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The use of Primula in folk medicine is described in the literature. Investigation of the biological and pharmacological activities of Primula are reported.
This study aims at providing a collection of publications on the genus Primula along with a critical revision of literature data. It focuses on the possible taxonomic significance of the secondary metabolites and on their ecological role as attractors for pollinators and deterrents against herbivores and parasites, in order to build the base for further studies.

*Graphical Abstract

Phytochemistry of European *Primula* species

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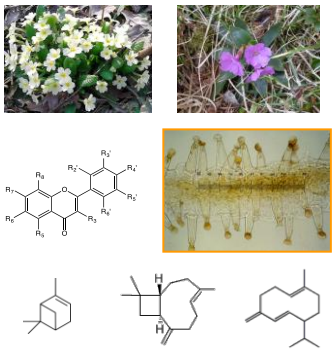

The secondary metabolites of the European species belonging *Primula* genus are reviewed. The main chemicals are tissue and epicuticular flavonoids, saponins and VOCs. Folk medicine and biological activities are reported.

***Highlights (for review)**

- Presentation and sorting of data related to secondary metabolites from European species of *Primula*.
- Flavonoids: researching compounds common to different species.
- Phytochemistry: researching biological activities and ecological role of metabolites.

Phytochemistry of European *Primula* species

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Abstract

The genus *Primula* is the largest among the Primulaceae and is widespread mainly in the cold and temperate regions of the Northern Hemisphere.

Since the beginning of the Twentieth century, several studies on the phytochemical composition of different species of *Primula* have been carried out. The main constituents examined were tissue and epicuticular flavonoids and saponins, which are of therapeutic significance. Only in recent years have studies of the volatiles emitted by leaves and flowers been carried out as well, but they are restricted to a small number of species. Only a few authors have documented the morphology and function of glandular trichomes in relation to the production of flavonoids and volatile organic compounds (VOCs).

The use of *Primula* in folk medicine is described in the literature. Investigation of the biological and pharmacological activities of *Primula* are reported.

This study aims at providing a collection of publications on the genus *Primula* along with a critical revision of literature data. It focuses on the possible taxonomic significance of the secondary metabolites and on their ecological role as attractors for pollinators and deterrents against herbivores and parasites, in order to build the base for further studies.

Keywords: genus *Primula*, Primulaceae, Europe, chemotaxonomy, flavonoids, saponins, volatile compounds.

Contents

1. Introduction

The genus *Primula* is the most widespread among the Primulaceae family, accounting for 430 species classified in 7 subgenera and 38 sections (Richard, 2003). It is chiefly distributed in the temperate and cold regions of the Northern Hemisphere, where it is particularly concentrated in the Himalayan area. Europe accounts for 33 species (**Table 1**) subdivided into 3 subgenera [*Primula*, *Aleuritia* (Duby) Wendelbo, *Auriculastrum* Schott - Tutin et al., 1993]. Here the genus is concentrated mainly in the Alps. Italy houses almost two thirds of the whole European *Primula* spp.: Pignatti (1982) recognised 20 species, and three further species endemic to the Alps, *P. albenensis*, *P. grignensis* and *P. recubariensis*, were recently discovered (Aeschimann et al., 2004), while on the Apennines two endemic species were identified: *P. apennina* and *P. palinuri* (Pignatti, 1982).

The genus is composed of herbaceous plants with a basal rosette of leaves and flowers on top of a naked scape (sometimes absent, as in *P. vulgaris*) gathered in lateral or perpendicular to the axis umbels.

The classification is traditionally based on morphological characteristics: flower colour, bract and calyx size and shape, seed appearance, leaf shape, consistency and, if present, scent, glandular hairs, exudates and farina disposition and morphology.

The interest in the genus *Primula* is evidenced by the publication, starting from the beginning of the nineteenth century, of extended monographs -Duby (1844), Lehmann (1817), Lüdi (1926), Pax (1889), Pax and Knuth (1905), Richards (1993; 2003), Schott (1851), Wendelbo (1961), Wright Smith and Fletcher (1941-1950), Wright Smith and Forrest (1928)-, an interest which is still active, as shown by the great number of works published on this topic in the past few years (Aronne et al., 2015; Boucher et al., 2016; Brys and Jacquemyn, 2015; Elser et al., 2016; Hashimoto et al., 2015; Nowak et al., 2015; Triest et al., 2015). These studies bear witness to the attention towards several aspects of scientific relevance: phytogeography, phytochemistry, biochemistry, phytotherapy, etc.

The current work presents an up-to-date critical review of publications on the genus *Primula* with the aim of laying the groundwork for further scientific investigations.

2. Chemical constituents

2.1. Flavonoids - A historical overview

Flavonoids are among the most studied plant secondary metabolites. These compounds may be accumulated both into the various organ tissues and on the aerial parts as epicuticular secretion, where they exert a number of useful functions for the plant.

Several phytochemical studies on the flavonoid composition have been carried out on the genus *Primula*.

The first studies on the flavonoids from genus *Primula* can be traced back to the beginning of the Twentieth century (Blansdale 1945, 1947; Brunswik, 1922; Müller, 1915). In particular, we mention the work by Jeffrey B. Harborne (1968) on the correlation between the pigment content and the systematics of the Primulaceae family and the numerous studies by Ekhard Wollenweber and colleagues on the flavonoid composition of the farina in *Primula* species. (Wollenweber, 1974; Wollenweber and Mann, 1986; Wollenweber and Schnepf, 1970; Wollenweber et al., 1988a; 1988b; 1989; 1990).

Furthermore, we cite the work by Schöpker et al. (1995), who localised PAL (phenylalanine ammonia-lyase) and CHS (calchone synthase) in the glandular trichomes of *P. kewensis*, and thus postulated that the gland cells could be involved in the biosynthesis of flavonoids.

2.1.1. Epicuticular flavonoids

Many plants accumulate flavonoids on their external surfaces. The ecological role of these chemicals has not been completely clarified. Some authors proposed an UV-screening function (Tattini et al., 2005). Other have postulated that they may act as antioxidants or for parasites/pathogen resistance (Valkama et al., 2003). Furthermore, unsubstituted flavone from the farina was proved to produce an improvement in freezing tolerance in some *Primula* spp. (Isshiki et al., 2014).

From the chemical point of view, it can be noted that the characterised compounds are generally aglycones differently substituted (1 to 5 substituents) with hydroxy, methoxy, methylenedioxy and acetyl groups. They are mainly flavones and flavonols, together with a few chalcones, substituted on all the position of ring B, preferably on 2' and 3' carbons. Ring A is normally less substituted, mainly on 5, 7 and 8 position.

Several references to these compounds in genus *Primula* can be found in the literature. **Table 2** shows the data from recent studies on the epicuticular flavonoids from European *Primula* species.

Traditionally, a pathway different from the usual, which involves the synthesis of flavones via the acetate and phenylalanine (Wollenweber et al., 1990), had been hypothesysed for primulas; such peculiar flavones were denominated "*Primula*-type flavones". The data collected in this review,

pertaining to the most recent publications, suggest the presence of the 7-hydroxyl group and hydroxylations in 5, 3'and 4', partially proving what said above wrong.

Recently, Elser et al. (2016) showed that in eight species of subsect. *Euauricula* the occurrence of "*Primula*-type flavones" and of flavonoids derived from the regular biosynthetic pathway seems to be mutually exclusive.

2.1.2. Tissue flavonoids

The most polar flavonoids (i.e. glycosyl derivatives) are normally stored in the tissues, usually within the vacuole (Debeaujon et al., 2001).

