CHEMICAL ASSESSMENT AND BIOLOGIC POTENTIAL OF A SPECIAL *LESPEDEZA CAPITATA* EXTRACT

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CHEMICAL ASSESSMENT AND BIOLOGIC POTENTIAL OF A SPECIAL LESPEDEZA CAPITATA EXTRACT (Abstract): Secondary metabolites are natural compounds synthesized by plants as an adaptation to environmental factors. Humans use such components for the prevention and treatment of minor or mild conditions. *Lespedeza capitata* is a medicinal and melliferous plant known for its various beneficial properties (antioxidant, antiaging, antiedematous, etc.). **The objectives** of this study were to identify and quantify the secondary metabolites from a selective *L. capitata* extract. Moreover, the evaluation of the antioxidant activity was part of the present research. **Material and methods:** Identification and semi-quantification were assessed by liquid chromatography (HPLC/MS), and the total flavonoids and polyphenols were determined by spectrophotometry according to European Pharmacopeia indications. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and iron chelation test were used to establish the antioxidant putative effects of the investigated extract. **Results**: Among the identified compounds different quercetin derivatives, chlorogenic acid, and rosmarinic acid were found. The total polyphenols amounted to 174.24 mg/g GAE and flavonoids measured 95.65 mg/g rutoside equivalents. The antioxidant activity showed a good capacity to scavenge free radicals and a higher potential to chelate ferrous ions. The intensity of the effect was correlated to concentration. All in all, the investigated *L. capitata* extract demonstrated a polyphenol and flavonoid-rich content with good antioxidant potential. **Keywords**: LESPEDEZA, HPLC/MS, TOTAL POLYPHENOLS, ANTIOXIDANT ACTIVITY.

Actual living conditions are commonly related to a variable exposure to polluted environments which determines the alteration of homeostasis, the overloading of body defense mechanisms, and immunosuppression with a negative impact on health and lifestyle. In this context, plant secondary metabolites represent frequent options as antioxidants that can counteract the harmful effects of pollution and overexpression of oxygen-reactive species (1, 2, 3). The chemical conformation of flavonoids and polyphenols presents chemical patterns that allow them to donate protons to quench free radicals. Various studies confirm the antioxidant and antiradical properties of natural

and synthetic flavonoids (1, 4, 5). Moreover, certain compounds were found to have possible applications in the prevention of hypertension, vascular problems, degenerative disorders, and gastrointestinal and urinary tract diseases (6, 7, 8).

In the last decade, forage legumes come into the spotlight due to their beneficial effects on domesticated animals and the environment (phytoremediation). Hence, many plant species belonging to the Fabaceae family were screened for their rich content of polyphenols, a chemical group of metabolites, generally recognized as antioxidants (4, 5). *Lespedeza* species represent a group of wild plants used as legumes, native to the Americas and oriental countries as part of their traditional remedies. Some of the reports showed that five species (*L. tomentosa* (Thunb.) Maxim, *L. bicolor* Turcz., *L. hedysaroides* (Pall.) Kitag., *L. cyrtobotria* Miq., and *L. davurica* (Laxm.) Schindl.) are also native to Russia (5, 9, 10, 11). These species were found to contain flavonoid glycosides, lignans, sterols, alkaloids, organic acids, coumestans, and terpenoids.

Lespedeza capitata Michx. (Roundhead *Lespedeza)*, native to eastern North America and common in meadows, is an understudied legume with a tradition in folk medicine as a diuretic, anti-inflammatory, and antirheumatic both in the USA, Korea, and China. Native Americans used the roots or extracts from roots as an antidote for poisoning (5). Among the *Lespedeza* species, *L. capitata* is less studied, although extracts from leaves and stems are used for urinary tract and kidney disorders.

This study aimed to evaluate the chemical spectra of an industrial extract obtained from the aerial parts of *Lespedeza capitata* and to establish the existence of an antioxidant profile.

MATERIALS AND METHODS

Plant extract: The plant extract was obtained by direct collaboration with Vanelli company, which are single importer of *Lespedeza capitata* dried extract (4:1) in our country.

