COMPARATIVE MORPHOLOGIC AND CHEMICAL PRELIMINARY ASSESSMENT OF THREE ROMANIAN *AGASTACHE* SPECIES

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COMPARATIVE MORPHOLOGIC AND CHEMICAL PRELIMINARY ASSESSMENT OF THREE ROMANIAN *AGASTACHE* SPECIES (Abstract): Phenolic compounds, such as flavonoids and phenolic acids, are classified as secondary metabolites in plants. The increasing interest in utilizing these components in traditional medicine for the prevention or treatment of diverse pathologies based on their antioxidant properties. **The objectives** of this study were to identify the microscopic characteristics and to quantify the levels of total phenolics and flavonoids of three different *Agastache* species: *A. foeniculum*, *A. mexicana*, and *A. urticifolia*. **Material and methods:** Microscopy followed 10th European Pharmacopoeia (Ph. Eur.) provision regarding the evaluation of plant products. The quantification of phenolic content was conducted using the Folin-Ciocalteu method, while the determination of total flavonoids was conducted using the aluminum chloride and sodium nitrate method. **Results**: Microscopic features showed similar structures for leaves and flowers. Differences were given by the density of stomata and glandular or surface trichomes. The total phenolic content of the extracts varied from 213 to 642 mg % and the maximum was determined for *A. foeniculum*. Also, the measured total flavonoid content was found to be between 29.4 mg % and 58 mg %, the same sample having the highest amount. **Conclusions:** The methanolic extracts of this plant exhibits a notable concentration of phenolic and flavonoid compounds, suggesting its potential as a therapeutic agent for various diseases. However, further investigation is required to fully understand its efficacy and safety profile. **Keywords**: MICROSCOPY, TOTAL PHENOLS, TOTAL FLAVONOID CONTENT.

The taxonomic classification of the plant genus *Agastache* situates it within the botanical family *Lamiaceae*, encompassing a collective assemblage of 22 distinct species of enduring aromatic herbs that possess notable medicinal characteristics. The *Agastache* genus is known for its notable prevalence of phenylpropanoid and terpenoid specialized metabolites, a trait frequently observed within the *Lamiaceae* botanical family. One classification of chemical compounds encompasses flavonoids, free phenolic acids and depsides, alongside lignans, while the other significant classification is based on terpenoids, which can be discerned as both volatile and nonvolatile fractions (1, 2).

Agastache foeniculum, known by vari-

ous common names such as anise hyssop, blue giant hyssop, fragrant giant hyssop, or the lavender giant hyssop, is indigenous to the north-central and northern regions of North America. It possesses a chemical composition that includes volatile oils, phenolic compounds (specifically phenolic acids and flavonoids), as well as derived flavonoids, alcohols, cinnamic aldehydes, terpenoids, and other similar substances, with concentrations ranging from 0.9 g % to 1.8 g % (3, 4, 5). Various components of the plant are utilized for the management of cardiovascular, nervous system, and gastrointestinal ailments, in addition to their application in the treatment of common colds, fevers, and bodily discomfort. Additionally, the plant exhibits antivomitive, antibacterial, and antifungal properties (6, 7).

Agastache mexicana, a vascular plant indigenous to Mexico, exhibits the characteristic morphology of the *Lamiaceae* family, including opposite, petiolate leaves, a four-angled stem, and abundant trichomes. This plant has been traditionally recognized in Asia for its culinary and medicinal properties. Research findings have demonstrated that certain components of the plant exhibit properties associated with antioxidant, antifungal, anti-inflammatory, antiobesity, and anti-photoaging effects (8, 9).

Agastache urticifolia is a perennial plant that is strongly aromatic, and it is known by wo varieties of the species var. *urticifolia* and var. *glaucifolia*, however, only native populations of the former exist in Utah mountain ranges (10, 11). Though native to the Intermountain West (USA), this species has been cultivated throughout the world and even used for ethnobotanical practices in Bangladesh (12, 13).

The majority of the published studies primarily concentrate on the analysis of essential oils. Additionally, a considerable number of scholarly articles have been published documenting the process of isolating and comprehending the molecular structure of diverse phytochemical compounds (14, 15, 16, 17).

The aim of this study was to conduct a comparative analysis of three *Agastache* species, namely *A. foeniculum*, *A. mexicana*, and *A. urticifolia*, following microscopic features of the leaves and flowers and the total polyphenolic and flavonoid compounds obtained from methanolic extracts.