In genus *Primula* these chemicals have been poorly studied (**Table 3**). Based on the information we could retrieve from the literature the tissue flavonoids characterising flowers and leaves are mainly flavonols. The most frequently described aglycones are kaempferol, quercetin and isorhamnetin, but also apigenin, luteolin and, less frequently, limocitrin, myricetin and tamarixin have been reported. Tissue flavonoids are usually linked to 2-3 sugar units, typically glucose and rhamnose as gentiobiose, neohesperidose and sophorose. However, also arabinose, mannose, galactose and xylose have been identified.

The *O*-glycosylation is the most common in *Primula* genus. The sugar moiety of *O*-glycosides is always linked to C3 in flavonols. In flavones, glycosylation may occur at different positions, mainly on the hydroxyl group at C7. The aglycone moieties of the glycosides contain hydroxy- and sometimes methoxy-groups. Glycosylation on the B-ring is very rare among European species, it has been reported for *P. albenensis* and *P. farinosa* on 4' and 3' positions, respectively.

It is particularly interesting to draw attention to the case of *P. spectabilis*, unique in the genus *Primula* as, besides *O*-glycosides, also some glycoflavonoids **(13t-15t)** have been characterised, which have the typical glycosylation (glucose or arabinose) at the 6- and/or the 8-position of the flavonoid. Whithin this group, also a less frequent *O*-glycosylflavonol (**15t**) has been reported.

In general, among the aglycones, the flavones apigenin and luteolin are the most represented. Glycoflavones may be an interesting feature of *P. spectabilis*, as their biosynthesis appears to be different with respect to *O*-glycosides. Indeed, *C*-glycosylation is likely to be part of the flavonoid skeleton synthesis, while *O*-glycosylation generally occurs as a final stabilising step (Courts and Williamson, 2015).

An overview of the studies is presented in **Tables 3** and **4**.

2.2. Quinones

Primin (2-methoxy-6-pentyl-1,4-benzoquinone) and its benzoquinone derivatives were detected by Elser et al. (2016) in exudates from some European *Primula* species.

2.3. Saponins

The saponins from European *Primula* species have been poorly characterised.**Table 5** shows the compounds identified so far. *Primula* saponins were first reported from *P. vulgaris* (Hohnjec-Mihaljinac and Benzinger, 1962a, b, c). In 1975 Tschesche and Ballhorn recognised protoprimulagenin A (PSap1) as the aglycone of the main saponin in *P. elatior*; Tschesche and coauthors also studied the structure of the sugar chain, identifying two different saponins (PSap2 and PSap3) (Tschesche and Wiemann, 1977; Tschesche et al., 1983). Recently, the application of chromatographic techniques hyphenated with mass spectrometry and light scattering detection allowed separation of three further saponins from *P. elatior* and *P. veris* (Müller et al., 2006) identified as priverosaponin-B-22 acetate (PSap4), primulasaponin II (PSap5) and primulasaponin I; the latter corresponds to the main saponin characterized by Tschetsche et al., in 1983 (PSap2). Moreover, research was dedicated to the development of innovative methods for the purification, extraction and expression in cell cultures of the main derivatives characterised, as reported by Coran and Mulas, 2012; Hahn-Deinstrop et al., 2000; Okršlar et al., 2007.

2.4. Volatile compounds and essential oils

Studies on *Primula* VOCs or essential oils are scarce and limited to few species. These compounds seem to be characterising, but studies should be performed on a higher number of species and in different phenological stages. Indeed, the composition of the volatiles could change due to climatic and environmental pressures and herbivore or parasite attacks. In *Primula* spp., volatiles are derived mainly from the terpenoid and the phenylpropene pathways. The oldest investigation on volatiles from this genus dates back to 1980, when *P. veris* (Nilsson,1980) was found to produce the terpenoids *(E,E)*-α-farnesene, limonene and linalool. More recently, Gaskett et al. (2005) investigated the headspace of *P. elatior* and *P. farinosa* flowers and found that the two species emitted two completely different bouquets: the former released mainly monoterpenes, in particular limonene, while the latter produced benzenoids together with the quinone 4-oxoisophorone. *P. farinosa* was also investigated by Colombo et al. (2014a), although in this work the leaves were taken into consideration, together with those of *P. albenensis*, *P. auricula* and the leaves and flowers of *P. halleri*. The main VOCs sampled by SPME from *P. farinosa* were sesquiterpene hydrocarbons. A very similar behaviour was observed for *P. auricula* and the leaves of *P. halleri*, although the main sesquiterpene hydrocarbons of the respective

bouquets were different or present in different amounts. The volatile emission of the flowers of *P. halleri* was completely different, being mainly composed of non-terpene derivatives both for calyx and corolla. *P. albenensis* showed yet again a completely different emission pattern, being almost exclusively composed of the phenylpropanoid-related paeonal.

Another SPME study on the headspace of the leaves, whole flowers and flower parts was performed on *P. spectabilis* (Vitalini et al., 2011). The leaves emitted mainly non-terpene derivatives, while the whole flowers produced mainly monoterpene hydrocarbons, followed by non-terpene compounds.

The essential oil was obtained and characterised exclusively from the non-European species *P. obconica* (Nan et al., 2002).

2.5. Other compounds

Please, refer to paragraph **4.1** for other compounds such as phenolic glucosides.

3. Glandular trichomes

The morphology of glandular trichomes was examined in few studies only. So far, a morphological analysis has been performed on the following species: *P. albenensis* (Colombo et al., 2014a), *P. auricula* (Colombo et al., 2014a; Fico et al., 2007), *P. daonensis* (Fico et al., 2007), *P. farinosa* (Colombo et al., 2014a), *P. halleri* (Colombo et al., 2014a), *P. hirsuta* (Fico et al., 2007), *P. recubariensis* (Prosser and Scortegagna, 1998), *P. spectabilis* (Vitalini et al., 2011), *P. veris* (Länger and Saukel, 1993), *P. vulgaris* (Bhutia et al., 2012; Wollenweber and Schnepf, 1970). According to these studies, the trichome always follows the same morphological pattern, with a glandular head on a monoseriate stalk (which consists of a rectangular neck and a cylindric/conic stalk). A difference was observed, instead, in the dimensional ratio of the different segments. This seems to be a useful tool when it comes to discriminating the species, as shown in the works by Fico et al., 2007 and Colombo et al., 2014a.

A peculiar situation occurred in *P. veris* in which branched and unbranched trichomes with an apical inverse pear-shaped head were described on leaves (Länger and Saukel, 1993). However, further in-deepth micromorphological investigation are needed to uncertain the glandular or non-glandular nature of these structure.

4. Taxonomy

There are several studies, both phylogenetic and chemotaxonomic, on genus *Primula*. Since the beginning of the past century, a number of biochemical (Müller, 1915; Brunswik, 1922; Hegnauer, 1969), cytologic (Bruun, 1932), karyological (Wanner, 1943), cytotaxonomic (Kress, 1969),

palynological (Spanowsky, 1962) and biomolecular (Scudiero et al., 1980) works have been published. We cite in particular the systematic studies on section *Auricula* by Zhang and Kadereit (2004; 2005) and later by Crema and Cristofolini (2012), who, using molecular markers, could show that this Section is divided into two monophyletic groups. Lastly, Boucher et al. (2016) suggest the subdivision of section *Auricula* in 4 clades.

We also mention the phylogenetic studies on chloroplast sequences by Mast et al. (2001), which retrace the phylogenetic history of the group; the phytochemical works by Valant-Vetschera et al. (2010) to include genus *Dionysia* in genus *Primula* and by Mast and Reveal (2007) to move *Dodecatheon* to *Primula*; the study by Harborne (1968) on the connection between the flavonoid content and the systematic of family Primulaceae; the works by Bhutia et al. (2013; 2012), Bhutia and Valant-Vetschera (2012), Elser et al. (2016) and Valant-Vetschera et. al. (2009) for the analysis and comparison of the epicuticular flavonoid profile of over 60 species of primulas.

