Chapter 10
Antioxidant Potential of Wild Plant Foods

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10.1 Oxidative Stress and Antioxidant Defenses

10.1.1 Reactive Species and the Condition of Oxidative Stress

A free radical is defined as any species containing one or more unpaired electrons (electrons singly occupying an atomic or molecular orbital), whereas reactive species is the collective term for radicals and some other non-radical derivatives of oxygen, nitrogen, or sulfur that can easily generate free radicals and/or cause oxidative damage (Halliwell 2012).

As shown in Table 10.1, reactive oxygen species (ROS) include free radicals such as hydroperoxyl radical ($HO_2^{\bullet}$), superoxide anion radical ($O_2^{-\bullet}$), hydroxyl radical ($HO^{\bullet}$) and peroxyl radical ($ROO^{\bullet}$; e.g., lipid derived), and other species such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), and peroxide (ROOR; Lü et al. 2010; Carocho and Ferreira 2013).

The "primary" ROS $O_2^{-\bullet}$ is formed by the addition of one electron to molecular oxygen; this addition occurs in or outside mitochondria and involves different endogenous enzymatic systems such as NADPH oxidases or xanthine oxidases (Ferreira et al. 2009). At pH 7, $HO_2^{\bullet}$, also formed from molecular oxygen, dissociates to $O_2^{-\bullet}$. This radical is not very active, but it can interact with other molecules generating "secondary" ROS, such as H_2O_2 (by superoxide dismutase, SOD in Haber–Weiss reaction) and then $HO^{\bullet}$ (by Fenton reaction—electron transfer from

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Table 10.1 Radical and non-radical reactive oxygen, nitrogen, and sulfur species involved in oxidative stress

Reactive species		Formation
Reactive oxygen species (ROS)		
Radicals	Superoxide anion ($O_2^{-\bullet}$)	$O_2 + e^- \rightarrow O_2^{-\bullet}$ $HO_2^\bullet \rightarrow H^+ + O_2^{-\bullet}$ (pH 7.4) Mitochondria electron transport chain; NADPH oxidases; Xanthine oxidase
	Hydroperoxyl radical ($HO_2^\bullet$)	$O_2 + e^- + H^+ \rightarrow HO_2^\bullet$
	Hydroxyl radical ($HO^\bullet$)	$H_2O_2 + Fe^{2+} \rightarrow HO^- + HO^\bullet + Fe^{3+}$ (Fenton reaction)
	Peroxyl radical ($ROO^\bullet$)	$RH + O_2^{-\bullet} \rightarrow R^\bullet$ $R^\bullet + O_2 \rightarrow ROO^\bullet$
Non-radicals	Hydrogen peroxide (H_2O_2)	$2O_2^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$ (SOD)
	Singlet oxygen ($^1O_2$)	$OCl^- + H_2O_2 \rightarrow Cl^- + H_2O + {}^1O_2$
	Hypochlorous acid (HOCl)	$Cl^- + H_2O_2 \rightarrow OCl^- + H_2O$ (myeloperoxidase) $OCl^- + H^+ \rightarrow HOCl$
	Hydroperoxide (ROOH)	$ROO^\bullet \rightarrow ROOH$
Reactive nitrogen species (RNS)		
Radicals	Nitric oxide ($NO^\bullet$)	Arginine + NADPH + $H^+ \rightarrow NO^\bullet$ + Citrulline + $NADP^+$ Nitric oxide synthases
Non-radicals	Peroxynitrite ($ONOO^-$)	$NO^\bullet + O_2^{-\bullet} \rightarrow ONOO^-$
Reactive sulfur species (RSS)		
Radicals	Thiyl radical ($RS^\bullet$)	$RSH \rightarrow RS^\bullet + e^- + H^+$ (ROS; RNS)
	Sulfoxyl radical ($RSOO^\bullet$)	$RS^\bullet + O_2 \rightarrow RSOO^\bullet$
	Sulfinyl radical ($RSO^\bullet$)	$RSOO^\bullet + RSH \rightarrow RSO^\bullet + RSOH$
	Sulfonyl peroxyl radical ($RSO_3O^\bullet$)	$RSOO^\bullet + O_2 \rightarrow RSO_3O^\bullet$
Non-radicals	Thiol (RSH; e.g. cysteine)	$RSH + NO^\bullet \rightarrow RSNO$ $RSNO + GSH \rightarrow RSH + GSNO$
	Disulfide (RSSR)	RSH + (RSOH or ROS) → RSSR
	Sulfenic acid (RSOH)	RSH + (ROS) → RSOH $RSH \rightarrow RS^- + H^+$; $RS^- + H_2O_2 + H^+ \rightarrow RSOH + H_2O$
	Thiosulfinate (disulfide-S-monoxide) (RS(O)SR)	RSOH + (ROS) → RSOOH (Sulfinic acid) RSOOH + (ROS) → RSO_2OH (Sulfonic acid) $2RSOH \rightarrow RS(O)SR + H_2O$ $2RS^- + H_2O_2 + H^+ \rightarrow RS(O)SR$ RSSR + (ROS) → RS(O)SR
	Thiosulfonate (disulfide-S-dioxide) ($RS(O)_2SR$)	$RS(O)SR + (ROS) \rightarrow RS(O)_2SR$

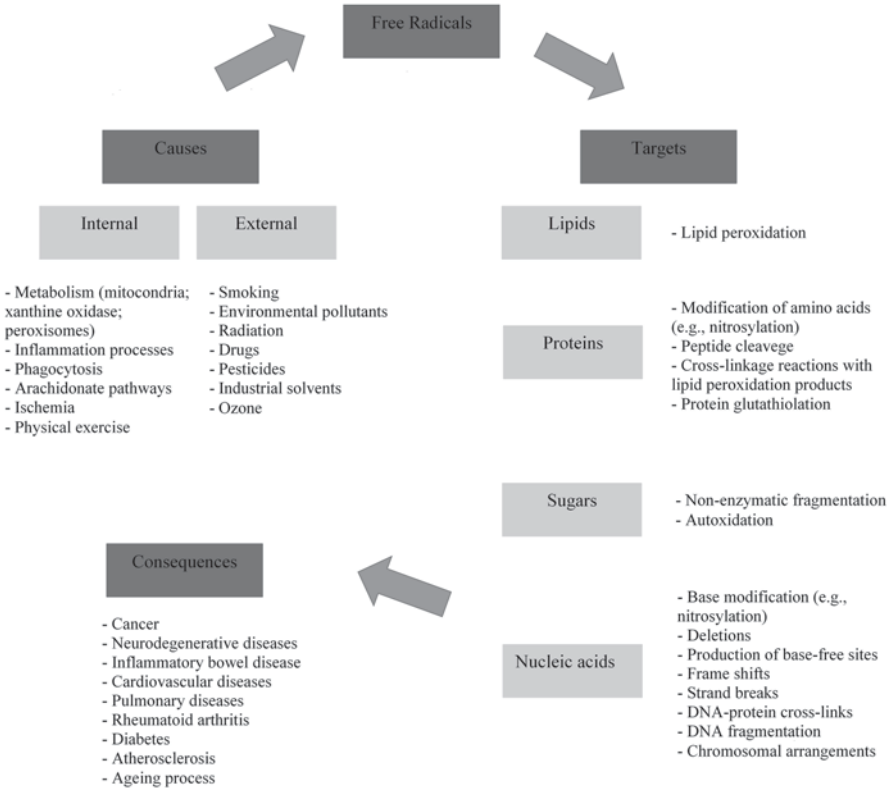

Fig. 10.1 Internal and external causes for overproduction of free radicals, main cellular targets, and related consequences

