originated from Iran was active against clinical isolates of *Klebsiella pneumoniae* (Raei et al. 2014). Interestingly, *Teucrium* *polium* and *T. marum* essential oils were active against some phytopathogenic bacteria and fungi (Ricci et al. 2005; Purnavab et al. 2015). Essential oil of *Teucrium marum* inhibits the growth of *Rhyzoctonia solani* at concentration of 250μg/ml. Furthermore, *Teucrium orientale*, *T. africanum*, *T. ramosissimum*, *T. mascatense* and *T. yemense* essential oils showed excellent activity against tested bacteria (Table 12.3). As promising antibacterial agents can be *Teucrium africanum* essential oil against *Streptococcus pyogenes* (MIC of 0.16 mg/ml), *Teucrium ramosissimum* against *Salmonella enterica, S. typhimurium, Escherichia coli* (MIC of 0.28–0.24 mg/ml), *Teucrium mascatense* and *T. yemense* against *Staphylococcus aureus* and *Bacillus subtilis* (MIC of 156μg/ml). Multi-drug resistant strain of *Enterobacter aerogenes* was susceptible to *Teucrium massiliense* and *T. scordonia* essential oil (MIC of 0.2–0.4 mg/ml) (Djabou et al. 2013). On the other side, *Teucrium stocksianum*, *T. kraussii*, *T. trifidum*, *T. leucoclada*, *T. chamedrys* and *T. arduini* exhibited low antibacterial activity (Table 12.3).

Antifungal activity of *Teucrium* essential oils was, especially, evaluated against *Candida* species and dermatophytes (Table 12.3). Essential oils of *Teucrium orientale* subsp. *orientale* and *T. orientale* subsp. *puberulens* could be promising anti-*Candida* agents. They were active at concentrations range of 12.5–50μg/ml (Kucukbay et al. 2011). In addition, *Teucrium polium* and *T. sauvagei* were active against tested dermatophytes (Salah et al. 2006; Ben Othman et al. 2017).

Essential oils obtained from *Teucrium* species possess significant broad-spectrum antimicrobial activity, however, there is limited information available about their mechanisms of action. Better understanding the mechanisms of action will give more detailed information about their potency and potential application as antimicrobial agents.

12.7 Conclusion

This chapter provides a detailed review of the antimicrobial activity of *Teucrium* species, their active constituents, and their potential as sources of antibacterial and antifungal agents. The relevant literature summary showed that *Teucrium* species exhibited a diverse range of antimicrobial properties. The promising activity and potential application in control of bacteria and fungi possess the following species: plant extracts of *Teucrium polium*, *T. flavum*, *T. fruticans*, *T. siculum*, *T. yemense*, *T. sokotranum*, *T. persicum*, *T. scordium*, and essential oils from *Teucrium polium, T. orientale*, *T. africanum*, *T. ramosissimum*, *T. mascatence*, *T. yemense*, *T. massiliense*, *T. scordonia*. They were active against important pathogenic bacteria (*Staphylococcus* sp., *Bacillus* sp., *Enterococcus* sp., *Streptococcus* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella* sp.) and fungi (*Candida* sp., *Trychophyton* sp.). However, these results are conducted on the basis of in vitro studies. The future studies on mechanisms of action of plant extracts, essential oils, and pure active compounds will contribute to the development of new *Teucrium* antimicrobial agents. Furthermore, in vivo testing of activity, toxicity and bioavailability will determine their actual relevance for treatment of infectious diseases or used as food preservatives.

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Chapter 13
Anticancer Activity of Secondary Metabolites of *Teucrium* Species

Milena Milutinović and Danijela Cvetković

Abstract Cancer is one of the life-threatening diseases and currently leading human health problem. Conventional use of chemotherapeutics in anticancer therapy is faced with some problems, such as their being harmful for the organism and development of the cancer cell resistance to the current therapeutic agents. There is a great need to discover and develop new anticancer drugs, more effective and less toxic for healthy cells, such as plant-derived compounds. There is a variety of experimental and epidemiological evidence that plant extracts and secondary metabolites from them achieve anticancer activity. Many of the species from the genus *Teucrium* have been used extensively in the traditional medicine as medicinal plants for treatment of numerous diseases, due to a variety of their therapeutic properties. Bearing in mind the advantages of utilization of plant products as anticancer agents, we revised recent literature and searched bibliographic databases about anticancer properties of *Teucrium* species, with an aim to give survey of their investigation and emphasize the need for further research in order to improve the anticancer treatment. This paper presents an overview of the current *in vitro* and *in vivo* data that support their anticancer potential. The extracts of *Teucrium* species and their secondary metabolites showed antiproliferative effects on multiple cancer cell lines, multiple signaling pathways, and altered the expression of genes and proteins involved in cancer development, cell cycle, apoptosis, angiogenesis, metastasis. The comparative review of results may contribute to better understanding of possible mechanisms of action and stimulate further examination for application of *Teucrium* species as adjuvant chemotherapeutics.

Keywords Apoptosis · Angiogenesis · Cancer · Cytotoxicity · Invasion · Migration · ROS · *Teucrium* species

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Abbreviations

AO/EB	Acridine Orange/Ethidium Bromide
Bcl-2	B-cell lymphoma 2
Bid	BH3 interacting-domain death agonist
Cdks	Cyclins dependent kinases
CKIs	Cdk inhibitors
CLL	Chronic Lymphocytic Leukemia
CXCL-12	CXC Motif Chemokine Ligand 12
CXCR-4	CXC Receptor 4
DISC	Death-Inducing-Signaling Complex
ECM	Extracellular Matrix
EMT	Mesenchymal-epithelial Transition
FGF	Fibroblast Growth Factor
GSH	Glutathione
HIF-1α	Hypoxia Inducible Factor-1α
ICAM-1	Intracellular Adhesion Molecule
iNOS	Inducible Nitric Oxide Synthase
MMP	Matrix metalloproteinase
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
NCI	National Cancer Institute
$O_2^{\cdot-}$	Superoxide anion radical
PARP	Poly ADB Ribose Polymerase
PI	Propidium Iodide
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
Smac/DIABLO	Second mitochondria-derived activator of caspase/Direct Inhibitor of Apoptosis-Binding protein
STAT3	Signal Transducer and Activator of Transcription 3
tBid	Truncated Bid
TNF	Tumor Necrosis Factor
VCAM1	Vascular Cell Adhesion Molecule 1
VEGF	Vascular Endothelial Growth Factor
XIAP	Inhibitor of caspase function proteins
γ-GCS	γ-glutamyl cysteine synthetase

13.1 Introduction

Cancer can be explained as uncontrolled cell growth, due to disorders in the basic cell processes (Cooper 2000; Ma and Yu 2006). It begins with changes in genetic material, i.e. mutations, but also may be the result of epigenetic interactions, mainly in protooncogenes, tumor suppressor, DNA repair genes and genes which regulate apoptosis. Changes in those genes are the most common causes of cancer development, together with impact of hereditary factors (Levine and Puzio-Kuter 2010; Ngo et al. 2015). Cancer cells are characterized by multiple molecular, biochemical, morphological and behavioral characteristics. Some of them are dysregulation and abnormality in cell cycle and division, production of own growth signals, evasion of apoptosis and contact inhibition, changes in communication with surrounding cells, altered metabolism, angiogenic, invasive and metastatic potential, etc. (Hanahan and Weinberg 2000).

Cancer is one of the leading health problems, with growing incidence in last years (Siegel et al. 2019). There are many strategies and attempts to overcome and treat this disease with very heterogeneous symptoms, depending on its primary and/or secondary location. Numerous *in vitro* and *in vivo* investigations have been carried out in order to find an effective anticancer treatment strategy and potential way to overcome this disease (Cvetković et al. 2017; Zeng 2018). The main approach to cancer treatment is chemotherapy, which is desirable to be fully effective and safe. However, currently used drugs have many side effects, thus affecting normal cells and developing drug resistance in cancer cells. In search for new drugs the most important goal is their high potential of drugs against cancer cells, together with the safe targeted therapy and avoidance of harmful effects after the treatment (Artemov et al. 2015; Zugazagoitia et al. 2016).

13.1.1 Plant-Derived Anticancer Compounds

Plants have been traditionally used for cancer prevention and they are a valuable source of potentially active substances in the anticancer therapy. The literature data indicate that consumption of fruits and vegetables, teas from medicinal plants, various tinctures and plant extracts, as well as numerous compounds isolated from plants have impact on chemo-prevention and/or chemo-therapy of cancer (Kotecha et al. 2016; Roy et al. 2017).

Primary positive effects of plants are related to their chemo-preventive role in the stage of cancer development. The preventive role of plants includes their antioxidative activity and reduction of free radicals, protection of biomolecules by preventing carcinogens to bind on targeting size through modulation of carcinogen-activation and carcinogen-detoxification enzymes, thus disabling their interaction with DNA, RNA or proteins. In this way, they protect biomolecules from harmful effects of cancer causative agents, like chemical carcinogen, radiation, infective agents, thus inhibiting the DNA damage (Desai et al. 2008; Kotecha et al. 2016).

An important role of plants and plant-derived compounds is their ability to inhibit initiation, promotion and progression of cancer, due to targeting key molecules involved in cancer cell signaling. The most important mechanisms of their anticancer success are stimulation and modulation of immune system, modulation of redox status, inhibition of growth and survival of malignant transformed cells, modulation of inner signal transmission paths, induction of apoptosis, suppression of promotion and progression of cancer, and antiangiogenic, antiinvasive antimigratory potentials (Demain and Vaishnav 2011; Newman and Cragg 2016; Blowman et al. 2018). Besides their own anticancer properties, naturally derived compounds, due their additive or synergistic effects, can be used in combination with chemotherapeutics, thus leading to increased efficacy of therapy. They can increase cell response sensitivity to induction of apoptosis or inhibition of proliferation and decrease harmful effects of chemotherapy. Their potential is based on selective cytotoxic activity on cancer cells, as well as low toxicity on normal cells (Greenwell and Rahman 2015; Milutinović et al. 2015a).

Based on the mentioned properties of plants, the investigation of plant extracts as the mixture of secondary metabolites or their individual consideration is one of the most important aspects in discovering new compounds with possible anticancer activity. It has been the focus of many studies (Ćurčić et al. 2012a; Stanković et al. 2012; Milutinović et al. 2015b; Alimpić et al. 2015, 2017a). This actual field of research started with discovery of vinblastine and vincristine from *Catharanthus roseus*, paclitaxel from *Taxus brevifolia*, followed by campotechin and podophyllotoxins and many other discovered natural products or their derivatives (Prakash et al. 2013; Newman and Cragg 2016; Thomford et al. 2018). Currently, many of these drugs are involved in advanced clinical trial and clinically used as anticancer agents (Newman and Cragg 2004; Butler 2008; Seca and Pinto 2018). Among currently used chemotherapeutics in the treatment of cancer, the secondary metabolites from plant and their semi-synthetic derivates take a significant place. Many of them, mainly phenolic compounds, which include flavonoids (flavanols, isoflavones, flavanones, anthocyanins), stilbenes and phenolic acid, terpenoids and alkaloids have been identified as potent anticancer agents. Some of them have been used directly or after chemical modifications in different strategies of cancer therapy, with more pronounced activity and lower toxicity compared to synthetic drugs (Fabricant and Fransworth 2001; Rayan et al. 2017; Shin et al. 2018; Seca and Pinto 2018).

13.2 *Teucrium* Species and Their Anticancer Properties

The genus *Teucrium* consists of diverse species with variety of habitats and medicinal use. Due to their positive effects on human health, many were traditionally known as medicinal and aromatic plants. They were used for diverse applications, mainly in pharmaceutical industry and treatments of many pathological conditions (Bahramikia and Yazdanparast 2012). Because of their bitter taste, some were used for improvement of digestion as tea or tonic, then in treatment of gastric pain, ulceration and generally, digestive tract (Sarkhail 2011; Sghaier et al. 2012). Diverse therapeutic and biological activities of *Teucrium* species have been reported such as antibacterial, antioxidant, antifungal, antidiabetic, antispasmodic, antioxidant, antiinflammatory, antiulcer, hypoglycemic, anti-acetylcholinesterase, and hepatoprotective (Ulubelen et al. 2000; Ljubunčić et al. 2006; Jaradat 2015; Purnavab et al. 2015; Othman et al. 2017; Khazaei et al. 2018). Various groups of bioactive

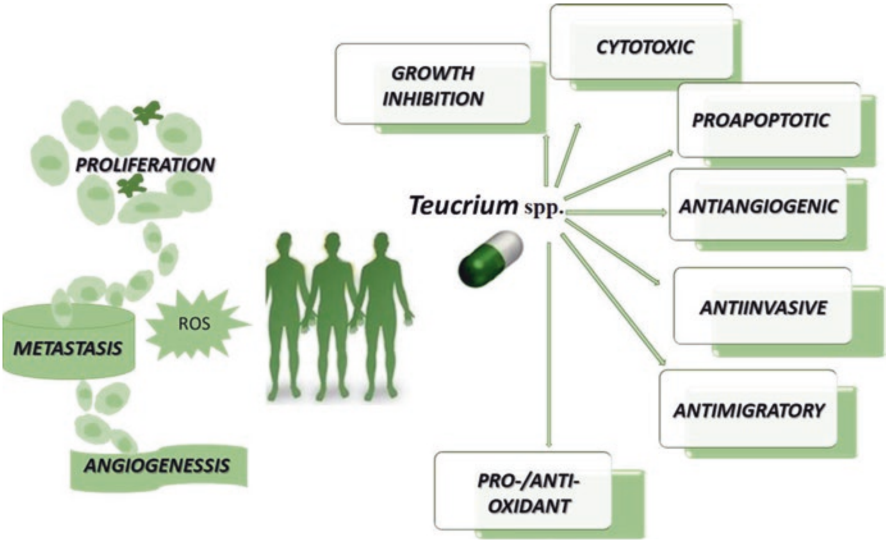

Fig. 13.1 The most important mechanisms of anticancer activity of extracts, essential oils and secondary metabolites from *Teucrium* species. *Teucrium* species have impact on multiple signaling pathways, genes and/or proteins involved in cancer development, cell cycle, apoptosis, redox status, migration, angiogenesis, etc

molecules were isolated from *Teucrium* species, but terpenoids and flavonoids were the most potent for achievement of its pharmacological and therapeutic effects (Bahramikia and Yazdanparast 2012). Besides, many other compounds were detected and isolated, such as phenolics, flavonoids, iridoids, sterols, tannins, saponins, terpenoids, alkaloids and glycosides of many others (Bedir et al. 1999; Bahramikia and Yazdanparast 2012; Elmasri et al. 2015, 2016; Fatima 2016; Milutinović et al. 2019). The observed anticancer properties of *Teucrium* species, and their various mechanisms of anticancer actions (Fig. 13.1), were the consequence of chemical contents and presence the high concentration and wide range of secondary metabolites. There were many reports that confirmed positive correlation between qualitative and/or quantitative content of phenolic compounds in plants and their biological activity (Stanković et al. 2012; Alimpić et al. 2017b; Duletić-Laušević et al. 2018).

In the focus of investigation are the extracts from *Teucrium* species obtained by different solvents as the mixture of total chemical constituents. Plant extracts consist of various secondary metabolites with different chemical structures, so they may produce diverse therapeutic effects in comparison to an individual compound. Their anticancer properties are not only the result of activities of individual components, but also of their interactions, since they act in a synergistic, additive or antagonistic manner (Freeman et al. 2010). Crude extracts were the most investigated, however, several phytochemicals obtained from different species from the genus *Teucrium* showed anticancer properties (Stanković et al. 2011; Shah et al. 2014; Ali et al. 2017). The essential oils from the genus were also evaluated (Guesmi et al. 2018), because essential oils were reported as potent anticancer agents *in vitro* and *in vivo* models against different cell lines (Blowman et al. 2018). Some of biological activities of *Teucrium* species were attributed to components of essential oils (Ali et al. 2008).

13.2.1 Antiproliferative and Cytotoxic Activity

Preclinical investigations of anticancer potential of plant extracts include them *in vitro* testing on various human cancer cell lines. There are different human cell line panels, used for development of anticancer drugs and screening for potential activity by using proliferation assays. One of the panels, with 60 tumor cell lines, was recommended by NCI (Shoemaker 2006). This panel was required to include the most common tumors, like lungs, prostate, colorectal, stomach and liver in the population of men and breast, colorectal, lungs, cervix and stomach in women (Torre et al. 2015). In the discovery of new cancer drug, it is important to select candidates for phase II and III trials. Besides investigation in cytotoxicity in phase I, it is important to examine the mechanism of their action (Zhou et al. 2000) and evaluation *in vivo* model systems as a xenograft model, hollow fiber assay, genetically engineered mouse model, etc. (Kinghorn et al. 2009; Kumar et al. 2016).

Table 13.1 shows the effects of tested extracts and essential oils from various *Teucrium* species on different cell lines, according to our knowledge and available literature data. The available results about cytotoxic activity, expressed as the IC_{50} values, the concentration which inhibits 50% of cell growth.