The issues in the recognition in the field and in the taxonomic determination are chiefly due to the species tendency to develop transitional plants and form fertile hybrids.

4.1 Chemotaxonomy

This review is aimed to assess whether it is possible to characterize and classify the genus *Primula* according to the chemodiversity of chemical profiles. Recently, El Morchid et al., 2014 observed that the flavonoid profile could be used to differentiate among wild population of *P. veris*.

Flavonoids are the most studied secondary metabolites in genus *Primula* as they are well represented, although the presence of saponins characterises the genus as well.

In the past, genus *Primula* was examined with a chemotaxonomic interest for its content in secondary metabolites: anthocyanins in flowers, flavonoids in leaves, saponins in roots and rhizomes, sugars and sugar alcohols in leaves, as well as polyphenols, salicylates, quinones, ascorbic acid, etc.

Some sugar alcohols and their corresponding sugars were found to be restricted to genus *Primula* and some of its sections and subsections: these are volemitol, hamamelitol, the alcohol corresponding to hamamelose, 2-hydroxymethyl-D-ribose, clusianose, the α-galactoside of hamamelitol (Kremer, 1978; Sellmair et al., 1977).

Based on the content in hamamelose, hamamelitol and clusianose, Sellmair et al. (1977) suggested a taxonomic separation of subsections *Auricula* and *Erythrodrosum* from the four subsections of section *Auricula*, but no further up-to-date data has been published on this topic so far.

Kremer (1978) and Häfliger et al. (1999) investigated the content and the metabolism of volemitol with a focus on its possible taxonomic role.

Müller et al. (2006) found phenolic glycosides, mainly primeverin (Benzoic acid, 4-methoxy-2-[(6-*O*-β-D-xylopyranosyl-β- D-glucopyranosyl)oxy]-, methyl ester) and primulaverin (Benzoic acid, 5-methoxy-2-[(6-*O*-β-D-xylopyranosyl- β-D-glucopyranosyl)oxy]-, methyl ester) in *P. elatior* and *P. veris* and confirmed their identity and purity through NMR.

Also stearidonic acid, a particular primary metabolite, has been suggested to be a possible chemotaxonomic marker for the genus *Primula*, but this hypothesis has not been proved yet, as its presence was investigated in non-European species only (Aitzetmüller and Werner, 1991).

4.1.1 Epicuticular flavonoids

Since 2005, 38 epicuticular flavonoids have been identified from different European *Primula* species (**Table 2**). In particular, 26 compounds were isolated from subgenus *Auriculastrum*, while subgenera *Aleuritia* and *Primula* gave 14 and 16 derivatives, respectively.

The main aglycone is flavone, B-ring was found to be the most substituted, as substitution can occur at any positions (2'-6'). On the A-ring substitution normally occurs at C5-position, but C6, C7 and C8 can be involved as well. The principal substituents are OH, OMe, OAc, MetDiox, benzoate.

Flavone, 2'-hydroxyflavone and 5-hydroxyflavone were found to be common to all the subgenera.

Subgenus *Auricula*

No compound is common to all the species belonging to the subgenus. The most distributed are flavone (**5**), 5-hydroxyflavone (**21**), 2'-hydroxyflavone (**6**) and 5,8-dihydroxyflavone (**27**).

Subgenus *Aleuritia*

Four compounds are shared by most of the species belonging to this subgenus: **5, 6, 21, 27.**

Subgenus *Primula*

The subgenus is characterised by compounds **7**, **11**, **13**, **14** and **17**, which occur in all investigated species, but the subgenus is distinguished from the other subgenera by the presence of compounds **9**, **14**, **15**, **16**, **17**, **19**, **24** and **25**, which have not been found in species of subgenera *Auricula* and *Aleuritia*.

At the species level, *P. palinuri*, *P. spectabilis*, *P. farinosa*, *P. halleri*, *P. elatior* and *P. veris* are characterised by unique compounds.

The most common compounds are flavone (**5**), 2'-hydroxyflavone (**6**), 5-hydroxyflavone (**21**), 5,8-dihydroxyflavone (**27**), 2'-methoxyflavone (**7**) and 5,2'-dihydroxyflavone (**22**). Some compounds are also shared with other non-European *Primula* species (Bhutia et al., 2013; Bouillant et al., 1971; Hausen et al., 1983; Kosenkova et al., 2008; Isshiki et al., 2014; Tokalov et al. 2004; Wollenweber and Mann, 1986; Wollenweber et al., 1988a; 1988b; 1989).

Comparing the data presented to those found in works on molecular biology from literature (Boucher et al., 2016; Crema and Cristofolini, 2012; Zhang and Kadereit, 2004), referred exclusively to section *Auricula*, we can conclude that the lack of phytochemical data do not allow any speculation on the possibility of supporting the biomolecular data. The only exception is *P. palinuri*, which, based on biomolecular data, does not have any similatity to any current species.

Such condition might be confirmed by the phytochemical data we presented prove the existence of compounds exclusive to this species (**Table 2**).

4.1.2 Tissue flavonoids

Since 1981, 38 tissue flavonoids have been described for European *Primula* species (**Table 3, 4**). 18 compounds have been isolated from subgenus *Auriculastrum*, while subgenera *Aleuritia* and *Primula* gave 7 and 18 derivatives, respectively.

Each species is well characterised: only compounds **4t**, **5t**, **12t**, **17t** are common to *Auriculastrum* and *Primula* subgenera, and **24t** to *Aleuritia* and *Primula* subgenera.

Subgenus *Auriculastrum*

It is characterised by mono- and diglycosylated flavonoids. Among aglycones flavone, tamarixetin, apigenin and luteolin are produced only in subgenus *Auriculastrum*.

The monosaccharides are glucose, rhamnose, xylose and arabinofuranose. Neohesperidose and sophorose, linked to the 3-position, are exclusive of this subgenus.

Subgenus *Aleuritia*

It is characterised by a prevalence of triglycosylated derivatives at 3-position. The aglycones are kaempferol, quercetin and isorhamnetin. The main sugar units are glucose, galactose and rhamnose.

Subgenus *Primula*

Typical compounds of this subgenus are mono-, di- and triglycosylated flavonols, mainly kaempferol, quercetin and isorhamnetin linked to glucose, galactose and rhamnose. Robinobiose, linked to 3-position, is another interesting feature of this subgenus.

Table 4 shows the common compounds at the species level.

Tamarixetin is unique to *P. daonensis* and luteolin to *P. spectabilis*. These aglycones have not been isolated in other species yet, neither European nor non-European.

Some species show similar glycosylation patterns in position 3 of the flavonoid nucleus: neohesperidose is common to *P. daonensis, P. hirsuta* and *P. latifolia; O*-Gal-Rha-Rha to *P. farinosa* and *P. halleri; O*-Rut and *O*-Glc to *P. elatior* and *P. veris*. 7-glycosylation is exclusive to *P. auricula* (*O*-Glc), while *C*-glycosylation in positions 6 and 8 is exclusive to *P. spectabilis*.

B-ring glycosylation is a peculiarity of *P. albenensis* (*O*-Glc) and *P. farinosa* (*O*-Gal).

The same remarks made for the epicuticular flavonoids apply to the comparison with the data presented in the molecular biology papers mentioned above.

5. Ecological role of *Primula* secondary metabolites

There are serveral studies citing the pests and predators of different species of European primulas (Brys and Jacquemyn, 2009; Hambler and Dixon, 2003; Jacquemyn et al. 2009; Taylor and Woodell, 2008). Nevertheless, the role of secondary metabolites in the reduction of the detrimental interactions with these organisms is still unclear and should be further investigated.

