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Review Article

Astragalus glycyphyllos L.: Phytochemical constituents, pharmacology, and biotechnology

Ivan Stambolov[1], Aleksandar Shkondrov[1], Ilina Krasteva[1]

1 *Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, 2 Dunav st., 1000 Sofia, Bulgaria*

Corresponding author: Ilina Krasteva (ikrasteva@pharmfac.mu-sofia.bg)

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Abstract

Astragalus glycyphyllos is a widely distributed plant found in Bulgaria that has been used in the folk medicine of the country for decades as an antihypertensive, diuretic, and anti-inflammatory. This review article is focused on the traditional usage, phytochemical studies, pharmacological activity, and biotechnology of this species. Recent progress in the phytochemical investigation led to the identification of different flavonoids, triterpenoid saponins, sterols, volatiles, etc. Many pharmacological studies, performed on extracts and pure compounds, revealed promising antiproliferative, cytotoxic, immunomodulatory, antiviral, and neuroprotective activities *in vitro* and an *in vivo* hepatoprotective effect on a model of CCl_4-induced liver damage. Based on its popularity in traditional Bulgarian medicine, the species represents a promising subject for further investigations.

Keywords

Astragalus glycyphyllos, saponins, flavonoids, pharmacology, biotechnology

Introduction

The genus *Astragalus* (Fabaceae) is comprised of more than 3500 species distributed worldwide, except Antarctica (Zarre and Azani 2013). In Bulgarian flora, the genus is represented by 29 species (Asyov et al. 2012). *Astragalus glycyphyllos* (wild liquorice, liquorice milk-vetch) is an herbaceous, perennial flowering plant with creeping, deep roots. The stems are branched, ascending, and up to 150 cm tall. Stipules are 15–25 mm long, all free. The leaves are 10–20 cm long, with short petioles that are unpinnate. The leaflets are 4–8 pairs, 20–50 mm, ovate or elliptical, on the upper side glabrous, and on the underside loosely covered with white hairs. The flowers are up to 16 mm long. The bracts are lanceolate, with sparsely ciliate white edges. The calyx is 5–6 mm long, glabrous or loosely covered with appressed black hairs. The petals are up to 15 mm long and yellowish-green. The wings are 10–13 mm long and rounded. The keel is 9–11 mm long and curved. The fruits (legumes) are 30–40 mm long and 4–5 mm wide, rounded ventrally and dorsally, glabrous, or loosely covered with hairs. The seeds are kidney-shaped, smooth, and reddish brown. The plant grows on rocky soils, in meadows, and in open places in the forests throughout Bulgaria up to 1800 m. a. s. l. The species flowers from June to August and gives fruits from July to August (Valev et al. 1976). It is widespread in Europe and the temperate regions of Asia and is considered to play an important role in maintaining the proper functioning of the ecosystem (Gnat et al. 2014). Due to the specific shape of the legume, the Bulgarian name of the plant means "eagle's claws" (Fig. 1).

The plant is widely used in the traditional medicine of many countries. In Western Europe, the French use the herbs of *A. glycyphyllos* as an emollient, diuretic, and refreshing agent. The main body of information on

the ethnopharmacological data is from nations living in North-eastern and South-eastern Europe. In the Caucasian region, the leaves and the seeds are used in cases of urolithiasis, oliguria, scrofulosis, dermatitis, and as a laxative. In Belarus, a decoction from the aerial parts is ingested to treat leucorrhoea, uteroptosis, and gastrointestinal disorders (dysentery). The decoction is applied topically against fungal infections of the scalp. A similar aqueous preparation is used in Ukraine as a laxative, diuretic, and mucoactive agent; against sexually transmitted infections, rheumatism, and dermatitis. Along the Volga River, the plant is used to treat diseases of the central nervous system. People from the Carpathian region also apply a decoction as a diuretic, as an expectorant, against rheumatism (arthralgia), diarrhoea, dermatitis, and syphilis; in gynaecology the preparation is given to stimulate labour and to accelerate placenta separation (third phase). This information led to clinical trials of a 10% infusion of *A. glycyphyllos* in Russia, which showed that it had hypotensive, anticoagulant, and diuretic activity (Lysiuk and Darmohray 2016). In Bulgaria, the species has been widely used in folk medicine as an antihypertensive, diuretic, and anti-inflammatory. The herb can be administered as an infusion in cases of cardiac insufficiency, renal inflammation, calculosis, increased blood pressure, tachycardia, etc. (Krasteva 2023).