Many *Teucrium* species have been investigated for possible antiproliferative activity and inhibition of cancer cell growth. According to results presented in the Table 13.1 it is evident that *Teucrium polium* is the most investigated; the literature data are available for some others as well, while many species in this genus have not been investigated yet. In the genus *Teucrium,* the most active secondary metabolites against tumor cells *in vitro* and *in vivo* are diterpenoids and flavonoids. The presence of diterpenoids was confirmed by many authors (Bruno et al. 1987, 2002; Avula et al. 2003). Neo-clerodane diterpenoids, Teucrins A, B, C, D, E and F were isolated from *Teucrium oliveranum* (de la Torre et al. 1991; Al-Yahia et al. 2002) and characterized as potential anticancer agents (Islam 2017). *Teucrium* diterpenoids were also reported against P 388 lymphocytic leukemia in mice (Nagao et al. 1982). Saponins from *Teucrium stocksianum* were recommended for future investigation of anticancer activity (Shah et al. 2014). Constituents from *Teucrium chamaedrys* were also specified, and the flavonoids, various glycosides, saponins, numerous neo-clerodane diterpenoids and the most dominant phenylethanoid glycosides (Forsythoside B, Samioside, Teucroside and Teucreoside) were found (Popa and Reinbold 1972; Savona et al. 1982; Piozzi et al. 1997; Milutinović et al. 2019). The content of essential oils from some *Teucrium* species was also evaluated; they contained high concentration of compounds with confirmed cytotoxic activity on some

Table 13.1 IC_{50} values (μg/ml) of *Teucrium* species in different cell lines, available in the literature data

Teucrium species	Type of extract	Experimental method	Time of exposure	Cell line	Effects IC_{50} (μg/ml)	References
T. persicum	M	MTT	48	Jurkat – T cell leukemia	> 200	Amirghofran et al. (2010)
				K562 – Chronic myelogenous leukemia	> 200	
				Raji – Burkitt's lymphoma	> 200	
				Fen – Bladder carcinoma	196	
				HeLa – Cervix epitheloid carcinoma	69	
T. persicum	M	MTT	48	PC-3 – Prostate cancer	142	Tafrihi et al. (2014)
				SW480 – Colon adenocarcinoma	79	
				T47D – Ductal epithelial breast tumor	50	
				NIH-3 T3 – Fibroblast	143	
T. sandrasicum	M	MTT	72	HeLa – Cervix epitheloid carcinoma	513	Artun et al. (2016)
				Vero – Normal kidney cell	593	
T. ramosissimum	M	MTT	48	K562 – Chronic myelogenous leukemia	150	Sghaier et al. (2012)
	CH				200	
	TOF				550	
	EA				600	
	AQ				800	

(continued)

Table 13.1 (continued)

Teucrium species	Type of extract	Experimental method	Time of exposure	Cell line	Effects IC_{50} (µg/ml)	References
T. mascatense	HE	ABA	24	MCAS – Ovarian mucinous cystadenocarcinoma	15.24	Said et al. (2017)
				MDA-MB-231 – Breast adenocarcinoma	61.08	
	CH			MCAS – Ovarian mucinous cystadenocarcinoma	> 100	
				MDA-MB-231 – Breast adenocarcinoma	33.80	
	EA AQ			MCAS – Ovarian mucinous cystadenocarcinoma and MDA MB-231 – Breast adenocarcinoma	> 100	
T. mascatense	M	MTT	72	MCF-10A – Normal epithelial cell line	45.83	Panicker et al. (2019)
				MCF-7 – Breast adenocarcinoma	227.00	
				MDA-MB-231 – Breast adenocarcinoma	232.80	
				HeLa – Cervix epithelioid carcinoma	196.40	
T. brevifolium	EO	MTT	48	CACO-2 – Colorectal adenocarcinoma	164	Mencini et al. (2009)
				C32 – Amelanotic melanoma	> 200	
				COR-L23 – Large lung cell carcinoma	80.7	
				RAW246-7 – Murine monocytic macrophage	> 100	
T. oliverianum	E	MTT	24	MCF-7 – Breast adenocarcinoma	> 1000	Ali et al. (2014)
T. orientale	E	MTT	48	PC-3 – Prostate cancer cells	> 300	Asadi-Samani et al. (2018)

(continued)

Table 13.1 (continued)

Teucrium species	Type of extract	Experimental method	Time of exposure	Cell line	Effects IC_{50} (µg/ml)	References
T. yemense				Du-145 – Prostate cancer cell	> 300	
	EO	XTT	72	HT-29 – Colorectal adenocarcinoma	43.7	Ali et al. (2017)
		MTT	48	MCF-7 – Breast adenocarcinoma	24.4	
				MDA-MB-231 – Breast adenocarcinoma	59.9	
T. arduini	M	MTT	72	HeLa – Cervix epithelioid carcinoma	137.51	Stanković et al. (2015)
				Fem-X – Malignant melanoma	> 200	
				K562 – Chronic myelogenous leukemia	113.38	
				MDA-MB-361 – Breast adenocarcinoma	~ 200	
T. arduini	M	MTT	24	HCT-116 – Colorectal carcinoma	114.16	Stanković et al. (2011)
			72		0.37	
T. botrys	M	MTT	72	HeLa – Cervix epithelioid carcinoma	164.23	Stanković et al. (2015)
				Fem-X – Malignant melanoma	~ 200	
				K562 – Chronic myelogenous leukemia	98.78	
				MDA-MB-361 – Breast adenocarcinoma	~ 200	
T. botrys	M	MTT	24	HCT-116 – Colorectal carcinoma	116.38	Stanković et al. (2011)
			72		183.15	
T. flavum	EO	MTT	48	CACO-2 – Colorectal adenocarcinoma	> 200	Mencini et al. (2009)
				C32 – Amelanotic melanoma	> 200	
				COR-L23 – Large lung cell carcinoma	104.2	
				RAW246-7 – Murine monocytic macrophage	> 100	

(continued)

Table 13.1 (continued)

Teucrium species	Type of extract	Experimental method	Time of exposure	Cell line	Effects IC_{50} (µg/ml)	References
T. montberietii	EO	MTT	48	CACO-2 – Colorectal adenocarcinoma	92.2	Mencini et al. (2009)
				C32 – Amelanotic melanoma	135	
				COR-L23 – Large lung cell carcinoma	143	
				RAW246-7 – Murine monocytic macrophage	> 100	
T. scordioides	M	MTT	72	HeLa – Cervix epithelioid carcinoma	139.96	Stanković et al. (2015)
				MDA-MB-361 – Breast adenocarcinoma	196.5	
				Fem-X – Malignant melanoma	> 200	
				K562 – Chronic myelogenous leukemia	96.63	
T. scordium subsp. *scordioides*	M	MTT	24	HCT-116 – Colorectal carcinoma	143.46	Stanković et al. (2011)
			72		72.83	
T. scordium	M	MTT	24	HCT-116 – Colorectal carcinoma	17.04	Stanković et al. (2011)
			72		59.02	
T. scordium	M	MTT	72	HeLa – Cervix epithelioid carcinoma	144.82	Stanković et al. (2015)
				Fem-X – Malignant melanoma	~ 200	
				K562 – Chronic myelogenous leukemia	102.48	
				MDA-MB-361 – Breast adenocarcinoma	~ 200	
T. scordium	CX	KBR	72	MDA-MB-361 – Breast adenocarcinoma	130.33	Kundaković et al. (2011)
	DM				189.89	
	M				> 500	
	CX			MDA-MB-453 – Breast carcinoma	367.28	
	DM				131.01	
	M				371.55	

(continued)

Table 13.1 (continued)

Teucrium species	Type of extract	Experimental method	Time of exposure	Cell line	Effects IC_{50} (µg/ml)	References
T. chamaedrys	M	MTT	72	HeLa – Cervix epithelioid carcinoma	146.47	Stanković et al. (2015)
				Fem-X – Malignant melanoma	190.16	
				K562 – Chronic myelogenous leukemia	102.71	
				MDA-MB-361 – Breast adenocarcinoma	188.28	
T. chamaedrys	M	MTT	24	HCT-116 – Colorectal carcinoma	< 50	Stanković et al. (2011)
			72		190.07	
T. chamaedrys	M	MTT	24	SW480 – Colon adenocarcinoma	53.27	Milutinović et al. (2019)
	EA				126.89	
	A				163.44	
	M		72		20.87	
	EA				63.57	
	A				36.13	
T. montanum	M	MTT	72	HeLa – Cervix epithelioid carcinoma	152.34	Stanković et al. (2015)
				Fem-X – Malignant melanoma	196.44	
				K562 – Chronic myelogenous leukemia	99.15	
				MDA-MB-361 – Breast adenocarcinoma	> 200	
T. montanum	M	MTT	24	HCT-116 – Colorectal carcinoma	< 50	Stanković et al. (2011)
			72		75.73	
T. montanum	M	MTT	24	SW480 – Colon adenocarcinoma	135.79	Nikodijević et al. (2016)
			72		372.69	
			24	MDA-MB-231 – Breast adenocarcinoma	199.32	
			72		174.39	
T. polium	AQ	MTT	72	REYF-1 – Glioblastoma multiforme	> 1000	Eskandary et al. (2007)
	M				95	
T. polium	M	MTT	24	HT-29 – Colorectal adenocarcinoma	4	Khodaei et al. (2018)
			48		3	
			72		6	

(continued)

Table 13.1 (continued)

Teucrium species	Type of extract	Experimental method	Time of exposure	Cell line	Effects IC_{50} (μg/ml)	References
T. polium	M	MTT	72	MCF-7 – Breast adenocarcinoma	118.5	Abu-rish et al. (2016)
				T47D – Ductal epithelial breast tumor	98.5	
				CACO-2 – Colorectal adenocarcinoma	102.2	
				HRT18 – Rectum adenocarcinoma	89.78	
				A375.S2 – Malignant melanoma	61.1	
				WM1361A – Malignant melanoma	99.64	
T. polium	M	MTT	24	HCT-116 – Colorectal carcinoma	77.83	Stanković et al. (2011)
			72		253.39	

(continued)

Table 13.1 (continued)

Teucrium species	Type of extract	Experimental method	Time of exposure	Cell line	Effects IC_{50} (µg/ml)	References
T. polium	90% E	ABA	48	MCF-7 – Breast adenocarcinoma	184	Alzeer et al. (2014)
				B16F10 – Malignant melanoma	803	
				HeLa – Cervix epithelioid carcinoma	420	
	80% M			MCF-7 – Breast adenocarcinoma	104	
				B16F10 – Malignant melanoma	426	
				HeLa – Cervix epithelioid carcinoma	460	
	A			MCF-7 – Breast adenocarcinoma	140	
				B16F10 – Malignant melanoma	129	
				HeLa – Cervix epithelioid carcinoma	173	
	5% AA			MCF-7 – Breast adenocarcinoma	360	
				B16F10 – Malignant melanoma	> 1000	
				HeLa – Cervix epithelioid carcinoma	> 1000	
	AV			MCF-7 – Breast adenocarcinoma	400	
				B16F10 – Malignant melanoma	> 1000	
				HeLa – Cervix epithelioid carcinoma	> 1000	
	GV			MCF-7 – Breast adenocarcinoma	360	
				B16F10 – Malignant melanoma	> 1000	
				HeLa – Cervix epithelioid carcinoma	> 1000	
	AQ			MCF-7 – Breast adenocarcinoma	650	
				B16F10 – Malignant melanoma	> 1000	
				HeLa – Cervix epithelioid carcinoma	> 1000	

(continued)

Table 13.1 (continued)

Teucrium species	Type of extract	Experimental method	Time of exposure	Cell line	Effects IC_{50} (µg/ml)	References
T. polium (seeds)	M	MTT	72	MCF-7 – Breast adenocarcinoma	20.2	El-Naggar et al. (2018)
				HepG-2 – Hepatocellular carcinoma	143.1	
T. polium	M	MTT	24	Skmel -3 – Malignant melanoma	83	Rajabalian (2008)
				SW480 – Colorectal adenocarcinoma	139	
				MCF-7 – Breast adenocarcinoma	174	
				EJ – Bladder carcinoma	108	
				Saos-2 – Osteoblastoma	109	
				A431 – Epidermoid carcinoma	93	
				KB – Oral cavity epidermal cells	174	
T. polium	M	MTT	24	SW480 – Colorectal adenocarcinoma	242.93	Nikodijević et al. (2016)
			72		292.13	
			24	MDA-MB-231 – Breast adenocarcinoma	429.37	
			72		118.26	
T. polium	E	MTT	24	HUVEC – Umbilical vein endothelial cells	98.7	Sheikhbahaei et al. (2018)
			72		~ 100	
T. polium spp. *capitatum*	EO	MTT	48	CACO-2 – Colorectal adenocarcinoma	52.7	Menichini et al. (2009)
				C32 – Amelanotic melanoma	91.2	
				COR-L23 – Large lung cell carcinoma	104	
				RAW246-7 – Murine monocytic macrophage	> 100	
T. polium	E	MTT	48	A549 – Lung adenocarcinoma	90	Nematollahi-Mahani et al. (2007)
				BT20 – Breast ductal carcinoma	106	
				MCF-7 – Breast adenocarcinoma	140	
				PC12 – Mouse pheochromocytoma	120	

(continued)

Table 13.1 (continued)

Teucrium species	Type of extract	Experimental method	Time of exposure	Cell line	Effects IC_{50} (μg/ml)	References
T. polium	M	MTT	72	HeLa – Cervix epithelioid carcinoma	148.02	Stanković et al. (2015)
				Fem-X – Malignant melanoma	199.79	
				K562 – Chronic myelogenous leukemia	116.75	
				MDA-MB-361 – Breast adenocarcinoma	~ 200	

MTT cell viability assay, *TBS* Trypan Blue Staining, *KBR* Kenacid Blue R assay, *ABA* Alamar Blue assay, *M* methanolic, *E* ethanolic, *A* acetone, *AQ* aqueous, *HE* hexane, *CH* chloroform, *EA* ethyl-acetate, *TOF* total flavonoid enriched extract, *AA* acetic acid, *AV* apple vinegar, *GV* grape vinegar, *CX* cyclohexane extract, *DM* dichloromethane extract, *EO* essential oil

cancer cell lines, like (*E*)-caryophyllene, *α*-humulene and *α*-cadinol on MCF-7, HT-29 and other cell lines (Jun et al. 2011; Sylvestre et al. 2007). Additionally, many other compounds were detected in *Teucrium yamense* essential oil, such as *α*-pinene, *β*-pinene, linalool, 3-octanol, *α*-cadinol, caryophyllene oxide, 8-cedren-13-ol, and (*E*)-*β*-farnesene (Ali et al. 2017).

The criteria of cytotoxic activity for crude extracts was recommended by NCI (Suffnes and Pezuto 1990). According this criterion, the extract which inhibited 50% of cell growth, in the concentration less than or approximately 30 μg/ml was considered highly cytotoxic and presented promising anticancer potential. The results obtained by many authors (Table 13.1) indicated that some of the observed IC_{50} values for the most investigated *Teucrium polium* on MCF-7 cells (El-Naggar et al. 2018) and HT-29 cells (Khodaei et al. 2018) were very close to this criterion. Among other *Teucrium* species, a significant cytotoxic activity was observed for *Teucrium yamense* essential oil on breast MCF-7 and HT-29 colon cancer cells (Ali et al. 2017), *Teucrium arduini*, *T. chamaedrys*, *T. scordium* and *T. montanum* on HCT-116 cells (Stanković et al. 2011), *Teucrium chamaedrys* on SW480 cells (Milutinović et al. 2019) and *Teucrium mascateus* on MCF-10A cells (Panicker et al. 2019). Variety of human tumor cell lines obtained from different origins have been selected for investigation of anticancer activity of *Teucrium* species. The presented IC_{50} values indicate that *Teucrium* species induced higher or lower cytotoxicity, depending on the origin and nature of cancer cell lines. A significant cytotoxic activity has been noticed in cells of colorectal carcinomas, thus indicating that *Teucrium* species show organ-specific activity, which is in correlation with traditional use of this plant in the treatment of digestive disorders.

In addition to the results presented in Table 13.1, some other studies indicate significant inhibition of cancer cells growth induced by *Teucrium* species, where IC_{50} values were not calculated. So, aqueous extract of *Teucrium polium* inhibited growth of hepatocellular carcinoma Hep2 cells, (Abdulrazzaq 2017), and lung cancer cells H322 and A549, 48 h after treatment (Haidara et al. 2011). Also, the methanol extract of *Teucrium polium* inhibited growth of human leukemia cell lines MOLT-4 and JMV-13 and chronic lymphocytic leukemia CLC, mainly in concentrations higher than 100 μg/ml, 24 and 48 h after treatment (Zarić et al. 2019). Dominant secondary metabolites, as the bioactive compounds in *Teucrium polium* were flavonoids like rutin and apigenin and neo-clerodane diterpenoids (Piozzi et al. 1997; Kadifkova et al. 2005). Chemically identified and isolated saponins were also present and showed cytotoxic activity on several cell lines (Elmasri et al. 2015). Additionally, many other compounds were identified in *Teucrium polium* extract with potential anticancer properties such as tannins, terpenoids, saponins, flavonoids, sterols, β-caryophyllene, monoterpenes, diterpenoids and many others (Hassan et al. 1979; Vokou and Bessiere 1985; Khazaei et al. 2018). *Teucrium polium* had the high level of phenolics, including flavonoids, anthocyanins and saponins which were involved in cytotoxic activity on MCF-7 and HepG-2 cells (El-Naggar et al. 2018). *Teucrium sandrasicum* was reported to inhibit cell proliferation of HeLa, MCF-7, and L929 cells (Tarhan et al. 2016). Hydrophobic and hydrophilic fraction of essential oils from *Teucrium alopecurus* inhibited cell proliferation of multiple myeloma U266 cells, pancreatic carcinoma Panc28, chronic myelogenous leukemia KBM-5, tonque squamosus carcinoma SCC4, breast cancer MCF-7 and colorectal cancer HCT-116 cells, 1, 3 and 5 days after treatment. Breast cancer MCF-7 cells and Panc28 cells were the most sensitive, while treatments induced moderate sensitivity over KBM-5 and HCT-116 cells (Guesmi et al. 2018). Chemical composition of essential oil from *Teucrium alopecurus* was evaluated, which confirmed that sesquiterpenes were detected in predominant percentages, like δ-cadinene, α-humulene, nerolidyl acetate and others (Hachicha et al. 2007). The cytotoxic activity correlated with the presence of these sesquiterpene compounds, which inhibited proliferation of several cell lines (El-Hadri et al. 2010; Sharma et al. 2013).

The potential of *Teucrium* species to inhibit cancer cell growth was examined with additional methods. Thus, authors report that methanol extract from *Teucrium polium* reduces colony forming efficacy in BT20, A549, MCF-7 and PC12 cell lines (Nematollahi-Mahani et al. 2007) and clonogenic potential of HCT-116 and KBM cells, 9 days after treatment (Guesmi et al. 2018). The same extract induced cell growth inhibition and decreased colony formation in Saos-2, Skmel-3 and EJ cell lines (Rajabalian 2008). The inhibition of REYF-1 cell growth, treated by *Teucrium polium* methanol extract was evaluated by soft agar clonogenic assay. The study reported its clonogenic efficiency of 70.77 ± 5.27% (Eskandary et al. 2007).

The cell cycle is regulated by control mechanisms which provide the cycle progression in a desirable direction. Cyclins, Cdks and CKIs have an important role in the cell cycle regulation (Fisher et al. 2012). The changes in cyclin levels lead to activation of cyclin-Cdk complex, which affects its own target proteins and induces specific cellular events (Lim and Kaldis 2013). Some of *Teucrium* species achieve antiproliferative effects by deregulation of cell cycle progression, like *Teucrium polium* in lung cancer cell lines H322 and A549. The increased number of treated cells, stained by PI were in Sub-G1 phase of the cell cycle, detected by Flow cytometry analysis, 48 h after treatment (Haidara et al. 2011). Fractions of essential oil from *Teucrium polium* also exert an effect on the *c-myc* gene with role in cell cycle progression. Their expression was reduced in treated HCT-116, MiaPaca-2, Panc28, KBM-5, SCC4, MCF-7, U266, HL-60, RAW 264.7 cells compared to nontreated controls, thus leading to growth arrest (Guesmi et al. 2018). The essential oil from *Teucrium alopecurus* inhibited cell cycle progression in G2/M phase after 24 h. The identified compound from EO called thymol was reported to induce cell cycle arrest at the G0/g1 phase (Yin et al. 2012). In HCT-116 cells treated by hydrophobic and hydrophilic fractions of *Teucrium alopecurus* Cyclin D1 and *p21*, cell cycle regulator protein decreased, thus contributing to reduced cell proliferation (Guesmi et al. 2018). These results showed that fractions of *Teucrium alopecurus* inhibited STAT-3 phosphorylation and induced repression of STAT-3 activation as regulator of diverse cellular processes, whose inhibition led to reduced proliferation and induction of apoptosis (Sherry et al. 2009).

