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Medicinal Importance of *Ajuga* Species in Iran: Ethnobotanical and Traditional Applications, Phytochemical, and Pharmacological Studies

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Abstract

Context: Five species of the genus *Ajuga* (*Lamiaceae*) having the common name of "bugle" are found in Iran. In Persian medicine (PM), the genus *Ajuga* (*Kamaphytus*) is used for treating jaundice, joint pain, gout, amenorrhea, sciatica, and wound healing. This study aimed to review the ethnobotanical, phytochemical, and biological activities of *Ajuga* species that grow in Iran to determine their therapeutic potentials and suggest further studies on the healing properties of this genus in Iran.

Evidence Acquisition: Electronic databases such as PubMed, Scopus, and Google Scholar were comprehensively searched for studies on *Ajuga* species in Iran, including "*Ajuga austro-iranica*," "*Ajuga chamaecistus*," "*Ajuga comata*" (Syn.: "*Ajuga chia*," "*Ajuga chamaepitys* subsp. *Chia*"), "*Ajuga orientalis*," and "*Ajuga reptans*." The search period was from 1966 to February 2021. The related articles were selected according to the inclusion and exclusion criteria of the current study.

Results: Several ethnobotanical and pharmacologic reports have verified the traditional uses of the genus *Ajuga* for anti-inflammatory, hypoglycemic, hypolipidemic, analgesic, anabolic, anti-arthritis, antipyretic, and hepatoprotective activities. Numerous phytochemicals have been identified from *Ajuga* species involving phytoecdysteroids, neo-clerodane-diterpenes, iridoids, flavonoids, withanolides, phenylethyl glycoside, and essential oils.

Conclusions: Due to the beneficial therapeutic effects of *Ajuga* genus, it can be considered in future clinical studies as a source of natural antioxidants, dietary supplements in the pharmaceutical industry, and stabilizing food against oxidative deterioration.

Keywords: *Ajuga*, Persian Medicine, Pharmacological Activities, Phytochemical Compositions, Ethnobotanical Uses

1. Context

The genus *Ajuga* of the *Lamiaceae* family, commonly known as bugle or bugleweed, ground pine, and carpet bugle, is a large plant genus that grows in Europe, Asia, Africa, Australia, and North America. The genus *Ajuga* is one of the 266 genera of the family *Lamiaceae*. Five species of the genus *Ajuga* are growing in the plant flora of Iran, including *Ajuga austro-iranica* Rech f., *Ajuga chamaecistus* Ging., *Ajuga comata* Stapf. (Syn.: *Ajuga chia*, *Ajuga chamaepitys* subsp. *chia*), *Ajuga orientalis* L., and *Ajuga reptans* L. (1-8). Many plants of the *Ajuga* genus are used traditionally as a remedy for rheumatic fever, dysentery, malaria, hypertension, diabetes, and gastrointestinal ailments, in addition to anti-inflammatory, astringent, diuretic, and antifungal activities (1, 2, 8-11). Different phytochemical compounds have been isolated and identified from several species of this genus, including phytoecdysteroids (12, 13), diterpenoids (14), and iridoids (8, 12, 15). Numerous biological studies showed that some of these compounds have beneficial biological effects such as antibacterial, antifungal, anti-plasmodial, cytotoxic, antitumor, analgesic, anti-inflammatory, antidiabetic, and antioxidant effects (16). In this review, *Ajuga* plants growing in Iran have been studied for their ethnobotanical, phytochemical, and pharmacological properties.

2. Evidence Acquisition

Electronic databases including PubMed, Scopus, and Google Scholar were explored for investigating *Ajuga*. The

search period was from 1966 to February 2021. The search keywords included "*Ajuga*," "*Ajuga austro-iranica*," "*Ajuga chamaecistus*," " *Ajuga comata*," "*Ajuga chia*," "*Ajuga pseudochia*," "*Ajuga chamaepitys* subsp. *chia*", "*Ajuga orientalis*," and "*Ajuga reptans*." Inclusion criteria were in vitro, in vivo, or phytochemical evaluation and traditional and ethnobotany use of five Iranian *Ajuga* species in papers with available full texts in English. Exclusion criteria were review articles and papers with non-English full-texts.

3. Results

3.1. Traditional and Ethnobotanical Uses

The genus *Ajuga* (*Kamaphytus*) is used traditionally for the management of jaundice, joint pain, gout (2, 17, 18), amenorrhea, sciatica, and wound-healing in traditional Persian medicine (19, 20). It is also utilized as a carminative and diuretic agent (3, 10, 20, 21). Iranian physicians believe that this plant can be effective for liver (22, 23) and spleen disease. They also used this remedy for treating constipation (24). The critical point of "*Kamaphytus*" mentioned in many books of Persian physicians is the wound-healing effects of these plants (20, 21, 24-27).

Table 1 shows the traditional uses of some species of the genus *Ajuga* growing in different regions of the Iranian plateau. Most species of this genus are used for treating gastrointestinal diseases such as constipation, skin diseases, blood sugar control, rheumatism, menstrual cramps, hypertension, gout, and wound healing (28).

3.2. Phytochemicals

Much phytochemical research has assessed the separation of *Ajuga* ingredients. These studies have led to the isolation of several secondary metabolites, including neoclerodane_diterpenes and diterpenoids, phytoecdysteroid components, flavonoid derivatives, iridoids, sesquiterpenoid compounds, withanolides, phenylethanoid glycoside derivatives, sterols, anthocyanins, and O-coumaric acid compounds (3, 8, 12, 13, 15, 23, 29, 34, 39, 40, 45-51). Isolated compounds from different *Ajuga* species growing in Iran are shown in Table 2. Also, the phytochemical structures of the main secondary metabolites of *Ajuga* species in Iran are presented in Figures 1 - 3. According to the results, phytochemical compounds have been determined in several species, especially *A. chamaecistus*, *A. chamaepitys*, and *A. reptans*. The major constituents in different species were phenolic compounds like phenylethanoid glycosides, neo-clerodane-type diterpenes, iridoids, anthocyanin glycosides, and phytoecdysteroids. Phytochemical studies of *A. austro-iranica* and some subspecies have not been reported and need to be studied.

