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Phytoecdysteroids from *Silene* plants: distribution, diversity and biological (antitumour, antibacterial and antioxidant) activities

[Fitoeocdiesteroides de plantas Silene: distribución, diversidad y actividades biológicas (antitumorales, antibacterianas y antioxidantes)]

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**Abstract**

*Silene* is a genus of the Caryophyllaceae family, contains more than 700 species, which are widely distributed in Northern Hemisphere, but also in Africa, Asia and South American. Phytochemical investigations of *Silene* species have revealed that many components from this genus are highly bioactive. More than 400 compounds has been isolated, among them major are phytoecdysteroids. The paper reviews the biological (antitumour, antibacterial and antioxidant) activities and the phytoecdysteroids of genus *Silene*. We summarized the phytoecdysteroids content referring to 171 species from the genus *Silene* and list 93 phytoecdysteroids isolated over the past few decades. There are also reports on the mentioned folk and traditional effects of *Silene* plants.

**Keywords:** *Silene*, phytoecdysteroids, plants, distribution, flora, traditional uses, pharmacological properties

**Resumen**

*Silene* es un género de la familia Caryophyllaceae, conteniendo más de 70 especies, ampliamente distribuidas no solo en el hemisferio norte, sino que también en África, Asia y sud América. Investigaciones fotoquímicas de las especies de *Silene* han revelado que muchos componentes de este género son altamente bioactivos. Más de 400 compuestos han sido aislados, entre ellos los mayoritarios correspondientes a fitoeocdiesteroides. El manuscrito revisa las actividades biológicas (antitumorales, antibacterianas y antioxidantes) y los fitoeocdiesteroides del género *Silene*. Resumimos el contenido de fitoeocdiesteroides en 171 especies del género *Silene* y listamos 93 fitoeocdiesteroides aislados desde hace unas pocas décadas. Hay también reportes mencionados acerca de los efectos tradicionales y folclóricos de la plantas del género *Silene*.

**Palabras Clave:** *Silene*; fitoeocdiesteroides; plantas; distribución; flora; usos tradicionales; propiedades farmacológicas.

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INTRODUCTION
The genus Silene, which belongs in the family of the Caryophyllaceae and distributed mainly in the Northern Hemisphere, but also in Africa, Asia and South America. Genus of Silene includes more than 700 species (which allocated to 39 sections) of annuals, biennials, and perennials with a worldwide distribution and its taxonomy appears very complex. Most of species are hermaphrodite, but a few are dioecious or gynoecious (Grauter, 1995). Common names of Silene include campion (S. dioica), white campion (S. latifolia, S. alba) and bladder campion (S. vulgaris) are common wildflowers throughout Europe.

In this review article, the phyoecdysteroids isolated from the genus Silene are listed, and the phytochemical investigation in this genus is summarized. The structures of phyoecdysteroids are shown below, i.e., their names (Figure 1) and the corresponding plant sources are collected in Table 1. The traditional and medicinal uses of Silene plants and biological (antitumour, antibacterial and antioxidant) activities of phyoecdysteroids isolated from these plants are also discussed.

Figure 1
Chemical structures of phyoecdysteroids isolated from Silene plants

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<tr>
<th>N</th>
<th>Name</th>
<th>Empiric formula</th>
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<th>Substituents in steroidal core</th>
<th>Substituents in side-chain</th>
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Phyoecdysteroids from Silene plants: distribution, diversity, and biological activities

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<th>Name</th>
<th>Empirical formula</th>
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<th>Substituents in steroidal core</th>
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<td>89</td>
<td>Rubrosterone</td>
<td>C₁₉H₂₆O₅</td>
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<td>90</td>
<td>Makisterone C-2,3;20,22-diacetonide</td>
<td>C₃₅H₅₆O₇</td>
<td>1146696-54-9</td>
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</table>
RESULTS AND DISCUSSION

Chemical constituents

Chemical investigations of the genus Silene have led to the isolation of phytoecdysteroids, triterpene glycosides (Gaidi et al., 2002), terpenoids, benzenoids, flavonoids (Darmograi, 1977), anthocyanins, N-containing compounds (Dotterl et al., 2005), fatty acids (Kucukboyaci et al. 2010; Mamadalieva et al., 2010a; Mamadalieva et al., 2010b), amino acids (Terrab et al., 2007), polysaccharides (Ovodova et al., 2000), sugars (Mogosanu et al., 2011), sterols, vitamins, organic acids and microelements (Eshmirzayeva et al., 2005; Arnetoli et al., 2008).