According to the presented IC_{50} values, time dependence was not always observed. In some cases, the extension of exposure time did not show the increased activity. On the contrary, after longer time cells recovered and adapted to treatments. This was noticed in *Teucrium polium* treatment of HT-29 cells, where the IC_{50} value for 72 h was higher compared to 24 and 48 h (Khodaei et al. 2018), and in HCT-116 cells (Stanković et al. 2011), SW480 cells (Nikodijević et al. 2016) and HUVEC (Sheikhbahaei et al. 2018), after 72 compared to 24 h. Similarly, the time dependence for cytotoxicity was not observed for *Teucrium bortys*, *T. scordium*, *T. chamaedrys* on HCT-116, and for *Teucrium montanum* in HCT-116 and SW480 cells (Stanković et al. 2011; Nikodijević et al. 2016).

Some data indicate cell selectivity of *Teucrium* species, apropos their cytotoxic activity on cancer cells, while they did not influence normal cells as the healthy control. *Teucrium mascatense* was not cytotoxic on normal fibroblasts (Said et al. 2017), *Teucrium alopecurus* on HUVECs cells (Guesmi et al. 2018), *Teucrium polium* on peripheral blood mononuclear cells (PBMCs) isolated from 16 healthy peoples (Zarić et al. 2019), *Teucrium persicum* on adherent fibroblasts (Tafrihi et al. 2014) and *Teucrium chamaedrys* on normal HaCaT keratinocytes (Milutinović et al. 2019). This selectivity and absence of effect on normal cells are significant results for further evaluation of these plants related to anticancer activity.

Differences in effects are observed for various extracts depending on the solvent used in extraction procedure. Dissolving of natural compounds with anticancer activities depends on their structure, therefore the selection of solvent is the first step in natural product processing. Methanol and ethanol extracts, able to extract polar and non-polar compounds were mainly investigated. Many authors reported that methanol was the most potent solvent for extraction of secondary metabolites with anticancer activity (Dai and Mumper 2010; Gupta et al. 2012; Mitrović et al. 2011; Milutinović et al. 2019). In addition, hot and cold water, acetone and ethylacetate extract were also commonly used solvents. Lipophilic compounds were most often extracted by petrol and chloroform. Healthy safe apple and grape vinegar of *Teucrium polium* were investigated as well. In the study of Alzeer et al. (2014), acetone was the most potent organic solvent for extraction of secondary metabolites from *Teucrium polium* with antiproliferative activity on MCF-7, B16F10 and Hela cell lines, while the coconut water was not recommended for natural compound extraction.

13.2.2 Proapoptotic Activity

Apoptosis is natural highly regulated cell mechanism of programmed cell death, essential for development and maintaining of tissue homeostasis. Different conditions, extra- and intra-cellular signals may induce apoptosis. Besides physiological condition, apoptosis is involved in molecular basis of many apoptosis-related diseases, such as cancer (Kroemer et al. 2007; Fuchs and Steller 2011). The dysregulation and reduced rate of apoptosis contribute to pathogenesis of cancer (Elmore 2007; Fulda 2009). In addition, apoptosis is relevant targeting mechanism in anticancer treatment. Targeting of apoptosis is effective by modulation of its signaling pathways, especially since cancer cells develop multiple mechanisms to avoid apoptosis. The direct targeting of apoptosis could offer new possibilities for improvement of cancer therapy (Pfeffer and Singh 2018).

Among the investigated anticancer properties of natural compounds, the potential to modulate apoptosis is one of the most significant, as a desirable way of induced cytotoxicity in cancer cells. Many studies suggest that secondary metabolites delivered from plants, separately or in combination with chemotherapeutic drugs promote apoptosis in different cell lines (Milutinović et al. 2015a; Wang et al. 2018). Their ability to interfere and regulate key-proteins on gene and/or protein level provide novel opportunities for development of cancer drugs (Hassan et al. 2014; Wang et al. 2018).

Apoptosis is characterized by common, well-known morphological changes and biochemical events (Fig. 13.2). These characteristics, like DNK fragmentation and condensation, membrane blebbing, loss of adhesion to extracellular matrix, size reduction, and activation of the most important caspases have been used for apoptosis detection (Kalinichenko and Matveeva 2008; Filipović et al. 2014).

There are two major pathways for apoptosis induction, the receptor-mediated (extrinsic) and the mitochondrial-mediated (intrinsic) pathways. Both ways result in activation of protein family of enzymes, named caspases, which are initiator or effector molecules in apoptosis signaling (Green and Llambi 2015; Pfeffer and Singh 2018). The involvement of the death responsible receptors, such as FAS and TNF receptor superfamily, include formation of the protein complex named DISC, with appropriate adapter proteins resulting from activation of caspase-8 and/or -10. Once-activated caspase-8 down-regulates cascade of caspases, including effector caspase-3, or alternatively, cleaves Bid into tBid (Pobezinskaya and Liu 2012). Bid is the protein of the Bcl-2 protein family, which is translocated as tBid to mitochondria after cleavage by caspase-8, where it stimulates permeabilization of mitochondrial membrane (Kroemer et al. 2007). The intrinsic pathway includes mitochondria

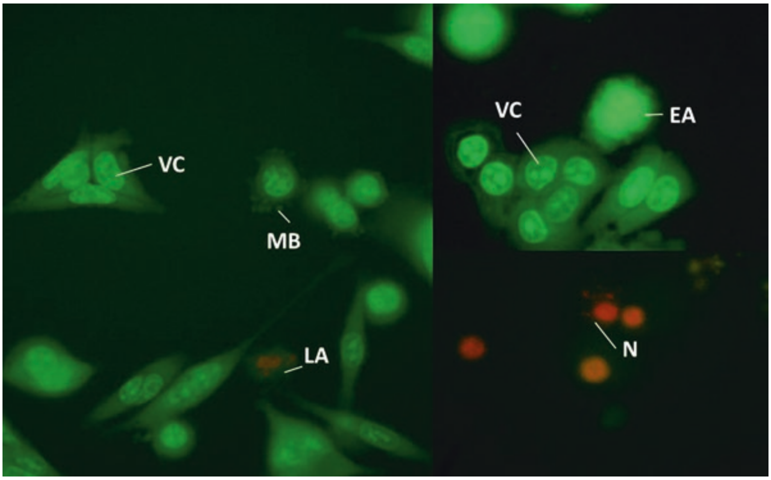

Fig. 13.2 Morphological features of viable, apoptotic and necrotic cells detected by AO/EB assay. *VC* viable cells, *EA* early apoptosis, *MB* membrane blebbing, *LA* late apoptosis, *N* necrosis

and mitochondrial proteins. It is regulated by Bcl-2 family of proteins, which controls and modulates the release of apoptotic factors. There are different structures and functionally distinct proteins within the family, divided into anti-apoptotic (Bcl-2, Bcl-xL, Bcl-W; MCL-1) and pro-apoptotic (Bax, Bak, Bim, Bid, Puma and Noxa) members (Yip and Reed 2008; Hata et al. 2015). They regulate permeability of outer mitochondrial membrane, releasing cytochrome *c* into the cytosol, formation of the complex named apoptosome which causes the activation of the initiator caspase-9, followed by activation of effector caspase-3 (Elmore 2007). The crucial event may be also releasing of Smac/DIABLO, with ability for bind to endogenous XIAPs (Lopez and Tait 2015). Contrary to apoptosis as regulated and controlled cell death, necrosis is passive, uncontrolled process which includes the release of inflammatory cellular substances, inflammation and effects on the surrounding cells (Fink and Cookson 2005).

13.2.3 **Teucrium-***Targeting Molecules and Key Proteins Involved in Apoptosis Process*

Some mechanisms related to how apoptosis pathways are targeted by extracts of *Teucrium* species and how apoptosis can be utilized for cancer therapy are presented. There are many extracts from *Teucrium* species or their secondary metabolites that target various molecules in both intrinsic and extrinsic pathways (Nikodijević et al. 2016; Milutinović et al. 2019; Panicker et al. 2019; Zarić et al. 2019). A significant strategy for cancer treatment is increase and stimulation of proapoptotic and/or reduction and inhibition of antiapoptotic molecules (Wang et al. 2018). There are many surveys on proapoptotic activity of extracts from *Teucrium* species detected by different methods, based on staining, fluorescence observation, morphology, DNK labeling, etc. (Rajabalian 2008; Stanković et al. 2011; Sheikhbahaei et al. 2018). The changes on the membrane of apoptotic cells include exposition of phosphatidylserine on the cell surface during apoptosis, aimed at recognition by phagocytes. This may be detectable by Annexin V/PI staining on Flow cytometer, as has been detected in *Teucrium polium* treated HT-29 cells compared to nontreated control (Khodaei et al. 2018). The induced cell death was due to increased mitochondrial membrane permeability. The extract of *Teucrium polium* also induces proapoptotic activity in human lung cancer H322 and A549 cell lines (Haidara et al. 2011). According to morphological observation by DAPI staining, it was observed that *Teucrium polium* methanol extract induced apoptosis in Skmel-3, Saos-2, MCF-7, A431, SW480 and EJ cells (Rajabalian 2008). Methanol extracts of *Teucrium chamaedrys, T. montanum*, *T. scordium*. *T. scordium* spp. *scordioides*, *T. botrys* and *T. arduini* from Serbian flora predominantly induced proapoptotic activity, according to their cytotoxic effects on HCT-116 cells and dose and time dependence (Stanković et al. 2011). Treatments with extracts of these *Teucrium* species caused morphological changes characteristic for early and late apoptosis, observed by AO/EB method. Methanol, ethyl-acetate and acetone extracts from *Teucrium chamaedrys* also induce apoptosis in SW480 cells 24 and 72 h after treatment, detected by AO/EB methods (Milutinović et al. 2019). In this study apoptosis was dominantly induced via activation of Fas receptors and caspase-8 in the receptor-mediated pathway, but increased caspase-9 activity indicate some role and mitochondria in induced apoptosis. Apoptotic morphological changes and increased number of early and late apoptosis were observed in the treatment on HUVECs cells by *Teucrium polium* extract, detected by AO/EB assay after 72 h, which was achieved by increased proapoptotic *Bax* and decreased *Bcl-2* gene expression (Sheikhbahaei et al. 2018). *Teucrium persicum* achieved the antiproliferative activity on SW480 and T47D cells by induction of apoptosis and detected by Flow cytometry and DNK fragmentation experiment (Tafrihi et al. 2014). One of the main events in apoptosis was activation of various caspases and downstream effector protein PARP (Ba and Garg 2011; Milutinović et al. 2015a). The extracts from *Teucrium montanum* and *T. polium* showed a significant proapoptotic activity in SW480 and MDA-MB-231 (Nikodijević et al. 2016). In this study, apoptosis was induced via activation of extinct pathway, by increased Fas receptor expression and caspase-8 activity and increased caspase-9 activity, thus suggesting an inclusion of mitochondrial pathway in MDA-MB-231 cells. The death of SW480 cells occurred in caspase-independent way. Panicker et al. (2019) reported that dichloromethane fraction of *Teucrium mascatense* induced caspase dependent apoptosis in MCF-7 cells by cleaved caspase-7 and PARP protein, as well as activation of caspase-8 and -9. This treatment induced mainly early apoptosis 24 h after treatment. Cytotoxic activity of *Teucrium polium* methanol extracts was induced by apoptosis in CLL lymphocytes isolated from 28 patients (Zarić et al. 2019). This extract induced Bax translocation and decreased the level of cellular Bcl-2 protein, where the ratio of Bax:Bcl-2 proteins was in favor of Bax that caused release of cytochrome *c* to cytosol and activation of caspase-3. Tarhan et al. (2016) also reported that hydro-ethanolic and ethyl-acetate extract of *Teucrium sandrasicum* induced apoptosis in HeLa and MCF-7 cells, by affecting mitochondrial membrane permeability and activating caspase-9, while only ethyl-acetate extract activated caspase-3 in HeLa cells.

Some studies report the necrotic activity of *Teucrium* species. This may be related to the results obtained about hepatotoxicity of some constituents of *Teucrium* (Chitturi and Farrell 2008; Lin et al. 2009). Hepatotoxicity of *Teucrium chamaedrys* was investigated and discussed in many studies (Forouzandeh et al. 2013; Nencini et al. 2014). *Teucrium polium* also induced hepatotoxicity in *Swiss albino* mice after prolonged herb administration (Krache et al. 2015). Several neo-clerodanes diterpenes were isolated from leaf extracts of *Teucrium polium* and evaluated for their hepatotoxicity, for which low toxicity was confirmed to hepatoblastoma cancer HepG2 cells (Fiorentino et al. 2011). Thus *Teucrium ramosissimum* extract inhibited K562 cells proliferation, but DNA ladder profile suggested that cell death was not caused by apoptotic mechanism (Sghaier et al. 2012). Other authors reported that extract of *Teucrium polium* induced necrosis by increased ROS production. High amount of ROS induced necrosis of HT-29 cells, through instinct pathway and modifications of Sirt3 as an important member of Sirtuin family of mitochondrial regulatory proteins (Khodaei et al. 2018). The impact on mitochondria and induction of oxidative stress led to cell death in cells of colorectal cancer HT-29, mainly by necrosis, too.

The greatest number of studies report that apoptosis was mainly induced by polyphenols (Sharma et al. 2018). However, antitumor properties of essential oils and their bioactive constituents were well examined in many repots (Blowman et al. 2018). Essential oil from *Teucrium alopecurus* was also investigated for its pro-apoptotic potential (Guesmi et al. 2018). It induced apoptosis in a time- and concentration dependence in HCT-116, U266, SCC4, Panc28, KBM5 and MCF-7 cells. It was shown that essential oil from *Teucrium alopecurus* triggered the increase of cleaved PARP expression, activation of initiator caspases-8 and -9, and consequently caspase-3 in HCT-116 cells. Downstream expression levels of Bcl-2 genes were also observed.

13.2.4 Impact on Redox Status in Cancer Cells

Oxygen and nitrogen derived species such as superoxide radical, singlet oxygen, hydrogen peroxide, hydroxyl radical, nitric oxide, peroxynitrite, and others have been related to the etiology of many human diseases, including cancer (Waris and Ashan 2006). Overgeneration of ROS and RNS in cells induce oxidative and nitrosative stress, often with modifications of biomolecules – proteins, lipids, DNA and RNA as the consequence. Their damages can lead to activation and changes in the key proteins necessary for initiation and progression of carcinogenesis. Overexpressed levels of ROS are associated with various human cancers (England

and Cotter 2005; Gibellini et al. 2010). Redox regulation has an impact on basic cellular processes such as proliferation, regulation of various signaling pathways in the cell and many transcription factors, apoptosis, ageing, etc. (Mates et al. 2008; Gibellini et al. 2010). It is very important to understand and examine the ROS and RNS as important mediators in the cancer cells, which offer various opportunities for pharmacological intervention (Waris and Ashan 2006).

There are numerous attempts, experiments *in vitro* and *in vivo* to reduce ROS and RNS generation by exogenous antioxidants (Marković et al. 2011; Di Meo et al. 2016). Plants are the most studied and accessible source of exogenous antioxidants, rich with numerous secondary metabolites with antioxidant properties (Kasote et al. 2015). Phenol antioxidants directly reduce reactive ROS and RNS intermediaries, and in this way, they may stop the chain of oxidative propagation via their scavenging. The antioxidative activity of plant phenolics depend of their structure, number and position of substituents (hydroxyl and methoxy groups). Some natural antioxidants act as prooxidants in certain conditions, such as in presence of transition metals (Sakihama et al. 2002; Ullah et al. 2013; Ćurčić et al. 2014). Prooxidative and antioxidative potential also depends on oxidative potential inside the target cell. Cancer cells contains higher level of endogenous oxidative stress and changes oxidative status compared to normal cell. Thus, cancer cells can be more sensitive to phenolics in comparison to normal cells, which leads to additional increase in production of reactive oxygen intermediaries and induction of apoptosis. So, the phenolics-induced ROS generation contribute to their cytotoxicity and proapoptotic activity (Milutinović et al. 2015a). The role of natural polyphenol in cancer therapies is widely and intensively discussed in the current literature data (Mileo and Micadei 2015).