Moreover, the constituents of volatile oil isolated from the aerial parts of the *Ajuga* plant (leaves and flowers) are shown in Table 3. Phytochemical studies of essential oils of almost all species of Iran have been performed, and the major compounds in different species were identified, such as germacrene-D, α- and β-pinene, 1-octen-3-ol, p-cymene, thymol, and hexadecanoic acid.

3.2.1. Biological Importance of Phytochemicals Identified in *Ajuga* Species

3.2.1.1. Neo-clerodane Diterpenes

Neo-clerodane diterpenes are the characteristic type of phytochemicals from the genus *Ajuga* L. besides valuable pharmacological properties (28). Neo-clerodane diterpens identified in *Ajuga* species from Iran are represented in Table 2 and Figure 1. Some biological activities of neo-clerodane diterpens have been reported like moderate neuroprotective (60), antifeedant (41), antiproliferative (61), anti-inflammatory (62, 63), antinociceptive (64), and vasorelaxant effects (65). Also, inhibitory activities on LPS-induced NO production (66) and RANKL-induced Osteoclastogenesis (67) have been shown in previous studies.

3.2.1.2. Iridoids

Iridoids are a group of secondary metabolites with a wide range of biological activities present in the genus *Ajuga* L. (68). Among these compounds, 8-acetylharpagide has been reported to show a strong antitumor-promoting activity (69). Also, iridoids such as ajugoside, asperulosidic acid, and deacetyl-asperulosidic acid isolated from *A. chamaepitys* subsp. *chia* showed antiprotozoal activity (70). In addition, 6-deoxyharpagide exhibited anti-inflammatory effects via COX-2 inhibition (71). Chemical structures of isolated iridoids from *Ajuga* species in Iran are illustrated in Figure 2 and Table 2.

3.2.1.3. Phytoecdysteroids

The ecdysteroids are a class of polyhydroxysteroids existing in the genus *Ajuga*. This group of secondary compounds exhibits a wide range of biological activities in mammals and adaptogenic, anabolic (72), hypoglycemic (73), hepatoprotective, immunoprotective, wound-healing (72), and free radical scavenging effects (74). Besides, 20-Hydroxyecdysone, cyasterone, and *ajugalactone* are the most common phytoecdysteroids in *Ajuga* specie (75).

3.3. Biological Effects

3.3.1. *Ajuga chamaecistus*

Ajuga chamaecistus is the most extensive species of *Ajuga* in Iran, with four subspecies including *scoparia*, *euphrasioides*, *chamaecistus*, and *tomentella* that are all exclusive to Iran. The plant is distributed in Afghanistan, Central Asia, eastern Turkey, the Caucasus, and Iraq and usually grows on mountainous or rocky slopes. Also, *A. chamaecistus* subsp. *scoparia* is a habitat of Iran's western, central, and southern provinces. Moreover, *A. chamaecistus*

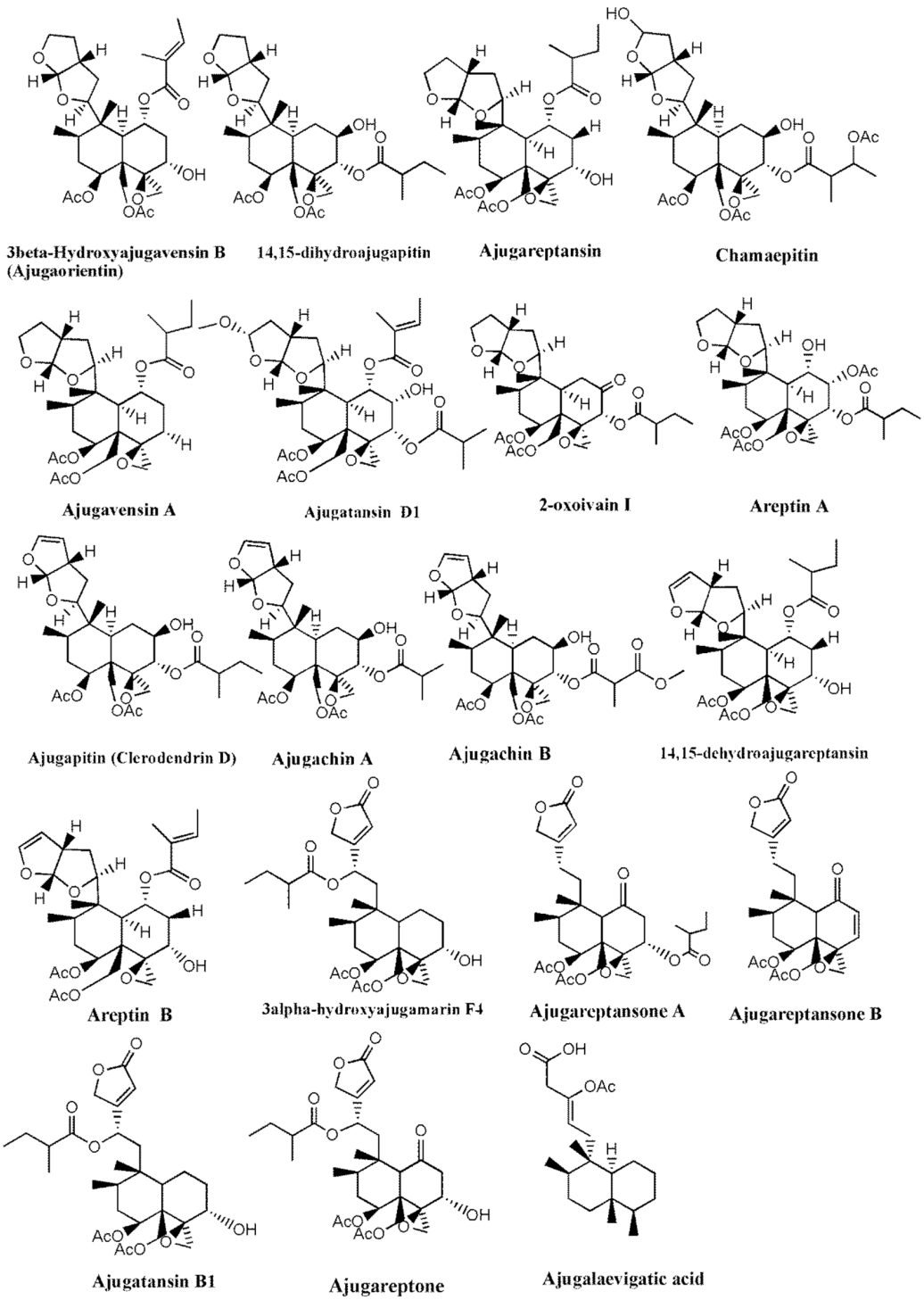

Figure 1. Chemical structure of neo-clerodane diterpenoids identified in *Ajuga* species growing in Iran