Figure 1. *A. glycyphyllos*.

The current review is aimed at summing up the literature data from the first report until the present time on the phytochemistry, pharmacology, and biotechnology of *A. glycyphyllos*.

Chemical compounds

More than 40 years ago, phytochemical research on *A. glycyphyllos* was initiated in Bulgaria (Krasteva et al. 2016). The species contains secondary metabolites such as triterpenoid saponins, flavonoids, sterols, etc. Volatiles from the plant have also been studied in detail (Platikanov et al. 2005). A lot of research is still being conducted to elucidate new compounds from this species and to understand their pharmacological action in order to deepen our knowledge of this valuable medicinal plant (Pistelli 2002; Ionkova et al. 2014; Krasteva et al. 2016).

Saponins

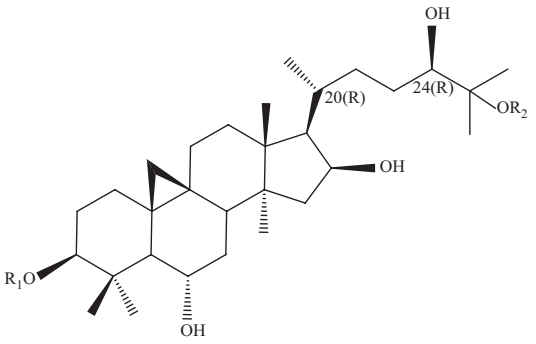
Triterpenoid saponins are an important group of secondary metabolites, isolated from species of the genus *Astragalus*, and known for their structural variety and high polarity. They are classified into two large groups: tetracyclic and pentacyclic. It is known that most *Astragalus* species found in Bulgaria contain predominately oleanane-type ones (Krasteva et al. 2016). The earliest report on the plant is on sapogenins, identified after acid hydrolysis of the aerial parts. The pentacyclic sapogenins soyasapogenol B and 3*β*,22*β*,24-trihidroxyolean-12-en-19-one were reported (Elenga et al. 1986; Elenga et al. 1987). Noteworthy, to date, no corresponding saponins of these two oleananes have been isolated. Unlike these, *A. glycyphyllos* accumulates mainly tetracyclic cycloartane triterpenoid saponins (Table 1). From the roots of the plant, askendoside C and F were isolated (Linnek et al. 2008; Linnek et al. 2009). Recently, from the aerial parts 17(*R*),20(*R*)-3*β*,6*α*,16*β*-trihydroxycycloartanyl-23-carboxilic acid 16-lactone 3-*O*-*β*-D-glucopyranoside was obtained (Shkondrov et al. 2020a).

Flavonoids

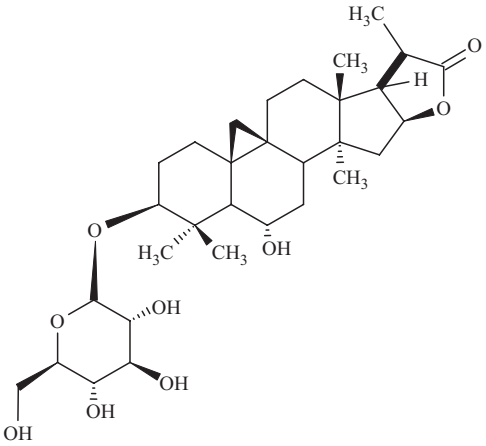
The earliest report on the chemical composition of the plant is on its flavonoid content. The species was reported to accumulate mainly flavonols (glycosides of kaempferol, quercetin, and isorhamnetin) as well as flavones (apigenin glycosides). Initially, cosmosin (apigenin-7-*O*-*β*-D-glucoside), astragalin (kaempferol-3-*O*-*β*-D-glucoside) and isorhamnetin-3-*O*-*β*-D-glucoside were identified in its aerial parts by paper chromatography with reference substances (Nikolov et al. 1984). Later in the eighties, kaempferol, quercetin, apigenin-7-*O*-arabinosyl-glucoside, kaempferol-3-*O*-xylosyl-glucoside, kaempferol-7-*O*-galactoside, and rutin were identified in the same plant parts by the same technique (Elenga 1986). In recent studies, kaempferol triglycosides have been reported in its aerial parts. The rare camelliaside A (kaempferol-3-*O*-[2-*O*-*β*-D-galactopyranosyl-6-*O*-*α*-L-rhamnopyranosyl]-*β*-D-glucopyranoside) was isolated for the second time from a plant source (Shkondrov et al. 2020a). In a total extract from its herbs, mauritianin (kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*α*-L-rhamnopyranosyl-(1→6)]-*β*-D-galactopyranoside) was identified and quantified by ultra-high performance liquid chromatography-high resolution electrospray ionization mass spectrometry (UHPLC-MS) analysis using an authentic reference substance. The species could be considered a reliable source of this kaempferol triglycoside (Shkondrov et al. 2020b). Both camelliaside A and mauritianin occur rarely in plants, and their accumulation in this taxon is of future interest. The flavonoids from the species are presented in Table 2.