Teucrium species consist of many secondary metabolites with antioxidative activity (Alwahsh et al. 2015). Ardestani et al. (2008) showed that *Teucrium polium* processed antioxidative activity via presented quercetin in the extract that protected lipid and proteins from oxidation in pancreatic tissue in the rats. *Teucrium* species *in vitro* condition, measured by DPPH and many other assays show antioxidative activity (Vlase et al. 2014; Golfakhrabadi et al. 2015; Özer et al. 2018). However, in cancer cell lines they may behave and act differently. Beside the ability to scavenge free radicals and enhance antioxidative defenses by stimulation of synthesis and activity of antioxidative enzymes, some constituents from the *Teucrium* species can stimulate ROS production. In this way, stimulated oxidative stress in the cancer cells showed to be effective for their removal (Vallejo et al. 2017). Measured by Flow cytometry, the oily fraction of *Teucrium alopecurus* stimulated ROS generation in HCT-116 colon cancer cells, while its hydrophilic fraction induced nonsignificant amount of ROS (Guesmi et al. 2018). Khodaei et al. (2018) showed that *Teucrium polium* extracts increased ROS levels in HT-29 cells 6 and 12 h after the treatment. This hyperproduction of ROS was responsible for anticancer properties via mitochondria alternation, increased Sirt3 activity that correlated with ROS overproduction and induction of necrosis. The other authors suggested that plant extracts, including the *Teucrium* species induced oxidative stress by enhanced ROS production, followed by loss of cell function and induction of apoptosis, mainly by mediated mitochondrial permeability and cytochrome *c* release associated with caspase activation (Stanković et al. 2011; Milutinović et al. 2015a; Zhang et al. 2016). It seems that the concentration of produced ROS is responsible for cell choice, i.e. whether the cells will die with apoptosis or necrosis. The methanol extract of *Teucrium chamaedrys* induced apoptosis partially by increasing of $O^{2.-}$ level and activation of caspase 9 in SW480 cells (Milutinović et al. 2019). In HCT-116 cells seven different species *Teucrium polium*, *T. chamaedrys*, *T. montanum*, *T. scordium*, *T. scordium* spp. *scordioides*, *T. arduini* and *T. botrys*, also induced prooxidant effects and increased $O^{2.-}$ level, which was probably responsible for their observed proapoptotic activity (Stanković et al. 2011). Higher production of $O^{2.-}$ was observed after 24 h in comparison to longer exposure time (72 h), when some of them acted as antioxidants, which correlated with cell recovery, adaptation to treatments and lower cytotoxity of extracts. Similarly, Živanović et al. (2016) reported that *Teucrium polium* and *T. montanum* intensified $O^{2.-}$ concentrations in treated SW480 colon and MDA-MB-231 breast cancer cells, thus indicating prooxidant effects of these species after 24 h; after 72 h they induced antioxidant activity in some concentrations. The reason for observed activities may lie in chemical structure and properties of *Teucrium* constituents, which reduce and/or oxidize substrates depending on the environmental conditions, the cell endogenous protective system, and adaptation to oxidative stress. The level of NO, as an important cell signaling molecule involved in many processes was also examined in some studies. Thus, literature data shows that extract of *Teucrium polium* significantly reduced NO production in HUVECs (Sheikhbahaei et al. 2018), as well as *Teucrium polium* and *T. montanum* in SW480 and MDA-MB-231 cells (Živanović et al. 2016) and several *Teucrium* species in HT-116 cells (Stanković et al. 2011). The reports of other authors confirmed the investigated effects of plant extracts on NO in cancer cell lines; they noted that many plant phenolics had scavenger activity for RNS such as NO and peroxynitrites (Ćurčić et al. 2014; Jeong and Jeong 2010; Cvetković et al. 2019). These findings indicate that plant constituents, associated with endogenous generation of NO may be related to synthesis by reduced or increased expression of iNOS (Ćurčić et al. 2014; Cvetković et al. 2019). The effects of *Teucrium* species on redox status in cancer cells require examination of antioxidant defense system, as the protective system that may be insufficient or attenuated in cancer cells. Živanović et al. (2016) reported that GSH, as the major component of these systems decreased in SW480 cells treated by *Teucrium polium* and *T. montanum*, as the consequence of induced oxidative stress. On the contrary, *Teucrium polium* increased the level of reduced GSH in HepG2 cells, as well as glutathione peroxidase and glutathione reductase activities (Shtukmaster et al. 2010). The results of these authors indicate that hepatoprotective activity of *Teucrium polium* was due to enhanced intracellular GSH level. Also, *Teucrium polium* ethyl-acetate extract exhibited preventive and antioxidative properties in K562 erythroleukemic cells, in which oxidative stress was pre-induced by 2-deoxy-d-ribose (Yazdanparast and Ardestani 2009). This antioxidative activity was mainly due to enhanced GSH content and its increased synthesis by γ-GCS.

13.3 Antiinvasive, Antiangiogenic and Antimigratory Potential

Metastatic potential is the ability of malignant cells to separate from primary cancer site, invade through ECM of surrounding tissues, blood and lymphoid vessels and migrate to other tissues and organs, thus forming secondary cancers (Friedl and Wolf 2010; Gupton and Gertler 2007). These features of cancer cells are one of the main problems in cancer therapy (Friedl and Wolf 2010). Invasive potential is the ability of cancer cells to have an effect on basement membrane and modulate the ECM. The initial transformation of malignant cells shows changes in shape and polarity of cells that acquire invasive phenotype. The changes occur through reorganization of cell cytoskeleton and impaired extracellular bounding and adherence. *β*-Catenin is a component of cell adherens junction, whereby its intracellular and intranuclear localization promotes cancer initiation and progression (Pećina-Šlaus 2003). *E*-Cadherin is one of the proteins required for the cell adherents and connectivity into the epithelial tissues (Jeanes et al. 2008). One of crucial events and initial steps of invasion and metastasis is the destruction of *E*-Cadherin/*β*-Catechin complex, which takes part in the important process called EMT (Fan et al. 2012; Tafrihi and Nakhaei Sistani 2017). The downregulation of *E*-Cadherin results in loss of epithelial integrity, leading to EMT. In cancer patients, the therapy takes the direction of reverse EMT. The cells with invasive potential have the ability to overproduce proteolytic enzymes – MMPs which degrade basement membrane and ECM and thus facilitate the penetration of malignant cells into the surrounding tissues (Gupton and Gertler 2007; Friedl and Wolf 2010; Munson et al. 2013; Weinberg 2014).

Angiogenesis, that creates new from the pre-existing blood vessels, has the important role in cancer progression. In poorly differentiated cancers the vascular network cannot support intensive cell proliferation. Thus "*angiogenic switch*" is initiated in the cancer due to rapid reduction of pO_2, lack of nutrients and other factors, after which numerous factors induce production of chemotaxis and movement of endothelial cells towards the tumor mass (Folkman 2007). In hypoxic condition *HIF-1α* is stabilized; it activates expression of genes with role in angiogenesis. The increase of VEGF expression leads to increased vascular permeability, while FGF allows growth of endothelial cells. HIF-1*α*, together with VEGF, thus inducing increased expression of MMPs, especially MMP-9 (van Hinsbergh and Koolwijk 2008; Cvetković et al. 2017). The factors that are released in microenvironment activate tumor-associated macrophages which produce angiogenic factors such as VEGF and MMPs and additionally contribute to angiogenesis (Guo et al. 2013). Under the influence of VEGF, stromal fibroblasts in tumor tissues produce CXCL-12, bonded to CXCR-4 and other cancer cell receptors, which initiate formation of the new blood vessels and mobilization of progenitor cells from bone marrow (Zheng et al. 2007; Chavakis et al. 2008).

Plant-derived products have been widely examined as antiinvasive, antiangiogenic and antimigratory agents, and great number of strategies was devised aimed at the prevention and treatment of cancer (Šeklić et al. 2016; Lu et sar. 2016; Cvetković et al. 2019). The reason of their therapeutic potential lies in their ability to modulate these processes through their impact on different molecular signaling, limited toxicity and availability (Klongkumnuankarn et al. 2015). Since the cancer invasion and metastases lead to death of cancer patients, drugs with antiinvasive, antiangiogenic and antimigratory potential are significantly challenging for scientific research. In this regard some of *Teucrium* species were evaluated.

The results of wound healing assay, which measures the movement of cell population, indicate the antiinvasive activity of *Teucrium persicum*, where the extract induces decrease in wound healing PC-3 cell sheets. The gelatin zymography assay also indicates its antiinvasive potential due to the reduced level of MMP-2 and -9 for approximately 50–60% in PC-3 cells, measured 48 h after treatment (Tafrihi et al. 2014). The extract of *Teucrium persicum* enhanced *E*-Cadherin and *β*-Catenin expression and membrane localization, thus inducing the formation of different phenotype and reverse EMT in less than 20% of the treated PC-3 cell population. *Teucrium persicum* changed morphology of invasive and poorly differentiated PC-3 cells, which lost their mesenchymal phenotype under the treatment and transformed into mesenchymal epithelial phenotype, with closely attached epithelial-like cells (Tafrihi et al. 2014). Kandouz et al. (2010), report that the extract from *Teucrium polium* inhibited invasion and migration of prostate PC-3 and DU-145 carcinoma cell lines. It inhibited the invasion and motility of human prostate cancer cells via the restoration of *E*-Cadherin/Catenin complex. Other authors also reported that *Teucrium polium* induced differentiation of epithelial phenotype, EMC, and reduced cell invasion and motility of H322 and A549 lung cell lines (Haidara et al. 2011). The mechanism of these actions included re-localization of *E*-Cadherin and *β*-Catenin phosphorylation due to Src dephosphorylation. Tafrihi and Nakhaei Sistani (2017) reported that *Teucrium persicum* and *T. polium* downregulated MMPs, *E*-Cadherin and inhibited EMT and metastases. *Teucrium polium* showed ability to inhibit micro-vessel formation in concentrations of 50 and 25 μg/ml, on EA.hy926 human endothelial cell line, which suggested its antiinvasive potential (Abdallah et al. 2018). Essential oil from *Teucrium alopecurus* also has an impact on crucial component involved in invasion and metastasis due to reduction of CXCR-4 level, ICAM-1, VCAM-1 and MMP-9 expression in Panc28, Miapaca, and HL60 cancer cells (Guesmi et al. 2018). The VEGF and ICAM-1 are also inhibited in HCT-116 cells (Guesmi et al. 2018).

The examination of antimigratory potential of methanol extract from *Teucrium montanum* on SW480 and MDA-MB-231 cells showed some opposite results considering the used concentrations. The concentration of 1 μg/ml reduced cell migration, while the 10 and 50 μg/ml had the promigratory potential on both cell lines, monitored in the real time on RTCA xCELLigence system (Živanović et al. 2016). In the same study, *Teucrium polium* caused antimigratory effects on both SW480 and MDA-MB-231 cells, in treatment periods longer than 12 h. Sheikhbahaei et al. (2018) also reported that *Teucrium polium* had antimigratory activity on HUVECs cells, measured by wound healing (scratch method).

13.4 *Teucrium* Species in Combined Treatments

Many studies propose combined therapy that includes two or more therapeutic agents. There are attempts for application of chemo-therapeutics together with plant extracts or plant-derived compounds. Some secondary metabolites from plants can reduce side effects of chemotherapy and radiotherapy, when applied in cotreatment (Chung et al. 2004; Mokhtari et al. 2017; Zhang et al. 2018). A combined therapy may achieve many benefits compared to administration of drugs alone. Primarily, it can increase cytotoxicity by synergistic or additive interactions between combined drugs. A higher cell sensitivity in combined treatments is also observed, as well as enhanced efficacy or more potent cytotoxicity, due to targeting on different pathways, induction of apoptosis rather than necrosis, reduction of cancer cell drug resistance, etc. (Khdair et al. 2010; Ćurčić et al. 2012b; Milutinović et al. 2015a). One of the main benefits is prevention of healthy cells from toxic effects (Blagosklonny 2005). There are some statistical tests and calculations of interactions, such as *CalcuSyn*, combination index analysis, *CI index* (Rajabalian 2008; Milutinović et al. 2015a).

Among *Teucrium* species only the *Teucrium polium* was evaluated for anticancer testing in combined therapy. Sheikhbahaei et al. (2018) evaluated the effects of ethanol extract of *Teucrium polium* in combination with *tranilast* on HUVECs cells. The results showed synergistically enhanced cytotoxic activity in combined treatment, as well as increased apoptosis due to changed ratio between Bax and Bcl-2 gene expression, observed 72 h after treatment. This combined treatment also showed the ability to reduce NO concentration, as well as antimigratory potential of HUVECs cells (Sheikhbahaei et al. 2018). Other authors also investigated *Teucrium polium* in combination with other drugs (Rajbalian 2008). The combined treatments of *Teucrium polium* with *vinblastine*, *vincristine* and *doxorubicine* also induced increased proapoptotic activity compared to single treatments on MCF-7, A431, SW480, Skmel3, KB and EJ cell lines, without synergistic effects on normal fibroblasts. The calculated CI index indicated synergistic interaction of these drugs with methanolic extract of *Teucrium polium*.

13.5 *In Vivo* Anticancer Evaluation of *Teucrium* Species

Although *Teucrium* species, as medicinal plants are widely used for therapy of different disorders and extensively investigated in preclinical *in vitro* experiments, there are insufficient data about their anticancer properties based on *in vivo* studies. Movahedi et al. (2014) investigated protective role of *Teucrium polium* on liver cells against hepatocellular carcinoma on animal model system, in which cancerogenesis was induced. According to the investigated biochemical parameters, the results suggest that *Teucrium polium* may have had protective role for liver cells in 40 male rats, 8 ± 1 weeks old. Similar investigation was conducted by Shahat et al. (2016), who reported data about *Teucrium oliverianum* anticancer potential in chemically-induced hepatocellular carcinoma in rats. These results indicate hepatoprotective, antiproliferative activity and antiangiogenic potential of *Teucrium oliverianum*. The antioxidative activity of *Teucrium polium* im adult male Wistar rats was also observed and comparable with α-tocopherol, as positive control for antioxidant activity (Hasani et al. 2007). Considering that increased concentrations of ROS and RNS and oxidative damage are in the basis of almost all kinds of cancers, the antioxidative role of *Teucrium polium* may have a protective effect (Liou and Storz 2010). Guesmi et al. (2018) reported anticancer properties of essential oil from *Teucrium alopecurus in vitro*, but also showed its nontoxic effects and no damage on histological review between treated and nontreated groups of mice, as the experimental model.

13.6 Conclusions

There are reports of many authors about anticancer properties of different species from the genus *Teurium*, that influence multistep cancerogenseis. Various preclinical findings and results of several *in vitro* and *in vivo* studies report preventive and pronounced anticancer properties of different *Teucrium* species, where the most investigated was *Teucrium polium*. In conclusion, after the reviewed literature data on anticancer properties, the genus *Teucrium* is a promising source for new anticancer plant-derived compounds, that exhibit anticancer activity through antiproliferative activity, activation of the apoptotic pathways, modulation of apoptotic key molecules and redox status in the cancer cells, with antiinvasive, antimetastatic and antiangiogenic activities. The most investigated effect was antiproliferative activity on variety of cell lines *in vitro*; there are fewer data about their possible mechanism of action, impact on migration, angiogenesis and invasion. Therefore, more data about these activities on greater number of model systems, especially on *in vivo* models are desirable. Also, given the diversity of the genus *Teucrium*, it is necessary to examine other species of this genus that have not been examined yet. Additional preclinical studies and clinical trials are certainly still required in order to elucidate the full spectrum of anticancer properties of the *Teucrium* species alone, or in synergistic combination with others for their commercial utilization and future perspective in drug design.

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Chapter 14
Anticholinesterase, Antidiabetic and Anti-inflammatory Activity of Secondary Metabolites of *Teucrium* Species

Nenad Zlatić and Milan Stanković

Abstract The chapter reviews the data about anticholinesterase, antidiabetic, and anti-inflammatory activity of the active substances from *Teucrium* species. Extracts and essential oils from the species of *Teucrium* genus possess a wide range of secondary metabolites that exhibit biological activity. Discussed examinations are focused on the determination of the qualitative and quantitative composition of the active substances from *Teucrium* species, as well as the anticholinesterase, antidiabetic and anti-inflammatory activity. Many authors state that Alzheimer's disease, diabetes mellitus, and inflammatory diseases correlate because they are precursors of neurodegenerative diseases. Secondary metabolites isolated from plants of the genus *Teucrium* have been shown to be potential inhibitors of acetylcholinesterase. Also, examined metabolites possess antihyperglycemic activity and reduce blood glucose levels. Extracts of the species from the genus *Teucrium* exhibit anti-inflammatory activity in in vitro and in vivo conditions by inhibiting carrageenan-induced inflammation and significantly reduced serum levels of triglyceride and cholesterol. As a result, it has been shown that the species of the genus *Teucrium* are rich in compounds such as flavonoids, phenolic acids and terpenoids that directly contribute to anticholinesterase, antidiabetic and anti-inflammatory activity. Species of the genus *Teucrium* are good candidates for further examination in order to the treatment of the previously mentioned disorders.

Keywords *Teucrium* species · Acetylcholinesterase · Alzheimer's disease · Diabetes mellitus · Inflammation

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Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
AChI	Acetylcholine iodide
AD	Alzheimer's disease
COX	Cyclooxygenase
DTNB	5,5-dithio-bis-2-nitrobenzoic acid
FDA	Food and drug administration
HLA	Human leukocyte antigen
IC_{50}	Half maximal inhibitory concentration
iNOS	Inducible nitric oxide synthase
LDL	Low density lipoproteins
mRNA	Messenger ribonucleic acid
NO	Nitric oxide
STZ	Single dose streptozotocin
TNB	2-nitro-5-mercaptobenzoic acid

14.1 Introduction

Genus *Teucrium* L. belongs to the family Lamiaceae. Some species belonging to the genus *Teucrium* are known medicinal plants that have wide application. Medicinal species of the genus *Teucrium* are used in the treatment of rheumatism, diabetes, inflammatory and gastrointestinal diseases. The most common groups of secondary metabolites from the *Teucrium* species are flavonoids, phenolic acids, monoterpenes, diterpenes, sesquiterpenes, and others. Due to the specific quantitative and qualitative composition of secondary metabolites, plant species of the genus *Teucrium* exhibit various types of biological activity such as antioxidant, anticancer, anti-inflammatory, antidiabetic, antimicrobial and antiviral activity (Stanković 2012.

This chapter describes the main features of medicinal plants from the genus *Teucrium* on acetylcholinesterase inhibition, diabetes mellitus, and inflammatory processes. Additionally, a brief description of the methods used in the examination of anticholinesterase, antidiabetic and anti-inflammatory activity will be shown.

14.2 Acetylcholinesterase

Acetylcholinesterase (AChE) is serine hydrolysis belonging to the group of enzymes that affect different types of carboxylic esters (Čolović et al. 2013). Acetylcholine is a monomeric molecule consisting of 12 collected beta plates surrounded by 14 alpha helices and possesses a molecular weight of about 60,000. The acetylcholinesterase molecule consists of several essential parts, peripheral anionic site, the catalytically active site and the aromatic throat (aromatic "gorge"). The peripheral anionic site plays an important role in recognizing the conformation of residues in the aromatic throat and in the catalytic site. The catalytic active site consists of an esterase and anion subunit. Esterase subunit is composed of three amino acids (Ser200, His440, and Glu327). The anionic sub spot plays a role in binding the positively charged quaternary amine of the choline group of acetylcholine (Nađpal 2017).

Acetylcholine (Fig. 14.1) is one of the essential neurotransmitters in the brain. In the central nervous system, it is found in interneurons and some cholinergic nerve fibers. Also, acetylcholine has an important role in the processes of memory and learning, as well as the maintenance of consciousness (Sisodia and Tanzi 2006; Topcu and Kusman 2014). In the peripheral nervous system, acetylcholine participates in the transmission of nerve signals in the motor plate and causes muscle contraction, slows down the pulse, enlarges blood vessels, influences the increased secretion of gastric glands, enhances peristaltic intestines (Takeda et al. 2004).

Science has not yet explained in the best way how choline is brought to the nerve endings, while acetyl groups are fed through the choline acetyltransferase enzyme derived from coenzyme A. Acetylcholine is found in presynaptic nerve endings in a state that can be immediately released or store in the form of surplus (Orhan et al. 2007; Čolović et al. 2013). Depending on whether there is a pulse, it remains unclear how acetylcholine is released from presynaptic endings. Although it is considered that calcium plays a major role in the process. Acetylcholine is released from the presynaptic neuron into the synaptic crack during transmission and binds to acetylcholine receptors (muscarinic and nicotinic) in the postsynaptic membrane, transmitting the neuron signal. The acetylcholine degradation enzyme is called acetylcholinesterase (Takeda et al. 2004; Sisodia and Tanzi 2006; Vladimir-Knežević et al. 2014). The acetylcholinesterase enzyme separates the acetylcholine to the choline an acetate group, which prevents the recurrence of muscular irritation. Released choline is transported to the presynaptic neuron and it is used for the resynthesis of acetylcholine by the activity of choline acetyltransferase enzyme (Čolović et al. 2013). Each molecule of acetylcholinesterase enzyme inhibits an average of 25,000 acetylcholine molecules per second, thereby enzyme having an appreciable catalytic activity. The choline formed in the process of decomposition of acetylcholine is recycled to recover acetylcholine. The main form of acetylcholinesterase is found in the brain and muscles. Acetylcholinesterase is encoded by AChE in humans (Čolović et al. 2013; Nađpal 2017).

