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Review

Ragged Robin (*Lychnis flos-cuculi*) - a plant with potential medicinal value

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A B S T R A C T

Lychnis flos-cuculi L., Caryophyllaceae, contains a number of active compounds belonging to several chemical groups. Previous studies have led to the identification of phytoecdysteroids, triterpenoids saponins, volatile compounds, fatty acid derivatives, phenolic acids and flavonoids. Research on pharmacological activity showed that plant extracts inhibited the growth of bacteria and fungi. The antimitotic properties of preparations from the herb *L. flos-cuculi* were also reported. The phytochemical analyses demonstrated that this taxon contains pharmaceutically promising compounds, but more phytochemical and pharmacological studies of *L. flos-cuculi* are needed for further information regarding this plant. This review summarizes reports regarding chemical composition and biological activity of *L. flos-cuculi* as well as several cognate species, which pose opportunities related to *in vitro* propagation and cell and tissue cultures. *In vitro*-regenerated plantlets could be a good source of genetically uniform plant material for future research.

Introduction

There is a rising interest in new, recently unknown plant materials with potential therapeutic activity. It results in worldwide phytochemical and pharmacological screening research, conducted on plant species used in local or foreign traditional medicine, in order to find new sources of pharmaceutically active substances. A number of plant species rarely known in the past are also being investigated for possibly having medicinal properties.

The vast genus *Silene*, the largest in the Caryophyllaceae family, includes weeds, ornamental plants and species of known medicinal value. Some classifications consider closely related genus *Lychnis* a part of *Silene* sp. The genus *Lychnis*, belonging to the tribe *Sileneae* of the family Caryophyllaceae, comprisess 25-30 species growing in Northern and Eastern Africa, Central and Eastern Asia, and Europe (Eggens, 2006; Ghahremaninejad et al., 2014). The genus name *Lychnis* comes from the Greek diminutive of *lychnos* meaning "lamp" in reference to the use of grey-felted leaves of *L. coronaria* (L.) Desr.

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as lamp-wicks. The name was already used by Theophrastus in 370-285 BC according to The New Royal Horticultural Society Dictionary of Gardening (Huxley, 1992).

There are numerous reports describing various medicinal properties of the species belonging to *Lychnis* sp. In this review, *Lychnis flos-cuculi* L. (Ragged Robin) is introduced, and its potential use as a source of pharmaceutically active compounds is investigated. Being phylogenetically related, other species of genus *Lychnis* and their known biological activity are also described.

Taxonomy and systematic position

The taxonomy of the tribe *Sileneae* is complicated and has had a long history of controversial reclassifications on genus level. The genus *Lychnis* was first described by Linnaeus, based on morphological characteristics. Later, all genera from the tribe *Sileneae,* with exception of genus *Agrostemma*, have been merged by Greuter into a single genus Silene. The resulting genus *Silene* (*sensu lato*) encompasses over 700 different species distributed almost worldwide (Greuter, 1995).

Most recently, molecular phylogenetic studies on nuclear ITS (internal transcribed spacer) and plastid *rps16* intron sequences have provided sound evidence that type species of genus *Lychnis*, including *L. flos-cuculi,* constitute a monophyletic clade within the tribe *Sileneae* and can be separated in a genus of its own (Oxelman et al., 1997; 2001; Ullbors, 2008). *Silene* sp. *s.l.* can therefore be split into genera *Lychnis, Atocion, Eudianthe, Heliosperma, Viscaria* and *Silene (sensu stricto)*. However, *Silene* will be still incomparably larger than the other genera (*ca.* 650 species), with *Lychnis* as the second largest (*ca.* 30), and each of the other genera consisting of only a few species. Contrary to the worldwide distribution of *Silene s.s.,* the other genera occur mainly in Eurasia (Eggens, 2006).

The phylogenetic studies helped overcome the inconsistent classifications relying on highly variable morphological characteristics even within the species. However, though the results allowed elucidate the main branches of the *Sileneae* phylogenetic tree, there are still cases where results are contradictory, mostly between plastid and nuclear DNA analyses. This might suggest various mechanisms that occurred during evolution, related to horizontal gene transfer, hybridization, gene duplication, or gene loss (Eggens, 2006).

