Phytochemical Characterization of Phenolic Compounds by LC-MS/MS and Biological Activities of *Ajuga reptans* L., *Ajuga salicifolia* (L.) Schreber and *Ajuga genevensis* L. from Turkey

**ABSTRACT**

**Objectives:** In this study, it was aimed to characterize the phenolic contents of *Ajuga reptans* L., *Ajuga salicifolia* (L.) Schreber, and *Ajuga genevensis* L. and to investigate their *in vitro* antioxidant and antimicrobial activities.

**Materials and Methods:** Air dried aerial parts of *A. reptans* L., *A. salicifolia* (L.) Schreber, and *A. genevensis* L. collected from Turkey were extracted with methanol (70%), and the phenolic composition of the crude extracts was analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. To determine the total phenolic content the Folin-Ciocalteu method was used. The radical scavenging activities of the extracts were evaluated by the photometric 1,1-diphenyl-2-picrylhydrazyl radical, and trolox equivalent antioxidant capacity assays (TEAC). Furthermore, *Ajuga* sp. extracts were tested against *Escherichia coli* NRRL B3008, *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 13311, *Bacillus cereus* NRRL B-3711, *Candida albicans* ATCC 90028, *Candida tropicalis* ATCC 1369, and *Candida parapsilosis* ATCC 22019 using the *in vitro* broth dilution assay.

**Results:** The LC-MS/MS analyses identified 19 compounds. The amount of total phenolics ranged from 30.0 to 42.2 mg gallic acid equivalent/g in all extracts. According to the results of TEAC assay, the tested extracts were found to have relatively high activity at 1.2-1.5 mM concentrations. *Ajuga* sp. extracts inhibited all tested microorganisms; however, *C. albicans*, *C. tropicalis*, and *C. parapsilosis* exhibited relatively more susceptibility (minimum inhibitory concentration: 156.25 μg/mL) compared to the bacteria tested.

**Conclusion:** The antioxidant activities of all extracts were determined for the first time by the TEAC method, and the *in vitro* antimicrobial activity of *A. salicifolia* was investigated for the first time against selected strains.

**Key words:** *Ajuga reptans*, *Ajuga salicifolia*, *Ajuga genevensis*, LC-MS/MS, antioxidant activity, antimicrobial activity

**ÖZ**

Amaç: Bu çalışmada Türkiye'de yetişen *Ajuga reptans* L., *Ajuga salicifolia* (L.) Schreber ve *Ajuga genevensis* L.'nin fenolik içeriklerinin karakterizasyonu, *in vitro* antioxidan ve antimikrobiyal aktivitelerinin araştırılması amaçlanmıştır.

**Gereç ve Yöntemler:** *A. reptans* L., *A. salicifolia* (L.) Schreber ve *A. genevensis* L.'nin toprak üstü kısımları metanol (%70) ile ekstre edilmiş ve liyofilize edilerek sıvı kromatografi tandem kütle/kütle spektrometre (LC-MS/MS) ile karakterizasyonları yapılmıştır. Toplam fenolik madde miktarları Folin-Ciocalteu yöntemi ile belirlenmiştir. Ekstrelerin radikal süpürücü etkileri, 1,1-difenil-2-picrilhidrazil ve trolox eşdeğeri antioxidant
INTRODUCTION

The Lamiaceae family includes more than 245 genera and 7886 species distributed worldwide.1 Ajuga L. is a genus of annual and perennial herbaceous flowering plants in the Lamiaceae family, with most species native to Asia, Africa, and Europe.2 Ajuga is represented by 14 species and 27 taxa in Turkey.2 Ajuga has a long history of use for wound healing preparation, and although little used today, it is well known in Anatolia as "Mayasil otu". Some Ajuga species are widely consumed as diuretic, diaphoretic, astringent, antipyretic, and tonic in Turkish traditional medicine.3 Ajuga sp. plants are reported for their in vitro antimalarial, antimicrobial, antioxidant, anti-inflammatory, lipoxygenase, acetylcholinesterase, and butyrylcholinesterase inhibition, antipyretic, and antiproliferative activities.4,5,6,7,8

