# Turkish Astragalus Species: Botanical Aspects, Secondary Metabolites, and Biotransformation

# Authors

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#### **Keywords**

Astragalus, Leguminosae, botany, phytochemistry, cycloartenols, biological activity, biotransformation

received		April 2, 2024
accepted after revision		September 30, 2024
Bibliography		
Planta Med 2024		
DOI	10.1055/a-2444-3252	
ISSN	0032-0943	

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#### Supplementary Material is available under https://doi.org/10.1055/a-2444-3252

#### ABSTRACT

Astragalus is a widespread genus comprising approximately 3500 species, both annual and perennial, found across Asia, Europe, Africa, and the Americas. In Turkey, it is represented by 63 sections and 485 taxa with a high endemism ratio (51%). In traditional medicine, the roots of various Astragalus species represent very old and well-known drugs used for antiperspirant, diuretic, and tonic purposes, as well as for the treatment of nephritis, diabetes, leukemia, and uterine cancer. The genus Astragalus is the richest source of cycloartanetype compounds, which display a diverse range of bioactivities, such as wound healing, immunomodulatory, antitumor, hepatoprotective, antimutagenic, antiviral, and antiprotozoal activities. Moreover, cycloastragenol, the main sapogenol of many cycloartane-type glycosides found in the Astragalus genus, has gained attention as a potent telomerase activator over the past decade. The preparation of cycloastragenol derivatives could be significant in the near future due to their unique bioactivity. This review covers the botanical aspects of Astragalus L., as well as the phytochemical and biological activity studies conducted on Turkish Astragalus species, with a special focus on cycloartenols. It contains 36 articles reporting the phytochemistry of 29 Astragalus species and 111 new compounds, including 104 triterpene saponins. In addition to the phytochemical studies, this review summarizes the biotransformation studies on Astragalus cycloartanes via endophytic fungi isolated from the tissues of Astragalus species.

# Introduction

Astragalus L. (Leguminosae) is one of the most widely distributed genera in the plant kingdom, with about 3500 annual and perennial species found in Asia, Europe, Africa, and North and South America. In the flora of Turkey, the genus Astragalus is represented by 479 species [1–4]. The species known as geven in Turkey are typically annual herbaceous or perennial thorny and pillow-shaped plants. It is known by names such as milkvetch and locoweed in foreign sources.

Some species of *Astragalus* are used in the production of "Tragacanth Gum" (Tragacantha), a valuable traditional drug employed as a thickener, stabilizer, or emulsifier in the food, pharmaceutical, and cosmetics industries. The name 'tragacanth' derives from the Greek words *tragos* (meaning "goat") and *akantha* ("thorn"). Turkey and Iran are the primary producers of tragacanth globally. *A. microcephalus*, Willd. *A. gummifer* Labill, and *A. kurdicus* Boiss. are used in the production of tragacanth in Turkey [5–8]. In addition, it is recorded that the roots of some *Astragalus* species were used as substitutes for Çöven (*Gypsophila* L. root) in the preparation of Turkish Tahin Helva [6, 7]. In Turkey, *Astragalus* species are also known by the names *cekme, gön, kavan, ketire, ketre,* and *geven* [7].

The roots of *Astragalus* species have been used in Chinese medicine as an immunomodulator, diuretic, vasodilator, antiper-

spirant, and tonic since ancient times. They have also been used in the treatment of nephritis, diabetes, respiratory tract infections, and uterine cancers. *Astragalus membranaceus* Bunge (Huang-Qi) is a well-known species used in TCM for its diverse medicinal effects [9]. Although this drug has been used in the Far East since ancient times, its clinical applications and pharmacological effects have only recently gained attention in Western medicine.

In the early 1990 s, the utilization of the roots from one of the *Astragalus* species for treating leukemia in Turkey prompted us to direct our research toward the Turkish *Astragalus* species.

Here, we summarized our extensive studies on the Turkish Astragalus species from botanical, phytochemical, and bioactivity perspectives, as well as the reports of other research groups. Moreover, whole-cell biotransformation studies on some of the Astragalus sapogenins by endophytic fungi were included.

# Botanical Aspects of Astragalus L.

The genus *Astragalus* L. is a member of the Fabaceae and one of the largest genera in the world. According to the International Plant Names Index (IPNI), the number of taxa is 6200. Also, according to Maassoumi (1998) [10], it is represented with 8 subgenera, 245 sections, and 2530 taxa (subspecies and varieties); 102 of them are annual, and 2428 are perennial in the Old World (IPNI, January 2024). It consists of 2398 taxa and 136 sections in the Old World [10, 11]. The members of the genus are dominant plants of the steppe vegetation. The diversity center of the genus is Turkey (with 480 species), Russia (c. 735 species), and Iran (840 species) [10–12].

In Turkey, it is represented by 480 taxa representing 63 sections, and the rate of endemism is 51% [3,4,13].

Taxonomical characters used in the key for the determination of the *Astragalus* species are based on whether the plants are annuals or perennials, which can be scapose or caulescent, and herbaceous or woody, with spiny shrubs. Additional morphological features are the shapes of leaves (paripinnate or imparipinnate), leaflets (simple hairy or bifurcate, glabrous), flowers (sessile or pedicellate), structure of bracts and bracteoles, calyx (inflated or not), standard stenekoid or platanocoid, and the type of legume (stipitate unilocular or bilocular, one to many seeds, cylindiric, oblong, ovate, inflated or not, glabrous or hairy) (**> Fig. 1**).

Among the 480 taxa represented in the flora of Turkey, there are more than 20 annual (-biannual) species, which are classified in nine sections (**Fig. 1S**, Supporting Information). Sect. Oxyglottis Bunge is represented by eight species. *A. triradiatus* (or *A. stella*) is one of the eight species in this section. Sections Harbilobus Bunge and Platyglottis Bunge are represented by two species, while the others are represented by only one species. Perennial plants are represented by 54 sections (**Fig. 2S**, Supporting Information). In both figures, some *Astragalus* species are given as a representative of some sections selected.

# Chemical Compounds in Astragalus Species

## Toxic Astragalus species

Apart from the *Astragalus* species used medicinally, there are also some species that contain toxic compounds, which can be categorized into three groups: species that synthesize aliphatic nitro compounds, species that cause locoweed toxicity, and species that can accumulate selenium [14].

#### Aliphatic nitro compounds

Nitro compounds [1 (1-O-[5-oxotetrahydrofuran-3-yl]acetyl-6-O-[3-nitropropanoil]- $\beta$ -D-glucopyranose) and 2 (3-Nitro-1-propil- $\beta$ -D-laminaribioside)] are nitropropionic acid (3 nitropropyl)-glucose derivatives (**> Fig. 2**). When they are hydrolyzed, 3-nitropropionic acid becomes free, and this causes methemoglobinemia [15, 16].

#### Indolizidine alkaloids

The compounds that cause "locoweed" poisoning, known as mountain sickness in animals, are indolizidine alkaloids. One of these compounds, which is mostly found in the species of sections *Astragalus* and *Oxytropis*, is an alkaloid, swainsonine (**3**) ( $\succ$  **Fig. 2**). This compound alters glycoprotein synthesis by inhibiting acidic  $\alpha$ -mannosidases, leading to fetal degeneration in pregnant livestock, and may cause miscarriage [14].

#### Selenium compounds

Selenium compounds cause chronic toxicity. This condition results in hair thickening and thinning, pathological formations in the hooves, disabilities, weakness, and loss of appetite, as well as a weakening and slowing of the functions. *Astragalus bisulcatus, A. saurinus, A. flavus*, and *A. tenellus* are the species rich in selenifereous compounds such as seleno-cysteine, seleno-cystine, selenomethionine, and seleno-cystathionine [14].

#### Medicinal Astragalus species

Several species of *Astragalus* are used for medicinal purposes, particularly A. *membranaceus* being the most widely studied and utilized. *Astragali* radix (*Huang-Qi*), one of the most commonly used traditional Chinese crude drugs, is prepared from the roots of *Astragalus membranaceus* and *Astragalus mongholicus*, which were described as an immunostimulant, hepatoprotective, antiperspirant, diuretic, or tonic (adaptogenic) in traditional Chinese medicine (TCM) [17, 18]. The main bioactive compounds of the underground part of the *Astragalus* species with medicinal value are polysaccharides and cycloartane-type glycosides. Other major compounds responsible for their pharmacological action include oleanane-type triterpene glycosides, flavonoids, pterocarpanes, and ionone glycosides.

#### Polysaccharides

The polysaccharides derived from the roots of *A. membranaceus* are well known and have been reported by several research groups. These polysaccharides are considered a group of potential bioactive components that contribute to the medicinal properties of the genus *Astragalus*. It has been demonstrated that



▶ Fig. 1 Key used in the determination of Astragalus Species according to their morphological characteristics [2].

*A. membranaceus* polysaccharides (APSs) have various biological properties including immunomodulation, antioxidant, antidiabetic, antiviral, hepatoprotective, and anti-inflammatory ones [9, 19–21]. Fang et al. (1982) reported three polysaccharides, astragalan I, II, and III from the aqueous etract of the roots of *A. membranaceus* var. *mongolicus* [22]. Astragalan I was a polysaccharide composed of D-glucose, D-galactose, and L-arabinose in a molar ratio of 1.75:1.63:1, with a molecular weight of 36300 D, while astragalan II and III were composed of D-glucose only, with molecular weights of 12300 D and 34600 D, respectively.

In another study, an acidic polysaccharide (AMon-S) was obtained from the roots of *A. mongolicus* Bunge. composed of L-arabinoside, D-galactose, D-galacturonic acid, and D-glucuronic acid in a molar ratio of 18:18:1:1, along with small amounts of acetyl groups and peptide moieties. According to the authors, AMon-S is the second example of reticuloendothelial-system-activating acidic polysaccharides that possess terminal glucuronic acid units [23].

In 1992, a glycan (AMem-P) was isolated from the roots of *A. membranaceus*. This glycan was primarily composed of L-arabinoside, D-galactose, L-rhamnose, and D-galacturonic acid in a molar ratio of 6:9:8:30. The authors reported that AMem-P exhibited significant reticuloendothelial-system-potentiating activity in the carbon clearance test [24].

#### Cycloartanes

Cycloartanes, which are widely distributed in the plant kingdom, are formed by the cyclization of squalene-2,3-epoxide and play a role in the biosynthesis of other plant sterols in higher plants and algae. In most plant steroids, the cyclopropane ring has been reopened. In animals, lanosterol is a precursor for cholesterol and other sterois [25].



Fig. 2 Nitropropionic acid-glucose derivatives (1 and 2) and swainsonine (3).



**Fig. 3** Structures of cycloartanes (4–15).

A literature survey indicated that the families rich in cycloartane-type triterpenoids are mainly Meliaceae (*Heynea* Roxb, *Aglaia* F. Allam and *Sweitenia* Jacg. species), Orchidaceae (*Cirrhopetalum* Lindl, *Pholidota* Lindl, and *Cymbidium* Sw. species), Passifloraceae (*Passiflora* L. species), Combretaceae (*Combretum* Loefl. species), Araliaceae (*Acanthopanax* (Decne. & Planch.) Miq *species*), Ranunculaceae (*Thalictrum* L, *Cimicifuga* L. ex Wernisch, *species*), and Fabaceae (*Astragalus*, *Abrus* Adans. species). Moreover, cycloartanes have been the most studied compounds in the genus *Astragalus* since their first report in 1981 [26–28].

Cyclosieversigenin (= cycloastragenol) (4), cycloasgenin A (5), and dasyanthogenin (6) are the first cycloartanes (**> Fig. 3**) reported from *A. sieversianus* Pall. [26], *A. taschkendicus* Bunge. [27], and *A. dasyanthus* Pall. [28], respectively. The first cycloartane glycosides from *Astragalus membranaceus* were first reported in 1983 [29]. Cycloartanes can be free, or they can be found as mono-, bi-, and tridesmosidic glycosides. Glycosidation sides are the secondary and tertiary alcohol groups in different positions of the cycloartenol skeleton. The most common sugar found on the structure of glycosides is  $\beta$ -D-xylose. Additional monosaccharides are  $\beta$ -D-glucose,  $\alpha$ -L-rhamnose,  $\alpha$ -L-arabinose, and rarely,  $\beta$ -D-apiose. In addition to the presence of hydroxyl and ketone functions at different positions in the A, B, C, and D rings and the side chain linked from the 17th carbon, the epoxidations observed in the side chain and between the side chain and the D ring cause a rich structural diversity in the cycloartane skeleton [30]. Therefore, it is possible to classify the cycloartanes into three groups according to the side-chain structure (**> Fig. 3**):

- Cycloartanes with an acyclic side chain [7 (3-dehydrocycloasgenin), 8 (cyclofoetigenin B), 9 (cyclocantogenin), and 10 (macrophyllogenin)].
- 20,24-epoxycycloartanes and 20,25-epoxycycloartanes [4 (cyclosieversigenin), 11 (cyclogalegigenin), and 12 (cyclocephalogenin)].