Chemical profile: The identity of the compounds found in the investigated extract was evaluated by liquid chromatography using a Transcend TLX-1 Vanquish Flex system coupled with the Orbitrap Exploris 480 high-resolution mass spectrometer and Compound Discoverer 3.3.1 software for the identification of small compounds, part of the CENEMED platform. The method used formic acid 0.1% (A) and methanol (B) in a ramp from 95:5 to 10:90 in 15 minutes, partial loop injection of 10 µL, a debit flow of 300 µL, Hypersil Gold C18 column (50 x 2.1 mm, 1.9 µm) at 40^{o}C, and the instrument settings were electrospray ionization (ESI), negative ion mode (-p), mass range m/z from 100 to 1500 at 5 Hz frequency, negative voltage of 3.0 kV and an ionization temperature of 350^{o}C. Integration and detection were subjected to Compound Discoverer 3.2 software for evaluation in targeted and untargeted modules. The peaks with a rating of at least 7.4 were taken into account and verified against Thermo m/z Vault, NIST, and Chem spider databases.

Total phenolic content evaluation: A colorimetric method (proposed by Singleton and Rossi) using the Folin-Ciocalteu reagent was conducted to determine the total amount of polyphenols that are capable of forming blue complexes in alkaline conditions, following a method previously described (13, 14). Gallic acid (amounts of 3-8 µg/mL) was used to obtain a calibration curve and the final results were expressed as gallic acid equivalents (GAE), quantified as milligrams of gallic acid per g dry

extract (3, 14).

Total flavonoid content evaluation: Similarly**, t**he quantification of total flavonoids used a spectrophotometric method using aluminum chloride in alkaline conditions, when flavones and their derivatives form yellow fluorescent complexes that can be measured at λ = 430 nm (12). Rutoside was used for calibration and the results were denoted as rutoside equivalents (mg rutoside/ g dry extract).

Antioxidant potential: The antioxidant effects of the extracts were evaluated by two well-known methods DPPH radical assay and iron chelation assay. For this, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was used to estimate the radical scavenging effect of *Lespedeza capitata* extract. previously described (3, 14). Ion ions (Fe^{2+}) may be complexed by ferrozine to form a magenta complex measured at 562 nm. The compounds that have an iron chelation effect block the complex formation thus resulting in a lower absorbance. All steps were followed as previously described by Nani *et al.* and Iancu *et al.* (15, 16), Ascorbic acid was used as a positive standard in both tests.

All determinations were in triplicate, and the presented results were expressed as mean ± standard deviation error.

RESULTS AND DISCUSSION

Chemical profile

The general compound profile showed the major presence of flavonoid derivatives (especially quercetin derivatives) and some polyphenol carboxylic acids [chlorogenic acid (m/z = 353.08) and rosmarinic acid (m/z = 359.16)]. The general aspect of the chromatogram is given in first figure.

Different studies indicated the major presence of similar compounds, in particular flavonoid glycosides and condensed tannins (2, 5, 6, 17), however a more elaborate chemical composition of this species is not yet available. Some of the aforementioned studies are dated before 2005. For our results, it should be taken into account that the data is strictly related to the investigated extract (which is a commercial, industrially obtained, and selective extract) and cannot comprise the full spectra of components that belong to the plant.

Total phenols amounted to almost 175 mg GAE for each gram of extract, whereas flavonoids were less (95.65) (tab. II).

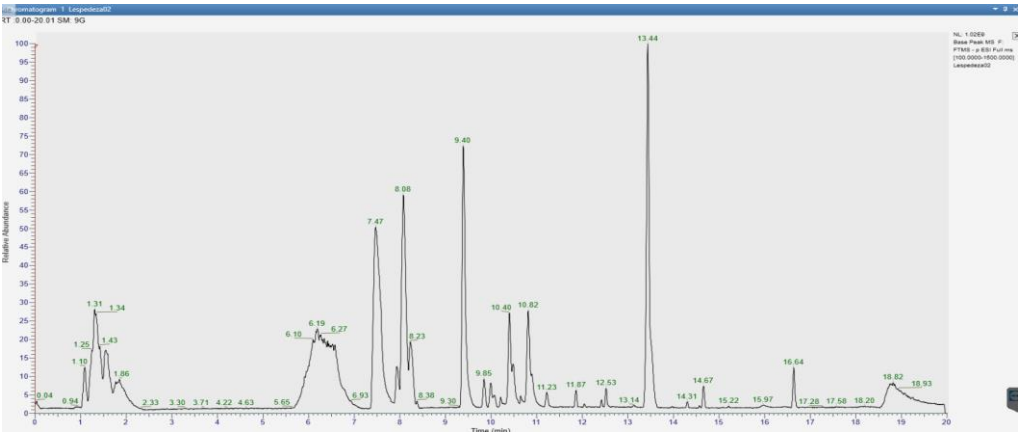

Fig. 1. General LC-ESI-MS chromatogram for *Lespedeza capitata* extract

TABLE I.