transition metals to H_2O_2), with the latter considered the most toxic ROS (Valko et al. 2007; Ferreira et al. 2009; Flora 2009; Carocho and Ferreira 2013). In the presence of H_2O_2 and the chloride ion (Cl^-), the enzyme myeloperoxidase produces OCl^- and then HOCl; the latter species can generate $HO^\bullet$ by reacting with $O_2^{-\bullet}$ or Fe^{2+}. OCl^- can also react with H_2O_2 to make singlet O_2 ($^1O_2$) (Halliwell 2006). Lipid peroxidation promotes the production of different types of ROS such as $R^\bullet$ that can react with O_2 to form $ROO^\bullet$; if not neutralized, these radicals react with other adjacent lipids producing hydroperoxide lipids (ROOH) that can easily be decomposed to form new $R^\bullet$, initiating a process that is known as chain propagation reaction (Ferreira et al. 2009).

Table 10.1 also lists the reactive nitrogen species (RNS), including nitric oxide radical ($NO^\bullet$), generated in biological tissues by specific nitric oxide synthases (NOS) that transform arginine to citrulline. $NO^\bullet$ reacts with $O_2^{\bullet-}$ to yield peroxynitrite ($ONOO^-$), a non-radical RNS (Ghafourifar and Cadenas 2005; Ferreira et al. 2009).

As shown in Fig. 10.1, reactive species are produced in mitochondria or peroxisomes within metabolic processes or by xanthine oxidase activity, inflammation processes, phagocytosis, arachidonate pathway, ischemia, and physical exercise. Smoking, environmental pollutants, radiation, drugs, pesticides, industrial solvents,

and ozone are examples of external factors that promote the production of free radicals (Halliwell 2011; Carocho and Ferreira 2013).

The main targets of reactive species are lipids, proteins, sugars, and nucleic acids (Lü et al. 2010). Lipid peroxidation (attack on membrane lipids) occurs mainly as a result of the action of $HO^{\bullet}$ or $^{1}O_{2}$, but also of $ONOO^{-}$. Proteins can be oxidatively modified in specific amino acids (e.g., nitrosylation with $NO^{\bullet}$) by free radical-mediated peptide cleavage or by formation of protein cross-linkages due to reaction with lipid peroxidation products (Ferreira et al. 2009; Carocho and Ferreira 2013). In particular, *S*-nitrosation of glutathione (GSH) produces *S*-nitrosoglutathione (GSNO), which itself is capable of *S*-nitrosating cysteine residues in proteins to make cysteine-*S*-nitrosothiol (Table 10.1). Protein glutathionylation is a prominent consequence of RSS exposure and consists in the redox reaction of protein cysteinyl residues with the tripeptide glutathione, resulting in a protein–glutathione mixed disulfide (Giles et al. 2001; Gruhlke and Slusarenko 2012). The formation of ROS could also contribute to glycoxidative damage; during the initial stages of nonenzymatic glycosylation, sugar fragmentation produces short-chain species such as glycolaldehyde, whose chain is too short to cyclize and is therefore prone to autoxidation (Benov and Beema 2003; Carocho and Ferreira 2013). The damage in nucleic acids induced by reactive species includes production of base-free sites, deletions, modification of all bases, frame shifts, strand breaks, DNA–protein cross-links, and chromosomal arrangements. $HO^{\bullet}$ is known to react with all the components of the DNA molecule, intervening also in DNA oxidation, whereas $ONOO^{-}$ is related to DNA fragmentation (Ferreira et al. 2009; Carocho and Ferreira 2013).

The damage to cells and tissues caused by reactive species, mostly ROS, is called oxidative damage and is a consequence of oxidative stress, a serious imbalance between the generation of ROS and antioxidant protection in favor of the former (Halliwell 2012). Recently, the mentioned author answered the question "does the oxidative stress that is likely to occur as a result of the tissue damage play any role at all in the disease pathology?", with a firm yes for cancer and neurodegenerative diseases, with a probably yes for inflammatory bowel disease, rheumatic arthritis, chronic granulomatous disease, and with a maybe for atherosclerosis and diabetes.

10.1.2 Endogenous Antioxidant Defenses

As shown in Fig. 10.2, humans produce many endogenous antioxidant systems (enzymes such as SOD (superoxide dismutases), CAT (catalases), Prx (peroxiredoxins), GPx (glutathione peroxidase), GRed (glutathione reductase) and GST (glutathione-S-transferases), or nonenzymatic antioxidant defenses, namely GSH (reduced glutathione), Q10, and uric acid) but also obtain some other antioxidants from the diet, such as vitamin E, vitamin C, polyphenols, and carotenoids (Halliwell 2011; Carocho and Ferreira 2013).

SOD converts $O_{2}^{\bullet -}$ into $H_{2}O_{2}$ through a dismutation reaction, which is then detoxified to water either by CAT in the peroxisomes or by GPx in the mitochon-