Phytochemical constituents diterpenoids, such as phenylethanoid glycosides, sterols, phytoecdysteroids, flavonoids, and iridoids were reported as the main active compounds in Ajuga L. species.11 Ajuga salicifolia sterol glycosides were isolated and tested for antimicrobial and cytotoxic activity.12 Iridoid, ionone, and phenylethanoid glycosides from the same group were also reported for this species.13 Phytochemical profile of Romanian Ajuga genevensis L. and A. reptans reptans were recently reported.6 A summary of phytochemical investigations on A. salicifolia, A. reptans, and A. genevensis species are listed in Table 1.6-36

Bulgular: LC-MS/MS analizleriyle 19 fenolik bileşik tanımlanmıştır. Tüm ekstrelerde toplam fenol miktarı 30,0-42,2 mg gallik asit eşdeğeri/g arasında bulunmuştur. TEAK antioksidan aktive sonucunda ekstreler (1,2-1,5 mM) konsantrasyonlarda nispeten yüksek aktive göstermiştir. Ajuga sp. ekstreleri, test edilen tüm mikroorganizmaları karşı antimikrobiyal aktive göstermiştir. Ancak ekstreler, test edilen bakterileri kıyaslada C. albicans, C. tropicalis ve C. parapsilosis suşlarına karşı nispeten daha fazla etkili (minimal inhibisyon konsantrasyonu: 156,25 µg/mL) bulunmuştur.

Sonuç: TEAK yöntemi ile tüm ekstrelerin ilk defa antioksidan aktiveşleri belirlenmiştir ve A. salicifolia’nın in vitro antimikrobiyal aktivesi seçilen suşlara karşı ilk kez incelenmiştir.

Anahtar kelimeler: Ajuga reptans, Ajuga salicifolia, Ajuga genevensis, LC-MS/MS, antioksidan aktive, antimikrobiyal aktive

| Table 1. Literature data on phytochemical profile for Ajuga species |
|-----------------------|--------------------------|-------------------|
| Species               | Compounds                | References |
| Ajuga reptans         | Iridoid glycoside (ajureptaside) | 19           |
| Ajuga reptans         | Iridoid glucosides (ajureptaside A-D) | 24           |
| Ajuga salicifolia     | Iridoid, ionone and phenylethanoid glycosides (8-O-acetylharpagide corchoionoside C, leonosides A) | 13           |
| Ajuga genevensis      | Neo-clerodane diterpenoids (ajugavensins A-C) | 18           |
| Ajuga salicifolia     | Clerodane diterpine (ajugachin a derivative) | 20           |
| Ajuga reptans         | Neo-clerodane diterpenes (ajugatansins) | 21           |
| Ajuga salicifolia     | Sterol glycosides (ajugasalicioside A-E) | 12           |
| Ajuga reptans         | Phytoecdysteroids (28-Epi-sengosterone) | 36           |
| Ajuga salicifolia     | Stigmastane sterols (ajugasalicigenin) | 22           |
| Ajuga reptans         | Anthocyanins (cyanidin) | 25           |
| Ajuga reptans         | Anthocyanins (delphinidin) | 26           |
| Ajuga reptans         | Anthocyanins (cyanidin and delphinidin glucosides) | 27           |
| Ajuga genevensis      | Hydroxycinnamic acids (cafeic acid, chlorogenic acid), flavonoids (apigenin and luteolin-7-O-glucoside) | 17           |
| Ajuga genevensis      | Hydroxycinnamic acids (cafeic acid, p-coumaric acid, ferulic acid), flavonoids (hyperoside, isoquercitrin, rutin, quercitrin, luteolin, apigenin) | 6            |
| Ajuga reptans         | Hydroxycinnamic acids (p-coumaric acid, ferulic acid), flavonoids (isoquercitrin, rutin, quercitrin, luteolin, apigenin) | 6            |
A. genevensis was used in traditional Austrian medicine and consumed as medicinal tea in treating respiratory tract disorders, and in vitro anticancer activity studies were reported from Europe, Asia, and America.

A. reptans grows natively in Europe and have bluish-purple flowers colored with anthocyanin pigments. It was used in Mediterranean traditional medicine for cardiovascular complications and skin disorders and in traditional Austrian medicine as a medicinal tea for the treatment of respiratory tract disorders. A previous study showed that A. reptans L. is used due to the anti-inflammatory effects of its polyphenols, its wound healing properties, and antidiarrhea, antiulcerogenic, and hepatoprotective effects due to the presence of iridoids.