Tentative identification of the components from *Lespedeza capitata* extract

No	$[M-H]^-$ (m/z)	MS/MS (m/z)	Compounds
1	353.08	353.08, 191	Chlorogenic acid
2	179.02	341, 179, 135	Caffeic acid-3-glucoside
3	301.03	301.03, 179.0, 150.9	Quercetin-3-D-galactoside
4	515.10	515.10, 353, 191, 179	1,3-Dicaffeoylquinic acid
5	287.05	333, 287.05, 151	Dihydroluteolin
6	269.04	269.04	3,3',4'-Trihydroxyflavone syn. 5,7-Dideoxyquercetin
7	317.06	317, 299, 175	6-Methyldihydroquercetin
8	359.16	359, 161.02, 197	Rosmarinic acid

TABLE II.

Major classes of compounds for the *Lespedeza capitata* extract

Sample	Total phenols (mg gallic acid/g)	Total flavonoids (mg rutoside/g)
Lespedeza capitata	174.24± 0.63	95.65± 0.31

Results expressed as mean ± standard error of the mean (n =3) of three determinations

Despite the long tradition and use in folk and modern medicine, *Lespedeza capitata* has no scientific data available regarding the total polyphenols and flavonoids. By searching Scopus/Elsevier databases for *lespedeza AND capitata AND total AND polyphenols OR flavonoids* no matching documents were found. Nevertheless, other Lespedeza species such as Korean *L. cuneata* were proven to contain 102.7 mg gallic acid/g and 62.8 mg quercetin/g (18). Also, data on the aerial plant product (*L. bicolor*) from Pakistan indicated the presence of 3.321 mg GAE/g of dried weight and 0.148 mg/g of dried weight (19).

Antioxidant potential

The obtained data for both antioxidant tests are given in fig. 2 and are expressed as the inhibitory concentration of 50 % of DPPH radicals and iron complexes respectively.

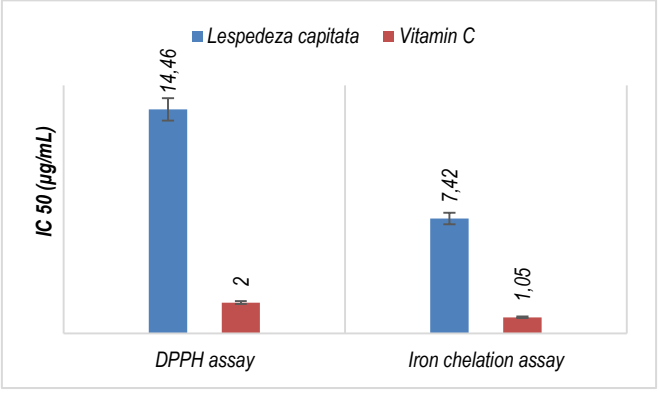

Fig. 2. Results for the antioxidants assays (represented as IC50) for *L. capitata* extract

The results indicated that the investigated extract has a lower activity than ascorbic acid used as a positive standard, however, the IC50 values were expressed in μg/mL of extract. Such amounts are extremely low and prove that the selective *L. capitata* extract has great potential as an antioxidant, but the intensity of the effect depends on the mechanism of action. The phytocomplex found in the extract exhibited twice as much activity against ferrous ions than against DPPH radicals. This is an interesting aspect since ferrous ions are involved in the Fenton reaction that generates hydroxyl radicals in living organisms, such radicals are known to be aggressive and damaging (15, 16).

Other *Lespedeza* species (*L. bicolor*) showed good antioxidant activity, with IC50 values ranging from 50 to 200 μg/mL, but the root samples were better than the aerial parts (19).

More recent research investigated the capacity of an *L. capitata* extract to inhibit collagenase activity which is related to skin cell ageing. The results showed that the extract possesses potential benefits for dermatologic use and wound healing similar to some other Brazilian plants (12).