Table 1. Traditional Uses, Pharmacological, and Clinical Studies of *Ajuga* Genus Grown in Iran

Plant Name	Traditional Use	Region	Pharmacological and Clinical Studies	References
***Ajuga orientalis* L.**	Skin diseases, diabetes, digestive, rheumatism	Anatolia, Palestinian area	Antibacterial and antioxidant effect, plant antifungal	(6, 29, 30)
***Ajuga austro-iranica* Rech.f.**	Women infertility, gynecological problems, cardiac problems, hypertension, constipation	Kohghiluyehva Boyer Ahmad	-	(30)
***Ajuga* comate synonym of *Ajuga chamaepitys* subsp. *Chia* (Schreb.) Arcang.**	Cancer antiarthritic external, wound healing, diarrhea, hemorrhoids, and various intestinal infections	Iran, Eurasia, Jordan, Europe, Turkey	Antibacterial effect	(22, 31)
***Ajuga chamaepitys* (L.) Schreb. subsp. *Chia* (Schreb.) Arcang. Var. ciliate Briq.**	Menstruation, diaphoretic	Turkey	Anticolitis effect	(32, 33)
Ajuga chamaepitys	Diuretic, tonic, emmenagogue agent for wound-healing and perspiration; treating scorpion and snake bites, hemorrhoids, stomachache, jaundice, inflammatory diseases, such as gout and joint pains, and common colds; antimicrobial, antiviral, and antifeedant	Iran	Antioxidant activity, antiproliferative activity, analgesic effect	(17, 34-38)
Ajuga chamaecistus	Treatment of edema, jaundice, joint pains, and sciatica. Topically, it has been used for wound healing, and breast hardness; diuretic and emmenagogue agent	Iran	*Ajuga chamaecistus* ssp. tomentella: Anti-inflammatory and analgesic, cytotoxic, antibacterial, antioxidant, blood sugar lowering, toxicity studies larvicidal, antibacterial; *Ajuga chamaecistus* subsp. scoparia: Antimicrobial effect antidiabetic, skincare, and neuroprotective effect	(2, 3, 8-11, 23, 39, 40)
***Ajuga reptans*L.**	Anti-inflammatory, wound healing, hepatoprotective properties, mild analgesic	The center and, especially, the eastern part of Europe, northern Iran, Caucasus	Antioxidant and antibacterial effect, antifeedant effect; anti-colitis effect	(41-44)

subsp. *euphrasioides* grows in central Iran and *A. chamaecistus* subsp. *chamaecistus* grows in the northwestern, western, and central provinces of Iran.

In addition, *A. chamaecistus* subsp. *tomentella*, also known as Sefid Moshkak and Mash Daro, is a shrub and perennial herb that grows mainly in mountainous areas and sporadically in other steppe and semi-steppe areas. Its habitat is located in the west, center, and south of Iran (2, 4, 76).

3.3.1.1. Ajuga chamaecistus Ging. ssp. tomentella

3.3.1.1.1. Anti-inflammatory and Analgesic Effects

As demonstrated in the study by Khanavi et al., the oral administration of different doses of methanolic and aqueous extracts and their fractions (200, 400, and 600 mg/kg) had analgesic effects in the chronic phase (15 - 60 min after formalin injection) in mice. Besides, total water and diethyl ether extracts at a dose of 400 mg/kg presented a very significant analgesic action (2).

3.3.1.1.2. Cytotoxic Effects

In the study by Sadati et al., the cytotoxic effect of the main compounds isolated from plant methanolic extract, including 20-hydroxyecdysone, cyasteron, and 8-acetylharpagide, on cancer cells (T47D, Caco-2 , and HT-29) and normal cells (NIH 3T3) was investigated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay. It was found that the main components of this plant had no cytotoxic effects up to 400 μg/mL (23).

3.3.1.1.3. Antibacterial Effects

In another study, the antibacterial effect of different extracts and fractions of *A. chamaecistus* ssp. *tomentella* was investigated on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aerorginas*. All fractions were effective on the tested bacterial species. The diethyl ether fraction had the greatest effect on *Staphylococcus* and *Bacillus* species. The greatest effect of butanol fraction was on *E. coli* (8).

In the study by Shams Ardekani et al., the antibacterial effect of volatile oil from aerial parts of *A. chamaecis-*