In the present study, 70% methanol extract of aerial parts of A. reptans L., A. salicifolia (L.) Schreber, and A. genevensis L. from Turkey were evaluated for their phytochemical profiles, total phenol, and total flavonoid contents, as well as their in vitro antioxidant and antimicrobial activities. LC-MS/MS techniques were used to analyze the extracts.

### Table 2. Literature survey of antimicrobial activity for Ajuga salicifolia, Ajuga genevensis, and Ajuga reptans

| Compounds/species | Microorganisms | MIC (mg/mL) inhibition zone (mm) | References |
|-------------------|----------------|----------------------------------|------------|
| Ajuguasalicioside A, B, C, D, E compounds from A. salicifolia | B. cereus ATCC 10702, S. epidermidis ATCC 12228 | No activity was found | 12 |
| MeOH extract of A. reptans | F. oxysporum, F. verticillioides, P. brevum, P. expansum, A. flavus, A. fumigatus, F. oxysporum, F. verticillioides, P. brevum | Range of 2.65 mm-31.65 mm | 37 |
| Water extract from aerial parts of A. genevensis | S. aureus 209, S. aureus (Makarov), S. aureus Type, E. coli 675, S. gallinarum, P. vulgaris, B. subtilis L2, B. anthracoides | Range of 7 mm-15 mm | 38 |
| MeOH extract from A. reptans | B. subtilis ATCC 6633, E. coli ATCC 25922 | 8.5 mm-10.00 mm | 39 |
| Water, MeOH and EtOH extracts from aerial parts of A. reptans | E. coli ATCC 25922, P. aeruginosa ATCC 27853, S. typhimurium ATCC 14028, S. marcescens ATCC 8100, P. vulgaris ATCC 13315, E. cloacae ATCC 23355, K. pneumoniae ATCC 13883, S. pyogenes ATCC 19615, S. aureus ATCC 25923, S. epidermidis ATCC 12228, S. aureus ATCC 49444, P. aeruginosa ATCC 27853, L. monocytogenes ATCC 19114, E. coli ATCC 25922, S. typhimurium ATCC 14028 | MIC value of 1.56-6.25 | 29 |
| MeOH and EtOH extracts from flowers of A. genevensis | S. aureus ATCC 49444, P. aeruginosa ATCC 27853, L. monocytogenes ATCC 19114, E. coli ATCC 25922, S. typhimurium ATCC 14028 | MIC value of 1.56-6.25 | 28 |
| MeOH and EtOH extracts from flowers of A. reptans | S. aureus ATCC 49444, P. aeruginosa ATCC 27853, L. monocytogenes ATCC 19114, E. coli ATCC 25922, S. typhimurium ATCC 14028 | MIC value of 1.56-6.25 | 28 |
| EtOH, PE and Chl. extracts from aerial parts of A. genevensis | A. flavus ATCC 9643, A. niger ATCC 6275, C. albicans ATCC 10231, C. parapsilosis ATCC 22019, P. funiculosum ATCC 56755, A. flavus ATCC 9643 | MIC value of 0.05-0.1 | 6 |
| EtOH, PE and Chl. extracts from aerial parts of A. reptans | A. niger ATCC 6275, C. albicans ATCC 10231, C. parapsilosis ATCC 22019, P. funiculosum ATCC 56755 | MIC value of 0.05-0.025 | 6 |
were used for phytochemical analyses. In vitro, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, and trolox equivalent antioxidant capacity (TEAC) assays were performed. Additionally, antimicrobial properties of Ajuga extracts were assessed against microbial strains of Escherichia coli NRRL B3008, Staphylococcus aureus ATCC 6538, Salmonella typhimurium ATCC 13311, Bacillus cereus NRRL B-3711, Candida albicans ATCC 90028, Candida tropicalis ATCC 1369, and Candida parapsilosis ATCC 22019.

To the best of our knowledge, this is the first study to perform the TEAC antioxidant activity for all extracts and the in vitro antimicrobial activity of A. salicifolia.

**MATERIALS AND METHODS**

**Chemicals**

Antimicrobial standards, Mueller Hinton Broth, and RPMI-1640 medium were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO). All chemicals and solvents used were of analytical grade.

