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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF AQUEOUS AERIAL EXTRACT OF *MENTHA ARVENSIS* LINN FROM KASHMIR

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ABSTRACT

Preliminary phytochemical screening by standard methods showed presence of various phytoconstituents in aqueous aerial extract of *Mentha arvensis* Linn. Antioxidant activity of aqueous aerial extract of *Mentha arvensis* Linn were evaluated by different assays. The extract was containing 21.01 mg/g total phenolic and 44.12 mg/g of total flavonoid content. The percentage inhibition of DPPH and Nitrous oxide scavenging was found to be 42.97% and 57.7% at concentration of 500 µg/mL respectively. The percentage inhibition of H_2O_2 scavenging was 25.31% at concentration of 1000 µg/mL. At concentration of 500 µg/mL, the percentage inhibition of metal chelating activity was 61.99%. The reducing power of extract was found to be 0.12 at 100 µg/mL and increased to 0.99 at 500 µg/mL. The results indicate that aqueous extract from aerial parts of *Mentha arvensis* L has moderate antioxidant activity.

Keywords: *Mentha arvensis;* DPPH; reducing power; nitrous oxide; metal chelating; H_2O_2.

INTRODUCTION

The importance of reactive oxygen species (ROS) has attracted attention globally over the past decade. Superoxide anion radical, hydroxyl radical and hydrogen peroxide are produced in human body by many enzymatic systems through oxygen consumption [1]. Free radicals are essential part of aerobic life and metabolism [2]. Over a hundred of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome are caused by reactive oxygen species

(ROS) by their ability to attack numerous molecules such as proteins and lipids. ROS include superoxide, anion, hydrogen peroxide (H_2O_2), peroxyl (ROO-) radicals, and reactive hydroxyl (OH-) radicals; the nitrogen derived free radicals are nitric oxide (NO_2) and peroxynitrite anion (ONOO-) [3, 4]. The diseases/disorders are mainly linked to oxidative stress due to ROS [5].In past plants and plant products are being used as a source of medicine.

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, less toxicity and economic viability [6]. Flavonoids and phenolic compounds widely distributed in medicinal plants which have been reported to possess multiple biological activities, like antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc [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. *Mentha arvensis* L. (Family: *Lamiaceae*) known as Pudina in Hindi, Sanskrit and Kannada.

In English it is called as Marsh mint. The common names of the plant are Field mint, Corn mint, Podina. 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, 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[12], hepatoprotective and antioxidant activity[13] , antibacterial [14] and antifertility action[15]. The plant consist essential oils of monoterpenes like menthol, menthone, carvone and pulegone major constituents. It is used in food products for its mint flavour and in oral products (e.g. tooth paste and mouth fresheners) for its physiological cooling effect. Thus it is also used as fragrance component in soaps, detergents, cosmetics and perfumes, toothpastes and industrial fragrances[9]. This plant also possesses anti-Candida [16] and also radio protective activity against gamma radiation [17].

The locals use the powder of aerial parts mixed with dilute curd to cure cough, sore throat, indigestion and constipation [18] also the leaves are used in Diarrhoea and Asthma [19].

MATERIALS AND METHODS

Collection of plant material and preparation of various extracts of *M. Arvensis*

The plant 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 aerial parts (stem and leaf) was separated (200 g) and dried under shade and crushed to coarse powder and the powdered drug material was taken in a percolator for (cold extraction) using water as solvent. After 48 hours, the filtrate was filtered through Whattman filter paper No.2. The extract was evaporated to dryness on water bath. The yield of dried fraction was 7.56g%.The extract 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 the analytical grade.

Phytochemical evaluation

Phytochemical screening of the extract was carried out by using standard phytochemical methods. The results for phytochemical screening of aqueous 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 aqueous 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 was added followed by serial dilutions (100µg/mL to 500µg/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 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 (extract) 100, 200, 300, 400 and 500µg/mL of aqueous extract 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 aqueous aerial extract of *Mentha arvensis* L was determined using standard method [31]. Crude aqueous extract was 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 plant 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 flavanoids in extract was expressed in terms of rutin equivalent (mg of RU/g of extract) [33].

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction [34]. In this assay, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *Mentha arvensis* extract (100µg/mL to 500µg/mL) or standard solution (rutin, 0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Rutin was used as a standard.

