Thieme

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

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## 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 cycloartane-type 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 *çekme, 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]-*β*-D-glucopyranose) and **2** (3-Nitro-1-propil-*β*-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**) (▶ **Fig. 2**). This compound alters glycoprotein synthesis by inhibiting acidic *α*-mannosidases, leading to fetal degeneration in pregnant livestock, and may cause miscarriage [14].

#### Selenium compounds

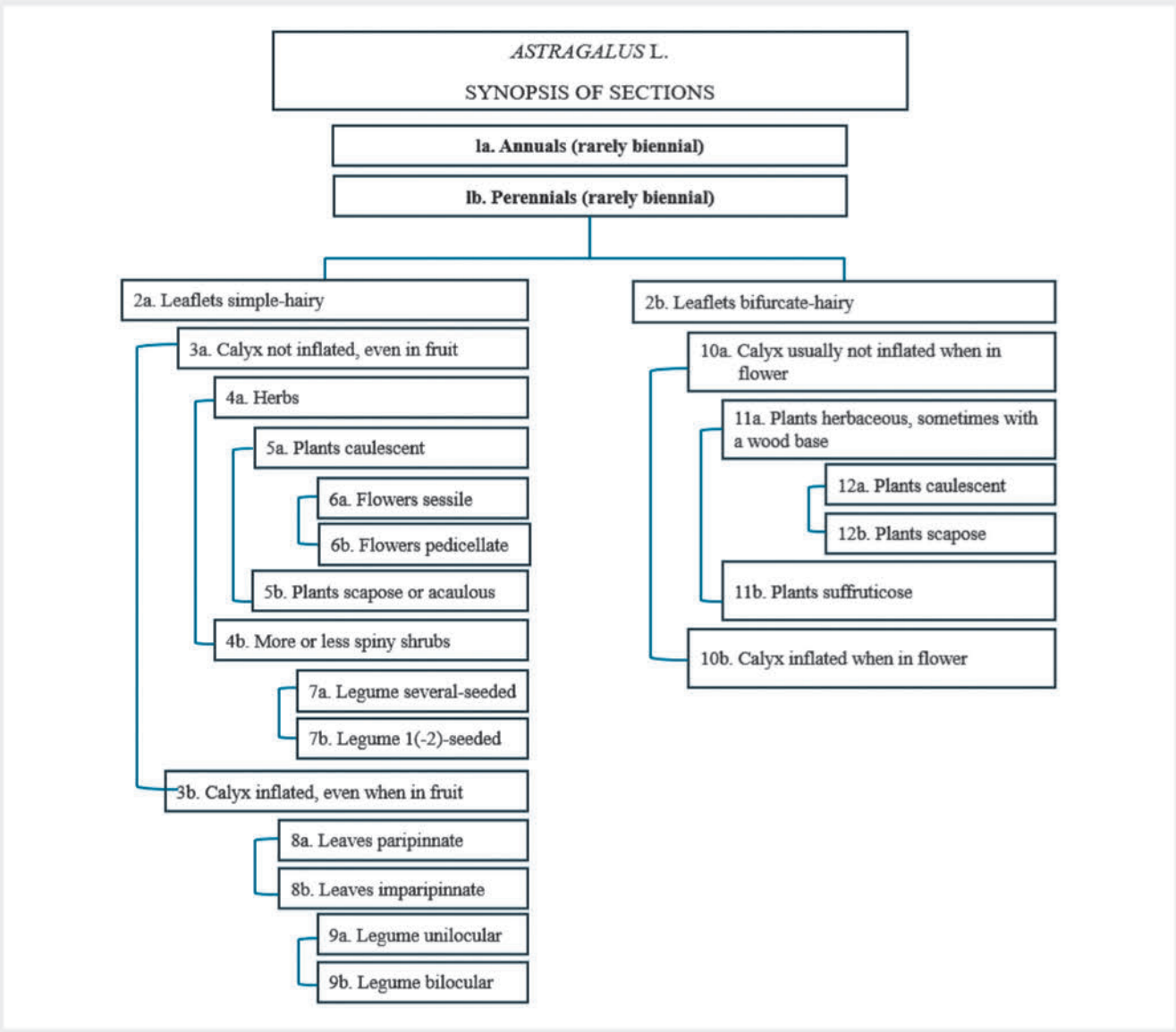
Selenium compounds cause chronic toxicity. This condition results in hair thickening and thinning, pathological formations in the hooves, disabilities, weakness, and loss of appetite, as well as a weakening and slowing of the functions. *Astragalus bisulcatus, A. saurinus, A. flavus*, and *A. tenellus*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

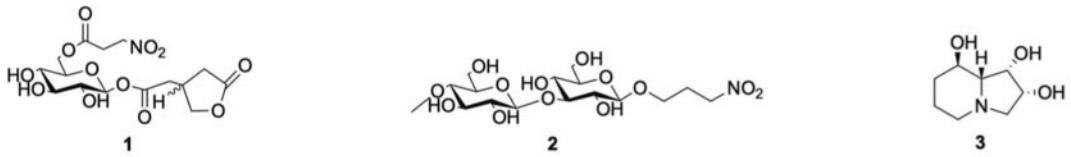
*A. membranaceus* polysaccharides (APSs) have various biological properties including immunomodulation, antioxidant, antidiabetic, antiviral, hepatoprotective, and anti-inflammatory ones [9, 19–21]. Fang et al. (1982) reported three polysaccharides, astragalan I, II, and III from the aqueous etract of 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 36 300 D, while astragalan II and III were composed of D-glucose only, with molecular weights of 12 300 D and 34 600 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].

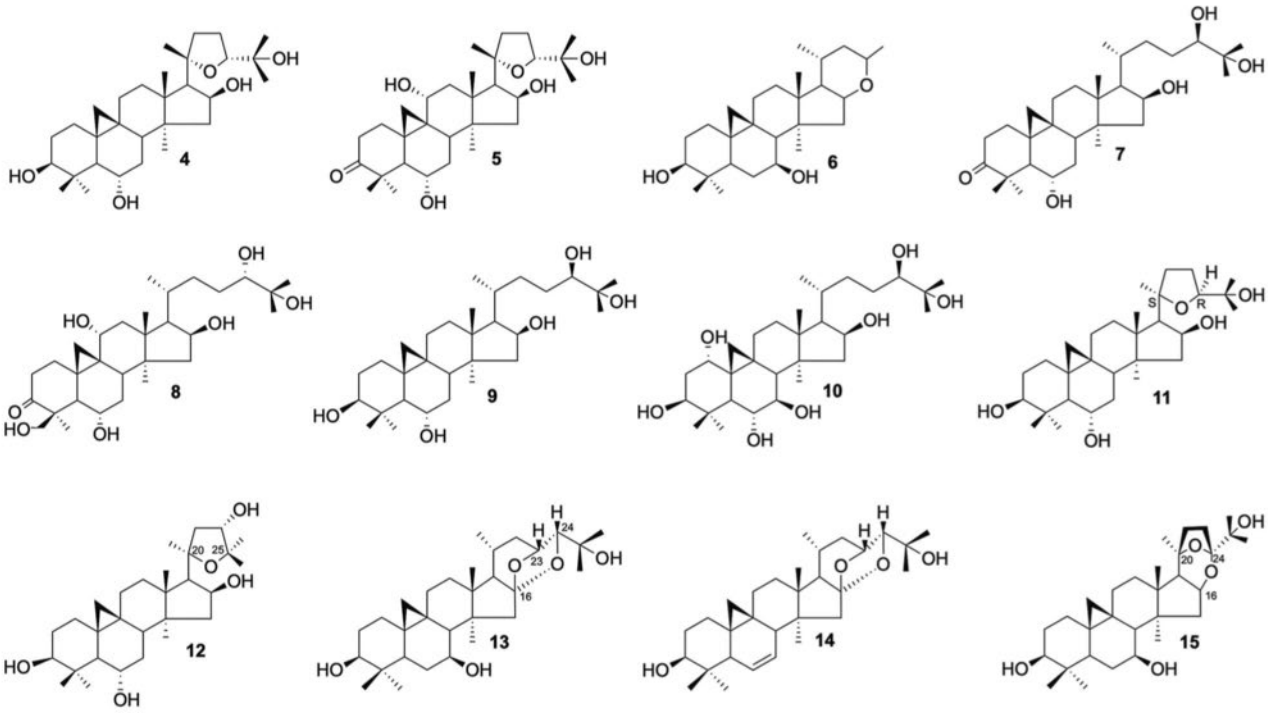
In 1992, a glycan (AMem-P) was isolated from the roots of *A. membranaceus*. This glycan was primarily composed of L-arabinoside, D-galactose, L-rhamnose, and D-galacturonic acid in a molar ratio of 6 : 9 : 8 : 30. The authors reported that AMem-P exhibited significant reticuloendothelial-system-potentiating 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 sterols [25].