Phytoecdysteroids

Phytoecdysteroids are contentedly widespread in the plant world. They are isolated from the main types of higher plants - ferns, gymnosperms and angiosperms, but their function in plants are yet studied insufficiently. One can conclude that the role of ecdysteroids in various plants and plant families may differ: when the ecdysteroid content is low, these exert a determinative effect on the plant growth and development; when it is high (several percent), these may be involved in the biochemical processes of storage, transportation, and metabolism of sterols. In this case, such metabolic products are not excluded that can defense the plant against the harmful factors of the environment (Baltaev, 2000). High concentrations of phytoecdysteroids have found in the reproductive organs, the anthers, apical leaves, and roots. Up to date, phytoecdysteroids isolated from more than 400 species of the plants and fungus belonging to 52 families (Mamadalieva, 2012a). As can be seen, phytoecdysteroids is the predominant constituents within the genus Silene (Table 1). Given the complexity of ecdysteroid cocktails existing in many Silene species, it has been proposed that ecdysteroids have a chemotaxonomic value in this genus (Zibareva et al., 2009). Silene plants accumulate high levels of phytoecdysteroids and have therefore been exploited as an industrial source for the production of these compounds.
production of phytoecdysteroids. The qualitative and quantitative composition of phytoecdysteroid cocktails depends considerably on the plant species, but possibly also on soil climatic conditions and on the developmental stage of the plant.

More than 170 Silene species have been analyzed for their phytoecdysteroid content, and 140 of them were found to be positive and 93 different ecdysteroids (ca. 25% of the known phytoecdysteroids) have been detected from these plants (Table 1). Some of them contain a high concentration of 20-hydroxyecdysone, such as Silene oitites (almost 1%) and Silene multiflora (1.9%) (Bathori et al., 1987). In the Table 1 we can see, that some species of Silene genus characterized by the absence of phytoecdysteroids. Zibareva et al., (2009) explained that by the assumption that some sections of Silene (for example, Siphonomorpha, Chloranthae, Coronatae, Graminiformes, Otites, Silene, Dipterosperma, Lasioalyceae, Holopetalae) consist of only ecdysteroid-containing species, whereas other sections (Behen, Atocion, Psammophilae, Odontopetalae, etc.) contain only ecdysteroid-negative species. In these sections, it is possible, with high probability, to predict ecdysteroid presence or absence in so as yet uninvestigated species.

The major phytoecdysteroids identified in Silene plants include: 20-hydroxyecdysone, polypodine B, 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone, inokosterone, integristereone A and ecdysone. Natural derivatives of phytoecdysteroids are also considered as phytoecdysteroids in the definite sense. Silene spp. plants are characterized by the highest diversity of ecdysteroid derivatives, such as acetates, acetonides, benzoates, cinnamates, coumarates, crotonates, glycosil-ferulates, glucosides, galactosides, ramnoses, xylosides, sulfates, etc. The presence of furan, epoxy and lactone ring contains derivatives of phytoecdysteroids is typical for this genus (Lafont et al., 2002).

Table 1
Distribution of phytoecdysteroids in the genus of Silene

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Isolated phytoecdysteroids</th>
<th>Reference</th>
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<tbody>
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<td>Silene acaulis (L) Jac.</td>
<td>34</td>
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S. uralensis (Rupr.) Bocquet 34 Zibareva, 2009
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(-) - denotes no phytocysteoylcontent; (+) - phytocysteoyls were detected but not identified.