Metal chelating Activity

The ferrous level was monitored by measuring the formation of the ferrous ion-ferrozine complex [35]. The reaction mixture containing 1.0 ml of different concentrations aqueous extract of *Mentha arvensis* (100µg/mL to 500µg/mL) were added to 0.1 ml of 2 mM ferrous chloride and 0.2 ml of 5 mM ferrozine to initiate the reaction and the mixture was shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. The positive controls were those using ascorbic acid and all tests and analysis were run in triplicate. The percentage chelating effect of Ferrozine-Fe2+ complex formation was calculated. The chelating activity was calculated as

$$\% \text{ Chelating Activity} = [(A1 - A2) / A0] \times 100$$

Where A0 represents the absorbance of the control (without extract) and A1 represents the absorbance of reaction mixture, A2 represents the absorbance without $FeCl_2$.

Scavenging of Hydrogen Peroxide

The ability of the extract to scavenge hydrogen peroxide was determined according to our recently published papers [36-37]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extract (200, 400, 600, 800 and 1000µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compounds was calculated as follows: % Scavenged [H2O2] = [(Ao − A1)/Ao] × 100

Where Ao is the absorbance of the control and A1 is the absorbance in the presence of the sample of extract and standard [36] [38].

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of flavonoids, saponins, steroids, terpenoids, glycosides, carbohydrates, proteins, tannins and phenolics (Table 1).

Table 1 Results of phytochemical screening

Tests	Aqueous Extract
Alkaloids	-
Tannins	+
Phenolics	+
Flavonoids	++
Cardiac Glycosides	+
Terpenoid	+
Steroid	+
Saponins	+
Carbohydrates	+
Proteins	+
Fats	-

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

Antioxidant compounds act as free radical scavengers, initiator of the complexes of prooxidant metals, reducing agents and quenchers of singlet oxygen formation [39]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess the above functions [40]. Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities [41]. Recent studies showed that many flavonoids & related polyphenols contribute significantly to the total antioxidant activity of many plants [42]. The content of phenolic compounds (mg/g) in Gallic acid equivalent and total Flavonoid content (mg/g) in Rutin equivalent of aerial parts of *Mentha arvensis* was 21.01 and 44.12 mg/g respectively (Table 2 and Fig. 1), which may be responsible for its antioxidant behavior.

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

Total phenolics mg/g plant extract (in GAE)	Total flavonoid mg/g plant extract (in RE)
21.01 ± 0.52	4.12 ± 2.11

(a): average of three determinations

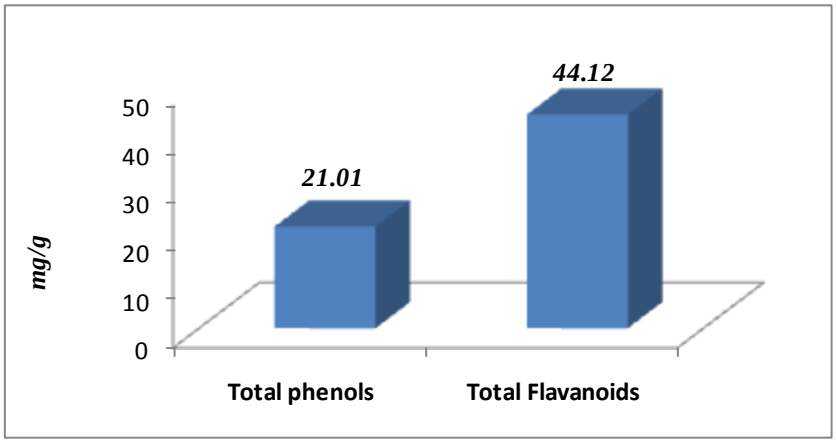

Figure 1. Total phenolic and Total flavanoid content of aqueous aerial extract of *Mentha arvensis*.

The DPPH free radical scavenging test is carried out to measure the hydrogen atom or electron donor capacity of the extracts to the stable DPPH radical formed in solution [43]. This method is a widely used method to evaluate the free radical scavenging ability of various samples [44]. DPPH free radical scavenging of aqueous aerial extract of *Mentha arvensis* was found to be 42.97% at concentration 500 µg/mL, where as percentage inhibition of ascorbic acid at the same concentration was 99.16% (Fig. 2).