To this day, various synonyms are used interchangeably, at the same time considering other names as illegitimate, causing confusion among scientists and hindering the search for scientific data (GRIN, 2014; ITIS, 2014; USDA, 2014). Most common synonyms are *Silene flos-cuculi* (L.) Greuter & Burdet, *Coronaria flos-cuculi* (L.) A. Braun and *Coccyganthe flos-cuculi* (L.) Rchb. This review will follow this recent trend and refer to the species using a Linnaean basionym: *Lychnis flos-cuculi* L.

Botanical description

L. flos-cuculi is a diploid polycarpic perennial hemicryptophyte. This herbaceous plant, commonly called Ragged Robin, is native to Europe and Northwestern Asia. It grows in sunny and moist open habitats, such as marshes, floodplains, wet meadows, roadsides, ditches and light alder woods, preferring deep soils, rich in nutrients (Aavik et al., 2014). The reddish stem with barbed hairs grows 30-80 cm high. The basal leaves forming a vegetative rosette are oblong and stalked, whereas the stem leaves are lanceolate and stalkless (Fig. 1). *L. flos-cuculi* blooms from May to August. The flowers are arranged in open panicles and have five petals, each divided into four linear lobes. The fruits consist of small spherical capsules opening at the top by five teeth. Small brown seeds are dispersed mechanically when the capsules split open in August. *L. flos-cuculi* reproduces both by seeds and vegetatively, using underground stolons. It is also able to create secondary daughter rosettes from axillary buds, which usually remain attached to the mother rosette longer than one growing season, forming an interconnected clone (Bailey, 1949; Biere, 1995; 1996; Chaloupecká and Lepš, 2004; Galeuchet et al., 2005a,b).

Phytochemical profile

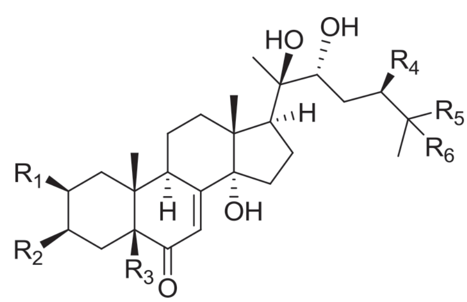
Relatively little is still known about the chemical compounds in aerial and underground parts of *L. flos-cuculi.* Several studies allowed the identification of the different compounds listed below.

Phytoecdysteroids

Phytoecdysteroids are a class of active compounds isolated and identified from Ragged Robin extracts. These substances

Figure 1 – A flowering specimen of *Lychnis flos-cuculi* L., Caryophyllaceae.

are synthesized by the plant as defense against phytophagous insects by mimicking ecdysteroids, hormones involved in moulting or ecdysis; hence the name. Phytoecdysteroids are structurally similar or even identical to arthropod ecdysteroids. Chemically, these compounds are related to triterpenes, synthesized in plant cells from mevalonic acid through the mevalonate pathway but then cleaved, forming a cholest-7-en-6-one backbone (C27), upon which the majority of phytoecdysteroids is based. The highest ecdysteroid concentrations are probably found in tissues that are the most significant for the survival of the plant. Also, their amount depends on season, vegetation period, habitat and developmental stage (Dinan, 1992, 2001; Grebenok and Adler, 1991). The highest concentrations of these compounds in the Ragged Robin are found in the roots (Zibareva et al., 2003), they are also reported to peak in the reproductive organs of the plants of the interrelated genus *Silene* during budding and flowering (Zibareva, 2000). Many species within the genus *Silene s.l.* are described as particularly rich in phytoecdysteroids of diverse structure (93 different compounds reported), being perhaps their richest source in the entire plant kingdom (Speranza, 2010; Mamadalieva, 2012; Mamadalieva et al., 2014). Previous studies have led to the isolation and identification of eleven ecdysteroids present in *L. flos-cuculi*. Among them, 20-hydroxyecdysone (**1**) (the major phytoecdysteroid), polypodine B (**2**), 26-hydroxypolypodine B and dihydrorubrosterone occur in significant quantities. The presence of poststerone, 20,26-dihydroxyecdysone (**3**), 2-deoxy-20-hydroxyecdysone (**4**), rubrosterone, makisterone A (**5**), taxisterone, 20-hydroxyecdysone 2-acetate (**6**) and 20-hydroxyecdysone 3-acetate (**7**), and viticosterone E (**8**) are also indicated (Girault et al., 1990; Báthori et al., 2001).