Table 1. Sapogenins and saponins isolated from *A. glycyphyllos*.

Cycloartane-type saponins



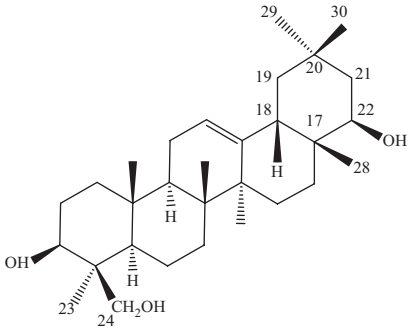
20(*R*),24(*R*)-3*β*,6*α*,16*β*,25-pentahydroxycycloartane



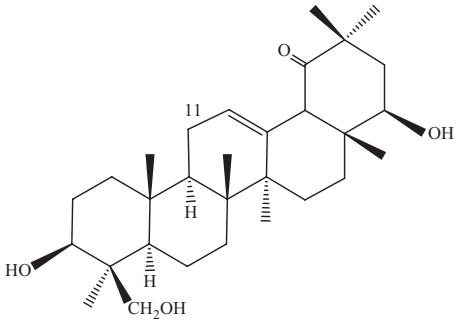
17(*R*),20(*R*)-3*β*,6*α*,16*β*-trihydroxycycloartanyl-23-carboxylic acid 16-lactone 3-*O*-*β*-D-glucopyranoside

	R_1	R_2
Askendoside C	*β*-D-ara(1→2)-*β*-D-xyl	H
Askendoside F	*β*-D-xyl(1→2)-*β*-D-ara	*β*-D-glc

Oleanane-type sapogenins

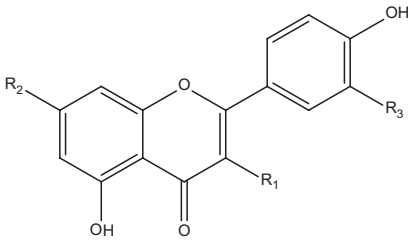


Soyasapogenol B



Sapogenin II

Table 2. Flavonoids in *A. glycyphyllos*.



R_1	R_2	R_3	Flavonoid
H	*O*-*β*-D-glc	OH	Cosmosin
O-*β*-D-glc	OH	H	Astragalin
O-*β*-D-glc	OH	OCH_3	Isorhamnetin-3-*O*-*β*-D-glucoside
OH	*O*-ara-glc	H	Apigenin-7-*O*-arabinosyl-glucoside
O-xyl-glc	OH	H	Kaempferol-3-*O*-xylosyl-glucoside
OH	*O*-gal	H	Kaempferol-7-*O*-galactoside
O-*α*-L-rha-*β*-D-glc	OH	OH	Rutin
O-[2-*O*-*β*-D-gal-6-*O*-*α*-L-rha]-*β*-D-glc	OH	H	Camelliaside A
O-*α*-L-rha-(1→2)-[*α*-L-rhal-(1→6)]-*β*-D-gal	OH	H	Mauritianin

Sterols

The information on this group of secondary metabolites in this species is still quite limited. There are no reports on their presence in the roots, which are the usual source of these compounds. A mixture of sterols from the overground parts was obtained by column chromatography with Al_2O_3. Using their chromatographic characteristics, and the IR and MS spectra, the presence of *ß*-sitosterol, stigmasterol, and campesterol was elucidated (Elenga et al. 1986).

Volatiles

A complex study of four *Astragalus* species grown in Bulgaria was conducted. Among the samples, leaves, flowers, and fruits of *A. glycyphyllos* were investigated. Volatiles from the samples were obtained by steam distillation, and their chemical composition was studied by gas chromatography-mass spectrometry (GC-MS). The influence of the phenological stage on their chemical composition was examined as well. Hydrocarbons, as well as hydroxy-, aldoxy-, carboxy-, and other functional classes, were quantified. A significant change in the amount of hydrocarbons from leaf

development to fructification was proven. It was notable that terpenes were not present in the fruit sample, unlike in the initial stages of leaf development, where these compounds were the predominant ones (Platikanov et al. 2005). The volatiles, proven in the plant are summarized in Table 3.