▶ Fig. 4 Oleanane and lanostane triterpenoids (16–24).

 16β, 23;16α, 24-diepoxycycloartanes and 16,24;20,24-diepoxycycloartanes [13 (cycloorbigenin), 14 (cycloorbigenin A), and 15 (cycloalpigenin)].

#### Oleanane-type triterpene glycosides

Oleanane-type saponins are less common in the genus Astragalus compared to cycloartanes. They are found in the Astragalus species and are based on soyasapogenol B as their aglycone, and their sugar chains typically have a  $\beta$ -D-glucuronic acid directly linked to the aglycone at C-3 and carry  $\beta$ -D-glucose,  $\beta$ -D-galactose, or  $\beta$ -D-xylose bonded to C-2' [31]. Glycosides (**16–21**) obtained from the seeds of *A. sinicus* [32] and *A. complanatus* R.Br. ex Bunge [33] can be shown as an example of this group. An unusual oleanane-type saponin lactone, 19-hydroxyolean-12-ene-28,21- $\beta$ -D-xylopyranoside (**22**), was isolated from the ethanolic extract of *A. corniculatus* Bieb. (**> Fig. 4**) [34].

#### Lanostane-type triterpenoids

Lanostane-type triterpenoids are not common in the genus *Astragalus*. Orbigenin (23) and orbicoside (24) are lanostane-type triterpenes obtained from the aboveground organs of *A. orbiculatus* Ledeb (**> Fig. 4**) [35].

# Initial Studies on Astragalus Species

The notable improvements observed in the blood profile of a leukemia patient who utilized an extract derived from a folkloric medicinal plant initiated our research on Turkish *Astragalus* species. In the early 1990 s, the first plant material, which consisted of the roots of a plant used for this purpose, was provided to us by the Gülhane Military Medical Academy (GMMA) for examination. The origin of these roots was unknown, and the user referred to it as Gune root (Turkish name: Gune Kökü).

At the end of the study conducted on three pieces of roots, three compounds were isolated as the major constituents of the methanolic extract. The structure elucidation studies revealed that these compounds were glycosides with a novel skeleton containing 24S-cycloartane-1 $\alpha$ ,3 $\beta$ ,7 $\beta$ ,24,25-pentol aglycone. Despite the absence of the isolated compounds in chromatographic studies, the morphological structure of the roots, the high polysaccharide content, and the plant samples sent by the patient from Şanlıurfa, a city in Southeastern Turkey, along with the structures of the compounds identified as cycloartane-type glycosides, suggested that the roots belong to an *Astragalus* species. The novel aglycone structures of these three glycosides, which were previously unknown in the scientific world, prompted us to conduct further research on the genus *Astragalus*.

Our phytochemical studies on the genus of Astragalus focused on 14 species belong to 8 sections, viz, Macrophyllium Bunge. (A. oleifolius DC.), Christiana Bunge. (A. melanophrurius Boiss.), Rhacophorus Bunge. (A. microcephalus, A. cephalotes Banks & Sol, A. zahlbruckneri Hand.-Mazz, A. prusianus Boiss.), Pterophorus Bunge. (A. brachypterus Fischer, A. trojanus Stev, A. baibutensis Bunge.), Dissitiflori DC. (A. elongatus Willd, A. campylosema (Syn. A. pendulus DC.), Vulneraria Bunge. (A. vulneraria DC.), Stereocalyx Bornm. (A. stereocalyx Bornm.), and Erophaca Boiss. (A. lusitanicus Lam.).

# Astragalus oleifolius DC. (Sect. Macrophyllium Bunge.)

The three glycosides obtained from the drug sent by GATA were found only in the methanol extract of *A. oleifolius* collected from Ahlatlıbel, Ankara, among the many *Astragalus* species collected from various regions of our country. Subsequently, two more gly-



cosides were isolated and named macrophyllosaponins A–E (25–29) (► Fig. 5) [36,37]. Studies conducted by the NCI (National Cancer Institute–Maryland, USA) revealed that these compounds did not exhibit any significant cytotoxic activity against the 60 human cancer cell lines.

In continuation of our studies on this species, two new cycloartane-type glycosides oleifoliosides A (**30**) and B (**31**), along with three known compounds cyclocanthoside E (**32**), astragaloside II (**33**) and IV (**34**), were obtained [29, 38] from the lower stem parts of *A. oleifolius*, which was collected from Şırnak: Uludere-Habur junction toward Hakkari (**> Fig. 5**) [39]. The potential cytotoxicity of the isolated compounds on primary mammalian (L6) cells was evaluated along with their *in vitro* trypanocidal, leishmanicidal, and antiplasmodial activities. All the compounds, with the exception of astragaloside IV, exhibited a significant growth inhibitory activity against *Leishmania donovani* with IC<sub>50</sub> values in the range of 13.2 to 21.3 µg/ml. Weak activity against *Trypanosoma brucei*  rhodesiense was observed with the known compounds astragaloside II (4,  $IC_{50}$  66.6 µg/ml) and cyclocanthoside E (3,  $IC_{50}$ 85.2 µg/ml), whereas all compounds were inactive against *Trypanosoma cruzi* and *Plasmodium falciparum*. None of the compounds showed toxicity to mammalian cells. In this study, the leishmanicidal and trypanocidal activity of cycloartane-type triterpene glycosides were reported for the first time.

# Astragalus melanophrurius Boiss. (Sect. Christiana Bunge.)

The studies conducted on *A. melanophrurius*, an endemic species collected from Ankara, Ahlatlıbel, resulted in the isolation of eight known saponins: astragalosides I (**35**) [29], II (**33**) [29], IV (**34**) [29], and VI (**36**) [40]; astrasieversianins II (**37**) [41] and X (**38**) [41]; and cyclocanthosides E (**32**) [38] and G (**39**) [38] ( $\blacktriangleright$  Fig. 5) [42]. Notably, the majority of these compounds are cycloastrage-nol glycosides, many of which have been reported previously from

A. membranaceus, a plant used in Far Eastern traditional medicine [29, 40]. In subsequent *in vitro* bioassays, these isolates were evaluated for cytotoxicity, estrogenic-antiestrogenic properties, antimalarial activity, antimicrobial activity, and immunomodulatory effects. The screening studies revealed that all compounds showed significant immunomodulatory activity, in addition to moderate antibacterial activity. Furthermore, in the lymphocyte stimulation test, all compounds were found to stimulate proliferation in human lymphocytes at a concentration of 0.01 to  $10 \,\mu$ g/ml. These findings were confirmed in a similar study conducted by another research group using cycloartane and oleanane-type saponins obtained from the *Astragalus* species [43].

# Astragalus microcephalus Willd. (Sect. Rhacophorus Bunge.)

A. microcephalus is one of the species used for the production of tragacanth in Turkey. This species was collected from Mucur-Avanos, Nevşehir, Central Anatolia. Cycloastragenol (4) [29], cyclocantoside E (**32**) [38], astragaloside IV (**34**) [29], and two new compounds, cyclocephaloside I (**40**) and II (**41**), were isolated from the roots of *A. microcephalus* [44, 45]. Notably, cyclocephaloside I (**40**) (**• Fig. 5**) was a novel cycloartane-type glycoside with a structure of 20,25-epoxy, $3\beta$ -( $\beta$ -D-xylopranosyl)oxy- $6\alpha$ -( $\beta$ -D-glucopranosyl)oxy-cycloartane-16 $\beta$ ,24 $\alpha$ -diol. It was the first structure bearing an epoxide group between the 20th and 25th carbons in the side chain [44].

# Astragalus brachypterus Fischer (Sect. Pterophorus Bunge.)

A. brachypterus was collected from Mucur-Avanos, Nevşehir, Central Anatolia. In addition to astragalosides I (**35**) [29], II (**33**) [29], IV (**34**) [29], and cyclocantoside E (**32**) [38], three new compounds named brachyosides A (**42**), B (**43**), and C (**44**) were isolated from this species (▶ **Fig. 5**) [45].

# Astragalus trojanus Stev. (Sect. Pterophorus Bunge.)

In another study, both the roots and aerial parts of A. trojanus, an endemic species collected from Hacıbozlar Village, Burhaniye-Balikesir, West Anatolia, were studied [46–48]. Six novel cycloartane type qlycosides (45-50), together with a new oleanane glycoside (astrojanoside A) (51) and tryptophan derivative (52) [(Achillamide)=N-(3-hydroxy-3-methyl-glutaroyl)-tryptophan], were obtained from the roots of the plant. Trojanoside A (45) and B (46) were found to contain (20*R*,24*S*)-epoxy-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,25 tetrahydroxycycloartane as the aqlycone, whereas trojanosides C (47), D (48), E (49), and F (50) had 3β,6α,16β, (24S),25-pentaahydroxycycloartane as the aglycone [47]. In addition, four new compounds [trojanosides H (53), I (54), J (55), and K (56)] along with the known cycloartane glycosides astragalosides I (35) [29], II (33) [29], IV (34) [29], VII [49], astrasiversianins IX (49) [41], XV (50) [41, 50], and brachyosides B (43) [45] and C (44) [45], and a pterocarpane derivative macianin (maackianin) (59) [51] were isolated from the aerial parts of the plant (> Fig. 6) [47, 48].

# Astragalus cephalotes Bangs & Sol. var. brevicalyx Eig. (Sect. Rhacophorus Bunge.)

A. cephalotes var. brevicalyx, traditionally used for wound healing in southeastern Anatolia, was collected from Borgaç village, Hilvan, Şanlıurfa. Mono- (cyclocantoside A) [52], bi-(cyclocantosides D and E) [38] and tridesmosidic (cephalatoside A (60)) glycosides of cyclocantogenin were obtained in this study [53]. Cephalotoside A (60) (► Fig. 6), a new tridesmosidic cycloartane type glycoside, was isolated from the roots of *A. cephalotes* var. brevicalyx. Tridesmosidic glycosides are rarely encountered in nature and have only been isolated from the *Astragalus* species.

# Astragalus zahlbruckneri Hand.-Mazz. (Sect. Rhacophorus Bunge.)

The study on the roots of A. zahlbruckneri, collected from Sivrice, Elâzığ, Eastern Anatolia, resulted in the isolation of six compounds (61–66) (> Fig. 7). The apolar fractions of the ethanolic extract afforded two cycloartane derivative triterpenes, 20(R),25-epoxy- $3\beta$ , $6\alpha$ , $16\beta$ , $24\alpha$ -tetrahydroxycycloartane (61) and 20(R),24(S)-ep $oxy-3\beta$ ,  $6\alpha$ , 25-trihydroxycycloartan-16-one (62), together with cycloastragenol [29]. Compound 62 was previously reported as a cycloartane derivative obtained by chemical oxidation of cycloastragenol [29,40,49]. A new lignan [(+)-*neo*-olivil-4-*O*-β-apiofuranosyl- $(1 \rightarrow 2)$ - $\beta$ -glucopyranoside (63)] and three phenolic glycosides [7,8-dihydro-7-hydroxyconiferyl alcohol 4-O-β-apiofuranosyl- $(1 \rightarrow 2)$ - $\beta$ -glucopyranoside (64), 2-methoxyphenol-4-O- $\beta$ -apiofuranosyl- $(1 \rightarrow 2)$ - $\beta$ -glucopyranoside (65), and 3-hydroxy-5-methoxyphenol-2-O- $\beta$ -apiofuranosyl- $(1 \rightarrow 2)$ - $\beta$ -glucopyranoside (66)] were isolated from the polar fractions of A. zahlbruckneri [54].

# Astragalus prusianus Boiss. (Sect. Rhacophorus Bunge.)

Continuing our research on the genus Astragalus, A. prusianus was collected from Kale, Muğla, West Anatolia. In this study, two novel cycloartane-type triterpene glycosides, 16-O- $\beta$ -D-glucopyrano-syl-20(S),24(R)-5 $\alpha$ ,9-diepoxy,2 $\alpha$ ,3 $\beta$ ,16 $\beta$ ,25-tetrahydroxy-9,10-se-co-cycloarta-1(10),6(7)-diene (**67**) and 3-O- $\beta$ -D-xylopyranosyl-16-O- $\beta$ -D-glucopyranosyl-20(S),24(R)-epoxy-3 $\beta$ ,16 $\beta$ ,25-trihydroxy-cycloartane (**68**), were obtained (**Fig. 7**). The 5 $\alpha$ ,9-epoxy structural feature in prussianoside A (**67**) was reported for the first time in triterpene chemistry [55].

