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Treasure from garden: chemical profiling, pharmacology and biotechnology of mulleins

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Abstract The genus *Verbascum* (mulleins), belonging to the family Scrophulariaceae, comprises about 360 species of flowering plants. The leaves, flowers and whole aerial parts of *Verbascum* spp. have been widely used in traditional medicine for the treatment of respiratory and inflammatory disorders and also display powerful wound healing activity. *Verbascum* species are found to accumulate several groups of bioactive molecules, therefore they might be utilized as attractive sources of new (drug) leads. The present review attempts to provide an up-to-date comprehensive overview on phytochemical and pharmacological aspects of *Verbacum* spp. research along with some successful examples of growing (and transforming) mulleins in vitro.

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Introduction

Verbascum species, commonly known as "mulleins" (also named "koroviaks" in Russia and "sığirkuyruğu" in Anatolia), are well-known herbs with long-standing use in the folk medicine. Various applications of leaves, flowers and roots of *Verbascum* spp. for treatment of respiratory disorders, eczema, rheumatism, wounds and anal fistula in traditional Turkish medicine have been thoroughly descrbied (Baytop 1999; Tatli and Akdemir 2006). Moreover, mulleins are used in the European folk medicine as antiseptic, astringent and expectorant agents, and frequently applied in the treatment of inflammations, migraine, asthma and spasmodic coughs (Grieve 1995). Although mulleins have been used as remedy since ancient times, their popularity increased commercially in the past few years. Nowadays, the dried leaves and flowers, swallow capsules, alcoholic extracts and the flower oil of common mullein (*Verbascum thapsus* L.) can be found in the USA health stores (Turker and Gurel 2005). According to the assesment report of European Medicines Agency (EMEA; www.ema.europa.eu), written in 2008 on *V. thapsus*, *V. densiflorum* and *V. phlomoides* flos with

traditional use, on the European market *Verbascum* flowers are included in combination or in mono-preaparation products—herbal substances for tea preparation, liquid exracts prepared with ethanol or syrup products.

The genus *Verbascum* L. belongs to the Scrophulariaceae family. The Latin name *Verbascum* is considered to be a corruption of barbascum from the Latin 'barba' (beard) in allusion to the shaggy foliage and was bestowed on the genus by Linnaeus. Mulleins are biennial or perennial, rarely annual plants, with deep tap roots (Turker and Gurel 2005). According to Heywood (1993) it comprises about 360 species, predominantly distributed in Asia, Europe and North America. West and Central Asia (especially Anatolia) are the main centres of diversity of the genus (Kaynak et al. 2006). In Turkey, *Verbascum* is represented by about 232 species, 84 % of whom are endemics (Huber-Morath 1978). The distribution of the species in the region has also been thoroughly described: 51 species in Russia, 49 in *Flora Iranica* and 20 in *Flora Palaestina* (Celebi et al. 2009), while in *Flora Europaea* 95 *Verbascum* species are included (Ferguson 1972). Bulgaria is situated in the zone of speciation of the genus, which has resulted in a considerable number of endemic species, as among the distributed in the country 46 species half are endemic (Stefanova-Gateva 1995). The genus *Verbascum* bears a very complicated taxonomy. To the best of our knowledge, untill now, there is no adequate taxonomic scheme, reflecting the relationships between the taxa. Huber-Morath (1971, 1978) divided the genus into 13 informal groups (from A to M), while Ferguson in *Flora Europaea* (1972) used two informal groups, named A and B. Recently, the taxonomic status of group A (*Verbascum*) in Turkey has been clarified (Celebi et al. 2009).

The extensive use of *Verbascum* spp. in traditional medicine and the interest from taxonomic point of view have resulted in-depth research on the genus in both phytochemical and pharmacological directions.

Chemical constituents of mulleins

The joint efforts of several groups globally resulted in the identification of over 200 compounds, which can be classified into several main groups: iridoids, phenylethanoids, flavonoids and neolignan glycosides along with saponins and spermine alkaloids.

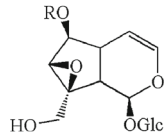
Iridoids

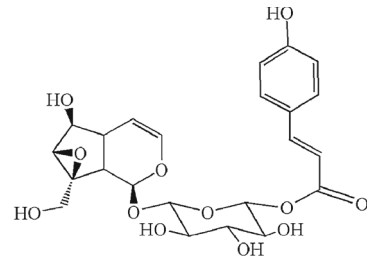
The iridoids are widespread secondary metabolites in Scrophulariaceae family and, at present, the largest group of compounds found in *Verbascum* species. Over 70 iridoid glycosides (**1**–**77**) and several non-glycosidic compounds (**78**–**87**) have been isolated from flowers, leaves, roots and whole *Verbascum* plants (Figs. 1, 2, 3). The main group of these compounds and the most numerous representatives found in mulleins are C_9-type iridoids: catalpol (**1**), aucubin (**37**) and their acylated derivatives (with variable position of the ester group). Ajugol (**60**) and harpagide (**68**) and their derivatives have been also identified in many of the investigated plant sources (Figs. 1, 2, 3). Harpagoside, among others, is a high-value molecule and the major constituent in pharmaceutical preparations of devil's claw (*Harpagophytum procumbens*). Harpagoside is used for standartization of commercial devil's claw products and according to the European Pharmacopoeia these products should contain at least 1.2 % harpagoside (Georgiev et al. 2013a). Therefore, *Verbascum* species, accumulating harpagoside, might serve as an alternative source of this pharmaceutically important molecule.

Only five C_{10}-type iridoids: geniposidic acid (**75**), lychnitoside (**76**), genipin (**79**), α-gardiol (**80**) and β-gardiol (**81**) have been isolated from mulleins so far (Fig. 4). The isolation of rehmaglutin D (**77**) and glutinoside (**78**), possessing $\Delta^{3,4}$ saturated iridoid aglycone with chlorine atom and an unusual epoxy function, has been reported only for *V. wiedemannianum* (Abou Gazar et al. 2003a).

The distribution of iridoids in *Verbascum* spp. is presented in Table 1. Mulleins are widely studied species regarding their iridoid constituents, though the iridoids composition of some *Verbascum* species has been established only by thin-layer chromatography (TLC) (Grabias and Swiatek 1987). Several studies have been focused on *V. phlomoides, V. thapsiforme,* and *V. thapsus*, the sources of the drug Verbasci flos according to the European Phramacopoeia. Among these, *Verbascum thapsus* appeared to be best studied member of the genus—different parts of the taxon of different origin, extracted by various solvents, have been described. For instance, Warashina et al. (1991) reported on the isolation of 23 iridoids, mostly of catalpol type: **11**–**26**, **30** and **32**, from the water extract of the whole plant, while Pardo et al. (1998), focusing

Fig. 1 Structures of catalpol-type iridoid glycosides found in *Verbascum* spp





36 Picroside IV

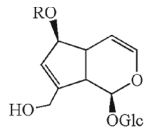
Compounds	**R**
1 Catalpol	H
2 Methylcatalpol	CH_3
3 Catalposide	*p*-OH-Benzoyl
4 Specioside	*p*-Coumaroyl
5 6-*O*-Rhamnopyranosylcatalpol	Rha
6 6-*O*-*β*-D-Glucopyranosylcatalpol	Glc
7 6-*O*-*β*-D-Xylopyranosylcatalpol	Xyl
8 6-*O*-(2″-*O*-*(E)*-Cinnamoyl)-*α*-L-rhamnopyranosylcatalpol = Verbaspinoside	2″-*O*-*(E)*-Cinnamoyl-*α*-L-Rha
9 6-*O*-(3″-*O*-*(E)*-Cinnamoyl)-*α*-L-rhamnopyranosylcatalpol	3″-*O*-*(E)*-Cinnamoyl-*α*-L-Rha
10 6-*O*-(4″-*O*-*(E)*- Cinnamoyl)-*α*-L-rhamnopyranosylcatalpol	4″-*O*-*(E)*-Cinnamoyl-*α*-L-Rha
11 Saccatoside	2″-*O*-*p*-Coumaroyl-*α*-L-Rha
12 6-*O*-(3″-*O*-*p*- Coumaroyl)-*α*-L-rhamnopyranosylcatalpol	3″-*O*-*p*-Coumaroyl-*α*-L-Rha
13 6-*O*-(4″-*O*-*(E)*-*p*- Coumaroyl)-*α*-L-rhamnopyranosylcatalpol	4″-*O*-*p*-Coumaroyl-*α*-L-Rha
14 6-*O*-(2″-*O*-*(E)*-*p*-Methoxycinnamoyl)-*α*-L-rhamnopyranosylcatalpol	2″-*O*-*(E)*-*p*-Methoxycinnamoyl-*α*-L-Rha
15 6-*O*-(3″-*O*-*(E)*-*p*- methoxycinnamoyl)-*α*-L-rhamnopyranosylcatalpol	3″-*O*-*(E)*-*p*-Methoxycinnamoyl-*α*-L-Rha
16 Verbascoside A = 6-*O*-(4″-*O*-*(E)*-*p*-methoxycinnamoyl)-*α*-L-rhamnopyranosylcatalpol	4″-*O*-*(E)*-*p*-Methoxycinnamoyl-*α*-L-Rha
17 6-*O*-(2″-*O*-*(E)*-Caffeoyl)-*α*-L-rhamnopyranosylcatalpol	2″-*O*-*(E)*-Caffeoyl-*α*-L-Rha
18 6-*O*-(3″-*O*-*(E)*- Caffeoyl)-*α*-L-rhamnopyranosylcatalpol	3″-*O*-*(E)*-Caffeoyl-*α*-L-Rha

