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In Vitro Antioxidant Activity of Methanol Aerial Extract of *Mentha Arvensis* Linn from Kashmiri Himalaya

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ABSTRACT

To evaluate phytochemical constituents and antioxidant potential of methanol aerial parts (leaf and stem) extract of *Mentha arvensis* L from Kashmiri Himalaya region. The extract shows the presence of flavonoids, saponins, alkaloids, steroids, carbohydrates, proteins, tannins and phenolics. The antioxidant activity of methanol aerial extract of *Mentha arvensis* L was evaluated by using 1,1-diphenyl, 2-picrylhydrazyl (DPPH) scavenging and reducing power assays. The total phenolic and total flavonoid content (mg/g) was found to be 672.11 and 283.1 respectively. The percentage inhibition of DPPH scavenging was found to be 81.33% at concentration of 0.5mg/ml. The reducing power was found to be 0.76 at 0.1mg/ml and increased to 2.42 at 0.5mg/ml. The results obtained revealed that methanol extract from aerial parts of *Mentha arvensis* L can be promising candidates for natural sources of antioxidants with high activity.

Keywords: *Mentha arvensis;* DPPH; antioxidant potential; reducing power; phenolics; flavanoids.

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INTRODUCTION

In past, the utilization of herbal plants has drawn immense interest as they could accommodate therapeutic response and are promising candidate to be developed as pharmaceutical products like antioxidants, antibacterial agents etc[1, 2]. Free radicals have been responsible for initiating many serious diseases [3, 4], such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and also dementias [5]. Antioxidants cause protective effect by neutralizing free radicals, which are toxic byproducts of natural cell metabolism both in plants and animals. Metabolism in humans leads to the production of free radicals which are neutralised by body's natural antioxidant mechanism but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age[6]. Antioxidant compounds can minimize the incidence of these diseases by decreasing oxidative stress. The suppression of reactive oxygen species formation either by inhibition of the enzymes or by chelating of trace elements involved in free radical production, scavenging of reactive species and up- regulating or protecting antioxidant defence is the mechanism by which antioxidants act[7].

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 commonly used in traditional system of medicine for various purposes like carminative, digestive, expectorant, cardiotonic, diuretic, dentifrice, jaundice, hepatalgia, inflammation of liver, peptic ulcer, diarrhoea, bronchitis and skin diseases [8-11].The plant has been shown to possess anti–inflammatory and sedative–hypnotic activity, hepatoprotective and antioxidant activity, antibacterial and antifertility action[11-15]**.** The plant is the source for essential oils of monoterpenes like menthol, menthone, carvone and pulegone major constituents. This plant also possesses anti-Candida [16] and also radio protective activity against gamma radiation [17]. The locals in Kashmir uses the powder of aerial parts mixed with dilute curd to cure cough, sore throat, indigestion and constipation [18] and also the leaves are used in Diarrhoea and Asthma [19]. The focus of current study is to evaluate phytochemical constituents and antioxidant potential of methanolic aerial extract of *Mentha arvensis* L.

MATERIALS AND METHODS

Collection of Plant Material and Preparation of Various Extracts of *M. arvensis* L

The whole plant of *Mentha arvensis* L. was collected in July-August 2012 from the fields and orchids of Narabal, Budgam, J&K. The plant was authenticated by the centre of Plant taxonomy, Department of Botany, University of Kashmir, Hazratbal, and specimen was kept in KASH herbarium under a specific voucher number. The plant material aerial parts (stem and leaf) 500 g was dried under shade and crushed to coarse powder and the powdered drug material was taken in a percolator for (cold extraction) using methanol solvent and was kept as such for 48 hours with constant shaking. Then crude extract were filtered through Whatt man No-2 filter paper. The extract was evaporated to dryness at 40ºC by Rotary vacuum evaporator to obtain the dried extract. The yield of dried fraction was 13.5g%.The extract was stored in a refrigerator for further analysis.

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 were of the analytical grade.

Phytochemical evaluation

Various chemical tests were carried out on methanol aerial extract using standard procedures to identify the constituents such as alkaloids, glycosides, phenolics, terpenoids and steroids, flavonoids, saponins, carbohydrates, proteins, fats and tannin. The results for presence of various constituents in methanol aerial extract of *Mentha arvensis* L. are given in (Table 1).

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 [20].

Alkaloids

Alkaloid solution produces white yellowish precipitate when few drops of Mayer's reagents are added [21]. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent [22].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 [23].

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 [23].

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 [23].

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 [24].

Phenolics

To 2 ml of alcoholic or aqueous extract, 1 ml of 1% ferric chloride solution was added. Blue or green colour indicates phenols [25].

Carbohydrates

To 2ml of test solution add 2-3 drops of Molish reagent; add 2ml of conc. H_2SO_4 along the sides of test tube to form two layers. Violet ring at the junction of two liquids indicate the presence of carbohydrates [26].

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 [27].

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 [28].