# Astragalus vulneraria DC. (Sect. Vulneraria DC.)

A. vulneraria was the only species from which no cycloartane derivative has been isolated in our studies. However, two flavonol glycosides were isolated from the aerial parts of the plant material collected from Polatlı, Ankara, Central Anatolia. One of the glycosides was a new compound (**69**) [isorhamnetin 3-*O*- $\beta$ -D-apiofuranosyl-(1  $\rightarrow$  2)-[ $\alpha$ -L-rhamnopyranosy-(1  $\rightarrow$  6)]- $\beta$ -D galactopyranoside], and the other was a known isorhamnetine derivative (**70**) [56], isorhamnetin 3-*O*- $\beta$ -D-apiofuranosyl-(1  $\rightarrow$  2)- $\beta$ -D-galactopyranoside (**> Fig. 7**) [57].

# Astragalus baibutensis Bunge (Sect. Pterophorus Bunge)

As a result of our studies on the chemistry of *A. baibutensis*, (20R, 24S)-3-O- $[\beta$ -D-apiofuranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-xylopyranosyl]-6-O-



▶ Fig. 6 Structures of compounds 45–60.

β-D-glucopyranosyl-3β,6α,16β,25-tetrahydroxy-20,24-epoxycycloartane, named baibutoside (71) ( $\succ$  Fig. 7), a new cycloartanetype glycoside together with four known glycosides, acetylastragaloside I [29], astragaloside I (35) [29], II (33) [29], and IV (34) [29], were reported [58]. The antiprotozoal activities of the isolated compounds were also evaluated against some parasites, including *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*. All the tested compounds were inactive against *L. donovani* and *P. falciparum*. In addition, the selective toxicity tests on primary L6 mammalian cells (rat skeletal myoblasts) demonstrated that only acetylastragaloside I had a cytotoxic effect with narrow selectivity index values of 2.5 and 4.8.

# Astragalus campylosema Boiss. ssp. campylosema (Astragalus pendulus DC. (Sect. Dissitiflori DC.), Astragalogia: 232 (1802) [11])

In the course of studies on the Turkish Astragalus species, four new cycloartane glycosides,  $3-O-[\alpha-L-arabinopyranosyl-(1 \rightarrow 2)-\beta-D-xylopyranosyl]-3\beta,6\alpha,16\beta,23\alpha,25-pentahydroxy-20(R),24(S)-epoxycycloartane (72), <math>3-O-[\alpha-L-arabinopyranosyl-(1 \rightarrow 2)-\beta-D-xylopyranosyl]-16-O-hydroxyacetoxy-23-O-acetoxy-3\beta,6\alpha,25-trihydroxy-20(R),24(S)-epoxycycloartane (73), <math>3-O-[\alpha-L-arabinopyranosyl-(1 \rightarrow 2)-\beta-D-xylopyranosyl]-25-O-\beta-D-glucopyranosyl-3\beta,6\alpha,16\beta,25-tetrahydroxy-20(R),24(S)-epoxycycloartane (74),$ 

and 3-O-[ $\alpha$ -L-arabinopyranosyl-( $1 \rightarrow 2$ )- $\beta$ -D-xylopyranosyl]-3 $\beta$ ,6 $\alpha$ ,23 $\alpha$ ,25-tetrahydroxy-20(R),24(S)-16 $\beta$ ,24;20,24-diepoxycycloartane (**75**) ( $\blacktriangleright$  **Fig. 8**), together with three previously isolated cycloartane glycosides, namely, 3-O-[ $\alpha$ -L-arabinopyranosyl-( $1 \rightarrow 2$ )- $\beta$ -D-xylopyranosyl]-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,25-tetrahydroxy-20(R),24 (S)-epoxycycloartane [59], askendoside C [60], and askendoside G [61], were obtained from the MeOH extract of the roots of *A. pendulus*, collected from Tutak, Ağrı, East Anatolia [62].

# Astragalus elongatus (Sect. Dissitiflori DC.)

Continuing of our work on the genus Astragalus, the roots of Astragalus elongatus, collected from Central Anatolia, Ahlatlıbel, Ankara, were also studied. In this study, a new monodesmosidic cycloartane-type glycoside, elongatoside (76) (3-O-[ $\alpha$ -arabinopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -xylopyranosyl]-cycloastragenol) (**Fig. 8**), was isolated in addition to two known cycloartane-type glycosides: askendosides D (3-O-[ $\alpha$ -arabinopyranosyl-(1  $\rightarrow$  2)- $\beta$ -xylopyranosyl]-6-O- $\beta$ -xylopyranosyl-cycloastragenol [59] and G (3-O- $[\alpha$ -arabinopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -xylopyranosyl]-16-O- $\beta$ -glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24(R),25-pentahydroxycycloartane) [61]. These compounds were assessed for their effects on cell proliferation and ICAM-1 expression using the human microvascular endothelial cell line HMEC-1. The results showed that compound 76 exhibited weak activity in the ICAM-1 assay [63].



**Fig. 7** Structures of compounds **61–71**.

# Astragalus stereocalyx Bornm. (Sect. Stereocalyx Bornm.)

As part of our ongoing studies on the Turkish Astragalus species, 3-O-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-xylopyranosyl]-16-O- $\beta$ -Dglucopyranosyl-3 $\beta$ ,6 $\alpha$ , 16 $\beta$ ,20(5),24(R),25-hexahydroxycycloartane (77), 3-O-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-xylopyranosyl]-3 $\beta$ ,6 $\alpha$ , 16 $\beta$ ,20(5),24(R),25-hexahydroxycycloartane (78), 3-O-[ $\alpha$ -Larabinopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranosyl]-3 $\beta$ ,6 $\alpha$ , 16 $\beta$ ,20(5), 24(R),25-hexahydroxycycloartane (79), 3-O-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranosyl]-24-O- $\beta$ -D-glucopyranosyl]-3 $\beta$ ,6 $\alpha$ , 16 $\beta$ ,24(R),25-pentahydroxycycloartane (80), 3-O-[ $\alpha$ -Larabinopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranosyl]-16-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ , 16 $\beta$ ,24(R),25-pentahydroxycycloartane (81), and 3-O-{ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  4)-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranosyl]]-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24(R),25-pentahydroxycycloartane (82) were isolated from the MeOH extract of *A. stereocalyx* (**> Fig. 8**). Additionally, the known compounds askendoside C [60], askendoside F [64], askendoside G [61], 3-O $\beta$ -D-glucopyranosyl-16-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24(R),25 pentahydroxycycloartane [43], elongatoside (76) [63], and trojanoside H (53) [47] were also obtained from the roots of *A. stereocalyx*. In addition, the isolated compounds were evaluated for their cytotoxicity against different cell lines including human cervical cancer (Hela), human colon cancer (HT-29), human leukemia (U937), and human lung cancer (H446). Only a few com-



**Fig. 8** Structures of compounds 72–82.

pounds exhibited a weak cytotoxic activity in the concentration of  $1-50 \ \mu M$  [65].

#### Astragalus lusitanicus Lam. (Sect. Erophaca Boiss.)

Ongoing studies are being conducted on *A. lusitanicus*, a species known for its toxicity in Turkey and the countries bordering the Mediterranean Sea. So far, only kersetol and kaempferol derivative flavonol glycosides have been obtained. It is believed that aliphatic nitro compounds are responsible for the toxicity in animals caused by this species [14].

In 2000, due to the well-known immunostimulatory activity of saponins together with our earlier investigation on some of the compounds for their bioactivity [42], 19 cycloartane-type triterpene glycosides were tested for their immunostimulatory effects on macrophage activation and expression of inflammatory cytokines. Macrophlyllosaponins B-D (25-29) [36, 37], askendoside G [61], cyclocanthoside D [38] and E (32) [38], cephalotoside A (60) [53], astrasieversianin II (37) [41] and X (38) [41], astragaloside I (35) [29], II (33) [29], IV (34) [29], VI (36) and VII [40], trojanoside A (45) [46] and H (46) [47], cycloastragenol (4) [29], brachyoside B (43) [45], and cyclocephaloside I (40) [44] were evaluated using a transcription-based bioassay for nuclear factor kappa B (NF-kappa B) activation in THP-1 human monocyte cells. Only astragaloside I was active at 100 µg/ml, which increased NF-kappa-B-directed luciferase expression up to 65% compared with maximal stimulation by Escherichia coli lipopolysaccharide (LPS) at 10 µg/ml. At low concentrations, all the compounds were inactive in the presence of 50 ng/ml LPS. In addition, astragaloside I increased mRNA expression of the inflammatory cytokines interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [66].

In 2005, another study was conducted to evaluate the immunostimulating activity of cycloartane- and oleanane-type saponins, namely, brachyoside A (42) [45], brachyoside B (43) [45], brachyoside C (44) [45], cyclocephaloside I (40) [44], cyclocephaloside II (41) [45], cycloastragenol (4) [29], astragaloside I (35) [42], astragaloside II (33) [47], astragaloside IV (34) [42], astragaloside VII [47], trojanoside A (45) [47], trojanoside H (53) [47], and astrojanoside A (51) [47], isolated from Turkish Astragalus species. Additionally, methanol extracts from the roots of three Astragalus species (Astragalus cephalotes Banks and Sol. var. brevicalyx Eig, Astragalus oleifolius DC. and Astragalus trojanus Stev.) were also examined. Cytokine concentrations of interleukins IL-1 and IL-8, and TNF- $\alpha$  after bacterial lipopolysaccharide (LPS) stimulation, and IL-2, IL-4, and INF-y after phorbolacetate (PHA) stimulation were determined via commercially available enzymelinked immunosorbent assay (ELISA) kits. All of the compounds tested in this study exhibited a significant IL-2-inducing activity between 35.9% for brachyoside A and 139.6% for astragaloside VII. Among the extracts tested, Astragalus oleifolius DC. showed the highest activity score, at 141.2%. In general, glycosides of 20,24-epoxy and 20,25-epoxy cycloartanes exhibited higher IL-2inducing activity compared to those of acyclic cycloartanes [67].

The evaluation of the gastroprotective effect of astragaloside IV (**34**), obtained from *Astragalus zahlbruckneri*, was studied [68]. Ulceration was induced by intragastric instillation of ethanol (1 ml/ rat). The rats were orally administered with astragaloside IV, which was found to reduce gastric hemorrhagic lesions in a dose-dependent manner when compared to the control group. The maximum percentage inhibition of ulcers (% gastroprotection) obtained with 30 mg/kg astragaloside IV following oral administration was 52%. Furthermore, the results demonstrate that endogenous NO (nitric oxide) plays an important role in the gastroprotective mechanism of astragaloside IV on ethanol-induced gastric lesions.

In a recent study, the antitumor properties of five Astragalus cycloartanes, namely, astragaloside IV (34) [29], cyclocanthoside



**Fig. 9** Structures of compounds **83–101**.

E (32) [38], astrasieversianin X (38) [41], and macrophyllosaponin B (26) [36] and D (28) [36], were evaluated in MCF-7 and MDA-MB-231 breast cancer cell lines. This was the first study to investigate the antitumor properties of different saponin extracts from *Astragalus* species in breast cancer. The results demonstrated that *Astragalus* saponins can inhibit the proliferation of breast cancer cells in a dose- and time-dependent manner. These findings indicated that saponins obtained from *Astragalus* species have important antiproliferative and antiapoptotic effects in the MCF-7 cell line [69].

Apart from our studies on the Turkish *Astragalus* species, there have been several reports by other research groups.

Six known cycloartane-type saponins, astrasieversianins I [41], II (37) [41], VI [41], VIII [41], X (38) [41], and astragaloside IV (34)

[29], were isolated from the roots of *Astragalus gilvus* Boiss. (Sect. Christiana). In addition to *A. gilvus*, only *Astragalus melanophrurius* was found to be rich in acylated cycloartane-type glycosides [49]. It is noteworthy that *A. gilvus* and *A. melanophrurius* were both members of the Christiana section. This suggests that the presence of acylated glycosides found in the Christiana section could be of taxonomic importance [70].