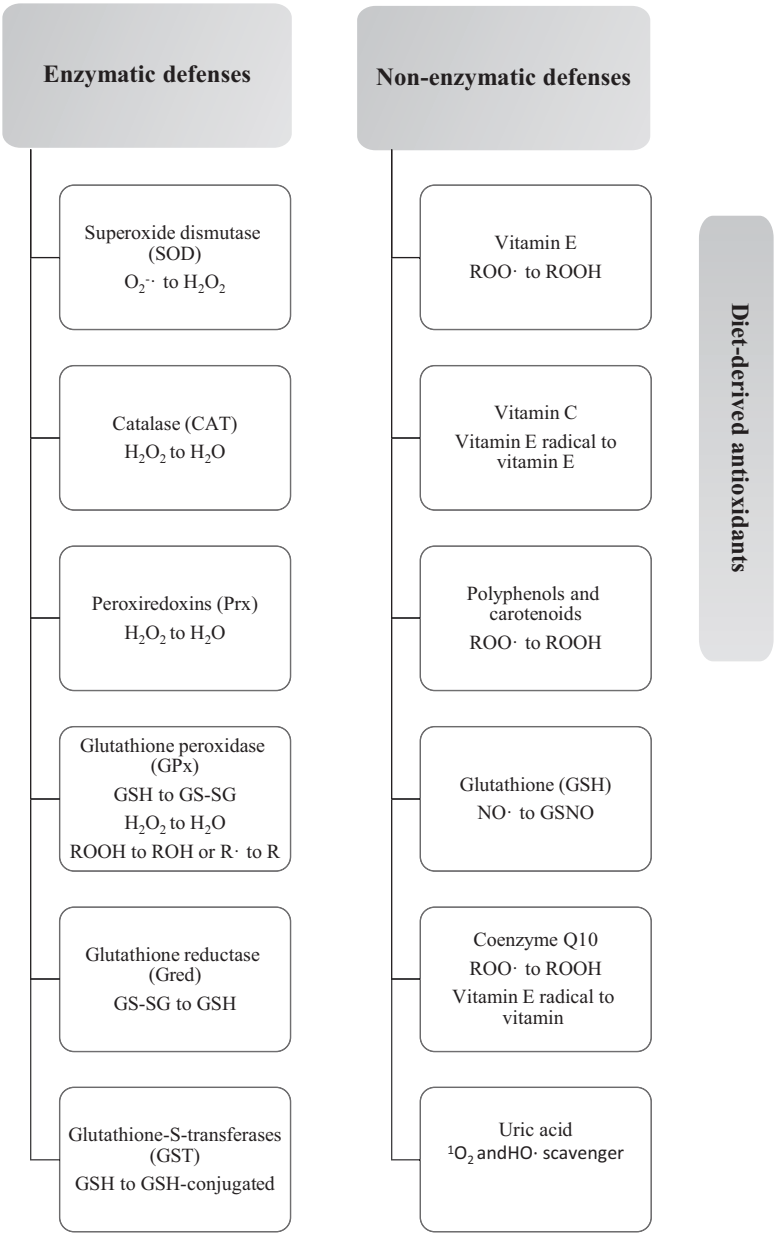

Fig. 10.2 Major endogenous and exogenous enzymatic and nonenzymatic antioxidant defenses. *GS-SG* glutathione disulphide, *GSNO* S-nitrosoglutathione, *R* (e.g., lipid)

dria, cytosol, or nucleus. Other enzymes that reduce H_2O_2 are peroxiredoxins (with cysteine in the active site). GRed regenerates GSH that is used as a hydrogen donor by GPx; the latter can also transform hydroperoxide lipids into alcohols (ROH).

GSH effectively scavenges ROS ($HO^{\bullet}$, H_2O_2, $LOO^{\bullet}$ and $ONOO^{-}$) either directly or indirectly as a cofactor of several detoxifying enzymes, for example, GPx and GST. In the neutralization process of ROS, GSH is oxidized to glutathione disulphide (GS-SG), which can be further reduced to two GSH molecules by the enzyme GRed. GSH is also able to regenerate other antioxidant molecules such as vitamins C and E and react with a variety of electrophilic xenobiotics in reactions catalyzed by GST, generating products with higher solubility (thus easier to eliminate). Finally, GSH can also neutralize $NO^{\bullet}$, resulting in the formation of S-nitrosoglutathione (GSNO; Valko et al. 2007; Ferreira et al. 2009).

Coenzyme Q10 acts by preventing the formation of or by neutralizing lipid peroxyl radicals, but its ability to regenerate vitamin E has also been reported. Uric acid prevents the overproduction of oxo-heme oxidants that result from the reaction of hemoglobin with peroxides. It also prevents the lysis of erythrocytes by peroxidation, and it is a potent scavenger of $HO^{\bullet}$ or $^1O_2$ (Carocho and Ferreira 2013).

10.1.3 Contribution of Plants as Exogenous Antioxidant Defenses

Plants, used since ancient times due to their medicinal properties, may be considered as a source of bioactive compounds with antioxidant potential. These properties have been studied in the past few years, proving their potential to act as functional foods (Krishnaiah et al. 2011).

As shown in Fig. 10.2, different compounds, such as vitamin E, vitamin C, polyphenols, and carotenoids, have been reported to help the endogenous antioxidant defense system as exogenous sources. Vitamin E, a liposoluble vitamin present in the membranes, plays an important role in the prevention of lipid peroxidation. ROS (e.g., $HO^{\bullet}$ and $LOO^{\bullet}$) react with vitamin E, generating vitamin $E^{\bullet}$. Then, vitamin C reacts with vitamin $E^{\bullet}$ (Fig. 10.3) producing vitamin $C^{\bullet}$ (that could be eliminated by semidehydroascorbate reductase), regenerating vitamin E. Both radicals (vitamin $E^{\bullet}$ and vitamin $C^{\bullet}$) are poorly reactive species (Ferreira et al. 2009).

The antioxidant properties of polyphenols, mostly flavonoids and phenolic acids, are conferred by the phenolic hydroxyl groups attached to the ring structures. They can act as reducing agents; hydrogen donators; singlet oxygen quenchers; peroxynitrites, superoxide, hydroxyl, and peroxyl radical scavengers; and even as metal chelators. They also activate antioxidant enzymes, reduce vitamin E radicals,

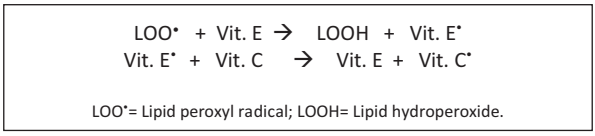

Fig. 10.3 Vitamin E regeneration mediated by vitamin C

inhibit oxidases, mitigate nitrosative stress, and increase levels of uric acid and low-molecular-weight molecules (Procházková et al. 2011; Carocho and Ferreira 2013).

The main antioxidant potential of carotenoids is due to singlet oxygen quenching. The only free radicals that completely destroy these pigments are peroxyl radicals. Carotenoids are relatively unreactive but may also decay and form nonradical compounds that may terminate free radical attacks by binding to these radicals (Paiva and Russel 1999; Carocho and Ferreira 2013).

According to Halliwell (2012), our endogenous antioxidant defenses are inadequate to prevent oxidative damage completely; hence, dietary sources of antioxidants are especially important to avoid diseases related to oxidative stress. Nevertheless, the contribution of some of them (e.g., polyphenols and carotenoids) to the beneficial dietary effect of plants is uncertain, as suggested by the limited and confusing literature on their in vivo effects, except possibly in the stomach, small intestine, and colon (Halliwell 2011, 2012).

The extracts obtained from plant materials (whole herb or roots, young stems, leaves, basal leaves, shoots, aerial parts, flower buds, flowers, inflorescences, fruits, seeds, and wood) might be used as antioxidants due to the chemical diversity of their phytochemicals and synergistic effects. In fact, the beneficial effects of diet-derived antioxidants may be maximally exerted when they are consumed at currently recommended dietary intakes, rather than in large amounts. Plants are a rich source of antioxidants, nonetheless the protective effect may not be the same by pulling up one or two individual antioxidant molecules into a high-dose pill (Halliwell 2012).

In this perspective, the antioxidant potential of several plants from Portugal and Spain has been extensively reviewed (Barros et al. 2009, 2010a, b, 2011a, b, c; Martins et al. 2011; Morales et al. 2012, 2013a, b; Pereira et al. 2011), revealing very promising results.