MATERIAL AND METHODS

Plant material and reagents: Agastache foeniculum (AG1) comes from Cluj-Napoca 46°46′42.24″ N, 23°37′01.92″ E, *Agastache foeniculum* (AG2) and *Agastache mexicana* (AGM) were harvested from Brașov (Dacia Plant), 45°39′ N 25°36′E, while *Agastache urticifolia* (AGU) was harvested from the Republic of Moldova, Chisinau, 47°01′0″N 28°52′0″E. All chemicals and reagents used for extraction and characterization were of analytical grade, provided by Sigma Aldrich Chemical Co. (Germany) and were used without further purification.

For each of the three species, the air-dried plants (5 g) were pulverized into a fine powder using a mill. The resulting powder was then subjected to extraction using 50 mL of 50% methanol (V/V) at 70°C for 1 hour under reflux. Following the cooling process, the extracts underwent filtration. The isolated products were subsequently rinsed with a solution consisting of 10 mL of 50% (V/V) methanol. The resulting filtrates were combined in a volumetric flask and further diluted to a final volume of 100 mL using the same solvent. A portion of 80 mL was taken from each extract and was concentrated using a rotary

evaporator (Buchi R 210, Switzerland) in order to eliminate the solvent. The residues were subjected to a drying process and subsequently stored at a temperature of 4°C in order to facilitate further analysis.

Microscopic investigation: The powdered plant material was clarified with chloralhydrate (80% in water) directly on the microscopic plate. All steps were according to the indications of Ph. Eur. 10 (18). The visualization and photography of the observed elements were realized with an Optika (WF10X-11MM) microscope with built-in camera (Canon PS A450).

Determination of total phenolic content: The determination of the total phenolic content of each extract of *Agastache* was conducted using the Folin-Ciocalteu reagent, following the method proposed by Singleton and Rossi (19), with gallic acid serving as the standard. A standard curve was generated by determining the concentrations of gallic acid within the range of 3-6 µg/mL. The outcomes were quantified in terms of gallic acid equivalents (GAE), specifically measured as milligrams of gallic acid per 100 g dry plant material (20, 21).

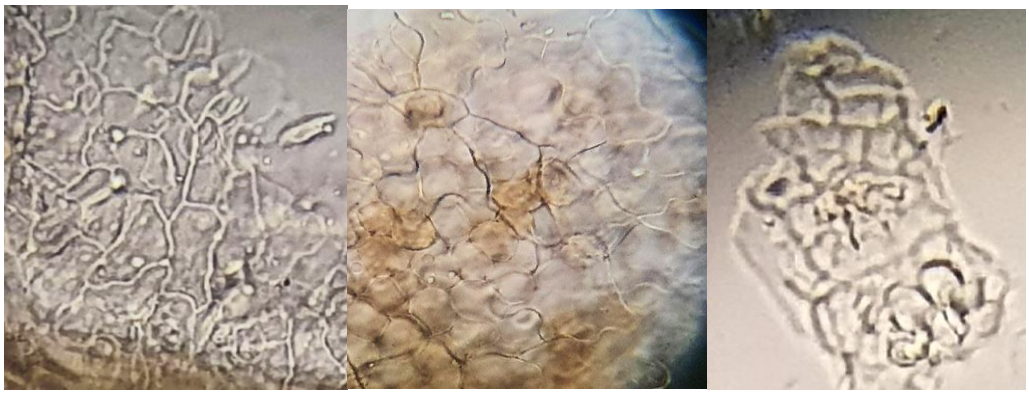
Determination of flavonoid content: The quantification of the overall flavonoid content was conducted using a colorimetric assay as previously (20, 21). The quantification of the total flavonoid content in the extract was denoted as rutoside (mg rutoside (RUE)/100 g dry plant product).

For all determinations, samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Morphologic characterization