Phytochemical investigation of the roots of Astragalus flavescens (Sect. Eustales) resulted in the isolation of six new triterpene saponins  $[3-O-\alpha-L-rhamnopyranosyl-(1 \rightarrow 2)]-\beta-D-glucopyrano$  $syl-(1 \rightarrow 2)]-\beta-D-glucuronopyranosyl-21-epi-kudzusapogenol A;$  $<math>3-O-\alpha-L-rhamnopyranosyl-(1 \rightarrow 2)]-\beta-D-xylopyranosyl-(1 \rightarrow 2)]-\beta D-glucuronopyranosyl-22-O-\beta-D-glucopyranosyl-21-epi-kudzusa$  $pogenol A; <math>3-O-\alpha-L-rhamnopyranosyl-(1 \rightarrow 2)]-\beta-D-glucopyra-$  nosyl-(1 → 2)]-β-D-glucuronopyranosyl-22-*O*-β-D-glucopyranosyl-21-epi-kudzusapogenol A; 3-*O*-α-L-rhamnopyranosyl-(1 → 2)]-β-D-ylocuronopyranosyl-22-*O*-α-L-arabinopyranosyl-21-epi-kudzusapogenol A; 3-*O*-α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 2)]-β-D-glucuronopyranosyl-22-*O*-α-L-arabinopyranosyl-21-epi-kudzusapogenol A] [71] along with five previously isolated compounds (trojanoside B (**46**) [46], azukisaponin V [72], astragaloside IV (**34**) [29], astragaloside VII [49], and VIII [49]).

Another phytochemical study was performed on Astragalus amblolepis Fischer (Sect. Rhacophorus), which resulted in the isolation and structural elucidation of five new cycloartane-type triterpene glycosides, including  $3-O-\beta-D-xy$ lopyranosyl- $3\beta$ ,  $6\alpha$ , 16β,24(S),25-pentahydroxycycloartane (83), 3-O-[β-D-glucuronopyranosyl-  $(1 \rightarrow 2)$ - $\beta$ -D-xylopyranosyl]-25-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24(S),25-pentahydroxycycloartane (84), 3-O- $\beta$ -Dxylopyranosyl-24,25-di-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24(S),25pentahydroxycycloartane (85), 6-O- $\alpha$ -L-rhamnopyranosyl-16,24- $O-\beta$ -D-glucopyranosyl-3 $\beta$ ,  $6\alpha$ ,  $16\beta$ , 24(S), 25-pentahydroxycydicloartane (86), and  $6-O-\alpha$ -L-rhamnopyranosyl-16,25-di- $O-\beta$ -Dglucopyranosyl- $3\beta$ , $6\alpha$ ,  $16\beta$ , 24(S), 25-pentahydroxycycloartane (87), together with a known compound,  $3-O-\beta-D-xy|opyranosy|-16-O \beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24(S),25-pentahydroxycycloartane [73] (> Fig. 9). The researchers noted that cycloartane glycosides without a sugar residue at the C-3 position, such as compounds 86 and 87, are quite uncommon in nature. In addition, the presence of a rhamnosyl unit at the C-6 position in the cyclocanthogenol skeleton was reported for the first time, which is one of the most common aglycons in the genus Astragalus, along with cycloastragenol. The glucuronic acid moiety in cycloartanes was encountered for the first time in this study [74].

Polat et al. (2010) reported on the isolation and structural elucidation of three new cycloarte-type saponins,  $3-O-[\alpha-L-rham$ nopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucopyranosyl]-25-O- $\beta$ -D-glucopyranosyl-20(R),24(S)-epoxy-3β,6α,16β,24(S),25-tetrahydroxycycloartane, 3-O-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-xylopyranosyl]-6-O- $\beta$ -D-glucopyranosyl-24-O- $\alpha$ -(4'-O-acetoxy)-L-arabinopyranosyl-16-O-acetoxy- $3\beta$ , $6\alpha$ , $16\beta$ ,24(S),25-pentahydroxycycloartane, and 3-O-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-xylopyranosyl]-6-O- $\beta$ -D-glucopyranosyl-24-O- $\alpha$ -L-arabinopyranosyl-16-O-acetoxy-3 $\beta$ , 6 $\alpha$ , 16 $\beta$ , 24 (S),25-pentahydroxycycloartane, from Astragalus wiedemannianus Fischer (Sect. Pterophorus) [75], along with eight known compounds (cycloastragenol [30], cycloascauloside B [76], astragaloside IV (34) [29], astragaloside VIII [49], brachyoside B (43) [45], astragaloside II (33) [29], astrachrysoside A [50], and astrasieversianin X (38) [41]). The authors stated that an arabinose moiety on the acyclic side chain was reported for the first time.

In another study, six new cycloartane-type triterpene glycosides were isolated from the MeOH extract of the whole plant of *A. icmadophilus* (Sect. Acanthophace) together with eight known secondary metabolites, namely, oleifolioside B (**31**) [39], astragaloside I (**35**) [29], azukisaponin V [72], azukisaponin V methyl ester [77], astragaloside VIII [49], astragaloside VIII methy ester [33], 22-*O*-[ $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)-*O*- $\alpha$ -L-arabinopyranosyl]-3 $\beta$ ,22 $\beta$ ,24-trihydroxy-olean-12-ene [78], and narcissin [79]. The structures of the new compounds were established as 3-*O*-[ $\alpha$ -Larabinopyranosyl-(1  $\rightarrow$  2)-*O*-3-acetoxy- $\alpha$ -L-arabinopyranosyl]-6 $\begin{array}{l} O-\beta-D-glucopyranosyl-3\beta, 6\alpha, 16\beta, 24(S), 25-pentahydroxycycloartane; 3-O-[\alpha-L-rhamnopyranosyl-(1 \rightarrow 2)-O-\alpha-L-arabinopyranosyl-(1 \rightarrow 2)-O-\beta-D-xylopyranosyl]-6-O-\beta-D-glucopyranosyl-3\beta, 6\alpha, 16\beta, 24(S), 25-pentahydroxycycloartane; 3-O-[\alpha-L-arabinopyranosyl-(1 \rightarrow 2)-O-3, 4-diacetoxy-\alpha-L-arabinopyranosyl]-6-O-\beta-D-glucopyranosyl-3\beta, 6\alpha, 16\beta, 24(S), 25-pentahydroxycycloartane; 3-O-[\alpha-L-arabinopyranosyl-(1 \rightarrow 2)-O-3-acetoxy-\alpha-L-arabinopyranosyl]-6-O-\beta-D-glucopyranosyl-3\beta, 6\alpha, 16\beta, 25-tetrahydroxy-20(R), 24(S)-epoxycycloartane; 3-O-[\alpha-L-arabinopyranosyl]-6-O-\beta-D-glucopyranosyl-3\beta, 6\alpha, 16\beta, 25-tetrahydroxy-20(R), 24(S)-epoxycycloartane; 3-O-[\alpha-L-arabinopyranosyl]-6-O-\beta-D-glucopyranosyl-(1 \rightarrow 2)-O-\beta-D-xylopyranosyl]-6-O-\beta-D-glucopyranosyl-(1 \rightarrow 2)-O-\alpha-L-arabinopyranosyl-(1 \rightarrow 2)-O-\alpha-L-arabinopyranosyl-(1 \rightarrow 2)-O-\beta-D-xylopyranosyl]-6-O-\beta-D-glucopyranosyl-3\beta, 6\alpha, 16\beta, 24\alpha-tetrahydroxy-20(R), 25-epoxycycloartane, and 3-O-[\alpha-L-rhamnopyranosyl]-6-O-\beta-D-glucopyranosyl-3\beta, 6\alpha, 16\beta, 24\alpha-tetrahydroxy-20(R), 25-epoxycycloartane, and 3-O-[\alpha-L-rhamnopyrano$ 

Phytochemical investigation of *A. ptilodes* Boiss. var. *cariensis* Boiss. (Sect. Pterophorus) resulted in the isolation of five previously isolated compounds [81], i.e., astragaloside VII (**36**) [49], cyclosiversioside E [82], cyclosiversioside F [82], astragaloside I (**35**) [29], and cyclosiversioside A [83].

Studies on A. aureus Willd (Sect. Adiaspastus) resulted in the isolation of eight new cycloartane-type triterpene glycosides. The structures of the new compounds were established as 3-O- $[\alpha$ -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - $\alpha$ -L-arabinopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -Dxylopyranosyl]-6-O- $\beta$ -D-xylopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24(S),25-pentahydroxycycloartane (88), 3,6-di-O- $\beta$ -D-xylopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ , 24(S),25-pentahydroxycycloartane (89), 3,6-di-O-β-D-xylopyranosyl-25-O-β-D-glucopyranosyl-3β,6α,16β,24(S),25-pentahydroxycycloartane (90), 3-O-β-D-xylopyranosyl-6,25-di-O-β-D-qlucopyranosyl- $3\beta$ , $6\alpha$ , $16\beta$ ,24(S),25-pentahydroxycycloartane (**91**), 6- $O-\beta$ -D-glucopyranosyl-3 $\beta$ ,  $6\alpha$ ,  $16\beta$ , 24(S), 25-pentahydroxycycloartane (92), 3-O-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-xylopyranosyl]- $3\beta$ ,  $6\alpha$ ,  $16\beta$ ,  $24\alpha$ -tetrahydroxy-20(R), 25-epoxycycloartane (93), 6-O-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(R),25epoxycycloartane (94), and 6-O- $\beta$ -D-xylopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,  $24\alpha$ -tetrahydroxy-20(R),25-epoxycycloartane (95) (> Fig. 9), in addition to 10 known compounds,  $3-O-[\alpha-L-rhamnopyranosyl (1 \rightarrow 2)$ -O- $\alpha$ -L-arabinopyranosyl- $(1 \rightarrow 2)$ -O- $\beta$ -D-xylopyranosyl]-6- $O-\beta$ -D-glucopyranosyl-3 $\beta$ ,  $6\alpha$ ,  $16\beta$ , 24(S), 25-pentahydroxycycloartane [78], oleifolioside B (31) [39], cyclocanthoside E (32) [38], cyclocanthoside G (39) [38], 3-O-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $O-\alpha$ -L-arabinopyranosyl- $(1 \rightarrow 2)-O-\beta$ -D-xylopyranosyl]-6- $O-\beta$ -Dglucopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(R),25-epoxycycloartane [80], 3-O-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  2)-O- $\beta$ -D-xylopyranosyl]-6-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24 $\alpha$ -tetrahydroxy-20 (R),25-epoxycycloartane [80], cyclocanthoside F [84], cyclocephaloside I (40) [44], cyclotrisectoside [85], and macrophyllosaponin B (26) [36]. According to the authors, aminoglycosides of cyclocanthogenin (84) and cyclocephalogenin (94,95) were reported for the first time. In addition, the isolated compounds were tested for their cytotoxic activity against different cancer cell lines. Compound 95 was the only one that showed moderate activity against the human breast cancer cell line (MCF7) at a concentration of 45 µM [86].

In another study on the Turkish Astragalus species, four new cycloartanes (hareftoside A–D) and a new oleanane-type triterpenoid (hareftoside E) were isolated and characterized from the MeOH extract of the whole plant of Astragalus hareftae (Sect. Acanthophace), along with 11 known cycloartane-type glycosides [87], namely, cyclocanthoside E (**32**) [38], macrophyllosaponin B (**26**) [36], cyclocephaloside I (**40**) [44], oleifolioside B (**31**) [39], astrasieversianin X (**38**) [41], trojanoside B (**46**) [46], cycloastragenol (**4**) [29], astragaloside IV (**34**) [29], brachyoside B (**43**) [45], cyclodissectoside [85], and 3-*O*- $\beta$ -D-xylopyranosyl-6,25-di-*O*- $\beta$ -D-glucopyranosyl-3 $\beta$ , $6\alpha$ , 16 $\beta$ ,24(*S*),25-pentahydroxycycloartane (**91**) [86].

Phytochemical investigation of *A. schottianus* Boiss. (Sect. Rhacophorus) resulted in the isolation of three new cycloartane type glycosides. Their structures were determined as 20(R),25-epoxy-3-O- $\beta$ -D-xylopyranosyl-24-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ , 24 $\alpha$ -tetrahydroxycycloartane, 20(R),25-epoxy-3-O-[ $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)]- $\beta$ -D-xylopyranosyl-24-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24 $\alpha$ -tetrahydroxycycloartane, and 3-O- $\beta$ -D-xylopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,20(S),24(S),25-hexahydroxycycloartane (96) ( $\triangleright$  Fig. 9). The authors stated that compound 96 was the second cycloartane-type compound in the genus *Astragalus* that possesses a 20-OH functional group [88].

A new cycloartane-type saponin, namely, 3-O-[ $\beta$ -D-xylopy-ranosyl-(1  $\rightarrow$  2)- $\beta$ -D-xylopyranosyl]-6-O- $\beta$ -D-glucuronopyranosyl- $3\beta$ , $6\alpha$ ,  $16\beta$ , 24(S), 25-pentahydroxycycloartane, was obtained from *A. erinaceus* (Sect. Rhacophorus) together with five known compounds. According to the authors, this new compound represents the second example of a cycloartane-type compound that possesses a glucuronic acid moiety [89]. Known compounds were identified as cyclodissectoside [85], cycloastragenol (4) [29], oleifolioside B (**31**) [39], 3,6-di-O- $\beta$ -D-xylopyranosyl- $3\beta$ , $6\alpha$ ,  $16\beta$ , 24(S), 25-pentahydroxycycloartane (**89**) [86], and 6-O- $\beta$ -D-glucopyranosyl- $3\beta$ , $6\alpha$ ,  $16\beta$ , 24(S), 25-pentahydroxycycloartane (**92**) [86].

