

Antioxidant potential of methanol Root extract of *Mentha arvensis* L. from Kashmir Region

M. Akbar Dar^a, Mubashir H. Masoodi^{a*}, Adil F. Wali^a, Mudasar A. Mir^a, Nida S. Shapoo^b

^aDept. of Pharmaceutical Sciences, University of Kashmir, Srinagar-190006, J&K, India.

^bDept. of Biochemistry, University of Kashmir, Srinagar-190006, J&K, India.

ARTICLE INFO

Article history:

Received on: 11/01/2014

Revised on: 17/02/2014

Accepted on: 06/03/2014

Available online: 30/03/2014

Key words:

Mentha arvensis; DPPH;
reducing power; nitrous
oxide; metal chelating; H₂O₂.

ABSTRACT

The study was carried out to evaluate the phytochemical constituents and antioxidant potential of methanolic root extract of *Mentha arvensis* L. from Kashmir region. The antioxidant activity of methanol extract of *Mentha arvensis* L. was evaluated by using 1, 1-diphenyl, 2-picrylhydrazyl (DPPH) scavenging, reducing power, metal chelating, nitrous oxide scavenging and hydrogen peroxide scavenging assays. The total phenolic content and total flavonoid content was found to be 9.12 and 32.14 mg/g respectively. The percentage inhibition values of DPPH scavenging for methanol extract were found to be 35.83%. The percentage inhibition values of Nitrous oxide scavenging for extract were found to be 39.11%, the percentage inhibition values of H₂O₂ scavenging for extract were found to be 21.72%, the percentage inhibition values of metal chelating activity of methanol extract at the concentration of 500 µg/ml was found to be 79.31% and that for standard ascorbic acid was found to be 96% at same concentration and the reducing power of extract was found to be 0.75 at concentration of 0.25mg/ml. The results indicate that the methanolic root extract of *Mentha arvensis* L. has good antioxidant potential and it can be regarded as promising candidates for natural plant sources of antioxidants with high.

INTRODUCTION

The byproduct of normal metabolism, drugs or ionizing radiations leads to the formation of free radicals or reactive oxygen species, (ROS) or activated oxygen species (AOS) (Freeman and Crapo, 1982). In plants ROS generated performs various functions like programmed cell death, pathogen defence, and stomatal behaviour (Apel and Hirt, 2004). There is a greater impact on humans both from within the body and the environment due to free radicals, particularly reactive oxygen species (ROS). During metabolism, ROS such as superoxide (O²⁻), hydroxyl (OH) and hydrogen peroxide (H₂O₂) can arise normally or sometimes by the immune cells in order to neutralize the foreign bodies. Moreover, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also generate free radicals. These ROS can damage essential proteins, DNA and lipids can be damaged by ROS and cause various human diseases like atherosclerosis, cancer, liver injury, cardiovascular disease, neurodegenerative

disorders and rheumatism as a result of 'oxidative stress' which is an imbalance between the oxidant and antioxidant ratio of body. The body possesses defence mechanisms in the form of enzymes and antioxidant nutrients, which prevents the damaging properties of ROS (Wu *et al.*, 1998; Liao and Yin, 2000; Halliwell, 1999; Halliwell, 1995; Sies, 1993). The amount of free radicals is increased in the body beyond its ability to control and cause irreversible oxidative damages due to continuous exposure to chemicals and contaminants (Tseng *et al.*, 1997). Therefore, the ability of antioxidants to scavenging free radicals may have great role to play in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares *et al.*, 1997). In this respect, the correlation between polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Brown and Rice-Evans, 1998; Gil *et al.*, 1999; Kahkonen *et al.*, 1999; Vinson *et al.*, 1995). The synthetic antioxidants currently have various possible toxicities which have been criticized. It is generally assumed that frequent consumption of plant derived phytochemicals like phenolics and flavonoids from vegetables, fruit, tea, and herb have good antioxidant status (Halliwell, 1996).

* Corresponding Author

Dr. Mubashir Hussain Masoodi

Senior Assistant Professor Department of Pharmaceutical Sciences,
University of Kashmir, Hazratbal, Srinagar-190006, J&K, India

Thus, the interest in research of natural antioxidant, especially of plant origin, has greatly increased in recent years (Jayaprakash and Rao, 2000). *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 (Sola, 1995; Kiritkar and Basu, 1998; Chopra and Chopra, 1994; Khare, 2004). The plant has been shown to possess anti-inflammatory and sedative-hypnotic activity, hepatoprotective and antioxidant activity, antibacterial and antifertility action (Verma *et al.*, 2003; Kowti *et al.*, 2013; Coutinho *et al.*, 2009; Kanjanapothi *et al.*, 1981). The plant consist essential oils of monoterpenes like menthol, menthone, carvone and pulegone major constituents. This plant also possesses anti-Candida and also radio protective activity against gamma radiation (Marta *et al.*, 2005; Ganesh and Manjeshwar, 2002). In Kashmir, the powder of aerial parts mixed with dilute curd is given to cure cough, sore throat, indigestion and constipation; also the leaves are used in Diarrhoea and Asthma (Akhtar *et al.*, 2011; Towseef *et al.*, 2012). The focus of current study is to evaluate phytochemical constituents and antioxidant potential of methanolic root extract of *Mentha arvensis* L.

MATERIALS AND METHODS

Collection of Plant material, identification and authentication

The plant *Mentha arvensis* L. was collected in July-August 2012 from the fields and orchards of Narabal, Budgam, J&K. The plant was authenticated by the centre of Plant taxonomy, Department of Botany, University of Kashmir, Hazratbal.