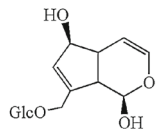
Fig. 1 continued

19 6-*O*-(4″-*O*-*(E)*- Caffeoyl)-*α*-L-rhamnopyranosylcatalpol	4″-*O*-*(E)*-Caffeoyl-*α*-L-Rha
20 6-*O*-(2″-*O*-Feruloyl)-*α*-L-rhamnopyranosylcatalpol	2″-*O*-Feruloyl-*α*-L-Rha
21 6-*O*-(4″-*O*- Feruloyl)-*α*-L-rhamnopyranosylcatalpol	4″-*O*-Feruloyl-*α*-L-Rha
22 6-*O*-(2″-*O*- Isoferuloyl)-*α*-L-rhamnopyranosylcatalpol	2″-*O*-Isoferuloyl-*α*-L-Rha
23 6-*O*-(3″-*O*- Isoferuloyl)-*α*-L-rhamnopyranosylcatalpol	3″-*O*-Isoferuloyl-*α*-L-Rha
24 6-*O*-(4″-*O*- Isoferuloyl)-*α*-L-rhamnopyranosylcatalpol	4″-*O*-Isoferuloyl-*α*-L-Rha
25 6-*O*-(2″-*O*-*(E)*-3,4-Dimethoxycinnamoyl)-*α*-L-rhamnopyranosyl	2″-*O*-*(E)*-3,4-Dimethoxycinnamoyl-*α*-L-Rha
26 6-*O*-(3″-*O*-*(E)*-3,4- Dimethoxycinnamoyl)-*α*-L-rhamnopyranosyl	3″-*O*-*(E)*-3,4-Dimethoxycinnamoyl-*α*-L-Rha
27 Buddlejoside A_8	4″-*O*-*(E)*-3,4-Dimethoxycinnamoyl-*α*-L-Rha
28 Buddlejoside A_5	2″-*O*-Acetyl 3″-p-Methoxycinnamoyl-*α*-L-Rha
29 6-*O*-(2″, 3″-*O*-Diacetyl)-*α*-L-rhamnopyranosyl catalpol	2″-3″-*O*-diacetyl-*α*-L-Rha
30 Pulverulentoside I	3″-*O*-acetyl-2″-*O*-*(E)*-*p*-Methoxycinnamoyl-*α*-L-Rha
31 6-*O*-(3″-*O*-Acetyl-2″-*O*-*(Z)*-*p*-methoxycinnamoyl)-*α*-L-rhamnopyranosylcatalpol	3″-*O*-Acetyl-2″-*O*-*(Z)*-*p*-methoxycinnamoyl-*α*-L-Rha
32 6-*O*-(4″-*O*-Acetyl-2″-*O*-*(E)*-*p*-methoxycinnamoyl)-*α*-L-rhamnopyranosylcatalpol	4″-*O*-Acetyl-2″-*O*-*(E)*-*p*-methoxycinnamoyl-*α*-L-Rha
33 Pulverulentoside II	2″-*O*-Acetyl-3″-*O*-isoferuloyl-*α*-L-Rha
34 Scrospioside A	2″,4″-di-*O*-Acetyl-3″-*O*-*(E)*-cinnamoyl-*α*-L-Rha
35 Scropolioside B	2″-*O*-Acetyl-3″,4″-di-*O*-*(E)*-cinnamoyl-*α*-L-Rha

on the ethanolic extract of mullein roots, succeeded in isolation of four iridoid glycosides, among these aucubin (**37**). Furthermore, the methanolic extract of the whole plant (collected in Pakistan) was found to be a source of some minor constituents (**79**–**81**) along with previously reported iridoids (**36**, **60**, **70** and **71**;

Fig. 2 Structures of aucubin-type iridoid glycosides reported in *Verbascum* spp

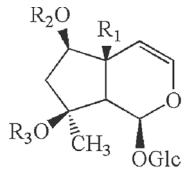


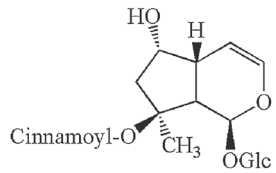


59 Aucuboside

Compounds	**R**
37 Aucubin	H
38 Sinuatol	*α*-L-Rha
39 6-*O*-*β*-D-Glucopyranosylaucubin	*β*-D-Glc
40 6-*O*-*β*-D-Xylopyranosylaucubin	*β*-D-Xyl
41 6-*O*-*p*-Coumaroylaucubin	*p*-Coumaroyl
42 6-*O*-*p*-Methoxycinnamoylaucubin	*p*-Cethoxycinnamoyl
43 Nigroside I	3″-*O*-Cinnamoyl-*α*-L-Rha
44 Nigroside II	2″-*O*-Cinnamoyl-*α*-L-Rha
45 Nigroside III	2″-*O*-(*E*)-*p*-Coumaroyl-*α*-L-Rha
46 6-*O*-(3″-*O*-*p*-Coumaroyl)-*α*-L-rhamnopyranosylaucubin	3″-*O*-*p*-Coumaroyl-*α*-L-Rha
47 6-*O*-(4″-*O*-*(E)*-*p*-Coumaroyl)-*α*-L-rhamnopyaranosylaucubin = Lasianthoside 1	4″-*O*-(*E*)-*p*-Coumaroyl-*α*-L-Rha
48 6-*O*-(6″-*O*-*(E)*-*p*-coumaroyl)-*β*-D-glucopyaranosylaucubin	6″-*O*-(*E*)-*p*-Coumaroyl-*β*-D-Glc
49 Phlomoidoside	4″-*O*-*p*-Coumaroyl-*β*-D-Xyl
50 Unduloside II	3″-*O*-*(E)*-*p*-Dimethoxycinnamoyl-*α*-L-Rha
51 Unduloside III	3″-*O*-*(E)*-*p*-Methoxycinnamoyl-*α*-L-Rha
52 6-*O*-(4″-*O*-*(E)*-*p*-Methoxycinnamoyl)-*α*-L-rhamnopyranosylaucubin	4″-*O*-*(E)*-*p*-Methoxycinnamoyl-*α*-L-Rha
53 Unduloside	2″-*O*-*(E)*-Feruloyl-*α*-L-Rha
54 6-*O*-(3″-*O*-*(E)*-Feruloyl)-*α*-L-rhamnopyranosylaucubin	3″-*O*-*(E)*-Feruloyl-*α*-L-Rha
55 Nigroside IV	3″-*O*-(*E*)-*p*-Isoferuloyl-*α*-L-Rha
56 Nigroside V	2″-*O*-*(E)*-Isoferuloyl-*α*-L-Rha
57 6-*O*-(3″-*O*-acetyl-2″-*O*-*p*-Methoxy- *(E)*-cinnamoyl)-*α*-L-rhamnopyaranosylaucubin	3″-*O*-Acetyl-2″-*O*-*p*-methoxy- *(E)*-cinnamoyl-*α*-L-Rha
58 Sinuatoside	3″-*O*-*β*-Xyl-α-D-Gal

Fig. 3 Ajugol and harpagide-type iridoid glycosides found in *Verbascum* spp





71 Laterioside

Compounds	**R_1**	**R_2**	**R_3**
60 Ajugol	H	H	H
61 6-*O*-Benzoylajugol	H	Benzoyl	H
62 6-*O*-*p*-Hydroxybenzoylajugol	H	*p*-OH-enzoyl	H
63 6-*O*-*p*-Methoxybenzoylajugol	H	*p*-OCH_3-benzoyl	H
64 6-*O*-Vanilloylajugol	H	Vanilloyl	H
65 6-*O*-Syringoylajugol	H	Syringoyl	H
66 Ajugoside	H	Ac	H
67 8-Cinnamoyl myoporoside	H	H	Cinnamoyl
68 Harpagide	OH	H	H
69 8-*O*-Acetylharpagide	OH	H	Ac
70 Harpagoside	OH	H	Cinnamoyl

Hussain et al. 2009). Several rare non-glycosidic iridoids (**82**–**86**; Fig. 5) were identified from the 70 % aqueous acetone extract of the mulleins aerial parts (the samples were collected in Southwest China; Zhao et al. 2011).

It should be, however, mentioned that the presence of catalpol (**1**), aucubin (**37**), ajugol (**60**), harpagide (**66**) and their derivatives in *V. phlomoides* and *V. thapsiforme* has been proved mainly by TLC analysis, while catalpol and aucubin type glycosides in extracts have been quantified spectrometrically (at 605 nm through forming a colored product with Ehrlich reagent) using aucuboside as a standart (Swiatek and Adamczyk 1983, 1985; Swiatek et al. 1984). Afterward, the isolation of specioside (**4**), phlomoidoside (**49**) (Klimek 1991a, 1996a), catalpol (**1**), saccatoside (**11**), aucubin (**37**) and 6-*O*-xylosylaucubin (**40**) from *V. phlomoides* (Gvazava and Kikoladze 2009) has been reported. The same authors identified catalpol (**1**), verbascoside A (**16**), aucubin (**37**), harpagide (**68**) and acetylharpagide (**69**) in *V. densiflorum* (Gvazava and Kikoladze 2009), a synomim of *V. thapsiforme* according to Flora Europaea.

The scientific interest on the iridoids distribution within Scrophulariaceae species has resulted to extensive investigations of several mulleins, besides the recognised sources of Verbasci flos. For instance, 12 iridoid glycosides from *V. nigrum* (Seifert et al. 1982, 1985; Vesper and Seifert 1994) and 11 iridoids from the aerial parts of *V. sinuatum* (Bianco et al. 1980, 1981a, b; Falsone et al. 1982; Eribekyan et al. 1987) have been reported. Moreover, 15 iridoids from the roots and

