



Phytochemical and antioxidant Analysis of the five genus *Mentha* in AL-Jabal AL-Akhder – Libya

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Abstract

In this study, significant differences were observed between the antioxidant values statistically analyzed by the standard curve obtained using different concentrations of the Prussian blue method to the extent that the five species can be divided into four groups and it confirmed by the statistical method used (standard deviation). The first group with the highest antioxidant value is *M. pulegium* followed by *M. spicata*, which occupied the second position (second group). The third group *M. piperita*, The last position with the lowest value was represented by *M. aquatic* and *M. longifolia* (the fourth group). Chemical tests for the presence of some secondary metabolites revealed that. The presence of Tannins, Sterols and Flavonoids in different amounts in the five species. *M. aquatic* and *M. longifolia* lack the presence of alkaloids, while there is a negative result for saponins in *M. longifolia*.

Keywords: Antioxidant; Chemical Taxonomy ; *Mentha*; Phytochemical.

INTRODUCTION

Mentha L. is the most important genus of the Lamiaceae family because there are many species that produce economically valuable essential oils. This genus is the second most oil-producing species after *Citrus* L (Mucciarelli, et al., 2001), and is of great medicinal and commercial importance. Typically, mint plants' leaves, flowers, and stems are utilized. These parts are commonly employed in herbal teas or as an addition to spice blends for various foods to enhance their flavor. Furthermore, mint is widely recognized as a popular remedy for conditions such as anorexia, flatulence, bronchitis, nausea, ulcerative colitis, and liver diseases. Among the numerous mint species, *M. piperita*, *M. spicata* and *M. Canadensis* are commercially the most significant. (Bhat, et al., 2002) Among these species, they are cultivated only for oil production. Pepper mint essential oil is one of the most popular and used essential oils, especially for its menthol, menthone and Carvone content. Wild mint is the best source of natural menthol. It is used to flavor pharmaceutical and oral preparations, such as toothpastes, creams, and mouthwashes. Its pleasant taste makes it an excellent gastric stimulant. Fresh and dried mint plants are widely used in various applications. Since ancient times, Eastern and Western cultures have used mint as a medicinal and aromatic plant. In terms of biological uses, mint is also used as an antimicrobial and antioxidant agent. Due to its antioxidant, anti-radical, and chelating properties, the inclusion of mint in foods can help maintain the balance of the



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redox state in the body and improve the safety and the effect on human welfare. Mint is widely used in cosmetics and soaps (Hajlaoui, et al., 2009). In addition, essential oil is used for its carminative, hepatoprotective, antiviral, and anticancer properties.

The antimicrobial properties of essential oils have found applications in various fields, including food preservation, pharmaceuticals, alternative medicine, and natural therapies. This is particularly significant due to the increasing prevalence of antibiotic-resistant bacteria and the limitations of traditional food preservation. Essential oils have demonstrated potent activity against a wide range of microorganisms, including both gram-positive and Gram-negative bacteria, as well as fungi and human pathogens. For instance, they have shown effectiveness against highly resistant strains of bacteria such as *Escherichia coli*, *Streptococcus pneumoniae*, *Salmonella laenteritidis* and *Salmonella typhi*, which often exhibit resistance to synthetic drugs. Furthermore, essential oils have exhibited significant antifungal properties, particularly against *Candida albicans* and dermatomycetes. Numerous laboratory studies have confirmed the antimicrobial activity of various *Mentha* species, both cultivated and wild.

MATERIALS AND METHODS

The plant species used in this research were collected from different areas in Al-Jabal Al-Akhder of Libya. They were identified in the Silphium Herbarium Department of Botany, Faculty of Science, Omer AL-Mukhtar University.

Table: (1). Location and details of the populations of *Mentha* Species studied

Scientific name	Common name	Collection site	Collection date
<i>M. aquatica</i> L.	Water mint	Wadi Darna	2022-5-24
		Al-Wasita	2021-12-29
<i>M. pulegium</i> L.	Wild mint	Ain Al-dabosia	2022-6-11
<i>M. spicata</i> L.	Nanaa	Wadi Darna	2022-4-28
<i>M. piperita</i> L.	Magriby mint	Wadi Darna	2022-4-28
		Al-Wasita	2021-12-29
<i>M. longifolia</i> L.	Saudi mint	Al-Bayda	2022-5-26

The fresh samples were stretched between newspaper sheets and pressed inside a herbarium press, and allowed to dry. To avoid rotting of the plant material, the sheets were changed after two days from the time of collection. The samples were then mounted, labeled, and deposited with the other species at the Herbarium of the Botany Department.