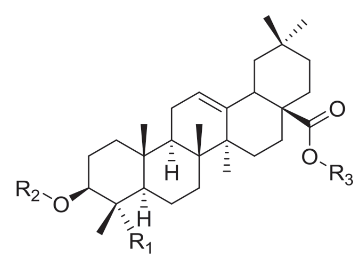


1 $R_1=R_2=R_6=OH$; $R_3=R_4=R_5=H$
2 $R_1=R_2=R_3=R_6=OH$; $R_4=R_5=H$
3 $R_1=R_2=R_5=R_6=OH$; $R_3=R_4=H$
4 $R_1=R_2=R_6=OH$; $R_3=R_4=R_5=H$
5 $R_1=R_2=R_6=OH$; $R_3=R_5=H$; $R_4=CH_3$
6 $R_1=CH_3CO_2$; $R_2=R_6=OH$; $R_3=R_4=R_5=H$
7 $R_1=R_6=OH$; $R_2=CH_3CO_2$; $R_3=R_4=R_5=H$
8 $R_1=R_2=OH$; $R_3=R_4=R_5=H$; $R_6=CH_3CO_2$

An interesting result was also obtained with the sterol composition of *Lychnis alba* Mill., which have been shown by Salt and Alder (1986). They reported that the predominant 4-desmethyl sterols are 24-ethyl-D^7-sterols such as spinasterol (24α-ethylcholesta-7,22E-dien-3β-ol), 22-dihydrospinasterol (24α-ethylcholest-7-en-3β-ol) and avenasterol (24-ethylcholesta-7,24(28)*Z*-dien-3β-ol). Predominant 4-desmethylsterols in angiosperms typically contain D^7-unsaturated bond. It seems that 4-desmethylsterols with less common D^7-unsaturated bond, found particularly in Caryophyllales, serve as precursors for ecdysteroids (Corio-Costet et al., 1998).

Triterpenoid saponins

Among other active substances identified in extracts of the Ragged Robin herb are triterpenoid saponins. Analysis of extracts from *L. flos-cuculi* revealed the presence of gypsogenin (**9**) and hederagenin (**10**); glycosides, including coronoside A (**11**) and coronoside B (**12**) (Bucharow et al., 1974; Kondratenko et al., 1981). The other species belonging to Caryophyllaceae family are rich in oleanolic acid, gypsogenic acid and quillaic acid. The amount of saponins depends on the vegetation period, the part of the plant, and the season (Böttger and Melzig, 2011). The highest concentration of saponins is usually found in the roots or seeds (Kołodziejski and Stecka, 1965). The dominant class of saponins occurring in this family belongs to the oleanane-type (Böttger and Melzig, 2011). Typical sugar moieties constituting the oleanane-type saponins include apiose, arabinose, fucose, galactose, glucose, glucuronic acid, *N*-acetyl glucosamine, quinovose, rhamnose, ribose and xylose (Vincken et al., 2007).



9 R_1=CHO; $R_2=R_3$=H
10 $R_1=CH_2OH$; $R_2=R_3$=H
11 R_1=CHO; R_2=D-GlcUA; R_3= 2 D-Xyl, 3 L-Rha
12 $R_1=CH_2OH$; R_2=D-GlcUA, 2 D-Gal, 2 D-Xyl; R_3=2 D-Xyl, 3 L-Rha

Volatile compounds

Studies on the volatile compounds contained in Ragged Robin flowers revealed the presence of benzenoids. Scrupulous analysis allowed the identification of the following compounds, shown in Table 1.

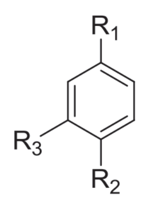
Relatively large amounts of aromatic compounds, including phenylacetaldehyde, benzaldehyde and methyl benzoate suggest plant adaptation to lure butterflies involved in pollination (Andersson et al., 2002). The pollinators of Ragged Robin flowers are hymenoptera (Hymenoptera), flies (Diptera) and butterflies (Lepidoptera) (Van Rossum and Triest, 2010).