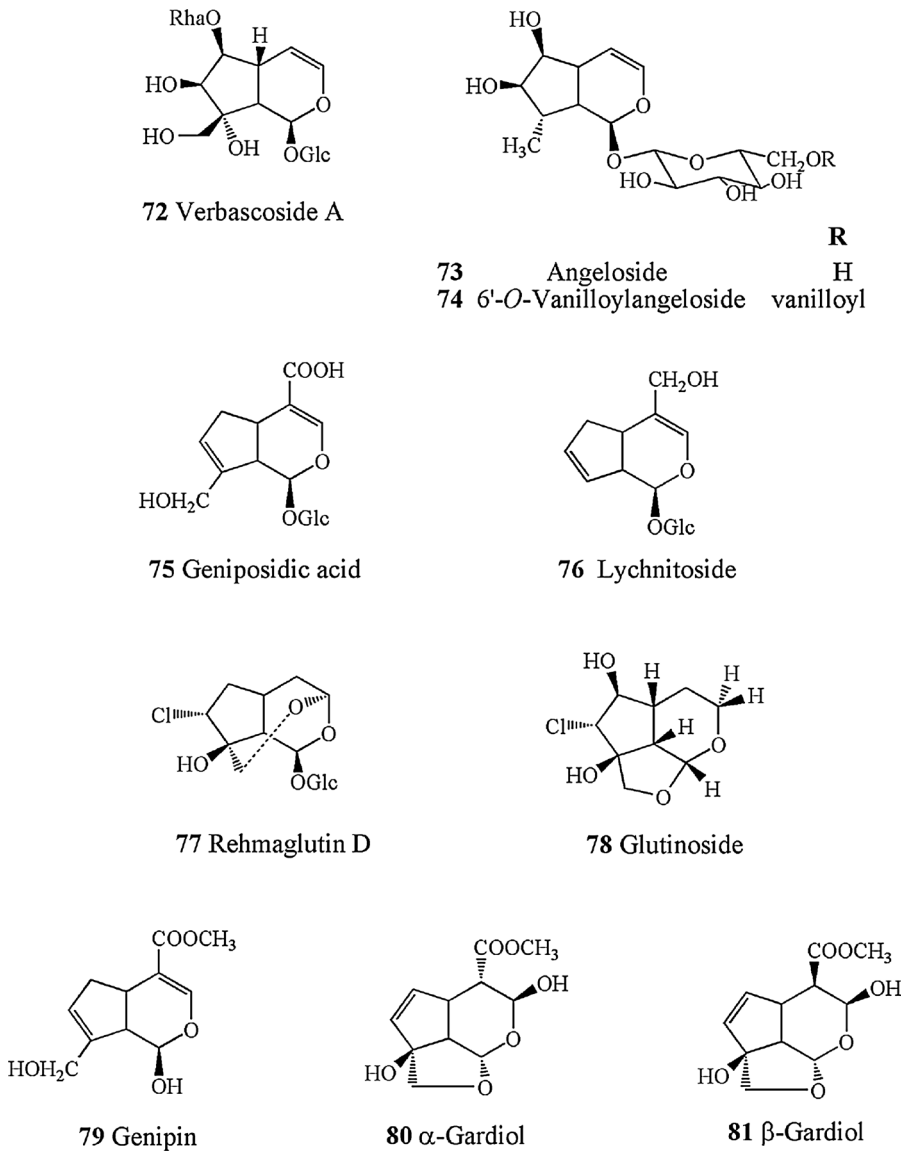


Fig. 4 Structures of C_{10}-type and unusual iridoids

flowers of *V. lasianthum* have been found (Akdemir et al. 2004a, b; Tatli et al. 2006), while systematic investigations of *V. undulatum* led to the isolation of nine iridoid glycosides mainly of aucubin type (Skaltsounis et al. 1996; Magiatis et al. 1998, 2000).

Phenolic compounds

The occurrence of three primary groups of phenolic compounds including phenylethanoids, flavonoids and neolignas in *Verbascum* spp. has been reported.

More than 20 phenylethanoid (C_6–C_2) and two phenylpropanoid (C_6–C_3) glycosides have been isolated from various mulleins so far, as most of these being triglycosides containing apiose, arabinose, glucose, rhamnose and xylose as a third glycosidic moiety in the molecules, attached to C-6 of the core glucose (Fig. 6). According to the published data verbascoside (=acteoside; **87**) is widely distributed compund within the group, it has been isolated from nearly all *Verbascum* species studied, followed by poliumoside (**91**) and forsythoside B (**101**). Verbascoside was recently reported to possess an

Table 1 Distribution of iridoids in *Verbascum* spp

Species	Iridoids	References
V. apentulium Heldr.	**70, 71**	Seifert et al. (1985)
	37, 38, 39, 40, 60, 66, 68, 70	Grabias and Swiatek (1987)
V. ballii (Batt.) M. Qaiser	**34**, **35**	Arrif et al. (2006)
V. blattaria L.	**1, 37, 38, 39, 40, 60, 68, 69, 71,**	Grabias and Swiatek (1987)
V. blattarioides Link	**1, 37, 39, 40, 60, 66, 68, 69, 70, 71**	Grabias and Swiatek (1987)
V. boerhaavii L.	**37, 38, 39, 40, 60, 68, 69, 70**	Grabias and Swiatek (1987)
V. bombyciferum Boiss.	**1, 37, 39, 40, 60, 68, 69, 70**	Grabias and Swiatek (1987)
V. capitis-viridis Hub.-Mor.	**1, 37, 38, 39, 40, 60, 66, 68, 69, 70**	Grabias and Swiatek (1987)
V. chaixii Vill	**37, 38, 39, 40, 43, 60, 66, 68, 70, 71**	Grabias and Swiatek (1987)
V. cheirantifolium Bioss.	**1, 37, 39, 40, 60, 66, 68, 69**	Grabias and Swiatek (1987)
	1, 37, 41, 42	Eribekyan et al. (1989)
V. cilicicum Bioss.	**1, 8, 9, 10**, **11**, **12**	Tatli et al. (2003)
V. densiflorum Bertol.	**70**	Seifert et al. (1985)
	1, 16, 37, 68, 69	Gvazava and Kikoladze (2009)
V. dentifolium Del.	**30, 31, 37, 68, 70, 73, 74**	Arrif et al. (2008)
V. dudleyanum	**1, 5, 11, 12, 37, 60**	Tatli et al. (2008a)
V. georgicum Benth.	**1, 5, 16, 37**	Agababyan et al. (1982)
	1, 37, 39, 40, 60, 66, 69, 68, 70, 71	Grabias and Swiatek (1987)
	72	Arutyunyan et al. (1983)
V. gnaphalodes MB	**37, 39, 40, 60, 66, 68, 69, 70, 71**	Grabias and Swiatek (1987)

Table 1 continued

Species	Iridoids	References
V. lagurus Fisch. Mey.	**1, 37, 40, 60, 66, 68**	Grabias and Swiatek (1987)
V. laxum	**1, 37, 38, 46, 57, 70**	Agababyan et al. (1987)
V. lasianthum Boiss.ex Bentham	**5, 16, 28, 29, 37, 51**	Akdemir et al. (2004a)
	64, 69, 70	Akdemir et al. (2004b)
	1, 37, 38, 47, 52, 60, 75	Tatli et al. (2006)
V. letourneuxii	**5, 30, 51, 65, 70**	Emam (2010)
V. leucophyllum Griseb.	**1, 37, 39, 40, 60, 66, 68, 69, 70**	Grabias and Swiatek (1987)
V. lychnitis L.	**1, 2, 3, 59**	Serdyuk et al. (1976)
	1, 37, 76	De Pascual et al. (1982)
	71, 70	Seifert et al. (1985)
	1, 7, 37, 39, 40, 60, 66, 68, 70, 71	Grabias and Swiatek (1987)
	4	Klimek (1991a)
V. macrurum	**11, 37, 41, 46, 60, 75**	Aligiannis et al. (2003)
V. mallophorum Boiss et Held.	**37, 38, 39, 40, 60, 68, 69, 70**	Grabias and Swiatek (1987)
V. mucronatum Lam.	**1, 37, 46, 60**	Akdemir et al. (2011)
V. nigrum L.	**37, 38, 43, 44, 71, 70**	Seifert et al. (1982)
	1, 2, 37	Seifert et al. (1985)
	37, 39, 40, 43, 60, 66, 68, 69, 70, 71	Grabias and Swiatek (1987)
	45, 46, 54, 55	Vesper and Seifert (1994)
V. niveum Ten. subsp. *garganicum*	**1, 37, 38, 39, 40, 58, 60, 68, 69, 70**	Grabias and Swiatek (1987)
V. olympicum Boiss	**37, 38, 39, 40, 60, 66, 68, 69, 70, 71**	Grabias and Swiatek (1987)
	75	Grabias et al. (1989)

Table 1 continued

Species	Iridoids	References
V. oreophilum C. Koch. var. *oreophilum*	**1, 37, 39, 40, 60, 68, 70**	Grabias and Swiatek (1987)
V. ovalifolium Donn.	**1, 37, 39, 40, 60, 66, 68, 69, 70**	Grabias and Swiatek (1987)
V. phoeniceum L.	**1, 7, 37, 39, 40, 60, 66, 68, 69**	Grabias and Swiatek (1987)
V. phlomoides L.	**1, 37**	Osvath et al. (1982)
	1, 7, 37, 40, 68, 69, 70, 71	Świątek et al. (1984)
	1, 37, 39, 40, 60, 66, 68, 70, 71	Grabias and Swiatek (1987)
	4	Klimek (1991a)
	4, 49	Klimek (1996a)
	1, 11, 37, 40	Gvazava and Kikoladze (2009)
V. pterocalycinum var. *mutense* Hub-Mor.	**36, 60**	Tatli et al. (2004)
V. pulverulentum Vill.	**70**	Seifert et al. (1985)
	1, 37, 39, 40, 60, 68, 70, 71	Grabias and Swiatek (1987)
	30, 33, 70	Seifert et al. (1989)
V. pyramidatum Bieb.	**1, 37, 38, 39, 40, 60, 68, 70**	Grabias and Swiatek (1987)
V. pycnostachyum	**37, 60, 66, 70**	Tatli et al. (2007)
V. roripifolium (Hal.) L.K. Ferg.	**1, 37, 38, 39, 40, 60, 66, 68, 69, 70, 71**	Grabias and Swiatek (1987)
V. saccatum	**5, 11, 37**	Mnatsakanyan et al. (1983)
V. salviifolium Boiss.	**5, 6, 8, 27, 30, 39, 48**	Akdemir et al. (2005)
V. sinuatum L.	**37, 39, 40, 68**	Bianco et al. (1980)
	38	Bianco et al.(1981a)
	58	Bianco et al. (1981b)
	29, 30, 37, 70	Falsone et al. (1982)
	1, 12, 37, 38	Eribekyan et al. (1987)
	1, 37, 38, 39, 40, 58, 60, 66, 68, 70	Grabias and Swiatek (1987)
V. songaricum Schrenk.	**1, 7, 37, 39, 40, 60, 66, 68, 69, 70, 71**	Grabias and Swiatek (1987)
V. speciosum Schrad	**37, 39, 40, 60, 66, 68, 70, 71**	Grabias and Swiatek (1987)
V spectabile Bieb.	**37, 39, 40, 60, 66, 68, 70**	Grabias and Swiatek (1987)
V. spinosum Lin.	**1, 8, 37, 60**	Kalpoutzakis et al.(1999)
V. thapsiforme Schrad.	**1, 7, 37, 40, 71, 68, 69, 70**	Świątek et al. (1984)
	37	Seifert et al. (1985)
	1, 7, 37, 39, 60, 66, 68, 69, 70, 71	Grabias and Swiatek (1987)
V. thapsus L.	**71, 70**	Seifert et al. (1985)
	1, 37, 39, 40, 60, 66, 68, 69, 70, 71	Grabias and Swiatek (1987)
	37, 40	Khuroo et al. (1988)
	11-26, 30, 32, 61, 62, 63, 65, 70, 71	Warashina et al. (1991)
	37, 60, 70, 71	Pardo et al. (1998)
	36, 60, 70, 71, 79, 80, 81	Hussain et al. (2009)
	60, 67, 70, 82, 83, 84, 85, 86	Zhao et al. (2011)
V. undulatum Lam.	**70**	Seifert et al. (1985)
	37, 38, 39, 40, 60, 66, 68, 69, 70	Grabias and Swiatek (1987)
.	**38, 44, 48, 53**	Skaltsounis et al. (1996)
	43, 46, 54, 60	Magiatis et al. (1998)
	50, 51	Magiatis et al. (2000)
V. virgatum Spr.	**11, 12, 37, 60**	Pardo et al. (2004)

Table 1 continued

Species	Iridoids	References
V. wiedemannianum Fisch et Mey.	**70, 71**	Seifert et al. (1985)
	1, 37, 60, 73, 77, 78	Abou Gazar et al. (2003a)
V. xanthophoeniceum Griseb.	**37, 45, 60, 70**	Dimitrova et al. (2012)

insecticidal activity against *Drosophila melanogaster* and *Spodoptera frugiperda* (Munoz et al. 2013), besides abundant biological properties (see below).

