Hop and Medicinal Plants, Year XXX, No. 1-2, 2022
ISSN 2360-0179 print, ISSN 2360-0187 electronic

Comparative Study of Polyphenolic Content and Antioxidant Activity of Four *Agastache* Species (*Lamiaceae*)

NECHITA Mihaela-Ancuţa[1], Daniela BENEDEC[1], Daniela HANGANU[1]*, Vlad-Ionuţ NECHITA[1], Rodica VÂRBAN[2], Anca TOIU[1], Adriana NISTOR[1], Ilioara ONIGA[1]

[1]"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Faculty of Pharmacy, 8 V. Babeş, 400012, Cluj-Napoca, Romania

[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, 3-5 Calea Mănăştur, 400372, Cluj-Napoca, Romania

[*]Corresponding author: dhanganu@umfcluj.ro

Abstract: *Agastache* species (*Lamiaceae*) are aromatic plants that are used in traditional medicine for the treatment of gastrointestinal diseases and bacterial infections. Their phytochemical profile is characterized by the presence of essential oil, phenolic derivatives and terpenoid compounds. In the present study, the polyphenolic composition and antioxidant potential of four *Agastache* species (*A. foeniculum, A. mexicana, A. scrophulariifolia, A. rugosa*) and one cultivar (*A. rugosa 'After Eight'*) were investigated. The total polyphenols content (TPC), caffeic acid derivatives content (TCADC) and flavonoids content (TFC) were evaluated spectrophotometrically. Also, the antioxidant capacity of *Agastache* extracts was measured using 2,2–diphenyl-picrylhydrazil (DPPH) and ferric-reducing antioxidant power (FRAP) methods. This study showed a TPC ranging from 1.92 to 3.64 mg GAE/g, TCADC ranging from 1.35 to 3.58 mg CAE/g, and TFC between 0.58 and 0.72 mg RE/g. The phenolic determinations are in accordance with the antioxidant activity. Thus, a higher TPC (3.64 mg GAE/g dried plant material) for *A. mexicana* methanolic extract is related to a higher DPPH radical scavenging activity (IC_{50} = 109.08 μg/mL) and a higher ferric ion reducing antioxidant capacity (2346.88 μM TE/100 mL extract). The results open new directions in the pharmacognostic analysis of *Agastache* species and provide important preliminary information for further pharmacological research.

Keywords: *Agastache sp.*, antioxidant activity, caffeic acid derivatives, flavonoids, polyphenols.

Introduction

Lamiaceae species are known worldwide as medicinal and aromatic plants, such as basil, peppermint, sage, and many others. *Agastache* genus, although less known, also belongs to the *Lamiaceae* family. Plants of this genus are used as spices, medicinal and ornamental plants or as sources of volatile oil. *Agastache* species are native to North America, with one species originating from East Asia (*A. rugosa*) (Zielińska et al., 2014). They are empirically used for the treatment of various digestive issues such as nausea, vomiting, anorexia, cholera, diarrhea, miasma or bacterial infections (Park et al., 2016; Yeo et al., 2021).

Agastache species contain phenolic compounds (including flavonoids, phenolic acids, lignans) and terpenic compounds (Zielińska et al., 2014). The most abundant non-volatile polyphenols identified in different *Agastache* species are flavonoids (tilianin, acacetin) and caffeic acid derivatives (caffeic acid, chlorogenic acid, rosmarinic acid) (Desta et al., 2016; Bielecka et al., 2019; Hong et al., 2021). Polyphenols are known to have strong antioxidant properties (Olszowy et al., 2019).

Agastache species have a wide range of therapeutic properties, such as antihypertensive (Hernández-Abreu et al., 2009), anti-adipogenic (Hwang et al., 2021), anti-osteoporotic (Hong et al., 2021), gastroprotective (Nam et al., 2020), anti-inflammatory, barrier-protective and antiwrinkle properties (Lee et al., 2020). Lee et al. showed that antiwrinkle properties of *A. rugosa* could be related to its antioxidant activity, reducing NO and ROS levels in HaCat keratinocytes (Lee et al., 2020). Also, the vasodilator effect of *A. mexicana* extract can be related to the presence of tilianin, which showed vasorelaxant and antihypertensive properties when administered to male Wistar rats (Hernández-Abreu et al., 2009).

Given the importance of polyphenols for antioxidant (Olszowy *et al.*, 2019) and other pharmacological properties, a comparative analysis was performed to evaluate TPC (total polyphenolic content), TFC (total flavonoid content) and TCADC (total caffeic acid derivatives content), along with antioxidant capacity of four Romanian cultivated *Agastache* species and one cultivar. To the best of our knowledge, there are no studies evaluating the polyphenolic content and antioxidant capacity of *A. scrophulariifolia* and *A. rugosa 'After Eight'*.