Gülcemal et al. (2012) reported on the isolation and characterization of six new cycloartane-type triterpenoids,  $3-O-[\alpha-L-rham$ nopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucopyranosyl]-16-O-hydroxyacetoxy- $3\beta$ ,  $6\alpha$ ,  $16\beta$ , 25-tetrahydroxy-20(R), 24(S)-epoxycycloartane, 3-O- $[\alpha$ -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucopyranosyl]-16-O-hydroxyacetoxy- $3\beta$ , $6\alpha$ , $16\beta$ ,  $23\alpha$ ,25-pentahydroxy-20(R),24(S)-epoxycycloartane, 3-O-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranosyl]-3 $\beta$ ,6 $\alpha$ ,25-trihydroxy-20(R),24(S)-epoxycycloartane-16-one, 3-O-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranosyl]-3 $\beta$ , $6\alpha$ , 16 $\beta$ , 25tetrahydroxy-20(R),24(R)-epoxycycloartane (97), 3-O-β-D-xylopyranosyl-6-*O*-α-L-rhamnopyranosyl-3β,6α,16β,25-tetrahydroxy-20(R), 24(R)-epoxycycloartane (98), and 6-O- $\alpha$ -L-rhamnopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,25-tetrahydroxy-20(R),24(R)-epoxycycloartane (99), from A. angustifolius (Sect. Melanocercis) (> Fig. 9), along with four oleanane-type triterpenoids, namely,  $3-O-[\alpha-L-rhamno$ pyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-xylopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-3 $\beta$ ,21 $\beta$ ,22 $\alpha$ ,24,29-pentahydroxyolean-12-ene, 3-O-[ $\alpha$ -Lrhamnopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-xylopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-qlucuronopyranosyl]-3*β*,22*β*,24-trihydroxyolean-12-en-29-oic acid, 3- $O-[\alpha-L-rhamnopyranosyl-(1 \rightarrow 2)-\beta-D-xylopyranosyl-(1 \rightarrow 2)-\beta-D$ glucuronopyranosyl]-22-O-α-L-arabinopyranosyl-3β,22β,24-trihydroxyolean-12-ene, and 29-O-β-D-glucopyranosyl-3β,22β,24,29tetrahydroxyolean-12-ene, and five known triterpene glycosides (astrojanoside A (51) [47], astragaloside VIII [49], 25-O-glucopyranosylcycloastragenol [49], 3-O-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranosyl]-25-O-β-D-glucopyranosyl-20(R),24(S)-epoxy3β,6α,16β,24(*S*),25-tetrahydroxycycloartane [75], and cycloaraloside D [90]). According to the authors, compounds **89–91** possessed the C-24 epimer of cycloastragenol as their aglycone, which was reported for the first time. The compounds were evaluated for their ability to inhibit cell growth in cell lines including Hela, H-446, HT-29, and U937. Of these compounds, only one compound (3-O-[α-L-rhamnopyranosyl-(1 → 2)-β-D-xylopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-3β,22β,24-trihydroxyolean-12-en-29-oic acid) showed a weak inhibitory effect with IC<sub>50</sub> values of 36 and 50 μM in the Hela and HT-29 cell lines, respectively [91].

A new cycloartane-type glycoside (20*R*,24*S*)-3-*O*-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-xylopyranosyl]-20,24-epoxy-16-*O*- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,25-tetrahydroxycycloartane, and a new glycoside (**100**) ( $\triangleright$  **Fig. 9**), 3-*O*-[ $\beta$ -D-apiofuranosyl-(1  $\rightarrow$  2)- $\beta$ -Dglucopyranosyl]maltol, were isolated from the whole plant of *A. halicacabus* (Sect. Halicacabus), together with seven known cycloartane-type glycosides, namely, cyclocanthoside D [38], askendoside D [59], askendoside F [64], askendoside G [61], elongatoside (**76**) [63], cyclosieversioside G [92], and cyclostipuloside A [73]. Authors reported that a maltol glycoside (**100**) was encountered for the first time in the Leguminosae family [93].

In 2013, the results of an online screening by HPLC-ESIMS<sup>n</sup> led to the isolation of 22 oleanane-type triterpene glycosides from A. tauricolus (sect. Malacothrix), including 10 new compounds, 3-*O*-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-xylopyranosylnamely,  $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-29-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,22 $\beta$ , 24-trihydroxyolean-12-ene-29-oic acid, 3-O-[α-L-rhamnopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-29-O- $\beta$ -D-qlucopyranosyl-3 $\beta$ ,22 $\beta$ ,24,29-tetrahydroxyolean-12-ene, 3-O- $[\alpha$ -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-xylopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-21-O- $\alpha$ -L-rhamnopyranosyl-3 $\beta$ ,21 $\beta$ ,22 $\alpha$ ,24-tetrahydroxyolean-12-ene,  $3-O-[\alpha-L-rhamnopyranosyl-(1 \rightarrow 2)-\beta-D$ glucopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-21-O- $\alpha$ -L-rhamnopyranosyl-3 $\beta$ ,21 $\beta$ ,22 $\alpha$ ,24-tetraydroxyolean-12-ene, 3-O-[ $\alpha$ -Lrhamnopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-29-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,22 $\beta$ ,24-trihydroxyolean-12-ene-29-oic acid, 3-O-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -Dxylopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-22-O- $\alpha$ -L-rhamnopyranosyl-3 $\beta$ ,22 $\beta$ ,24-trihydroxyolean-12-ene, 3-O-[ $\alpha$ -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-3β,24-dihydroxyolean-12-ene-22-oxo-29-oic acid, 3-O-[α-Lrhamnopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-3 $\beta$ ,21 $\beta$ ,22 $\alpha$ ,24,29-pentahydroxyolean-12-ene, 3- $O-[\beta-D-qlucopyranosyl-(1 \rightarrow 2)-\beta-D-qlucuronopyranosyl]-29-O-\beta-$ D-glucopyranosyl-3 $\beta$ ,22 $\beta$ ,24-trihydroxyolean-12-ene-29-oic acid, 3-O-[ $\beta$ -D-xylopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucuronopyranosyl]and 29-O-β-D-qlucopyranosyl-3β,22β,24-trihydroxyolean-12-ene-29oic acid. Known compounds were identified as astrojanoside A (51) [47], astragaloside VIII [49], azukisaponin II [72], azukisaponin V [72], 3-O-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-xylopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]- $3\beta$ ,  $21\beta$ ,  $22\alpha$ , 24, 29-pentahydroxyolean-12-ene [91], melilotus-saponin O2 [94], wistariasaponin B1 [95], wistariasaponin B2 [95], wistariasaponin D [96], cloversaponin IV [97], dehydroazukisaponin V [98], and  $3-O-\beta$ -D-glucuronopyranosyl-soyasapogenin B [99]. It is noteworthy that cycloartane-type triterpene glycosides, which are the main constituents of Astragalus spp., were not found. This unique feature is present only in a small group of Astragalus species, including A. hamosus [100], A. sinicus [32], A. complanatus [33], and A. corniculatus [34]. Moreover, the antiproliferative activity of the isolated compounds was evaluated against four human cell lines: MCF-7 (breast cancer), A549 (lung adenocarcinoma), PC-3 (prostate cancer), and U937 (leukemia). Only one compound (3-O-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucuronopyranosyl]-3 $\beta$ ,21 $\beta$ ,22 $\alpha$ ,24,29-pentahydroxyolean-12-ene) exhibited moderate activity, with an IC<sub>50</sub> of 22  $\mu$ M against the U937 cell line at concentrations ranging from 1 to 50  $\mu$ M [101].

Sixteen cycloartane glycosides were obtained from the methanol extract of A. plumosus var. krugianus Chamb. & Matthews (Sect. Rhacophorus). Among them, krugianoside A,  $3-O-[\alpha-L$ rhamnopyranosyl- $(1 \rightarrow 2)$ - $\alpha$ -L-arabinopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-glucuronopyranosyl]-25-O-β-D-xylopyranosyl-3β,6α,16β,24(S),25-pentahydroxycycloartane, was a new compound. Known compounds were characterized as oleifolioside B (31) [39], cyclocephaloside I (40) [44], cyclocanthoside E (32) [38], cycloastragenol (4) [29], brachyoside B (43) [45], elongatoside (76) [63], astragaloside IV (34) [29], cycloaraloside D [90], cycloaraloside A [102], cyclogaleginoside B [103], 3-O-[ $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  2)-O- $\beta$ -D-xylopyranosyl]-6-O-β-D-qlucopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(R),25epoxycycloartane [80],  $3-O-[\alpha-L-rhamnopyranosyl-(1 \rightarrow 2)-O-\alpha-L$ arabinopyranosyl- $(1 \rightarrow 2)$ -*O*- $\beta$ -D-xylopyranosyl]-6-*O*- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24(S),25-pentahydroxycycloartane [80], 3-O-[ $\alpha$ -Larabinopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-xylopyranosyl]-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24 $\alpha$ -tetrahydroxy-20(R),25-epoxycycloartane (93) [86], and 6-O- $\beta$ -D-glucopyranosyl-3 $\beta$ ,6 $\alpha$ ,16 $\beta$ ,24 $\alpha$ -tetrahydroxy-20(R),25-epoxycycloartane (94) [86]. In this study, the cytotoxic activity of the isolated compounds was evaluated in human skin fibroblast cells (WS1). Compounds that did not significantly affect WS1 viability were tested for their antioxidant potential. Krugianoside A and oleifolioides B prevented the elevation of reactive oxygen species (ROS) induced by t-BOOH, indicating their potential to protect fibroblasts from oxidative stress [104].

Phytochemical investigation of A. pennatulus (Sect. Rhacophorus) resulted in isolation of four new cycloartane-type glycosides,  $3-O-\beta-D-xylopyranosyl-6-O-\beta-D-glucopyranosyl-3\beta, 6\alpha, 16\beta-trihy$ droxy-24-oxo-20(R),25-epoxycycloartane (101) (> Fig. 9), 3-0- $[\beta$ -D-qlucuronopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-xylopyranosyl]- $3\beta$ ,  $16\beta$ ,  $24\alpha$ trihydroxy-20(*R*),25-epoxycycloartane, 3-O-[β-D-glucuronopyranosyl- $(1 \rightarrow 2)$ - $\beta$ -D-xylopyranosyl]-3 $\beta$ ,16 $\beta$ ,25-trihydroxy-20(R),24 (S)-epoxycycloartane, 3,25-di-O-β-D-glucuronopyranosyl-6-O-β-D-xylopyranosyl-3 $\beta$ ,  $6\alpha$ ,  $16\beta$ , 25-tetrahydroxy-20(R), 24(S)-epoxycycloartane, and a new oleanane-type glycoside, 29-O- $\alpha$ -Lrhamnopyranosyl-abrisapogenol B, along with five previously isolated cycloartane-type compounds (6-*O*-β-D-glucopyranosyl- $3\beta$ , $6\alpha$ , $16\beta$ , $24\alpha$ -tetrahydroxy-20(*R*),25-epoxycycloartane (94) [86], cyclodissectoside [85], hareftoside C [87], cyclocephaloside I (40) [44], and astragaloside IV (34) [29]). According to the authors, the aglycone of compound 101 was encountered for the first time. In addition, the cytotoxic activity of the compounds was tested on three cell lines including A549 (human lung adenocarcinoma), A375 (human melanoma), and DeFew (human B lymphoma) cells. The results showed that none of the tested compounds exhibited significant cytotoxicity [105].

# Biotransformation of *Astragalus Sapogenins* by Endophytic Fungi

Biotransformation, a biochemical reaction catalyzed by whole cell systems or isolated enzymes, is a powerful tool for generating libraries of structurally diverse compounds. These transformations, mediated by biocatalysts, offer several advantages over conventional chemical synthesis, including being environmentally friendly, having highly selective catalytic abilities, and allowing transformation at non-active sites of the compounds [106–108]. In drug discovery and development studies, biotransformation has many applications, including the synthesis of drug metabolites for prediction of mammalian metabolism, lead optimization, and the generation of chemically diverse screening libraries for structure-activity relationship (SAR) and bioactivity studies [109].

In particular, whole cell systems can convert a broad range of substrates through multistep reactions with cofactor regeneration, which makes them a cost-effective alternative to isolated enzyme systems [109–111].