Evidently, *V. thapsus* and *V. wiedemannianum* are the most comprehensively investigated species regarding their phenolics content. The presence of the phenylpropanoid glucosides coniferin (**112**) and syringin (**113**) in *V. letourneuxii* has been only reported recently (Emam 2010). The distribution of phenylethanoid and phenylpropanoid glycosides within mulleins is summarized in Table 2.

Verbascum species appear also to be a rich source of various flavonoids, as several flavanones (**114** and **115**), flavones (**116–135**) and flavonols (**136–147**) and their *O*-glycosides have been identified. In addition, four isoflavonoids in *V. sinaiticum* (**148–151**), two *C*-glycosides (**152** and **153**) in *V. cheirantifolium* and a bisflavonoid named amentoflavone (**154**) in *V. thapsus* have been found within the genus. Apigenin (**116**), luteolin (**118**) and their 7-*O*-glucosides (**120**) and (**123**) are common flavones in the genus. The chemical structures of the above mentioned compounds are presented in Figs. 7, 8, 9, 10, while their distribution within *Verbascum* species is given in Table 3.

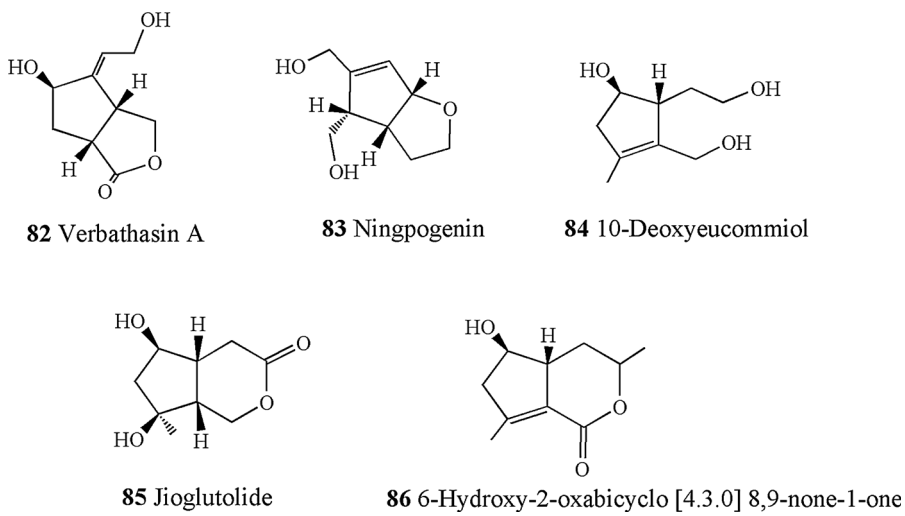
The presence of several neolignan glucosides (Fig. 11) with dehydroconiferyl alcohol sceleton has been reported only in *V. thapsus* (**155–159**; Warashina et al. 1992), *V. salviifolium* (**160** and **161**; Akdemir et al. 2004c) and *V. letourneuxii* (**161** and **162**; Emam 2010).

Saponins

The occurrence of triterpenic saponins mainly of oleanane type (**163–184**) in mulleins has been also reported. Up-to-date, the aerial parts of Turkish endemic *V. wiedemannianum* are the only source of ursane type saponins rosamutin (**186**) and nigaichigoside F1 (**187**; Abou Gazar et al. 2003a). Ilwensisaponin A (**166**) and ilwesisaponin C (**180**) are the most frequently detected compounds from this group (Fig. 12a–c). The aerial parts of *V. songaricum* (Seifert et al. 1991; Hartleb and Seifert 1994, 1995), *V. sinaiticum* (Miyase et al. 1997) and *V. thapsifrome* (Miyase et al. 1997) appeared to be most abundant sources of saponins (see Table 4).

Alkaloids

The distribution of alkaloids in mulleins is restricted to several species. The presence of alkaloids in *V. nobile*

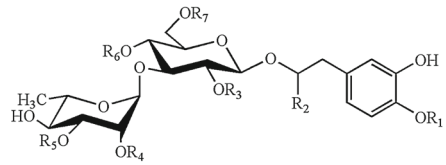


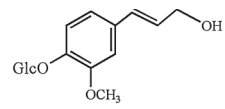
82 Verbathasin A **83** Ningpogenin **84** 10-Deoxyeucommiol

85 Jioglutolide **86** 6-Hydroxy-2-oxabicyclo [4.3.0] 8,9-none-1-one

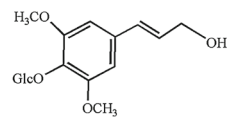
Fig. 5 Non-glycosidic iridoids from *V. thapsus*

Fig. 6 Phenylethanoid and phenylpropanoid glycosides found in *Verbascum* spp





112 Coniferin



113 Syringin

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7
87 Verbascoside (acteoside)	H	H	H	H	H	Caff	H
88 Acetylacteoside	H	H	Ac	H	H	Caff	H
89 *β*-Hydroxyacteoside	H	OH	H	H	H	Caff	H
90 Martynoside	CH_3	H	H	H	H	Fer	H
91 Poliumoside	H	H	H	H	H	Caff	Rha
92 Eukovoside	H	H	H	H	H	Isofer	H
93 Angoroside A	H	H	H	H	H	Caff	Ara
94 Arenarioside	H	H	H	H	H	Caff	Xyl
95 Wiedemannioside A	CH_3	H	H	H	H	Fer	Ac
96 Wiedemannioside B	CH_3	H	H	Ac	Ac	Fer	Ac
97 Wiedemannioside C	H	H	H	H	H	Fer	Glc
98 Wiedemannioside D	H	H	Ac	H	H	Fer	Rha
99 Wiedemannioside E	H	H	Ac	Ac	H	Fer	Rha
100 Echinacoside	H	H	H	H	H	Caff	Glc
101 Forsythoside B	H	H	H	H	H	Caff	Api
102 Angoroside C	CH_3	H	H	H	H	Fer	Ara
103 Alyssonoside	H	H	H	H	H	Fer	Api
104 Leucosceptoside B	CH_3	H	H	H	H	Fer	Api
105 1′-*O*-*β*-*D*-(3,4- Dihydroxy-phenyl)-ethyl-*O*-*α*-*L*-rhamnopyranosyl-(1→3′)- *β*-*D*-xylopyranosyl (1→6′)-4′-*O*-feruloyl-glucopyranoside	H	H	H	H	H	Fer	Xyl
106 1′-*O*-*β*-*D*-(3-Hydroxy-4- methoxy-phenyl)-ethyl-*O*-*α*-*L*-rhamnopyranosyl-(1→3′)- *β*-*D*-	CH_3	H	H	H	H	Fer	Xyl

Fig. 6 continued

xylopyranosyl (1→6′)-4′-*O*- feruloyl-glucopyranoside							
107 1′-*O*-*β*-*D*-(3-Hydroxy-4- methoxy-phenyl)-ethyl-*O*-*α*-*L*-rhamnopyranosyl-(1→3′)- *β*-*D*-xylopyranosyl (1→6′)-4′-*O*-caffeoyl-glucopyranoside	CH_3	H	H	H	H	Caff	Xyl
108 6″-*O*-*β*-D-Glucopyranosylmartynoside	CH_3	H	H	H	H	Fer	Glc
109 1′-*O*-*β*-*D*-(3,4- Dihydroxy-phenyl)-ethyl-*O*-*α*-*L*-rhamnopyranosyl- (1→3′)- *β*-*D*-glucopyranosyl (1→6′)-4′-*O*-isoferuloyl-glucopyranoside	H	H	H	H	H	Isofer	Xyl
110 1′-*O*-*β*-*D*-(3-Hydroxy-4- methoxy phenyl)-ethyl-*O*-*α*-*L*-rhamnopyranosyl-(1→3′)- *β*-*D*-glucopyranosyl (1→6′)-4′-*O*- isoferuloyl-glucopyranoside	CH_3	H	H	H	H	Isofer	Glc
111 1′-*O*-*β*-*D*-(3,4- Dihydroxy-phenyl)-ethyl-*O*-*α*-*L*-rhamnopyranosyl-(1→3′)-3‴-hydroxy-4‴-*O*-*β*-*D*-glucopyranosyl-cinnamoyl(1→6′) glucopyranoside	H	H	H	H	H	H	4-*O*-Glc-Cinn

Ac: Acetyl, Api: Apiofuranosyl, Ara: Arabinopyranosyl, Glc: Glucopyranosyl, Rha: Rhamnopyranosyl, Xyl: Xylopyaranosyl, Caff: Caffeoyl, Fer: Feruloyl, Isofer: Isoferuloyl, Cinn: Cinnamoyl

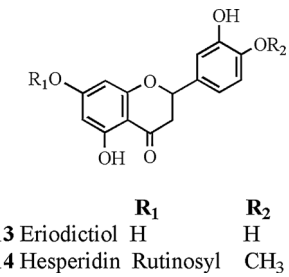
and *V. songoricum* has been reported for the first time in early 70es (Ninova et al. 1971; Ziyaev et al. 1971). Although, the hypotensive and spasmolitic effect of total alkaloid-containing extract *V. pseudonobile* Stoj et Stef has been established in the 1960s (Drandarov and Hais 1996) the isolation and structural elucidation of alkaloids from the species have been published much later (Koblicova et al. 1983), followed by isolation, separation of *E-Z* isomers and synthesis of macrocyclic spermine alkaloids from *V. pseudonobile* and *V. phoeniceum* (Drandarov 1995; Drandarov and Hais 1996; Drandarov 1997; Drandarov et al. 1999; Youhnovslki et al. 1999; Drandarov and Hesse 2002). The structures of the main—naturally occurring—alkaloids are given in Fig. 13.