**Traditional uses of Silene plants**

Some plants of this genus such as *S. acaulis, S. multifida* and *S. regia* as ornamental plants and have beautiful flowers and have been cultured as garden plants in many countries (Erturk et al., 2006). The root of *S. latifolia* is used as a soap substitute for washing clothes etc. The soap is obtained by simmering the root in hot water (Uphof, 1968; Usher, 1974). Also *S.*

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**Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas/487**
**Phytoecdysteroids from Silene plants: distribution, diversity, and biological activities**

Mamadalieva

**acaulis** and *S. conoidea*, used as fodder as well as a substitute for soap (Ahmad et al., 1998; Nasir and Ali, 1986).

The use of edible wild species *S. acaulis*, *S. cucubalis* and *S. vulgaris* has been reported by several authors (Fernald et al., 1996; Hadjichambis et al., 2007; Guarrella, 2003; Alarcon et al., 2006). Several ethnobotanical studies reveal that especially young shoots and the leaves of *S. vulgaris* is very much appreciable in the traditional gastronomy of Turkey, Italy, Austria, Germany and Spain (Hadjichambis et al., 2007).

**Traditional medicinal uses of Silene plants**

The plant of *S. acaulis* has been used in the treatment of children with colic (Moerman, 1998). Some members of this genus are used in folk medicine, mainly as an emollient and used as a fumigant (Ali, 1998). Juice of *S. cucubalis* L. is prescribed in ophthalmia (Chadha, 1972). The flowers of *S. nigrescens* used in Tibetan medicine in the treatment for hearing loss, blocking otic canal and volvulus (Tsarong Tsewang, 1994). The seeds of *S. dioica* have also been used to cure snakebites [http://en.wikipedia.org]. *S. szechuensis* Williams has been used in Chinese medicine as antipyretic, analgesic, diuretic (Zhang et al., 1997). *S. vulgaris* also widely used in medicine as antianemic (Conforti et al., 2011), sedative, anti-inflammatory and antitoxic agent (Ballero and Fresu, 1993, Golovko and Bushneva, 2007). The plant *S. undulata* Aiton is used as a medicine in treating many diseases particularly fevers and delirium (Sobiecki, 2008). Root barks of *S. undulata* (syn. *S. capensis*) is used by the Zulu and Xhosa of Africa people as an oneirogenic agent (Hirst, 1997; Hirst, 2005; Sobiecki, 2008). Other *Silene* species such as *S. bellidioides* Sond and *S. pilosellifolia* Cham. & Schltdl. used by the Zulus and taken as a love charm emetic, treatment of scrofula, to combat sleepiness, in tonic baths after severe illness, to produce dreams relating to the ancestral spirits in South Africa (Sobiecki, 2008).

**Biological activities of phytoecdysteroids**

Biological investigations on phytoecdysteroids have indicated their anabolic (Syrov, 2000), adaptogenic, tonic (Syrov and Kurnukov, 1977), cardiotoxic (Kurnukov and Yermishina, 1991), hypoglycemic, hypolipidemic, hepatoprotective (Kurtepova et al., 2001; Syrov et al., 1983) and other activities. Drugs derived from phytoecdysteroids can regulate mineral, carbohydrate, lipid and protein metabolism (Dinan and Lafont, 2006). Also these compounds appear in many plants mostly as a protection agent (toxins or antifeedants) against herbivore insects (Kubo and Klocke, 1983). Several reviews dealing with mentioned pharmacological effects of phytoecdysteroids have been reviewed recently (Slama and Lafont, 1995; Bathori, 2002; Lafont and Dinan, 2003; Bathori and Pongracz, 2005; Dinan and Lafont, 2006) and we will describe in this paper only antitumour, antibacterial and antioxidant effects of phytoecdysteroids.

