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## PHYTOCHEMICAL ANALYSIS AND REDUCING PROPERTY OF HYDROALCHOLIC AERIAL EXTRACT OF *MENTHA ARVENSIS* LINN FROM KASHMIR

Mohammad Akbar Dar, Mubashir H. Masoodi\*, Parampreet Kour

Department of Pharmaceutical Sciences, University of Kashmir, Srinagar-190006, J&K, India

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### For Correspondence:

**Dr. Mubashir Hussain Masoodi**

Department of Pharmaceutical  
Sciences, University of  
Kashmir, Srinagar-190006,  
J&K, India

### E-mail:

[mube5090@gmail.com](mailto:mube5090@gmail.com),  
[akbardr297@gmail.com](mailto:akbardr297@gmail.com)

### ABSTRACT

Medicinal plants contain bioactive compounds capable of preventing and fighting oxidative related diseases and other various diseases. These phytoconstituents must be screened and assayed before effective drugs are developed. Thus, phytochemical constituents and reducing property of hydroalcoholic (water : methanol, 1:1) extract of aerial parts (leaf and stem) of *Mentha arvensis* L from Kashmir region were evaluated, and revealed that the extract contained flavonoids, phenols, terpenoids, alkaloids, steroids, saponins, carbohydrates, proteins, fats and tannins. The total phenolics and total flavanoids were qntitatively estimated. The extract showing more phenolic content than flavanoids content 321.78 and 142.23 mg/g respectively. The reducing power of extract was found to increase with concentration of extract and was 0.31 at 0.1mg/ml and increased to 1.97 at 0.5mg/ml. Thus, hydroalcoholic extract of aerial parts (leaf and stem) of *Mentha arvensis* L could serve as sources of antioxidants and bioactive compounds for nutrition and therapeutic purposes.

## INTRODUCTION

The major source of drugs and natural products are medicinal plants on the basis of their therapeutics in virtually all cultures <sup>[1, 2]</sup>. The use of plants as medicines has involved the isolation of secondary metabolites and bioactive compounds. The active principle is usually found in specific part of plant such as aerial parts, leaves, flowers, fruits, seed, root, rhizome and bark and the crude plant extract of these parts is a complex mixture of phytochemical constituents <sup>[3]</sup>. It is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. These phytoconstituents work with nutrients and fibers to form an integrated part of defence system against various diseases and stress conditions, and are basically divided into two groups, i.e. primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acid, proteins and chlorophyll while secondary constituents consist of alkaloids, phenolics, tannins, terpenoids, steroids and flavonoids so on. These natural compounds formed the base of modern drugs as we use today <sup>[4, 5]</sup>.

*Mentha arvensis* Linn belonging to family Lamiaceae is native to the temperate regions of Europe and western and central Asia, east to the Himalaya and eastern Siberia, and America. It is a herbaceous perennial plant growing to 10–60 cm (rarely to 100 cm) tall. The leaves are in opposite pairs, simple, 2–6.5 cm long and 1–2 cm broad, hairy, and with a coarsely serrated margin. The flowers are pale purple (occasionally white or pink), in clusters on the stem, each flower 3–4 mm long. The plant is widely distributed throughout India and leaves of the plant are extensively used in traditional system of medicine for various ailments like carminative, digestive, expectorant, cardiogenic, diuretic, dentifrice, jaundice, hepatalgia, inflammation of liver, peptic ulcer, diarrhoea, bronchitis and skin diseases. The locals use the powder of aerial parts mixed with dilute curd to cure cough, sore throat, constipation etc <sup>[6]</sup>. The focus of this study is to evaluate preliminary phytochemical constituents, quantitative determination of phenolics, flavonoids and reducing potential of hydroalcoholic aerial extract of *Mentha arvensis* L.

## MATERIALS AND METHODS

### Collection of Plant material and preparation of various extracts of *M. arvensis* L.

The *Mentha arvensis* L. was collected in July-August 2012 from Narabal, Budgam, J&K. The plant was authenticated by the centre of Plant taxonomy, Department of Botany, University of Kashmir, Hazratbal, Srinagar. The plant material (250 g) was dried under shade and crushed to coarse powder and the powdered drug material was taken in a percolator for extraction (cold extraction) using hydroalcohol (water : methanol, 1:1) as solvent. The extract were semi-dried

under reduced pressure and then on water bath to get the crude dried fraction. The yield of dried fraction 13.06 g% and was stored in a refrigerator for further use.

#### **Source of Chemicals**

All the chemicals were purchased from a local dealer and were HiMedia Laboratories Pvt. Ltd. Mumbai and Central Drug House Ltd. New Dehli, India made and was of analytical grade.

#### **Phytochemical evaluation**

**Tannins:** To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins<sup>[7]</sup>.

**Alkaloids:** Alkaloid solution produces white yellowish precipitate when few drops of Mayer's reagents are added<sup>[8]</sup>. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent<sup>[9]</sup>. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

**Saponins:** 20 ml Water is added to 150mg extract and shaken vigorously, layer of foam formation indicates the presence of saponins<sup>[10]</sup>.