Other compounds

Figure 14 summarizes the structures of sesquiterpenes buddlindeterpen A (**196**) and buddlindeterpen B (**197**) isolated from *V. thapsus* along with a diterpene buddlindeterpen C (**198**; Hussain et al. 2009). The same figure also bears the structures of a macrocyclic dimer lactone, verbalactone (**199**), isolated from *V. undulatum* (Magiatis et al. 2001) and picein (**200**) from *V. dudleyanum* (Tatli et al. 2008a).

Table 2 Distribution of phenylethanoid glycosides in *Verbascum* spp

Species	Phenylethanoids	References
V. blattaria L.	**91**	Tatli and Akdemir (2004)
V. boerhaavii L.	**91**	Tatli and Akdemir (2004)
V. chaixii Vill	**91**	Tatli and Akdemir (2004)
V. georgicum	**87**	Agababyan et al. (1985)
V. lasianthum	**87, 91**	Akdemir et al. (2004b)
V. letourneuxii	**90, 92, 112, 113**	Emam (2010)
V. lychnitis	**87**	Klimek (1991b)
	101	Klimek (1996b)
V. macrurum	**87, 90, 102, 106**	Aligiannis et al. (2003)
V. mucronatum Lam	**87**	Akdemir et al. (2011)
V. nigrum	**87**	Klimek (1991b)
	101	Klimek (1996b)
V. phlomoides	**91**	Tatli and Akdemir (2004)
	87	Klimek (1991b)
	87, 101	Klimek (1996b)
V. pterocalycinum var. *mutense*	**87**	Tatli et al. (2004)
V. pycnostachyum	**87**	Tatli et al. (2007)
V. salviifolium Boiss.	**87, 90, 93, 89, 101**	Akdemir et al. (2004c)
	89, 90, 93, 101	Tatli et al. (2008b)
V. sinaiticum	**87**	Elgindi et al. (1999)
	88, 94, 109, 110	Elgindi and Marby (2000)
V. sinuatum	**87**	Scarpati and Delle Monache (1963)
	91	Tatli and Akdemir (2004)
V. spinosum	**87, 93, 102**	Kalpoutzakis et al. (1999)
V. thapsiforme	**87**	Klimek (1991b)
	101	Klimek (1996b)
V. thapsus	**91**	Tatli and Akdemir (2004)
	94, 101, 103, 104, 105, 106, 107, 108, 111	Warashina et al. (1992)
	87	Hussain et al. (2009)
V. undulatum	**87**	Skaltsounis et al. (1996)
	90, 94	Magiatis et al. (1998)
V. wiedemannianum	**87, 90, 95, 96, 97, 98, 99, 100, 104**	Abou Gazar et al. (2003b)
V. xanthophoeniceum Griseb.	**87, 101, 104**	Dimitrova et al. (2012)

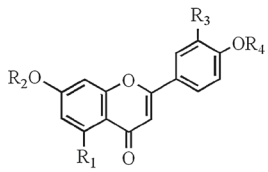


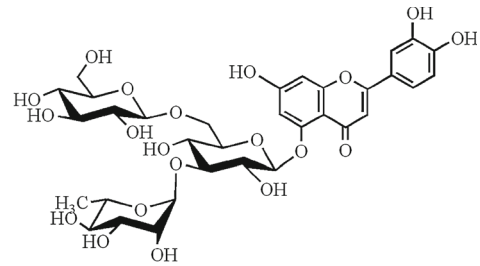
	R_1	R_2
113 Eriodictiol	H	H
114 Hesperidin	Rutinosyl	CH_3

Fig. 7 Structures of flavanones

Considering all reported data, several groups of bioactive metabolites from *V. lychnitis*, *V. nigrum*, *V. phlomoides*, *V. thapsiforme* and *V. thapsus* have been exhaustively studied. Other systematically examined species as *V. salviifolium* (Akdemir et al. 2004c, 2005; Tatli et al. 2008b), *V. lasianthum* (Akdemir et al. 2004a, b; Tatli et al. 2006; Kupeli et al. 2007), *V. mucronatum* (Akdemir et al. 2011) and *V. wiedemannianum* (Abou Gazar et al. 2003a, b) could be also considered as rather well studied. On the other hand, the knowledge on some species as *V. songaricum* is relatively limited. The taxon has been studied in details regarding its saponin content in aerial parts (Seifert et al. 1991; Hartleb and Seifert 1994, 1995) and flavonoids in the roots (Yuldashev 1996), while the iridoids presence has been proven only by TLC (Grabias and Swiatek 1987).

Fig. 8 Flavones reported in *Verbascum* spp





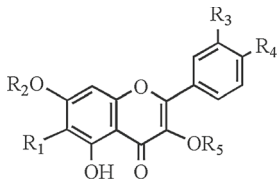
135 Verbacoside

Compounds	R_1	R_2	R_3	R_4
116 Apigenin	OH	H	H	H
117 Apigenin-4′-methylether	OH	H	H	CH_3
118 Luteolin	OH	H	OH	H
119 Chrysoeriol	OH	H	OCH_3	H
120 Apigenin-7-*O*-glucoside = apigetrin	OH	Glc	H	H
121 Apigenin-7-*O*-glucuronide	OH	Gluc	H	H
122 Luteolin-5-*O*-glucoside	OGlc	H	OH	H
123 Luteolin-7-*O*-glucoside	OH	Glc	OH	H
124 Luteolin-7-*O*-glucuronide	OH	Gluc	OH	H
125 luteolin-3′-*O*-glucoside	OH	H	OGlc	H
126 Luteolin-7-methylether	OH	CH_3	OH	H
127 Diosmetin	OH	H	OH	CH_3
128 Diosmetin-7-*O*-glucuronide	OH	Gluc	OH	CH_3
129 Diosmetin-7-*O*-rutinoside	OH	Rut	OH	CH_3
130 7-Hydroxy-4′-*O*-*α*-*L*-rhamnopyranosylflavone	H	H	H	Rha
131 Apigenin-4′-*O*-rhamnoside	OH	H	H	Rha
132 Acacetin-7-*O*-glucoside	OH	Glc	H	CH_3
133 Acacetin-7-*O*-galactoside	OH	Gal	H	CH_3
134 Chrysoeriol-7-*O*-glucoside	OH	Glc	OCH_3	H

Metabolomics of *Verbascum* spp

Despite all applications (see below) the knowledge of the metabolites, accumulated in some *Verbascum* species, could be considered as still limited and based mainly on determination of the major compounds. Moreover to explore the chemodiversity of the genus, aiming to distinguish between species and to establish differences in metabolite profiles and chemical fingerprints, an application of emerging comprehensive

Fig. 9 Flavonols reported in *Verbascum* spp

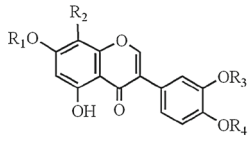


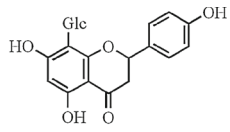
Compounds	R_1	R_2	R_3	R_4	R_5
136 Kaemferol	H	H	H	OH	H
137 3,5-Dixydroxy-6,7-dimethoxyflavone	OCH_3	CH_3	H	H	H
138 Quercetin	H	H	OH	OH	H
139 Isorhamnetin	H	H	OH	OCH_3	H
140 Quercetin-7-*O*-glucoside	H	Glc	OH	OH	H
141 Quercetin-7-*O*-glucuronide	H	Gluc	OH	OH	H
142 Rutin	H	Rut	OH	OH	H
143 Quercetin-3,7-*O*-diglucoside	H	Glc	OH	OH	Glc
144 Tamarixetin-7-*O*-glucoside	H	Glc	OH	OCH_3	H
145 Tamarixetin-7-*O*-rhamnoside	H	Rha	OH	OCH_3	H
146 Tamarixetin-7-*O*-rutinoside	H	Rut	OH	OCH_3	H
147 Patuletin	OCH_3	H	OH	OH	H

analytical platforms (*e.g.* metabolomics) might be very useful. Metabolomics is a holistic approach, defined as systematic identification and quantification of all metabolites in an organism (Kim et al. 2010), at given conditions. Several platforms and techniques for high throughput analyses of targeted molecules have been developed (mainly mass spectrometry and nuclear magnetic resonance spectroscopy) during the past 15 years. Nuclear magnetic resonance (NMR) has been already proven as quite suitable and adequate method to perform metabolomics, as it allows simultaneous detection of abundant primary metabolites along with diverse groups of secondary metabolites (Verpoorte et al. 2007; Kim et al. 2010). Moreover ^{1}H NMR-scpectroscopy possesses a great advantage over the other analytical platforms (*e.g.* mass spectrometry), as the signal intensity is only dependent on the molar concentration in the solution, which enables the direct comparison of concentrations of all compounds, which are present in a particular sample (Kim et al. 2010, 2011).

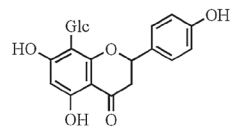
NMR-based metabolomics approach was applied to study metabolic differentiations of five *Verbascum* species (Georgiev et al. 2011a). ^{1}H NMR fingerprinting in combination with multivariate data analysis (*e.g.* principal component analysis, PCA) allows classification of *Verbascum* species in two groups: group A (*V. phlomoides* varieties 7 and 33, and *V. densiflorum*) and group B (*V. xanthophoeniceum*, *V. nigrum* and *V. phoeniceum*). Further, it was found that the plants in group B synthesize higher amounts of bioactive iridoid glycosides [*e.g.* pharmaceutically important harpagoside (0.5 % on dry weight basis) **70**] and phenylethanoid glycosides (in total about 6 % on dry weight basis)—verbascoside (**87**), forsythoside B (**101**) and leucosceptoside B (**104**). ^{1}H NMR

Fig. 10 Isoflavonoids, C-glycosides and bisflavonoid

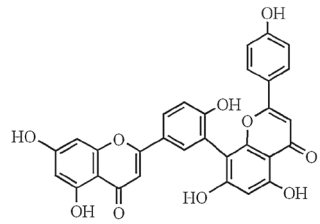




152 Vitexin



153 Swertisin



154 Amentoflavone

Compounds	R_1	R_2	R_3	R_4
148 Orobol	H	H	H	H
149 Orobol-7-*O*-glucoside	Glc	H	H	H
150 5,3′,4′-Trihydroxy-8-methylisoflavone-7-*O*-glucoside	Glc	CH_3	H	H
151 5-Hydroxy-3′,4′-dimethoxyisoflavone-7-*O*-rhamnoside	Rha	H	CH_3	CH_3