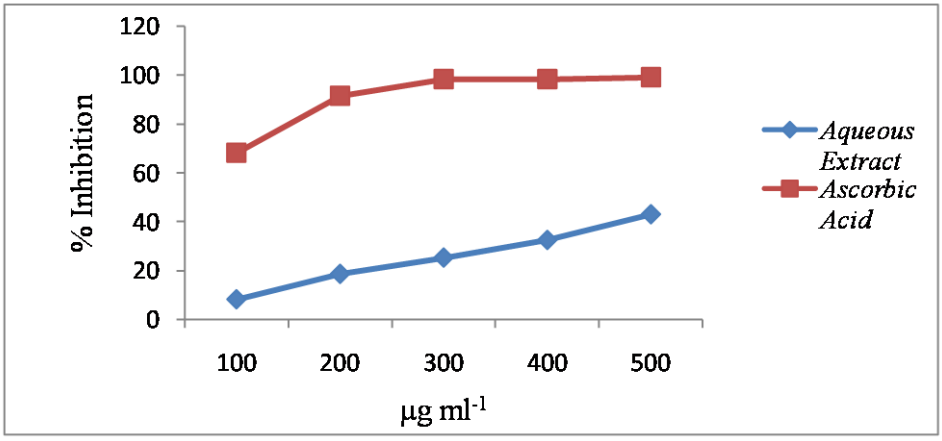

Figure 2. DPPH scavenging activity of aqueous aerial extract of *Mentha arvensis*.

The ability of aqueous aerial extract to reduce the $Fe^{3+/}$ferricyanide complex by forming ferrous products. Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue coloration at 700 nm. Increased absorbance at this wavelength indicates stronger reducing power. Fig. 3 shows the reductive capability of aqueous aerial extract of *Mentha arvensis* L which shows an increase in reducing property with concentration compared to ascorbic acid as standard. The reducing action is an important mechanism of phenolics and can be strongly correlated with the presence of reductones, which have the ability to donating hydrogen atom and exert antioxidant action by breaking the free radical chain [45, 46].

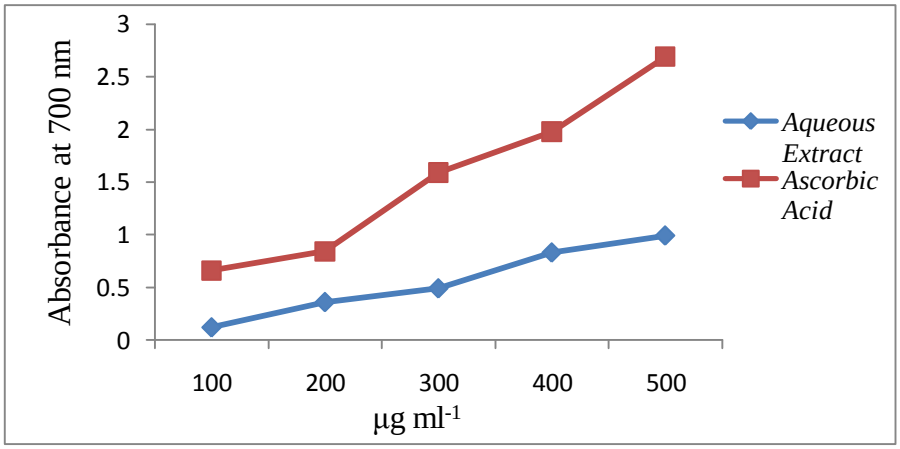

Figure 3. Reducing power of aqueous aerial extract of *Mentha arvensis*.

Sodium nitrprusside in phosphate buffer solution after incubation produces nitrite ion which was reduced by the extract. Aqueous extract of *Mentha arvensis* to scavenge nitric oxide radical was determined by percentage inhibition which was found to be 57.7% at concentration 500 µg/mL, where as percentage inhibition of standard rutin at the same concentration was 98.74% (Fig. 4). This may be due to the antioxidant principles in the

extract which compete with oxygen to react with nitric oxide and thus inhibit the generation of nitrite [47].

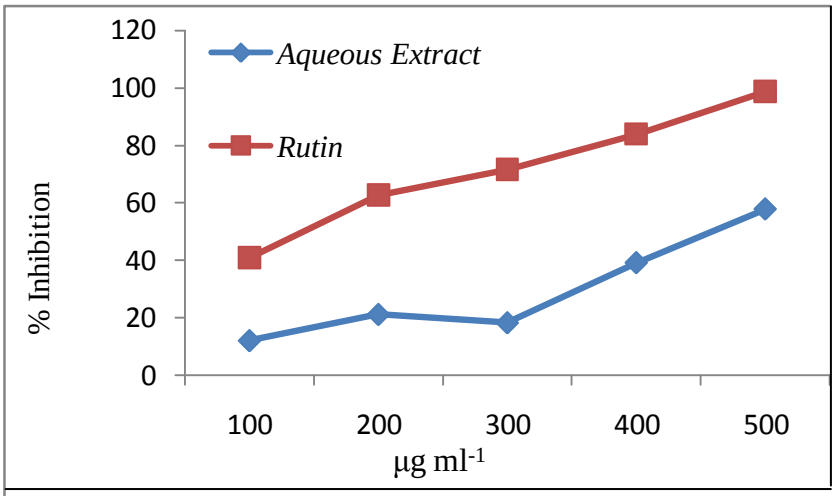

Figure 4. Nitric oxide radical scavenging ability of aqueous aerial extract of *Mentha arvensis*.