**Glycosides:** To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer<sup>[10]</sup>.

**Terpenoid and Steroid:** Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids<sup>[10]</sup>.

**Flavonoids:** 2 g plant material was extracted in 10 ml alcohol or water. To 2 ml filtrate few drops of concentrated HCl followed by 0.5 g of zinc or magnesium turnings was added. After 3 minutes magenta red or pink colour indicated the presence of flavonoids<sup>[11]</sup>.

**Phenolics:** To 2 ml of alcoholic or aqueous extract, 1 ml of 1% ferric chloride solution was added. Blue or green colour indicates phenols<sup>[12]</sup>.

**Carbohydrates:** To 2ml of test solution add 2-3 drops of Molish reagent; add 2ml of conc. H<sub>2</sub>SO<sub>4</sub> along the sides of test tube to form two layers. Violet ring at the junction of two liquids indicate the presence of carbohydrates<sup>[13]</sup>.

**Proteins:** To 2ml of test solution add 2ml of 4% NaOH, to this add few drops of biuret reagent. Violet or pink colour indicates the presence of proteins<sup>[14]</sup>.

**Fats & oils:** 1 ml of the extract was added to a filter paper. These extract was allow it for evaporation on filter paper and the appearance of transparency on filter paper indicates the presence of fats & oils <sup>[15]</sup>.

**Table 1: Results of phytochemical screening of hydroalcoholic aerial extract of *Mentha arvensis* L.**

| Tests              | Hydroalchol (1:1, water: methanol) |
|--------------------|------------------------------------|
| Alkaloids          | +                                  |
| Tannins            | +                                  |
| Phenolics          | ++                                 |
| Flavonoids         | +                                  |
| Cardiac Glycosides | -                                  |
| Terpenoid          | +                                  |
| Steroid            | +                                  |
| Saponins           | +                                  |
| Carbohydrates      | +                                  |
| Proteins           | +                                  |
| Fats               | +                                  |

+ = Slight coloration; ++ = Deep coloration; - = Absent

#### **Determination of the Total Phenolic and Flavonoid content**

The concentration of phenolics in hydroalcoholic aerial extract of *Mentha arvensis* L. was determined using standard method <sup>[16]</sup>. Crude extract of *Mentha arvensis* were dissolved in the concentration of 1mg/ml. The reaction mixture was prepared by mixing 0.5 ml of methanol solution of extracts, 2.5ml of 10% Folin's-Ciocalteu's reagent dissolved in water and 2.5ml of 7.5% NaHCO<sub>3</sub>. Blank was concomitantly prepared, containing 0.5ml methanol 2.5ml of 10% Folin's-Ciocalteu's reagent dissolved in water and 2.5ml of 7.5% NaHCO<sub>3</sub>. The samples were then incubated for 45mins at a temperature of 45degrees. Absorbance was measured at 765nm. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for standard solution of Gallic acid and for control all reagents except extract was used <sup>[17]</sup>. The content of flavanoids in hydroalcoholic aerial extract of *Mentha arvensis* L. was determined using standard procedure. The sample contained 1ml of

methanol solution of the extract in the concentration of 1mg/ml and 1ml of 2%  $\text{AlCl}_3$  solution dissolved in methanol. Same procedure was repeated for other extracts of *Mentha arvensis*. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at 415nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The content of flavanoids in extract was expressed in terms of rutin equivalent (mg of RU/g of extract) <sup>[18]</sup>.

#### **Determination of reducing power**

The reductive capability of the extract was quantified by the method of Oyaizu <sup>[19]</sup>. One ml of 100, 200, 300, 400 and 500µg/ml of extract was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ]. Similar concentrations of standard ascorbic acid were used as standard. The mixture was incubated at 50°C for 20 min. Then, the reaction was terminated by adding 2.5 ml of 10% trichloroacetic acid. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of 0.1%  $\text{FeCl}_3$ . Blank reagent is prepared as above without adding extract. The absorbance was measured at 700 nm in a spectrophotometer against a blank sample. Increased absorbance of the reaction mixture indicated greater reducing power.

