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The Evaluation of Polyphenolic Content and Antioxidant Activity of *Ajuga reptans* and *Ajuga genevensis* Extracts (*Lamiaceae*)

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Abstract: Several *Ajuga* species (*Lamiaceae*) are used in Romanian traditional medicine for their anti-inflammatory, wound healing and hepatoprotective properties. The aerial parts of *Ajuga reptans* and *A. genevensis* contain phenolic compounds, diterpenoids and iridoids. In the present research, the polyphenolic composition and antioxidant potential of *Ajuga reptans* and *A. genevensis* aerial parts extracts were investigated. The total polyphenolic content (TPC), flavonoids content (TFC) and iridoids content (TIC) were determined. The antioxidant capacity of *Ajuga sp.* extracts was measured using 2,2–diphenyl-picrylhydrazil (DPPH) and ferric-reducing antioxidant power (FRAP) methods. The results showed that the TPC ranged between 18.96-20.07 mg GAE/g, TIC from 17.93-21.17 mg AE/g, and TFC between 10.25-14.68 mg RE/g. The phenolic determinations are in accordance with the antioxidant activity, therefore a higher TPC for *A. genevensis* aerial parts extract is related to a higher DPPH radical scavenging activity and a higher ferric ion reducing antioxidant capacity. The results provide important preliminary information for further pharmacological research on *Ajuga* species aerial parts extracts.

Keywords: *Ajuga genevensis*, *Ajuga reptans*, antioxidant activity, flavonoids, iridoids, polyphenols.

Introduction

The genus *Ajuga* (*Lamiaceae* family) contains about 50 species and 300 taxa, distributed all over the world (Atay et al., 2016). Many *Ajuga* species are traditionally used for their anti-

inflammatory, hypoglycemic, hepatoprotective, antifungal, antimalarial, and antioxidant properties (Israili and Lyoussi, 2009).

Various phytochemical researches on *Ajuga* species have shown that the main secondary metabolites of the genus are: iridoids, flavonoids, diterpenes, phytoecdysteroids, sterol glycosides and phenylethanoid glycosides (Atay et al., 2016; Ono et al., 2011; Toiu et al., 2018).

In spontaneous Romanian flora there are six *Ajuga* species mentioned, and they are used in our traditional medicine for anti-inflammatory, wound healing, hepatoprotective and anti-diarrhoeal potential. There are few data on the chemical composition of Romanian species, especially on *A. reptans, A. genevensis* and *A. laxmannii* (Ciocârlan, 2000; Toiu et al., 2016; Toiu et al., 2017).

A. reptans L. (bugle or common bugle) is an herbaceous flowering plant native to Europe. The extracts obtained from the aerial parts are used due to the content of polyphenols and iridoids as antidiarrhoeaic, antileucoreic, hepatoprotecting, and vulnerar (Toiu et al., 2017).

A. genevensis L. (blue bugle or blue bugleweed) is an erect pubescent herb which grows in the grasslands of Romania and other parts of Europe. The species is used for its anti-inflammatory, sedative, antihemorrhagic properties, in treatment of diarrhoeal diseases and in topic remedies for its wound-healing and epithelization capacity (Israili and Lyoussi, 2009).

In this regard, in order to continue our research on traditionally used *Ajuga* species, this research aimed to evaluate the total polyphenolic content (TPC), total flavonoid content (TFC) and total iridoid content (TIC), as well as the antioxidant capacity of *A. reptans* and *A. genevensis* aerial parts extracts.

Materials and methods

Plant materials

A. reptans and *A. genevensis* aerial parts were collected from Cluj County (Romania) spontaneous flora during flowering stage. Voucher specimens were deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, voucher number Ar18 (*A. reptans*) and Ag22 (*A. genevensis*).

Sample preparation

The air-dried powder of herbal material was extracted with 50% methanol. The methanol extract was obtained from 5 g herbal material and 50 mL solvent, after extraction on a water bath at 60°C for 30 min (Toiu et al., 2018).

Chemicals and Apparatus

Ethanol, methanol, sodium acetate, aluminum chloride, hydrochloric acid, sodium hydroxide, sodium carbonate, Folin-Ciocâlteu reagent, DPPH·radical, acetic acid, copper II sulfate, concentrated hydrochloric acid were purchased from Sigma-Aldrich and Merck (Germany). The spectrophotometric determinations were performed using a Cary 60 ultraviolet–visible (UV–Vis) spectrophotometer from Agilent Technologies.