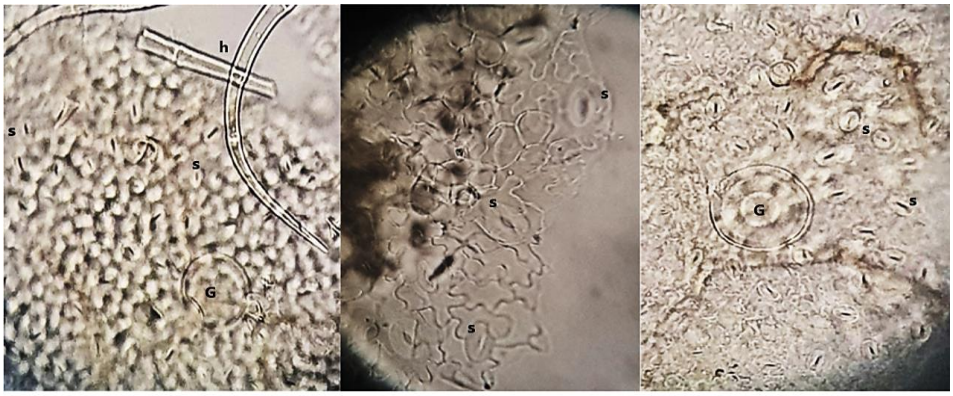
Our observations indicated the presence of common tissue (upper and lower epidermis, stomata, vascular bundles) and similar structures (octocellular glandular trichomes and surface hairs) with taxonomic relevance in all investigated samples, as indicated in fig 1-3. Such microscopic characteristics prove that the investigated species belong to Lamiaceae family (22).



Agastache foeniculum *Agastache mexicana* *Agastache urticifolia*

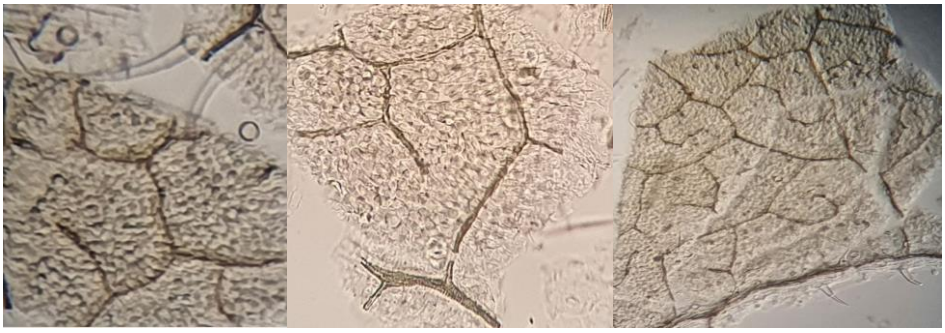
Fig. 1. Front view of upper epidermis (adaxial surface) showing elongated cells with thicker and almost straight walls in the investigated *Agastache* samples

Numerous small stomata were present in *A. foeniculum* and *A. urticifolia* abaxial surface, whereas bigger and fewer stomata were noted in the *A. mexicana* sample (fig. 2).



Agastache foeniculum *Agastache mexicana* *Agastache urticifolia*

Fig. 2. Front view of lower epidermis showing cells with thin and wavy walls: s - stomata, G - octocellular glandular trichomes, h - pluricellular surface trichomes



Agastache foeniculum *Agastache mexicana* *Agastache urticifolia*

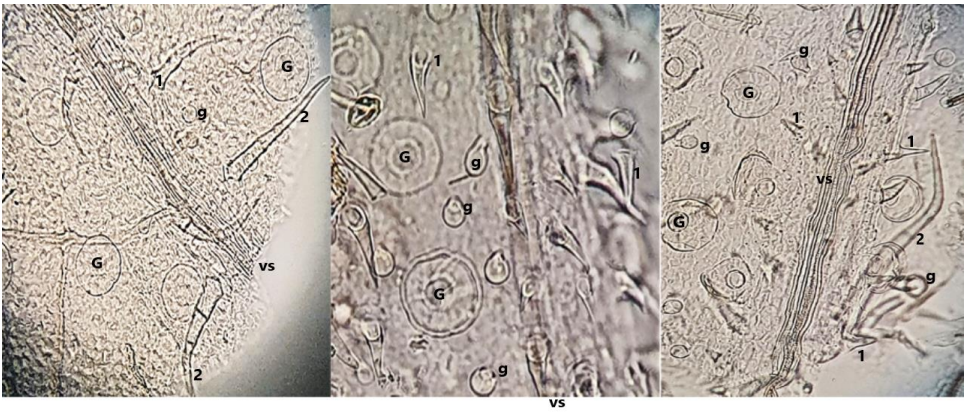
Fig. 3. Front view of leaf mesophyll with vascular bundles (vascular network is represented by the dark brown veins

For all three species, the microscopic examination highlighted the presence of numerous short, single cell surface hairs, monocellular head and sessile octocellular glands denser on the flower epidermis and on the abaxial surface of the leaves (fig. 4). Moreover, long pluricellular (3-5 cells) surface hairs were noted in *A. foeniculum* and *A. urticifolia*, but not as frequent as the short trichomes.