**Antitumour activity**

There are some data on the inhibition effect of sarcoma and other types of cancer cells growth by phytoecdysteroids. Burdette and Richards (1961) observed proliferation-inhibitory effects on sarcoma cells in vitro, which they used extract from silkworm pupae containing ecdysteroids. But later investigations with individual phytoecdysteroids showed no effects (Burdette, 1974). Also, El-Mofti (1987, 1994) reported that ecdysone was able to induce neoplastic lesions in toads and mice; other researchers reported that ecdysteroid-containing extract of *Silene viridisflora* exerted antitumour activity in vivo (Zibareva, 2003). Some phytoecdysteroids isolated from *Ajuga* species showed antitumour activities in a mouse-skin model in vivo in a two-stage carcinogenesis trial, using 7,12-dimethylbenz[a]anthracene as initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as the promoter (Takasaki et al., 1999). However, Lagova and Valueva (1981) reported that 20-hydroxyecdysone was mainly ineffective in preventing tumour growth in mice, but it stimulated the growth of mammary gland carcinomas. Also in the our in vitro experiments phytoecdysteroid 2,3-diaceatate-22-benzoate-20-hydroxyecdysone showed a moderate inhibition against HeLa and HepG-2 cells [IC<sub>50</sub> values (127.97 ± 11.34) and (106.76 ± 7.81) μM, respectively], while 2-deoxy-20-hydroxyecdysone was most active against MCF-7 cells [IC<sub>50</sub> (126.54 ± 12.09) μM]. As compared to doxorubicin (IC<sub>50</sub> between 0.28 to 1.07 μg/mL) the phytoecdysteroids showed moderate cytotoxicity (Mamadalieva et al., 2011). Compare the results from others and our experiments are difficult, because of used different tumor models, phytoecdysteroids and assay methods. Most likely, since phytoecdysteroids structurally resemble sex hormones, they may bind to steroid hormone receptors in mammals and stimulate the growth of hormone-dependent tumours.

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**Antibacterial activity**

We studied the antimicrobial activity of different extracts and phytoecdysteroids from *Silene* plans towards the pathogenic microorganisms. The plant extracts obtained from *S. wallichiana* were tested at various concentrations ranging from 0.6 – 5 mg/ml and the evaluated MIC values (Mamadalieva et al., 2012b). The *Acinetobacter* sp., *Enterococcus faecalis*, *Klebsiella oxytoca*, *Pantoea agglomerans*, *Proteus rettgeri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains were inhibited by the methanol extract of *S. wallichiana* at MIC = 2.5 mg/ml, while *Escherichia coli* and *Klebsiella pneumoniae* was inhibited at MIC = 1.25 mg/ml. The butanol extract of *S. wallichiana* showed activity against the pathogenic bacterium *Acinetobacter* sp., *E. coli*, *K. pneumoniae*, *P. agglomerans*, *P. aeruginosa* (MIC = 2.5 mg/ml), and *P. rettgeri* (MIC = 1.25 mg/ml), although with weaker action respect to the methanol extract. The chloroform extract had minimum activity against all bacterial strains and only inhibited *Citrobacter freundii*, *E. coli* (MIC = 2.5 mg/ml) and *P. aeruginosa* (MIC = 1.25 mg/ml). The *S. wallichiana* aqueous extract also showed low antimicrobial activity against two strains only, *E. coli* and *P. aeruginosa* (MIC = 2.5 and 1.25 mg/ml, respectively). But pure phytoecdysteroids (viticosterone E, 20-hydroxyecdysone-22-benzoate, 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone, 20-hydroxyecdysone and integristerone A) isolated from *S. wallichiana* exhibited very low activity against the bacteria (Mamadalieva et al., 2012b).

The CHCl₃ extract of *S. brachuica* inhibited growth of three Gram-negative (*Enterococcus faecalis*, *Proteus rettgeri*, and *Pseudomonas aeruginosa*) and one Gram-positive (*Micrococcus luteus*) bacterial strain. The CHCl₃ extract of *S. viridisflora* was active against *M. luteus*, *P. rettgeri*, *Klebsiella pneumoniae*, and *P. aeruginosa*, whereas the extract of *S. wallichiana* exhibited activity against only pathogenic bacteria *M. luteus* and *P. aeruginosa* (Mamadalieva et al., 2010b). Also preliminary screening of the CHCl₃ extract from the aerial part of *S. guntensis* found that it exhibited antibacterial effects against *Escherichia coli*, *P. aeruginosa*, and *Acinetobacter* sp. (Mamadalieva et al., 2010c).