A hint on their possible defensive role is given in the work by Aronne et al. (2010): studying the post-germination phases of the seeds of *P. palinuri*, they observed an unusual accumulation of phenolic compounds in the hypocotyl and inferred that it might be part of a strategy to protect seeds against herbivores and predators. A preliminary study on the possible allelopathic interference of the flavonoids deposited on the leaf of *P. auricula* was carried out in vitro by Vignati et al. (2008).

Flowers emit volatiles to attract species-specific pollinators in association with the visual cues provided by pigments. A wide spectrum of insect visitors and pollinators has been observed in European *Primula* spp. (Brys and Jacquemyn, 2009; De Micco and Aronne, 2012; Fisogni et al., 2011; Hambler and Dixon, 2003; Jacquemyn et al., 2009; Minuto et al., 2014; Taylor and Woodell, 2008). Andersson et al. (2002) explored a possible convergence in the composition of the volatiles emitted by 22 butterfly-pollinated plant species, among which *P. farinosa*. The flowers of this species contain large quantities of the irregular terpene oxoisophorone. The authors suggest that these compounds might be involved in the attraction of pollinating butterflies.

P. elatior and *P. veris* were studied by Gaskett et al. (2005). These species present heterostyly to prevent self-pollination. Consequently, pollen transfer between different individuals requires the presence of pollinators. This might help preventing pollinators from preferring one morph to the other. Interestingly, no intraspecific variation in scent between the floral morphs in either *Primula* species was evidenced.

Vitalini et al. (2011) studied the emission of *P. spectabilis* and found that flowers emit large amounts of benzenoids and of single compounds, such as α-pinene and methyl salicylate. The authors suggested that this might be a double strategy to attract both butterflies and bees. Furthermore, each flower part produced a very different bouquet, supporting the hypotesis that the formation of odor gradients may represent an orientation cue for proper pollination by insects landed to visit the flowers (Flamini, 2012; Flamini and Cioni, 2010).

6. Folk medicine - Ethnobotany and traditional use

In the folk medicine, the hypogeal parts (rhizome and root, *Primulae radix*) and the flowers (*Primulae flos)* from *P. veris* and *P. elatior* are used in the form of infusion, decoction or tincture as antitussives and mucolytics/secretolitic (Da Legnano, 1973; Della Loggia, 1993; Firenzuoli, 2002). They are known to be rich in saponins (Coran and Mulas, 2012) and are included in the European Pharmacopoeia (EDQM, 2011; EMA, 2012). The leaf (*Primulae folium)* infusion of these two species is also recommended for vitamin C deficiency (Dinulica e Borz, 2013). In Denmark, the distilled water obtained from the flowers and the infusion of fresh or dry plants are used against headache, epileptic seizures and insomnia (Jäger et al., 2006).

7. Biological activities

Among all the biological activities of primroses, the one which has been known for the longest time is the sensitising action. Primroses can cause allergic contact dermatitis (Aplin et al., 2000). The most representative species is *P. obconica*, a non-European species, whose sensitising effects have been known and studied for more than a century (Aplin and Lovell, 2001). The allergens are concentrated in the glandular hairs of the aerial parts. Other than by contact, the allergic reactions can be elicited by inhalation of the particles emitted into the air (Christensen and Larsen, 2000). The main sensitiser is primin (2-methoxy-6-pentyl-1,4-benzoquinone), but its biosynthetic precursor, miconidin (2-methoxy-6-pentyl-1,4-dihydroxybenzene), has proved to be a possible allergen as well (Connolly et al., 2004).

Some interesting pharmacological activities of extracts, phytocomplexes, pure compounds, etc. from European species are:

- Antioxidant and antiinflammatory: this activity was proved for the flavonoids extracted from aerial parts of *P. elatior* and isolated from the EtOAc fraction (Mostafa et al., 2014).
- Antimicrobic: the ether and ethanol extract of *P. veris* flowers inhibit the growth of Gram positive and Gram negative bacteria (Başbülbül et. al., 2008). Methanol and cold water extracts of *P. vulgaris* leaves and roots were tested with good results against *Escherichia coli* and *Pseudomonas aeruginosa* (Majid et al., 2014). The ethanol extracts of *P. vulgaris* subsp. *sibthorpii* leaves and flowers have proved active against *Mycobacterium tuberculosis* (Tosun et al., 2005).
- Antimitotic: the decoction in distilled water of *P. veris* flowers inhibits root growth in *Allium cepa* (Başbülbül et. al., 2008).

8. Discussion and conclusions

The studies on the European primulas need being supplemented by further research on the chemical composition of the species.

Only 37% of European *Primula* species were studied with regard to epicuticular flavonoids: 33% of the species belonging to section *Auricula*, 43% to section *Aleuritia* and the three species belonging to section *Primula*. The number of species examined for their tissue flavonoid content is even smaller and corresponds to 29% of total *Primula* spp.: 25% of the species from section *Auricula*, 28% from section *Aleuritia* and the three species belonging to section *Primula*.

Only an in-depth work on the characterisation of the flavonoid composition will allow further chemotaxonomic speculation.

Quinones, mainly primin and miconidine, have been studied in non-European *Primula* spp.

Recent findings show that some exudates from European species contain the benzoquinone primin and some of its derivatives (Elser et al., 2016).

Most of the studies on pigments found in literature date back to 1968 (Harborne). Studies on VOCs content are lacking as well: only 7 species have so far been examined to assess their emission.

No European *Primula* species was studied to test the composition in essential oil.

Saponins, the second largest group of secondary metabolites characterising the genus, were assessed only in section *Primula*, in *P*. *elatior* and in *P*. *veris*.

Some evidence of other compounds less frequently detected was also documented.

Some critical issues with respect to the studies suggesting the use of flavonoids as taxonomic markers emerged: a) homogeneity in starting botanical material is lacking (material collected in the wild or cultivated or purchased/dried or fresh material or herbarium specimens); b) in some cases the taxonomic characterisation is not rigorous and the species were identified by a botanist only seldom (in some cases the authors refer to hybrids or commercial samples); c) the compounds compared are often produced by different parts of the plant (flowers, leaves, aerial parts); d) harvesting times are different; e) in most cases the plant material originates from a single entity; only rarely entities of the same species were evaluated throughout the years or entities belonging to the same species, but coming from different areas, were compared.

From a chemical point of view, the most relevant observations concern the fact that in some works a structural identification by NMR is lacking (only a comparison with reference standards is performed) and in some cases it is not possible to discriminate between epicuticular and tissue flavonoids.

It is evident that some epicuticular compounds characterise the species, but the critical issues we found suggest the need to conduct further investigations on the flavonoid profiles of *Primula* before we can affirm that they have a value in the context of chemodiversity studies.

Tissue flavonoids seem to be generally characterising at the species level, but the small presence of literature data makes it impossible to formulate taxonomical hypotheses aimed to understand the similarities between species belonging to the same subgenus, section or subsection.

There are still unanswered questions related to the high productivity of volatile compounds, which allow botanists to recognise the different species even from their smell thanks to their peculiar scents. Data analysis of the literature showed that the morphology of the glandular hairs is identical in every species belonging to the genus *Primula*, while differences were found in the size ratios of the components of the single trichome.