Plant specimens were identified by comparison with the description mentioned in Libyan flora books under the supervision of the Silphium Herbarium team at Omar Al-Mukhtar University.

Determination of Antioxidant Capacity by Prussian Blue Method

One gram of the powdered sample was dissolved in ether oil. The dissolved powder is then extracted by mixing twice with 10 ml of methanol, then with 10 ml of 1% hydrochloric.

Acid: methanol (v/v). The three combined extracts were subjected to evaporation under reduced pressure. The resulting residue was subsequently dissolved in 10 ml of methanol. Half a milliliter of the solution was diluted with 3 ml of distilled water, 3 ml (0.008 M) $K_3Fe(CN)_6$ (potassium ferric cyanide), 3 ml of 0.1 M HCl, and 1 ml of 1% $FeCl_3$ (ferric chloride). The blue dye was allowed to develop for five minutes the absorbance is measured at 720 nm against the blank (Wangensteen, et al., 2004). A standard curve was prepared using different concentrations of ascorbic acid. Samples dilution was required in some samples before treatment with the Prussian blue reagent.

Phytochemical Analysis

Preparation of Crude Plant Extracts

The leaves of the studied plants were separated and washed several times with distilled water. The samples were then dried in a dark and dry place. Then the samples were ground by mortar and stored in polyethylene bottles until analysis.

The crude plant extracts were prepared by dissolving 100g each of the samples separately in 500 ml of successive solvents with increasing polarities (chloroform, ethanol, and ethyl acetate).

The plant materials were soaked overnight in the solvent, filtered and evaporated to dryness under reduced pressure in a rotary evaporator. The extracts were then evaporated and weighed. Three replicates were used for each sample.

Phytochemical Screening of Extracts

All the phytochemical screening tests were carried out according to standard methods. The methods are described as follows:

Chemical Tests

Preliminary screening for the major classes of secondary metabolites was conducted according to the techniques described by (Harborne, 1973).

1) Tannins

One ml of the reagent 1% FeCl₃ (ferric chloride solution) was added two ml of the ethanol extract were taken in a test tube. Blue color develops in cases of the presence of tannins.

2) Saponins:

Five ml of tap water is added to 1 ml of each extract, then shaken vigorously for five minutes, a froth develops having 1cm high and persists for 15 minutes indicating the presence of Saponin.

3) Sterols

One ml of the chloroform extract of each sample with 0.3 ml of acetic anhydride was mixed, and then a few drops of concentrated sulfuric acid were added along the side of the dry test tube. A red-dish-violet color is produced at the junction of the two layers, and chloroform solution acquires a green color in case of the presence of sterols.

4) Flavonoid glycosides

The extracts (alcohol and aqueous) of the tested herbal plants were further extracted with 1% hydrochloric acid. Each extract was subjected to the following test: 10 mL of each extract was rendered alkaline, where a faint yellow color is produced in the case of the presence of flavonoids.

Two ml of the extract is mixed with 0.2 ml ethanolic-naphthol (2%), and 2 ml of concentrated sulfuric acid is added to the side of the dry test tube. A violet ring is observed at the junction of the two layers, indicating the presence of glycosides.

5) Alkaloids

The extracts of the tested herbal plants were further extracted with 20 ml of dilute hydrochloric acid, cooled and rendered alkaline with dilute ammonium hydroxide solution, then extracted with chloroform. The chloroform extract is subjected to the following tests:

Dragendorff: The preparation of the reagent:

Solution a. Solution a. Basic bismuth nitrate was dissolved in a mixture of 10 ml of acetic acid and 40 ml of distilled water.

Solution b. About (8g) of potassium iodide was dissolved in 20ml water.

Stock solution: Equal volumes of solutions (a) and (b) are mixed.

A few drops of chloroform extract were applied to filter paper, allowed to dry, and sprayed with the reagent orange color is observed in cases of the presence of alkaloids.