Endophytic organisms, which inhabit healthy plant tissues for at least a part of their life cycle, possess specific enzymes that enable them to colonize their hosts [112]. Among the microorganisms, endophytic fungi have gained considerable attention in biotransformation studies due to their ability to modify complex molecules with high selectivity, including chemo-, regio-, and stereoselectivity [113–115].

In addition to the previous works on *Astragalus* cycloartanes, biotransformation studies were conducted on *Astragalus*-derived sapogenins (cyclocanthogenol, cycloastragenol, astragenol, and 20(27)-octanor cycloastragenol) by endophytic fungi isolated from *Astragalus* species.

Initially, fresh samples of *A. angustiflorus* C. Koch. and *A. condensatus* Ledeb. were collected from Spil Mountain, Manisa, Turkey. Subsequently, fungal endophytes were isolated from the roots, leaves, and stems of the plant samples. As a result of the isolation studies, a total of 15 fungal endophytes were obtained and characterized by molecular identification based on ITS analysis [116].

Following analytical scale biotransformation experiments, four endophytic isolates (*Alternaria eureka*, *Camarosporium laburnicola*, *Neosartorya hiratsukae*, and *Penicillium roseopurpureum*) were selected for further studies. The biotransformations of the abovementioned sapogenins by these fungal endophytes resulted in the isolation of 45 metabolites (▶ Figs. 10–13) [116–119]. These transformations involved various reactions including hydroxylation, oxidation, epoxidation, *O*-methylation, ring expansion, methyl migration, ring cleavage-methyl migration, dehydrogenation, and Baeyer–Villiger-type oxidation reactions.

Given that cycloastragenol is the first commercialized natural telomerase activator on the market [120], the potential of the biotransformation products to increase telomerase activity in neonatal cells was also evaluated using PCR-based ELISA testing. As a result, 16 compounds displayed activity ranging from 1.2- to 11.3-fold (at 0.5 and 300 nM doses) in comparison to the control group treated with DMSO [118–120]. Notably, the most potent



▶ Fig. 10 Biotransformation products of cycloastragenol by Neosartorya hiratsukae, Alternaria eureka, and Camarosporium laburnicola.



**Fig. 11** Biotransformation products of astragenol by Alternaria eureka and Camarosporium laburnicola.



**Fig. 12** Biotransformation products of cyclocanthogenol by *Alternaria eureka*.



▶ Fig. 13 Biotransformation products of 20(27)-octananor cycloastragenol by Neosartorya hiratsukae, Alternaria eureka, Camarosporium laburnicola, and Penicillium roseopurpureum.



Fig. 14 Biotransformation products of cyclocephagenol by Alternaria eureka.

molecules were A-ring-modified cycloastragenol derivatives catalyzed by the fungus *Camarosporium laburnicola*.

In 2022, Küçüksolak et al. [121] reported 21 biotransformation products of cyclocephagenol, a novel cycloartane-type sapogenin from Astragalus microcephalus, using the endophytic isolate Alternaria eureka (> Fig. 14). In addition, neuroprotective activities of the metabolites and the starting compound were investigated against H<sub>2</sub>O<sub>2</sub>-induced cell injury. As a result, 11 metabolites exhibited promising neuroprotective activity, and 6 were chosen for further analyses. The authors suggested that monooxygenation in C-11 and 12 played a significant role in the observed bioactivity, whereas oxidation at position 12 enhanced the neuroprotective activity. Conversely, a further increase in hydrophobicity and hydrophilicity was found to decrease the bioactivity. In addition, due to their conformational flexibility,  $3(10)\beta$ -epoxy-9,10seco-cycloartane products showed potential as neuroprotective agents.

On the basis of our previous studies, which revealed that the biotransformation products of cycloastragenol and astragenol obtained from the *C. laburnicola* study had potent telomerase activity, Küçüksolak et al. (2023) made a further attempt on cyclocephagenol and its 12-hydroxy derivatives to obtain new activators [122]. In this recent study, seven new metabolites were isolated as a result of the biotransformation reactions (**> Fig. 15**), including oxidation, Baeyer–Villiger oxidation, ring opening, and dehydration. These metabolites were evaluated for their effects on telomere activation using TeloTAGGG assay, revealing that the tested biotransformation products exhibited potent telomerase activation compared to the positive control cycloastragenol, with activity ranging from 1.02- to 1.46-fold.

# Conclusion

Turkish Astragalus species have been extensively studied since the early 1990 s. During the almost 28 years of research on these Astragalus species, about 200 compounds were isolated and identified, including 104 new triterpene saponins, a new tryptophan derivative, a new maltol glucoside, and 5 new phenolic glycosides.

Over the last two decades, more than 50 new *Astragalus* species have been discovered and added to the flora of Turkey [2, 123]. *Astragalus ihsancalisii* is one of the recent records [124]. Furthermore, the rich diversity of the genus *Astragalus* in Turkey, with over 470 species, highlights its potential as a source for the discovery of new bioactive compounds.

The phytochemical studies mentioned above demonstrate that the genus *Astragalus* is abundant in cycloartane-type triterpenoids. Cycloartanes and their derivatives have been reported to exhibit a variety of biological activities, such as immunostimulating [42,66,67], anti-protozoal [39], antiviral [125], and cytotoxic [126] activities. In Turkish folk medicine, the aqueous root extracts of some *Astragalus* species have been used to treat leukemia and for wound healing [42,66]. Cycloartenol-type glycosides



Fig. 15 Biotransformation products of cyclocephagenol and 12α-hydroxycyclocephagenol (171) by Camarosporium laburnicola.

isolated from Turkish *Astragalus* species exhibited weak or no cytotoxic activity, as reported by preliminary cytotoxicity panels. Consequently, additional studies were conducted to investigate the immunomodulatory properties of these compounds, which yielded promising results for their potential as vaccine adjuvants and immunotherapeutic agents *in vitro* and *in vivo* [127, 128]. Furthermore, the discovery of cycloastragenol, the main sapogenol of numerous cycloartane-type glycosides in the genus *Astragalus*, as a telomerase activator in a systematic screening of natural product extracts from traditional Chinese medicine in 2000 [120], was a significant development for cycloartanes. The preparation of cycloartane derivatives with such activity could have future significance due to their activity on telomerase activation.

Biotransformation is an effective tool for creating structural diversity in a natural product library to produce new potent molecules. Microbial-catalyzed biotransformation studies were conducted on Astragalus cycloartanes using endophytic fungi isolated from Astragalus species. The results of the biotransformation studies revealed that fungal endophytes have a promising potential to transform plant-derived natural products. Hydroxylation, oxidation, epoxidation, O-methylation, ring expansion, methyl migration, ring cleavage-methyl migration, dehydrogenation, and Baeyer-Villiger type oxidation reactions were observed on the starting compounds, which would be difficult to achieve through conventional synthetic methods. These studies prove that microbial transformation via plant-derived fungal endophytes is a highly efficient method for modifying the structure of natural products to expand chemical libraries and predicting potential mammalian metabolites of traditionally used crude drugs.

Taken together, the genus *Astragalus* remains a highly intriguing group for further phytochemical, biological, and pharmacological studies. To fully explore the potential of this remarkable genus, a multidisciplinary research approach is needed, which includes advanced phytochemical analysis, mechanistic studies on bioactive metabolites, the synthesis and modification of cycloartane-type compounds to improve their pharmacological properties and clinical potential, and comprehensive clinical investigations.

#### **Contributors' Statement**

Conceptualization, I.Çalis, G. Ekiz Dinçman, and Z. Aytaç; writing– original draft preparation, G. Ekiz Dinçman and I. Çalis.; writing– review and editing, G. Ekiz Dinçman, Z. Aytaç, and I.Ç.

#### Acknowledgements

The authors thank A. A. Dönmez and H. Duman for donating plant materials. We also thank A. A. Dönmez and F. Taeb for providing photographs of some *Astragalus* species. The authors are grateful to E. Bedir and team and Ö. Alankuş and team for their contributions to the progress of *Astragalus* research in Turkey.

#### Conflict of Interest

The authors declare that they have no conflict of interest.

- Pistelli L. Secondary metabolites of genus Astragalus: Structure and biological activity. In: Atta-Ur-Rahman, ed. Studies in Natural Products Chemistry (Bioactive Natural Products, Part H). Amsterdam: Elsevier Science; 2002: 443–545
- [2] Chamberlain DF, Matthews VA. Astragalus L. In: Davis PH, ed. Flora of Turkey and the East Aegean Islands, Vol. 3. Edinburgh: Edinburgh University Press; 1970: 49–254
- [3] Aytaç Z. Astragalus L., Flora of Turkey and the East Aegean Islands. Vol 11. Edinburgh: Edinburgh University Press; 2000: 79–88
- [4] Aytaç Z, Ekici M, Akan H. Astragalus L. In: Güner A, Aslan S, Ekim T, Vural M, Babaç MT, eds. Türkiye bitkileri listesi (Damarlı bitkiler) (List of Turkish plants [vascular plants]). İstanbul: Nezahat Gökyiğit Botanik Bahçesi ve Flora, Araştırmaları Derneği Yayını; 2012: 427–456
- [5] Doğan M, Ekim T, Anderson MW. The production of gum tragacanth from Astragalus microcephalus in Turkey – A contribution towards a balanced environment. Biol Agric Hortic 1985; 2: 329–334
- [6] Baytop T. Türkiye'de Bitkiler ile Tedavi, Geçmişte ve Bugün (Therapy with medicinal plants in Turkey, Past and Present). 2nd edition. İstanbul: Nobel Tıp Kitabevi; 1999
- [7] Baytop T. Türkçe Bitki Adları Sözlüğü. Atatürk Kültür, Dil ve Tarih Yüksek Kurumu. Türk Dil Kurumu Yayınları: 578. Ankara: Türk tarih Kurumu Basım Evi; 1994
- [8] Anderson DM, Bridgeman MME. The composition of the proteinaceous polysaccharides exuded by Astragalus microcephalus, A. gummifer and A. kurdicus-The sources of turkish gum tragacanth. Phytochemistry 1985; 24: 2301–2304
- [9] Tang W, Eisenbrand G. Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine. Heidelberg: Springer; 1992
- [10] Maassoumi AA. Astragalus in the old world: Check list. Research Institute of Forests and Rangelands. Iran; 1998. ISBN: 9644730348, 9789644730344
- [11] Podlech D, Zarre SH. A taxonomic revision of the genus Astragalus L. (Leguminosae) in the old world. Vol. 1–3. Nat Hist Mus Vienna 2013; 1–3. Vol. 1: 6–822, Vol. 2: 823–1640, Vol. 3: 1642–2372, 2439
- [12] Ghahremaninejad F, Joharchi MR. 840th species of genus Astragalus (Fabaceae) for the flora of Iran from Khorassan Province as a new record: A. globiceps Bunge. Iran J Bot 2020; 32: 910–914
- [13] Duman H, Aytaç Z, Özbek F. Astragalus aybarsii a new species of sect. Onobrychoidei DC. (Fabaceae) from Turkey. Turk | Bot 2020; 44: 661–669
- [14] Ríos JL, Waterman PG. A Review of the pharmacology and toxicology of Astragalus. Phytotherapy Res 1997; 11: 411–418
- [15] Benn M, Bai Y, Majak W. Aliphatic nitro-compounds in Astragalus canadensis. Phytochemistry 1995; 40: 1629–1631
- [16] Benn M, Majak W. 3-Nitro-1-propil-b-D-laminaribioside from Astragalus miser var. serotinus. Phytochemistry 1989; 28: 2369–2371
- [17] Fu J, Wang Z, Huang L, Zheng S, Wang D, Chen S, Zhang H, Yang S. Review of the botanical characteristics, phytochemistry, and pharmacology of *Astragalus membranaceus* (Huangqi). Phytother Res 2014; 28: 1275–1283
- [18] Li X, Qu L, Dong Y, Han L, Liu E, Fang S, Zhang Y, Wang T. A review of recent research progress on the *Astragalus* genus. Molecules 2014; 19: 18850–18880
- [19] Jin M, Zhao K, Huang Q, Shang P. Structural features and biological activities of the polysaccharides from Astragalus membranaceus. Int J Biol Macromol 2014; 64: 257–266
- [20] Wang XY, Wang RC, Qu ZY, Zhu YZ, Li YL. Advances on immunoregulation effect of astragalus polysaccharides. Front Nat Prod 2022; 7: 971679