**Plant materials**

A. reptans L.: [A1(E) Kırklareli: İğneada, Fidanlık kavşağı, 350 m, N 41° 52’ 25.3” E 27° 56’ 11”, 21 iv 2009], A. salicifolia (L.) Schreber: (B3, Eskişehir: Çalgan köyü, 1000 m, K 390 39’ 971” D 300 31’ 185”, 31 vi 2010), and A. genevensis L. [A1(E) Kırklareli: Derek köyü, 449 m, K 410 50’ 6.13” D 270 18’ 3.18”, 22 iv 2009] were collected and identified by Dr. Y.B Köse, and herbarium materials were deposited in the Herbarium of Anadolu University, Faculty of Pharmacy under herbarium code YBK1560, YBK1575, and YBK 1561, respectively.

**Preparation of extracts**

The aerial parts of the plants were dried in the shade at room temperature and ground to powder in a mechanical grinder. Each species (1 g) was extracted with methanol (70%, 100 mL) for 24 h, three times a day. After filtration, the solvents were evaporated under vacuo.

**Phytochemical analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS)**

The phytochemical analyses were performed using LC-MS/MS techniques.5

**Determination of phenolic compounds**

The total phenols contained in the extracts were calculated using the Folin-Ciocalteu method equivalent to gallic acid (GA).30 A sample solution (100 μL) and Folin-Ciocalteu reagent (500 μL) were added to a 10 mL scale vessel containing 6 mL of distilled water. After 1 min, 1.5 mL of 20% aqueous Na2CO3 was added and completed with water to reach 10 mL. The reagent-free of extracts was used as the control. After incubation at 25°C for 2 h, the absorbance was read at 760 nm and compared with the GA calibration curve. The total amount of phenolic was calculated as equivalent to GA. Three parallel experiments were performed, and the results were reported as mean values.

**Biological activity**

**DPPH radical scavenging assay**

The DPPH radical scavenging activity was performed according to Kumarasamy et al.31 For this purpose, 100 μL of methanol and samples were transferred to the first column of 96-well microtitre plates. A 10-fold dilution was made in an equal amount of MeOH via a multi-channel pipette and stirred in the vortex for 5 min. The DPPH stock solution was prepared by dissolving 2 mg of DPPH+ in 25 mL of MeOH, and solution was added to each well and left in a dark place for 30 min. Butylated hydroxy toluene (BHT) and GA at the same concentration were used as positive controls, and ultraviolet (UV) absorbance was measured at room temperature using a Biotek microplate spectrophotometer at 517 nm.

The following equations using 50% inhibition concentration (IC50) (equation 1) and percentage (%) inhibition values (equation 2) were calculated as follows:

\[
IC_{50} = \left( \frac{A_{0} - A_{t}}{A_{0}} \right) 
\]

\[
\text{Percentage Inhibition} = \left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100 \]  

**TEAC assay**

Experiments were performed as declared by Papandreou et al.32 sweeping ABTS+ (2,2’-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical and vitamin E. It is based on the comparison of water-soluble analog with trolox. The mixture of 7 mM ABTS+ and 2.5 mM sodium persulfate was kept in the dark for 12-16 h, resulting in the formation of blue-colored radicals. A sample (10 μL) and ABTS+ solution (990 μL) were mixed, and absorbance was measured at 734 nm per minute intervals for 30 min. To find out the TEAC activity results, the ABTS+ radical was plotted using Trolox’s 2.5-2-1.5-1-0.5-0.1 (mM) concentrations, according to the % inhibition values. For quantification, a Trolox calibration curve was used where all experiments were repeated in triplicates.

**Antimicrobial activity**

Antimicrobial activity testing was performed according to the guidelines of broth microdilution methods.33-35 Standard strains, E. coli NRRL B3008, S. aureus ATCC 6538, S. typhimurium ATCC 13311, B. cereus NRRL B-3711, C. tropicalis ATCC 1369, C. parapsilosis ATCC 22019, and C. albicans ATCC 90028, as well as antimicrobial standards, such as ampicillin, tetracycline, ketocanazole, and oxiconazole, were used in this study. Methanol extracts were prepared at 1250-2.44 μg/mL concentrations and dissolved in dimethyl sulfoxide and initial test solutions. Serial dilutions were prepared at 64-0.125 μg/mL for ampicillin, tetracycline, and ketocanazole. All experiments were evaluated in triplicates, and mean values were reported.