Table 2. Phytochemicals from *Ajuga* Species Growing in Iran

Ajuga Species	Chemical Compound	Part Used	References
***Ajuga orientalis* L.**	Neo-clerodane-type diterpenes: Ajugorientin	Aerial parts	(45)
Ajuga chamaepitys	Phenylethanoidglycoside: Acteoside; flavone glycosides: Chrysoeriol 7-Oglucopyranoside; (3'-methoxy-luteolin 7-O-glucopyranoside) and apigenin 7-Orhamnopyranoside, isovitexin, orientin, flavonol, and cyaniding; iridoid: Ajugoside, reptoside, 8-O-acetylharpagide, harpagide, 6-O-β-D-glucopyranosyl-harpagide, asperulosidic acid and deacetylasperulosidic acid. 6-O-β-, D-glucopyranosyl-8-O-acetylharpagide; phytoecdysteroids: Cyasterone, ecdysterone (20-hydroxyecdysone); neo-clerodanediterpenoids: *ajugapitin* (Clerodendrin D), 14,15-dihydro*Ajuga*pitin, chamaepitin, *ajugachin* A, *ajugachin* B, *ajugalaevigatic* acid	Aerial parts; flowering aerial; parts and root	(35, 36, 51, 52)
***Ajuga chamaepitys* subsp. *chia* (syn. *A. comata*)**	Neo-clerodanediterpenoids; *ajugachin* A, *ajugachin* B, *ajugapitin* and 14,15_*dihydroajugapitin*; iridoidglycoside: Ajugoside, asperulosidicacid and deacetyl-asperulosidic acid	Aerial parts	(32, 53)
Ajuga chamaecistus* ssp. *tomentella	Coumaric acid derivative: Cis-melilotoside and trans-melilotoside; phenylethanoid glycosides: Lavandulifolioside, leonoside B, martynoside; ecdysteroids: 20-hydroxyecdysone, cyasterone, *ajugalactone*, makisterone A, 24-dehydroprecyasterone, *ajugalide*-E; iridoids: 8-acetylharpagide		(11, 23)
***Ajuga reptans* L.**	Anthocyanins: Delphinidin 3-(p-coumaroylferuloyl) sophoroside-5-malonylglucoside, delphinidin 3-(diferuloyl) sophoroside-5-malonylglucoside, cyanidin 3-(di-p-coumaroyl) sophoroside-5-glucoside, delphinidin 3-(diferuloyl) sophoroside-5-glucoside and cyanidin 3-(feruloyl-p-coumaroyl) sophoroside-5 malonylglucoside; iridoidglucosides: Ajureptaside A, Ajureptaside B, ajureptaside C, ajureptaside D, reptoside, harpagide, 6-epi-acetyl harpagide, acetyl harpagide; aliphatic alcohol glycoside: 1-octen-3-ol 3-O-glucopyranosyl-(1→2)-(48)-glucopyranoside; ecdysteroids: *ajugalactone*, 20-hydroxyecdysone, 20-hydroxyecdysone; 3-acetate, 29-Norsengosterone,2-Acetyl-29-norcyasterone, 3-Acetyl-29-norcyasterone,Sengosterone, *ajugasterone* B; neo-clerodanediterpenoids:14,15-dehydroajugareptansin, 3β-hydroxy *ajugavensin* B and 3α-hydroxy ajugamarin F4,*ajugareptansin*, *ajugareptansone* A, B, *ajugavensin* A, *ajugatansin* B1, D1, *ajugareptone*, *ajugalaevigatic* acid, 2-oxoivain I, areptin A, B,*ajugaorientin*,*ajugachin* A; abietanes: *ajugaside* A; phenylpropanoidglycosides: Teupolioside		(48, 51, 54, 55)

tus subsp. *chamaecistus* was assessed using the disk diffusion process against Gram-positive bacteria (*S. aureus* and B. *subtilis*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Results displayed no considerable antibacterial activities against Gram-positive and Gram-negative bacteria, with 25 microliters per liter (40).

3.3.1.1.4. Antioxidant Effects

In the study of Sadati Lamardi et al., the antioxidant effect, free radical scavenging activity, and total phenolic content of aqueous and methanolic extracts of aerial parts of the plant were investigated using ferric reducing antioxidant power, DPPH (diphenyl-picryl-hydrazyl) test, and Folin-Ciocalteu methods. The results showed that the butanol fraction had the highest phenolic content (26.5 mg GAE/g of extract) and the highest antioxidant power (346.7 mmol Fe/g of extract) and inhibited DPPH free radicals (IC50 = 15.34 μg/mL) (10).

3.3.1.1.5. Blood Sugar Lowering Effect

In another study, the hypoglycemic effects of aqueous and methanol extracts of this plant were investigated in the model of induced diabetes using streptozotocin in Syrian mice. The results showed that all doses of the extract (200, 400, and 800 mg/kg) in oral administration reduced plasma glucose on days 3, 14, and 28 of the study. A dose of 400 mg/mL of the methanolic extract more greatly decreased glucose levels on the 14th day than metformin (500 mg/kg) (10).

3.3.1.1.6. Toxicity Studies

Acute and chronic toxicity of hydroalcoholic extract of this plant were investigated in rat. The acute toxicity study showed that the ethanolic extract was non-toxic up to a dose of 6,000 mg/kg. Based on the results of sub-chronic toxicity, after using the extract (1000 mg/kg) for 23 and 45 days, a significant reduction was observed in cholesterol and triglycerides. Histopathology of animal tissues did not show significant differences in animal tissues after 23 and 45 days between the treated and control groups (10).

3.3.1.1.7. Larvicidal Effect

In a study by Khanavi et al., the lethal effect of different fractions of methanolic extract of the plant was investigated on the malaria vector Anopheles stephensi larvae. The results showed that the hexane fraction of the extract could kill 100% of the larvae at a concentration of 102 ppm (11).

3.3.1.2. Ajuga chamaecistus subsp. scoparia

3.3.1.2.1. Antimicrobial Effect

Haghir Ebrahimabadi et al. reported the antimicrobial effect of essential oil prepared from flowering samples of *A. chamaecistus* subsp. scoparia collected from the Kashan