The quantitative determinations of total bioactive compounds

Total polyphenolic content (TPC)

The evaluation of total polyphenolic content was performed using a spectrophotometric method based on the reaction of polyphenols with the Folin-Ciocâlteu reagent. The samples were kept in darkness for 30 min., and the absorbance was measured at 760 nm. Gallic acid was used as standard for the calibration curve (R^2=0.999), and the results were expressed as gallic acid equivalents (GAE)/g dry plant material (Toiu et al., 2018). Assay was performed in triplicate.

Total flavonoid content (TFC)

Total flavonoid content was using a spectrophotometric method based on the reaction of flavonoids with aluminiun chloride. The absorbance of the solution was measured at 430 nm. Rutin was used as standard for the calibration curve (R^2=0.999), and the results were expressed as rutin equivalents (RE)/g dry plant material (Toiu et al., 2018). Assay was performed in triplicate.

Total iridoid content (TIC)

The total iridoid content was determined by a photometric method based on a Trim-Hill reaction, and the absorbance was measured at 609 nm. The amount of iridoids was calculated using an

aucubin calibration curve, and the results were expressed as aucubin equivalents (mg AEs/g dw herbal material) (R^2=0.999) (Toiu et al., 2018). Assay was performed in triplicate.

Evaluation of *In Vitro* Antioxidant Properties

DPPH free radical scavenging activity

The analysis of the antioxidant capacity of *A. reptans* and *A. genevensis* extracts by using the DPPH method is a spectrophotometric method based on the reaction between DPPH ● and the antioxidant compounds from the plant extracts. The change of absorbance was measured at 517 nm after incubation at 40°C in a thermostatic bath for 30 minutes (R^2=0.997). DPPH scavenging ability % was calculated according to the formula: Antioxidant activity (AO) % = ($A_{control} - A_{sample} / A_{control}$) × 100, where $A_{control}$ is the absorbance of DPPH ● + methanol solution (all reagents except the sample) and A_{sample} is the absorbance of DPPH ● + sample extract. The DPPH radical scavenging activity was expressed as IC_{50} (μg/mL) (the inhibitory concentration = the concentration of extract that scavenge 50% of DPPH free radicals). A lower IC_{50} value is associated with a higher radical scavenging activity (Toiu et al., 2019). Assay was performed in triplicate.

Ferric-Reducing Antioxidant Power Assay

The FRAP method evaluates the antioxidant capacity of the sample and consists in the reduction of the ferric complex 2,4,6-tri(2-pyridyl)-1,3,5-triazine (Fe(II)-TPTZ) to ferrous complex (Fe(III)-TPTZ. The FRAP reagent is a mixture of 10 mM TPTZ in 40 mM HCl, 20 mM ferric chloride and acetate buffer. 4 mL of each *Ajuga* sp. extract were diluted with water and then 6 mL of FRAP reagent was added (R^2=0.992). By using the spectrophotometric method, the absorbance was measured at 450 nm and the results were expressed as μM Trolox equivalents/100 mL extract (Nechita et al., 2022). Assay was performed in triplicate.

Statistical analysis

The results were expressed as the mean ± standard deviation (SD) by using the Excel software package. All the quantitative determinations were performed in triplicate.

Results and discussion

Quantitative determinations

Several studies showed that phenolic compounds are widely distributed in the *Ajuga* species and these compounds could contribute to their antioxidant activity. The preliminary comparative overview of the total phenolic (TPC), flavonoid (TFC) and iridoid contents (TIC) from *A. reptans* and *A. genevensis* aerial parts is presented in Table 1.

The highest concentration of phenolic compounds was determined for *A. genevensis* methanol extract (20.07 ± 0.55 mg GAE/g dw), whereas for *A. reptans*, the quantity was lower (18.96 ± 0.28 mg GAE/g dw).

The results regarding TPC values of *A. reptans* are in accordance with a previous study performed by Toiu et al., (2017), which reported 20.86 ± 0.53 and 24.11 ± 0.57 mg GAE/g for methanol and ethanol flower extracts, respectively. Another research regarding chemical composition of *A. chamaecistus* subsp. *scoparia* (Boiss.) Rech.f., aerial parts, revealed a TPC value of 20.32 ± 0.39 mg GAE/g ethanol extract, which is lower than the *Ajuga* species considered in our study (Movahhedin et al., 2016).