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

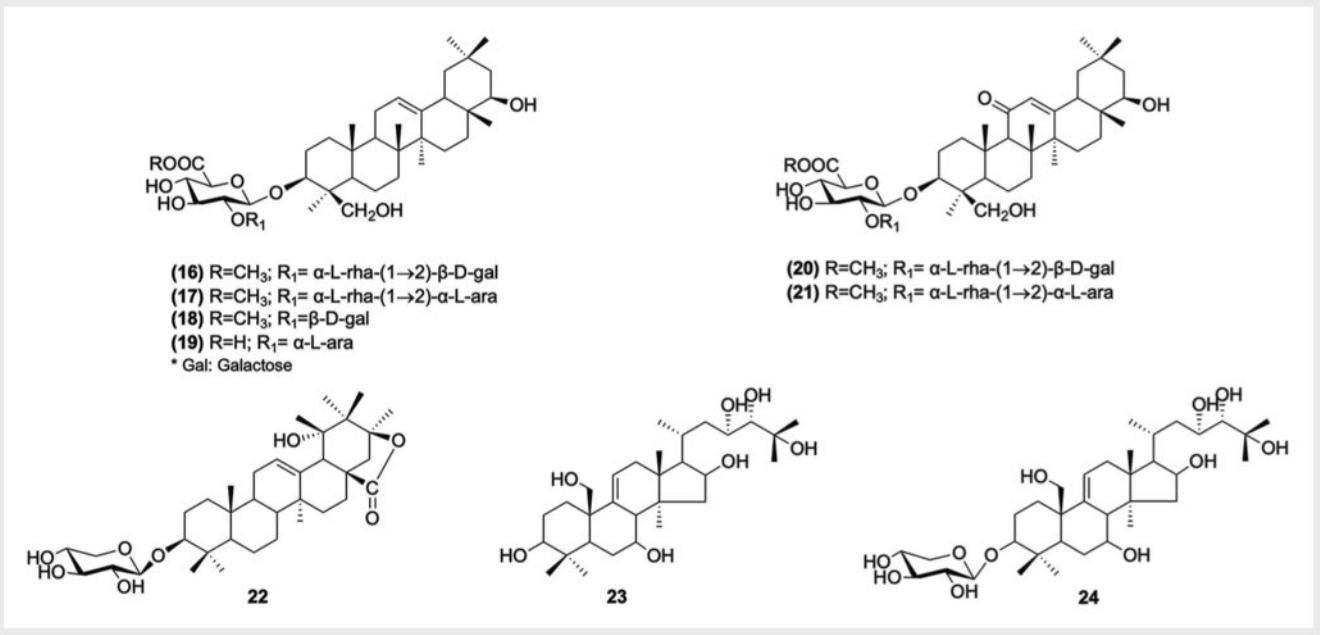



▶ **Fig. 3** Structures of cycloartanes (**4–15**).

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 *β*-D-xylose. Additional monosaccharides are *β*-D-glucose, *α*-L-rhamnose, *α*-L-arabinose, and rarely, *β*-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**).

- 16*β*, 23;16*α*, 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 *β*-D-glucuronic acid directly linked to the aglycone at C-3 and carry *β*-D-glucose, *β*-D-galactose, or *β*-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-*β*-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

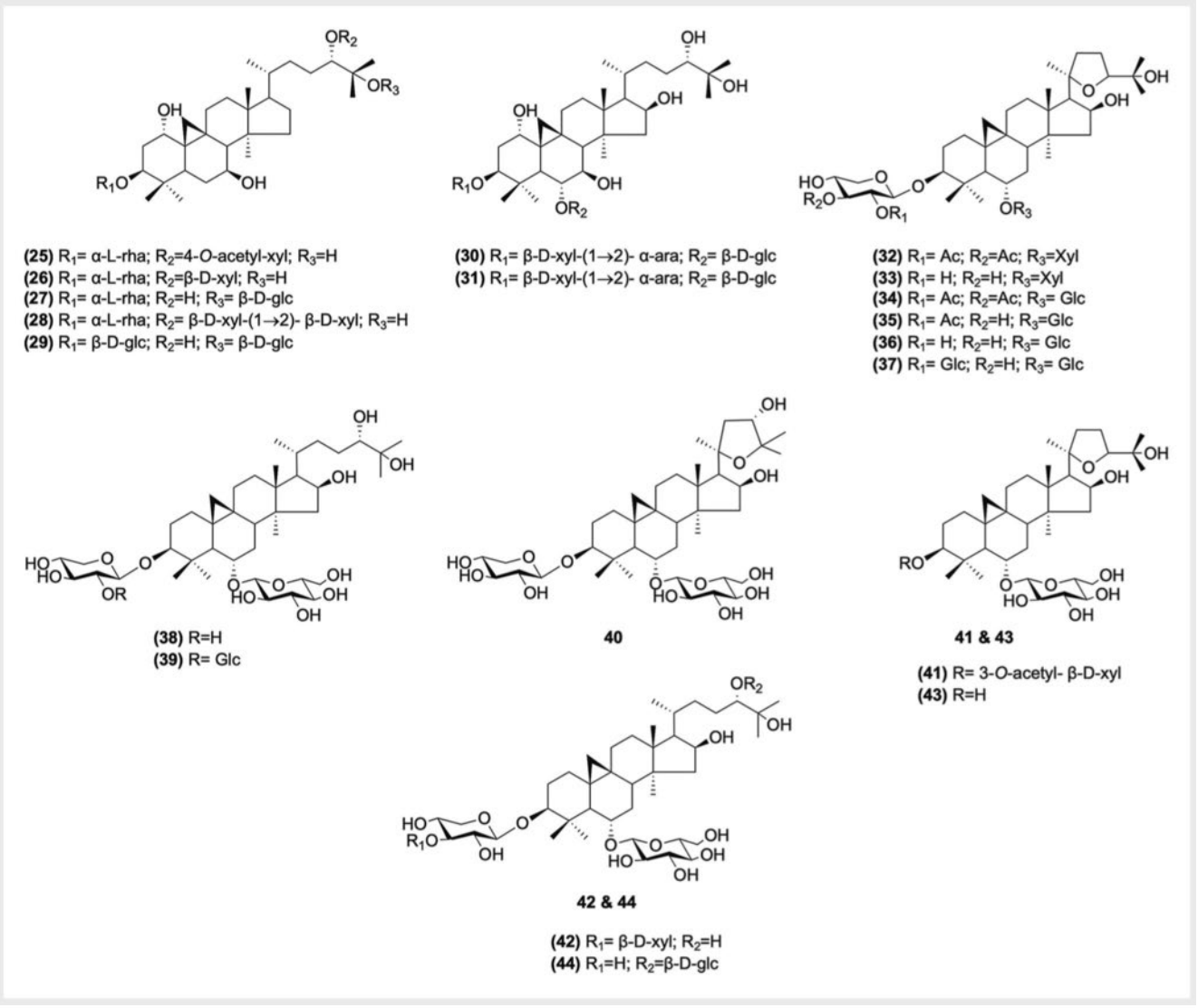
The notable improvements observed in the blood profile of a leukemia patient who utilized an extract derived from a folkloric medicinal plant initiated our research on Turkish *Astragalus* species. In the early 1990 s, the first plant material, which consisted of the roots of a plant used for this purpose, 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 24*S*-cycloartane-1*α*,3*β*,7*β*,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-




▶ **Fig. 5** Structures of compounds **25–44**.