There are also only few studies on the ecological role of secondary metabolites; for example, the reason for the particular disposition the farinas show on the aerial parts of the farinous species has not been understood, yet. In some cases (e.g. *P. auricula*) the farina produced by the glandular trichomes is deposited mainly on the edge of the leaf, in other species on the lower surface (e.g. *P. farinosa* and *P. halleri*) or even on both leaf surfaces, as in *P. albenensis*. A possible explanation for this kind of distribution is that the rosette structure of the leaves covered with UV-absorbing compounds and thus, visible to pollinators, could act as an enhanced visual cue the plant adopts to facilitate the processes of pollination. This hypothesis does not explain, though, why the farinas are sometimes deposited on the lower surfaces as well. The particular distribution of the farina might have different roles in different entities. The deposition on the lower surfaces of leaves could have a protective role against the soil and the microorganisms living therein. Literature lacks information and findings related to the presence of parasites, pests or soil microorganisms that pose a threat to any *Primula* spp. *P. vulgaris* represents an exception, as its visitors (Jacquemyn et al., 2009), including pests like the dipteran *Chromatomyia primulae* larva (Spencer, 1990; Hardy, 1849), are described. Also, the allelopathic role of such compounds (Vignati et al., 2008) was only poorly investigated. The main constituents of the farinas or exudates *Primula* spp. produce and deposit on leaf surfaces are flavone, 2'-hydroxy and 5-hydroxyflavone and are widespread in almost the whole genus; other compounds, on the contrary, are exclusive to some species. The latter might have a role in the species-specific recognition by pollinators, for example because of a different UV absorption. This hypothesis could be confirmed if species-specific pollination was ascertained, but it was so far observed in two Italian endemic species only (*P. apennina*, Fisogni et al., 2011 and *P. palinuri,* Giovannetti and Aronne, 2012).

For other species, such as *P. vulgaris* (Jacquemyn et al., 2009), *P. elatior* (Taylor and Woodell, 2008) and *P. farinosa* (Gaskett et al., 2005; Hambler and Dixon, 2003), numerous pollinators including butterflies, diurnal moths, diptera, bees and bumblebees, are reported.

A traditional use of *Primula* spp. roots, leaves and flowers is described. Little data is available on European species (*P*. *elatior*, *P*. *veris*), which are reported to have antitussive, mucolytic and secretolytic properties.

European species manifest interesting biological activities: antioxidant, antiinflammatory, antimicrobial and antimitotic, but the studies on this topic are limited as well.

There are several areas of application for the study of *Primula* species, which, considering the data presented, deserve attention from the scientific community. If there will be an active interest on the phytochemical and ecological, other than biological, role of *Primula* species, with the next review we will be able to propose chemotaxonomical hypotheses and the groundwork to a better understanding of the role of the different metabolites production in plant/environment interaction will be laid.

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Table 1
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Table 1
Primula genus - Taxonomy of the European species

Species	Subgenus	Section	Subsection
❋*P. albenensis* Banfi et Ferlinghetti	*Auriculastrum* Schott	*Auricula* Duby	*Brevibracteata* Widmer
❋*P. allionii* Loisel. in Desv.	*Auriculastrum* Schott	*Auricula* Duby	*Rhopsidium* Schott
❋*P. apennina* Widmer	*Auriculastrum* Schott	*Auricula* Duby	*Erythrodosum* Schott
❋*P. auricula* L.	*Auriculastrum* Schott	*Auricula* Duby	*Auricula*
❋*P. carniolica* Jacq.	*Auriculastrum* Schott	*Auricula* Duby	*Brevibracteata* Widmer
✦*P. clusiana* Tausch	*Auriculastrum* Schott	*Auricula* Duby	*Arthritica* Schott
❋*P. daonensis* (Leybold) Leybold	*Auriculastrum* Schott	*Auricula* Duby	*Erythrodosum* Schott
✦*P. deorum* Velen.	*Auriculastrum* Schott	*Auricula* Duby	*Cyanopsis* Schott
✦*P. glaucescens* Moretti	*Auriculastrum* Schott	*Auricula* Duby	*Arthritica* Schott
P. grignensis D. M. Moser	*Auriculastrum* Schott	*Auricula* Duby	*Erythrodosum* Schott
✦*P. glutinosa* Wulfen in Jacq.	*Auriculastrum* Schott	*Auricula* Duby	*Cyanopsis* Schott
❋*P. hirsuta* All.	*Auriculastrum* Schott	*Auricula* Duby	*Erythrodosum* Schott
✦*P. kitaibeliana* Schott	*Auriculastrum* Schott	*Auricula* Duby	-
✦*P. integrifolia* L.	*Auriculastrum* Schott	*Auricula* Duby	*Rhopsidium* Schott
❋*P. latifolia* Lapeyr.	*Auriculastrum* Schott	*Auricula* Duby	*Brevibracteata* Widmer
❋*P. marginata* Curtis	*Auriculastrum* Schott	*Auricula* Duby	*Brevibracteata* Widmer
✦*P. minima* L.	*Auriculastrum* Schott	*Auricula* Duby	*Chamaecallis* Schott
❋*P. palinuri* Petagna	*Auriculastrum* Schott	*Auricula* Duby	*Auricula*
❋*P. pedemontana* Thomas ex Gaudin	*Auriculastrum* Schott	*Auricula* Duby	*Erythrodosum* Schott
❋*P. recubariensis* Prosser et Scortegagna	*Auriculastrum* Schott	*Auricula* Duby	*Erythrodosum* Schott
✦*P. spectabilis* Tratt.	*Auriculastrum* Schott	*Auricula* Duby	*Arthritica* Schott
✦*P. tyrolensis* Schott	*Auriculastrum* Schott	*Auricula* Duby	*Rhopsidium* Schott
❋*P. villosa* Wulfen in Jacq.	*Auriculastrum* Schott	*Auricula* Duby	*Erythrodosum* Schott
✦*P. wulfeniana* Schott	*Auriculastrum* Schott	*Auricula* Duby	*Arthritica* Schott
P. egaliksensis Wormsk. in Hornem.	*Aleuritia* (Duby) Wendelbo	*Armerina* Lindley	-
P. farinosa L.	*Aleuritia* (Duby) Wendelbo	*Aleuritia* Duby	*Aleuritia*
P. frondosa Janka	*Aleur itia* (Duby) Wendelbo	*Aleuritia* Duby	*Aleuritia*
P. halleri G. F. Gmelin	*Aleuritia* (Duby) Wendelbo	*Aleuritia* Duby	*Aleuritia*
P. longiscapa Ledeb.	*Aleuritia* (Duby) Wendelbo	*Aleuritia* Duby	*Algida* A. J. Richards
P. nutans Georgi	*Aleuritia* (Duby) Wendelbo	*Armerina* Lindley	-
P. scandinavica Bruun	*Aleuritia* (Duby) Wendelbo	*Aleuritia*	*Aleuritia*
P. scotica Hooker in Curtis	*Aleuritia* (Duby) Wendelbo	*Aleuritia*	*Aleuritia*
P. stricta Hornem.	*Aleuritia* (Duby) Wendelbo	*Aleuritia*	*Aleuritia*
P. elatior (L.) Hill	*Primula*	*Primula*	-
P. veris L.[1]	*Primula*	*Primula*	-
P. vulgaris Hudson	*Primula*	*Primula*	-

[1]*P. veris* L. syn. *P. officinalis* (L.) Hill (Karl et al., 1981).

Notes: with reference to the classification introduced by Zhang and Kadereit, 2004: ❋ Subsection *Euauricula*; ✦ Subsection *Cyanopsis*. For recent reference to further biomolecular studies, see Boucher et al., 2016. *P. grignensis* was not mentioned in the work by Zhang and Kadereit, 2004.

Refs.: Pignatti, 1982; Richards, 2003; Tutin et al., 1993.