Materials and methods

Plant materials

The vegetable material, *A. foeniculum* (Pursh) Kuntze, *A. mexicana* (Kunth) Lint et Epling, *A. scrophulariifolia* (Willd.) Kuntze, *A. rugosa* (Fisch. et Mey.) Kuntze and one cultivar (*A. rugosa* 'After Eight') aerial parts were harvested in September 2019 from the experimental field of the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca. *A. rugosa* 'After Eight' is a creation of the breeder Brian Kabbes from the Netherlands (Kabbes, 2005). A sample of the plant material is held in the herbarium of the Pharmacognosy Department: voucher number 130 (*A. foeniculum*), 131 (*A. rugosa)*, 132 (*A. rugosa* 'After Eight'), 133 (*A. mexicana)*, 134 (*A. scrophulariifolia*).

Sample preparation

The aerial parts were air-dried at room temperature. After grinding, the dried sample powder (5 g) was extracted with 50 mL of 70% methanol, 30 min at 60°C (Epure et al., 2020).

Chemicals and Apparatus

Methanol, ethanol, aluminum chloride, sodium acetate, hydrochloric acid, sodium hydroxide, sodium carbonate, Folin-Ciocâlteu reagent, Arnow reagent, DPPH· radical were purchased from Sigma-Aldrich and Merck (Germany). All spectrophotometric determinations were performed using a Cary 60 ultraviolet–visible (UV–Vis) spectrophotometer from Agilent Technologies.

Analyses of the extracts

Total polyphenolic content (TPC)

Total polyphenolic content was determined using a spectrophotometric method based on the reaction of polyphenols with the Folin-Ciocâlteu reagent, in the presence of sodium carbonate. The samples were kept in darkness for 30 min, and then the absorbance was measured at 760 nm. Gallic acid was used as standard for the calibration curve (R^2 = 0.999). The results were expressed as gallic acid equivalents (GAE)/g dry plant material (Epure et al., 2020).

Total flavonoid content (TFC)

Total flavonoid content was determined by spectrophotometric assay, using a 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). The results were expressed as rutin equivalents (RE)/g dry plant material (Epure et al., 2020).

Total caffeic acid derivatives content (TCADC)

Total caffeic acid derivatives content was determined using a spectrophotometric method based on the reaction of caffeic acid derivatives with Arnow's reagent. The absorbance of the solution was measured at 500 nm and the results were expressed as caffeic acid equivalents (CAE)/g dry plant material (R^2 = 0.994) (Epure et al., 2020).

Evaluation of In Vitro Antioxidant Capacity

DPPH free radical scavenging activity

The analysis of the antioxidant capacity using the DPPH method is a spectrophotometric method based on the reaction between DPPH ● and the antioxidant compounds in the plant extract. After incubation at 40°C in a thermostatic bath for 30 minutes, the change in absorbance at 517 nm was measured (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. In order to evaluate the antioxidant capacity of the extracts, IC_{50} values (the inhibitory concentration = the concentration of extract that scavange 50% of DPPH free radicals) were also calculated. The lower IC_{50} value is associated with higher radical scavenging activity (Epure *et al.*, 2020).

Ferric-Reducing Antioxidant Power Assay

The FRAP method is a spectrophotometric method that 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 *Agastache* extract were diluted with

water at 1.8 mL and then 6 mL of FRAP reagent was added (R^2 = 0.992). The absorbance was measured at 450 nm and the results were expressed as μM Trolox equivalents/100 mL extract (Epure et al., 2020).

Statistical analysis

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

Results and discussion

Quantitative determinations

The total polyphenols, flavonoids and caffeic acid derivatives contents were determined spectrophotometrically, and the results obtained are presented in Table 1.

Our analysis showed a higher TPC for *A. mexicana* (3.64 ± 0.32 mg GAE/g dw) and lower values for *A. scrophulariifolia* (1.92 ± 0.63 mg GAE/g dw). Yeo et al. reported a TPC of 3.96 ± 0.06 mg/g dw for *A. rugosa* (Yeo et al., 2021), comparable to our results (3.25 ± 0.46 mg GAE/g dw). Others reported a TPC of 38.11 ± 0.88 mg/g ethanolic extract (Hwang et al., 2021), and 38.9 ± 1.7 mg GAE /g aqueous extract for *A. rugosa* (Lee et al., 2020), their results being expressed to the plant extract, not to dry plant material.