Table 3. Volatile compounds in *A. glycyphyllos*.

Compounds	Leaves	Flowers	Fruits
Alcohols			
3-Hexen-1-ol	+	-	+
1-Hexanol	+	-	-
1-Octen-3-ol	+	-	-
3-Ehyl-4-methylpentan-1-ol	+	-	-
Benzyl alcohol	+	-	+
2-Methoxy-4-vinyl phenol	+	-	-
4-Hydroxy-4-methyl-2-pentanone	-	-	+
Aldehydes			
Nonanal	+	+	-
Decanal	+	+	-
Benzaldehyde	-	-	+
Ketones			
3-Methyl-2(2-pentanyl)-2-cylcopenten-1-one	+	-	-
Acids			
Tetradecanoic acid	+	-	-
Pentadecanoic acid	+	-	-
Hexadecanoic acid	+	-	-
Palmitic acid	-	-	+
Esters			
3-Hexen-1-ol acetate	+	-	-
Hexadecanoic acid methyl ester	+	-	-
(18:1)-Mehyl ester	+	-	-
Hexanedioic acid ethylhexyl diester	-	+	-
Methyl dihydrojasmonate	-	-	+
1-Butanol-3-methoxy benzoate	-	-	+
Acetic acid ethylmethyl ester	-	-	+
Hydrocarbons			
Heptadecane	+	-	+
Octadecane	+	+	-
Nonadecane	+	+	-
Eicosane	+	+	+
Docosane	+	+	-
Pentacosane	+	+	+
Hexacosane	+	+	+
Heptacosane	+	+	+
Octacosane	+	-	+
Nonacosane	+	-	+
Triacontane	+	-	-
Dotriacontane	+	-	-
Hentriacontane	+	-	-
Squalene	+	-	-
Cyclotetradecane	-	+	-
Hexadecane	-	+	-
Heneicosane	-	+	+
Tetracosane	-	-	+
Aromatic hydrocarbons			
Phenanthrene	+	-	-
Terpenes			
Linalool	+	+	-
2-Terpineol	+	-	-
Geraniol	+	-	-
Hexahydrofarnesyl acetone	+	-	-
Phytol	+	+	-
Ethers			
1,1-Diethoxyethane	-	-	+
Amides			
N,*N*-Dibutyl-formamide	-	-	+
Halogenated compounds			
2-Bromo-1,1-dichloroethane	-	-	+
1,1-Diethoxy-2-chloroethane	-	-	+
1,1,1-Trichloropropane	-	-	+
Pentachlorethane	-	-	+
Hexachloroethane	-	-	+
Tetrachloroethane	-	-	+
Aromatic compounds			
Benzyl nitrile	-	-	+
Others			
2,3-Dihydro benzofurane	+	-	-

Biological activity

Pharmacological examination of extracts, purified fractions, and isolated compounds from *A. glycyphyllos* has been reported in many sources. The phytochemical content of the plant suggests that it possesses cytotoxic, antiproliferative, immunomodulatory, and antiviral activity, as well as antioxidant and hepatoprotective effects. In recent studies, a new neuroprotective action has been identified for some compounds isolated from the plant.

Cytotoxicity

Initially, the total volatile compounds from the overground parts of the plant were tested for cytotoxic activity *in vitro*. REH cells (human acute lymphoid leukaemia) were exposed to various concentrations of the total volatiles (0.1, 1, 10, 100, and 1000 μg/ml) and a MTT test was carried out to evaluate the results. The tested extract induced a concentration-dependent inhibition of the proliferation of REH cells (Momekov et al. 2007).