CONCLUSIONS

Our study represents a starting point for further research in assessing the putative activity of *L. capitata*. The investigated extract is already used in some formulations (along with silymarin) as food supplements recommended for maintaining good liver and kidney functionality. The obtained results indicated a good chemical profile and an interesting antioxidant potential, especially active against ferrous ions. In conclusion, more data is still needed to confirm the actual benefits of *Lespedeza capitata*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Fraga CG. Plant polyphenols: how to translate their in vitro antioxidant actions to *in vivo* conditions. *IUBMB life* 2007; 59(4-5): 308-315.
2. Wagner H, Elbl G. ACE-inhibitory procyanidins from *Lespedeza capitata*. *Planta medica* 1992; 58(03): 297-297.
3. Iliescu IA, Macovei I, Miron A. *Verbascum nigrum* L. *And ajuga chia* Schreb.: antioxidant activity and correlation with phenolic content. *Med Surg J - Rev Med Chir Soc Med Nat Iaşi* 2021; 125(4): 592-600.
4. Wink M. Evolution of secondary metabolites in legumes (Fabaceae). *South African Journal of Botany* 2013; 89: 164-175.

5. Cornara L, Xiao J, Burlando B. Therapeutic potential of temperate forage legumes: a review. *Critical reviews in food science and nutrition* 2016; 56(suppl. 1): S149-S161.
6. Elbl G, Schindler PW, Wagner H. Development of an in vitro screening test for angiotensin I-converting enzyme (ACE) inhibitors in plant extracts and isolation of an ACE-inhibiting, dimeric procyanidin from *Lespedeza capitata*. *Planta Medica* 1990; 56(6): 690 / doi: 10.1055/ s-2006-961368.
7. Kushnirenko SV, Ivanov DD, Rotova SA, Kushnirenko OV. Renoprotective opportunities of *Lespedeza capitata* in patients with chronic kidney disease and type 2 diabetes mellitus. *Kidneys* 2021; 10(3): 162-168.
8. Yarnell E. Botanical medicines for the urinary tract. *World Journal of Urology* 2002; 20: 285-293.
9. Burton T, Dunlap T, Dong H, Li G, Bolton J, Soejarto D, Van Breemen RB. American Indian botanicals: possible alternatives to hormone therapy during menopause. *Planta medica* 2015; 81(11): PM13.
10. Pokhilo ND, Fedoreyev SA, Tarbeeva DV, Veselova MV, Grigorchuk VP, Gorovoy PG. Flavonoid Glycosides from the Aerial Part of *Lespedeza tomentosa*. *Chemistry of Natural Compounds* 2021; 57(6): 1023-1028 / doi: 10.1007/s10600-021-03541-5.
11. Pokhilo ND, Tarbeeva DV, Grigorchuk VP, Starnovskaya SS, Gorovoy PG, Fedoreyev SA. Flavonoid Glycosides from the Aerial Part of *Lespedeza hedysaroides*. *Chemistry of Natural Compounds* 2023; 27: 1-3.
12. Pastorino G, Marchetti C, Borghesi B, Cornara L, Ribulla S, Burlando B. Biological activities of the legume crops *Melilotus officinalis* and *Lespedeza capitata* for skin care and pharmaceutical applications. *Industrial crops and products* 2017; 96: 158-164.
13. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am J Enol Vitic* 1965; 16: 144-158.
14. Yilmaz F, Pashayeva L, Tugay O. LC-ESI-MS/MS-based phytochemical profile, antioxidant and enzyme inhibition activities of extracts from *Cousinia aintabesis* Boiss. & Hausskn. *Farmacia* 2022; 70(2): 287-294.
15. Nani E, Hancianu M, Iancu C, Corciova A, Cioanca O, Stan C, Mircea C. Antioxidant and enzyme inhibitory effects of *Morus sp.* extracts. *Med Surg J - Rev Med Chir Soc Med Nat Iaşi* 2022; 126(4): 593-604.
16. Iancu C, Filip N, Mocanu M, *et al*. Antioxidant and anticancer effect of some *Pelargonium* species extracts. *Farmacia* 2023; 71(2): 312-321.
17. Linard A, Delaveau P, Paris RR, Dellamonica G, Chopin J. Isocarlinoside, a di-C-glycosylflavone from *Lespedeza capitata*. *Phytochemistry* 1982; 21(3): 797-799.
18. Park SI, Hwang HY, Kim JS, Park SW. Analysis of the Functional Components and Various Biological Activities of Korean Medicinal Herbs. *Journal of the Korean Society of Food Science and Nutrition* 2019; 48(12): 1345-1351.
19. Ullah S. Methanolic extract from *Lespedeza bicolor*: potential candidates for natural antioxidant and anticancer agent. *Journal of Traditional Chinese Medicine* 2017; 37(4): 444-451.