Phenolic acids

Previous investigations allowed the identification of phenolic acids present in *L. flos-cuculi* extracts: 4-hydroxybenzoic acid (**13**), caffeic acid (**14**), ferulic acid (**15**), vanillic acid **(16)**, *p*-coumaric acid (**15**) and protocatechuic acid **(16)** (Ferry and Darbour, 1979).

Table 1
Floral scent composition of *Lychnis flos-cuculi* L., Caryophyllaceae (Jürgens, 2004).

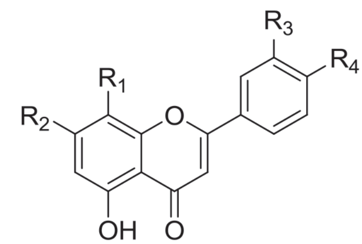
Compounds	Average relative amounts (%)
Fatty acid derivatives:	41.1
n-hexanal	8.2
2-heptanone	1.8
n-heptanal	5.0
n-octanal	7.0
cis-3-hexen-1-ol acetate	0.4
n-nonanal	13.4
n-decanal	5.3
Benzenoids:	45.1
ethenylbenzene	2.0
1,2-dimethylbenzene	3.9
benzaldehyde	30.1
propylbenzene	0.6
1,2,3-trimethylbenzene	0.6
phenyl acetate	0.5
phenylacetaldehyde	0.8
methyl benzoate	4.1
methyl salicylate	0.6
phenyl benzoate	0.8
dimethyl salicylate	1.1
Isoprenoids:	2.4
Monoterpenes:	
α-pinene	0.3
linalool	0.1
lilac aldehyde A	0.6
lilac aldehyde B	0.6
Sesquiterpenes:	
β-bourbonene	0.8
Nitrogen-containing compounds:	
N-acetyl-4(*H*)-pyridine	2.1



13 $R_1=CO_2H$; $R_2=OH$; $R_3=H$
14 $R_1=CH{=}CH\text{-}CO_2H$; $R_2=R_3=OH$
15 $R_1=CH{=}CH{=}CO_2H$; $R_2=OH$; $R_3=OCH_3$
16 $R_1=CO_2H$; $R_2=OH$; $R_3=OCH_3$
17 $R_1=CH{=}CH\text{-}CO_2H$; $R_2=OH$; $R_3=H$
18 $R_1=CO_2H$; $R_2=R_3=OH$

Flavonoid compounds

Research on the identification of polyphenolic compounds in *L. flos-cuculi* herb showed the presence of two flavonoid aglycones: apigenin (**19**) and luteolin (**20**). Two flavonoid C-glycosides: vitexin (apigenin 8-C-β-D-glucopyranoside) (**21**) and orientin (luteolin 8-C-β-D-glucopyranoside) (**22**) were also identified. The results confirmed a similar qualitative composition of polyphenolic compounds isolated from plants belonging to the Caryophyllaceae family (Tomczyk, 2008). On the other hand, the presence of flavonoids and related polyphenolics in tissue cultures as well as anthocyanidins and anthocyanins in flower petals of *L. senno* Siebold et Zucc. were reported (Kuwayama et al., 2005; Ogita et al., 2009). Recently, Devkota et al., (2013) have reported the presence in the aerial parts of *L. senno* of a new flavone C-glycoside, 5-O-acetyl-2”-α-rhamnopyranosylisovitexin, along with three known compounds, and observed their antioxidative activity by the 2,′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging method.



19 $R_1=H$; $R_2=R_3=R_4=OH$
20 $R_1=R_3=H$; $R_2=R_4=OH$
21 $R_1=$D-Glc; $R_2=R_3=OH$; $R_4=H$
22 $R_1=$D-Glc; $R_2=R_3=R_4OH$