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 $IC_{50}$ 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] (▶ **Fig. 5**) [42]. Notably, the majority of these compounds are cycloastragenol 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 µg/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*β*-(*β*-D-xylopranosyl)oxy-6*α*-(*β*-D-glucopranosyl)oxy-cycloartane-16*β*,24*α*-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-Balıkesir, West Anatolia, were studied [46–48]. Six novel cycloartane type glycosides (**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*β*,6*α*,16*β*,25 tetrahydroxycycloartane as the aglycone, whereas trojanosides C (**47**), D (**48**), E (**49**), and F (**50**) had 3*β*,6*α*,16*β*, (24*S*),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*β*,6*α*,16*β*,24*α*-tetrahydroxycycloartane (**61**) and 20(*R*),24(*S*)-epoxy-3*β*,6*α*,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 → 2)-*β*-glucopyranoside (**63**)] and three phenolic glycosides [7,8-dihydro-7-hydroxyconiferyl alcohol 4-*O*-*β*-apiofuranosyl-(1 → 2)-*β*-glucopyranoside (**64**), 2-methoxyphenol-4-*O*-*β*-apiofuranosyl-(1 → 2)-*β*-glucopyranoside (**65**), and 3-hydroxy-5-methoxyphenol-2-*O*-*β*-apiofuranosyl-(1 → 2)-*β*-glucopyranoside (**66**)] were isolated from the polar fractions of *A. zahlbruckneri* [54].

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

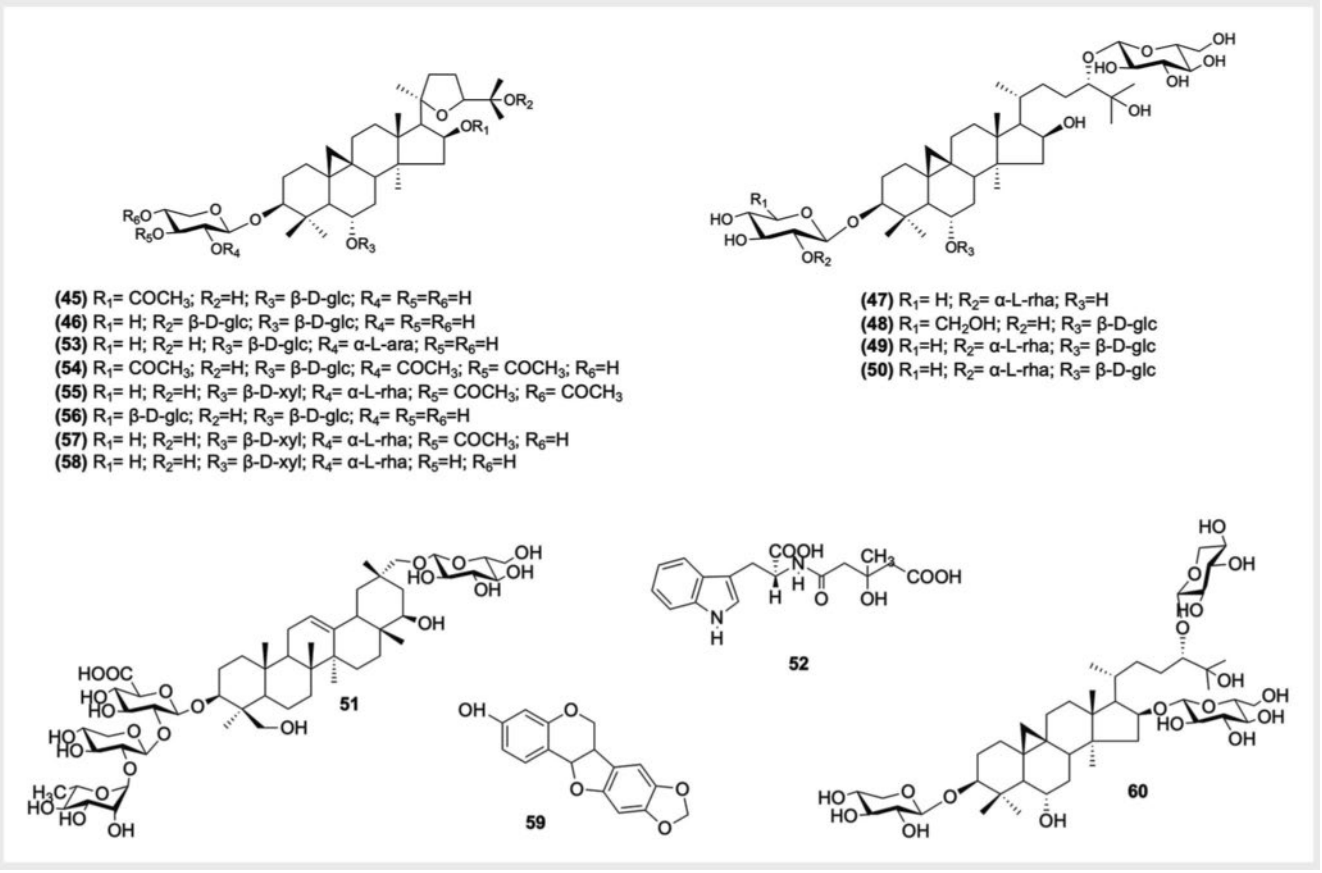
Continuing our research on the genus *Astragalus, A. prusianus* was collected from Kale, Muğla, West Anatolia. In this study, two novel cycloartane-type triterpene glycosides, 16-O-*β*-D-glucopyranosyl-20(*S*),24(*R*)-5*α*,9-diepoxy,2*α*,3*β*,16*β*,25-tetrahydroxy-9,10-seco-cycloarta-1(10),6(7)-diene (**67**) and 3-O-*β*-D-xylopyranosyl-16-O-*β*-D-glucopyranosyl-20(*S*),24(*R*)-epoxy-3*β*,16*β*,25-trihydroxycycloartane (**68**), were obtained (▶ **Fig. 7**). The 5*α*,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*-*β*-D-apiofuranosyl-(1 → 2)-[*α*-L-rhamnopyranosy-(1 → 6)]-*β*-D galactopyranoside], and the other was a known isorhamnetine derivative (**70**) [56], isorhamnetin 3-*O*-*β*-D-apiofuranosyl-(1 → 2)-*β*-D-galactopyranoside (▶ **Fig. 7**) [57].

### *Astragalus baibutensis* Bunge (Sect. Pterophorus Bunge)

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




▶ **Fig. 6** Structures of compounds **45–60**.