Table 3 Distribution of flavonoids in *Verbascum* spp

Species	Flavonoids	References
V. cheirantifolium	**152, 153**	Manav Yalcin (1989)
V. dudleyanum	**123**	Tatli et al. (2008a)
V. eremobium	**116, 120, 123, 134**	Kawashty (1997)
V. fruticulosum	**116, 118, 120, 123, 132**	Kawashty (1997)
V. letourneuxii	**120, 123**	Kawashty (1997)
V. lychnitis	**116, 117, 118, 122, 126, 147**	Serdyuk et al. (1976)
	121, 124, 138, 141	Klimek (1995)
V. nigrum	**121, 124, 128, 141**	Klimek (1995)
V. phlomoides	**144, 146**	Klimek and Królikiowska (1984)
	114, 116, 118, 119, 120, 123, 136, 138, 142	Papay et al. (1980)
	115, 129, 144, 145, 146	Tschesche et al. (1979)

Table 3 continued

Species	Flavonoids	References
V. salviifolium	**120, 123, 125, 134**	Tatli et al. (2008b)
V. scardicolum	**123**	Naumov et al. (1998)
V. schimperianum	**116, 120, 123, 132, 133, 134**	Kawashty (1997)
V. sinaiticum	**148, 149, 150, 151**	Elgindi et al. (1999)
	120, 123, 132, 133, 134	Kawashty (1997)
	118, 119	Afifi et al. (1993)
V. songaricum Schrenk.	**116, 118, 123, 138**	Yuldashev (1996)
V. thapsiforme	**116, 118, 120, 123, 140, 143**	Klimek and Krolikowska (1984)
V. thapsus subsp. *thapsus*	**137**	Tatli and Akdemir (2004)
	118, 135	Mehrotra et al. (1989)

Table 3 continued

Species	Flavonoids	References
	154	Hussain et al. (2009)
	118, 120	Zhao et al. (2011)
	130, 131, 139	Souleles and Geronikaki (1989)
V. wiedemannianum	**118**	Abou Gazar et al. (2003b)

metabolomics data and hierarchical clustering analysis revealed that *V. xanthophoeniceum* and *V. nigrum* species have a similar leaf metabolome, which is quite different from the other mullein species, recognized by the European Pharmacopoeia (Georgiev et al. 2011a). It was suggested that NMR spectroscopy can be used for the rapid quantification of pharmaceutically important harpagoside in plant samples, *e.g.* for quality control of pharmaceutical products and/or herbal supplements (Georgiev et al. 2013a).

Pharmacology of *Verbascum* spp

Up-to-date, *Verbascum* species have been reported to possess various biological activities. Some of the best recorded pharmacological properties of mullein species are mentioned in this part as a result of a literature survey in Pubmed and Scopus databases.

Anti-inflammatory activity

Anti-inflammatory effect of different mullein species—traditionally used against inflammatory diseases, asthma, coughs, diarrhea, and pulmonary problems (Turker and Gurel 2005; Tatli and Akdemir 2006; Kupeli et al. 2007; Speranza et al. 2009)—has been studied extensively. An early study on *V. thapsiforme* showed that the aqueous extract of the plant exhibits strong anti-inflammatory effect, through inhibition of elongation stage of protein biosynthesis in rat liver, and the saponin fraction was found to be responsible for the anti-inflammatory effect of the species (Paszkiewicz-Gadek et al. 1990). The methanolic extract of the flowers of *V. lasianthum*, subjected to carrageenan-induced hind paw edema

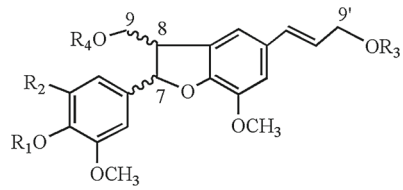
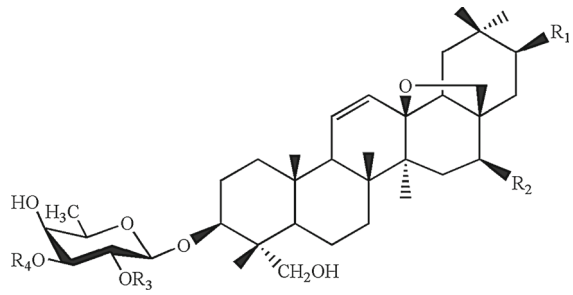


Fig. 11 Structures of neolignan glucosides

Compounds	R_1	R_2	R_3	R_4
155 Dehydrodiconiferyl glucoside E (*7R, 8S*)	H	H	H	Glc
156 Dehydrodiconiferyl glucoside D (*7S, 8R*)	Glc	H	H	H
157 Dehydrodiconiferyl glucoside G (*7R, 8S*)	H	OCH_3	H	Glc
158 Dehydrodiconiferyl glucoside (*7S, 8R*)	H	OCH_3	H	Glc
159 Dehydrodiconiferyl diglucoside	Glc	H	CH_3	Glc
160 Dehydrodiconiferyl alcohol-9′-glucoside	H	H	Glc	H
161 Dehydrodiconiferyl alcohol-9-glucoside	H	H	H	Glc
162 4-*O*-Methyl-dehydrodiconiferylalcohol-9′-glucoside	CH_3	H	Glc	H

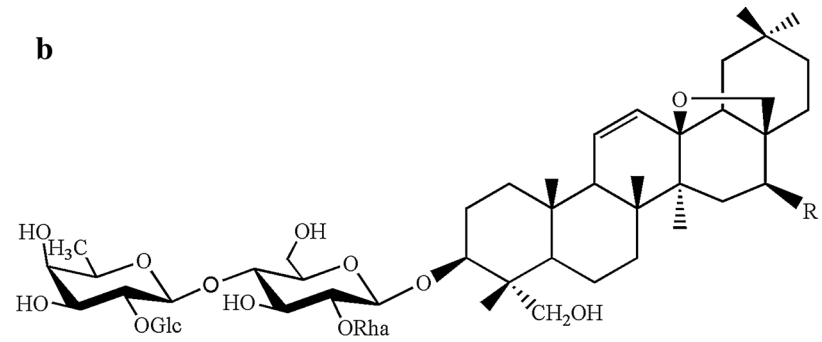
Fig. 12 **a–c** Saponins found in *Verbascum* spp

a

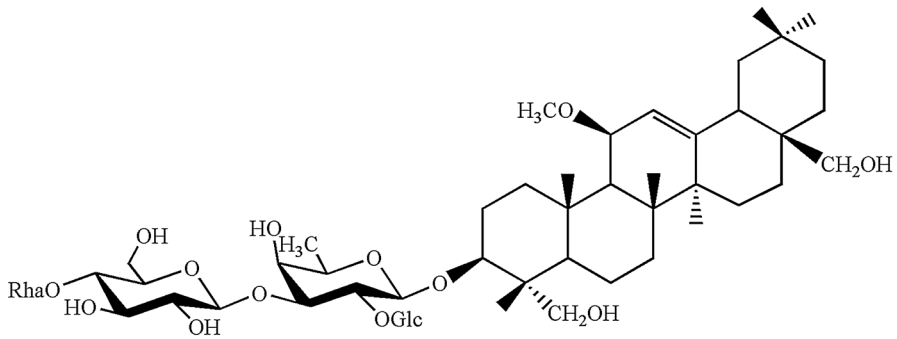


Compounds	R_1	R_2	R_3	R_4
163 3-*O*-β-Fucopyranosylsaikogenin F	H	OH	H	H
164 Saikosaponin A	H	OH	H	Glc
165 Desrhamnosyl verbascosaponin	H	H	Glc	Glc
166 Ilwensisaponin A=Mimengoside A	H	H	Glc	Rha(1→4)Glc
167 Buddlejasaponin I (Verbascosaponin B)	H	OH	Glc	Rha(1→4)Glc
168 Buddlejasaponin IV	H	OH	Glc	Glc
169 Mulleinsaponin I	H	H	H	Glc
170 Mulleinsaponin II	H	H	H	Rha(1→4)Glc
171 Mulleinsaponin III	H	OH	H	Rha(1→4)Glc
172 Mulleinsaponin IV	OH	OH	Glc	Rha(1→4)Glc
173 Mulleinsaponin V	OAc	OH	Glc	Rha(1→4)Glc
174 Mulleinsaponin VI	H	OAc	Glc	Rha(1→4)Glc
175 Mulleinsaponin VII	H	OGlc	Glc	Rha(1→4)Glc
176 Songarosaponin C	H	H	Glc	Glc(1→4)Glc
177 Songarosaponin D	H	OH	Glc	Glc(1→4)Glc

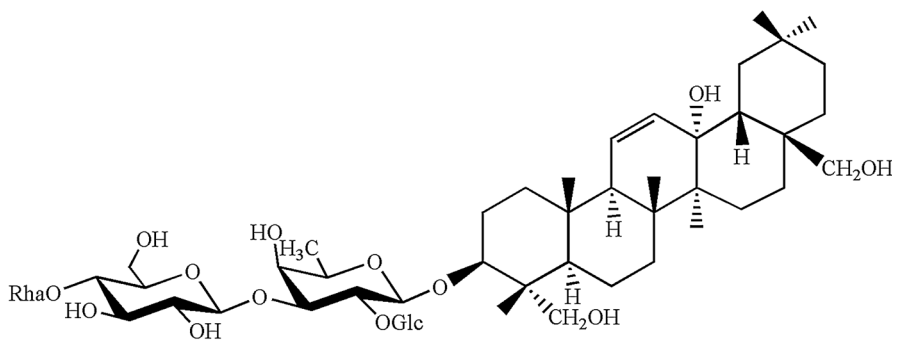
and to *p*-benzoquinone-induced writhings models in mice, demonstrated a significant anti-inflammatory and antinociceptive effect. Bioassay-guided fractionation of the extract resulted in the isolation of eight individual compounds, of whom aucubin (**37**) and ilwensisaponin A (**166**) were proven to posses remarkable anti-inflammatory and antinociceptive properties (Kupeli et al. 2007). The flower extract of *V. pterocalycinum* var. *mutense*, studied by the same research group (Kupeli Akkol et al. 2007) in carrageenan and PGE_1-induced hind paw edema along with 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema models, was found to display activity only in carrageenan and PGE_1-induced hind paw edema model, which led to a conclusion that the extract might act through inhibition of the cyclooxygenase (COX) activity. Ilwensisaponins A (**166**) and C (**180**) were proven to be the main active constituents in