10.1.4 Measurement of Antioxidant Activity in Plants

Regarding the study of antioxidant activity in plants, there is not one method that can provide unequivocal and defining results, which necessitates the use of various methods instead of a one-dimension approach; each method has its specific target within the matrix and its advantages and disadvantages. Some of these procedures use synthetic antioxidants or free radicals; some are specific for lipid peroxidation and require animal or plant cells (Carocho and Ferreira 2013).

One of the methods most frequently used to evaluate the antioxidant properties of different wild edible plants is the Folin–Ciocalteu assay. This method has been often used to evaluate total phenolic content in natural products. However, as it is based on the measurement of the reducing capacity of a sample, nowadays it is being used for antioxidant capacity determination (Huang et al. 2005). It follows the reaction below:

$$\mathrm{Mo(VI)(yellow)} + \mathrm{e-} \rightarrow \mathrm{Mo(V)(blue)}. \tag{10.1}$$

Molybdotungstate (Mo) reagent oxidizes phenols and yields a colored product with an absorption maximum at 745–750 nm. The reagent contains heteropolyphosphotungstates-molybdates that, under basic conditions, react with phenolic compounds to form a phenolate anion, possibly (phenol-$MoW_{11}O_{40})^{4-}$, by dissociation of a phenolic proton. This sequence of reversible one- or two-electron reduction reactions leads to blue-colored products (Huang et al. 2005; Prior et al. 2005).

The screening of antioxidant properties can also be measured using chemical assays (i and ii) or assays related to lipid peroxidation (iii and iv):

i. DPPH-scavenging activity:

$$X^{\bullet} + AH \rightarrow XH + A^{\bullet} \tag{10.2}$$

where $X^{\bullet}$ represents a DPPH radical and AH represents antioxidants present in the sample (plant tissue). Antioxidants donate a hydrogen atom to the DPPH radical, decreasing its absorbance at 517 nm (Antolovich et al. 2002).

ii. Reducing power:

$$Fe(CN)_6^{3-} + AH \rightarrow Fe(CN)_6^{4-} + AH^{+} \tag{10.3}$$

$$Fe(CN)_6^{4-} + Fe^{3+} \rightarrow Fe\left[Fe(CN)_6\right]^{-} \tag{10.4}$$

where $Fe(CN)_6^{3-}$ is the compound with the ferric form, and $Fe(CN)_6^{4-}$ is the compound with the ferrous form. Antioxidants present in the wild plants transfer an electron to ferricyanide complex, reducing Fe^{3+} to Fe^{2+}. The second reaction allows the measurement of the absorbance at 700 nm; higher absorbance corresponds to higher reducing power (Huang et al. 2005; Prior et al. 2005).

iii. β-carotene bleaching inhibition:

$$\beta - \text{carotene} - H(\text{orange}) + LOO^{\bullet} \rightarrow \beta - \text{carotene}^{\bullet}(\text{bleached}) + LOOH \tag{10.5}$$

$$\beta - \text{carotene} - H(\text{orange}) + LOO^{\bullet} + AH \rightarrow \beta - \text{carotene} - H(\text{orange}) + LOOH + A^{\bullet} \tag{10.6}$$

where $LOO^{\bullet}$ represents the linoleate free radical. Antioxidants present in the plants donate a hydrogen atom neutralizing the linoleate free radical formed in the system avoiding its attack on the highly unsaturated β-carotene and therefore inhibiting β-carotene bleaching (Prior et al. 2005).

iv. TBARS formation inhibition:

$$MDA + TBA \rightarrow MDA - TBA_2 \tag{10.7}$$

$$\mathrm{MDA} + \mathrm{TBA} + \mathrm{A} \rightarrow \mathrm{MDA} + \mathrm{TBA}_2 \quad (10.8)$$

where MDA represents malondialdehyde and TBA, thiobarbituric acid. The antioxidants present in the sample (plant tissue) will inhibit the formation of the MDA-TBA_2 complex. The TBARS assay measures the MDA formed as the split product of an endoperoxide of unsaturated fatty acids resulting from oxidation of a lipid substrate. The MDA reacts with TBA to form a pink pigment that is measured spectrophotometrically at 532 nm (Fernández et al. 1997).

As different methods measure the antioxidant activity through different mechanisms, the combined analysis of samples through different assays is often needed to have an overall idea of the properties of a given sample. Therefore, the interpretation of the data of antioxidant activity of foods is complex, because of the variety of assays available, and the different units used for expression of the results make comparison of the data difficult.

In this chapter, data about antioxidant properties of 32 different wild species widespread in the Mediterranean area have been reviewed and compared and information is gathered in Tables 10.2, 10.3, 10.4, and it will be carefully reviewed in the followed sections. For that purpose, they have been grouped according to the different plant parts traditionally used.

10.2 Overview of the Wild Food Plants with Antioxidant Potential

Since prehistoric times, when our ancestors used hunting and gathering to provide sustenance, wild food plants have played a central role in human diet and nutrition.

All over the world, wild edibles have been used as dietary supplements and are particularly important in times of famine and food shortage. Other plants were used to preserve food and for seasoning regional and traditional recipes. Natural flavors of some species have lent flavor to very poor, insufficient, and monotonous daily meals for decades. Therefore, these plants and the knowledge and practices associated with them are part of an interesting biocultural heritage (Carvalho 2010; Carvalho and Morales 2010; Barros et al. 2010a, 2011a, d).

Demand for natural products and ingredients of high quality has drawn people's attention to wild edibles, and in many different regions, their use and consumption are becoming more widespread (Łuczaj et al. 2012).

In recent years, experimental research based largely on ethnobotanical surveys and empirical traditional knowledge has shown that wild plants are interesting sources of nutrients and phytochemicals (see also Chap. 9), with significant antioxidant properties (e.g. Barros et al. and Morales et al. cited works) that prevent various illnesses, especially age-related diseases (The Local Food-Nutraceuticals Consortium 2005; Guarrera and Savo 2013).