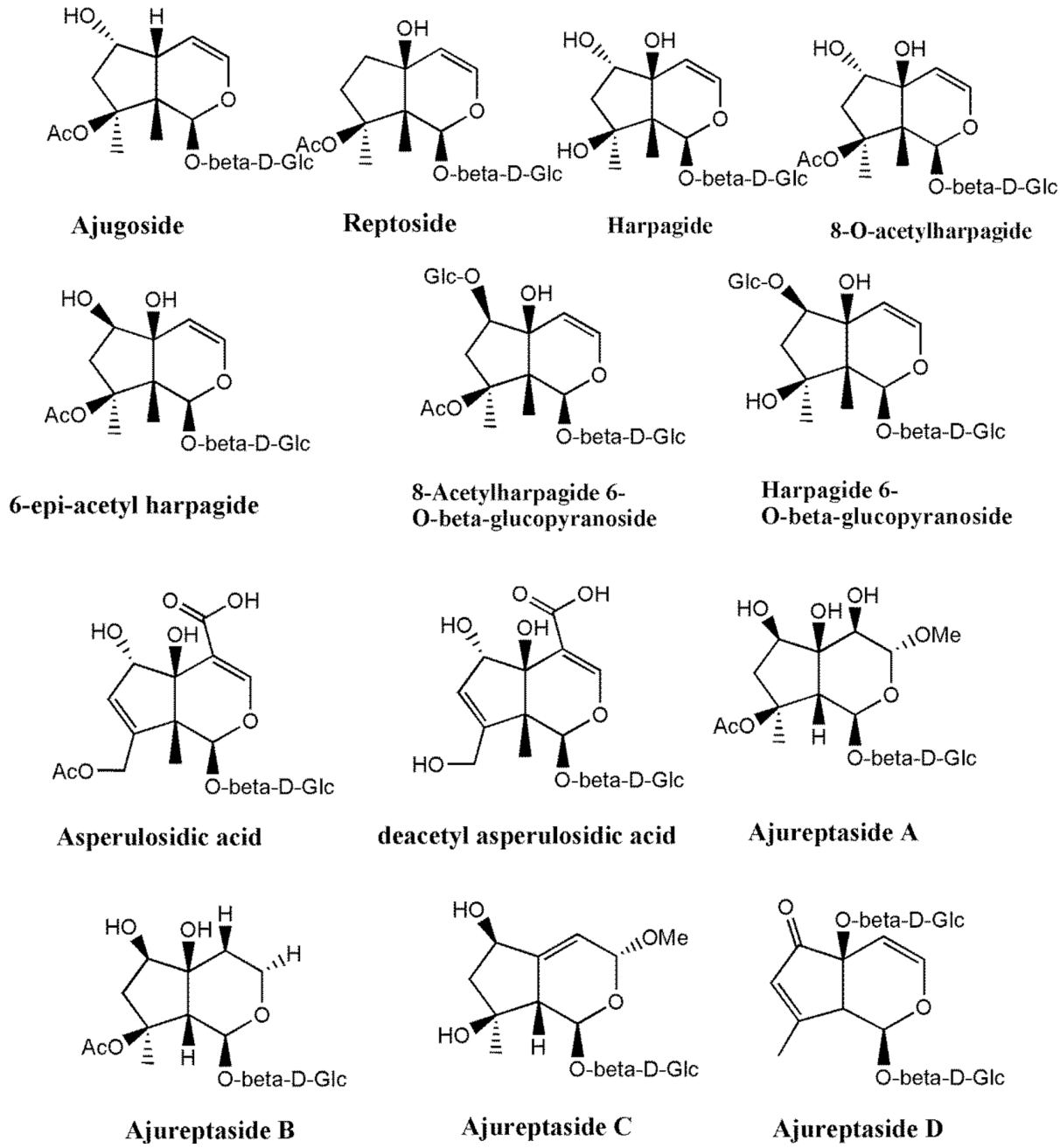

Figure 2. Chemical structure of iridoids identified in *Ajuga* species growing in Iran

area by agar disk diffusion method against 12 microorganisms, including Gram-negative and Gram-positive bacteria *P. aeruginosa*, *E. coli*, *B. subtilis*, *S. aureus*, *K. pneumonia*, *S. epidermidis*, *S. dysenteriae*, *P. vulgaris*, and *Salmonella paratyphi*-A serotype. Plant essential oil showed significant antimicrobial activity against all microorganisms. The minimum inhibitory concentration (MIC) values for microbial strains were 125 - 4000 μg/mL. The essential oil of this plant containing β-pinene, α-pinene, limonene, linalool, and eugenol as the main components showed significant antimicrobial potency compared to positive control antibiotics (9). Mohammadi-Bazargani et al., in an in vitro study, examined the antibacterial activity of the essential oil of this plant. The results exhibited antibacterial action on