Antioxidant Activity

Determination of DPPH frees radical scavenging

The free radical scavenging capacity of methanol aerial extract of *Mentha arvensis* was determined using DPPH [29]. Freshly prepared DPPH (2,2-diphenyl-1-picrylhydrazyl), solution was taken in test tubes and extract were added followed by serial dilutions (50µg/ml to 250µg/ml) to every test tube so that the final volume was 3 ml and after 30 min, the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid was used as standard. Control

sample was prepared containing the same volume without any extract and standard and the absorbance was read at 517 nm using a spectrophotometer. Methanol was served as blank.

Determination of reducing power

The reductive capability of the extract was quantified by the method of Oyaizu (1986) [30]. One ml of methanol extract 100, 200, 300, 400 and 500µg/ml was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K_3 Fe $(CN)_6$]. 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%FeCl3. 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.

Determination of the Total Phenolic and Flavonoid content

The concentration of phenolics in methanol aerial extract of *Mentha arvensis* was determined using standard method [31]. Crude methanol 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 extract, 2.5ml of 10% Folin's-Ciocalteu's reagent dissolved in water and 2.5ml of 7.5% $NaHCO_3$. 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% $NaHCO_3$. 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 [32].

The content of flavanoids in the methanol aerial extract 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% $AlCl_3$ solution dissolved in methanol. 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 flavonoids in extract was expressed in terms of rutin equivalent (mg of RU/g of extract) [33].

RESULTS AND DISCUSSION

DPPH frees radical scavenging activity

The ability of methanol aerial extract of *Mentha arvensis* to scavenge DPPH free radical was

calculated as percentage inhibition which was found to be 81.33% at concentration of 500 µg/ml, where as percentage inhibition of ascorbic acid at the same concentration was 99.16 (Figure 1).

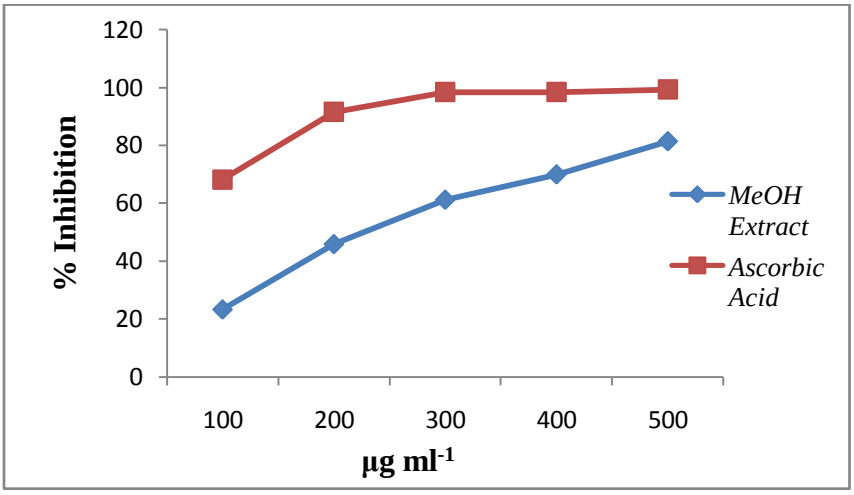

Figure 1: DPPH scavenging activity of methanol aerial extract of *Mentha arvensis*.

Reducing power

Methanol extract of aerial parts of *M. arvensis* showed good reducing power when compared with ascorbic acid (Figure 2). The extract showed good reducing power 2.42 at the concentration of 500 µg/ml, when compared to standard ascorbic acid which showed 2.69 at the same concentration.

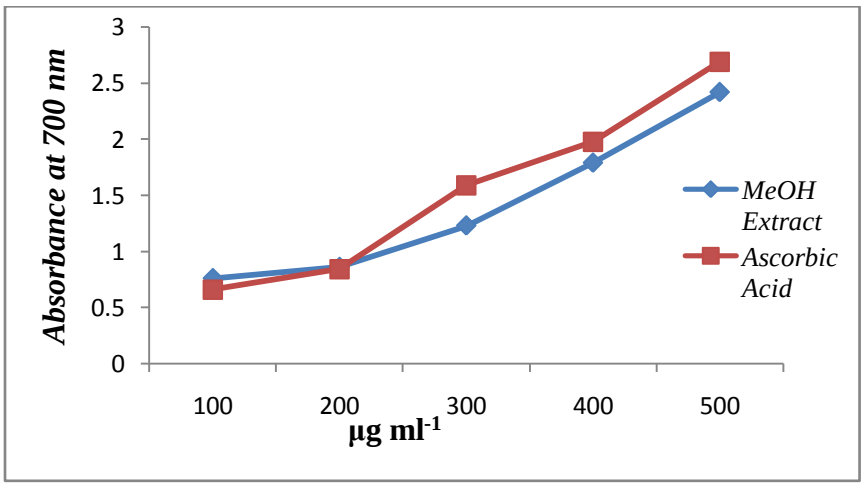

Figure 2: Reducing power of methanol aerial extract of *Mentha arvensis*

Total Phenolic and Flavonoid content

The results of Total Phenolic and Flavonoid content of methanol aerial extract of *Mentha arvensis* are give in (Table 2). The content of phenolic compounds (mg/g) in Gallic acid equivalent was found to be 672.11 mg/g and total Flavonoid content (mg/g) in Rutin equivalent was found to be 283.1 mg/g (Figure 3).