Table 2

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Table 2

European *Primula* species - Epicuticular flavonoids

Subgenus	Section	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	Refs.
			2',β-diOHchalcone	2',2-diOHchalcone	2'-OH-2-OMechalcone	2', 2,3-triOHchalcone	flavone	2'-OHflavone	2'-OMeflavone	2',5'-diOHflavone	2',5'-diOMeflavone	2'-OH-5'-OAcflavone	3'-OMeflavone	3',4'-diOHflavone	3',4'-diOMeflavone	3'-OH-4',5'-diOMeflavone	3'-OH-4',5'-MetDioxflavone	3'-OMe-4',5'-MetDioxflavone	3',4',5'-triOMeflavone	3,5-diOHflavone	3',5'-diOH-4'-OMeflavone	4'-OHflavone	5-OHflavone (Primuletin)	5,2'-diOHflavone	5-OH-2'-OMe-flavone	5,6,2',6'-tetraOMeflavone	5,6,2',3'6'-pentaOMeflavone	5,7-diOHflavone (Chrysin)	5,8-diOHflavone (Primetin)	5,8,2'-triOHflavone	7,3',4'-triOMequercetin	7,8-diOHflavone	8,2'-diOHflavone	8,2',5'-triOHflavone-5'-benzoate	Apigenin	Apigenin 7-Me	Kaempferol	Kaempferol 3-Me	Naringerin	Naringerin 7,4'-diMe	
Auriculastrum		*P. albenensis*					+	+					+		+							+																			a, d, e
		P. auricula					+	+						+	+							+	+	+				+													a, d, e
		P. glaucescens					+					+											+						+													b, d
		P. glutinosa		+		+																	+																			c, d
		P. hirsuta					+		+			+																	+						+	+	+	+	+		b, d
	Auricula	*P. latifolia*					+		+			+	+																+													d
		P. marginata					+	+															+						+													e, d
		P. minima					+					+											+						+													d
		P. palinuri	+	+	+		+	+	+	+		+											+	+						+				+	+						e, f
		P. spectabilis																													+											g
		P. villosa																																							+	d
Aleuritia		*P. farinosa*					+	+						+						+		+	+	+					+												a, e
	Aleuritia	*P. frondosa*					+	+															+	+																		e
		P. halleri					+	+	+	+													+	+	+	+			+	+			+									a, e
	Armerina	*P. nutans*		+			+	+		+				+									+																			e
Primula		*P. elatior*							+		+		+		+	+	+			+		+					+															e, h
	Primula	*P. veris*					+	+	+		+		+		+	+			+	+						+																e, i
		P. vulgaris					+		+			+	+		+	+				+				+		+																l

Note: Plant parts: a, c, d, g, h, i, l= leaves; b, e= aerial parts; f= inflorescences.

Refs.: (a) Colombo et al., 2014a; (b) Bhutia and Valant-Vetschera, 2012**;** (c) Bhutia et al., 2013; (d) Elser et al., 2016; (e) Valant-Vetschera et al., 2009; (f) Iinuma et al., 2006; (g) Vitalini et al., 2011; (h) Budzianowski and Wollenweber, 2007; (i) Budzianowski et al., 2005; (l) Bhutia et al., 2012.

Table 3
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Table 3
European *Primula* species - Tissue flavonoids[(a)]

Species	Plant part	No.	Compound	Refs.
P. albenensis	leaves	**1t**	4'-*O*-(β-glucopyranosyl)-3'-hydroxyflavone	Colombo et al., 2014a
P. auricula	leaves	**2t**	2'-hydroxyflavone 7-*O*-β-glucopyranoside (Macrophylloside)	Fico et al., 2007
		3t	Isorhamnetin 3-*O*-β-glucopyranosyl-(1→2)gentiobioside	
		4t	Quercetin 3-*O*-β-glucopyranosyl-(1→2)gentiobioside	
P. daonensis	leaves	**5t**	Isorhamnetin 3-*O*-β-glucopyranoside	Fico et al., 2007
		6t	Isorhamnetin 3-*O*-neohesperidoside	
		7t	Isorhamnetin 3-*O*-[(2-*O*-α-rhamnopyranosyl-6-*O*-β-glucopyranosyl)-β-glucopyranoside]	
		8t	Quercetin 3-*O*-neohesperidoside	
		9t	Tamarixin (Tamarixetin 3-*O*-β-glucopyranoside)	
P. hirsuta	leaves	**10t**	Kaempferol 3-*O*-α-rhamnopyranosyl-(1→2)-β-glucopyranoside (Kaempferol 3-*O*-neohesperidoside)	Fico et al., 2007
		11t	Kaempferol 3-*O*-α-rhamnopyranosyl-(1→2)-*O*-β-xylopyranosyl-β-glucopyranoside	
P. latifolia	leaves	**10t**	Kaempferol 3-*O*-α-rhamnopyranosyl-(1→2)-β-glucopyranoside (Kaempferol 3-*O*-neohesperidoside)	Colombo et al., 2014b
		12t	Quercetin 3-*O*-α-rhamnopyranosyl-(1→6)-β-glucopyranoside (Quercetin 3-*O*-rutinoside) (Rutin)	
P. spectabilis	leaves	**13t**	Apigenin 6-*C*-α-arabinofuranoside	Vitalini et al., 2010
		14t	Apigenin 6,8-di-*C*-β-glucopyranoside	
		15t	Kaempferol 8-*C*-β-glucopyranoside	
		16t	Luteolin 7-*O*-α-arabinofuranosyl-8-*C*-β-glucopyranoside	
		17t	Quercetin 3-*O*-gentiobioside	
		18t	Quercetin 3-*O*-sophoroside	
P. farinosa	leaves	**19t**	3'-*O*-(β-galactopyranosyl)-2'-hydroxyflavone	Colombo et al., 2014a
		20t	Kaempferol 3-*O*-α-rhamnopyranosyl-(1→3)-*O*-[α-rhamnopyranosyl-(1→6)]-*O*-β-galactopyranoside	
		21t	Kaempferol 3-*O*-α-rhamnopyranosyl-(1→3)-*O*-[α-rhamnopyranosyl-(1→6)]-*O*-β-glucopyranoside	
		22t	Kaempferol 3-*O*-(2",6"-di-*O*-α-rhamnopyranosyl)-β-glucopyranoside (Clitorin)	
P. halleri	leaves	**23t**	Isorhamnetin 3-*O*-α-rhamnopyranosyl-(1→3)-*O*-[α-rhamnopyranosyl-(1→6)]-*O*-β-galactopyranoside	Colombo et al., 2014a
		24t	Kaempferol 3-*O*-glucopyranosyl-(1→2)gentiobioside	
		25t	Quercetin 3-*O*-α-rhamnopyranosyl-(1→3)-*O*-[α-rhamnopyranosyl-(1→6)]-*O*-β-galactopyranoside	
P. elatior	aerial parts	**26t**	4'-methoxy kaempferol-3-*O*-β-glucuronopyranoside	Mostafa et al., 2014
		27t	Kaempferol-3-*O*-β-glucuronopyranoside	
		28t	Quercetin-3-*O*-β-glucuronopyranoside	
	flowers	**29t**	Quercetin 3-*O*-β-glucopyranoside (Isoquercitrin)	Petitjean-Freytet, 1993
		5t	Isorhamnetin 3-*O*-β-glucopyranoside	
		30t	Isorhamnetin 3-*O*-rutinoside	
		31t	Kaempferol 3-*O*-rutinoside	
		12t	Quercetin 3-*O*-α-rhamnopyranosyl-(1→6)-β-glucopyranoside (Quercetin 3-*O*-rutinoside) (Rutin)	
P. veris	flowers	**5t**	Isorhamnetin 3-*O*-glucopyranoside	Karl et al., 1981
		32t	Isorhamnetin 3-*O*-(rhamnopiranosyl)-robinobioside	
		33t	Isorhamnetin 3-*O*-robinobioside	
		34t	Kaempferol 3-*O*-robinobioside	

		31t	Kaempferol 3-*O*-rutinoside	
		35t	Limocitrin 3-*O*-glucopyranoside	
		4t	Quercetin 3-*O*-β-glucopyranosyl-(1→2)gentiobioside	
		17t	Quercetin 3-*O*-gentiobioside	
		36t	Quercetin 3-*O*-(rhamnopyranosyl)-robinobioside	
		37t	Quercetin 3-*O*-robinobioside	
		29t	Quercetin 3-*O*-β-glucopyranoside (Isoquercitrin)	
		30t	Isorhamnetin 3-*O*-rutinoside	
		12t	Quercetin 3-*O*-α-rhamnopyranosyl-(1→6)-β-glucopyranoside (Quercetin 3-*O*-rutinoside) (Rutin)	
		38t	3',4',5'-trimethoxyflavone	Huck et al., 1999; 2000
P. vulgaris	leaves	**24t**	Kaempferol 3-*O*-glucopyranosyl-(1→2)gentiobioside	Colombo et al., 2014b

[(a)]D-, L-, α- and β- are given only when mentioned in the original document.