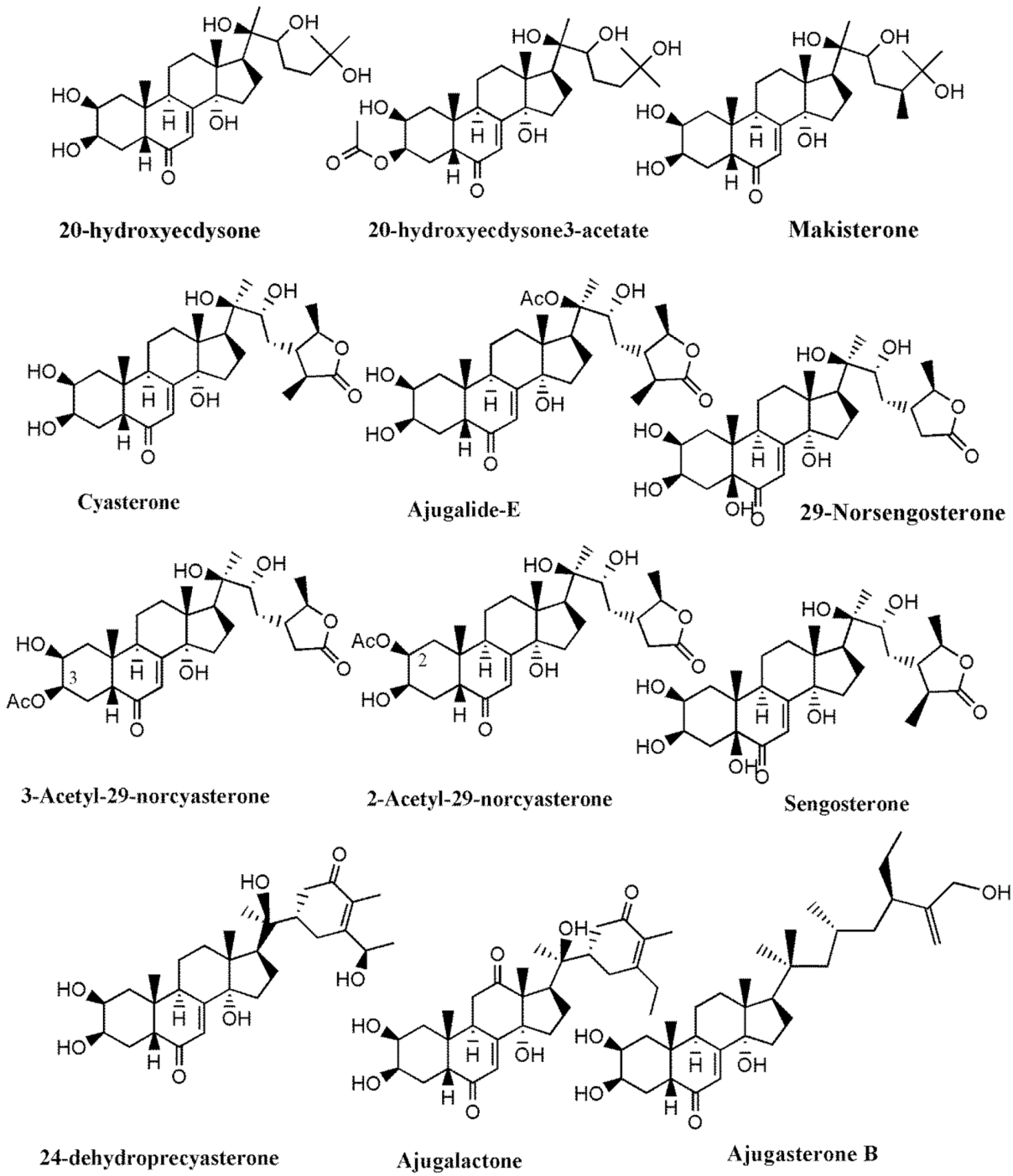

Figure 3. Chemical structure of phytoecdysteroids identified in *Ajuga* species growing in Iran

both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, while hexane extract showed antibacterial effects only against *S. aureus* (39).

3.3.1.2.2. Antidiabetic, Skin Care, and Neuroprotective Effect

Movahhedin et al. evaluated antidiabetic, cholinesterase inhibitory, tyrosinase inhibitory, and

Table 3. Essential Oil Analysis of *Ajuga* Species Growing in Iran [a]

Ajuga Species	Part Used	Method of Extraction-Method of Identification	Main Components (%) (K.I.)	References
***Ajuga orientalis* L.**	Aerial parts	Hydrodistillation by Clevenger-type apparatus for 3 h-GC/MS	Germacrene-D (24.2%) (1480), β-cubebene (18.3%) (1393), β-caryophyllene (16.9%) (1418),α-cubebene (5.3%) (1349), β-selinene (4.5%) (1489), bicyclogermacrene (4.4%) (1496) andα-humulene (4.2%) (1452)	(56)
Ajuga austro-iranica	Aerial parts	Hydrodistillation by Clevenger-type apparatus for 4 h-GC/MS	Trans-Verbenol (a pinene-type Monoterpenoid)(7%) (1144), caryophyllene oxide (6.8%) (1581), 6,10,14,-trimethyl-2-pentadecanone(6.5) (1844), myrtenol(6.3) (1196), 1-octen-3-ol (6.2) (980), β-pinene (6.1) (979),verbenone (5.7) (1208)	(57)
Ajuga chamaepitys	Aerial parts	Hydro-distillation method by the Clevenger apparatus for about 4 h,- GC-MS and GC-FID	α-pinene (23.66%) (931), β-pinene (9.33%) (971), 1-octen-3-ol (9.72%) (971), β-phellandrene (8.70%) (1022) and germacrene-D (7.92%) (1477)	(36)
Ajuga chamaepitys	Fresh aerial parts	Hydrodistillation-GC/FID and GC/MS	The monoterpene hydrocarbons α-pinene (16.1%) (936) and β-pinene (34.38) (981), γ-terpinene (7.7%) (1062), limonene (6.1%) (1032) and mycrene (1.4%) (994) The main sesquiterpene hydrocarbons identified were germacrene D (5.6%) (1487), γ-elemene (3.7%) (1439), β-cubebene (1.7%) (1396), α-copaene (1.8%) (1382) and β-bourbonene (1.5%) (1392)	(37)
Ajuga chamaepitys	Fresh flowering aerial parts	Aerial parts, hydrodistillation by a Clevenger type apparatus -GC-FID and GC-MS	Germacrene D (13.4%), kaurene (8.3%) and (E)-phytol (5.3%), ethyl linoleate (13.7%), and β-pinene (6.8%), oxygenated monoterpenes (4.1%), linalool (3.2%), globulol (3.3%), and 1-octen-3-ol (4.9%)	(36)
Ajuga chamaecistus* subsp. *scoparia	Crushed flowers	SDE (simultaneous distillation–extraction) and Clevenger apparatus for 3.5 h.	β-pinene (23.5%) (993), α-pinene (6.9%) (944), limonene (10.8%) (1042), linalool (8.3) (1113) and eugenol (7.7%) (1373).	(9)
Ajuga chamaecistus* subsp. *scoparia	The aerial parts	Hydrodistillation using a Clevenger-type apparatus for 3.5 h.	β-pinene (16.08%) (981), α-thojene (10.66%) (926), α-pinene (7.43%) (939), linalool (7.37%) (1101), bicyclogermacrene (6.71%) (1494), δ-cadinene (6.40%) (1538), limonene (4.95%) (1033) and spathulenol (3.09%) (1581)	(4)
***Ajuga chamaecistus Ging.* subsp. *tomentella*Rech. f.**	The aerial parts	Hydrodistillation using a Clevenger-type apparatus for 4 h-GC/MS	Thymol (34.45 %) (1288), exo-fenchol (15.58 %) (1120), β-pinene (8.26 %) (974), 1-octen-3-ol (5.92 %) (975), α-terpineol (3.88 %) (1158), 2-hexanol (3.85 %) (850), α-thujene (2.66%) (928), and α-pinene (2.54 %) (935).	(40)
***Ajuga reptans* L.**	The dried flowers and leaves	Hydrodistillation	Flowers: Hexadecanoic acid (38.0%) (1969), 6,10,14-trimethylpentadecan-2-one (16.4%) (1846), n-tetradecane (13.0%) (1400), and (Z,Z,Z)-9,12,15-octadecatrienoic acid methyl ester (7.3%) (2135). Leaves: 1-octen-3-ol (55.6%) (979), hexadecanoic acid (10.7%) (1969), terpinolene (5.6%) (1089), and 6,10,14-trimethyl-2-pentadecanone (5.2%) (1846).	(58)
***Ajuga reptans* L.**	Fresh aerial parts	Hydrodistillation using a Clevenger type apparatus for 4 h-GC/MS	1-octen-3-ol (40.7 %) (979), linalool (13.7%) (1101), n-hexadecanoic acid (11.7%) (1973), n-heptacosane (5.2%) (2700), 2-methylbenzofuran (4.6%) (1166)	(59)

[a] RI: Kovats retention indices.