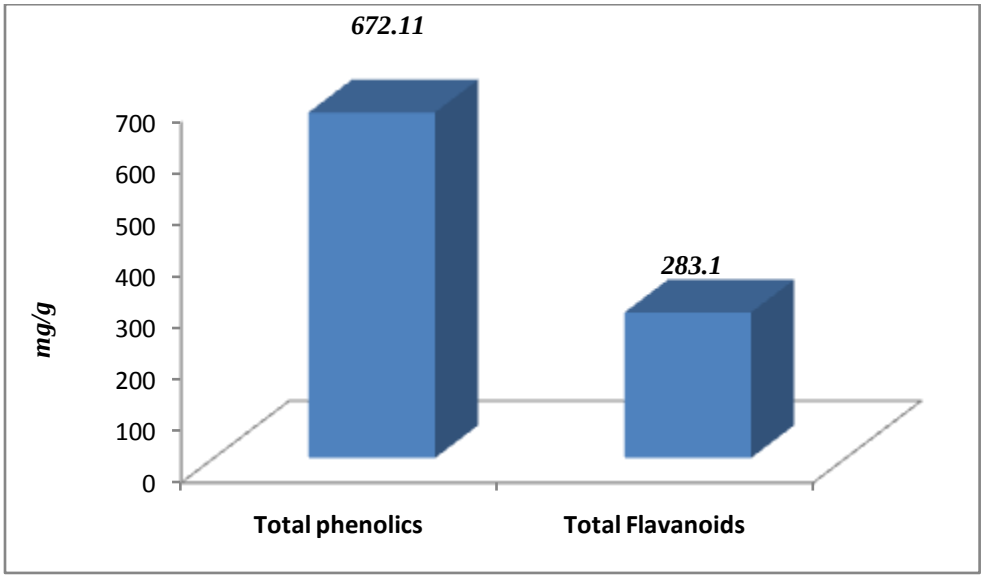

Figure 3. Total phenolic and Flavanoid content of methanol aerial extract of *Mentha arvensis.*

Preliminary phytochemical screening of methanol aerial extract of *Mentha arvensis* revealed the presence of flavonoids, saponins, alkaloids, steroids, carbohydrates, proteins, tannins and phenolics (Table 1).

Table1: showing the results of various phytoconstituents in methanol aerial extract of *Mentha arvensis* Linn.

Tests	Methanol aerial extract
Alkaloids	++
Tannins	+
Phenolics	+++
Flavonoids	+++
Cardiac Glycosides	+
Terpenoid	+
Steroid	+
Saponins	+
Carbohydrates	+
Proteins	+
Fats	+

+ = Slight coloration; ++ = Deep coloration; +++ = Very deep coloration.

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

Extract	Total phenolics mg/g plant extract(in GAE)	Total flavonoid mg/g plant extract(in RE)
Methanol	672.11 ± 2.5	283.1 ± 1.33

(a): average of three determinations

Recent studies have shown phenolic compounds from plants possess antioxidant activity [34, 35]. Therefore, the amount of total phenolic contents in the extracts was investigated by the Folin-Ciocalteu method. The content of total phenols is expressed as gallic acid equivalents (mg GAE/g extract). Recent studies have shown that many plant extract have antioxidant activity because of flavanoids which are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and also have anticancer activity [36-39]. Flavonoids and polyphenols found in *Mentha arvensis* extract could therefore be considered favourable for its antioxidant activity.

One of the most widely used methods for screening antioxidant activity of plant extracts is DPPH assay[40]. DPPH is a stable, nitrogen-centred free radical which produces violet colour in ethanol solution. It was reduced to a diphenylpicryl hydrazine, with the adding of the fraction in a concentration-dependent manner. The lessening in the number of DPPH molecules can be associated with the number of available hydroxyl groups. The methanol extract of aerial parts of *Mentha arvensis* showed good electron donating capacity towards DDPH radical showing percentage inhibition of 81.33% at concentration of 500 µg/ml as compared to standard ascorbic acid 99.16% at same concentration. The fraction showed significantly higher inhibition percentage (stronger hydrogen–donating ability) and positively correlated with total phenolic content. The conversion of Fe^{3+} into Fe^{2+} in the presence of various fractions was calculated to determine the reducing power ability. Reductones (antioxidants) are capable of donating electrons, which exert the antioxidant activity by breaking the free radical chain[41]. The reducing power was found to be 0.76 at 0.1mg/ml and increased to 2.42 at 0.5mg/ml while as ascorbic acid at the same concentration shows 2.62 (Figure 2). Methanol extract showed good reducing power when compared with standard ascorbic acid.

CONCLUSION

Thus from the results, it is concluded that methanol extract of the aerial parts of *Mentha arvensis* possess potent antioxidant action, when compared with standard ascorbic acid. Further studies to isolate, identify and characterize the active principle(s) are in progress to substantiate the present findings.

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