Pharmacological profile

Studies of the pharmacological properties of *L. flos-cuculi* demonstrate the multidirectional activity of extracts obtained from this plant. In the Central-Southern Italy or other Mediterranean countries the Ragged Robin is used to treat migraine, intestinal pain, and, in the past, also malaria. A number of botanical works report that the species is widespread throughout Central and Northern Europe. There is no medicinal use of *L. flos-cuculi*, and the ethnopharmacological literature contains only little information about this plant. In Britain and Ireland folk medicine, an ointment made from *L. flos-cuculi* was reportedly used as a remedy for snakebites (Allen and Hatfield, 2004). In Romanian traditional medicine, the aerial parts of *Lychnis* extracts have been used to treat wounds, especially those used as an infusion (Tită et al., 2009). Tomczyk (2008) has noted that previous pharmacological studies analysed only the potential cytostatic activity of the species, and the aqueous extracts of the plant were tested for antibacterial and antifungal properties. We were unable to find any pharmacological study demonstrating neuroprotective or anti-malarial properties of this species. However, there may be an interesting link to be found with a previous study carried out by Plotnikov et al. (2005), who was able to verify that the extract of *L. chalcedonica* L., exhibited anti-haemorrhaging and neuroprotective properties in rats with cerebral ischaemia: as above mentioned, our study cited anti-migraine as one of the uses of *L. flos-cuculi*.

It was proven that methanol and ethanol extracts inhibit the growth of microorganisms, both Gram-negative and Gram-positive bacteria: *Klebsiella oxytoca, Escherichia coli, Proteus rettgeri, Citrobacter freundii, Pseudomonas aeruginosa, Alcaligenes faecalis, Enterobacter hormaechei, Pantoea agglomerans, Bacillus cereus, Staphylococcus epidermidis, Micrococcus luteus* and *K. aerogenes*. A chloroform extract had an even wider range of bactericidal activity, it inhibited the growth of S.

aureus, Acinetobacter sp., and *S. saprophyticus* strains. From a microbiological point of view, the chloroform extract seems the most interesting because of the presence of phytoecdysteroids (Mamadalieva et al., 2008). The literature data indicate the antimicrobial activity of slightly polar acylated ecdysteroids, such as simple acetyl derivatives (Shirshova et al., 2006; Mamadalieva, 2012). The following pharmacological effects are attributed to *Leuzea carthamoides,* a plant containing high levels of ecdysteroids (especially 20-hydroxyecdysone, also identified in the Ragged Robin): roborant, adaptogenic and antidepressive (Slama and Lafont, 1995). It was proven that phytoecdysteroids significantly increase protein synthesis in skeletal muscle cells, both *in vitro* and *in vivo*, possibly through a PI3K (phosphoinositide-3-kinase)-mediated mechanism, thus contributing to increased physical performance (Gorelick-Feldman et al., 2008). They are also reported to induce the differentiation of human keratinocytes *in vitro*, which accounts for their wound-healing effects (Detmar et al., 1994). Currently, several ecdysteroids are used as ingredients in bodybuilding supplements and cosmetics, though evidence of their claimed activity is still considered inadequate (Lafont and Dinan, 2003). Phytoecdysteroids can be used to control diabetes (Yoshida et al., 1971; Najmutdinova and Saatov, 1999). 20-Hydroxyecdysone exhibits antioxidant activity and exerts neuroprotective effects by scavenging free radicals, modulating NF-κB (nuclear factor kappa-light chain enhancer of activated B cells) and JNK (c-Jun *N*-terminal kinase) pathways possibly interfering with mitochondrial apoptosis pathway, which makes it a potential candidate to protect neurons from hypoxic-ischemic injury, such as stroke (Hu et al., 2010; 2012).

Previous studies indicated that polypodine B (**2**), an ecdysteroid isolated from *Ajuga decumbens*, but also present in *L. flos-cuculi*, has a potential antitumor-promoting activity (Takasaki et al., 1999). Depending on their chemical structure, different ecdysteroids are reported to exert low to moderate cytotoxicity against various cancer cell lines, such as MCF-7, HeLa or HepG-2, with IC_{50} values above 100 µM (Mamadalieva, 2012). Acetonide derivatives of ecdysteroids were recently reported to disrupt multi-drug resistance, preventing the efflux of cytotoxic agent from the cancerous cell and improving the efficacy of chemotherapy. It is important to note that unmodified ecdysteroids had opposite effect (Martins et al., 2012).

Studies were also carried out on the antifungal properties of the extracts obtained from the Ragged Robin. They showed the fungistatic activity of extracts from this plant against strains of *Candida* sp. The compounds responsible for this activity are probably triterpenoid saponins (Chaumont and Bourgeois, 1978).