The variation in phenolic amounts could also be due to ecological factors, genetic factors and the status of different secondary metabolites in different growing locations, as shown by previous studies (Vittori et al., 2018).

A similar trend was observed for the total flavonoid content (TFC) for the two *Ajuga* sp. aerial parts. The highest values were obtained for *A. genevensis* (14.68 ± 0.55 RE/g dw), while for *A. reptans* methanol extracts the results were 10.25 ± 0.49 RE/g dw. In our previous study, a total flavonoid content value of 12.38 ± 0.22 mg RE/g dw for *A. reptans* flowers methanol extract was determined (Toiu et al., 2017), which is in accordance with the results presented in this study. A research performed by Rani et al., (2017) showed for *A. bracteosa* a total flavonoid content of 9.3 mg QE/g dw.

Concerning the total iridoid content of *Ajuga* species, *A. reptans* aerial parts contain a higher concentration of iridoids, compared to *A. genevensis* (21.17 ± 0.48 vs. 17.93 ± 0.59 mg AE/g dw). In a former research on *A. reptans* flowers, Toiu et al., (2017) the results showed that the methanol extract content in iridoids is lower than ethanol extract from the same species (22.17 ± 0.89 vs.

27.49 ± 0.94 mg AE/g dw). The same trend was observed in previous study for the *A. laxmannii*, the total iridoid content being 15.37 ± 0.77 and 16.28 ± 0.85 mg AE/g dw for methanol and ethanol extract, respectively (Toiu et al., 2018).

Table 1

TPC, TFC, TIC values and the evaluation of antioxidant activity for *A. reptans* (AR) and *A. genevensis* (AG) aerial parts extracts

Species	**TPC (mg GAE /g)**	**TFC (mg RE /g)**	**TIC (mg AE/g)**	**DPPH method IC_{50} (µg/mL)**	**FRAP method (µM TE / 100mL)**
AR	18.96 ± 0.28	10.25 ± 0.49	21.17 ± 0.48	56.39 ±2.01	2588.21 ±72.35
AG	20.07 ± 0.55	14.68 ± 0.55	17.93 ± 0.59	44.51 ±2.06	2741.09 ±84.52

Notes: Each value is the mean ± SD of three independent determinations; GAE: gallic acid equivalent; RE: rutin equivalent; AE: aucubin equivalent; TE: Trolox equivalent; TPC: total polyphenols content; TFC: total flavonoids content; TIC: total caffeic acid derivatives content; DPPH: 2,2-diphenyl-picrylhydrazil; IC_{50}: inhibitory concentration 50%; FRAP: ferric-reducing antioxidant power.

Antioxidant Activity

The antioxidant properties of *A. reptans* and *A. genevensis* methanol extracts was evaluated by using two *in vitro* methods: 2,2–diphenyl-picrylhydrazil (DPPH●) and ferric-reducing antioxidant power (FRAP) assays. Although both methods show the antioxidant capacity of the extracts, they assess different aspects: while the DPPH method shows the ability to neutralize free radicals, the FRAP analysis shows the ferric ion reducing activity (Toiu et al., 2018). The results of the antioxidant activity are also presented in Table 1.

The results from present research showed that *A. genevensis* had higher DPPH radical scavenging activity (IC_{50} = 44.51 ± 2.06), than *A. reptans* (IC_{50} = 56.39 ± 2.01). According to this method, the *Ajuga* sp. extracts exhibited a moderate antioxidant capacity.

Compared with other research, all extracts showed a lower antiradical activity. A previous study showed an antioxidant capacity value for *A. turkestanica* extract of 57.84±4.19 µg/mL (Mamadalieva

et al., 2013), while another research concerning *A. reptans* reported a slightly higher value (65.7±3.82 µg/mL) (Ono et al., 2011). Movahhedin et al., (2016) reported an antiradical activity for *A. chamaecistus* subsp. *scoparia* (Boiss.) Rech.f. of 22.69±1.30 µg/mL. A previous study focused on the methanol and ethanol flower extract of *A. genevensis* showed IC_{50} values of 72.08 ± 6.02 and 45.45 ± 3.27 µg/mL, respectively (Toiu et al., 2016).