Although the information regarding leaf and flower morphology of *Agastache* species is scarce, similar to our results but using scanning electron microscopy previous researchers have revealed that the upper surface of the leaf comprises both simple non-glandular and glandular (capitate and subsessile) trichomes, which possess the capability to yield essential oil through extraction processes. The essential oil samples of *A. urticifolia* from cultivated (Russia) and native (Oregon, USA) populations, which have been previously examined, have demonstrated a predominant composi-

tion of volatile compounds, namely limonene, menthone, and pulegone. It is worth noting that these compounds exist as enantiomers in the natural environment (23, 24).

In our samples, pollen grains were visible in two of the investigated samples (fig. 5).



Agastache foeniculum *Agastache mexicana* *Agastache urticifolia*

Fig. 4. Front view of flower epidermis: G - octocellular glandular trichomes, g - monocellular head glands, 1 - monocellular surface trichomes, 2 - pluricellular surface trichomes, *vs*. – vascular bundles (viewed by transparence)

Agastache foeniculum *Agastache mexicana*

Fig. 5. Pollen grains and pollen sacs indicate the presence of flowers in the powdered plant material

Secondary metabolites quantification

Total phenols content varied between 213 ± 3.21 mg% to 642 ± 3.12 mg%, *Agastache foeniculum* samples being the richest in such metabolites. Table I represents the analytical data for phenolics and total flavonoid content of the methanolic extract of the three *Agastache* species.

Plant extracts' total phenolic content has been linked to antioxidant activity due to their redox properties, which allow them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers. For our samples, the most promising samples seem both *A. foeniculum* species, the best being AG2, the sample harvested from Brasov. The lowest content was detected for AGM, a species which originates from

Mexico and most probably the environmental conditions do not offer proper conditions for a better biosynthetic capacity of the plants grown in our country. Similar results were obtained by Balanescu *et al.* for ultrasonicated ethanolic and methanolic extracts obtained from *A. foeniculum* cultivated in Romania (25). A better extractability was noted for the methanolic extracts as compared to ethanolic extracts.

TABLE I.
Total phenolic content for the three species of *Agastache*

Sample name	Total polyphenols (mg GAE %)	Total flavonoids content (mg RUE %)
AG1	455 ± 2.02	53 ± 1.78
AG2	642 ± 3.12	58 ± 2.43
AGM	213 ± 3.21	29.4 ± 1.08
AGU	396 ± 2.01	49.9 ± 1.11

In regard to the flavonoid content, the same sample, AG2, had the richest content of flavones, whereas *A. mexicana* registered the lowest amount. It was notable that the same variability was registered for flavonoids and total phenolic content. Taking into account that the extracted amounts were high, we can state that 50 % methanol was a good solvent for these classes of metabolites.

Currently, there are few studies pertaining to the phytochemical compositions and components found in *Agastache* species. Furthermore, a comprehensive comparative study is necessary in order to thoroughly understand the distinctions between these species. Furthermore, the characterization of primary and secondary metabolic compounds in *Agastache* species remains incomplete in terms of comparative study, with particular emphasis on the influence of flowering stage and cultivation time. Additionally, there is a lack of quantitative research that has investigated the alterations in composition throughout various stages of flowering, as well as the significance of each stage within the flowering process (7, 9, 14, 17).

CONCLUSIONS

The utilization of herbal products for the treatment of diseases has garnered significant attention in recent years. This is primarily attributed to the wide range of phytometabolic components found in these products, which possess diverse chemical structures and exhibit various biological activities. The utilization of *Agastache* species in traditional medicine for the treatment of diverse ailments has been prevalent for an extended period of time.

Plants exhibit a substantial presence of polyphenols and flavonoids which was demonstrated in this paper for *Agastache foeniculum*, *Agastache mexicana* and *Agastache urticifolia*, which contribute to their robust antioxidant capabilities. Consequently, these properties enable plants to engage in diverse defensive mechanisms and combat diseases effectively. Phenolic compounds are regarded as significant constituents of plants due to the presence of one or more hydroxyl groups on their aromatic ring, making them secondary metab-

olites. Due to the absence of adverse effects on human health, there has been a rise in the utilization of plant species with elevated levels of phenolic compounds within the food industry, with the objective of enhancing the overall quality of food products.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest.

Mihaela-Magdalena Flutur and Denisa Marin-Batir, had equal contribution and should be regarded as main authors of the manuscript.

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