	R
178 Thapsuin A	H
179 Hydroxythapsuin A	OGlc



180 Ilwensisaponin C = Verbascosaponin A

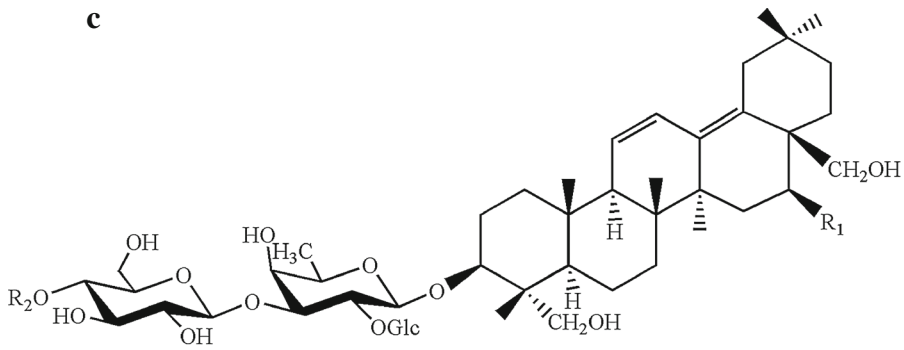


181 Songarosaponin B

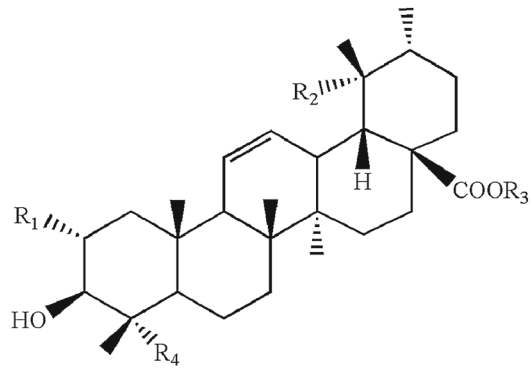
Fig. 12 continued

this plant species. The aerial parts of *V. salviifolium* were subjected to similar assays by the same research group and yielded *β*-hydroxyacteoside (**89**), apigenin 7-*O*-glucoside (**120**), luteolin 7-*O*-glucoside (**123**), luteolin and 3′-*O*-glucoside (**125**), as the active constituents in carrageenan and PGE_1-induced hind paw edema models in mice (Tatli et al. 2008b). In another study, authors assigned a significant anti-inflammatory effect to the flower extract of *V. mucronatum* by applying the same experimental models (Akdemir et al. 2011). The methanolic extract, iridoid- and phenylethanoid-containing fractions, and isolated pure compounds of *V. xanthophoeniceum* (collected from Bulgaria) were tested for their anti-

c



	R_1	R_2
182 Songarosaponin A=Ilwensisaponin B	H	Rha
183 Songarosaponin E	H	Glc
184 Songarosaponin F	OH	Glc



	R_1	R_2	R_3	R_4
185 Ursolic acid	H	H	H	H
186 Rosamultin	OH	OH	Glc	CH_3
187 Niga-ichigoside F1	OH	OH	Glc	CH_2OH

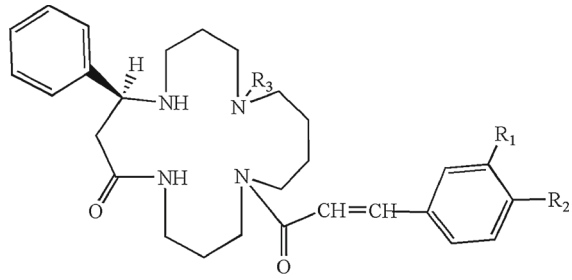
Fig. 12 continued

inflammatory effect by in vitro methods on nitric oxide and cytokine production by peritoneal macrophages accompanied by COX-1 and COX-2 expression and by in vivo method, using cobra venom factor (CVF)-induced edema in mice (Dimitrova et al. 2012). The extract was able to lessen paw-swelling at the doses of 40 and 200 mg/kg, while forsythoside B (**101**) showed the most pronounced inhibition of alternative pathway activity in CVF-induced edema model. Among the leaf and hairy root extracts obtained from several *Verbascum* species (*V. nigrum*, *V. densiflorum*, *V. phoeniceum* and *V. phlomoides*), grown in green sera

Table 4 Distribution of saponins in *Verbascum* spp

Species	Saponins	References
V. ballii	**166**	Arrif et al. (2006)
V. dudleyanum	**166, 180**	Tatli et al. (2008a)
V. fruticulosum	**167, 171, 172, 173**	Miyase et al. (1997)
V. lasianthum	**166**	Kupeli et al. (2007)
V. lychnitis	**173, 178, 179, 185**	De Pascual et al. (1982)
V. mucronatum Lam.	**166, 180**	Akdemir et al. (2011)
V. nigrum	**166, 180**	Anil (1980), Klimek et al. (1992)
V. phlomoides	**166**	Tschesche et al. (1980)
	165, 166, 180	Klimek (1996a)
V. pterocalycinum var. *mutense*	**166, 180**	Tatli et al. (2004)
V. roripifolium	**163, 164, 166, 167, 171**	Miyase et al. (1997)
V. sinaiticum	**164, 166, 167, 169, 170, 171, 174, 175**	Miyase et al. (1997)
V. songaricum Schrenk.	**176, 181, 182**	Seifert et al. (1991)
	177	Hartleb and Seifert (1994)
	169, 183, 184	Hartleb and Seifert (1995)
V. thapsiforme	**165, 166, 167, 168, 176, 177**	Miyase et al. (1997)
V. thapsus	**178, 179**	De Pascual et al. (1980)
V. wiedemannianum	**186, 187**	Abou Gazar et al. (2003b)

conditions, Dimitrova et al. (2013) reported noteworthy anti-inflammatory feature of *V. phoeniceum* in in vivo carrageenan-induced edema model by inhibiting COX-1 and COX-2 enzymes. Pure harpagoside (**70**) ameliorated the development of zymosan-induced arthritis and reduced pathological changes in joints as shown by the decreased histological score for cell infiltration in synovial cavity, cartilage loss and bone resorption. Moreover, molecular docking simulations of harpagoside suggested that it may function with increased specific affinity towards COX-1 than COX-2 (Dimitrova et al. 2013).



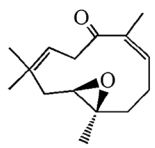
Compounds	R_1	R_2	R_3
188 (E) Verbacine	H	H	H
189 (Z) Verballocine	H	H	H
190 (E) Verbasitrine	OCH_3	OCH_3	H
191 (Z) Isoverbasitrine	OCH_3	OCH_3	H
192 (E) Verbasikrine	H	OCH_3	H
193 (Z) Isoverbasikrine	H	OCH_3	H
194 (E) Verbamedine	H	H	CHO
195 (Z) Isoverbamedine	H	H	CHO

Fig. 13 Structures of spermine alkaloids

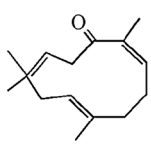
In another study of Georgiev et al. (2012a) the crude methanolic extract, its fractions as well as a number of individual constituents—of iridoid and phenylethanoid origins—from *V. xanthophoeniceum* were subjected to primary cultures of normal human keratinocytes along with a thorough investigation of their effect on pro-inflammatory chemokines (IL-8, MCP-1 and IP-10) and gene expression for possible anti-inflammatory effect. Among the tested samples, verbascoside (**87**) and forsythoside B (**101**) were found to be effective, dose-dependent inhibitors of gene expression and *de novo* synthesis of above mentioned chemokines. Therefore, *V. xanthophoeniceum*-derived phenylethanoid glycosides could be considered as potential active components for topical compositions aimed at the regulation of chronic inflammatory skin disorders, such as psoriasis and atopic dermatitis (being characterized by over-expression of IL-8, MCP-1, and IP-10; Georgiev et al. 2012a).

The anti-inflammatory potential of aqueous flower extract of *V. phlomoides* was recently examined in

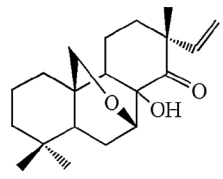
Fig. 14 Other compounds isolated from *Verbascum* spp



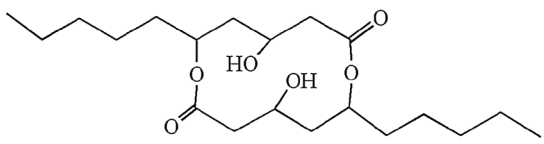
196 Buddlindeterpene A



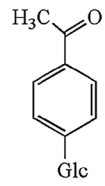
197 Buddlindeterpene B



198 Buddlindeterpene C



199 Verbalactone



200 Picein

in vitro and in vivo assays (Grigore et al. 2013). The findings from this study indicate that the extract was found to inhibit TNF-α-induced ICAM-1 expression significantly by 55–59 % on human umbilical vein endothelial cells at concentrations of 100 and 200 μg/mL, however, it did not display any effect in egg-white-induced rat paw edema model (Grigore et al. 2013). The authors concluded that in vitro anti-inflammatory effect of the extract could be corelated to its iridoid and phenylethanoid contents rather than polyphenolic constituents.

On the other hand, Speranza et al. (2009) examined the anti-inflammatory effect of verbascoside (the major phenylethanoid glycoside)-containing extract from *V. mallophorum* and reported that verbascoside *per se* exerted/had a significant anti-inflammatory action by causing a substantial diminution in the expression and activity of iNOS and extracellular O_2. Strong anti-inflammatory activity of verbascoside was also reported in an early study by inhibition of carrageenan-induced edema and histamine and bradykinin-induced contractions in guinea pig ileum (Schapoval et al. 1998).

Wound-healing activity

Süntar et al. (2010) screened the methanolic extracts of thirteen *Verbascum* species (*V. chionophyllum*, *V. cilicicum*, *V. dudleyanum*, *V. lasianthum*, *V. latisepalum*, *V. mucronatum*, *V. olympicum*, *V. pterocalycinum* var. *mutense*, *V. pycnostachyum*, *V. salviifolium*, *V. splendidum*, *V. stachydifolium*, and *V. uschackense*), grown in Turkey, for their in vivo wound-healing effect by linear incision and circular excision experimental models accompanied by histopathological examination. Among them, *V. olympicum*, *V. stachydifolium* and *V. uschackense* were found to display promising wound-healing effect in the models used. In another study, Korkina et al. (2007) reported verbascoside (56 %)-containing extract to have remarkable wound-healing activity on full thickness excision wound model, which has been linked to its inhibitory capacity on release of reactive oxygen species (ROS) from the whole blood granulocytes and monocytes as well as its metal-chelation ability (with Fe^{+2} ions). In some more recent studies (Mehdinezhad et al. 2011, 2012), the flower extract of *V. thapsus* was shown to exert notable healing effect on experimental coetaneous and zinc oxide wound models in rabbits with topical application of the extract.