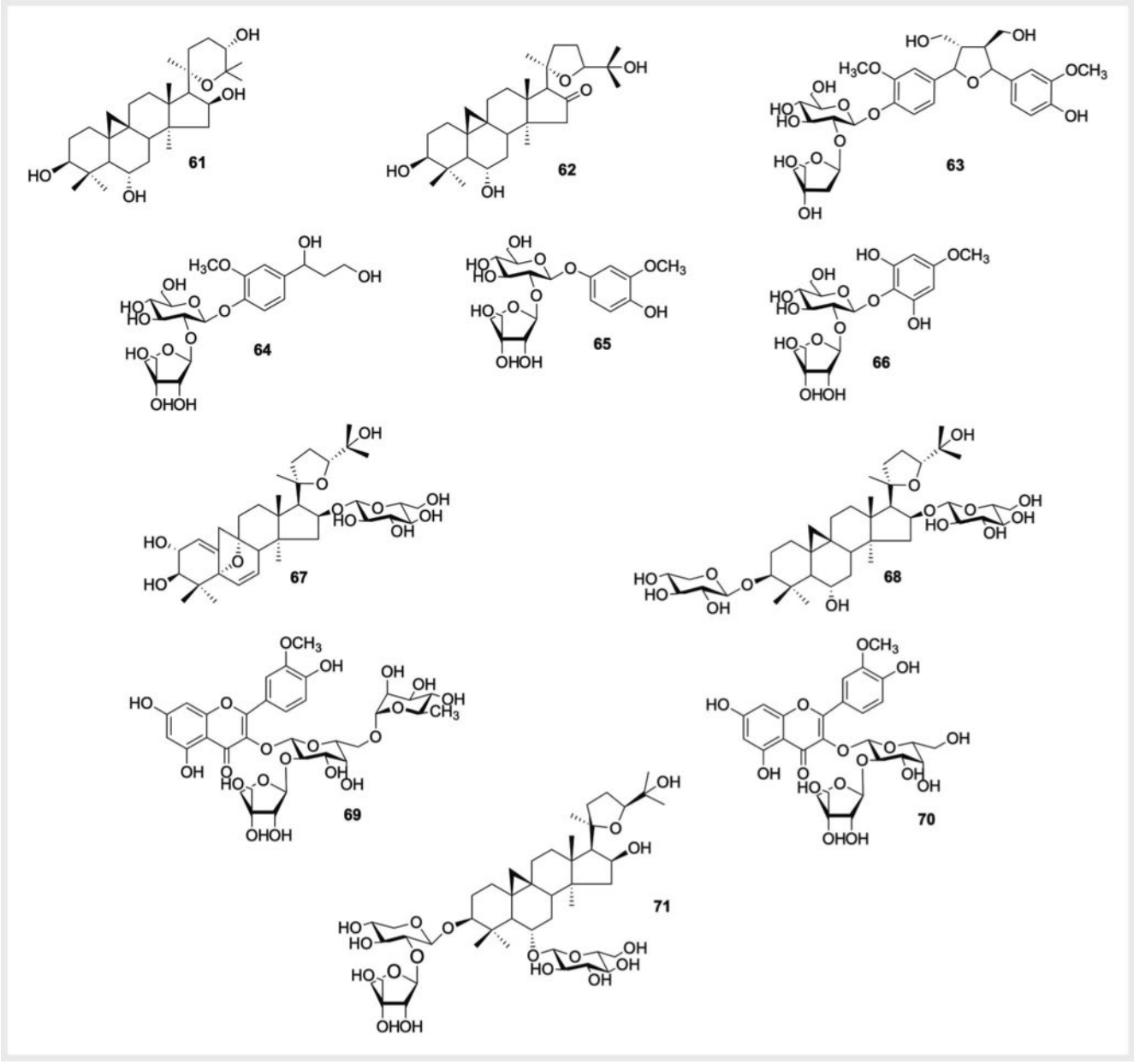
*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,25-tetrahydroxy-20,24-epoxycycloartane, named baibutoside (**71**) (▶ **Fig. 7**), a new cycloartane-type glycoside together with four known glycosides, acetylastragaloside I [29], astragaloside I (**35**) [29], II (**33**) [29], and IV (**34**) [29], were reported [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*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-3*β*,6*α*,16*β*,23*α*,25-pentahydroxy-20(*R*),24(*S*)-epoxycycloartane (**72**), 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-16-*O*-hydroxyacetoxy-23-*O*-acetoxy-3*β*,6*α*,25-trihydroxy-20(*R*),24(*S*)-epoxycycloartane (**73**), 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-25-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane (**74**), and 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-3*β*,6*α*,23*α*,25-tetrahydroxy-20(*R*),24(*S*)-16*β*,24;20,24-diepoxycycloartane (**75**) (▶ **Fig. 8**), together with three previously isolated cycloartane glycosides, namely, 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-3*β*,6*α*,16*β*,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.)

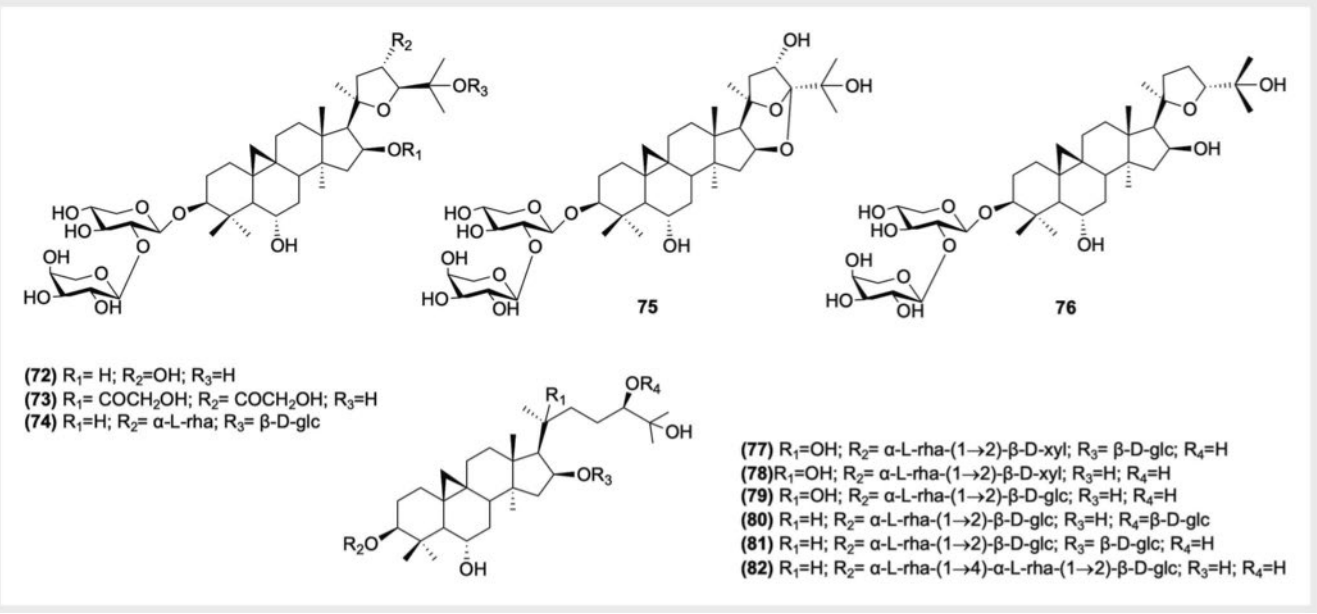
Continuing of our work on the genus *Astragalus*, the roots of *Astragalus elongatus*, collected from Central Anatolia, Ahlatlıbel, Ankara, were also studied. In this study, a new monodesmosidic cycloartane-type glycoside, elongatoside (**76**) (3-*O*-[*α*-arabinopyranosyl-(1 → 2)-*β*-xylopyranosyl]-cycloastragenol) (▶ **Fig. 8**), was isolated in addition to two known cycloartane-type glycosides: askendosides D (3-*O*-[*α*-arabinopyranosyl-(1 → 2)-*β*-xylopyranosyl]-6-*O*-*β*-xylopyranosyl-cycloastragenol [59] and G (3-*O*-[*α*-arabinopyranosyl-(1 → 2)-*β*-xylopyranosyl]-16-*O*-*β*-glucopyranosyl-3*β*,6*α*,16*β*,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*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-16-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,20(*S*),24(*R*),25-hexahydroxycycloartane (**77**), 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-3*β*,6*α*,16*β*,20(*S*),24(*R*),25-hexahydroxycycloartane (**78**), 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-3*β*,6*α*,16*β*,20(*S*),24(*R*),25-hexahydroxycycloartane (**79**), 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-24-*O*-*β*-D-glucopyranosyl]-3*β*,6*α*, 16*β*,24(*R*),25-pentahydroxycycloartane (**80**), 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-16-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*R*),25-pentahydroxycycloartane (**81**), and 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 4)-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]]-3*β*,6*α*,16*β*,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*-*β*-D-glucopyranosyl-16-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,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 µM [65].