Saponin-rich fractions produced from shoot cultures of *A. glycyphyllos* were tested on selected cell lines: T-24 (bladder carcinoma), CAL-29 (bladder transitional cell carcinoma), MJ (cutaneous T-cell lymphoma of the *Mycosis fungoides* type), and HUT-78 (cutaneous T-cell lymphoma of the Sézary syndrome type) *via* the standard MTT test. The saponin-rich fractions displayed high efficacy against urinary bladder cancer cells (IC_{50} = 168.4 μg/mL) with a constitutive high expression level of the xenobiotic pump gp170 (MDR1) (Shkondrov et al. 2019). The antiproliferative effects of a total saponin mixture, a purified saponin fraction, and a cycloartane saponin (17(*R*),20(*R*)-3*β*,6*α*,16*β*-trihydroxycycloartanyl-23-carboxylic acid 16-lactone 3-O-*β*-D-glucopyranoside) from the aerial parts of a wild-grown plant were tested in different concentrations (12.5-200 μg/mL) against the same cell lines. The fraction had the strongest cytotoxic activity on the examined cell lines. This is considered a normal result of the activity of all of the saponins in it. Interestingly, the saponin mixture had a lower antiproliferative effect, which can be partially explained by its lower purity

compared to the fraction obtained from it afterwards (Mihaylova et al. 2021).

A study to evaluate the possible *in vitro* and *in vivo* antiproliferative and cytotoxic activity of a purified saponin mixture (PSM) obtained from the species was also performed. The effects of PSM on the viability and proliferative activity on Graffi myeloid tumour cells were assessed by the MTT test. The cell viability values measured at two time intervals (on the 24th and 48th h) showed similar concentration-dependency. Moreover, no signs of reversibility of the cytotoxic action were proven. PSM induced a statistically significant decrease in cell viability ($p < 0.01$) compared to the control (non-treated Graffi tumour cells) on the 24th hour at concentrations higher than 31 μg/mL. On the 48th hour the values measured were slightly lower than those measured on the 24th. The *in vitro* results provided the basis for an *in vivo* evaluation of the antiproliferative effects of the PSM in Graffi-tumour-bearing hamsters (GTBH). The effects of per-oral application of the PSM resulted in decreased tumour size and increased mean survival time. Pathohistological examination of the tumours of GTBH treated with PSM revealed a well-formed stroma with an accumulation of mononuclear cells and a lower quantity of mitotic cells, than in the GTBH from the non-treated control. It was also found that the mixture exerted tumour-protective effects. They were proved by a prolonged mean survival time (with four days more, compared to the non-treated GTBH) and a lowering in mortality rates, unlike the higher ones in the non-treated group of animals (Georgieva et al. 2021).

Immunomodulation

It is known that saponins possess immunomodulatory action, and some are even used as adjuvants in vaccines (Lacaille-Dubois 1999). The *in vitro* immune modulating activity of the cycloartane saponin 17(*R*),20(*R*)-3*β*,6*α*,16*β*-trihydroxycycloartanyl-23-carboxylic acid 16-lactone 3-*O*-*β*-D-glucopyranoside, obtained from the aerial parts of *A. glycyphyllos*, was examined on a murine model of isolate mice lymphocytes and peritoneal marcophages at different concentrations (1, 10, 20, 60, 100, 200 μmol/L). A statistically significant dose-dependent stimulation on both immune cell types (in concentrations above 10 μmol/L) was proved (Mihaylova et al. 2021).

Hepatoprotective and antioxidant activity

The first *in vivo* investigation of *A. glycyphyllos* was performed on a model of CCl_4-induced liver damage in male Wistar rats. The levels of malonedialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) as biochemical markers of oxidative stress in the liver were studied in a pool of 36 animals. The extract was administered orally in a dose of 100 mg/kg against a control group of animals treated with silymarin (100 mg/kg). The extract decreased MDA production by 24% and led to an increase in GSH levels by 36%, GPx activity by 83%, GR activity by 68%, and in GST activity by 51%. These values were comparable to those found in the control group (treated with silymarin at the same dose) (Shkondrov et al. 2015).

The polysaccharides, obtained from an *n*-butanol extract of the aerial parts of the species, were tested on isolated rat liver microsomes in a model of non-enzyme- and enzyme-induced lipid peroxidation (LPO). The polysaccharides were applied at concentrations of 0.6, 6, and 60 μg/mL on the microsomes to test their antioxidant properties. A significant reduction in MDA production was observed after incubation. This was a sign of concentration-dependent antioxidant activity. It was most pronounced at the highest concentration level and commensurable to that of the control substance (silymarin) (Kondeva-Burdina et al. 2016).