The possibility of the potential use of *L. flos-cuculi* extracts in the treatment of cancer was also analyzed. Studies focused on the antimitotic properties were carried out using Levan's test on adventitious roots of *Allium cepa* L., Amaryllidaceae. However, it was considered that preparations of the plant are not of practical importance in oncology because 1% water extracts, ethanol and acetone showed antimitotic activity less than 60% (Grzycka et al., 1978). It can be hypothesized that while several compounds present in Ragged Robin may indeed exert antimitotic or cytotoxic activity, the others, acting as adaptogens, may neutralize this effect. In recent years, many studies have demonstrated the cytotoxic activity of both steroid and triterpenoid saponins against numerous cancer cell lines (Podolak et al., 2010). It seems that many oleanane-type monodesmosidic saponins exhibit moderate to high cytotoxicity (IC_{50} within low micromolar range), provided that the carboxylic group at C28 is free (Hai et al., 2012; Wang et al., 2013; Tian et al., 2013). Both coronosides from *L. flos-cuculi* unfortunately do not meet this structural requirement, but their activity and the exact structure of their sugar moieties are still unknown (Bucharow et al., 1974; Kondratenko et al., 1981). Apart from the cytotoxic activity, saponins were also reported to affect molecular phenomena related to crucial signaling pathways, exerting more intricate antitumor activity (Sparg et al., 2004). Nolle also reported that *L. flos-cuculi* preparations increased the excitability of uterine smooth muscle (Nolle, 1929).

Pharmacological activity of other *Lychnis* species

Among other plant species belonging to the genus *Lychnis* the pharmacological properties of *L. coronaria* and *L. chalcedonica* are noteworthy. The literature data reported the use of different parts of *L. coronaria* (common name - rose campion) in folk medicine. Diseases treated by this plant include diarrhea, leprosy, lung and liver ailments, and beriberi (Anonymus, 1962). It was shown that rose campion extract has hepatoprotective (Masoodi et al., 2007) and anti-inflammatory properties (Georgieva et al., 1982). Hot water extracts of the aerial parts of *L. coronaria*, as well as extracts from *L. flos-cuculi* were used for the treatment of hemorrhoids (Butoescu et al., 1987). In Northern India, the crushed roots of *L. coronaria* are macerated in water overnight and the resulting extract is administered orally as a medication for constipation and chronic cough (Lone and Bhardwaj, 2013). The aforementioned traditional uses may be attributed to the presence of secondary metabolites isolated from the whole plant of *L. coronaria*. They are identified as tricin 7-*O*-glucopyranoside, (+)-isoscoparin, epoxyactinidionoside, 1α, 20R-hydroxyecdysone (**1**), ecdysterone, polypodine B (**2**), ecdysterone 22-*O*-β-D-glucopyranoside, stigmast-5-ene-3-one, taraxerol, α-tocopherol, 10-eicosyl alcohol, nerol, dehydrodiconiferyl alcohol 4-*O*-β-D-glucopyranoside, 3-sitosterol, daucosterol which are all isolated for the first time from *L. coronaria* (Dai et al., 2002). Aliphatic alcohols were characterized as *n*-octacosanol, *n*-nonacosanol, *n*-tetracontanol and nonadecan-4,10-diene,6-one,1-ol in petroleum ether extract in this species (Masoodi et al., 2010).

Potential therapeutic properties of *L. chalcedonica* (scarlet lightning, maltese cross) to improve cerebral blood flow were examined in rats with artificially induced brain hypoxia. The results of this experiment indicated that a five-day treatment with an extract of *L. chalcedonica* at 150 mg/kg body weight significantly decreased abnormalities of cerebral blood flow, and normalized the bioelectric activity of this organ (Plotnikov et al., 2005). *L. chalcedonica* seeds contain lychnin, a type I ribosome inactivating protein (I RIP) (Fermani et al., 2003). RIP can be used to prepare immunotoxins by conjugating them with monoclonal antibodies (Chambery et al., 2007) or chimeric toxins by conjugating them with certain receptor ligands (e.g. EGF - epidermal growth factor) (Bachran et al., 2010a). Such type of compound is selectively toxic for a given type of target cell, which may be used to treat several types of cancer (Bolognesi and Polito, 2004). Moreover, triterpenoid saponins were reported to further improve the efficacy of such treatments, most likely by facilitating permeation of the conjugate through biological membranes (Bachran et al., 2010b). Therefore *L. chalcedonica* might be a potential source of both RIP protein and a valuable adjuvant. Fresh juice from the leaves of another species, *L. coronata* Thunb., as well as flavones isolated from the plant were found to inhibit the replication of herpes simplex virus I, reducing the pathological changes in infected cells at the same time (Hang et al., 1998).