The total flavonoid content was similar between species, with values that fell within the range of 0.58 to 0.72 mg RE/g dw. The highest TFC was determined in the methanolic extract of *A. scrophulariifolia*, while the other four extracts showed lower concentrations. The TFC value determined by us for *A. rugosa* (0.68 ± 0.13 mg RE/g dw) is consistent with that reported by other researchers (0.68 ± 0.01 mg/g dw) (Yeo et al., 2021).

The total content of caffeic acid derivatives in *Agastache* extracts determined in our study revealed the highest valued for *A. mexicana* (3.58 ± 0.43 mg CAE/g), and the lowest for *A. scrophulariifolia* (1.35 ± 0.17 mg CAE/g). No information regarding total caffeic acid derivatives content for *Agastache* species was available in the consulted literature.

The quantitative determinations for *A. rugosa* and the cultivar *A. rugosa* 'After Eight' show similar values, with no significant differences.

Table 1

Total content of polyphenols and the evaluation of antioxidant activity

No.	Samples	TPC (mg GAE /g)	TFC (mg RE /g)	TCADC (mg CAE/g)	DPPH method IC_{50} (μg/mL)	FRAP method AO (μM TE / 100)
1	AF	2.98±0.29	0.68±0.08	2.3±0.24	113.83±14.31	2333.2±271.35
2	AM	3.64±0.32	0.66±0.09	3.58±0.43	109.08±12.64	2346.9±296.41
3	AS	1.92±0.63	0.72±0.11	1.35 ±0.17	125.74±14.79	1490.5±301.47
4	AR	3.25±0.46	0.68±0.13	2.16±0.21	114.92±13.84	1668.5±191.52
5	ARA8	3.26±0.37	0.58±0.06	2.05±0.26	125.82±16.19	2007.1±213.15

Notes: Each value is the mean ± SD of three independent measurements; AF: *Agastache foeniculum*; AM: *Agastache mexicana*; AS: *Agastache scrophulariifolia*; AR: *Agastache rugosa*; ARA8: *Agastache rugosa 'After Eight'*.

GAE: gallic acid equivalent; RE: rutin equivalent; CAE: caffeic acid equivalent; TE: Trolox equivalent

TPC: total polyphenols content; TFC: total flavonoids content; TCADC: total caffeic acid derivatives content; DPPH: 2,2-diphenyl-picrylhydrazil; IC_{50}: inhibitory concentration 50%; FRAP: ferric-reducing antioxidant power; AO: antioxidant activity.

Antioxidant Activity

We used two *in vitro* methods to evaluate the antioxidant capacity of *Agastache* extracts: 2,2–diphenyl-picrylhydrazil (DPPH●) and ferric-reducing antioxidant power (FRAP) assays. Although both methods show the antioxidant activity of the extracts, they evaluate different aspects: the DPPH method shows the ability to neutralize free radicals, while the FRAP analysis shows the ferric ion reducing activity (Epure et al., 2020). The results of the antioxidant activity are presented in Table 1.

Our study showed that *A. mexicana* had the highest DPPH radical scavenging activity (IC_{50} = 109.08 ± 12.64 μg/mL), while *A. scrophulariifolia* and *A. rugosa 'After Eight'* had the lowest DPPH radical scavenging activity, with similar IC_{50} values (125.74 ± 14.79, and 125.82 ± 16.19 μg/mL respectively). According to this method, all *Agastache* extracts exhibited a moderate antioxidant capacity. Regarding *A. rugosa* extract, others reported a lower DPPH radical scavenging activity for the flower extract (IC_{50} = 142.42 ± 2.50 μg/mL) and a higher DPPH radical scavenging activity for the leaf and stem extracts (IC_{50} = 88.33 ± 0.82, and 71.63 ± 0.72 μg/mL

respectively) (Desta et al., 2016), compared to our results (IC_{50} = 114.92 ± 13.84 µg/mL).

In our experiments, *A. mexicana* extract demonstrated the highest ferric ion reducing antioxidant capacity (2346.88 ± 296.41 µM TE/100 mL extract), followed by *A. foeniculum* (2333.19 ± 271.35 µM TE/100 mL extract) and *A. rugosa 'After Eight'* (2007.05 ± 213.15 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. These results revealed that the phenolic constituents found in *Agastache* extracts are involved in the mechanisms of the antioxidant activity. Thus, a higher TPC (3.64 ± 0.32 mg GAE/g dw) for *A. mexicana* methanolic extract is related to a higher radical scavenging activity (IC_{50} = 109.08 ± 12.64 µg/mL) and with a higher ferric ion reducing antioxidant capacity (2346.88 ± 296.41 µM TE/100 mL extract).