A neurodegenerative disease such as Alzheimer's disease indicates a condition where neurons of the spinal cord and brain collapse (Mattson 2004). This leads to a loss of muscular coordination and cognitive dysfunction. Alzheimer's disease (AD), first described by neuropsychiatrist Alois Alzheimer in 1906, is a progressive

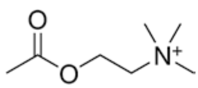

Fig. 14.1 Chemical structure of acetylcholine (ACh)

degeneration, which most often affects the elderly in developed countries (Nađpal 2017). The disease is recognized by the loss of short-term memory and disorientation (Akhondzadeh et al. 2003; Topcu and Kusman 2014). In morphological terms, Alzheimer's disease is characterized by the appearance of D-amyloid deposits and neurofibrillary loops produced by hyperphosphorylated τ-proteins (Adewusi et al. 2011). Neurochemical is characterized by loss of activity of cholinergic neurotransmitters, primarily acetylcholine (ACh) in the cerebral cortex (Orhan et al. 2007).

In people suffering from Alzheimer's disease, choline-acetyltransferase has been significantly reduced in the central nervous system. The loss of choline acetyltransferase is particularly pronounced in brain regions where the areas responsible for memory are located, i.e. in the cortex and hippocampus of the brain. Treatment of people suffering from Alzheimer's disease implied the use of acetylcholine, but such attempts to treat the disease have not shown great success (Nađpal 2017). Today's attempts to treat affected people are aimed at increasing cholinergic transmission by blocking the activity of acetylcholinesterase that degrades acetylcholine in synaptic cracks. Also, there are acetylcholinesterase inhibitors that are used in medicine but with varying degrees of efficacy in treatment (Mattson 2004).

14.2.1 Inhibition of Acetylcholinesterase

Pharmacological inhibitors of acetylcholinesterase are very important in the context of diseases where reduced neurotransmission activity mediates acetylcholine. In a disease such as Alzheimer's, there is a loss of cholinergic neurons in the brain, while some autoimmune diseases of the antibody lead to loss of nicotine acetylcholine receptors in the motor plate (Čolović et al. 2013). Alzheimer's disease is one of the most common forms of dementia, where there is a significant loss of brain mass. Despite numerous findings, it is still not known what causes Alzheimer's disease. It is considered that disease is developed by decreasing holing-acetyltransferase activity and inhibition of acetylcholinesterase enzyme is important for the treatment of the disease. The inhibition of acetylcholine increases its concentration in the synapses, which allows long-term stimulation of the receptors and the control of the disease (Soreq and Seidman 2001).

Depending on the mode of action, acetylcholine inhibitors are divided into irreversible and reversible. Reversible inhibitors are protagonists in the treatment of Alzheimer's disease symptoms. In the treatment of Alzheimer's disease, drugs that compensate for ACh deficiency, including ACh precursors, muscarinic and nicotinic receptor antagonists, and AChE inhibitors (Akhondzadeh et al. 2003). Inhibition of AChE is one of the most widely used treatment models for Alzheimer's disease (Akhondzadeh et al. 2003; Orhan et al. 2007). Some of the synthetic and natural remedies, such as galanthamine (Razadyne®, Reminyl®) donepezil (Aricept®), rivastigmine (Exelon®), and tacrine (Cognex®) are approved by the Food and Drug Administration, FDA) (Topcu and Kusman 2014; Nađpal 2017).

These drugs respond by enhancing the transmission of cholinergic neurons in the frontal region of the brain and compensating the loss of functional brain cells. Additionally, the treatment of Alzheimer's disease by drugs are used in the treatment of symptoms of neurological disorders such as Parkinson's disease, dementia with Levi's bodies and Myasthenia gravis. Irreversible acetylcholine inhibitors have a toxic activity whose function is reflected in the accumulation of acetylcholine in synaptic cracks, resulting in disorders in neurotransmission (Čolović et al. 2013). Synthetic acetylcholinesterase inhibitors exhibit side effects. Side effects are detected in the disorder of the gastrointestinal tract and hepatoxicity. There is still no answer in the science of developing acetylcholine inhibitors that manage in the brain without effect on other organs.

Commercially common inhibitors of acetylcholinesterase enzymes such as donepezil, tacrine, and rivastigmine have a damaging effect on human health, and now trying to find alternative substances of plant origin that will replace synthetic drugs. Tacrine has a short-acting time, unlike other drugs, and tablets should be taken several times a day, although it has been shown that taking this drug causes headache, muscle pain, loss of aptitude, nausea, diarrhea, etc. Rivastigmine and donepezil have similar effects because they are reversible acetylcholinesterase inhibitors, but their effect is reduced after prolonged usage (Martorana et al. 2010; Nađpal 2017).

14.2.2 Plants as Natural Sources of Anticholinesterase Agents

Nowadays, science is focused on the testing of plant extracts of certain species in order to inhibit acetylcholine and treat neurodegenerative diseases (Dastmalchi et al. 2007; Zhao et al. 2013). Galantamine is one of the most effective acetylcholinesterase inhibitors isolated from plant extracts of *Galanthus nivalis*. Galantamine does not exhibit adverse effects such as synthetic inhibitors and is used for clinical purposes. Moreover, other compounds that exhibit significant anti-acetylcholinesterase activity have been isolated (Mukherjee et al. 2007). The α-viniferin is isolated from *Caragana chamlagu* and exhibits significant anti-acetylcholinesterase activity, as well as huperzine-A which isolated from *Huperzia serrata*. Then, physostigmine isolated from the seeds of *Physostigma venenosum* is a reversible acetylcholinesterase enzyme inhibitor. Also, ursolic acid derived from commercial extract (*Origanum majorana*) exhibits significant anti-acetylcholinesterase activity (Mukherjee et al. 2007; Roseiro et al. 2012). *Ginkgo biloba* is used in traditional medicine for the prevention of degenerative brain disorders and helps maintain concentration. The chemical composition of this type of extract includes bilobalide, ginkgolide, and other compounds such as kaempferol and quercetin (Čolović et al. 2013; Nađpal 2017).

In addition to herbal extracts, there are data about using essential oils from herbs that are potent inhibitors of acetylcholinesterase enzymes due to the presence of certain compounds. Many medicinal plants are used in medicine as stimulants for cognitive stimulation, for the treatment of depression and Alzheimer's disease. Plant species that are used in traditional medicine in the treatment of dementia belong to the genus *Salvia*, which is at the same time one of the most numerous genera belonging to the family Lamiaceae (Vladimir-Knežević et al. 2014).

14.2.3 Lamiaceae Species as a Sources of Anticholinesterase Agents

Numerous plant species belonging to different families such as Amaryllidaceae, Lamiaceae, Papaveraceae have been investigated in order to find effective acetylcholinesterase inhibitors. Some plant alkaloids, ursolic and oleic acid have been shown to be good inhibitors of acetylcholinesterase (Topcu and Kusman 2014). Plant species belonging to the Lamiaceae family are known for their medicinal properties because they possess a wide diversity of secondary metabolites that exhibit their pronounced biological activity. The biological activity of plant secondary metabolites is based on their ability to react with many regulatory molecules and other cell and subcellular structures, thereby affecting, either positively or negatively, a large number of metabolic processes. Some Lamiaceae species possess essential oils, flavonoids and hydroxycinnamic acid which are one of the main bioactive compounds. Genera belonging to the Lamiaceae family, such as *Lavandula, Origanum, Mentha, Salvia, Rosmarinus, Thymus, Teucrium*, and *Calamintha* are well known for their neuroprotective effect and usage in traditional medicine in the treatment of many diseases (Topcu and Kusman 2014).

Plants of Lamiaceae family are rich in phenolic acids, primarily rosmarinic and chlorogenic acid, and also include gentisic, caffeine, proto-catheic, vanillic, ferulic and souric acid (*Lavandula, Nepeta, Rosmarinus, Teucrium, Salvia*). Rosmarinic acid is characteristic of the some species from Lamiaceae family (Vladimir-Knežević et al. 2014). Also, they are rich in triterpene derivatives, ursans and oleans, where ursol, betulin and oleic acid are present in the highest percentage (*Salvia, Rosmarinus*) (Jäger et al. 2007). The plants of the Lamiaceae family, as well as other plant species, synthesize flavones and flavonols that, in addition to other secondary metabolites, contribute to the biological activity of these plants (Vladimir-Knežević et al. 2014). The species of the Lamiaceae family were objects of numerous pharmacological studies showing that their extracts and essential oils show a number of biological effects in vitro and in vivo conditions. The plant's essential oils belonging to the family include components such as sesquiterpenes, monoterpenes, diterpenes, aliphatic and aromatic components. The biological activity of extracts and essential oils may be significant in the treatment of neurodegenerative disorders, in particular, Alzheimer's disease (Topcu and Kusman 2014).

Vladimir-Knežević et al. (2014) examined selected Lamiaceae species to the ability to inhibit acetylcholinesterase. In their results, it has been shown that extracts of several plant species such as *Teucrium chameadrys, Thymus vulgaris, Mentha* *piperita, Salvia officinalis, Saturea montana* inhibit over 75% acetylcholinesterase activity. The ethyl alcohol extract of *Salvia trilobacterium* inhibited acetylcholinesterase at 0.71 mg/ml, while the *Melissa officinalis* extract was completely inactive (Orhan and Aslan 2009). Ferreira et al. (2006) examined the extracts and essential oils of ten plants on the ability to inhibit acetylcholinesterase. They noticed that the species *Lavandula pedunculata*, *Mentha suaveolens* and *Melissa officinalis* show the inhibitory effect on acetylcholinesterase among the tested plant species. A high degree of acetylcholinesterase inhibition was observed by examining essential oil of *Mentha, Origanum* and *Saturea* species, while moderate and low inhibition of the activity of acetylcholinesterase enzymes was observed by examining the essential oil of plant species belonging to the genera *Ocimum, Lavandula*, and *Salvia* (Orhan et al. 2008). The results of the antiacetylcholinesterase effect of plant extracts and essential oils isolated from the species of the Lamiaceae family were presented in numerous studies (Orhan et al. 2007; Orhan and Aslan 2009; Topcu and Kusman 2014; Vladimir-Knežević et al. 2014). The studies show that plant species belonging to the Lamiaceae family are a natural source of acetylcholinesterase inhibitors and can be used in the prevention and treatment of neurodegenerative diseases (Vladimir-Knežević et al. 2014).

14.2.4 **Teucrium** *Species as a Sources of Anticholinesterase Agents*

Species of the genus *Teucrium* are known for their medicinal properties. Extracts of *Teucrium* species possess different secondary metabolites that exhibit biological activity. Secondary metabolites isolated from extracts of *Teucrium* species, have been shown to be potential inhibitors of acetylcholinesterase (Orhan and Aslan 2009; Vladimir-Knežević et al. 2014). Vladimir-Knežević et al. (2014) examined the ethanol extracts of *Teucrium* species on the ability to inhibit acetylcholinesterase using Ellman's colorimetric assay. The authors state that the extracts of the tested species such as *Teucrium arduini, T. chamaedrys, T. montanum*, and *T. polium* showed strong inhibitory activity against acetylcholinesterase. The percentage of the inhibition of acetylcholinesterase for the ethanolic extracts of these plants was obtained for tested concentrations of 0.25, 0.50 and 1 mg/ml, respectively. The same authors state that all investigated species of the genus *Teucrium* had a percentage of inhibition of acetylcholinesterase over 80% at 1 mg/ml. Among the investigated species, *Teucrium arduini* exhibits the highest ability to inhibit acetylcholinesterase enzymes over 95% at 1 mg/ml, followed by *Teucrium chamaedrys* that neutralize the enzyme near to 90% at the same concentration. *Teucrium polium* shows similar values as *Teucrium chamaedrys*, 86% at a concentration of 1 mg/ml. Among the tested species, *Teucrium montanum* shows the lowest ability of neutralizing acetylcholinesterase enzymes, about 80% at a concentration of 1 mg/ml. According to the ability to inhibit the enzyme acetylcholinesterase, the investigated plant species have the following order *T. arduini* > *T. chamaedrys* > *T. polium* > *T. montanum* at a concentration of 1 mg/ml. At a concentration of 0.50 mg/ml *Teucrium chamaedrys* and *T. polium* exhibit the highest ability to inhibit acetylcholinesterase enzyme above 60%. *Teucrium arduini* shows slightly less inhibitory capacity than the previous two species, 58%. *Teucrium montanum*, in this case, shows the lowest ability to neutralize acetylcholinesterase enzymes, about 45% at a concentration of 0.50 mg/ml. The order of plants for a concentration of 0.50 mg/ml would be *T. chamaedrys* > *T. polium* > *T. arduini* > *T. montanum*. At a concentration of 0.25 mg/ml *Teucrium chamaedrys* and *T. arduini*, the highest inhibition ability is greater than 35%. *Teucrium montanum* has a higher ability to inhibit acetylcholinesterase enzymes 32% from *Teucrium polium* 28% at a minimum concentration of 0.25 mg/ml. The investigated plant species of the genus *Teucrium* at a concentration of 0.25 mg/ml have the following order *T. chamaedrys* > *T. arduini* > *T. montanum* > *T. polium*.

Golfakhrabadi et al. (2015) investigated the influence of the methanol extract of *Teucrium hyrcanicum* on the inhibition of acetylcholinesterase enzymes at the same time compared to donepezil as a positive control. The results showed that the IC_{50} values for the methanol extract of *Teucrium hyrcanicum* and donepezil were 2.12 mg/ml and 0.013 mg/ml, respectively. At a concentration of 1 mg/ml, the inhibitory activity of the methanol extract of the tested species was 40% (Golfakhrabadi et al. 2015). The anticholinesterase activity was investigated for the *Teucrium royleanum*, using organic solvents of different polarities (Ahmad et al. 2007). The authors state that the greatest ability to neutralize the acetylcholinesterase enzyme is observed for the ethyl acetate fraction, 83.62%. The chloroform fraction with the ability to neutralize the enzyme is 70.5%. The n-butanol fraction showed moderate activity against acetylcholinesterase 59.55%, while crude extract of *Teucrium royleanum* also showed moderate activity against acetylcholinesterase enzymes, 52.4% (Ahmad et al. 2007). A qualitative examination of *Teucrium royleanum* essential oil identified compounds such as sesquiterpene hydrocarbons, *β*-caryophyllene, germacrene D, alpha-humulene and linalool (Mohan et al. 2010).

Preliminary investigations of the inhibitory activity of plant extracts of species belonging to the genus *Teucrium* on the enzyme acetylcholinesterase indicate that the extracts of these plants have a strong or moderate ability to inhibit this enzyme. By examining the composition of *Teucrium* phenolic compounds from the group of phenolic acids, the most common are rosmarinic and chlorogenic acid, as well as from the group of flavonoids the most common are luteolin and apigenin. Experiments showed that rosmarinic acid contributes to the inhibition of acetylcholinesterase enzymes (Vladimir-Knežević et al. 2014). For the species of this genus, the presence of a monoterpene and sesquiterpene is characteristic (Monsef-Esfahani et al. 2010). Plants of genus *Teucrium* are rich in terpene components which are known that possess inhibitory properties of the enzyme acetylcholinesterase (Yoo and Park 2012; Vladimir-Knežević et al. 2014).

14.2.5 Methods for Determination of the Anticholinesterase Activity

One of the most prominent methods present in scientific studies of acetylcholinesterase inhibition is Ellman's method (Ellman et al. 1961; Burčul 2008). It is a spectrophotometric method based on the reaction of Ellman's reagent DTNB (5,5-dithiobis-(2-nitrobenzoic acid)) with a thiol group of substrates formed by the action of acetylcholinesterase. The reaction of DTNB with thiol groups leads to the formation of TNB (2-nitro-5-mercaptobenzoic acid) which is further hydrolyzed in the water at neutral or alkaline pH. Free TNB has a yellow color. The maximum absorption DTNB is at a wavelength $\lambda = 320$ nm, while the amount of TNB is read at a wavelength at $\lambda = 412$ nm. Reaction mixture should consist of: 100 ml acetylcholinesterase concentration 0.3 U/mg dissolved in 0.1 M phosphate buffer at an alkaline pH = 8; then 50 ml DTNB; acetylcholine iodide concentration 0.3 mM dissolved in 0.1 M phosphate buffer at neutral pH = 7; 50 ml sample (inhibitor or alcohol extract) dissolved in methanol; 850 ml 0.1 M phosphate buffer alkaline pH = 8.

In order to determine the possible non-enzymatic hydrolysis of AChI, two reference solutions should be made. The first reference solution instead of AChE was buffered (1000 ml) instead of the ethanol inhibitor (50 ml) and DTNB and AChI (50 ml). The other reference solution contains everything the same as the first reference solution only that AChI is excluded so that it contains buffer (1000 ml), ethanol, AChE, and DTNB (50 ml). Additionally, it is necessary to prepare a control solution where the 50 ml inhibitor is replaced with 50 ml methanol. It is important to note that before the experiment it is necessary to check that methanol or some other organic solvent does not interfere with AChE inhibition. The reference solutions should be incubated at room temperature for a period of 30 min. The reaction begins with the addition of 50 ml AChI as the substrate and after 6 min the absorbance is measured at a wavelength $\lambda = 412$ nm.

14.3 Diabetes Mellitus

Early studies have shown that cerebral cortex and hippocampus neurons are characterized by an inadequate response and decreased secretion of insulin effect, which indicates the emergence of neurodegenerative diseases (Craft 2007; Chiung-Chun et al. 2010). Many authors consider that insulin plays an important role in central energy metabolism and that insulin regulation disorders can influence the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease. Numerous studies have shown a significant correlation between the occurrence of Alzheimer's disease and Type 2 diabetes mellitus (Craft 2007; Sims-Robinson et al. 2010).

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, or high blood glucose levels. The onset of the disease is the result of inadequate blood glucose control by insulin hormones synthesized in the pancreas (Zimmet et al. 2001). Hyperglycemia occurs due to partial or complete insulin deficiency. The onset of hyperglycemia affects the onset of long-term complications, with cardiovascular, peripheral and cerebrovascular disorders being distinguished (Alberti and Zimmet 1998).

Type 1 diabetes is characterized by the destruction of β-cells leading to an insulin deficiency. It is a condition where the pancreas does not create or creates very little insulin because the β-cells of the pancreas are destroyed by the autoimmune mechanism. Markers of immune destruction of β-cells are autoantibodies against β-cells when initially detected hyperglycemia. This disease is also associated with certain HLA genes, and the presence of certain alleles may be predominant or protective (Devendra et al. 2004).