In another our experiments the phytoecdysteroids of *A. turkestanica* had weak antimicrobial activity against Gram-positive bacteria, *Candida glabrata*, except *S. pyogenes*. Only cyasterone showed activity (MIC > 0.5 mM and MMC > 0.5 mM) against *C. glabrata* (Mamadalieva et al., 2012c).

From above mentioned results, we can conclude that antibacterial activity is more consistent in total extracts respect to the single tested phytoecdysteroids. In addition, this finding was consistent with the previous results of Ahmad et al., (1996), Volodin et al., (1999) and Shirshova et al., (2006), who claimed that most likely such compounds are not the major molecules responsible for the antibacterial activity of the plant extracts. Ahmad et al., (1996) and Shirshova et al., (2006) reported that some natural phytoecdysteroids, including 20-hydroxyecdysone, inokosterone, and ecdysone, did not exhibit antibacterial activity with respect to most standard test microbes cultures. However, introduction of the acetyl group into the 20-hydroxyecdysone molecule significantly increased the antibacterial activity with respect to microbes inducing inflammatory and purulent processes (Shirshova et al., 2006). In our case, besides phytoecdysteroids, extracts exhibiting antibacterial activity were related to the chemical nature of the solvents which play a key role in the extraction of different chemical compounds from the plant material.

**Antioxidant activity**

Drugs derived from phytoecdysteroids exhibit antioxidant properties and have a similar effect with vitamin D₃ (Kuzmenko et al., 1997). Miliauskas and coworkers (2005) reported mild radical scavenging activity for 20-hydroxyecdysone. These observations are contradictory to what was found in other studies (Osinskyaya et al., 1992).

The radical scavenging ability of the extracts and phytoecdysteroids of *S. guntensis* were evaluated by us using the reaction with the stable DPPH radical (Mamadalieva et al., 2011). In our experiments phytoecdysteroids were ineffective for DPPH radical scavenging activity (IC₅₀ value > 100 µg/mL) compared to that for quercetin (3.37 µg/mL). Maximum scavenging activity of DPPH was observed with the water extract (IC₅₀ 68.90 µg/mL) of *S. guntensis*, followed by the activity of the n-butanol, methanol, and chloroform extracts with IC₅₀ values of 69.12, 122.48, and 148.28 µg/mL, respectively. The activity of 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone, and 2,3-diacetate-22-benzoate-20-hydroxyecdysone were 144.75, 157.29, and 291.38 µg/mL, respectively.

However, we assume that the antioxidant effect of these extracts might be attributed to some co-
eluting phenolic compounds other than phytoecdysteroids. It is known that the presence of the ortho arrangement of two hydroxyl groups on the aromatic ring and 2,3-double bond in conjugation with 4-oxo function is essential for the antiradical activity of flavonoids. More effective is the ortho-arrangement of hydroxyl groups on the aromatic ring B (quercetin). Ecdysteroids are polyhydroxylated steroids, contain 7,8-double bond and 6-oxo function. The latter explanation seems to be more convincing, since the structure of ecdysteroid molecules is unlikely to exert an antioxidative effect, as compared to the common antioxidative flavonoids (Lu and Yeap, 2001).

CONCLUSION
This review summarizes and characterizes the diversity and distribution of phytoecdysteroids found from a wide range of Silene species. The plants of the genus Silene are well known as rich sources of phytoecdysteroids. Almost quarter of the known phytoecdysteroids have been detected from Silene plants. The studies on chemical constituents in recent years have disclosed many different activities for phytoecdysteroids, such as anabolic, adaptogenic, tonic and other activities. Plants of this genus may serve as a potential source for dietary supplements for humans as food supplements for sportsmens or for use as additives in medicine and cosmetics. From the above is obvious, that phytoecdysteroids could serve as new lead molecules having great expectations in the development of new classes of pharmaceuticals and dietary supplements.

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