RESULTS

Antioxidants Determination

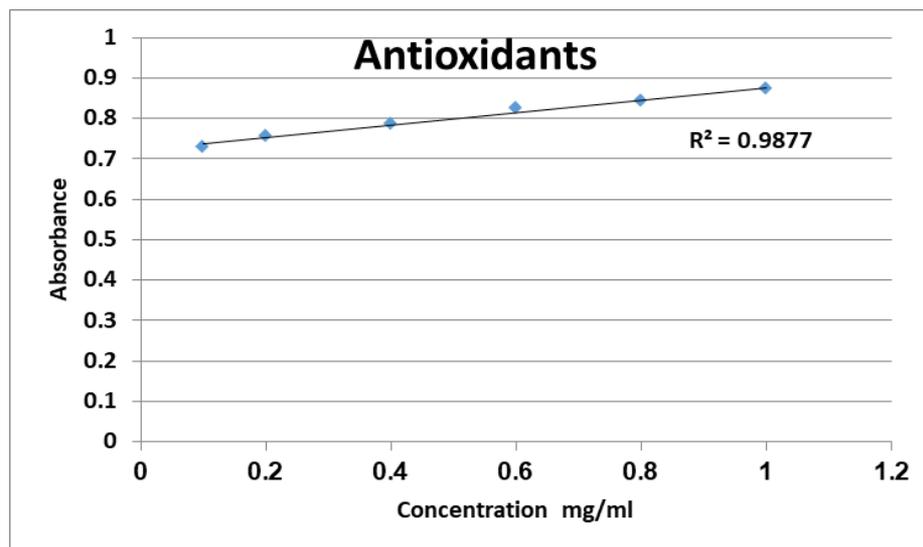


Fig:(1) . Standard Curve for the Estimation of Antioxidant (Conc.mg/ml against Absorbance nm)

Significant differences were observed between the antioxidant values statistically analyzed by the standard curve obtained using different concentrations of the Prussian blue method (fig10) , (Table7), to the extent that the five species can be divided into four groups as and it is confirmed by the statistical method used (standard deviation).

- 1-The first group with the highest antioxidant value is *M. pulegium*
- 2-Followed by *M. spicata* which occupied the second position (second group).
- 3-The third group *M. piperita*.
- 4-The last position with the lowest value was represented by *M. aquatic* and *M. longifolia* (the fourth group).

Table: (2). Levels of Antioxidants in Samples of *Mentha* Species (Mean \pm SD)

Samples	Mean \pm SD
<i>M. aquatica</i>	2.13400 \pm 0.01493 ^C
<i>M. piperita</i>	2.2223 \pm 0.0252 ^{BC}
<i>M. pulegium</i>	2.8443 \pm 0.0403 ^A
<i>M. longifolia</i>	2.1477 \pm 0.0573 ^C
<i>M. spicata</i>	2.3363 \pm 0.0677 ^B
Standerd	0.80200 \pm 0.00784 ^D

Values are expressed as means \pm SD; each sample group consisted of three replicates. Mean values within the same column that do not share the same superscript letter (a, b, c, d) were statistically significantly different from each other with $p < 0.05$.

Phytochemical Analysis:

Chemical tests for the presence of some secondary metabolites revealed that:
The presence of Tannins, Sterols and Flavonoids in different amounts in the five species.

M. aquatic and *M. longifolia* lack the presence of alkaloids, while a negative result for saponines is found in *M. longifolia*.

Table: (3). Levels of Chemical Tests in samples of *Mentha* Species

<i>Mentha Spp</i>	Tannins	Saponines	Sterols	Flavonoids	Alkaloids
<i>M. aquatic</i>	+	++	++	+++	-
<i>M. piperita</i>	++	++	+++	++	+
<i>M. pulegium</i>	+++	+++	++	+	+
<i>M. longifolia</i>	++	-	+++	+	-
<i>M. spicata</i>	+	+	+++	+++	+++

DISCUSSION

In this study, all essential oils derived from peppermint exhibited strong antioxidant properties. This high antioxidant activity can be attributed to the elevated levels of oxygenated mono terpenes present in all peppermint species. The difference in antioxidant activity found between species reflects differences in oil composition. The substance that played a major role in changing the antioxidant activity among the students was menthol. However, the lack of a significant relationship between the amount of other components and the antioxidant activity does not eliminate the antioxidant capacity, since its biological activities are well established. Therefore, it is often the secondary components, rather than the primary components of essential oils, that contribute significantly to their antioxidant activity. Furthermore, the synergistic interaction between small and large components within the oil can enhance its overall antioxidant potential. (Benabdallah, et al., 2018). Many medicinal plants, such as those belonging to the genus *Mentha*, are rich in antioxidants like ascorbic acid, phenolic compounds, and carotenoids. These compounds have the ability to delay or prevent cellular aging processes (Park et al., 2019). Phenolic compounds, in particular, function as free radical scavengers and inhibit lipid peroxidation (Rice-Evans et al., 1997).