Type 2 diabetes mellitus is predominantly insulin resistance with relative insulin deficiency and predominantly insulin secretory deficiency with insulin resistance. Type 2 diabetes accounts for 90 to 95% of the total number of diabetes cases in the world and is due to the presence of insulin resistance in skeletal muscles and liver and reduced insulin production, so there is a relative but rarely absolute insulin deficiency. Type 2 diabetes is a progressive disease where the production of insulin decreases as the disease progresses. Insulin resistance develops due to genetic defects in combination with environmental factors, primarily obesity, and physical inactivity. As the disease progresses, insulin resistance remains relatively stable, and insulin production declines progressively (Kahn 2003; Rewers 2012).

Treatment of diabetes involves a complex of factors that involve the exercise, diet, and drugs. Drugs used to treat diabetes of mainly synthetical origin and have certain negative side effects. Medicinal plants are used in the treatment of many disorders and have a significant role in the treatment of diabetes mellitus as a serious metabolic disorder. It has been shown experimentally that medicinal plants used in the treatment of diabetes do not show any negative effects. Plant species are rich in compounds such as phenolics, tannins, alkaloids and flavonoids that improve the efficiency of pancreatic tissues by increasing insulin secretion or decreasing intestinal absorption of glucose (Kooti et al. 2016).

14.3.1 Plants as Natural Sources of Antidiabetic Agents

Numerous plant species belonging to different families of Amaryllidaceae, Asteraceae, Apiaceae, Cucurbitaceae, Fabaceae, Gentianaceae, Lamiaceae, Liliaceae, Rosaceae, etc. were investigated in order to find effective substances for the purpose of treating diabetes (Moradi et al. 2018). Extracts of certain plant species have been shown to modulate the metabolic pathways such as glycogen synthesis, glycolysis, cholesterol synthesis and release of insulin (Prabhakar and Doble 2008). The extract of *Carthamus tinctorius* (Asteraceae) is used in the treatment of Type 1 and Type 2 diabetes, because the presence of flavonoids, such as quercetin and kaempferol, is determined by phytochemical analyzes, which are the causes of hypoglycemic effects of these compounds. *Swertia punicea* (Gentianaceae) was investigated in order to determine the hypoglycaemic effect, which can be used in the treatment of diabetes because it promotes insulin resistance in the diabetic mice (Moradi et al. 2018).

The aqueous root extract of *Sarcopoterium spinosum* (Rosaceae) was examined in order to determine the anti-diabetic effect of progressive hyperglycemia in mice that are genetically diseased with diabetes. The results showed that the aqueous extract of the roots of the investigated species showed positive results in the treatment of diseased mice. Also, the aqueous extract of *Liriope spicata* (Liliaceae) positively influences the reduction of blood glucose levels and promotes glucose tolerance and resistance of insulin in the mice (Moradi et al. 2018). Ethanol extract of *Momordica charantia* (Cucurbitaceae) possess antihyperglycemic and hypoglycemic effect in normal and streptozotocin-diabetic rats. A four-week experiment conducted on Type 2 diabetes mellitus rats showed that the *Allium sativum* extract (Alliaceae) enhanced glycemic control by increased insulin secretion and enhanced insulin sensitivity, which showed the antihyperglycemic and antihyperlipidemic effect of this type of extract (Moradi et al. 2018). The study of the effects of plant extracts on the treatment of diabetes was carried out by (Huseini et al. 2006; Rao and Nammi 2006; Das et al. 2009; Abeywickrama et al. 2011; George et al. 2011; Das and Barman 2012; Rabiei et al. 2014; Jiao et al. 2017; Moradi et al. 2018).

14.3.2 Lamiaceae Species as a Sources of Antidiabetic Agents

Some plant species belonging to the Lamiaceae family are used in the treatment of diabetes due to the presence of compounds that exhibit pronounced biological activity. Genera belonging to the Lamiaceae family, such as *Ocimum, Origanum, Salvia, Teucrium, Lamium*, etc. are known for their antidiabetic activity (Patil et al. 2011; Moradi et al. 2018). *Origanum vulgare* aqueous extract affects the reduction in glucose levels of glycosylated hemoglobin, and pancreatic amylase in diabetic rats. Extract usage in of dietary in diabetes rats influenced the content of glycogen in the liver, body weight and the level of urea. Plant parts of *Salvia nemorosa* contain glycosides such as salvionosides and megastigmands. It has been shown experimentally that in diabetes mice, insulin levels are increased using extracts, which increases the activity of insulin (Sadeghzadeh et al. 2008). By isolating a luteolin compound from a plant species belonging to the genus *Perilla* it has been shown a positive effect on the development of diabetic nephropathy (Moradi et al. 2018). Flavonoid luteolin reduces creatinine levels and diabetic rats and prevents the increase in urea protein in 24 h. Aqueous and ethanolic extract of the *Ocimum tenuiflorum* resulted in decreased levels of blood glucose, fatty acids, lipid peroxides and low-density lipoproteins (Hussain et al. 2001, 2015). A tetracyclic terpenoid isolated from a hydro alcoholic extract of *Ocimum tenuiflorum* exhibited potential antidiabetic properties (Patil et al. 2011). Hannan et al. (2006) showed that ethanol, butanol, aqueous and ethyl acetate fractions of *Ocimum tenuiflorum* stimulated insulin secretion.

14.3.3 **Teucrium** *Species as a Sources of Antidiabetic Agents*

Secondary metabolites isolated from extracts of *Teucrium* species are good agents against diabetes (Gharaibeh et al. 1988; Rasekh et al. 2001; Esmaeili and Yazdanparast 2004; Ardestani et al. 2008; Vahidi et al. 2010; Alamgeer et al. 2013; Dehghan et al. 2013; Sabet et al. 2013). Dehghan et al. (2013) have shown that the methanol extract of *Teucrium orientale* possesses antihyperglycemic activity. Using extract in the diabetic rat's diet it has been shown that serum glucose has decreased by 40%. Mechanisms that have influenced the decrease in serum glucose levels are strong anti-oxidant properties of *Teucrium orientale* compounds and increased peripheral glucose utilization. Studies have shown that *Teucrium orientale* is rich in flavonoids (Cakir et al. 2006) while flavonoids possess hypoglycemic properties as they increase the oxidative metabolisms of the diabetic states (Dehghan et al. 2013). Alamger et al. (2013) examined the effect of crude extract from *Teucrium stocksianum* on antidiabetic activity. The authors have shown that crude powder of tested species reduces blood glucose levels in diabetic rabbits at a dose of 250 and 500 mg/kg. Testing methanol, aqueous and ethyl acetate extract from *Teucrium stocksianum* noted that at a dose of 500 mg/kg reduce the blood glucose level in diabetes rabbits. The ethyl acetate extract showed better results than methanol and aqueous extract. Also, ethyl acetate extract produced a significant increase in the serum insulin level of diabetic rabbits.

The most noticeable *Teucrium* species in terms of antidiabetic effect is *Teucrium polium*. The *Teucrium polium* extract reduces the level of glucose and triglyceride in the serum at a dose of 4% (Vahidi et al. 2010). In vitro studies have shown that extracts of this species affect the increase in insulin levels and show a hypoglycemic effect in normal rats after only one dose. The highest hypoglycemic effect (50%) was noticed 8 h after a single administration of 125 mg/kg. Moghimi et al. (2017) state that the hypoglycemic activity of *Teucrium polium* extracts was referred to as the presence of secondary metabolites such as flavonoids, sterols, and volatile oils as the active compounds. Flavonoids have hypoglycemic effects by insulin release from the pancreas which stimulates glucose utilization.

14.3.4 Methods for Determination of the Antidiabetic Activity

Methods based on the study of antidiabetic activity consist of monitoring results before and after treatment in vivo and in vitro conditions. Basically, male Wistar rats are used in several groups. The first is a control group, the second is mice that are diabetic, and the third group consists of treated mice with diabetes. Type 1 diabetes was induced by autoimmune in mice by administering low-dose intraperitoneal injections of STZ (20 mg/kg body weight), dissolved in normal saline for several days. The presence of hyperglycemia is confirmed by diabetes. Depending on the duration of the experiment, after several days of the first STZ injection, plant aqueous extract is administered in a dose of 100 mg/kg for several weeks. The level of serum glucose is monitored at the beginning, mid and end of the experiment (Sabet et al. 2013).

14.4 Inflammation

Inflammation represents the defensive reaction of the organism to harmful exogenous or endogenous factors through the immune system (Abbas et al. 2010). The release of the organism from harmful agents includes a series of changes that take place in the intercellular matrix and blood vessels. The basic function of the inflammation process is to isolate the tissue and remove damaged cells (Medzhitov 2008; Soehnlein and Lindbon 2010; Ashley et al. 2012). There are several types of inflammation. Chronic inflammation leads to necrosis and tissue fibrosis. This type of inflammation affects the development of degenerative diseases, primarily Alzheimer's disease, diabetes, cancer, atherosclerosis, etc. (Murphy and Weaver 2017). Acute inflammation occurs after a few minutes or hours after tissue injury. This is a short-term process characterized by symptoms such as redness of the tissue. Acute inflammation is a good defense mechanism in the fight against bacteria.

Symptoms that are followed by inflammatory processes include tissue redness, pain, and high temperature (Brune and Hinz 2004). The onset of an inflammatory reaction is aimed at repairing the tissue after the elimination of the infectious agent. The transition of the inflammatory process into the tissue recovery process is the interruption of the production of pro-inflammatory cytokines and the start of the production of liposomes. Lipoxins activate monocytes that remove necrotic cells and inhibit neutrophil recruitment. Also, lipoxins initiate tissue remodeling (Medzhitov 2008). The inflammatory process involves the synthesis of interleukins, prostaglandins and certain hemotoxins. Inflammation occurs by stimulation activation on the membrane that activates the hydrolysis of the phospholipid membrane by phospholipase A into arachidonic acid, which further represents the substrate for cyclooxygenase and lipooxygenase enzyme.

It has been shown that inflammatory processes are involved in the etiology of many diseases as well as Alzheimer's disease (Breitner 1996). The drugs that are used today to relieve the symptoms of inflammation are non-steroidal anti-inflammatory drugs (Beale and Block 2010; Lemke et al. 2013).

14.4.1 Plants as Natural Sources of Anti-inflammatory Agents

Nowadays, plant extracts are used to reduce inflammatory processes (Mahesh and Sathish 2008). It has been shown experimentally that medicinal plants used in the treatment of inflammatory processes do not show any side effects. Numerous plant species belonging to different families of Aristolochiaceae, Apocynaceae, Asteraceae, Lamiaceae, Plantaginaceae, Salicaceae, etc. have been investigated in order to find effective substances to reduce the symptoms of inflammation. In addition to a number of medicinal properties, the anti-inflammatory activity of plants is appreciated in traditional medicine. The anti-inflammatory activity of herbal extracts reduces carrageenan-induced edema in the rats, with the oral application of these extracts. Similar results have been shown in numerous studies of medicinal plants (Dharmasiri et al. 2003; Li et al. 2003; Ojewole 2005; Rodriguez Silva et al. 2008; Kumar et al. 2009; Chandrashekar et al. 2010; Shah and Seth 2010; Sreejith et al. 2010; Garg and Paliwal 2011; Shah et al. 2011; Vishal et al. 2014; Verma 2016).

14.4.2 Lamiaceae Species as a Sources of Anti-inflammatory Agents

The Lamiaceae family includes about 250 genera with over 7500 species. A large number of species have been widely used in pharmacy. Some plant species belonging to the Lamiaceae family have been administered against COX. The biologically active compounds present in the species of this family are the main sources of COX inhibitors (Pang et al. 1996). Plant species belonging to the Lamiaceae genera, such as *Lavandula, Glechoma, Lamium, Mentha, Marrubium, Origanum, Ocimum, Rosmarinus, Salvia, Stachys, Sideritis* and *Teucrium* are known for their anti-inflammatory activity (Mihai et al. 2018).

Species of the genus *Ocimum* are used to treat inflammation and assuage of chronic pain in the joints. The anti-inflammatory property of *Ocimum basilicum* and *O. santanum* was confirmed by Singh et al. (1996). Phenolic compounds present in the investigated plants inhibit carrageenan-induced and arachidonic acid and leukotriene-induced paw edema, possibly by blocking the enzymatic activity of both COX and lipoxygenases (Singh et al. 1996). Phenolic compounds are responsible for the anti-inflammatory properties of several medicinal plants (Pongprayoon et al. 1991; Dewhirst 1980). The species belonging to the genus *Glechoma* have the ability to elaborate long-chain unsaturated fatty acids with anti-inflammatory potentials (Kuhn et al. 1989). *Thymus vulgaris* extracts possess inhibitory roles over the nitric oxide (NO) by limiting iNOS mRNA expression, and also are used in traditional medicine for inflammatory skin disorders (Vigo et al. 2004; Alabdullatif et al. 2017). Methanolic extracts derived from species which belong to the genus *Lamium*, such as *L. purpureum* and *L. garganicum*, reducing inflammatory pain in a model of ear edema and in carrageenan-induced paw edema (Akkol et al. 2008). Studies have shown that the hydrochloric extract of the species *Stachys* has better anti-inflammatory activity than a nonsteroidal anti-inflammatory drug, indomethacin. The extract of the investigated species was able to attenuate both early and delayed phases of carrageenan-induced inflammation over the 50–200 mg/kg dose range. The studies show that plant species belonging to family Lamiaceae is a natural source of anti-inflammatory compounds and that plants can be used in the treatment of inflammation (Mihai et al. 2018).

14.4.3 **Teucrium** ***Species as a Sources of Anti-inflammatory Agents***

Species belonging to the genus *Teucrium* possess secondary metabolites that, in addition to numerous biological activities, exhibit in vitro and in vivo anti-inflammatory activity (Tariq et al. 1989; Barrachina et al. 1995; Puntero et al. 1997; Radhakrishnan et al. 2001; Shakhanben 2001; Menichini et al. 2009; Cabral et al. 2010; Farshchi et al. 2010; Pourmotabed et al. 2010; Miri et al. 2015; Shah and Shah 2015; Rahmouni et al. 2017).

Amraei et al. (2018) examined the effect of *Teucrium polium* hydroalcoholic extract on anti-inflammatory activity. Administration of the extract significantly reduced the serum levels of triglyceride, cholesterol, and LDL-cholesterol. Additionally, the 170 mg/kg dose of extract was most effective in reducing serum levels of inflammatory markers. Authors Tariq et al. (1989) showed that a dose of 500 mg/kg of body weight of the ethanol extract of species *Teucrium polium* inhibited carrageenan-induced inflammation and reduced granuloma formation. According to a number of literary data, *Teucrium polium* contains bioactive compounds such as phenolic compounds and flavonoids. It has been shown that flavonoids reduce inflammatory processes by activating several pathways (Houshmand et al. 2015).

Pourmotabbed et al. (2010) examined the effect of aqueous extract of *Teucrium chamaedrys* on the anti-inflammatory activity, which showed that a dose-dependent inhibition of the edema was performed using 25–250 mg/kg administered 1 h before carrageenan-induced paw swelling. For extracts of *Teucrium hyrcanicum*, it has been shown to exhibit analgesic and anti-inflammatory activity in carrageenan-induced paw edema, and formalin pain tests (Farshchi et al. 2010). Extracts of *Teucrium stocksianum* significantly reduced the paw edema at a dose of 400 mg/kg in rats. However, the extract did not show any anti-inflammatory effect when administered orally in a sub-acute study using a cotton-pellet method but was effective orally in an acute study using rat paw edema (Radhakrishnan et al. 2001). Miri et al. (2015) examined aqueous extract from *Teucrium persicum* on anti-inflammatory activity. The authors have shown that aqueous extract of this species at concentrations of 100, 200 and 400 mg/kg shows an insignificant anti-inflammatory effect in the cotton pellet induced granuloma model in the mice. The authors assume that the activity was dependent on the dose, method, or period of time. El-Ashmawy (2018) investigate the anti-inflammatory effects and the phytochemical constituents of the methanol extract from *Teucrium oliverianum* using the carrageenan-induced rat paw edema (acute and sub-acute models) and the terpentine oil-induced granuloma pouch bioassay. The methanolic extract of *Teucrium oliverianum* exhibited anti-inflammatory activity in both phases of carrageenan-induced acute edema test in a dose-dependent manner.

14.4.4 Methods for Determination of the Anti-inflammatory Activity

To investigate the anti-inflammatory effect, there are many methods of which carrageenan-induced inflammatory edema is most commonly used in the hind paw of rats. Inhibition of carrageenan-induced inflammation is one of the most suitable test procedures for monitoring anti-inflammatory agents. Carrageenan is a muco-polysaccharide isolated from Sea moss *Chondrus* in order to experimentally induce arthritis. Carrageenan causes an acute inflammation that occurs in two stages. The first or early stage occurs after 1 h of injection of carrageenan. At this stage, edema develops due to the effects of serotonin and histamine. A second or late phase occurs after 2 h of injection of carrageenan where vascular permeability is maintained by bradykinin and prostaglandins. These mediators induce pain and contribute to the inflammatory response. Studies have shown that late edema is sensitive to anti-inflammatory drugs and is used to estimate the antiphlogistic effect of natural products (El-Ashmawy 2018).

14.5 Conclusions

This chapter is a review of anticholinesterase, antidiabetic and anti-inflammatory activity of several species of the genus *Teucrium.* Extracts from *Teucrium arduini, T. chamaedrys, T. montanum*, and *T. polium*, showed strong inhibitory activity against acetylcholinesterase. Extracts of some *Teucrium* species reduces blood glucose levels in diabetic rats and rabbits. Also, the use of plant extracts significantly reduce inflammatory markers and inhibited carrageenan-induced inflammation. By examining the qualitative and quantitative composition of secondary metabolites of *Teucrium* species, the presence of active phenolic acids was observed. The most common are rosmarinic and chlorogenic acid. Also, the most common are luteolin and apigenin from the group of flavonoids. It has been shown that these biologically active compounds contribute to anticholinesterase, antidiabetic and anti-inflammatory activity.

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Chapter 15
Application of *Teucrium* Species: Current Challenges and Further Perspectives

Dragana Jakovljević and Milan Stanković

Abstract Increased demand for products originating from natural sources lead to novel agricultural and biotechnological techniques and create the urgent need for implementations of these techniques to appropriate response for enlarged interests. The plants from the *Teucrium* L. genus are well-known sources of natural products with wide areas of application, primarily due to the high content of secondary metabolites with significant biological activities. Utilization of *Teucrium* species in diverse areas of the industry makes them suitable candidates for various biotechnological investigations as well as further applications of obtained results. The primary objective of this chapter is to summarize the most diverse areas of application from species of the *Teucrium* genus, regarding various biotechnological procedures that maximize the exploitation but also provide sustainable conservation and rational utilization of biodiversity both under in vivo as well as under in vitro conditions. With the high content of valuable compounds and a multidisciplinary approach, *Teucrium* species are the primary target for trait manipulation and offers major opportunities for the major application of plant biotechnology.