- [21] Wang J, Jia J, Song L, Gong X, Xu J, Yang M, Li M. Extraction, structure, and pharmacological activities of *Astragalus* polysaccharides. Appl Sci 2018; 9: 122
- [22] Fang SD, Chen Y, Xu XY, Ye CQ, Zhai SK, Shen ML. Studies of the active principles of Astragalus mongholicus Bunge. I. Isolation, characterization and biological effect of its polysaccharides. Chin J Org Chem 1982; 1: 26–31
- [23] Shimizu N, Tomoda M, Kanari M, Gonda R. An acidic polysaccharide having activity on the reticuloendothelial system from the root of Astragalus mongholicus. Chem Pharm Bull 1991; 39: 2969–2972
- [24] Tomoda M, Shimizu N, Ishii S. A reticuloendothelial system-activating glycan from the roots of Astragalus membranaceus. Phytochemistry 1992; 31: 63–66
- [25] Dewick PM. Medicinal Natural Products: A Biosynthetic Approach, 3rd edition. Hoboken: Wiley & Sons; 2009
- [26] Svechnikova AN, Umarova RU, Gorovits MB, Seitanudi KL, Rashkes YV, Yagudaev MR, Abubakirov NK. Triterpene glycosides of *Astragalus* and their genins. II. The structure of cyclosieversigenin. Chem Nat Compd 1981; 17: 60–67
- [27] Isaev MI, Gorovits MB, Abdullaev ND, Abubakirov NK. Triterpene glycosides of Astragalus and their genins. VI. Cycloasgenin C from Astragalus taschkendicus. Chem Nat Compd 1982; 18: 424–430
- [28] Evstratova RI, Sheichenko VI, Pakaln DA. Triterpenes of Astragalus dasyanthus. I. Structure of dasyanthogenin. Khim Prir Soedin 1981; 1: 102–103
- [29] Kitagawa I, Wang HK, Saito M, Takagi A, Yoshikawa M. Saponin and Sapogenol. XXXV. Chemical constituents of astragali radix, the root of Astragalus membranaceus bunge.(2) Astragalosides I, II and IV, Acetylastragaloside I and Isoastragalosides I and II. Chem Pharm Bull 1983; 31: 698–708
- [30] Isaev MI, Gorovits MB, Abubakirov NK. Progress in the chemistry of the cycloartanes. Khim Prir Soedin 1989; 2: 156
- [31] Verotta L, El-Sebakhy NA. Cycloartane and oleanane saponins from Astragalus sp. In: Atta-ur-Rahman ed. Studies in Natural Products Chemistry (Bioactive Natural Products, Part F). Karachi: Elsevier Science; 2001: 179–234
- [32] Cui B, Inoue J, Takeshita T, Kinjo J, Nohara T. Triterpene glycosides from the seeds of *Astragalus sinicus*. Chem Pharm Bull 1992; 40: 3330
- [33] Cui B, Sakai Y, Takeshita T, Kinjo J, Nohara T. Four new oleanane derivatives from the seeds of *Astragalus complanatus*. Chem Pharm Bull 1992; 40: 136
- [34] Krasteva I, Nikolov S, Kaloga M, Mayer G. A new saponin lactone from *Astragalus corniculatus*. Nat Prod Res 2007; 21: 941–945
- [35] Mamedova RP, Agzamova MA, Isaev MI. Triterpene glycosides of Astragalus and their genins. LXX. Orbicoside, the first lanostane glycoside from Astragalus plants. Chem Nat Compd 2003; 39: 583–585
- [36] Çalış İ, Zor M, Saracoğlu İ, Işımer A, Rüegger H. Four novel cycloartane glycosides from Astragalus oleifolius. J Nat Prod 1996; 59: 1019–1023
- [37] Bedir E, Çalış İ, Khan IA. Macrophyllosaponin E: A novel compound from the roots of Astragalus oleifolius. Chem Pharm Bull 2000; 48: 1081–1083
- [38] Isaev MI, Imomnazarov BA, Fadeev YM, Kintya PA. Triterpene glycosides of Astragalus and their genins. XLII. Cycloartanes of Astragalus tragacantha. Khim Prir Soedin 1992; 3: 360–367
- [39] Özipek M, Dönmez AA, Caliş İ, Brun R, Rüedi P, Taşdemir D. Leishmanicidal cycloartane-type triterpene glycosides from Astragalus oleifolius. Phytochemistry 2005; 66: 1168–1173
- [40] Kitagawa I, Wang HK, Saito M, Yoshikawa M. Saponin and Sapogenol. XXXVI. Chemical constituents of Astragali Radix, the root of Astragalus membranaceus Bunge.(3) Astragalosides III, V and VI. Chem Pharm Bull 1983; 31: 709–715
- [41] Li-Xian C, Xlao-Bing H, Yu-Qun C. The structures of thirteen astrasieversianins from Astragalus sieversianus. Phytochemistry 1986; 25: 2389– 2393

- [42] Çalış İ, Yürüker A, Taşdemir D, Wright AD, Sticher O, Luo YD, Pezzuto JM. Cycloartane glycosides from the roots of Astragalus melanophrurius. Planta Med 1997; 63: 183–186
- [43] Verotta L, Guerrini M, El-Sebakhy NA, Assad AM, Toaima SM, Radwan MM, Luo YD, Pezzuto JM. Cycloartane and oleanane saponins from Egyptian Astragalus spp. as modulators of lymphocyte proliferation. Planta Med 2002; 68: 986–994
- [44] Bedir E, Çalış İ, Zerbe O, Sticher O. Cyclocephaloside I: A novel cycloartane-type glycoside from Astragalus microcephalus. J Nat Prod 1998; 61: 503–505
- [45] Bedir E, Çalış İ, Aquino R, Piacente S, Pizza C. Cycloartane triterpene glycosides from the roots of Astragalus brachypterus and Astragalus microcephalus. J Nat Prod 1998; 61: 1469–1472
- [46] Bedir E, Çalış İ, Aquino R, Piacente S, Pizza C. Secondary metabolites from the roots of Astragalus trojanus. J Nat Prod 1999; 62: 563–568
- [47] Bedir E, Çalış İ, Aquino A, Piacente S, Pizza C. Trojanoside H: A cycloartane-type glycoside from the aerial parts of *Astragalus trojanus*. Phytochemistry 1999; 51: 1017–1020
- [48] Bedir E, Tatlı İ, Çalış İ, Khan IA. Trojanosides I–K: New cycloartane-type glycosides from the aerial parts of *Astragalus trojanus*. Chem Pharm Bull 2001; 49: 1482–1486
- [49] Kitagawa I, Wang HK, Saito M, Takagi A, Yoshikawa M. Saponin and sapogenol. XXXVII. Chemical constituents of astragali radix, the root of Astragalus membranaceus Bunge. (4). Astragalosides VII and VIII. Chem Pharm Bull 1983; 31: 716–722
- [50] Wang HK, He K, Xu HX, Zhang ZL, Wang YF, Kikuchi T, Tezuka Y. The structure of Astrachrysosid A and the study of 2D-NMR on Astrasieversianin XV and 7, 20-dihydroxy-30, 40-dimethoxy-isoflavane-7-O-beta-D-glycoside. Acta Pharmacol Sin 1990; 25: 445–450
- [51] Chaudhuri SK, Huang L, Fullas F, Brown DM, Wani MC, Wall ME. Isolation and structure identification of an active DNA strand scission agent, (+)-3, 4-dihydroxy-8, 9-methylenedioxypterocarpan. J Nat Prod 1995; 58: 1966–1969
- [52] Fadeev YM, Isaev MI, Akimov YA, Kintya PK, Gorovits MB, Abubakirov NK. Triterpene glycosides of *Astragalus* and their genins. 25. Cyclocantoside-D from *Astragalus tragantha*. Khim Prir Soedin 1988; 0: 73–76
- [53] Çalış İ, Yusufoğlu H, Zerbe O, Sticher O. Cephalatoside A: A tridesmosidic cycloartane type glycoside from *A. cephalotes* var. *brevicalyx*. Phytochemistry 1999; 50: 843–847
- [54] Çalış İ, Gazar HA, Piacente S, Pizza C. Secondary metabolites from the roots of Astragalus zahlbruckneri. J Nat Prod 2001; 64: 1179–1182
- [55] Bedir E, Çalış İ, Dunbar C, Sharan R, Buolamwini JK, Khan IA. Two novel cycloartane-type triterpene glycosides from the roots of Astragalus prusianus. Tetrahedron 2001; 57: 5961–5966
- [56] Burasheva GS, Mukhamedyarova MM, Chumbalov TK. Flavonoids of Alhagi kirgisorum. Khim Prir Soedin 1975; 11: 426
- [57] Bedir E, Çalış İ, Piacente S, Pizza C, Khan IA. A new flavonol glycoside from the aerial parts of Astragalus vulneraria. Chem Pharm Bull 2000; 48: 1994–1995
- [58] Çalış İ, Koyunoğlu S, Yeşilada A, Brun R, Rüedi P, Taşdemir D. Antitrypanosomal cycloartane glycosides from Astragalus baibutensis. Chem Biodivers 2006; 3: 923–929
- [59] Isaev MI, Gorovits MB, Abdullaev ND, Abubakirov NK. Triterpene glycosides of Astragalus and their genins. IX. Askendoside D from Astragalus taschkendicus. Chem Nat Compd 1983; 19: 170–174
- [60] Isaev MI, Gorovits MB, Gorovits TT, Abdullaev ND, Abubakirov NK. Triterpene glycosides of Astragalus and their genins. VIII. Askendoside C from Astragalus taschkendicus. Chem Nat Compd 1983; 19: 163–169
- [61] Isaev MI. Triterpene glycosides of Astragalus and their genins. Liv. askendoside G from Astragalus taschkendicus. Chem Nat Compd 1996; 32: 706–709

- [62] Çalış İ, Dönmez AA, Perrone A, Pizza C, Piacente S. Cycloartane glycosides from Astragalus campylosema Boiss. ssp. campylosema. Phytochemistry 2008; 69: 2634–2638
- [63] Çalış İ, Barbič M, Jürgenliemk G. Bioactive cycloartane-type triterpene glycosides from Astragalus elongatus. Z Naturforsch C Biosci 2008; 63: 813–820
- [64] Isaev MI. Triterpene glycosides of Astragalus and their genins. LII. Askendoside F from Astragalus taschkendicus. Khim Prir Soedin 1995; 6: 820– 823
- [65] Yalçın FN, Piacente S, Perrone A, Capasso A, Duman H, Çalış I. Cycloartane glycosides from *Astragalus stereocalyx* Bornm. Phytochemistry 2012, 73, 119–126
- [66] Bedir E, Pugh N, Çalış İ, Pasco DS, Khan IA. Immunostimulatory effects of cycloartane-type triterpene glycosides from Astragalus species. Biol Pharm Bull 2000; 23: 834–837
- [67] Yeşilada E, Bedir E, Çalış İ, Takaishi Y, Ohmoto Y. Effects of triterpene saponins from Astragalus species on in vitro cytokine release. J Ethnopharmacol 2005; 96: 71–77
- [68] Navarrete A, Arrieta J, Terrones L, Abou-Gazar H, Çalış İ. Gastroprotective effect of Astragaloside IV: Role of prostaglandins, sulfhydryls and nitric oxide. J Pharm Pharmacol 2005; 57: 1059–1064
- [69] Öğütçü G, Tülay P, Kükner A, Çalış İ, Şenol H. The effect of cycloartanetype of saponins from Astragalus species on the proliferation of MCF-7 and MDA-MB-231 breast cancer cells. Turk | Oncol 2023; 38: 350–357
- [70] Tabanca N, Bedir E, Alankus-Caliskan O, Khan IA. Cycloartane triterpene glycosides from the roots of *Astragalus gilvus* Boiss. Biochem Syst Ecol 2005; 33: 1067–1070
- [71] Avunduk S, Mitaine-Offer AC, Alankuş-Çalişkan Ö, Miyamoto T, Senol SG, Lacaille-Dubois MA. Triterpene glycosides from the roots of Astragalus flavescens. J Nat Prod 2008; 71: 141–145
- [72] Kitagawa I, Wang H, Saito M, Yoshikawa M. Saponin and sapogenol. XXXIII. Chemical constituents of the seeds of *Vigna angularis* (Willd.) Ohwi et Ohashi.(3). Azukisaponins V and VI. Chem Pharm Bull 1983; 31: 683–688
- [73] Karimov RZ, Umarova RU, Saatov Z, Levkovich MG, Abdullaev ND. Triterpene glycosides of *Tragacantha* and their genins. Cyclostipulosides A and B from *Tragacantha stipulosa*. Chem Nat Compd 1998; 34: 609–612
- [74] Polat E, Caliskan-Alankus O, Perrone A, Piacente S, Bedir E. Cycloartanetype glycosides from Astragalus amblolepis. Phytochemistry 2009; 70: 628–634
- [75] Polat E, Bedir E, Perrone A, Piacente S, Alankus-Caliskan Ö. Triterpenoid saponins from Astragalus wiedemannianus Fischer. Phytochemistry 2010; 71: 658–662
- [76] Alaniya MD, Kavtaradze NS, Faure R, Debrauwer L. Cycloascauloside B from Astragalus caucasicus. Chem Nat Compd 2008; 44: 324–326
- [77] Mohamed KM, Ohtani K, Kasai R, Yamasaki K. Oleanene glycosides from seeds of *Trifolium alexandrinum*. Phytochemistry 1995; 40: 1237–1242
- [78] Yoshikawa M, Wang H, Kayakiri H, Taniyama T, Kitagawa I. Saponin and sapogenol. XL. Structure of sophoraflavoside I, a bisdesmoside of soyasapogenol B, from Sophorae Radix, the root of *Sophora flavescens* AITON. Chem Pharm Bull 1985; 33: 4267–4274
- [79] Senatore F, D'Agostin M, Dini I. Flavonoid glycosides of *Barbarea vulgaris* L. (Brassicaceae). J Agric Food Chem 2000; 48: 2659–2662
- [80] Horo I, Bedir E, Perrone A, Özgökçe F, Piacente S, Alankuş-Çalışkan Ö. Triterpene glycosides from Astragalus icmadophilus. Phytochemistry 2010; 71: 956–963
- [81] Linnek J, Mitaine-Offer AC, Miyamoto T, Tanaka C, Paululat T, Avunduk S, Alankuş-Çalişkan Ö, Lacaille-Dubois MA. Cycloartane glycosides from three species of Astragalus (Fabaceae). Helv Chim Acta 2011; 94: 230– 237