Notes: Compound 3',4'-dihydroxyflavone-glucopyranoside found in *P. veris* (Karl et al., 1981) was excluded as in the article it is not clear whether it is a mono- or diglycosylated flavonol or not.

Compound **38t** is normally found on leaf surface; nonetheless, in this particular case it was obtained by methanol extraction after pulverisation, a technique used for tissue flavonoids.

Table 4
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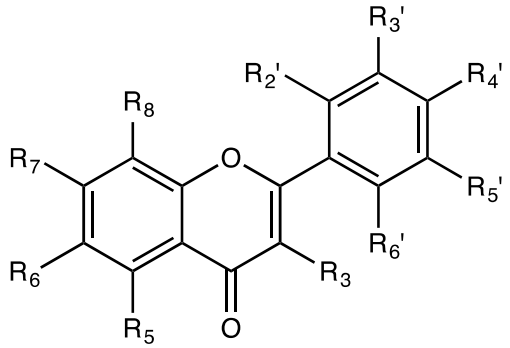
Table 4
European *Primula* species - Tissue flavonoid structures[(a)]



Subgenus *Auriculastrum*						Subgenus *Aleuritia*		Subgenus *Primula*												
P. albenensis	*P. auricula*	*P. daonensis*	*P. hirsuta*	*P. latifolia*	*P. spectabilis*	*P. farinosa*	*P. halleri*	*P. elatior*	*P. veris*	*P. vulgaris*	Aglycone	Compound	R_3	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$
+											Flavone	**1t**	H	H	H	H	H	H	OH	*O*-Glc	H
	+											**2t**	H	H	H	*O*-Glc	H	OH	H	H	H
						+						**19t**	H	H	H	H	H	OH	*O*-Gal	H	H
									+			**38t**	H	H	H	H	H	H	OMe	OMe	OMe
		+									Tamarixetin	**9t**	*O*-Glc	OH	H	OH	H	H	OH	OMe	H
	+										Isorhamnetin	**3t**	*O*-Gen-Glc	OH	H	OH	H	H	OMe	OH	H
		+						+	+			**5t**	*O*-Glc	OH	H	OH	H	H	OMe	OH	H
		+										**6t**	*O*-Neo	OH	H	OH	H	H	OMe	OH	H
		+										**7t**	*O*-Glc-Rha-Glc	OH	H	OH	H	H	OMe	OH	H
							+					**23t**	*O*-Gal-Rha-Rha	OH	H	OH	H	H	OMe	OH	H
								+	+			**30t**	*O*-Rut	OH	H	OH	H	H	OMe	OH	H
									+			**32t**	*O*-Rob-Rha	OH	H	OH	H	H	OMe	OH	H

										+			**33t**	*O*-Rob	OH	H	OH	H	H	OMe	OH	H
+										+		Quercetin	**4t**	*O*-Gen-Glc	OH	H	OH	H	H	OH	OH	H
	+												**8t**	*O*-Neo	OH	H	OH	H	H	OH	OH	H
			+						+	+			**12t**	*O*-Rut	OH	H	OH	H	H	OH	OH	H
				+						+			**17t**	*O*-Gen	OH	H	OH	H	H	OH	OH	H
				+									**18t**	*O*-Sop	OH	H	OH	H	H	OH	OH	H
							+						**25t**	*O*-Rha-Rha-Gal	OH	H	OH	H	H	OH	OH	H
									+				**28t**	*O*-Glu	OH	H	OH	H	H	OH	OH	H
									+	+			**29t**	*O*-Glc	OH	H	OH	H	H	OH	OH	H
										+			**36t**	*O*-Rob-Rha	OH	H	OH	H	H	OH	OH	H
										+			**37t**	*O*-Rob	OH	H	OH	H	H	OH	OH	H
		+	+									Kaempferol	**10t**	*O*-Neo	OH	H	OH	H	H	H	OH	H
		+											**11t**	*O*-Glc-Xyl-Rha	OH	H	OH	H	H	H	OH	H
				+									**15t**	OH	OH	H	OH	Glc	H	H	OH	H
						+							**20t**	*O*-Gal-Rha-Rha	OH	H	OH	H	H	H	OH	H
						+							**21t**	*O*-Glc-Rha-Rha	OH	H	OH	H	H	H	OH	H
						+							**22t**	*O*-Glc-Rha	OH	H	OH	H	H	H	OH	H
							+				+		**24t**	*O*-Gen-Glc	OH	H	OH	H	H	H	OH	H
									+				**26t**	*O*-Glu	OH	H	OH	H	H	H	OMe	H
									+				**27t**	*O*-Glu	OH	H	OH	H	H	H	OH	H
									+	+			**31t**	*O*-Rut	OH	H	OH	H	H	H	OH	H
										+			**34t**	*O*-Rob	OH	H	OH	H	H	H	OH	H
				+								Apigenin	**13t**	H	OH	Ara*f*	OH	H	H	H	OH	H
				+									**14t**	H	OH	Glc	OH	Glc	H	H	OH	H
				+								Luteolin	**16t**	H	OH	H	*O*-Ara*f*	Glc	H	OH	OH	H
										+		Limocitrin	**35t**	*O*-Glc	OH	H	OH	OMe	H	OMe	OH	H

[(a)]Glc= glucopyranoside; Glu= glucuronopyranoside; Gal= galactopyranoside; Rha= rhamnopyranoside; Xyl= xylopyranoside; Ara*f*= arabinofuranoside; Rob= robinobioside = -*O*-rhamnopyranosyl-(1→6)-galactopyranoside; Gen= gentiobioside = -*O*-[β-D-glucopyranosyl-(1→6)-β-glucopyranoside]; Rut= rutinoside = -*O*-α-rhamnopyranosyl-(1→6)-β-glucopyranoside; Neo= neohesperidoside = -*O*-α-L-rhamnopyranosyl-(1→2)-β-glucopyranoside; Sop= sophoroside = -*O*-β-glucopyranosyl-(2→1)-β-glucopyranoside.