Keywords *Teucrium* · Application · Biotechnology · Plant tissue culture · Useful compounds

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15.1 Introduction

Increase in world population and searching for novel products, together with the recognition that the human health is related to the nutritional quality of the food which is threatened under adverse climatic changes, create the urgent need for agriculture and plant biotechnologies implementations (Altman and Hasegawa 2012).

Among the members of the Lamiaceae family, genus *Teucrium* L. includes plant species which diverse areas of benefits for humans make them highly applicable traditionally as well as industrially. Traditionally, a large number of species belonging to the genus *Teucrium* have a long history of use in folk medicine and pharmacy. They are used in treatments of various disorders, mainly due to the high content of metabolites with significant biological activities. In addition, numerous studies suggest that extracts from *Teucrium* species and isolated bioactive compounds possess antioxidative, antibacterial, antidiabetic, anticancer and anti-inflammatory activities (Stankovic et al. 2011). From the industrial point of view, morphological and eco-physiological variability of the *Teucrium* species, and relatively high number of species included, emphasis the importance of plants from the genus *Teucrium* as biotechnological factories for new plant-based biomaterials.

Consumption of herbals from their natural sources is widespread and increasing, however, harvesting from the wild may lead to the loss of diversity and habitat destruction. Therefore, the use of controlled environments may successfully overcome these difficulties and could provide manipulation of phenotypic variation in bioactive compounds (Canter et al. 2005). The modern era of plant biotechnology coordinate with the ability to grow plant cells and tissues in vitro, to regenerate and clone new plants as well as to modify their genetic characteristics by traditional and molecular breeding, including genetic modification, and genome editing (Altman 2019). Having in mind diverse areas of applications of plants from the genus *Teucrium*, the main goal of this chapter is to provide information's about diverse areas of application of *Teucrium* species together with further implications of various biotechnological procedures that maximize the exploitation but also provide sustainable conservation and rational utilization of biodiversity both under in vivo as well as under in vitro conditions.

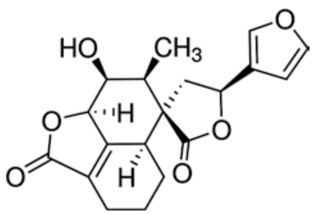
15.2 Diverse Areas of *Teucrium* Species Application

The species of the genus *Teucrium* have been popular since the very beginning of the use of herbs in the treatment of diseases. Data on healing properties can be found in Greek mythology and nowadays the species from this genus are used in the treatments of tuberculosis, scurvy, jaundice, and rheumatism. The high content of aromatic compounds with antimicrobial activity contributes to the treatment of many infectious diseases. Besides, the above-ground parts are very bitter, and due to the content of bitter aromatic compounds, plants from the genus *Teucrium* are used as an additive in cooking as well in the preparation of beverages (Keršek 2006). Among species of the genus *Teucrium* that are presented in the Flora of the Republic of Serbia, *Teucrium chamaedrys*, *T. montanum*, *T. polium*, and *T. scordium* are most often used for the preparation of teas, as well as for other medicinal preparations. In most cases, the green above-ground parts of plants with flowers are taken, which are then dried in the shade (Stanković 2012).

Teucrium chamaedrys is overall one of the most widely used species of the *Teucrium* genus. The benefits came from teas that have a very bitter taste, and for this purpose, the tops of twigs with the flowers are used *(Teucrii herba et folium)*. This plant parts are most often used for the treatment of digestive organs. Secondary metabolites that give this plant a bitter taste stimulative affect the digestive organs and intensify secretions, alleviate the effects of gastritis and increase the secretion of gastric acid. It is also used in the treatment of various infections and immunodeficiency (Pieroni et al. 2004; Bagci et al. 2010). *Teucrium montanum* is very often used to alleviate gastric problems and to improve digestion. This plant species is also effective as an additional therapy for the treatment of infectious diseases of the respiratory tract and tuberculosis. It can also be used as a medicinal bath (Stanković 2012). *Herba teucrii montani* can be used as a tincture but also in the form of wine and brandy (Keršek 2006). Teas, tinctures, and oils prepared from above-ground parts of *Teucrium polium* are used as an auxiliary in the treatment of hypertension, diabetes, and diseases of the digestive organs as well as for the control of hypercholesterolemia (Afifi et al. 2009; Vahidi et al. 2010). *Teucrium scordium* is used to stimulate the function of organs of the digestive system, in the treatments of infected wounds, but also the treatment of plague, cough, and fever (Redžić 2007).

In spite of all the confirmed advantages obtained from these species, it is necessary to keep in mind that after long-term use of teas and other medicinal preparations originated from *Teucrium polium* and *Teucrium chamaedrys* symptoms of mild hepatotoxicity may be established. After a large number of studies conducted, it has been concluded that secondary metabolites from the group of neoclerodane diterpenes, in particular, teucrin A, cause the symptoms of damage and liver inflammation (Starakis et al. 2006; Savvidou et al. 2007). However, after the dose and time-dependent testing, it has been established that hepatotoxic effect occurs only after higher doses in the body, and for a long period, while acute treatment has no negative effect on the organism (Khleifat et al. 2002). Therefore, careful and moderate use is recommended without any further health consequences. The structure of teucrin A (secondary metabolite from the group of neoclerodane diterpenes) is presented in Fig. 15.1.

Fig. 15.1 Chemical structure of teucrin A



The fact that many *Teucrium* species show antibacterial, antifungal and antioxidant activities regarding them useful as natural preservative ingredients with significant importance in food industries (Bagci et al. 2010). The foodborne diseases together with food spoilage caused by a variety of microorganisms at various stages (from production to sale and distribution) are among the most important concerns for the food industry. The increase of bacterial resistance to antibiotics leads to considerable interests in investigations of the antimicrobial effects of different extracts against a wide range of bacteria to develop other classes of antimicrobials useful for the food preservation (Belmekki et al. 2013). Özkan et al. (2007) demonstrated that extracts obtained from *Teucrium montbretii* preventing the autooxidation in oils and oil-bearing foods. According to these authors, the growth of food-borne pathogens or spoilage organisms can be inhibited when high concentrations of herb extracts were applied in food products.

According to Djabou et al. (2013), the foodborne pathogen *Campylobacter jejuni* was extremely sensitive to oils of *Teucrium* species. Authors found that oils from *Teucrium marum, T. massiliense, T. chamaedrys, T. scorodonia* subsp. *scorodonia*, *T. polium* subsp. *capitatum*, *T. flavum* subsp. *glaucum* and *T. flavum* subsp. *flavum* has the potential to be used as food preservatives and to prevent the growth of nosocomial bacteria. On the other hand, López et al. (2007) showed that aerial parts of *Teucrium chamaedrys*, in the view of their antioxidant and antifungal properties, possess potential to be used in order to obtain new active products with applications in the food industry.

Sadrizadeh et al. (2018) demonstrated that the addition of *Teucrium polium* essential oils to milk products significantly increased survival of *Lactobacillus casei* after 20-day storage at 4 °C. Besides, *Teucrium polium* essential oils and probiotic fermentation reduced the population of *Escherichia coli* during the storage time and the effects were stronger in combination than using them separately. These authors recommended combining effect of *Teucrium polium* extract and probiotic due to their complementary antimicrobial effects and practically no side effects. Also, because of good appearance without any signs of spoilage organisms, it is recommended to use this method to reduce the *Escherichia coli* growth and population in the food system.

15.3 Biotechnology of *Teucrium* Species

The area under medicinal and aromatic plants cultivation is increasing worldwide due to the use of natural therapies by a growing segment of the population, and at the same time, it is necessary to optimize the technologies to maximize production and to cover the market demand (Burducea et al. 2019). Environmental stresses (drought, salinity, high temperature, nutrients) usually decrease the yield of medicinal plants and increase the content of secondary metabolites (Hosseini et al. 2018; Jakovljević et al. 2019), and therefore, the studies focusing on agronomic conservation and management of plant species become necessary, particularly due to scarce information in literature about the cultivation of important plants (de Mesquita Arruda et al. 2018).

15.3.1 Plant Cell and Tissue Culture

Plant cell and tissue culture are among the most prominent and viable plant biotechnology techniques. Although is developed primarily for basic research on cellular and tissue differentiation and morphogenesis, tissue culture research in last decades and manipulation of cells and organs in aseptic conditions together with further growth in a medium under defined light intensity, humidity, and temperature allowed the increasing of uniformity and maintaining the same genetic characteristics of plants. In addition, the production of bioactive compounds under these conditions make additional efforts for sustainable conservation and rational utilization of biodiversity (Karuppusamy 2009; Altman and Hasegawa 2012; Dias et al. 2016). Based on the principle of cellular totipotency, plant cells and tissues may be cultured in terms of undifferentiated calli and cell suspensions, as well as organ culture (shoots and roots), and for producers, plant cell, tissue and organ culture are of great interest as an alternative for obtaining chemicals from plant species by reducing the time interval to harvest (Rodríguez-Sahagún et al. 2012).

15.3.1.1 In Vitro Seed Germination

The combination between biochemistry and biotechnology lead to significant improvement on production yield, however, initiation of in vitro culture and cultivation of some herbs has proven difficult due to low germination rates or lack of knowledge about the specific ecological requirements (Canter et al. 2005). It is demonstrated that seeds from *Teucrium* species have low germination capacities (Nadjafi et al. 2006; Luna et al. 2007; Moreira et al. 2010) and that germination characteristics of seeds in species which show lower germination using conventional techniques could be increased using in vitro methods (Pickens et al. 2003; Jakovljević et al. 2017). The effect of various pretreatments, culture conditions and storage time on in vitro germination of seeds from *T. capitatum* were examined by Papafotiou and Martini (2016). Pretreatments, such as cold stratification, scarification with sandpaper, dipping in concentrated sulfuric acid, or dipping in boiling water were tested on in vitro germinated seeds. It was demonstrated that seeds without any pretreatment germinated at lower than 10% whereas dipping in concentrated H_2SO_4 for 15 or 20 min was the most effective pretreatment. In addition to the better germination capacities, explants excised from in vitro-grown seedlings were established at higher rates compared with those collected from plants grown in a greenhouse.

15.3.1.2 Overcoming Habitat Encroachment

In addition to the low rate of seed germination which restrict massive propagation and further growth of *Teucrium* species, due to the all potential valuable properties, these plants are under various stresses (physical as well as anthropogenic) like unappropriated harvesting, overgrazing, and unsustainable development (Ahmad et al. 2002; Bouhouche and Ksiksi 2007). Because of irregular grazing and immoderate picking up for folk medicine usage, biodiversity of *Teucrium polium* decreased significantly in rapid eradication and extinction (Rad et al. 2014). Due to the selective collection for use in traditional medicine, and deforestation and habitat encroachment, wild populations of *Teucrium capitatum* are subjected to pressure (Papafotiou and Martini 2016). Recent investigations of *Teucrium stocksianum* area of cultivation revealed the need for urgent interventions due to the disappearing of this species at an alarming rate (Shinwari and Gilani 2003). Al-Qudah et al. (2011) pointed out that the biodiversity of *Teucrium polium* declined dramatically in recent years which could be attributed to habitat encroachment by urban and agricultural development, deterioration and deforestation, depletion of major water sources, as well as due to the illegal collection. Having all that in mind, the development of an in vitro protocols is of critical importance since it will provide plants that can be used for reintroduction in their natural habitats, to conserve it from being an endangered, and to maintain the germplasm (Bouhouche and Ksiksi 2007; Al-Qudah et al. 2011; Rad et al. 2014).

15.3.1.3 Micropropagation

As a large-scale clonal propagation, micropropagation is the most important (practical and economical) application of plant biotechnology (Da Silva et al. 2015) and it has been useful particularly in the case of valuable, rare or endangered plant species. This in vitro method is also an appropriate tool for multiplication of disease-free plants, faster cloning and the conservation of desired genotypes, in a very short time (Máthé et al. 2015). Plants can be regenerated either by the growth and proliferation of existing axillary and apical meristems or by the regeneration of adventitious shoots whereas adventitious buds and shoots are formed de novo and meristems are initiated from explants – leaves, petioles, hypocotyls, floral organs and roots (Iliev et al. 2010). Micropropagation was established for the *Teucrium fruticans* (Frabetti et al. 2009), *Teucrium stocksianum* (Bouhouche and Ksiksi 2007), *Teucrium capitatum* (Papafotiou and Martini 2016), *Teucrium polium* (Al-Qudah et al. 2011; Rad et al. 2014), *Teucrium scorodonia* (Makowczyńska et al. 2016). Habitus of *Teucrium fruticans* together with the complete micropropagation cycle of *Teucrium fruticans* (according to Frabetti et al. 2009) is presented in Figs. 15.2 and 15.3. Besides the fact that controlled growth system contributes to the reduction of the pressure on the natural populations, it also provides manipulation of phenotypic variation in the concentration of medicinally important compounds through the increase of uniformity and predictability of extracts (Canter et al. 2005).

Fig. 15.2 *Teucrium fruticans* L. during the flowering stage (Photo M. Stanković)

15.3.1.4 Improvement of Production Yield

The plants from the genus *Teucrium* is well-known for the production of a significant amount of biologically active secondary metabolites, however, the quantitative and qualitative characteristics of the produced metabolites are conditioned by the plant organs, as well as the physicochemical and morphological characteristics of the substrate. Additionally, the quantitative and qualitative composition of secondary metabolites of *Teucrium* species is characterized by the seasonal variations (Stanković et al. 2012a, b, 2014). Interpopulation variability in the total quantity of phenolic compounds and antioxidant activity was determined for *Teucrium camaedrys* and *T. montanum* in individuals from the different serpentine and calcareous habitats (Zlatić et al. 2017). For *Teucrium polium* essential oils it is established variability conditioned by the different latitudinal populations (Sadeghi et al. 2014). In addition to the variability in content and activity of biologically valuable secondary metabolites due to environmental conditions, there are several reports of hepatotoxicity caused by extracts obtained from *Teucrium* species (Larrey et al. 1992; Kouzi et al. 1994). According to Chitturi and Farrell (2008), *Teucrium*-associated hepatotoxicity was recognized with *Teucrium chamaedrys* (germander) and is attributed to reactive metabolites (epoxides) generated by CYP3A metabolism of its constituent neoclerodane diterpenoids (teucrin A) since the specific auto-antibody was identified from long-term drinkers of germander tea. In addition, *Teucrium capitatum* and *T. polium* was linked with the acute liver failure and acute hepatitis due to necrosis

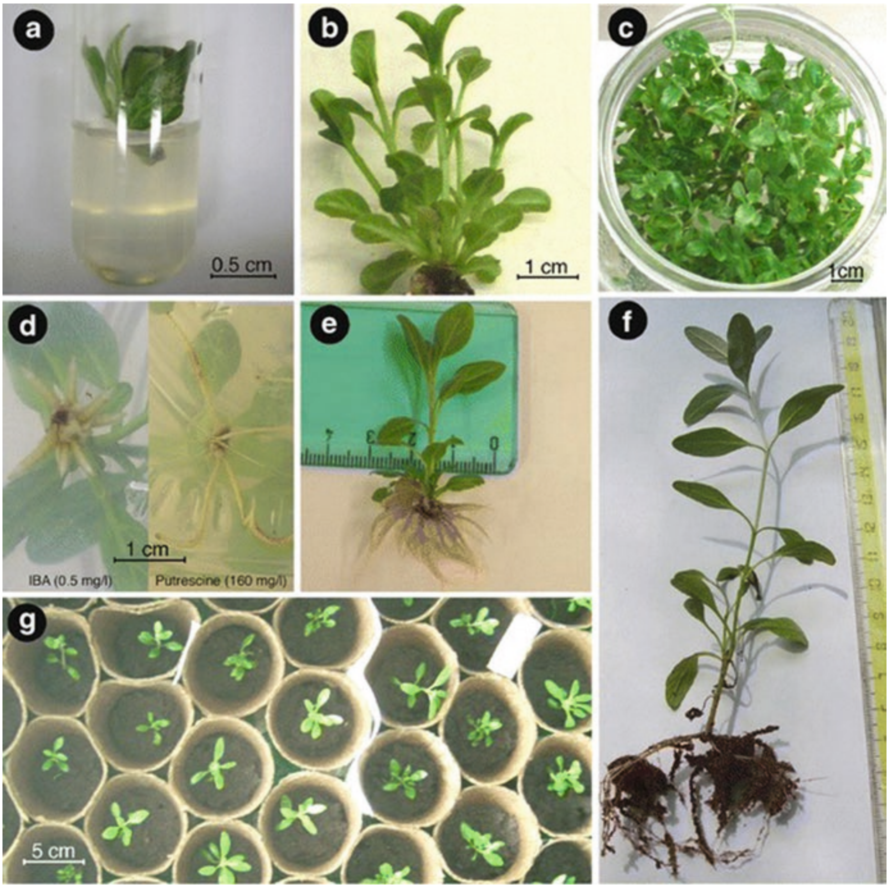


Fig. 15.3 Complete micropropagation cycle of *Teucrium fruticans* (according to Frabetti et al. 2009): (**a**) shoot initiation from nodal segments; (**b**) and (**c**) multiple shoots regenerating on medium; (**d**) root development after 14 days of incubation; (**e**) in vitro-rooted shoot ready for transfer into soil after 24 days of incubation; (**f**) root development after the acclimatization stage; (**g**) plant acclimatized to greenhouse conditions

or occasionally massive hepatocyte necrosis (Dourakis et al. 2002; Savvidou et al. 2007).

It is well established that biotechnological techniques can be applied to improve yield and uniformity, to modify potency or toxicity, as well as to increase the production of active phytochemical compounds (Canter et al. 2005). Additionally, the advanced biotechnological tools resulted in plants with improved agricultural benefits and expanded the use of plants to produce a variety of novel biomaterials (Altman and Hasegawa 2012). In vitro developed protocols for *Teucrium* species are advantageous in terms of the speed of plant regeneration since regenerated plants were obtained within 3 months after the initiation of the culture protocol; also, it is possible to use these plants as a source of tissues for the biochemical characterization of biologically active compounds (Bouhouche and Ksiksi 2007). According to Makowczyńska et al. (2016), it is possible to obtain approximately 300 and 400 in vitro derived *Teucrium scorodonia* plants from a single shoot or nodal explants. Additionally, these plants can be suitable for sustainable production of β-caryophyllene and germacrene D. Rad et al. (2014) demonstrated that amount of total flavonoids was different in tissue culture raised *Teucrium polium* plants comparing to the field-grown plants. These authors proved that the extracts of tissue culture plants contained the highest amount of flavonoid content and the greatest antioxidant activity compared to the field-grown wild plant.