Micropropagation and *in vitro* cultures

L. flos-cuculi is still a common taxon but the number of its populations is decreasing in some regions due to the conversion of the plant's natural habitat (*e.g.* meadows) by draining them and converting into arable or fallow land (Chaloupecká and Lepš, 2004). A specific habitat is required for the survival and growth of this species, therefore the availability of plant material is limited. Fortunately, advances in plant biotechnology over recent years have provided useful techniques, which can be applied to propagate and preserve the germplasms of rare and valuable plants. *In vitro* techniques allow for rapid, clonal multiplication of plants using minimum space, resulting in a steady supply of plant material, which in turn facilitates phytochemical investigations and studies on biological activity (Cruz-Cruz et al., 2013). Plant tissue cultures help overcome obstacles as dependence from climactic factors and seasons, insufficient abundance of pharmacologically active substance in intact plants, difficult cultivation or reproduction and limited availability from natural sources. Plant tissue culture offers a viable alternative for conventional propagation by seeds. Therefore plant tissue culture methods could play an important role in rapid *in vitro* propagation, used for secondary metabolite production, genetic improvement and germplasm conservation of these species (Rout et al., 2006). If such a need arises, plant cultures can be transferred under slow growth conditions to prolong the conservation time; an approach already tested on rare species belonging to the Caryophyllaceae family (Catana et al., 2010).

The protocol for clonal propagation through the development of axillary buds has been successfully established for *L. flos-cuculi,* as a plant with potential medicinal value (Thiem et al., 2013). Multiplication via buds, a tissue consisting of meristems, is suggested to be the preferred strategy to maintain genetic stability and used for micropropagation of medicinal plants (Bajaj et al., 1988). The ability of regenerated *L. flos-cuculi* plantlets to accumulate secondary metabolites in comparison with wild plants has been demonstrated (Maliński et al., 2014).

This biotechnological study for *L. flos-cuculi* reports a micropropagation procedure using shoot tips of axenic seedlings as explants that provide the genetically uniform *in vitro*-derived plantlets. Murashige and Skoog (MS) basal medium supplemented with plant growth regulators: benzyladenine (BA) and naphtalene-3-acetic acid (NAA) induced high plant regeneration efficiency, over 13 shoots per explant (Fig. 2). The *in vitro* regenerated shoots were successfully rooted and transferred into soil with high efficiency. Biotechnological studies carried out in our laboratory demonstrated the ability of the established *in vitro* cultures of *L. flos-cuculi* to accumulate secondary metabolites. Chromatographic analysis by thin layer chromatography (TLC) indicated that multiple shoots from *in vitro*-regenerated plants maintained the ability to produce flavonoids, phenolic acids and triterpenoid saponins similarly to the above ground part of the wild plants (Thiem et al., 2013). The presence of phytoecdysteroids, identified as 20-hydroxyecdysone (**1**) and polypodine B (**2**), was also recently confirmed, with especially high levels in the roots, but not in the callus (Maliński et al., 2014).

A wide range of strategies have been employed to modify plant metabolism in order to enhance the pharmaceutical productivity of plant cell and tissue culture. The most common methods are elicitation, precursor feeding, or establishing hairy root cultures. These strategies can be combined to reach better yield, as demonstrated in *Ajuga turkestanica* cultures as a source of phytoecdysteroids (Cheng et al., 2008).