Table 5
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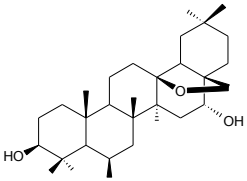
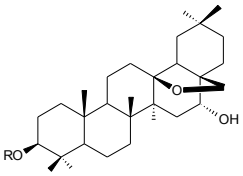
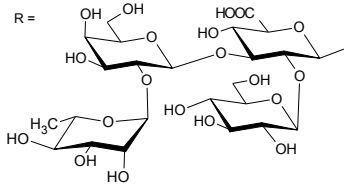
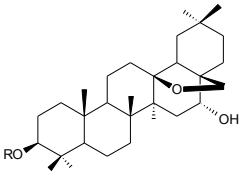
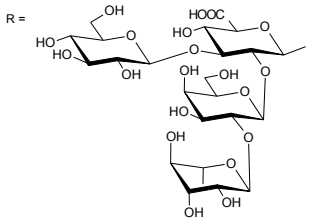
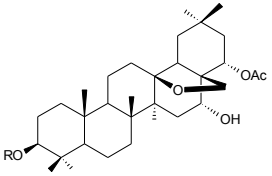
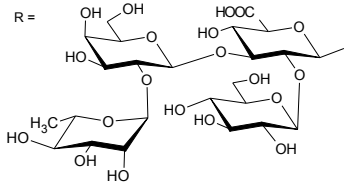
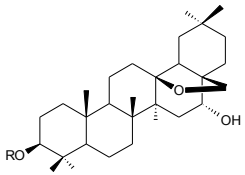
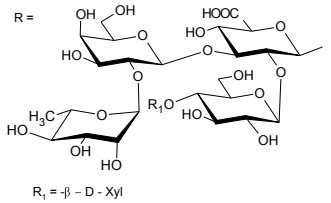
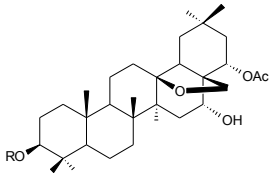
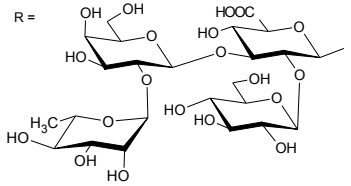
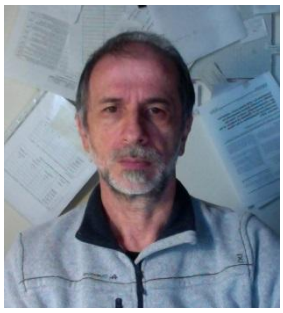
Table 5
European *Primula* species – Saponins

Species	Refs.	No.	Compound	Sapogenin	Sugar chain
P. elatior	Tschesche and Ballhorn, 1975.	**PSap1**	Protoprimulagenin A		-
	Tschesche and Wiemann, 1977; Tschesche et al, 1983; Müller et al., 2006	**PSap2**	Primulasaponin I		
	Tschesche et al, 1983	**PSap3**	Primulasaponin (minor)		
	Müller et al., 2006	**PSap4**	Priverosaponin B 22-acetate		

		PSap5	Primulasaponin II		
P. veris	Müller et al., 2006	**PSap4**	Priverosaponin B 22-acetate		

***Biography**

Paola Sira Colombo was born in Milan in 1953 and graduated in Natural Sciences at the University of Milan. From 1986 to 2009 she worked at the Documentation Centre of the Italian newspaper Corriere della Sera. Since 1987 she has been a member of CAI (Italian Alpine Club), whith whom she takes part to excursions and naturalistic activities.
She is currently collaborating with professor Gelsomina Fico (University of Milan) in her research on the genus *Primula*.



Guido Flamini was born in 1960. He graduated in Pharmacy at University of Pisa (Italy). At the same University he obtained the specialization degree in Science and Technique of Medicinal Plants. His research interests are the chemistry of secondary natural products, in particular volatile ones. His research also includes the characterization of the aroma profile from food of plant origin, in particular olive oil, and the influences of geographical origin and processing on the final products. At present he is Associate Professor at the Department of Pharmacy at the University of Pisa and actively collaborates with Italian, Nigerian, Turkish, Jordanian and Tunisian researchers. He is a member of the Italian Society of Phytochemistry.

Graziella Rodondi was born in 1951. She graduated at the University of Milan in 1977. Since 1983 she has been a researcher in Systematic Botany at the University of Milan (Department of Bioscience). Since 1990 she has been teaching Systematic Botany, Plants Phylogeny, Biodiversity and Evolution for students of Biology and Nature Science. Her scientific interest focuses on: 1.Ultrastructural and cytochemical studies on micro-organisms living in extreme environment. 2. Taxonomic-ultrastructural studies on the relationship between shape and function and between shape and phylogeny in plants, with particular interest in the micromorphology and ultrastructure of some critical systematic groups. She is now doing chorologic and biosystematic research on endemic Primula species in the Prealps of Lombardia (Italy), in order to clarify the taxonomic meaning of the secondary metabolites in some critical species. 3.Floristic-vegetation studies, with particular interest in different vegetation types with functional or environmental importance (grassland and bogs).

Claudia Giuliani was born in 1977. She studied plant biosystematics and ecology at the University of Florence. She finished her Ph.D. studies under the supervision of Laura Maleci Bini in 2008 at the Department of Biology (BIO) in Florence. Her research studies included the phytochemical and morphological characterization of aromatic and medicinal plants and the morpho-anatomical investigation of plant secretory tissues.
Afterward she worked as a post doctoral research fellow in the group of Dr. Bruno Foggi and Prof. Marta Mariotti Lippi at BIO in Florence, studying the alien invasive plants of Tuscany (Central Italy), with special focus on the reproductive strategies, the plant-pollinators interactions and the environmental impacts of leguminous trees (Regione Toscana QuiT Project

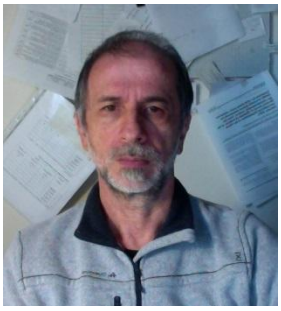
POR-FSE 2007-2013). Other central activities encompasses the impact assessment of the alien plants in Europe and the use of predictive modelling to evaluate the potential future distribution of invasive target species under the ongoing climate change scenario. She is currently a post doctoral research fellow under the supervision of Prof. Gelsomina Fico at the Department of Pharmaceutical Sciences of the University of Milan (Project IRIS Code: 2014-PDF-0363).

Laura Santagostini was born in . She graduated in Chemistry in 1996 at the University of the Studies of Milan (Italy), and obtained her PhD in Chemical Sciences in 2000 at the same University. Her research was formerly concerned to the application of instrumental analytical techniques to the direct study of copper oxygenases (tyrosinase) and multicopper oxidases (ascorbate oxidase, laccase), a class of copper enzymes, and to the study of biomimetic multicopper complexes with a tyrosinase- or oxidase-type reactivity.
In recent years the analytical study has become predominant and has been extended to the investigation of natural complex matrices, both from an elemental point of view, for a recognition of region of origin, and organic and phytochemical point of view, to determine their composition and evaluate the possible effects of human activities on them. In particular, her interest focuses on the polyphenol and flavonoid characterization of fruits and plants with potential pharmacological activity.

Gelsomina Fico was born in 1968. She received her Master's degree in Biology in 1994 at the University of Milan, and a PhD in “Science and technology of medicinal plants” in 2000 at the University of Pisa. In 2001 she joined the University of Milan (Department of Biology, Faculty of Pharmacy) as a researcher in the subject area BIO/15, Pharmaceutical Biology. In 2011 she became Associate Professor for the same subject area.
Her research activity covers several areas: 1. Morphology and Chemistry (extraction and isolation of secondary metabolites) of medicinal botanical species; 2. Productivity in secondary metabolites in relation to ecological and physiological aspects; 3. Biological activity of secondary metabolites; 4. Chemotaxonomy; 5. Investigation of effects of treatments with resistance inducers on the synthesis of secondary metabolites in species of medicinal and food interest; 6. Medicinal Plants: traditional use of herbal remedies; 7. Ethnobotany. With respect to her positions in scientific organizations: since 2003, she has been scientific coordinator of the Botanic Garden GE Ghirardi (Toscolano Maderno, Brescia), which belongs to the Department of Pharmaceutical Science of University of Milan and is dedicated to medicinal plants. Since 2008 she has been Vice president, and from 2012 to 2015 President, of the Network of Botanical Gardens of Lombardy. From 2009 to 2015 she was member of the Council of the Italian Society of Phytochemistry.

***Photo(s)**

Paola Sira Colombo



Guido Flamini

Graziella Rodondi

Claudia Giuliani

Laura Santagostini

Gelsomina Fico

Supplementary Information

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