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

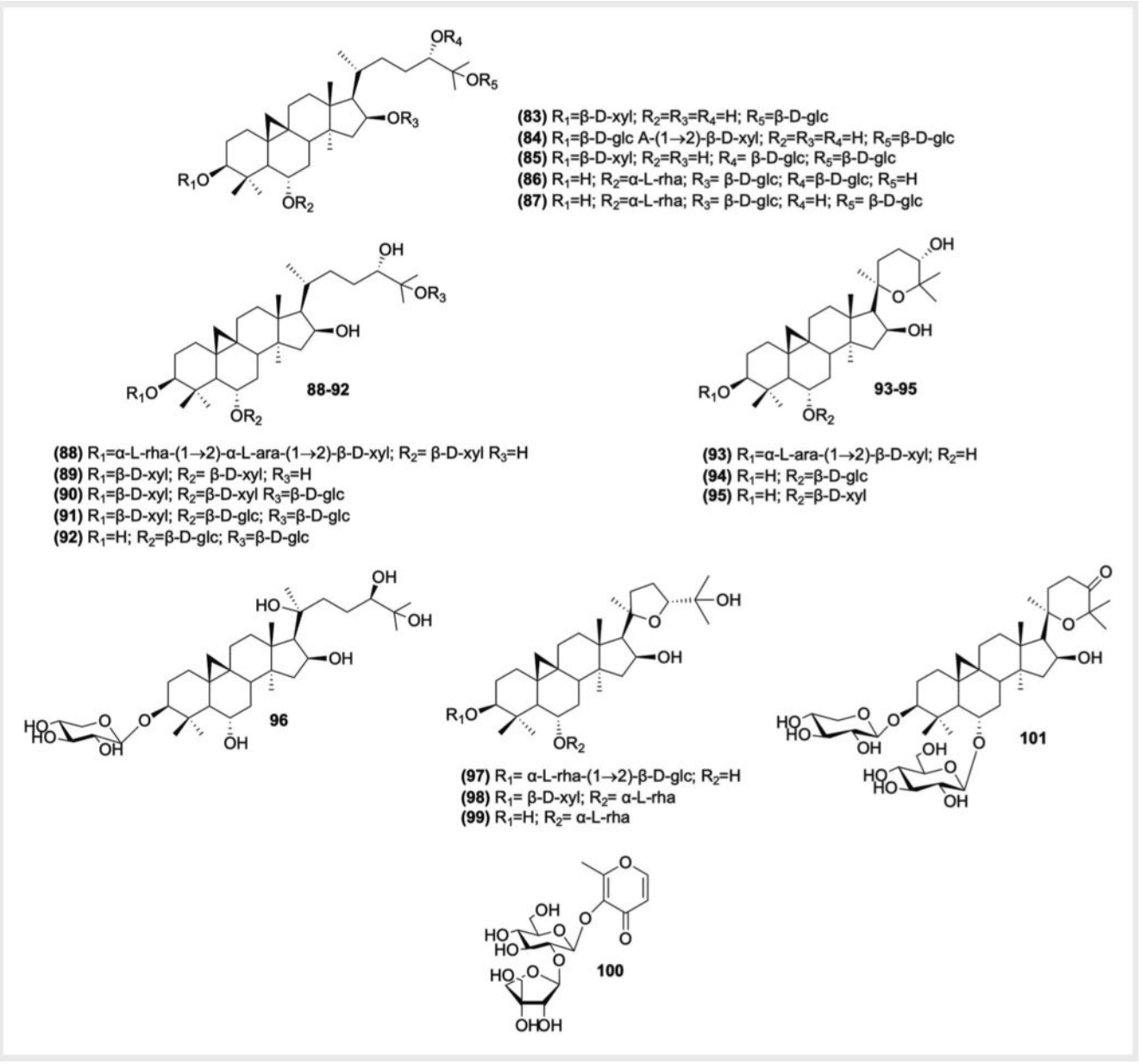
Ongoing studies are being conducted on *A. lusitanicus*, a species known for its toxicity in Turkey and the countries bordering the Mediterranean Sea. So far, only kersetol and kaempferol derivative flavonol glycosides have been obtained. It is believed that aliphatic nitro compounds are responsible 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-γ after phorbolacetate (PHA) stimulation were determined via commercially available enzyme-linked 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-2-inducing 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*-*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-glucopyranosyl-(1 → 2)]-*β*-D-glucuronopyranosyl-21-epi-kudzusapogenol A; 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-xylopyranosyl-(1 → 2)]-*β*-D-glucuronopyranosyl-22-*O*-*β*-D-glucopyranosyl-21-epi-kudzusapogenol A; 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-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-xylopyranosyl-(1 → 2)]-*β*-D-glucuronopyranosyl-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*-*β*-D-xylopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**83**), 3-*O*-[*β*-D-glucuronopyranosyl- (1 → 2)-*β*-D-xylopyranosyl]-25-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**84**), 3-*O*-*β*-D-xylopyranosyl-24,25-di-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**85**), 6-*O*-*α*-L-rhamnopyranosyl-16,24-di- *O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**86**), and 6-*O*-*α*-L-rhamnopyranosyl-16,25-di-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**87**), together with a known compound, 3-*O*-*β*-D-xylopyranosyl-16-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,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*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-25-*O*-*β*-D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3*β*,6*α*,16*β*,24(*S*),25-tetrahydroxycycloartane, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucopyranosyl-24-*O*-*α*-(4′-*O*-acetoxy)-L-arabinopyranosyl-16-*O*-acetoxy-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane, and 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucopyranosyl-24-*O*-*α*-L-arabinopyranosyl-16-*O*-acetoxy-3*β*,6*α*,16*β*,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*-[*β*-D-glucopyranosyl-(1 → 2)-*O*-*α*-L-arabinopyranosyl]-3*β*,22*β*,24-trihydroxy-olean-12-ene [78], and narcissin [79]. The structures of the new compounds were established as 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*O*-3-acetoxy-*α*-L-arabinopyranosyl]-6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane; 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*O*-*α*-L-arabinopyranosyl-(1 → 2)-*O*-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane; 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*O*-3,4-diacetoxy-*α*-L-arabinopyranosyl]-6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane; 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*O*-3-acetoxy-*α*-L-arabinopyranosyl]-6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane; 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*O*-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24*α*-tetrahydroxy-20(*R*),25-epoxycycloartane, and 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*O*-*α*-L-arabinopyranosyl-(1 → 2)-*O*-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24*α*-tetrahydroxy-20(*R*),25-epoxycycloartane [80].

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*-[*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-6-*O*-*β*-D-xylopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**88**), 3,6-di-*O*-*β*-D-xylopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**89**), 3,6-di-*O*-*β*-D-xylopyranosyl-25-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**90**), 3-*O*-*β*-D-xylopyranosyl-6,25-di-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**91**), 6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**92**), 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-3*β*,6*α*,16*β*,24*α*-tetrahydroxy-20(*R*),25-epoxycycloartane (**93**), 6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24*α*-tetrahydroxy-20(*R*),25-epoxycycloartane (**94**), and 6-*O*-*β*-D-xylopyranosyl-3*β*,6*α*,16*β*,24*α*-tetrahydroxy-20(*R*),25-epoxycycloartane (**95**) (▶ **Fig. 9**), in addition to 10 known compounds, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*O*-*α*-L-arabinopyranosyl-(1 → 2)-*O*-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane [78], oleifolioside B (**31**) [39], cyclocanthoside E (**32**) [38], cyclocanthoside G (**39**) [38], 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-O-*α*-L-arabinopyranosyl-(1 → 2)-O-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24*α*-tetrahydroxy-20(*R*),25-epoxycycloartane [80], 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*O*-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24*α*-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*-*β*-D-xylopyranosyl-6,25-di-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,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*-*β*-D-xylopyranosyl-24-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24*α*-tetrahydroxycycloartane, 20(*R*),25-epoxy-3-*O*-[*β*-D-glucopyranosyl-(1 → 2)]-*β*-D-xylopyranosyl-24-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24*α*-tetrahydroxycycloartane, and 3-*O*-*β*-D-xylopyranosyl-3*β*,6*α*,16*β*,20(*S*),24(*S*),25-hexahydroxycycloartane (**96**) (▶ **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*-[*β*-D-xylopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-6-*O*-*β*-D-glucuronopyranosyl-3*β*,6*α*,16*β*,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*-*β*-D-xylopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane **(89**) [86], and 6-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,24(*S*),25-pentahydroxycycloartane (**92**) [86].