Antiviral and antimicrobial activity

Verbascum species have been widely screened for their antimicrobial activity. Amongst them, several species were demonstrated to possess remarkable antiviral activity. The lyophilized flower infusion of *V. thapsus* exerted significant inhibitory effect against *Herpes simplex* virus (type 1) using the yield reduction test (Slagowska et al. 1987; Zgórniak-Nowosielska et al. 1991). On the other hand, combination of adamantanamine glucuronide and the lyophilized flower infusion of *V. thapsiforme* exerted a discernible effect against influenza virus in chicken embryo

fibroblast cell cultures (Serkedjieva 2000). In connection with these data, the methanolic extract of *V. thapsus* from Nepal was revealed to have a robust anti-influenza effect (Rajbhandari et al. 2009).

In a screening study on the mullein species from Argentina (Zanon et al. 1999), *V. thapsus* displayed the strongest inhibitory effect against *Pseudorabies* virus strain RC/79 (*Herpes suis*) in Vero cells (2 log) and in a follow-up study the same species was found to inhibit by 50 % plaque formation caused by *Pseudorabies* virus at concentration of 35 μg/mL, while incubation of the virus with the plant extract led to 99 % of inhibition during the adsorption phase (Escobar et al. 2012).

In addition, some early studies have revealed the antibacterial properties of some mullein species. For instance, verbalactone (**199**)—a macrocyclic dimer lactone derivative—isolated from *V. undulatum* was identified to display marked antibacterial effect (Magiatis et al. 2001). Turker and Camper (2002) showed strong antibacterial effect of the water extract prepared from the species against *Klebsiella pneumonia*, *Staphylococcus aureus*, *S. epidermidis*, and *Escherichia coli*, while the polar extracts of *V. sinuatum* (Senatore et al. 2007; Sener and Dulger 2009), *V. gypsicola* (Dulger and Gonuz 2004), *V. georgicum* (Sengul et al. 2005), *V. antiochium* (Ozcan et al. 2010), and *V. pinetorum* (Ozcan et al. 2011) exhibited significant activity towards a number of Gram (+) and Gram (−) bacteria. Several mullein species were tested against *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, *Enterococcus faecalis*, as well as the fungal strains of *Candida albicans*, *C. parapsilosis*, and *C. krusei* by disc diffusion methods, which led to identification of *V. mucronatum* and *V. olympicum* as demonstrating antibacterial activity against Gram (+) bacteria and *S. aureus* along with *V. latisepalum*, showing notable antifungal activity against *C. krusei* (Kahraman et al. 2011).

Anthelmintic activity

Up-to-date, only a few studies have been reported on anthelmintic activity of mulleins. The methanolic extract of *V. thapsus* was subjected to anthelmintic assays using adult roundworms (*Ascaridia galli*) and tapeworms (*Raillietina spiralis*) in which the time of paralysis and death was determined and compared to albendazole as reference drug. The results indicated that the methanolic extract has superior effect against *R. spiralis* than that of the reference drug (Ali et al. 2012). In a screening study, the extracts obtained from *V. lasianthum*, *V. latisepalum*, *V. mucronatum*, and *V. salviifolum* exerted a potent anthelmintic effect against *Aspiculuris tetraptera* at dose of 100 mg/kg in mice (Kozan et al. 2011).

Neuroprotective activity

Our literature survey pointed out that the knowledge on the neuroprotective effect of mulleins is still scarce. Inhibition of cholinesterases enzyme family has been one of the most accepted approaches for treatment of mild to moderate Alzheimer's disease and in this sense, the phenylethanoid-enriched fraction and some pure compounds from *V. xanthophoeniceum* were exposed to in vitro testing of cholinesterase enzyme inhibition. It was found that both methanolic extract and phenylethanoid-enriched fraction, at 200 μg/mL, along with forsythoside B (**101**), at 100 μg/mL, exerted significant butyrylcholinesterase inhibition (75, 84.3 and 98.3 %, respectively; Georgiev et al. 2011b). In a similar study, cholinesterase inhibitory activities of the aqueous extract of *V. mucronatum*, its fractions and several individual constituents were examined and only verbascoside was shown to possess mild cholinesterase-inhibiting effect (Kahraman et al. 2010).

Biotechnology of mulleins

The high importance of *Verbascum*-derived bioactive molecules imposes the development of alternative way to supply them. Plant in vitro technologies are considered as an attractive and cost-effective alternative to classical approaches and possess an immense potential for sustainable supply of value-added plant-derived metabolites ("chemical factories" concept; Georgiev et al. 2012b, 2013b). An efficient protocol for the establishment of transformed root culture (=hairy roots) of *V. xanthophoeniceum*, using sonication-assisted *Agrobacterium rhizogenes*-mediated transformation, was reported (Georgiev et al. 2011c). Ten days after the inoculation with *A. rhizogenes* ATCC 15834 suspension, and 45 s of ultrasound exposure, hairy roots appeared on 75 % of the *Verbascum* leaf explants. Moreover, most vigorous *V. xanthophoeniceum* hairy

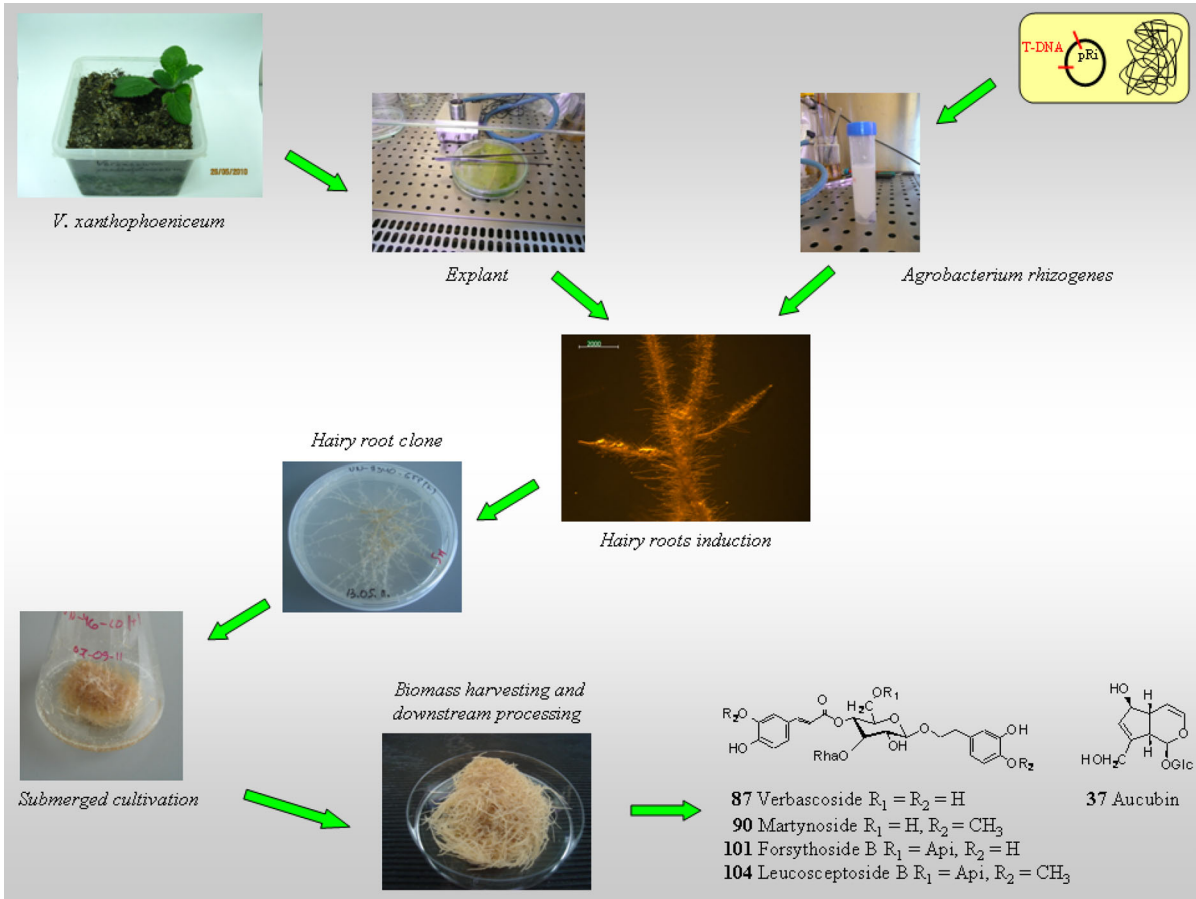


Fig. 15 Technological platform for *Verbascum* spp hairy roots induction and bioproduction of pharmaceutically important metabolites

root clones showed stable growth under submerged cultivation and accumulated high biomass amounts (13–14 g dry root mass/L). LC-APCI-MS metabolite profilling of the hairy roots revealed that verbascoside (**87**) was the most abundant secondary metabolite and its amounts were over 6-times higher than in the mother plant tissue (Georgiev et al. 2011c). Clearly, *Verbascum* transformed roots have an enormous biosynthetic potential and might therefore serve as an attractive source for bioproduction of pharmaceutically important metabolites (Fig. 15), though more detailed further research is pending.

Conclusion and further perspectives

Mulleins have a long tradition of use in folk medicine. While the clinical efficacy is yet to be established, various *Verbascum* extracts, fractions and purified secondary metabolites were shown to posess valuable pharmacological properties (in vitro and in vivo), suggesting that mulleins may be beneficial in the treatment (and prevention eventually) of respiratory, inflammatory and infectious disorders, in addition to their neuroprotective and wound healing activity. It should be, however, pointed out that some of these activities are observed at relative high concentrations, and therefore these studies should be considered with caution.

Mulleins are found to be a rich source of diverse groups of secondary metabolites. Untill now more than 200 compounds are identified, including iridoids, phenylethanoids, flavonoids, and saponins among others.

The genus *Verbascum* provides a wide range of research possibilities for the future. Key tasks as the lack of adequate taxonomic scheme and the incomplete or medley phytochemical data could be eventually solved by applying the modern 'omics' platforms (*inter alia* metabolomics). Further intensive studies (using

various animal models) are required to confirm *Verbascum*'s potential for treating various diseases, thereby enabling mulleins (or their bioactive principles) acceptance as therapeutic agents. For this, reliable standartization of mullein products is required and the chemical fingerprints need to be fully characterized.

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