Agastache species contain polyphenols, especially caffeic acid derivatives, which are important active principles, both for antioxidant activity and for other pharmaceutical properties.

Conclusions

In the present study, we performed a comparative chemical analysis between four species of *Agastache* and one cultivar, filling the gap in the literature regarding their polyphenolic content and antioxidant activity. The TCADC determination showed important quantities in all *Agastache* species, especially in *A. mexicana*. Among the four studied species, *A. mexicana* showed the strongest antioxidant activity, determined by both DPPH and FRAP methods. The antioxidant capacity was in accordance with the total polyphenolic content. Our results open new directions in the pharmacognostic analysis of *Agastache* species and provide important preliminary information for further pharmacological research.

References

Bielecka M., Zielińska S., Pencakowski B., Stafiniak M., Ślusarczyk S., Prescha A. and Matkowski A., 2019, Age-related variation of polyphenol content and expression of phenylpropanoid biosynthetic genes in Agastache rugosa, Industrial Crops and Products, 141:111743.

Desta K.T., Kim G.S., Kim Y.H., Lee W.S., Lee S.J., Jin J.S., El-Aty A.M., Shin H.C., Shim J.H. and Shin S.C., 2015, The polyphenolic profiles and antioxidant effects of *Agastache rugosa* Kuntze (Banga) flower, leaf, stem and root, Biomed Chromatogr, 30(2):225-31.

Epure A., Pârvu A.E., Vlase L., Benedec D., Hanganu D., Gheldiu A.M., Toma V.A. and Oniga I., 2020, Phytochemical Profile, Antioxidant, Cardioprotective and Nephroprotective Activity of Romanian Chicory Extract, Plants (Basel), 10(1):64.

Hernández-Abreu O., Castillo-España P., León-Rivera I., Ibarra-Barajas M., Villalobos-Molina R., González-Christen J., Vergara-Galicia J. and Estrada-Soto S., 2009, Antihypertensive and vasorelaxant effects of tilianin isolated from Agastache mexicana are mediated by NO/cGMP pathway and potassium channel opening, Biochem Pharmacol, 78(1):54-61.

Hong S., Cha K.H., Kwon D.Y., Son Y.J., Kim S.M., Choi J.H., Yoo G. and Nho C.W., 2021, *Agastache rugosa* ethanol extract suppresses bone loss via induction of osteoblast differentiation with alteration of gut microbiota, Phytomedicine, 84:153517.

Hwang J.M., Lee M.H., Lee J.H. and Lee J.H., 2021, Agastache rugosa Extract and Its Bioactive Compound Tilianin Suppress Adipogenesis and Lipogenesis on 3T3-L1 Cells, Applied Sciences, 11(16):7679.

Kabbes B., 2005, Agastache Plant Named 'After Eight'. U.S. Patent USPP15921P2. Available online: https://patents.google.com/patent/USPP15921P2/en (accessed on 1 November 2022).

Lee Y., Lim H.W., Ryu I.W., Huang Y.H., Park M., Chi Y.M. and Lim C.J., 2020, Anti-Inflammatory, Barrier-Protective, and Antiwrinkle Properties of Agastache rugosa Kuntze in Human Epidermal Keratinocytes, Biomed Res Int, 2020:1759067.

Nam H.H., Kim J.S., Lee J., Seo Y.H., Kim H.S., Ryu S.M., Choi G., Moon B.C. and Lee A.Y., 2020, Pharmacological Effects of Agastache rugosa against Gastritis Using a Network Pharmacology Approach, Biomolecules, 10(9):1298.

Olszowy M., 2019, What is responsible for antioxidant properties of polyphenolic compounds from plants?, Plant Physiology and Biochemistry, 144:135–43.

Park S., Kim N., Yoo G., Kim Y., Lee T.H., Kim S.Y. and Kim S.H, 2016, A new flavone glycoside from the leaves of *Agastache rugosa* (Fisch. & C.A.Mey.) Kuntze, Biochemical Systematics and Ecology, 67:17–21.

Yeo H.J., Park C.H., Park Y.E., Hyeon H., Kim J.K., Lee S.Y. and Park S.U., 2021, Metabolic profiling and antioxidant activity during flower development in *Agastache rugosa*, Physiol Mol Biol Plants, 27(3):445-455.

Zielińska S. and Matkowski A., 2014, Phytochemistry and bioactivity of aromatic and medicinal plants from the genus *Agastache* (*Lamiaceae*), Phytochem Rev, 13(2):391-416.