**Statistical analysis**

Data obtained from antioxidant and total phenolic content experiments were expressed as mean standard error. IC50 values were estimated using a non-linear regression algorithm.
RESULTS AND DISCUSSION

LC-MS/MS analysis of the extracts

Screening of the extracts by LC-MS/MS enabled the identification of phenolic acids, such as coumaroyl glucoside, flavonoids, and phenylethanoid glycosides. Figures 1-3 show the 280 nm UV chromatograms of *A. reptans*, *A. genevensis* and *A. salicifolia*, respectively. The compounds detected from *Ajuga* sp. methanol extracts are listed in Table 3.

**Figure 1.** HPLC-UV chromatogram (280 nm) of *A. reptans*
HPLC: High performance liquid chromatography, UV: Ultraviolet

**Figure 2.** HPLC-UV chromatogram (280 nm) of *A. genevensis*
HPLC: High performance liquid chromatography, UV: Ultraviolet
Table 3. Phytochemical composition of A. reptans, A. salicifolia and A. genevensis extracts

| No | Compound                        | Rt  | [M-H]- | Fragments          | Plant       | Reference |
|----|--------------------------------|-----|--------|--------------------|-------------|-----------|
| 1  | Caffeoyl glucose                | 6.4 | 341    | 179, 161, 133      | R           | 41        |
| 2  | Coumaroyl glucoside             | 8.5 | 325    | 163, 119           | S (major), G| 41,42     |
| 3  | Caffeic acid                    | 11.2| 179    | 135                | G           |           |
| 4  | Coumaroyl glucoside isomer      | 13.3| 325    | 163, 119           | S (major), G| 41,42     |
| 5  | Luteolin derivative             | 14.6| 487    | 285, 133, 117      | R (major), G| 43        |
| 6  | Apigenin-C-hexoside-C-pentoside| 14.7| 563    | 443, 383, 353      | G           | 44        |
| 7  | Echinocoside                    | 15.4| 785    | 623, 461, 161      | R (major)   | 44        |
| 8  | Forsythoside B                  | 15.6| 755    | 593, 461, 161      | S           | 45        |
| 9  | Forsythoside A                  | 16.3| 623    | 461, 315, 161      | R (major), G| 45        |
| 10 | Cistanoside A                   | 18.5| 799    | 623, 461, 175, 193 | S, R        | 45        |
| 11 | Leonosides A                    | 18.9| 769    | 593, 461, 315, 193, 175 | S | -         |
| 12 | Quercetin glucuronide           | 19.0| 477    | 301, 227, 133      | G           | 47        |
| 13 | Verbascoside                    | 19.2| 623    | 461, 315, 161      | R           | -         |
| 14 | Leucoseptoside A                | 19.6| 637    | 461, 175           | R, S        | 45        |
| 15 | Luteolin glucuronide            | 20.1| 461    | 285                | R, G (major)| 43        |
| 16 | Luteolin glucoside              | 21.6| 447    | 285                | S           | 46        |
| 17 | Luteolin                        | 35.4| 285    | 175, 133           | G           | 6,29      |
| 18 | Apigenin                        | 37.9| 269    | 149, 117           | G           | 29        |

R: A. reptans, S: A. salicifolia, G: A. genevensis
As a result, the only substance commonly identified in all Ajuga species was forsythoside A and luteolin glucuronide. The coumaroyl glucose and its glucoside isomer (Figure 4, 5) were determined for A. salicifolia extract. The echinacoside (Figure 6) was detected in A. reptans extract. The LC-MS/MS spectrum of luteoline derivative (Figure 7), forsytoside A (Figure 8), and luteoline glucuronide (Figure 9) were observed in both A. reptans and A. genevensis extracts.