Table 10.2 Antioxidant activity of several wild vegetables: wild leafy vegetables and wild asparaguses

Species	Origin	Folin–Ciocalteu (mg GAE/g dry extract)	EC_{50} values (mg dry extract/mL methanol)				References
			DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	TBARS inhibition	
Anchusa azurea Mill.	Spain	148.62±2.00	0.02±0.00	0.01±0.00	0.02±0.00	0.03±0.00	1
Apium nodiflorum (L.) Lag.	Spain	80.47±4.41	0.07±0.00	0.02±0.00	0.02±0.00	0.04±0.00	2
Beta maritima L.	Spain	61.91±7.51	1.35±0.03	0.47±0.00	0.38±0.00	0.05±0.00	1
Borago officinalis L.	Italy	97±1.03[a]	0.06±0.00[a]	–	0.004[a,b,c]	0.04±0.00[a]	3
	Portugal	113.58±0.92	0.07±0.00	0.23±0.01	0.13±0.02	0.14±0.00	4
Chondrilla juncea L.	Spain	37.66±2.40	1.64±0.15	0.34±0.01	0.38±0.02	0.12±0.00	1
Cichorium intybus L.	Italy	190.00±2.03	0.026[c]	–	0.10[b,c]	0.074[c]	5
	Spain	73.68±0.66	1.11±0.05	0.57±0.01	0.45±0.01	0.02±0.00	1
Foeniculum vulgare Mill. leaves	Italy	80±0.95	0.148[c]	–	0.046[b,c]	0.244[c]	5
	Portugal	39.49±0.62	6.88±0.70	1.17±0.07	1.14±0.03	0.22±0.02	6
F. vulgare young stems with leaves	Portugal	65.85±0.74	1.34±0.07	0.48±0.02	0.49±0.03	0.13±0.03	6
	Spain	42.16±0.98	2.75±0.06	1.10±0.02	0.47±0.00	0.02±0.00	2
F. vulgare stems	Portugal	8.61±0.09	12.16±0.94	2.82±0.04	2.38±0.12	0.27±0.01	6
Glechoma hederacea L.	Portugal	196.61±6.09	0.39±0.02	0.22±0.00	0.87±0.10	0.11±0.01	7
Montia fontana L.	Portugal	47.47±1.62	0.22±0.01	0.84±0.02	0.46±0.04	0.25±0.01	4
	Spain	75.53±7.05	1.49±0.07	0.36±0.01	0.48±0.01	0.02±0.00	2
Papaver rhoeas L.	Italy	72±0.76	0.049[c]	–	0.007[b,c]	0.283[c]	5
	Spain	25.86±3.52	1.28±0.03	0.40±0.00	0.56±0.11	0.02±0.00	1
Rorippa nasturtium-aquaticum (L.) Hayek	Portugal	50.42±2.77	0.13±0.03	0.74±0.02	0.85±0.16	0.38±0.06	4
	Turkey	74.18±1.72[d]	0.287[c]	0.20[c]	–	–	8
Rumex acetosella L.	Portugal	141.58±3.67	0.03±0.00	0.16±0.01	0.12±0.01	0.11±0.02	4
Rumex induratus Boiss. & Reut.	Portugal	117.08±2.54	0.03±0.00	0.22±0.01	0.19±0.03	0.10±0.01	4

Table 10.2 (Continued)

Species	Origin	Folin–Ciocalteu (mg GAE/g dry extract)	EC_{50} values (mg dry extract/mL methanol)				References
			DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	TBARS inhibition	
Rumex papillaris Boiss. & Reut.	Spain	104.18±4.17	2.45±0.09	0.60±0.01	0.30±0.01	0.03±0.00	1
Rumex pulcher L.	Spain	73.44±5.32	3.31±0.10	0.84±0.01	0.34±0.00	0.02±0.00	1
Scolymus hispanicus L.	Spain	21.51±1.51	4.97±0.08	5.97±0.04	0.65±0.01	0.04±0.00	1
Silene vulgaris (Moench) Garcke	Spain	26.72±1.63	3.31±0.07	0.84±0.01	0.62±0.08	0.02±0.00	2
Silybum marianum (L.) Gaertn.	Spain	3.72±0.36	13.09±0.04	1.82±0.01	0.44±0.03	0.02±0.00	1
Sonchus oleraceus L.	Italy	61±0.65	0.164[c]	–	0.065[b,c]	0.435[c]	5
	Spain	51.33±1.75	1.36±0.02	0.89±0.05	0.03±0.00	0.05±0.00	1
Taraxacum obovatum (Willd.) DC.	Spain	58.26±0.90	0.79±0.10	0.48±0.01	0.37±0.00	0.07±0.00	1
Asparagus acutifolius L.	Portugal	623.73±27.68	0.42±0.02	0.19±0.01	0.17±0.01	0.10±0.00	9
	Spain	17.60±0.29	4.87±0.38	1.62±0.00	0.47±0.04	0.07±0.02	2
Bryonia dioica Jacq.	Portugal	258.24±21.95	0.64±0.05	0.20±0.01	0.37±0.01	0.20±0.01	9
	Spain	35.10±2.43	4.43±1.29	1.44±0.01	0.47±0.03	0.08±0.01	2
Humulus lupulus L.	Spain	55.83±1.34	1.36±0.02	0.80±0.01	0.48±0.02	0.03±0.00	2
Tamus communis L.	Portugal	758.99±28.96	0.20±0.03	0.07±0.00	0.07±0.01	0.09±0.01	9
	Spain	49.51±4.07	3.59±0.93	1.32±0.01	0.49±0.15	0.05±0.01	2

GAE gallic acid equivalents; [a] ethanolic extract; [b] data obtained after 60 min of incubation; [c] data was showed as graphical values; [d] μg pyrocatechol/1000 mg extract. Data expressed as mean ± standard deviation. Plant names according to Flora Iberica (http://www.floraiberica.es), except for Asteraceae, which is according to The Plant List (2010) (http://www.theplantlist.org). References: 1:Morales et al. 2013a; 2:Morales et al. 2012; 3:Conforti et al. 2008; 4:Pereira et al. 2011; 5:Conforti et al. 2009; 6:Barros et al. 2009; 7:Barros et al. 2010a; 8:Özen 2009; 9:Martins et al. 2011.

Table 10.3 Antioxidant activity of several wild plants used for seasoning and flavoring

Species	Origin	Folin-Ciocalteu (mg GAE/g dry extract)	EC_{50} values (mg dry extract/mL methanol)				References
			DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	TBARS inhibition	
Foeniculum vulgare Mill.	Portugal	34.68±0.74	7.72±0.87	1.02±0.02	1.29±0.03	0.25±0.01	Barros et al. 2009
Mentha pulegium L.	Portugal	331.69±19.63	0.56±0.05	0.12±0.01	0.01±0.00	0.08±0.00	Fernandes et al. 2010
	Portugal	71.7±2.1[a,b]	0.025±0.00	–	0.165±0.00[b]	–	Mata et al. 2007
	Portugal	6[c]	0.1[c]	<0.140[c]	–	–	Teixeira et al. 2012
	Spain	–	0.037±0.00	–	–	–	Lopéz et al. 2007
Origanum vulgare L.	Portugal	368.58±18.18	0.16±0.03	0.18±0.00	0.45±0.05	0.01±0.00	Barros et al. 2010a
	Portugal	13.5±0.3[b]	0.233±0.06[b]	–	–	–	Teixeira et al. 2013
	Serbia	135±1.08[b]	0.035±0.00[b]	–	–	–	Ličina et al. 2013
	Spain	–	0.186±0.00	–	–	–	Lopéz et al. 2007
	Spain	120	0.014±0.00	–	–	–	Rodríguez-Meizoso et al. 2006
	Turkey	220	0.01±0.00	–	3.125[c]	–	Şahin et al. 2004
Rosa canina L.	Portugal	270.28±35.54	0.22±0.01	0.24±0.03	0.12±0.03	0.03±0.00	Barros et al. 2011a
Sambucus nigra L.	Portugal	92.73±4.66	0.57±0.03	0.27±0.01	0.16±0.01	0.12±0.01	Barros et al. 2011b
Thymus mastichina L.	Portugal	165.29±1.11	0.69±0.04	0.23±0.00	0.90±0.09	0.43±0.02	Barros et al. 2010a