Gülcemal et al. (2012) reported on the isolation and characterization of six new cycloartane-type triterpenoids, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-16-*O*-hydroxyacetoxy-3*β*,6*α*,16*β*,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-16-*O*-hydroxyacetoxy-3*β*,6*α*,16*β*, 23*α*,25-pentahydroxy-20(*R*),24(*S*)-epoxycycloartane, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-3*β*,6*α*,25-trihydroxy-20(*R*),24(*S*)-epoxycycloartane-16-one, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-3*β*,6*α*,16*β*,25-tetrahydroxy-20(*R*),24(*R*)-epoxycycloartane (**97**), 3-*O*-*β*-D-xylopyranosyl-6-*O*-*α*-L-rhamnopyranosyl-3*β*,6*α*,16*β*,25-tetrahydroxy-20(*R*),24(*R*)-epoxycycloartane (**98**), and 6-*O*-*α*-L-rhamnopyranosyl-3*β*,6*α*,16*β*,25-tetrahydroxy-20(*R*),24(*R*)-epoxycycloartane (**99**), from *A. angustifolius* (Sect. Melanocercis) (▶ **Fig. 9**), along with four oleanane-type triterpenoids, namely, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-3*β*,21*β*,22*α*,24,29-pentahydroxyolean-12-ene, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-3*β*,22*β*,24-trihydroxyolean-12-en-29-oic acid, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-22-*O*-*α*-L-arabinopyranosyl-3*β*,22*β*,24-trihydroxyolean-12-ene, and 29-*O*-*β*-D-glucopyranosyl-3*β*,22*β*,24,29-tetrahydroxyolean-12-ene, and five known triterpene glycosides (astrojanoside A (**51**) [47], astragaloside VIII [49], 25-*O*-glucopyranosylcycloastragenol [49], 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]-25-*O*-*β*-D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3*β*,6*α*,16*β*,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]-3*β*,22*β*,24-trihydroxyolean-12-en-29-oic acid**)** showed a weak inhibitory effect with $IC_{50}$ 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*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-xylopyranosyl]-20,24-epoxy-16-*O*-*β*-D-glucopyranosyl-3*β*,6*α*,16*β*,25-tetrahydroxycycloartane, and a new glycoside (**100**) (▶ **Fig. 9**), 3-*O*-[*β*-D-apiofuranosyl-(1 → 2)-*β*-D-glucopyranosyl]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$^n$ led to the isolation of 22 oleanane-type triterpene glycosides from *A. tauricolus* (sect. Malacothrix), including 10 new compounds, namely, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-29-*O*-*β*-D-glucopyranosyl-3*β*,22*β*, 24-trihydroxyolean-12-ene-29-oic acid, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-29-*O*-*β*-D-glucopyranosyl-3*β*,22*β*,24,29-tetrahydroxyolean-12-ene, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-21-*O*-*α*-L-rhamnopyranosyl-3*β*,21*β*,22*α*,24-tetrahydroxyolean-12-ene, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-21-*O*-*α*-L-rhamnopyranosyl-3*β*,21*β*,22*α*,24-tetraydroxyolean-12-ene, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-29-*O*-*β*-D-glucopyranosyl-3*β*,22*β*,24-trihydroxyolean-12-ene-29-oic acid, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-22-*O*-*α*-L-rhamnopyranosyl-3*β*,22*β*,24-trihydroxyolean-12-ene, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-3*β*,24-dihydroxyolean-12-ene-22-oxo-29-oic acid, 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-3*β*,21*β*,22*α*,24,29-pentahydroxyolean-12-ene, 3-*O*-[*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-29-*O*-*β*-D-glucopyranosyl-3*β*,22*β*,24-trihydroxyolean-12-ene-29-oic acid, and 3-*O*-[*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-29-*O*-*β*-D-glucopyranosyl-3*β*,22*β*,24-trihydroxyolean-12-ene-29-oic acid. Known compounds were identified as astrojanoside A (**51**) [47], astragaloside VIII [49], azukisaponin II [72], azukisaponin V [72], 3-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl]-3*β*,21*β*,22*α*,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*-*β*-D-glucuronopyranosyl-soyasapogenin B [99]. It is noteworthy that cycloartane-type triterpene glycosides, which are the main constitu-

ents 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*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-3β,21β,22α,24,29-pentahydroxyolean-12-ene) exhibited moderate activity, with an $IC_{50}$ of 22 µM against the U937 cell line at concentrations ranging from 1 to 50 µM [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*-[α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 2)-β-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*-[α-L-arabinopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(*R*),25-epoxycycloartane [80], 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane [80], 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,6α,16β,24α-tetrahydroxy-20(*R*),25-epoxycycloartane **(93)** [86], and 6-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-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*-β-D-xylopyranosyl-6-*O*-β-D-glucopyranosyl-3β,6α,16β-trihydroxy-24-oxo-20(*R*),25-epoxycycloartane (**101**) (▶ **Fig. 9**), 3-*O*-[β-D-glucuronopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,16β,24α-trihydroxy-20(*R*),25-epoxycycloartane, 3-*O*-[β-D-glucuronopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,16β,25-trihydroxy-20(*R*),24(*S*)-epoxycycloartane, 3,25-di-*O*-β-D-glucuronopyranosyl-6-*O*-β-D-xylopyranosyl-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane, and a new oleanane-type glycoside, 29-*O*-α-L-rhamnopyranosyl-abrisapogenol B, along with five previously isolated cycloartane-type compounds (6-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-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].

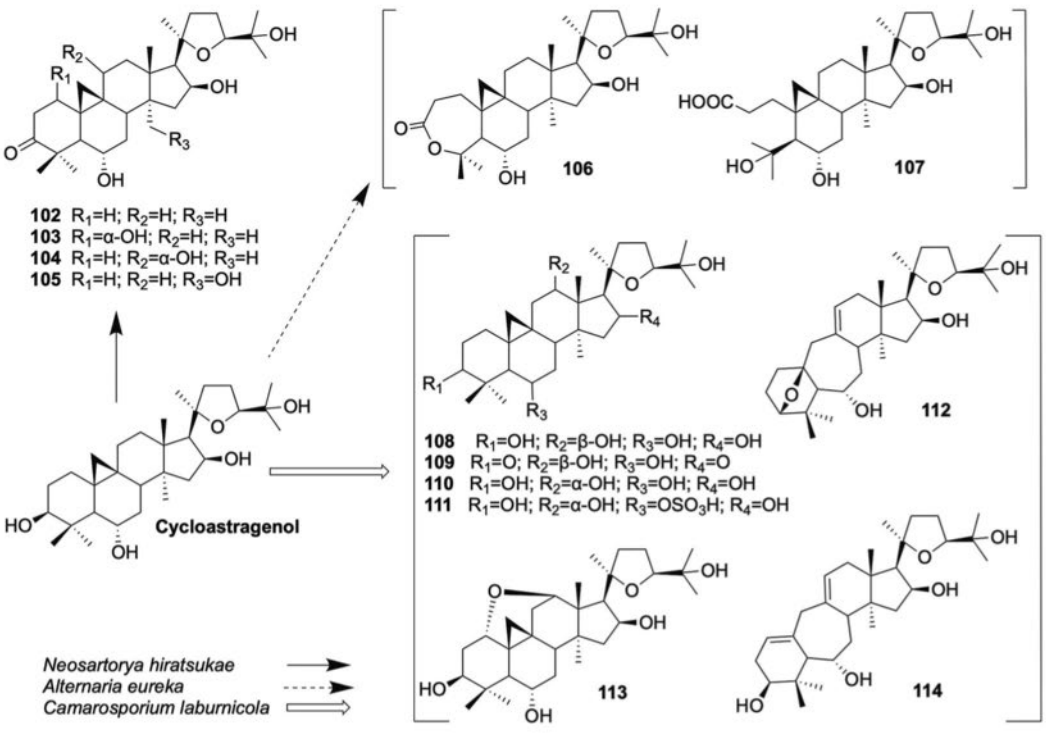
Endophytic organisms, which inhabit healthy plant tissues for at least a part of their life cycle, possess specific enzymes that enable them to colonize their hosts [112]. Among the microorganisms, endophytic fungi have gained considerable attention in biotransformation studies due to their ability to 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.