The presence of transition metal ions in a biological system could catalyse the Haber-Weiss and Fenton type reactions, leads to hydroxyl radicals (OH) formation. Antioxidants have the ability to form chelates with these transition metals, which results in the suppression of OH generation and inhibition of peroxidation processes of biological molecules [48]. The extract shows metal ion scavenging effects, which was increasing with the increase in the concentrations of the extract from 100-500 µg/mL. The percentage of metal scavenging capacity at the concentration of 500 µg/mL was 61.99%. The percentage inhibition for standard ascorbic acid was 99% at same concentration (Fig. 5).

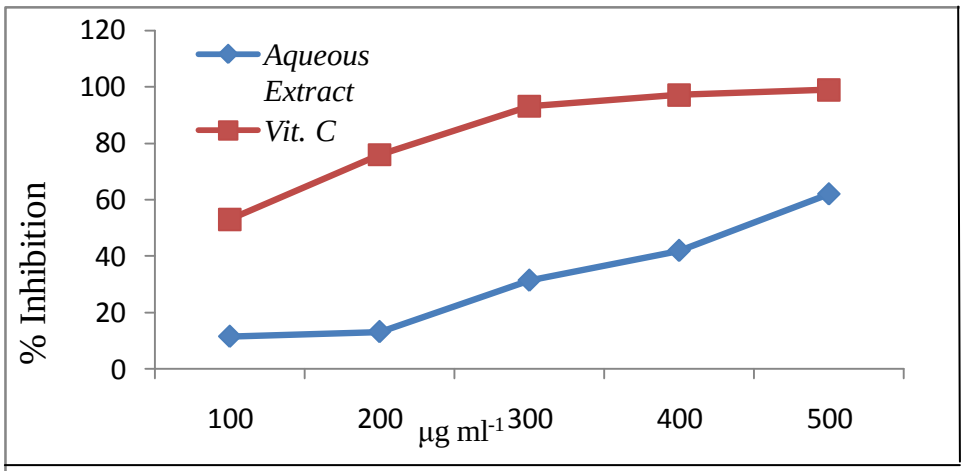

Figure 5. Chelating ability of aqueous aerial extract of *Mentha arvensis*

Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the cause of

many of its toxic effects [49]. The aqueous extract showed 25.31% percentage inhibition of hydrogen peroxide scavenging at concentration 1mg/ml as compared to standard ascorbic acid 96.43% at same concentration (Fig. 6).

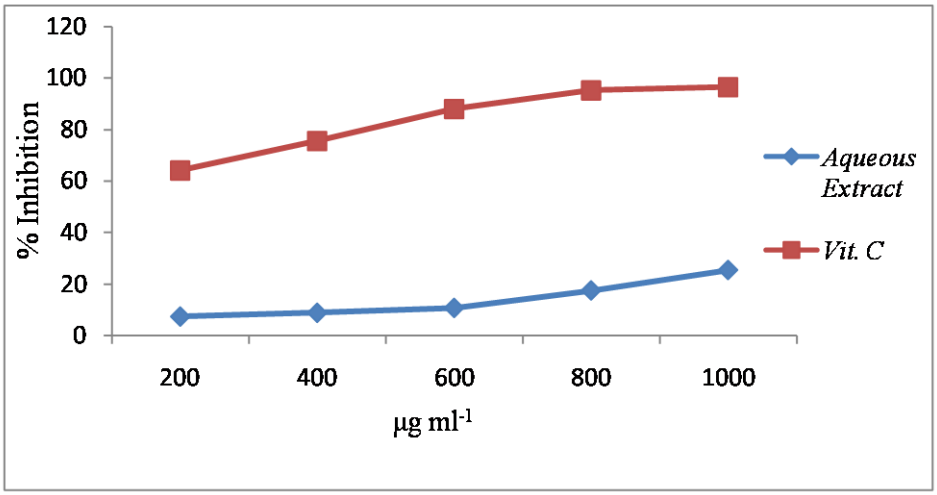

Figure 6. Hydrogen peroxide scavenging ability of aqueous aerial extract of *Mentha arvensis*.

CONCLUSION

On the basis of results it is concluded that aqueous extracts of aerial parts of *Mentha arvensis* L. possess antioxidant action, when compared with standards. Further studies to isolate, identify and characterize the active principle(s) will be carried out to substantiate the present findings.

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