Neuroprotective activity

The neuroprotective effect of 17(*R*),20(*R*)-3*β*,6*α*,16*β*-trihydroxycycloartanyl-23-carboxylic acid 16-lactone 3-*O*-*β*-D-glucopyranoside was tested in different models of cyanotoxin (anatoxin-α)-induced neurotoxicity on subcellular fractions (rat brain microsomes, mitochondria, synaptosomes) in concentrations of 1, 10, and 100 μM. The saponin protected the synaptosomal viability and maintained the GSH level at 89% and 70%, respectively (at a concentration of 100 μM), compared with the pure anatoxin-α. Also, on isolated brain mitochondria, it preserved the GSH level and decreased MDA production, by 64% and by 48%. In conditions of anatoxin-α-induced toxicity on rat brain microsomes, the saponin (100 μM) decreased MDA production by 48% in comparison with the control (Ilieva et al. 2020). Further, the saponin, along with camelliaside A, were tested on a 6-hydroxydopamine (6-OHDA)-induced neurotoxicity model on isolated rat brain synaptosomes *in vitro*. Both compounds showed statistically significant neuroprotective activity at 100 μM. It showed activities comparable to those of silibinin, while camelliaside A had a weaker effect on synaptosomal viability (98% for the saponin and 32% for the flavonoid) against 6-OHDA). The levels of GSH were retained as follows: 56% for the saponin and 36% for the flavonoid against the toxic agent (Shkondrov et at. 2020a).

In another study, the same compounds (1 μM concentration) were also tested for possible activity on human recombinant monoamine oxidase type B enzyme (*h*MAO-B). They had an inhibitory effect, 44% and 35%, respectively, in comparison to the reference selegiline (55%). Thus, this saponin could be considered a promising structure in the treatment of neurodegenerative diseases (Shkondrov et al. 2020a).

Antiviral activity

A defatted extract from the plant (DEAG) was standardized in respect of saponins and tested for its antiviral activity *in vitro* alone and in combination with acyclovir

against Simplexvirus humanalpha types 1 (acyclovir sensitive) and 2 (acyclovir resistant). DEAG was applied in concentrations ranging from 0.00625 mg/mL to 8 mg/mL and a maximal non-toxic concentration of 0.6 mg/mL was established. When used in that concentration, DEAG reached between 60% and 70% protection against both virus strains. The combination of the extract and acyclovir was tested against Simplexvirus humanalpha type 1 and led to antagonism, and it was concluded that they should not be used simultaneously (Shkondrov et al. 2023).

Biotechnological studies

Biotechnological studies of the species were performed, despite its wide distribution in the flora. The medicinal interest in *A. glycyphyllos* and its continuous depletion from its natural habitats are the main reasons to apply *in vitro* techniques for biotechnological production of biomass. In these studies, the saponin and flavonoid content of the developed cultures were monitored to select the most appropriate one in phytochemical terms.

Initially, after seed germination, callus, suspension, and shoot *in vitro* cultures were established. Their saponin content was studied by UHPLC-HRESIMS, using 17(*R*),20(*R*)-3*ß*,6*α*,16*ß*-trihydroxycycloartanyl-23-carboxylic acid 16-lactone 3-*O*-*ß*-D-glucopyranoside as the reference. A comparison to the wild-grown species was done. Noteworthy, *in vitro* shoot cultures accumulated double the amount of this saponin compared to the wild plant. The potential of *in vitro* cultivation as a source of pharmaceutically important metabolites such as cytotoxic triterpenoid saponins (Shkondrov et al. 2019).

It is known that many factors could induce the secondary metabolism of an *in vitro* culture (hormones, cultivation regimen, additives, etc.) (Ionkova et al. 2014). Shots, calli, and suspension cultures from the plant, grown on different media, supplemented or not with additives or hormones, and in different regimens (light or dark), were studied for their total flavonoid content and the quantity of rutin and camelliaside A as the specific flavonol markers. It was found that calli cultivated on a modified cultivation medium with double the amount of Ca^{2+} and Mg^{2+} showed a greater amount of total flavonoids. Suspensions cultures, cultivated on a modified medium with 10, 20, and 30 mg/mL quercetin also achieved higher total flavonoid content. For the differentiated flavonoid content of rutin and camelliaside A, shoot cultures were found to be the best, accumulating the highest quantity (Popova et al. 2020). These findings could serve as a basis for further exploration of the limitless possibilities of biotechnology.

Conclusion

Due to its wide distribution and various biological effects, *Astragalus glycyphyllos* is a source of valuable secondary metabolites such as flavonoids, saponins, etc. Pharmacological investigations of the plant reveal their promising antioxidant, hepatoprotective, antiproliferative, immunomodulatory, antiviral, and neuroprotective activities. These facts make the species interesting for further research in order to deepen our knowledge of its chemical composition and biological action.

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