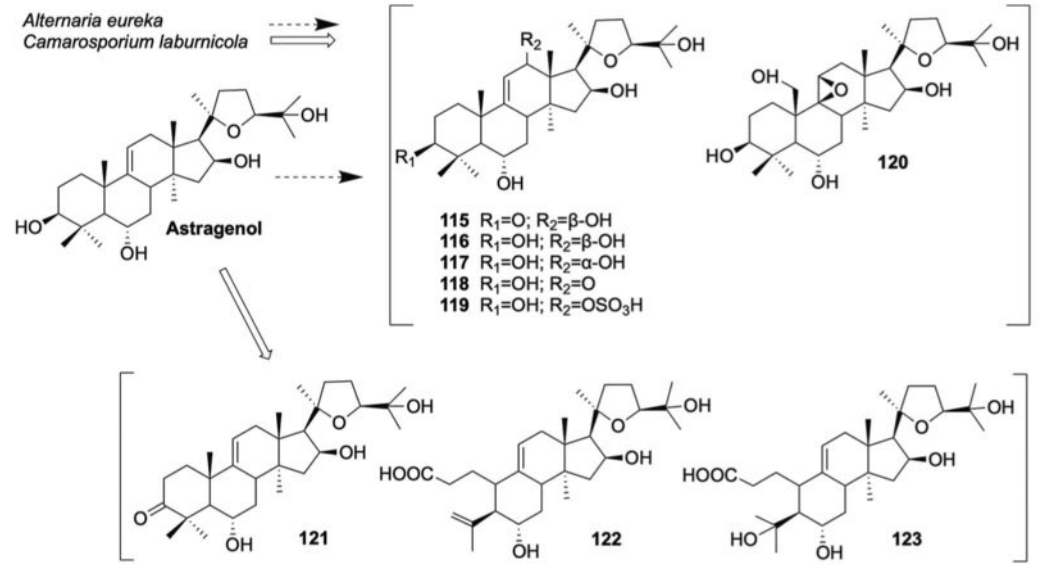
Initially, fresh samples of *A. angustiflorus* C. Koch. and *A. condensatus* Ledeb. were collected from Spil Mountain, Manisa, Turkey. Subsequently, fungal endophytes were isolated from the roots, leaves, and stems of the plant samples. As a result of the isolation studies, a total of 15 fungal endophytes 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 above-mentioned 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.

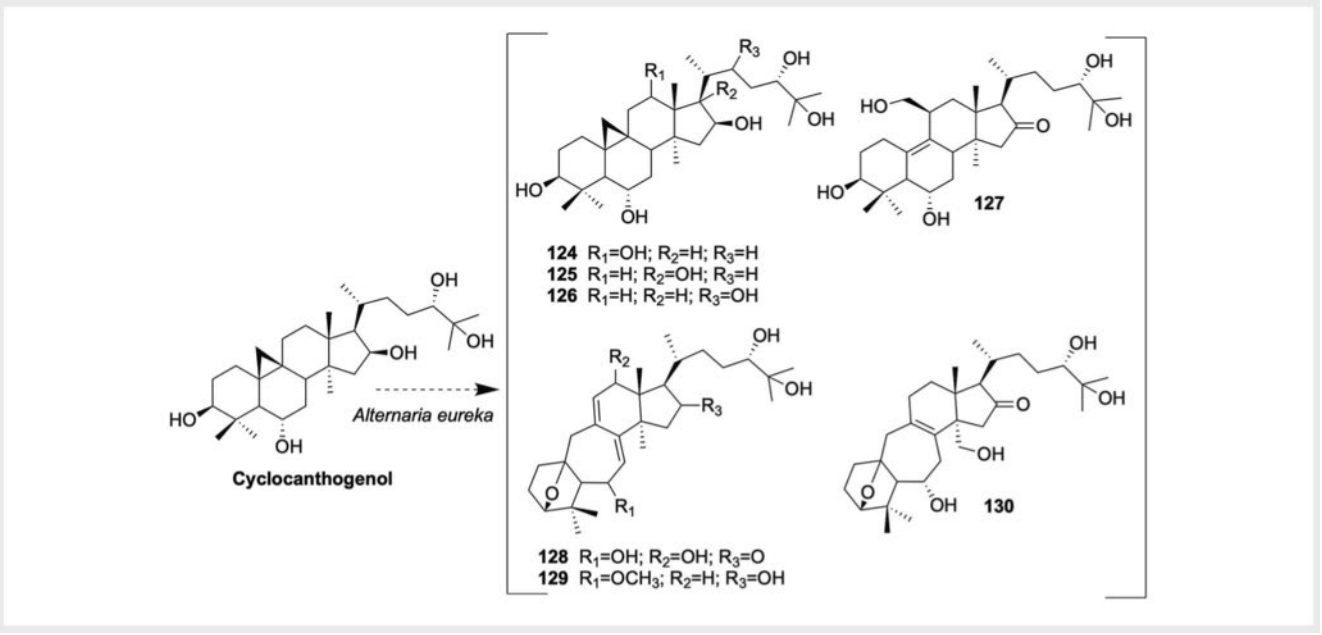
Given that cycloastragenol is the first commercialized natural telomerase activator on the market [120], the potential of the biotransformation products to increase telomerase activity in neonatal cells was also evaluated using PCR-based ELISA testing. As a result, 16 compounds displayed activity ranging 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



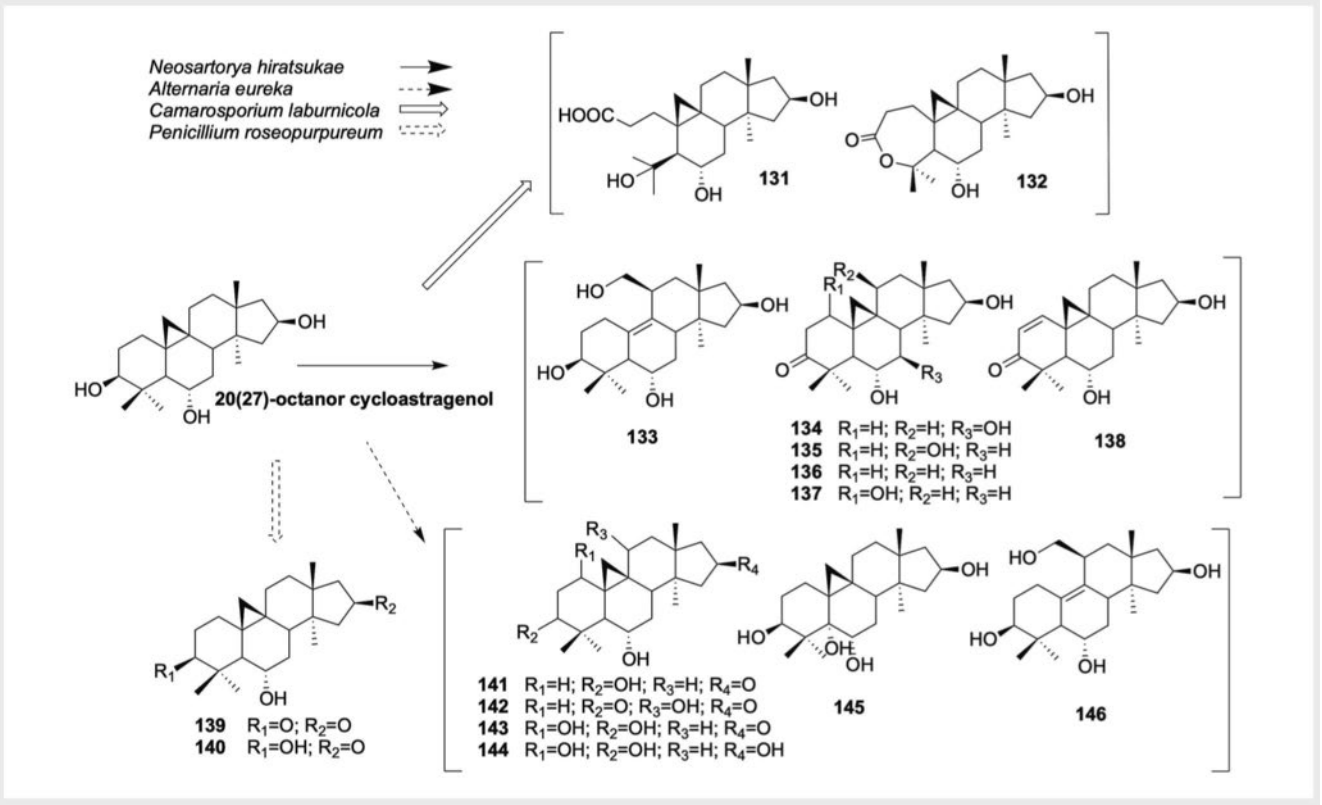

▶ **Fig. 10** Biotransformation products of cycloastragenol by *Neosartorya hiratsukae*, *Alternaria eureka*, and *Camarosporium laburnicola*.