GAE gallic acid equivalents

[a] mg pyrogallol per g of sample

[b] ethanol extract

[c] Data were showed as graphical values. Data expressed as mean ± standard deviation. Plant names according to The Plant List (2010) (http://www.theplantlist.org/)

Table 10.4 Antioxidant activity of edible fruits of several wild edible plants

Species	Origin	Folin-Ciocalteu (mg GAE/g dry extract)	EC_{50} values (mg dry extract/mL methanol)				References
			DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	TBARS inhibition	
Arbutus unedo L.	Algeria	104.98±4.59[a]	0.006±0.00[a]	0.001±0.00[a]	–	NA	Boulanouar et al. 2013
	Italy	922±38[b]	4.5±1.1[c]	–	–	–	Tuberoso et al. 2013
	Portugal	126.83±6.66	0.45±0.00	0.41±0.00	0.77±0.00	0.09±0.00	Barros et al. 2010b
	Portugal	14.6±0.9	–	–	–	–	Silva et al. 2001
	Portugal	48.26±4.49	0.37±0.02	1.09±0.05	–	–	Oliveira et al. 2011
	Portugal	16.7±0.4	0.790±0.016	2.894±0.049	0.732±0.452	–	Mendes et al. 2011
	Spain	–	–	–	0.10±0.00	0.03±0.00	Morales et al. 2013b
	Spain	16.53	–	–	–	–	Ruiz-Rodriguez et al. 2011
	Spain	586±53[d]	0.59±0.18	–	–	–	Ganhão et al. 2010
Crataegus monogyna Jacq.	France	1226.3±33.7[e]	5.40±0.40[e]	–	–	–	Froehlicher et al. 2009
	Lithuania	182	0.2[f]	–	–	–	Bernatonienė et al. 2008
	Portugal	247.03±9.32	0.13±0.01	0.08±0.00	0.10±0.01	0.05±0.00	Barros et al. 2011c
	Serbia	35.4±2.48[g]	1.470±0.00[g]	–	–	–	Tadić et al. 2008
	Spain	–	–	–	0.02±0.00	0.02±0.00	Morales et al. 2013b
	Spain	820.55[d]	1.54[h]			–	Ruiz-Rodriguez et al. 2014
	Spain	600±105[d]	0.70±0.16	–	–	–	Ganhão et al. 2010

Table 10.4 (Continued)

Species	Origin	Folin-Ciocalteu (mg GAE/g dry extract)	EC_{50} values (mg dry extract/mL methanol)				References
			DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	TBARS inhibition	
Prunus spinosa L.	Portugal	83.40 ± 2.75	0.60 ± 0.00	0.61 ± 0.00	0.99 ± 0.00	0.15 ± 0.00	Barros et al. 2010b
	Spain	–	–	–	0.09 ± 0.00	0.02 ± 0.00	Morales et al. 2013b
	Spain	326 ± 29[d]	1.98 ± 0.32	–	–	–	Ganhão et al. 2010
	Spain	2255.57[d]	1.14[h]	–	–	–	Ruiz-Rodriguez et al. 2014
Rosa canina L.	Portugal	143.17 ± 5.25	0.43 ± 0.00	0.17 ± 0.00	0.40 ± 0.00	0.09 ± 0.00	Barros et al. 2010b
	Spain	2377 ± 492[d]	0.18 ± 0.05	–	–	–	Ganhão et al. 2010
Rubus ulmifolius Schott	Spain	–	–	–	0.03 ± 0.00	0.02 ± 0.00	Morales et al. 2013b
	Spain	871 ± 80[d]	0.41 ± 0.05	–	–	–	Ganhão et al. 2010

NA not active. Data expressed as mean ± standard deviation. Plant names according to The Plant List (2010) (http://www.theplantlist.org/)
GAE gallic acid equivalents
[a] Hydro-alcoholic extract (70% ethanol)
[b] mg GAE/L
[c] DPPH (mmol TEAC/L) results are expressed as TEAC millimolar concentration, obtained from a Trolox solution having an antiradical capacity equivalent
[d] mg of GAE/100 g of fruit fresh matter
[e] Results expressed in dry weight basis
[f] Around 0.2 g/mL of ethanolic extract provide around 50% of inhibition effect; the measure was made after 5 min of incubation
[g] Ethanolic extract
[h] mmol of Trolox Eq/100 g fw

10.2.1 Wild Species Providing Vegetables

The Mediterranean flora is a combination of taxa of various biogeographical origins and evolutionary histories effected over time by climatic events and anthropogenic actions with increasing impact (Cowling et al. 1996). Besides floristic diversity, the richness of habitats, different cultures and mores, and landscape management and historic development around the Mediterranean Basin resulted in the common use of many different plants that met basic dietary needs and are interesting food resources, well adapted to local diets and folk traditions (Rivera et al. 2005; Hadjichambiset al. 2008).

Although it is reported that some tree organs (leaves, young shoots, and flowers) are also used (e.g. Tardío et al. 2006), vegetables are mainly the edible product of herbaceous plants and can be roots or underground stems (tubers, bulbs, or rhizomes), whole immature plants (sprouts), stems, whorled basal leaves, expanded leaves, leaf sheaths, midribs and veins, flower heads, and unripe fruits and seeds. Green, vibrant colored or yellowish white, vivid flavored or having very little taste, these edibles are traditionally used raw or cooked for preparing soups, broths, stews, stir-fries, accompaniments, salads, and sometimes desserts.

Several botanical families provide leafy vegetables that play a significant role in different local Mediterranean cuisines and have interesting phytochemical profiles and promising bioactive properties (Guarrera and Savo 2013). Therefore, species of the Asteraceae, Polygonaceae, Brassicaceae, and Amaryllidaceae families are some of the most gathered and consumed edible greens at least in the three large southern European peninsulas (e.g. Pieroni et al. 2005; Tardío et al. 2006; Hadjichambis et al. 2008; Łuczaj et al. 2012). Some examples are basal leaves, midribs, and soft leafy stems of genera such as *Chondrilla* L., *Cichorium* L., *Hypochaeris* L., *Scolymus* L., *Scorzonera* L. and *Sonchus* L. and species from Carduoideae and Asteroideae subfamilies; leaves of docks and sorrels (genus *Rumex* L.); tender leaves, stems and small flowers from annuals such as *Eruca vesicaria* (L.) Cav., *Raphanus raphanistrum* L., *Capsella bursa-pastoris* (L.) Medik., mustards and wild cabbage; the bulbs, bulbils and linear, channelled or flat leaf blades of *Allium* species (see Chaps. 4 and 13).