The results obtained for the chemical presence of secondary metabolites provided different points of support for both the similarities and differences among the species within the group. Tannins are present in the species. Identified a variety of plant sources for traditional tanning materials, including oak, willow, chestnut, sumac, alder, birch, hemlock, barberry, heather, bloodroot, alfalfa, tea, sweet galls, and certain ferns. Saponins, however, were not found in *M. longifolia*. In contrast, saponins are prevalent in many desert plants as reported by (Sparg et al., 2004; Alaila et al., 2017) further confirmed the presence of saponins in three species of Lamiaceae. (Kambouche et al., 2009) specifically analyzed *Anabasis articulata* and concluded that the saponins play a crucial role in blood sugar regulation.

The absence of detectable saponins in the two species examined in this study may be attributed to their low concentration. Flavonoids are present in different concentrations. The highest concentration was observed in *M. aquatic* and *M. spicata*; followed by *M. piperita*, while the lowest concentrations were detected in *M. pulegium* and *M. longifolia*. It has also been reported that, in general, a specific group of flavonoids (Malkin & Rabinowitz 1967) characterizes each of the species.

CONCLUSION

According to the results recorded in this study for the antioxidants, Phytochemicals in five species of *Mentha*, the difference in antioxidant activity found between species reflects differences in oil composition. Also, the results obtained for the chemical presence of secondary metabolites

provided different points of support for the similarities and differences regarding the species of the group, for example, Flavonoids are present in different concentrations. The highest concentration was observed in *M. aquatica* and *M. spicata*; followed by *M. piperita*, while the lowest. Concentrations were detected in *M. pulegium* and *M. longifolia*.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions :Contribution is equal between authors.

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REFERENCES

- Alaila, A. K., Hamad, H., Ali, R. F., & Adress Hasan, H. M. (2017). Phytochemical screening of some herbal plants (Menthe, Origanum, and Salvia) growing at al-gabal al-akhder region-Libya. *International Journal of Pharmacy & Life Sciences*, 8(4).
- Benabdallah, A., Boumendjel, M., Aissi, O., Rahmoune, C., Boussaid, M., & Messaoud, C. (2018). Chemical composition, antioxidant activity, and acetylcholinesterase inhibitory of wild Mentha species from northeastern Algeria. *South African Journal of Botany*, 116, 131-139.
- Bhat, S., Maheshwari, P., Kumar, S., & Kumar, A. (2002). Mentha species: in vitro regeneration and genetic transformation. *Molecular Biology Today*, 3(1), 11-23.
- Hajlaoui, H., Trabelsi, N., Noumi, E., Snoussi, M., Fallah, H., Ksouri, R., & Bakhrouf, A. (2009). Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. *World Journal of Microbiology and Biotechnology*, 25, 2227-2238.
- Harborne, J.B (1973). *Phytochemical methods. A guide to modern techniques of plants analysis*. Chapman and Hall Press. London. University of Reading.
- Kambouche, N., Merah, B., Derdour, A., Bellahouel, S., Bouayed, J., Dicko, A., & Soulimani, R. (2009). Hypoglycemic and antihyperglycemic effects of *Anabasis articulata* (Forssk) Moq (Chenopodiaceae), an Algerian medicinal plant. *African Journal of Biotechnology*, 8(20).
- Malkin, R. I. C. H. A. R. D., & Rabinowitz, J. C. (1967). Nonheme iron electron-transfer proteins. *Annual Review of Biochemistry*, 36(1), 113-148.
- Park, Y. J., Baek, S. A., Choi, Y., Kim, J. K., & Park, S. U. (2019). Metabolic profiling of nine Mentha species and prediction of their antioxidant properties using chemometrics. *Molecules*, 24(2), 258.
- Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in plant science*, 2(4), 152-159.
- Sparg, S., Light, M. E., & Van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of ethno pharmacology*, 94(2-3), 219-243.
- Wangensteen, H., Samuelsen, A. B., & Malterud, K. E. (2004). Antioxidant activity in extracts from coriander. *Food chemistry*, 88(2), 293-297.