antioxidant effects of essential oils and different extracts of *A. chamaecistus* subsp. *scoparia*, and identified their chemical compositions. The highest inhibitory effect on sugar-degrading enzymes was observed by volatile oils. The results exhibited that the essential oil significantly reduced the activity of α-glucosidase (equivalent to 4.3 mmol of acarbose per gram), α-amylase (2.8 mmol equivalents of acarbose per gram), acetyl cholinesterase (1.96 mg equivalent of gallic acid per gram), butyrylcholinesterase (2.2 mg gallic acid equivalent per gram), and tyrosinase (36 mg equivalent of kojic acid per gram); however, ethanolic and aqueous extracts displayed moderate effects on enzyme activity. Twenty-three phenolic compounds were extracted from the plant, and p-coumaric acid was the major component in both extracts. In addition, gallic acid and ferulic acid were the main compounds in aqueous and ethanolic extracts, respectively. Volatile oils of this plant showed longer anticholinesterase activity than aqueous decoction and ethanolic extract of the plant, so it may be used to treat Alzheimer's disease since the plant has high radical scavenging and metal chelating activity. Therefore, it has a good antioxidant capacity and can be used in skin

problems (3).

3.3.2. Ajuga chamaepitys (L.) Schreb.

3.3.2.1. Antiproliferative Effect

The cytotoxicity effects of *A. chamaepitys* essential oil, ethanolic, and aqueous extracts were tested by the MTT assay against human malignant melanoma (A375), human breast adenocarcinoma (MDA-MB 231), and human colon carcinoma (HCT116) cell lines. The results of this study showed that essential oil exhibited a moderate cytotoxic effect on MDA-MB 231, HCT116, and A375 cells with IC50s of 59.24, 64.12, and 67.44 μg/mL, respectively, while ethanolic extract was more effective on MDA-MB-231 than HCT116 cell line with IC50s of 36.88 and 60.48 μg/mL, respectively (35).

3.3.2.2. Analgesic Effect

In a study, Jaffal et al. investigated the analgesic effect of *A. chamaepitys* methanolic extract growing in Jordan with chemical and thermal pain-induced models in mice. The results showed that the extract of this plant significantly reduced the number of writhes in mice, comparable with the negative control group. The inhibitory effect of 300 mg/kg i.p. of the extract was similar to that of 300 mg/kg aspirin. Administration of 450 mg/kg (i.p.) of plant extract significantly reduced paw licking time in the early and late stages of the formalin test. The delay time in the hot plate test was also increased. Before the treatment, Naloxone injection having the extract reduced the analgesic effect of the extract. As a result, the analgesic effect of this plant can be affected by its effect on opioid receptors. The LC-MS analysis of the extract led to the identification of 19 compounds, the most important of which were isovitexin, orientin, flavonol, and cyanidin (52).

3.3.2.3. Antioxidant Activity

Venditti et al. studied the free radical scavenging activity of the essential oil, aqueous, and ethanolic extracts of *A. chamaepitys* aerial branches utilizing DPPH and ABTS+ free-radical-scavenging assays. According to the results, aqueous and ethanolic extracts displayed comparable radical scavenging properties in ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) test with IC50 = 50.0 and 54.9 μg/mL, about 27 folds higher than that of Trolox. Additionally, both aqueous and ethanolic extracts and the essential oil exhibited moderate antioxidant effects on DPPH radicals with IC50s from 83 to 300 times higher than that of Trolox (35). In the study by Delazar et al., the free-radical-scavenging potential of the extracts, fractions, and isolated compounds from A. chamaepitys was tested by the DPPH assay. The methanol extract presented the highest free-radical-scavenging activity (RC50: 1.15 $\times$ 10^{-1} mg/mL), and chrysoeriol 7-O-glucopyranoside was the most effective compound (RC50: 3.00 $\times$ 10^{-3} mg/mL) (36). Based on the antioxidant properties of *A. chamaepitys*, this plant can be suggested as a natural source of antioxidants, dietary supplements in pharmaceutical manufacturing, and stabilizing food against oxidative deterioration (34).

3.3.3. A. chamaepitys subsp. euphratica

3.3.3.1. Antimicrobial Activity

Turkoglu et al. examined the methanol, water, and chloroform extracts of *A. chamaepitys* subsp. *euphratica* using the disk diffusion method for antimicrobial properties. They tested the extracts on the growth of six bacterial species *E. coli*, *S. aureus*, *B. cereus*, *P. aeruginosa*, *K. pneumoniae*, and *E. aerogenes*, and two yeast species. The extracts showed antimicrobial effects on Gram-negative bacteria (inhibition zone diameter: *E. coli* 13 mm, *P. aeruginosa* 15 mm, *K. pneumoniae* 15 mm, and *E. aerogenes* 12 mm); however, they did not show any antimicrobial activity against Gram-positive bacteria and yeasts *Saccharomyces cerevisiae* and *Candida* sp. (34).

3.3.3.2. Antioxidant Activity

Several studies reported the antioxidant action of extracts and phenolic compounds of *A. chamaepitys* from different countries using the ABTS and DPPH antioxidant tests. Turkoglu et al. evaluated the antioxidant power of *A. chamaepitys* subsp. *euphratica* using ABTS, DPPH, hydrogen peroxide scavenging, metal chelating properties, butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), and α-tocopherol. The results exhibited that methanol, aqueous, and chloroform extracts had high antioxidant power in all tests compared to industrial antioxidants (34).

3.3.4. A. chamaepitys subsp. chia (Ajuga comata Stapf.)

3.3.4.1. Anti-colitis Effect

Ajuga comate Stapf. is the synonym of *A. chamaepitys* subsp. *chia* distributed in Golestan, Mazandaran, Guilan, Azerbaijan, Kordestan, Hamedan, and Tehran provinces, Iran (42). In folk medicine of Turkey, it is a plant used for diarrhea. In a study reported by Kupeli Akkol et al., the total methanolic extract (MeOH) and fractions of aerial fragments of *A. chamaepitys* subsp. *chia* were examined in a colitis model with acetic acid in rats. The MeOH extract and n-Buthanol fraction controlled the caspase-3, myeloperoxidase, TNF-α, IL-6 levels, and antioxidant factors. Furthermore, ajugoside, asperulosidic acid, and deacetyl-asperulosidic acid were isolated from the n-BuOH fraction (32).