Sweet immature pods and seeds from wild species of the Fabaceae family (e.g. genera *Vicia* L., *Lathyrus* L., *Astragalus* L.) are frequently considered as organoleptically interesting wild food (Carvalho and Telo 2012; Tardío et al 2006) with perceived health benefits.

Other noteworthy leafy vegetables are the vernal leaves of some species of Amaranthaceae (genera *Atriplex* L. and *Chenopodium* L.), Boraginaceae (e.g. *Borago officinalis* L. and *Anchusa* sp. pl.), and Apiaceae (such as *Foeniculum vulgare* Mill. and *Apium nodiflorum* (L.) Lag.).

Small amounts of many of these edibles can safely be eaten raw. Different cooking processes such as soaking or boiling are, sometimes, able to remove most traces of different toxins and alkaloids, for instance. However, some plants have both edible and toxic parts (e.g. bryonies); other botanical families such as Apiaceae have

species that can be very poisonous. Many leafy greens have high oxalic acid content (e.g. sorrels), and some accumulate chemicals from several contaminants. Thus, consumers must avoid potential hazards, act with extreme caution, and be informed about the risks and learn how to distinguish between edible and poisonous species, organs, or parts of plants.

Data of antioxidant activity in basal leaves as well as leaves accompanied by other aerial parts of 17 different edible species are shown in Table 10.2.

Anchusa azurea Mill. (bugloss) is a Boraginaceae, commonly consumed after cooking as well as for medicinal purposes against gastralgia, cold, kidney stones, pain, and skin problems (Benítez et al. 2008; Carvalho and Morales 2010; Carvalho 2010; Tardío 2010), and samples of this species gathered from Spain presented the lowest EC_{50} values (highest antioxidant activity) for DPPH scavenging activity and reducing power (0.02 and 0.01 mg/mL, respectively) (Morales et al. 2013) when compared with all the plant species shown in Table 10.2. Leaves of *Borago officinalis* L. (borage, also a consumed wild Boraginaceae) from Italy registered the best results in β-carotene bleaching inhibition assays (0.004 mg/mL), according to Conforti et al. (2008).

For the TBARS assay, the leaves of *Cichorium intybus* L. (chicory), *Papaver rhoeas* L. (poppy), *Rumex pulcher* L. (fiddle dock), and *Silybum marianum* (L.) Gaertn. (milk thistle), also gathered from Spain, presented the lowest EC_{50} values (0.02 mg/mL; Morales et al. 2013). Traditionally, most of them are eaten cooked; however, they are sometimes boiled and fried in olive oil with garlic, as the fleshy midribs of *Silybum marianum. Cichorium intybus* and *Papaver rhoeas* are eaten raw in salads as well. Chicory and poppy are also used in traditional medicine due to their effectiveness in digestive disorders, nervousness, insomnia, respiratory disorders, among others (Benítez et al. 2008; Carvalho and Morales 2010; Carvalho 2010; Tardío 2010). Furthermore, *Glechoma hederacea* L. from Portugal revealed the highest phenolic content (196.61 mg GAE/g).

Borago officinalis samples obtained from Italy had lower phenolic content (97 mg chlorogenic acid equivalents/g extract) but slightly higher DPPH scavenging activity and β-carotene bleaching inhibition capacity (Conforti et al. 2008) when compared with the samples of the same species obtained from Portugal (Pereira et al. 2011).

Cichorium intybus samples obtained from Italy presented higher phenolic content, expressed in gallic acid equivalents (GAE), DPPH scavenging activity, and β-carotene bleaching inhibition (190 mg GAE/g; 0.026 and 0.10 mg/mL, respectively) (Conforti et al. 2009) than those gathered from Spain and Greece. Moreover, chicory obtained from Spain had the highest reducing power and TBARS formation inhibition (0.57 and 0.02 mg/mL, respectively). *Foeniculum vulgare* Mill. (fennel), *Papaver rhoeas* L. and *Sonchus oleraceus* L. (smooth sow thistle) from Italy presented the highest DPPH scavenging activity (0.148, 0.049 and 0.164 mg/mL, respectively) and β-carotene bleaching inhibition capacity (0.046, 0.007 and 0.065 mg/mL, in that order) when compared with the same species from other countries (Table 10.2). Fennel and poppy leaves also presented the highest activity for TBARS assay (0.24 and 0.283 mg/mL, respectively), according to Conforti et al.

(2009), and *Papaver rhoeas* from Italy presented the highest levels of phenolics (72 mg GAE/g). *Rorippa nasturtium-aquaticum* (L.) Hayek (watercress) from Portugal showed higher phenolics (50.42 mg GAE/g extract) and DPPH scavenging activity (0.13 mg/mL) but lower reducing power (0.20 mg/mL) than the sample from Turkey (Özen 2009).

It is remarkable that the edible leaves of *Anchusa azurea, Borago officinalis, Cichorium intybus, Papaver rhoeas, Rumex pulcher*, and *Silybum marianum* exhibit higher antioxidant activity measured by one or more different assays than the other species. Special attention should be paid to *Anchusa azurea* leaves gathered in Spain (Morales et al. 2013), which revealed the highest antioxidant activity in all the assays reviewed.

10.2.2 Wild Species Providing Wild Asparaguses

Sprouts and young shoots (asparaguses) of different plants are also considered edible in southern countries. *Asparagus acutifolius* L. (Asparagaceae), *Bryonia dioica* Jacq (Cucurbitaceae), *Humulus lupulus* L. (Cannabaceae), *Tamus communis* L. (Dioscoreaceae), and *Rubus* species (Rosaceae) are some examples of these type of wild edibles usually gathered in early spring while tender and still lacking flower buds (Tardío et al. 2006; Carvalho 2010).

Data on antioxidant activity in young shoots of four wild Mediterranean species are also shown in Table 10.2. The highest phenolic content was found in *Tamus communis* L. (black bryony) from Portugal (758.99 mg GAE/g).

Asparagus acutifolius L. (wild asparagus), *Bryonia dioica* Jacq. (white bryony), and *Tamus communis* from Portugal (Martins et al. 2011) had higher antioxidant activity, with the exception of the TBARS assay, when compared with samples from Spain (Morales et al. 2012).

Edible parts of *Tamus communis* should be remarked for the coincident results found through the different antioxidant assays performed, revealing a high antioxidant potential.

10.2.3 Plants Used for Seasoning and Flavoring

Seasoning and preserving food are still common procedures that have an influence on the traditional cuisine and are fundamental to many regional recipes, particularly in rural areas. Many species with natural flavors are used as additives for enhancing the taste and smell of food but their bioactive properties are also important as food preserves (Dias and Dias 2006; Pardo-de-Santayana et al. 2007).

In former times, such species were related to nutritional needs, especially during those famine periods when wild edible plants were the main source of nourishment

for rural families. Moreover, different sauces and pastes were prepared with herbs and stored in glass bottles to use all through the year (Póvoa et al. 2009; Carvalho and Morales 2010). An interesting example is the traditional *piso* from southern Portugal, a paste made of *Mentha pulegium* L. or *M. cervina* L (Lamiaceae), crushed with garlic and salt and then covered with olive oil. Experimental assays proved that after a 6-month storage period, food sauces retained their physicochemical properties and could be used for seasoning (Póvoa et al. 2009).