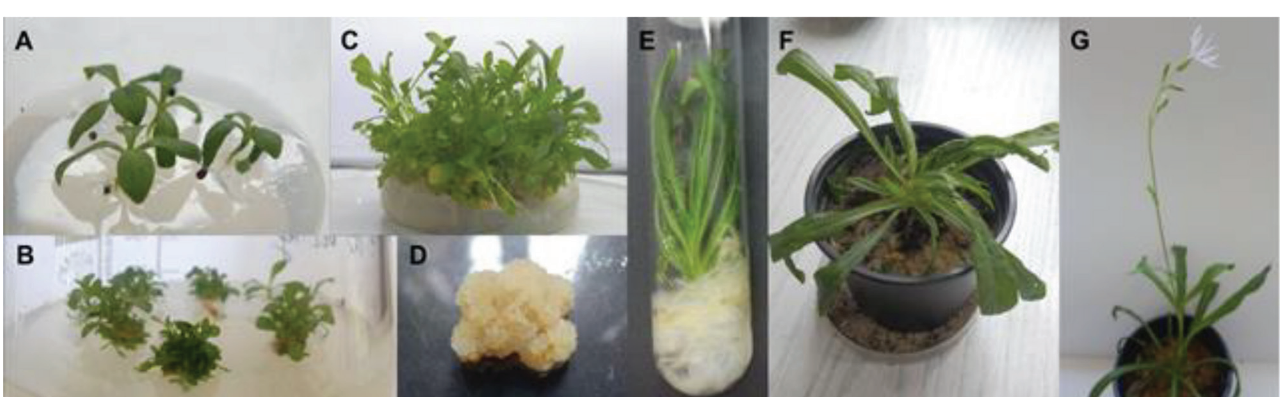


Figure 2 – *Lychnis flos-cuculi* L., Caryophyllaceae. **A**. Axenic seedlings; **B**. Young shoot cultures; **C**. Multiplied shoot culture - a source of biomass; **D**. Callus tissue; **E**. Regenerated rooted plant; **F**. *In vitro* regenerated plant transferred into a pot; **G**. A flowering plant

Another promising approach is the use of colchicine to obtain polyploid plants from *in vitro* cultures, so the production of valuable secondary metabolites could be increased. This approach was successful with *L. senno in vitro* cultures, yielding tetraploids differing from diploid plants, at least morphologically (Chen et al., 2006a). *L. senno* is an example of another rare species from genus *Lychnis* for which *in vitro* propagation system has been also established. Though mostly a plant of ornamental value, it is reported to be used traditionally as a natural blood-thinner (Chen et al., 2006b; Ogita et al., 2009). Recently, an effective propagation protocol has been also developed for *L. wilfordii* (Regel) Maxim, a critically endangered ornamental species endemic to Korea (Bae et al., 2014).

Since many secondary metabolites are tissue-specific, different tissue cultures could provide these compounds in higher quantities. For example, adventitious root cultures in liquid medium could be a good source of ecdysteroids. In case of *L. senno* cultures, 6 of 14 major polyphenolics detected are tissue-specific metabolites. It seems that the biosynthetic pathways responsible for the production of secondary metabolites are strongly influenced by conditions of *in vitro* culture, therefore it might be possible to selectively produce novel compounds of medicinal value by engineering a target tissue culture (Ogita et al., 2009). It should be noted, however, that the secondary metabolites produced by one tissue might be accumulated in another, and the parts of the plant containing high levels of a specific metabolite are not necessarily responsible for its biosynthesis.

In conclusion, plant *in vitro* cultures could provide plant material and give an opportunity to carry out phytochemical and biological investigation of rare and endangered plants, especially medicinal species, without collecting them from natural sites (Thiem et al., 2008).

Conclusion

Previous studies allowed the identification of only a part of the chemical compounds present in *Lychnis flos-cuculi*. Phytochemical analyses of various parts of this plant indicates naturally occurring phytoecdysteroids, triterpenoid saponins, phenolic acids, benzenoids, fatty acids derivatives and flavonoids. The potential pharmacological activity of preparations from *L. flos-cuculi* is multidirectional; recent studies showed bactericidal, fungistatic and antimitotic properties. There are also reports indicating the ability of Ragged Robin extracts to increase the excitability of uterine smooth muscle. Because of the abundance of compounds in *L. flos-cuculi*, these are probably not the only pharmacological properties of this plant. Plants of the genus *Lychnis* have a wide spectrum of therapeutic effects, *e.g.* hemorheological, neuroprotective, blood-thinning properties and anti-inflammatory activity. More phytochemical and pharmacological studies of *L. flos-cuculi* are needed for further information regarding this plant. *In vitro*-regenerated plantlets and organ cultures could be a good source of genetically uniform plant material for future investigations.

Authors' contributions

All authors contributed to the acquisition, analysis and interpretation of data for the manuscript. All authors participated in drafting the article and revising it critically. All the authors have read the final version of the manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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