In our experiments, *A. genevensis* aerial parts extract demonstrated higher ferric ion reducing antioxidant capacity (2741.09 ± 84.52 µM TE/100 mL extract), than *A. reptans* (2588.21 ± 72.35 µM TE/100 mL extract). The results of the antioxidant activity determined by FRAP assay are in reasonable agreement with the DPPH method values and with the phenolic contents, presented in Table 1.

The obtained results at the evaluation of antioxidant capacity of *A. genevensis* and *A. reptans* could be due to the presence of polyphenolic compounds in higher amounts in *A. genevensis* aerial parts methanol extracts.

Conclusions

In the present study, we evaluated the polyphenols and iridoid contents of *Ajuga reptans* and *A. genevensis* aerial parts extracts as well as their antioxidant activity. *A. reptans* had the higher iridoid content value, while *A. genevensis* was richer in polyphenols. *A. genevensis* aerial parts extract showed the strongest antioxidant activity, determined by both DPPH and FRAP methods. The antioxidant capacity was in accordance with the total polyphenolic content. Further *in vivo* experiments are recommended in order to develop efficient and safe phytopharmaceuticals.

References

Atay I., Kirmizibekmez H., Kaiser M., Akaydin G., Yesilada E. and Tasdemir D., 2016, Evaluation of in vitro antiprotozoal activity of Ajuga laxmannii and its secondary metabolites, Pharm. Biol., 54:1808–1814.

Ciocârlan V., 2000, Flora ilustrată a României. Pteridophyta et Spermatophyta, Bucureşti: Ed. Ceres.

Israili Z.H. and Lyoussi B., 2009, Ethnopharmacology of the plants of genus Ajuga, Pak. J. Pharm. Sci., 22:425—462.

Mamadalieva N.Z., El-readi M.Z., Ovidi E., Ashour M.L., Hamoud R., Sagdullaev S.S., Azimova S.S., Tiezzi A. and Wink M., 2013, Antiproliferative, antimicrobial and antioxidant activities of the chemical constituents of Ajuga turkestanica, Phytopharmacology, 4:1–18.

Movahhedin N., Zengin G., Bahadori M.B., Sarikurkcu C., Bahadori S. and Dinparast L., 2016, Ajuga chamaecistus subsp. scoparia (Boiss.) Rech.f.: A new source of phytochemicals for antidiabetic, skin-care, and neuroprotective uses, Ind. Crops Prod, 94:89–96.

Nechita M.A., Benedec D., Hanganu D., Nechita V.I., Vârban R., Toiu A., Nistor A. and Oniga I., 2022, Comparative study of polyphenolic content and antioxidant activity of four Agastache Species (Lamiaceae), *Hop and Medicinal Plants*, 30(1-2):268-275.

Ono M., Furusawa C., Ozono T., Oda K., Yasuda S., Okawa M., et al. Four new iridoid glucosides from Ajuga reptans, Chem. Pharm. Bull., 59:1065–1068.

Rani R., Khan M.A., Kayani W.K., Ullah S., Naeem I. and Mirza B., 2017, Metabolic signatures altered by in vitro temperature stress in Ajuga bracteosa Wall. ex. Benth, Acta Physiol. Plant, 39:1-10.

Toiu A., Vlase L., Arsene A.L., Vodnar D.C. and Oniga I., 2016, LC/UV/MS profile of polyphenols, antioxidant and antimicrobial effects of Ajuga genevensis l. extracts, Farmacia, 64(1):53-57.

Toiu A., Vlase L., Gheldiu A.M., Vodnar D. and Oniga I., 2017, Evaluation of the antioxidant and antibacterial potential of bioactive compounds from Ajuga reptans extracts, Farmacia, 65(3):351-355.

Toiu A., Mocan A., Vlase L., Pârvu A.E., Vodnar D.C., Gheldiu A.M., Moldovan C. and Oniga I., 2018, Phytochemical composition, antioxidant, antimicrobial and in vivo anti-inflammatory activity of traditionally used Romanian Ajuga laxmannii (Murray) Benth. ("nobleman's beard" – barba împăratului), Frontiers in Pharmacology, 9(7):1-15.

Toiu A., Mocan A., Vlase L., Pârvu A.E., Vodnar D.C., Gheldiu A.M., Moldovan C. and Oniga I., 2019, 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, 24(8):1597.

Vittori L.D., Mazzoni L., Battino M. and Mezzetti B., 2018, Pre-harvest factors in fluencing the quality of berries, Sci. Hortic. (Amsterdam), 233:310–322.