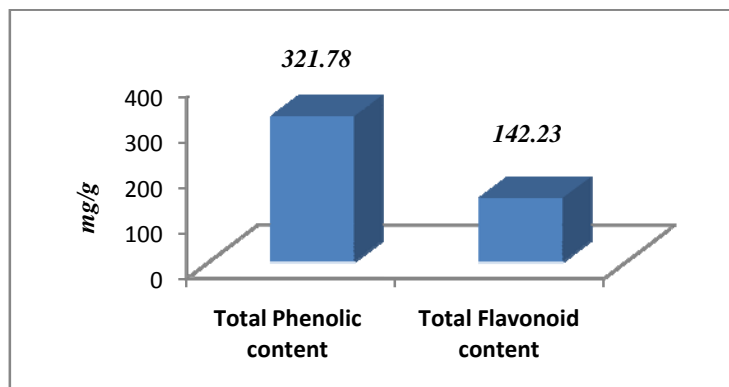
#### **RESULTS AND DISCUSSION**

The present study investigated the phytochemical constituent of hydroalchoic aerial extract of *Mentha arvensis* L. The results indicated presence of flavonoids, phenols, terpenoids, alkaloids, steroids, saponins, carbohydrates, proteins, fats and tannins (Table 1). Alkaloids are reported to have anti-inflammatory, antimicrobial and antifungal effect and also acts as an antihypertensive agent <sup>[20, 21]</sup>. Tannins from plants are reported to having healing properties<sup>[20]</sup>. Flavonoids and phenolics are most important groups of secondary metabolites and bioactive compounds in plants. Flavonoids have important roles in human life and health. Their function in human health is supported by the ability of flavonoids to induce human protective enzyme systems, and by number of epidemiological studies which suggests that flavonoid is protective against cardiovascular diseases, cancers and other related diseases <sup>[22]</sup>. Flavonoids and phenols are potent water soluble antioxidants which prevent oxidative cell damage and possess antiseptics, anticancer, anti-inflammatory effects and mild antihypertensive properties <sup>[23, 24]</sup>. Also phenolics are major group of compounds having capability of scavenging reactive oxygen species hence may act as antioxidants <sup>[25]</sup>. The content of phenolic compounds (mg/g) in Gallic acid equivalent and total Flavonoid content (mg/g) in Rutin equivalent of hydroalcholic aerial extract of *Mentha arvensis* L. was 321.78 and 142.23 mg/g respectively (Figure 1, Table 2).

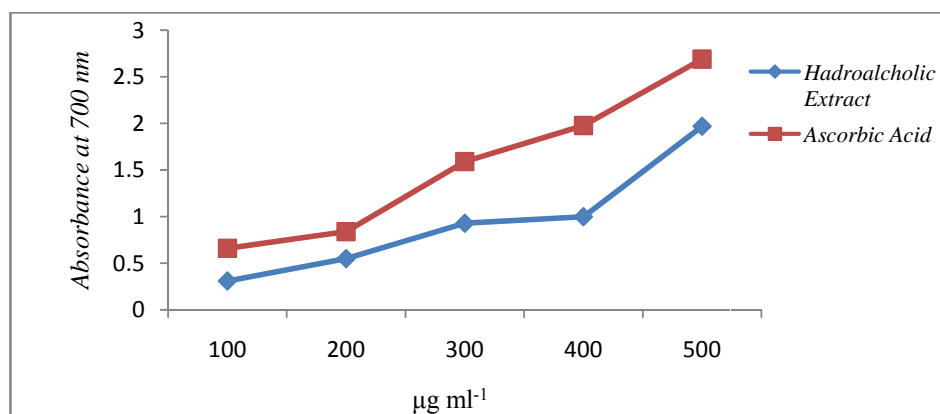
**Table 2: Total amount of phenolic and flavonoid content of the various extract of aerial parts of *Mentha arvensis*, [Mean  $\pm$  S.E.M. a]**

| Total phenolics mg/g plant extract (in GAE) | Total flavonoid mg/g plant extract (in RE) |
|---|--|
| 321.78 $\pm$ 1.41                           | 142.23 $\pm$ 3.92                          |

(a): average of three determinations

**Figure 1:** Total phenolic and Total flavanoid content of hydroalcoholic aerial parts of *Mentha arvensis* L.

The reducing capacity of the extract may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidants substances in the antioxidant samples causes the reduction of the  $\text{Fe}^{3+}$ /ferricyanide complex to the ferrous form. Therefore, the  $\text{Fe}^{2+}$  can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. For the measurements of the reductive ability, the  $\text{Fe}^{3+}$ -  $\text{Fe}^{2+}$  transformation was investigated in the presence of hydroalcoholic extract using the method of Oyaizu [19]. The reducing power of extract was found to concentration dependent and increased with concentration of extract and was 0.31 at 0.1mg/ml and increased to 1.97 at 100  $\mu\text{g/mL}$ , when compared with ascorbic acid which showed increase from 0.66 at 100 to 2.69 at 500  $\mu\text{g/mL}$  (Figure 2). This may be due the presence of phenolics and flavonoids in the extract.

**Figure 2.** Reducing power of hydroalcoholic aerial extract of *Mentha arvensis* L.

## CONCLUSION

The above results indicate that, the hydroalchoic aerial extract of *Mentha arvensis* L. is rich in alkaloids, flavonoids, steroids, terpenoids, phenols, saponins and tannins. Thus the hydroalchoic aerial extract of *Mentha arvensis* L. has medicinal potential and could be exploited as sources of free radical scavengers because of its reducing property and bioactive metabolites for nutritional, medicinal and commercial purposes.

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