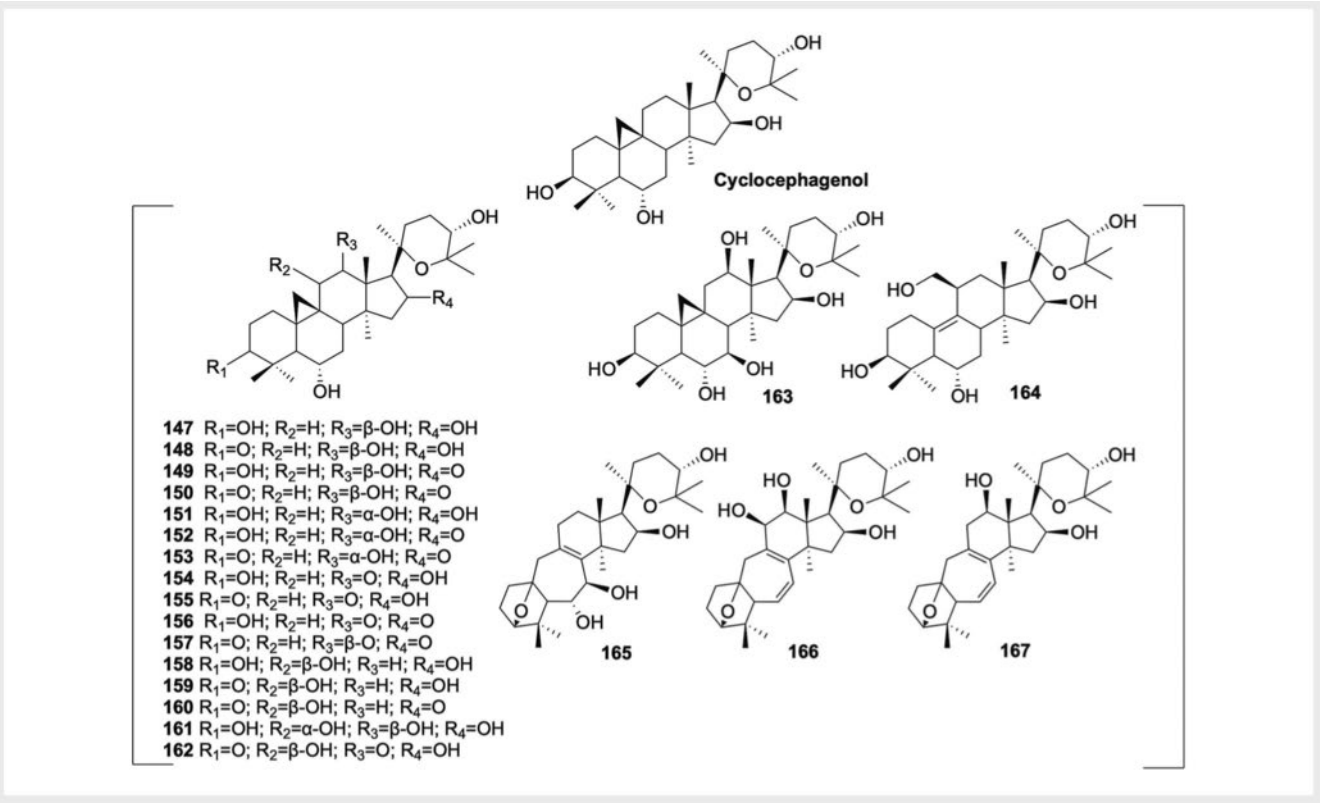

▶ **Fig. 11** Biotransformation products of astragenol by *Alternaria eureka* and *Camarosporium laburnicola*.




▶ **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 $H_2O_2$-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)*β*-epoxy-9,10-seco-cycloartane products showed potential as neuroprotective agents.

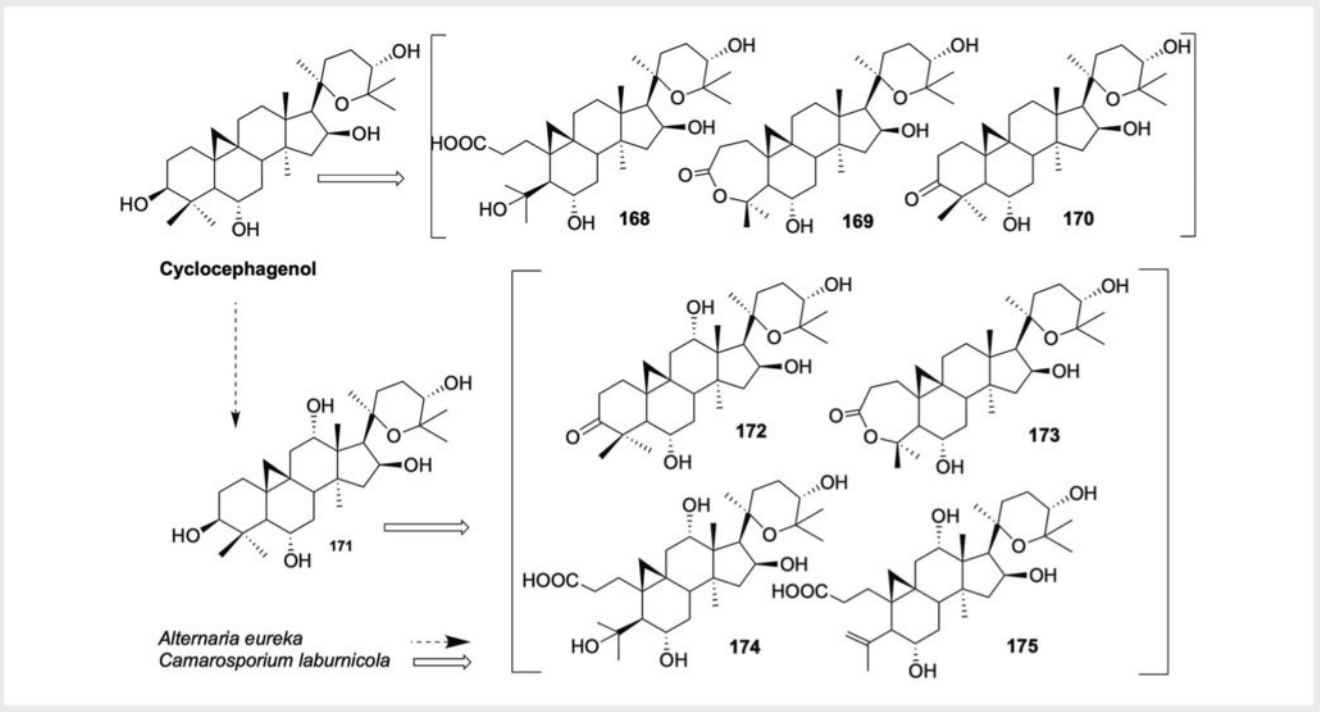
On the basis of our previous studies, which revealed that the biotransformation products of cycloastragenol and astragenol obtained from the *C. laburnicola* study had potent telomerase activity, Küçüksolak et al. (2023) made a further attempt on cyclocephagenol and its 12-hydroxy derivatives to obtain 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.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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