Several limitation factors of plant cell biotechnology that hampers its extensive commercialization can be overcome through the elicitation which implies exogenous addition of elicitors (chemical compounds from abiotic and biotic sources) in the growth medium. This procedure stimulates stress responses in plants and leading to the enhanced synthesis and accumulation of secondary metabolites or the induction of novel secondary metabolites since induced stress activates several defense-related genes or inactivates non-defense-related genes (Naik and Al–Khayri 2016; Narayani and Srivastava 2017).

Antognoni et al. (2012) established in vitro culture of *Teucrium chamaedrys* with the possibilities to elicit the production of phenylethanoid glycosides, particularly teucrioside in the levels higher than those found in the leaves of intact plants. The absence of neoclerodane diterpenoids in in vitro culture of *Teucrium chamaedrys* makes these results particularly significant having in mind that these diterpenes may induce symptoms of hepatotoxicity.

The high content of phenylpropanoid compounds (particularly phenolics) together with significant biological and bioactive characteristics of these metabolites in *Teucrium* species are well documented (Stanković 2012; Stanković et al. 2012a, b, 2014) and is usually part of the complex defense mechanism in an adverse environment. The phenylpropanoid compounds are mainly synthetized through the shikimate pathway and occurs via a number of enzymatic reactions (Jakovljević et al. 2017). In general, enhanced biosynthesis of phenylpropanoid compounds through the phenylpropanoid biosynthetic pathway is a characteristic of adaptation to the stressful environmental conditions which is resulting in accumulation of phenolic compounds (Jakovljević et al. 2017, 2019). Different elicitors (biotic and abiotic) is used in order to increase the production of phenolic compounds. In Fig. 15.4 (according to Dias et al. 2016) are presented some of the elicitors used to increase production of phenolic compounds and pointed out parts of the biosynthetic pathways were elicitors may enhance the synthesis of these valuable metabolites.

There is considerable interest in manipulating plant biosynthetic pathways to produce secondary metabolites, but there are significant interests in the production of drug precursors, food components or pesticides (Canter et al. 2005). Generally, exogenous supply of different elicitors to different culture systems of plants create stress like cellular environment and, therefore, the elicitors not only bring yield enhancement of existing biomolecules but also found to alter/synthesize secondary metabolites qualitatively through the discovery of entirely novel compounds (Giri and Zaheer 2016). Further, extensive appraisal of elicitor use under controlled

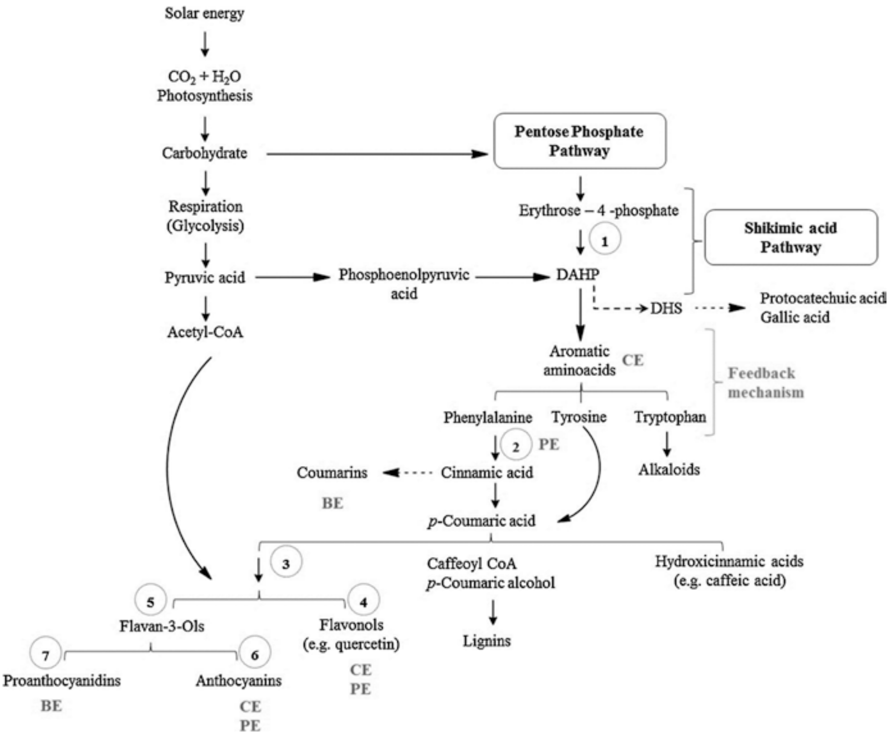


Fig. 15.4 Biosynthetic pathway of some phenolic compounds and the influence of elicitation (according to Dias et al. 2016). *CO_2* carbon dioxide, *H_2O* water, *Acetil-CoA* Acetil-Coenzyme A, *DAHP* 3-Deoxy-O-arabino-heptulosonate phosphate, *DHS* 3-Dehydroquinate, *BE* biological elicitation, *CE* chemical elicitation, *PE* physical elicitation; Enzymes involved in the biosynthesis are marked with rounded dashed black forms: 1-DAHP synthase (3-Deoxy-O-arabino-heptulosonate phosphate synthase); 2-PAL (Phenylalanine ammonia-lyase); 3-CHS (Chalcones synthase), CHI (Chalcones isomerase), F3H (Flavanone-3-hydroxylase); 4-FLS (Flavonol synthase); 5-LAR (Leucoanthocyanidin reductase); 6-LDOX (Leucoanthocyanidin dioxygenase)

conditions will be useful for the understanding of *Teucrium* secondary metabolites synthesis, as well as for the production and in vitro enhanced production of biotechnologically applicable metabolites.

15.3.2 Hydroponic Culture

Among the soilless cultivation technologies with natural or artificial media used and with possibilities for easy environmental control, the hydroponic systems offer several additional benefits such as the ability to reuse water and nutrients, prevention of soil-borne diseases and pests, as well as higher yields and higher quality products. Therefore, demand for the hydroponic system has increased dramatically (Lommen

2007; Brentlinger 2007; Lee and Lee 2015). Hydroponic *Teucrium polium* contains flavonoids, iridoids, as well as terpenoids and phenylpropanoid glycosides with phytoestrogen-like activity (Galstyan et al. 2010). In addition to phytoestrogen-like activity, extracts from hydroponic *Teucrium polium* demonstrated neuroprotective activity following bilateral ovariectomy (Simonyan and Chavushyan 2015). Cultivation of *Teucrium polium* in open hydroponics provides the ecologically clean yield and enrichment in the content of apigenin, luteolin, verbascoside, poliumoside and teupolioside (Oganesyan et al. 1991). Simonyan and Chavushyan (2016) investigated the effects of hydroponic *Teucrium polium* on hippocampal neuronal activity and morpho-histochemistry of bilateral ovariectomized (OVX) rats. Authors concluded that *Teucrium polium* reduced OVX-induce neurodegenerative alterations in entorhinal cortex-hippocamp circuitry and facilitated neuronal survival by modulating the activity of neurotransmitters and network plasticity.

15.4 Application of *Teucrium* Useful Compounds

Although the natural products originating from plants (in particular phenylpropanoid-derived products) has a broad spectrum of applications relevant to human health, key challenges still remain to be met in the application of biotechnology for improvement in phenylpropanoid and flavonoid natural product profiles of plants and in exploitation of their full biotechnological potential (Ververidis et al. 2007). The wealth of plant-derived metabolites further emphasizes the importance of plants as biotechnological factories for new biomaterials (Altman and Hasegawa 2012).

For extracts obtained from *Teucrium* species, significant antioxidant activity has been identified, and the results suggest that there is a correlation between the intensity of the antioxidant activity and the amount of phenolic compounds. The ability of *Teucrium* spp. extracts to neutralize harmful free radicals, determined through the total antioxidant activity of secondary metabolites, was established for ethanol, aqueous, chloroform, acetone and methanol extracts, both in vitro and in vivo conditions (Gülçin et al. 2003; Kadifkova-Panovska et al. 2005; Katalinic et al. 2005; Ljubuncic et al. 2006; Hasani et al. 2007). Potential allelopathic effects of neoclerodane diterpenes from *Teucrium chamaedrys* was estimated on germination and seedling growth of *Dactylis hispanica* Roth, *Petrorhagia velutina* (Guss.) Ball et Heyw., and *Phleum subulatum* (Savi) Asch et Gr. (Fiorentino et al. 2009).

The insecticidal activity of plants from the *Teucrium* genus was confirmed, both under in vivo and in vitro conditions (Masanori et al. 2000). The insecticidal activity of essential oils from *Teucrium polium* was confirmed by treating the *Musca domestica* L. (housefly), wherein the secondary metabolites of this plant species inhibit the activity of the digestive enzymes of the house fly larvae (Bigham et al. 2010). By treating potatoes (*Solanum tuberosum* L.) with secondary metabolites *Teucrium* spp., it has been found that ericcephalin, teucrin A, teucorolide and tequin affect the increase in potato plant resistance through the effects on Colorado Potato beetle (*Leptinotarsa decemlineata* Say) larvae (Ortego et al. 1995). In addition to insecticidal, *Teucrium* spp. secondary metabolites also possess antimicrobial activity (Gülçin et al. 2003; Šamec et al. 2010).

Based on the in vivo testing of essential oils obtained from *Teucrium polium*, *T. montanum*, *T. chamaedrys*, *T. flavum* and *T. arduini* it was established that *β*-caryophyllene and germacrene possess significant antiphytoviral activity. Results demonstrated that essential oils from *Teucrium* plants incibit tissue damages on *Chenopodium quinoa* Willd. infected with cucumber mosaic virus (CMV) (Dunkić et al. 2011; Bezić et al. 2011).

Based on the study of the anticancer effects of *Teucrium polium*, it has been found that metabolites from the extracts of this plant can be used in the treatment of prostate cancer because they have the ability to inhibit signal pathways leading to cancer metastasis (Kandouza et al. 2010). Treating the cellular culture of multiform glioblastoma, alveolar adenocarcinoma, breast cancer and adrenal gland with the extracts of *Teucrium polium*, it has been found that secondary metabolites of this plant exhibit significant cytotoxic activity under in vitro conditions (Eskandary et al. 2007; Nematollahi-Mahani et al. 2007). By examining the effects of an aqueous *Teucrium polium* extract on the NSCLS cell line of lung cancer, it has been found that the extract exhibits strong antiproliferative activity by inhibiting the cell cycle by causing cell death (Haïdara et al. 2011).

The methanolic extract of *Teucrium polium* enhances the effect of vincristine, vinblastine, and doxorubicin used in cancer therapy and has a significant antibacterial activity (Zerroug et al. 2011). During in vivo testing of the aqueous extract from the above-ground parts of the *Teucrium polium*, strong antioxidant activity in the pancreatic tissue was associated with a hypoglycaemic effects in artificially induced diabetes in rats (Gharaibeh et al. 1988; Yazdanparast et al. 2005; Shahraki et al. 2006; Ardestani et al. 2008; Esmaeili et al. 2009; Mirghazanfari et al. 2010). By examining the effects of the aqueous and ethyl acetate extract of *Teucrium polium* in in vivo conditions on some diagnostic parameters of the liver, significant hepatoprotective activity was demonstrated (Kadifkova-Panovska et al. 2005; Shtukmaster et al. 2010). In addition, through the in vivo testing of *Teucrium polium* on artificially induced stomach ulcer, the significant antiulcer activity was established (Mehrabani et al. 2009). Based on the in vivo testing of extracts and essential oil from *Teucrium polium*, it was demonstrated that secondary metabolites from this plant species have an antinociceptive effect (Abdollahi et al. 2003). Antiinflammatory and analgesic activity was determined during in vivo testing of water extract of *Teucrium chamaedrys* and *Teucrium polium* (Tariq et al. 1989; Pourmotabbed et al. 2010).

The testing with crude plant extracts and isolation and characterization of the constituents responsible for the activity of extracts is a very common starting approach in biotechnology of plant-derived bioactive compounds. In addition, preparation of plant extracts is relatively simple, low in cost and time investment. However, it is crucial to carefully document identity and processing of the plant material, and the extraction method, since the extraction method can strongly influence the biological activity as well as the composition of the extracts (Koehn and Carter 2005; Stankovic et al. 2014; Atanasov et al. 2015). These issues need to be included in order to maximize the exploitation potential of plants from the genus *Teucrium*.

15.5 Phytoremediation

Several anthropogenic activities, including mining, smelting, the burning of fossil fuels, as well as the manufacture of fertilizers and pesticides, may lead to heavy metal contamination. The heavy metal contamination can be toxic at very low concentration to both plants and animals, and due to its potentially harmful effects, there is a high level of interests in methods which aim to eliminate the heavy metals at minimal coast. Additionally, abandoned mining areas are a significant problem due to the high and toxic concentration of heavy metals and the absence of vegetation over most of the surface. Consequentially, the application of disruptive technologies is inappropriate due to the high cost and potential impacts on the environment, the modification of landscape and soil properties. Generally, the removal of heavy metals can be performed using a variety of high cost physical, thermal, and chemical treatments (which may cause secondary pollutions) or through the biological means (Cao et al. 2009; Rascioa and Izzo 2011; Ozkan et al. 2014; Yaman 2014).

The use of plant species to clean up soil and water is called phytoremediation. The phytoremediation is emerging technology and has gained importance since the use of plant species which are hyperaccumulators is a cost-effective, promising and environmentally friendly technology to remove, extract, or inactivate metal ions in the soil using plants (Fairbrother et al. 2007; Ozkan et al. 2014). Although widespread phytoremediation application is inhibited due to the long remediation time and the production of large amounts of metal-contaminated biomass (Stals et al. 2010; Ozkan et al. 2014), phytoremediation can be considered a good solution since the application of phytostabilization (a phytotechnology that allows stabilization of contaminants in soil) and phytoextraction technique and its optimization in order to lower reclamation times can provide effective protection (Cao et al. 2009).

The bioavailability of elements in plants depends on the characteristics of the soil, climatic conditions, and the genotype and agronomic behavior of the plant (Chojnacka et al. 2005; Martínez-López et al. 2014). Further remediation success of hyperaccumulator plants depends on several factors including the transfer of the metal from the root to the above-ground parts, the growth of the plant, as well as large biomass and the high uptake capacity of the plants (Shah and Nongkynrih 2007; Yaman 2014).

According to Martínez-López et al. (2014), species *Teucrium dunense* grow in sandy soils and loose soils in wadis and dunes close to the sea under intermediate content of arsenic. The soils with intermediate content of arsenic are formed of materials from the beds of ephemeral streams, as a result of hydric erosion of calcareous materials, mica schist and mining materials. However, when there is evidence of a strong mining influence, *Teucrium dunense* also grow in the soils that contain high concentrations of arsenic.

In addition to the soils with moderate or high arsenic content, for *Teucrium* spp. there are possibilities to be used in soils containing nickel, cadmium, lead, and zinc. According to Safari Sinegani and Dastjerdi (2008) *Teucrium polium*, grown at the metalliferous site of Iran, tolerated and absorbed moderate levels of cadmium and lead. On the other hand, Yaman (2014) analyzed the roots and above ground parts of the *Teucrium polium* plants grown in serpentine and non-serpentine soils in terms of nickel (Ni) and cobalt (Co) hyperaccumulation. The author demonstrated that the ratios of Ni/Co concentrations in the roots and above-ground parts of *Teucrium polium* grown in the serpentine soil were significantly higher than the ratios for *Teucrium polium* grown in the non-serpentine soils. Also, according to obtained results, species *Teucrium polium* has been suggested for phytoextraction of Ni in contaminated soils.

In order to investigate the possibility of using native Mediterranean plants species for the application of phytostabilization and assisted phytoextraction in the remediation of abandoned mining sites, *Teucrium flavum* subsp. *glaucum* was tested by Cao et al. (2009). This species is chamaephyte that grows on carbonatic substrata and can colonize the mining waste when pedogenetic process has started. Authors showed that *Teucrium flavum* subsp. *glaucum* possess a high tolerance for lead and zinc. However, besides the great ability to accumulate lead in the aerial parts, this plant species possess low survival and biomass production, and therefore, the further application of assisted phytoextraction needs to be optimized. Having in mind that the phytoremediation can be regarded as a promising low-cost tool for the remediation of the heavy metal-contaminated soils, further investigations of *Teucrium* species in terms of their growth, phytostabilization, and phytoextraction can find diverse practical applications.

15.6 Further Perspectives

As pointed out by Canter et al. (2005), on a large scale, wild harvesting and continual selection of the wild-growing individuals, or those with the desirable traits, may lead to degradation of the wild population, and therefore, appropriate use is not possible without cultivation and conservation of wild population. In this order, different strategies may be used, including the optimization of culture conditions, selection of high producing lines, explant modifications, as well as presence or absence of different environmental conditions. On the other hand, as is pointed out previously, the modern era of plant biotechnology coordinate with the ability to grow and to regenerate plant cells and tissues in vitro, to clone new plants or to modify plant genetic characteristics by genetic modification, and genome editing (Altman 2019).

With the high content of valuable compounds, *Teucrium* species are the primary target for trait manipulation and offers major opportunities for the major application of plant biotechnology. A multidisciplinary approach involving genetic and metabolic engineering will go a long way in enhancing the production of targeted metabolites for commercial applications and direct manipulation of DNA sequences to alter gene expression in medicinal plants is an area that is ripe for expansion (Canter et al. 2005; Bindu et al. 2018). In addition to the enhancement of concentration of the desired compounds, the introducing novel biosynthetic pathways to a variety of species allowing improvement of plant nutritional or commercial value, and therefore metabolic engineering can be used to manipulate flux through both primary and secondary metabolic pathways towards products of interest (Wilson and Roberts 2014).

15.7 Conclusions

Increased in world population and increasing demand for products originating from natural sources lead to the development of novel agricultural and biotechnological techniques and implementations of these techniques to appropriate responses. With the high diversity in morphological, anatomical, phytochemical, ecophysiological and genetic characteristics, as well as in quantity and quality of valuable compounds, the species from the genus *Teucrium* have become well-known for wide areas of application. The variations in form and habitual characteristics have enabled the domestication of different *Teucrium* species and the exploitation of their horticultural potential. The growth and development under unfavorable environmental conditions, particularly in sandy soils and in soils containing heavy metals, making *Teucrium* plants possible candidates to be used in phytoextraction and phytoremediation. The high content of phenolic compounds and essential oils with significant biological activities led to the use of species of this genus both traditionally and industrially. Isolated bioactive compounds possess antioxidative, antibacterial, antifungal, genotoxic, anticancer and numerous other biological activities. To reduce time interval to harvest or to reduce the pressure on the natural populations and to provide manipulation of variation in the concentration of important compounds, *Teucrium* species may be cultured in vitro as undifferentiated calli or cell suspensions, as well as the culture of shoots and roots. Finally, a multidisciplinary approach involving genetic and metabolic engineering can be used in the manipulation of products of interest.

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