3.3.4.2. Antibacterial Effect

In the study by Asgharian et al., the extracts of n-hexane, dichloromethane, and methanol extracts of aerial parts of the plant were studied against different microorganisms by the agar disk diffusion method. The effective extracts were then fractionated by the VLC technique and

determined by the MIC serial dilution method. Finally, *Ajuga comata* did not show any inhibitory effect against any of the microorganisms *S. aureus* and *B. subtilis* (31).

3.3.5. Ajuga orientalis

3.3.5.1. Antibacterial Effect

In the study of Goger et al., the antibacterial effect of a methanolic extract of *A. orientalis* in the flowering stage was evaluated against seven strains *E. coli*, S. aureus, *S. typhimurium*, *B. cereus*, *C. albicans*, *C. tropicalis*, and *C. parapsilosis*. The minimum inhibitory concentration (MIC) of the strains was assessed by broth microdilution methods compared to standard antimicrobial drugs such as ampicillin, tetracycline, ketoconazole, and oxiconazole. The results showed that *A. orientalis* extract had an antimicrobial effect against all tested microorganisms and was more effective against the yeast *C. tropicalis* (MIC = 78.12 μg/mL). Due to its antimicrobial activity, especially against *Candida*, the extract of this plant can be used for the natural prevention of *Candida* infections (29).

3.3.5.2. Plant Antifungal Effect

In an in vitro study, the effects of essential oils and extracts of *A. orientalis* were investigated against *Phytophthora capsicileon*, a pathogenic fungus causing common root throat burn red pepper disease. The results showed that the chloroform extract of the plant had the highest inhibitory effect (MIC 0.800 to 0.825 mg/mL) on this fungus (29).

3.3.6. Ajuga austroiranica Rech.f.

Ajuga austroiranica is a beautiful perennial plant widely distributed on rocky slopes and crevices in the mountainous regions of southern and southwestern Iran. The analysis of volatile essential oil compounds obtained from *A. austroiranica* collected from Fars province was performed by GC/MS. The result of the analysis showed that monoterpenes (30.5% pinene structure) were the main components of the essential oil (Table 3) (57).

3.3.7. Ajuga reptans L.

Ajuga reptans are usually known as bugle, bugleweed, and common bugle. *Ajuga reptans* L. is distributed in the center and especially the eastern part of Europe, northern Iran (Mazandaran and Guilan provinces), and the Caucasus (42). In folk medicine, *A. reptans* L. is used as antidiabetic, anti-hypertensive, diuretic, and hepatoprotective medicinal plant (77). Several phytochemicals have been identified in *A. reptans*, such as ecdysteroids, anthocyanidin glycosides, phenylpropanoid glycoside, neo-clerodane diterpenes, and iridoids that possess antioxidant, antimicrobial, and anti-inflammatory activity (43, 78).

3.3.7.1. Antifeedant Effect

Insect antifeedant evaluation of neo-clerodane diterpenoids isolated from the aerial parts of *A. reptans* showed that 14,15-dehydroajugareptans had a significant effect on sixth stadium larvae of Spodoptera littoralis (41).

3.3.7.2. Anti-colitis Effect

Di Paola et al. reported that teupolioside, a phenylpropanoid glycoside, produced by *A. reptans* cell line, meaningfully decreased diarrhea manifestations and body weight loss in a rat model of colitis by applying a significant reduction in colonic myeloperoxidase activity and malondialdehyde levels. Moreover, the reduced release of pro-inflammatory cytokines was observed. Therefore, teupolioside may be valuable for treating inflammatory bowel disease (44).

3.3.7.3. Antioxidant and Antibacterial Effect

The antioxidant and antibacterial potential of active compounds from *A. reptans* flowers were evaluated in a study by Toiu et al. The results indicated that total phenols, flavonoids, anthocyanins, and iridoids were higher in ethanol/water extraction with maceration and reflux heating than methanol/water extraction.

Besides, *A. reptans* flowers extracts showed different degrees of DPPH radical scavenging activity. The ethanol extract showed the highest radical scavenging effect (49.35 ± 2.91 μg/mL), and also a high antioxidant activity (IC50 $\leq$ 50 μg/mL).

The antibacterial assay of *A. reptans* flower extracts showed moderate antibacterial activity against *P. aeruginosa*, *L. monocytogenes*, *E. coli*, and *S. typhimurium*. In addition, *A. reptans* ethanol extract showed the most significant antimicrobial effect on *S. aureus* and *P. aeruginosa* (43).

4. Conclusions

The aim of this research was to investigate different species of *Ajuga* growing in Iran in terms of traditional uses, phytochemical compositions, and pharmacological or clinical studies. As known, *A. chamaecistus* is the most widespread species in Iran with five exclusive subspecies. Also, numerous phytochemical and pharmacological studies have been performed on various species, especially tomentella. Various pharmacological effects have been reported on these species, confirming the traditional use of the genus *Ajuga* in many cases, such as anti-inflammatory, hypoglycemic, and analgesic activities. Given that most pharmacological studies have been performed on animals, and on the other hand, toxicological studies have been assessed on some species, it has been shown that the plants of this genus are non-toxic, and there is a need for clinical studies on the use of medicinal products containing these plants or phytochemical compounds isolated from

them as an analgesic and anti-inflammatory agents. Since different species of these plants have valuable compounds such as phytoecdysteroids, which can be used in cosmetics for wound healing and hair growth, it seems that studies in these fields can be considered therapeutically and economically valuable.

The results of modern medicine were consistent with traditional medicine in many cases, such as analgesic, anti-inflammatory, and hypoglycemic properties. Due to the beneficial therapeutic effects of the *Ajuga* genus, it can be considered in future clinical studies and the production of complementary drugs. Also, given that preclinical and clinical studies are not yet sufficient, the results of the studies should be carefully interpreted and generalized.

Footnotes

Authors' Contribution: Original idea and the protocol, S.N. S.L.; Acquisition of data, A. R. and M. H.E.; Drafting of the manuscript, A. R. and S.N. S.L.; Critical revision of the manuscript for important intellectual content, M.H. A., H. H.G., and S.M.B. F. All authors read and approved the final manuscript.

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