The phenolic acids as caffeeic acid and flavonoids; apigenin-C-hexoside-C-pentoside, quercetin glucuronide, luteolin, and apigenin were identified only for A. genevensis. Furthermore, the phenylethanoid glycosides forsythoside B and leonosides A were identified only for A. salicifolia. The phytochemical research on Ajuga species focus on the isolation of flavonoids, caffeeic and chlorogenic acid type derivates, phenylethanoid glycosides, phytoecdysteroids, iridoids, and diterpenes. Some anthocyanins, delphinidin, and cyanidin 3-O-sophoroside-5-O-glucosides, were acylated with p-coumaric acid, while ferulic acid and malonic acid were isolated from the flowers of cell cultures of A. reptans.

Total phenolic amounts of the extracts

The amount of total phenolics ranged from 30.0 to 42.2 mg GA equivalent (GAE)/g of the extracts. The phenolic amounts equivalent to GA in all three methanol extracts are shown in Table 4. The highest total phenolic level was found in the methanol extract of A. reptans. In previous studies, the total phenolic content of methanol extracts of A. reptans and A. genevensis has been evaluated to be 20.86±0.53 mg RE/g dw and 22.63±0.61 mg GAE/g.

DPPH radical scavenging activity

DPPH radical scavenging activity results are presented in Table 4. The positive control, BHT with IC50 value of 0.06 mg/mL, was found as the most potent antioxidant. The highest radical scavenging activity were obtained for A. salicifolia (IC50: 0.28±0.01 mg/mL) and A. reptans (IC50: 0.30±0.01 mg/mL) extracts. A correlation was also found between radical scavenging capacity and total phenol content. Previous studies showed the antioxidant activity of the methanol extract of A. genevensis flowers as IC50: 72.08±6.02 μg/mL and A. reptans as IC50: 83.16±5.21. However, there have been no reports on the antioxidant activity of A. salicifolia. This study is the first to determine the antioxidant activity of A. salicifolia.

TEAC assay

The results obtained for the evaluation of the antioxidant activity using TEAC assay are presented in Table 4. ABTS•+ radical sweeping impact results are in parallel with the results of the DPPH radical scavenging effect. Extracts from all three plants show ABTS•+ radical scavenging activity at 1% concentrations, but these effects are not as high as the BHT used as the standard.
**Figure 5.** LC-MS/MS spectrum of coumaroyl glucoside isomer (4) in *A. salicifolia* extract

LC-MS/MS: Liquid chromatography with tandem mass spectrometry

**Figure 6.** LC-MS/MS spectrum of echinacoside (7) in *A. reptans* extract

LC-MS/MS: Liquid chromatography with tandem mass spectrometry
Figure 7. LC-MS/MS spectrum of luteoline derivative (5) in *A. reptans* and *A. genevensis* extracts

LC-MS/MS: Liquid chromatography with tandem mass spectrometry

Figure 8. LC-MS/MS spectrum of forsytoside A (9) in *A. reptans* and *A. genevensis* extracts

LC-MS/MS: Liquid chromatography with tandem mass spectrometry
There have been no reports on the antioxidant activity performed by a TEAC assay. Antioxidant activity was performed for the first time in this study for *A. reptans* L., *A. salicifolia* (L.) Schreber, and *A. genevensis* L. species found in Turkey.

**Antimicrobial activity**

The seven different strains tested in this study are presented in Table 5. *Ajuga* L. extracts showed antimicrobial effect against all microorganisms tested and were more effective against yeasts than bacteria [minimum inhibitory concentration (MIC): 156.25 μg/mL]. The MIC was 312.5 μg/mL for *E. coli* NRRL B-3008, *S. areus* ATCC 6538, *S. thyphimurium* ATCC 13311, and *B. cereus* NRRL B-3711 for methanol *Ajuga* extracts. As a result, the antimicrobial activity was observed in *Ajuga* extracts, especially against *Candida* strains.

Data from previous studies on the antimicrobial activity of *A. reptans*, *A. genevensis*, and *A. salicifolia* are listed in Table 2. To the best of our knowledge, this is the first report on the antimicrobial evaluation for methanol extract of *A. salicifolia*, which was more effective against yeast than bacteria.

**CONCLUSION**

We disclose the phytochemical profiles of *A. reptans*, *A. salicifolia*, and *A. genevensis* collected from Turkey.
The extracts were found to contain valuable metabolites; phenolics acids, coumaroyl glucoside, flavonoids, and phenylethanoid glycosides. The phytochemicals could be employed as potential chemotaxonomic markers because different phytochemicals were observed between the three *Ajuga* species.