- [82] Svechnikova AN, Umarova RU, Gorovits MB, Abubakirov NK. Triterpene glycosides of Astragalus and their genins. 4. Cyclosiversiosid-E, a new diglycoside from Astragalus sieversianus. Khim Prir Soedin 1982; 2: 204–208
- [83] Svechnikova AN, Umarova RU, Abdullaev ND, Gorovits MB, Abubakirov NK. Triterpene glycosides of *Astragalus* and their genins. 7. Structure of cyclosiversioside-A and cyclosiversioside-C. Khim Prir Soedin 1982; 5: 629–632
- [84] Agzamova MA, Isaev MI. Triterpene glycosides of Astragalus and their genins LIX. Structure of cyclocanthoside F. Chem Nat Compd 1999; 35: 314–319
- [85] Sukhina IA, Mamedova RP, Agzamova MA, Isaev MI. Triterpene glucosides of Astragalus and their genins. LXXIV. Cyclotrisectoside, the first trisdesmoside of cyclocephalogenin. Chem Nat Compd 2007; 43: 159–161
- [86] Gülcemal D, Alankuş-Çalışkan Ö, Perrone A, Özgökçe F, Piacente S, Bedir E. Cycloartane glycosides from Astragalus aureus. Phytochemistry 2011; 72: 761–768
- [87] Horo I, Bedir E, Masullo M, Piacente S, Özgökçe F, Alankuş-Çalışkan Ö. Saponins from Astragalus hareftae (NAB.) SIRJ. Phytochemistry 2012; 84: 147–153
- [88] Karabey F, Khan IA, Bedir E. Cycloartane-type glycosides from Astragalus schottianus. Phytochem Lett 2012; 5: 320–324
- [89] Savran T, Gülcemal D, Masullo M, Karayıldırım T, Polat E, Piacente S, Alankus-Çaliskan Ö. Cycloartane glycosides from Astragalus erinaceus. Rec Nat Prod 2012; 6: 230–236
- [90] Isaev MI. Triterpene glycosides of Astragalus and their genins XXXIX.
  Cycloaraloside D from Astragalus amarus. Chem Nat Compd 1991; 27: 4, 457–459
- [91] Gülcemal D, Masullo M, Bedir E, Festa M, Karayıldırım T, Alankus-Caliskan O, Piacente S. Triterpene glycosides from *Astragalus angustifolius*. Planta Med 2012; 78: 720–729
- [92] Svechnikova AN, Umarova RU, Gorovits MB, Abdullaev ND, Abubakirov NK. Triterpene glycosides of *Astragalus* and their genins. 11. Cyclosiversioside-G-triglycoside from *Astragalus sieversianus*. Khim Prir Soedin 1983; 3: 312–315
- [93] Djimtombaye BJ, Alankuş-Çalışkan Ö, Gülcemal D, Khan IA, Anıl H, Bedir E. Unusual secondary metabolites from Astragalus halicacabus Lam. Chem Biodivers 2013; 10: 1328–1334
- [94] Hirakawa T, Okawa M, Kinjo J, Nohara T. A new oleanene glucuronide obtained from the aerial parts of *Melilotus officinalis*. Chem Pharm Bull 2000; 48: 286–287
- [95] Konoshima T, Kozuka M, Haruna M, Ito K, Kimura T, Tokuda H. Studies on the constituents of leguminous plants XII. The structures of new triterpenoid saponins from *Wistaria branchybotrys* Sieb. et Zucc. Chem Pharm Bull 1989; 37: 2731–2735
- [96] Konoshima T, Kozuka M, Haruna M, Ito K. Constituents of leguminous plants, XIII. New triterpenoid saponins from *Wistaria brachybotrys*. J Nat Prod 1991; 54: 830–836
- [97] Sakamato S, Kofuji S, Kuroyanagi M, Ueno A, Sekita S. Saponins from Trifolium repens. Phytochemistry 1992; 31: 1773–1777
- [98] Mohamed KM, Ohtani K, Kasai R, Yamasaki K. Oleanene glycosides from seeds of *Trifolium alexandrinum*. Phytochemistry 1995; 40: 1237–1242
- [99] Udayama M, Ohkawa M, Yoshida N, Kinjo J, Nohara T. Structures of three new oleanane glucuronides isolated from *Lathyrus palustris* var. *pilosus* and hepatoprotective activity. Chem Pharm Bull 1998; 46: 1412–1415
- [100] Ionkova I. Production triterpene saponins by conventional and transformed cultures of Astragalus hamosus (Fabaceae). Problem Pharm Pharmacol 1991; 5: 32–38
- [101] Gülcemal D, Masullo M, Napolitano A, Karayıldırım T, Bedir E, Alankuş-Çalışkan Ö, Piacente S. Oleanane glycosides from Astragalus tauricolus:

Isolation and structural elucidation based on a preliminary liquid chromatography-electrospray ionization tandem mass spectrometry profiling. Phytochemistry 2013; 86: 184–194

- [102] Isaev MI, Gorovits MB, Abubakirov NK. Triterpene glycosides of Astragalus and their genins XXX. Cycloaraloside A from Astragalus amarus. Chem Nat Compd 1989; 25: 684–687
- [103] Alaniya MD, Isaev MI, Gorovits MB, Abdullaev ND, Kemertelidze EP, Abubakirov NK. Astragalus triterpene glycosides and their genins. XVI. Cyclogaleginosides A and B from Astragalus galegiformis. Khim Prir Soedin 1984; 4: 477–481
- [104] Denizli N, Horo I, Gülcemal D, Masullo M, Festa M, Capasso A, Koz Ö, Piacente S, Alankuş-Çalışkan Ö. Cycloartane glycosides from Astragalus plumosus var. krugianus and evaluation of their antioxidant potential. Fitoterapia 2014; 92: 211–218
- [105] Un R, Horo I, Masullo M, Falco A, Senol SG, Piacente S, Alankuş-Çalıskan
  Ö. Cycloartane and oleanane-type glycosides from Astragalus pennatulus. Fitoterapia 2016; 109: 254–260
- [106] Borges KB, de Souza Borges W, Durán-Patrón R, Pupo MT, Bonato PS, Collado IG. Stereoselective biotransformations using fungi as biocatalysts. Tetrahedron Asymmetry 2009; 20: 385–397
- [107] Pollard DJ, Woodley JM. Biocatalysis for pharmaceutical intermediates: The future is now. Trends Biotechnol 2007; 25: 66–73
- [108] Ishige T, Honda K, Shimizu S. Whole organism biocatalysis. Curr Opin Chem Biol 2005; 9: 174–180
- [109] Lam KS. Application of Whole-Cell Biotransformation in the Pharmaceutical Industry. In: Tao J, Lin GQ, Liese A, eds. Biocatalysis for the Pharmaceutical Industry: Discovery, Development, and Manufacturing. Singapore: Wiley & Sons; 2009: 214–227
- [110] de Carvalho CC. Whole cell biocatalysts: Essential workers from nature to the industry. Microb Biotechnol 2017; 10(2): 250–263
- [111] de Carvalho CC. Enzymatic and whole cell catalysis: Finding new strategies for old processes. Biotechnol Adv 2011; 29: 75–83
- [112] Kusari S, Hertweck C, Spiteller M. Chemical ecology of endophytic fungi: Origins of secondary metabolites. Chem Biol 2012; 19: 792–798
- [113] Choudhary M, Gupta S, Dhar MK, Kaul S. Endophytic fungi-mediated biocatalysis and biotransformations paving the way toward green chemistry. Front Bioeng Biotechnol 2021; 9: 664705
- [114] Wang Y, Dai CC. Endophytes: A potential resource for biosynthesis, biotransformation, and biodegradation. Ann Microbiol 2011; 61: 207– 215
- [115] Borges WD, Borges KB, Bonato PS, Said S, Pupo MT. Endophytic fungi: Natural products, enzymes and biotransformation reactions. Curr Org Chem 2009; 13: 1137–1163
- [116] Ekiz G, Duman S, Bedir E. Biotransformation of cyclocanthogenol by the endophytic fungus *Alternaria eureka* 1E1BL1. Phytochemistry 2018; 151: 91–98
- [117] Ekiz G, Yılmaz S, Yusufoglu H, Kırmızıbayrak PB, Bedir E. Microbial transformation of cycloastragenol and astragenol by endophytic fungi isolated from Astragalus species. J Nat Prod 2019; 82(11): 2979–2985
- [118] Duman S, Ekiz G, Yılmaz S, Yusufoglu H, Kırmızıbayrak PB, Bedir E. Telomerase activators from 20(27)-octanor-cycloastragenol via biotransformation by the fungal endophytes. Bioorg Chem 2021; 109: 104708
- [119] Küçüksolak M, Ekiz G, Duman S, Yılmaz S, Ballar-Kırmızıbayrak P, Bedir E. Telomerase activators derived from Astragalus sapogenins via biotransformation with the recently discovered endophytic fungus Camarosporium laburnicola. Planta Med 2019; 85: P-062
- [120] Harley C, Khor S, Ramaseshan M, Ramiya P, Pirot Z, Fauce S, Lin T. Composition and methods for increasing telomerase activity. US Patent 7846904B2; 2010
- [121] Küçüksolak M, Üner G, Ballar-Kırmızıbayrak P, Bedir E. Neuroprotective metabolites via fungal biotransformation of a novel sapogenin, cyclocephagenol. Sci Rep 2022; 12: 18481

- [122] Küçüksolak M, Yılmaz S, Ballar-Kırmızıbayrak P, Bedir E. Potent telomerase activators from a novel sapogenin via biotransformation utilizing *Camarosporium laburnicola*, an endophytic fungus. Microb Cell Fact 2023; 22: 66
- [123] Davis P, Miller R, Kit T. Flora of Turkey and the East Aegean Islands. Vol. 10. Edinburgh: Edinburgh University Press; 1988
- [124] Dönmez AA, Aydin ZU. Astragalus ihsancalisii (Fabaceae), a new species from Erzurum province, E Turkey. Willdenowia 2018; 48: 399–404
- [125] Gariboldi P, Pelizzoni F, Tato M, Verotta L, El-Sebakhy NA, Asaad AM, Abdallah RM, Toaima SM. Cycloartane triterpene gylcosides from Astragalus trigonus. Phytochemistry 1995; 40: 1755–1760
- [126] Tian Z, Yang M, Huang F, Li K, Si J, Shi L, Chen S, Xiao P. Cytotoxicity of three cycloartane triterpenoids from *Cimicifuga dahurica*. Cancer Lett 2005; 226: 65–75
- [127] Nalbantsoy A, Nesil T, Yılmaz-Dilsiz O, Aksu G, Khan S, Bedir E. Evaluation of the immunomodulatory properties in mice and in vitro anti-inflammatory activity of cycloartane type saponins from Astragalus species. J Ethnopharmacol 2012; 139: 574–581
- [128] Yakubogullari N, Cagir A, Bedir E, Sag D. Astragalus Saponins, Astragaloside VII and newly synthesized derivatives, induce dendritic cell maturation and T cell activation. Vaccines 2023; 11: 495