At least in the Iberian Peninsula, Lamiaceae and Apiaceae are undoubtedly the botanical families providing a great number of species used as seasoning and flavoring agents although many others are also used, such as some Fabaceae like *Pterospartum tridentatum* (L.) Willk. and *Cytisus* sp. pl., *Alliaria petiolata* (M. Bieb.) Cavara & Grande (Brassicaceae) and *Allium ampeloprasum* L., *A. schoenoprasum* L. and *A. triquetum* L. (Amaryllidaceae) (Pardo-de-Santayana et al. 2007; Carvalho 2010). Many of these species are also included in the preparation of different fresh beverages, liqueurs, and herbal teas drunk daily or after meals (Carvalho 2010; Sõukand et al 2013)

Data regarding flowers and inflorescences of *Foeniculum vulgare, Mentha pulegium* L. (pennyroyal), *Origanum vulgare* L. (oregano), *Rosa canina* L. (dog rose), *Sambucus nigra* L. (elder), and *Thymus mastichina* L. (mastic thyme), widespread Mediterranean perennial herbs traditionally used for medicinal purposes and seasoning, are also listed in Table 10.3.

Of all the species mentioned in Table 10.3, *Mentha pulegium* and *Origanum vulgare* are two of the most studied species. *Mentha pulegium* from Portugal presented the highest reducing power and β-carotene bleaching inhibition capacity (0.12 and 0.01 mg/mL, respectively, Fernandes et al. 2010); *Origanum vulgare* from Portugal had the highest amount of total phenolics (368 mg GAE/g) and the lowest EC_{50} values (highest antioxidant activity) for the TBARS assay (0.01 mg/mL, Barros et al. 2010a), whereas the same species from Turkey presented the highest DPPH scavenging activity (0.01 mg/mL, Şahin et al. 2004). The flowers of these two species should be highlighted for their high antioxidant capacity measured by different assays, with coincidences among studies performed with samples from different origins, showing their higher antioxidant potential compared with flowers of other species.

10.2.4 Wild Edible Fruits

Regarding wild Mediterranean fruits, five wild species were reviewed: *Arbutus unedo* L. (strawberry-tree), *Crataegus monogyna* Jacq. (common hawthorn), *Prunus spinosa* L. (blackthorn), *Rosa canina* L., and *Rubus ulmifolius* Schott (blackberry), and the results are summarized in Table 10.4.

Arbutus unedo from Algeria presented the highest antioxidant potential for radical scavenging activity and reducing power (0.006 and 0.001 mg/mL, Boulanouar et al. 2013), but *Crataegus monogyna* from Spain showed the lowest EC_{50} values

for lipid peroxidation assays, such as β-carotene bleaching inhibition and TBARS assay (0.02 mg/mL, Morales et al. 2013a). The sample from Portugal revealed the highest phenolic content, expressed in mg per g of extract (247 mg GAE/g, Barros et al 2011c). The different units used for expression of the results of antioxidant activity of plant material make the comparison of data difficult (Table 10.4). However, *Arbutus unedo* and *Crataegus monogyna* are two of the fruits which reported the highest values in the Folin–Ciocalteu assay.

10.2.5 Underutilized and Underexploited Species

Many plant resources, growing as wild plants or that have been naturalized growing on their own, are well adapted to different ecological situations and have great potential to be exploited. This is the case of many leafy vegetables from the Fabaceae, Brassicaceae, and Amaryllidaceae families that have a surprising number of edible species and varieties. A number of them are naturalized from old crops and long-time introduced specimens. Lentils, peas and wormseed (*Chenopodium ambrosioides* L.) are some examples. Leeks and wild garlic, for instance, also have great potential that sometimes is forgotten and not used (Carvalho 2010).

Wild fruits and aromatic species used to be commonly preserved and stored for consumption during long and hard winters. These are species, such as *Rosa* sp. pl. and many others from woods, scrubland, riversides, and natural prairies or meadows, that have become underutilized because various staple products from the retail market or cultivated for daily meals are now offered or more accessible (Carvalho and Morales 2010). However, experimental research shows that some of them have great antioxidant potential (Barros et al. 2010b, 2011a, b).

In rural areas from the Iberian Peninsula, people have brought some of the most popular wild plants used as food additives and beverages from the wild to grow in their home gardens, in order to make them easily available (Carvalho and Morales 2010). This behavior shows that some species can be easily adapted to cultivation providing sustainable use without endangering wild populations (see Chap. 5).

10.3 Concluding Remarks

A widespread traditional use of many wild botanicals (e.g. leafy vegetables, flowers, fruits and seeds) as food was the starting point of experimental research on the antioxidant potential of several plants in Mediterranean regions. The diversity of phytochemicals, present in different edible parts of selected species, provides antioxidant properties with potential health benefits. Whenever possible, data obtained were systematically compared with other studies already published. The antioxidant activity results, using the same methodology, can be expressed differently (e.g. EC_{50}

or as trolox equivalents); therefore, in some cases it is very difficult to compare results.

Overall, leaves of *Anchusa azurea, Apium nodiflorum, Borago officinalis, Cichorium intybus, Papaver rhoeas, Rumex pulcher*, and *Silybum marianum*; young shoots of *Tamus communis*; flowers and inflorescences of *Mentha pulegium* and *Origanum vulgare*; and fruits of *Arbutus unedo* and *Crataegus monogyna* stand out among others for their antioxidant potential. Moreover, it can be highlighted that, in general, fruits were the most active plant part (EC_{50} values of all the assays ranged between 0.001 and 5.4 mg/mL), whereas in most cases, leaves reported lower antioxidant activity (EC_{50} values between 0.01 and 13 mg/mL).

There are also numerous publications of the antioxidant potential and bioactive compounds present in the same parts of the reviewed 32 species from other countries that do not belong to the Mediterranean. *Asparagus acutifolius* from Brazil (Tiveron et al. 2012), China (Shou et al. 2007), USA (Sun et al. 2007a and b), and Poland (Vinson et al. 1998); *Borago officinalis* from Lithuania (Bandoniene and Murkovic 2002; Bandoniene et al. 2005); *Crataegus monogyna* fruits from France (Froehlicher et al. 2009); *Foeniculum vulgare* from Iran (Motamed and Naghibi 2010); *Rorippa nasturtium-aquaticum* from Iran (Bahramikia and Yazdanparast 2010), Denmark (Justesen and Knuthsen 2001), Brazil (Hassimotto et al. 2009), and Australia (Lako et al. 2007); *Rosa canina* from Austria (Wenzig et al. 2008) and Denmark (Kirkeskov et al. 2011); *Sambucus nigra* from Austria (Rieger et al. 2008) and USA (Wu et al. 2004) are some examples of wild species studied out of the Mediterranean area.

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