The scope of this study included the biological potential of methanol extracts of *A. reptans, A. salicifolia*, and *A. genevensis* evaluated for the first time against some pathogenic strains. *Ajuga* species may be considered a valuable natural source against Candida infections and candidal resistance. However, further *in vitro* and *in vivo* experiments using different alternative Candida and fungal species are required to validate these screening results.

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**REFERENCES**

1. The Plant List, 2013 Version 1.1. Last Accessed Date: 07.11.2020. Available from: http://www.theplantlist.org/

2. Kösse Ayuga YB. In: Güner A, Aslan S, Ekim T, Vural M, Babaç MT, (eds). Türkiye Bitkileri Listesi (Damarlı Bitkiler). İstanbul: Flora Araştırmaları Derneği ve Nezahat Gökyiğit Botanik Bahçesi Yayınları; 1999.

3. Baytop T. Türkiye’de Bitkiler ile Tedavi: Geçmişte ve Bugün. Nobel Tip Kitabevleri: Ankara; 1999.

4. Njoroge GN, Bussmann RW. Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya). J Ethnobiol Ethnomed. 2006;2:8.

5. Göger F, Kösse YB, Göger G, Demirci F. Phytochemical characterization of phenolics by LC-MS/MS and biological evaluation of *Ajuga orientalis* from Turkey. Bangladesh J Pharmacol. 2015;10:639-644.

6. Toiu A, Mocan A, Vlase L, Parve AU, Vodnar DC, Ghegliu AM, Oniga I. Comparative phytochemical profile, antioxidant, antimicrobial and in vivo anti-inflammatory activity of different extracts of traditionally used Romanian *Ajuga genevensis* L. and *A. reptans* L. (Lamiaceae). Molecules. 2019;24:1597.

7. Matu EN, Van Staden J. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. J Ethnopharmacol. 2003;87:35-41.

8. Riaz N, Nawaz SA, Mukhtar N, Malik A, Azza N, Ali S, Choudhary MI. Isolation and enzyme-inhibition studies of the chemical constituents from *Ajuga bracteosa*. Chem Biodivers. 2007;4:72-83.

9. Debell A, Makonnen E, Zerihun L, Abebe D, Teka F. *In-vivo* antipyretic studies of the aqueous and ethanol extracts of the leaves of *Ajuga remota* and *Lippia adonisa*. Ethiop J Med. 2005;43:111-118.

10. Mamadalieva NZ, El-Readi MZ, Ovidi E, Ashour ML, Hamoud R, Sagdullaev SS, Wink M. Antiproliferative, antimicrobial and antioxidant activities of the chemical constituents of *Ajuga turkestanica*. Phytopharmacology. 2013;4:1-18.

11. Luan F, Han K, Li M, Zhang T, Liu D, Yu L, Lv H. Ethnomedical uses, phytochemistry, pharmacology, and toxicology of species from the genus *Ajuga*: a systematic review. Am J Chin Med. 2019;47:959-1003.

12. Akbay P, Gertsch J, Çalis I, Heilmann J, Zerbe O, Sticher O. Novel antileukemic sterol glycosides from *Ajuga salicifolia*. Helv Chim Acta. 2002;85:1930-1942.

13. Akbay P, Çalıç I, Heilmann J, Sticher O. Ionone, iridoid and phenylethanoid glycosides from *Ajuga salicifolia* Z. Naturforsch C. 2003;58:177-180.

14. Vogl S, Picker P, Mihaly-Bison J, Fakhruedin N, Atanasov AG, Heiss EH, Kopp B. Ethnopharmacological *in vitro* studies on Austria’s folk medicine-an unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. J Ethnopharmacol. 2013;149:750-771.

15. Israili ZH, Lyoussi B. Ethnopharmacology of the plants of genus *Ajuga*. Pak J Pharm Sci. 2009;22:4.

16. Gonzalez-Tejero MR, Casares-Porcel M, Sánchez-Rojas CP, Ramiro-Gutiérrez JM, Molero-Mesa J, Pieroni A, El Juhrig S. Medicinal plants in the Mediterranean area: synthesis of the results of the project Rubia. J Ethnopharmacol. 2008;116:341-357.