Preparation of methanolic root extract of *M. arvensis* Linn

The plant (Root) material (500 g) was dried under shade and crushed to coarse powder and the powdered drug material was taken in a percolator for (cold extraction) extraction using methanol as solvent. The fraction after filtration was dried under reduced pressure to get the crude dried fraction. The yield of dried fraction of methanol extract of methanolic root extract of *Mentha arvensis* L. was 38.7 g.

Source of Chemicals

All the chemicals were purchased from a local dealer and were HiMedia Laboratories Pvt. Ltd. Mumbai, India made.

Phytochemical evaluation

Various chemical tests were carried out on methanolic root extract of *Mentha arvensis* L. 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 extract methanolic root 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 (Iyengar, 1995).

Alkaloids

Alkaloid solution produces white yellowish precipitate when few drops of Mayer's reagents are added (Siddiqui and Ali, 1997). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Evans, 2002). 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 (Siddiqui and Ali, 1997).

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 (Siddiqui and Ali, 1997).

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 (Siddiqui and Ali, 1997).

Flavonoids

To 2 ml of methanolic 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 (Oguyemi and Sofowora, 1979).

Phenolics

To 2 ml of alcoholic or aqueous extract, 1 ml of 1% ferric chloride solution was added. Blue or green colour indicates phenols (Edeoga *et al.*, 2005).

Carbohydrates

To 2ml of test solution add 2-3 drops of Molish reagent; add 2ml of conc. H₂SO₄ along the sides of test tube to form two layers. Violet ring at the junction of two liquids indicate the presence of carbohydrates (Krishnaveni *et al.*, 1984).

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 (Khandelwal, 2003).

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 (Prasanth *et al.*, 2011).

Table. 1: Showing presence/absence of various phytoconstituents.

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

(-) absent; (+) present in a negligible quantity; (++) present in moderate quantity; (+++): present in a considerable quantity

Antioxidant Activity

Determination of DPPH frees radical scavenging

The free radical scavenging capacity of methanolic root extract of *Mentha arvensis* L. was determined using DPPH (Braca *et al.*, 2001). Freshly prepared DPPH (2,2-diphenyl-1-picrylhydrazyl), solution was taken in test tubes and extracts 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 (Oyaizu 1986). One ml of (Extract) different concentrations of methanol extract was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K₃ 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 methanolic root extract of *Mentha arvensis* L. was determined using standard method (Singleton and Rossi, 1965). Methanol extract of *Mentha arvensis* L.were dissolved in the concentration of 1mg/ml. The reaction mixture was prepared by mixing 0.5 ml of methanol solution of extracts, 2.5 ml of 10% Folin's-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5ml methanol 2.5 ml of 10% Folin's-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% NaHCO₃. The samples were then incubated for 45 mins at a temperature of 45 °C. Absorbance was measured at 765 nm. 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 (Milan, 2011).

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% AlCl₃ 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) (Quettier *et al.*, 2000).

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction (Garratt, 1964). In this assay, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *Mentha arvensis* L. extract (50 to 250 µg/ml) or standard solution (rutin) 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 (Dinis *et al.*, 1994). The reaction mixture containing 1.0 ml of different concentrations

of *Mentha arvensis* L. methanol extract (1.0 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 % Chelating Activity = $[(A_1 - A_2) / A_0] \times 100$ Where A_0 represents the absorbance of the control (without extract) and A_1 represents the absorbance of reaction mixture, A_2 represents the absorbance without FeCl₂.

Scavenging of Hydrogen Peroxide

The ability of the extract to scavenge hydrogen peroxide was determined according to our recently published papers (Nabavi et al., 2008a; Nabavi et al., 2009a). 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 (100-500 µg/ml) in distilled methanol 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:

$$\% \text{ Scavenged } [H_2O_2] = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard (Nabavi et al., 2008a; Nabavi et al., 2009a; Nabavi et al., 2008b).

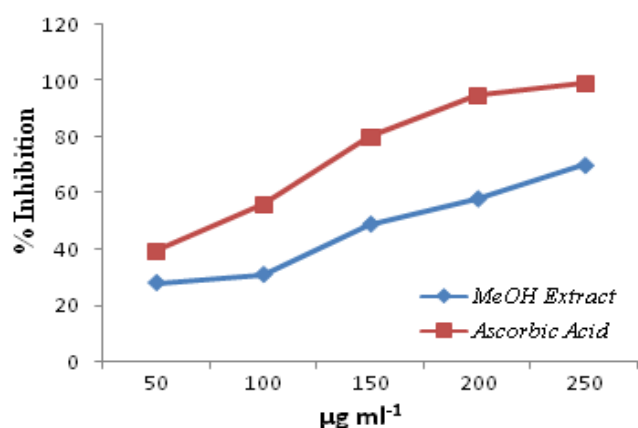


Fig. 1: DPPH scavenging activity of methanol root extract of *Mentha arvensis* L.

RESULTS

DPPH frees radical scavenging activity

The ability of methanolic root extract of *Mentha arvensis* L. to scavenge DPPH free radical was calculated as percentage inhibition which was found to be 70.08 at concentration 250 µg/ml, where as percentage inhibition of ascorbic acid at the same concentration was 99.16 (Figure 1).

Reducing power

The methanolic root extract of *Mentha arvensis* L. showed good reducing power when compared with standard ascorbic acid. The reducing power shown by methanolic root extract of *Mentha arvensis* L. was 1.98 at concentration of 0.25 mg/ml as compared to 2.69 shown by standard ascorbic acid at same concentration (Figure 2).

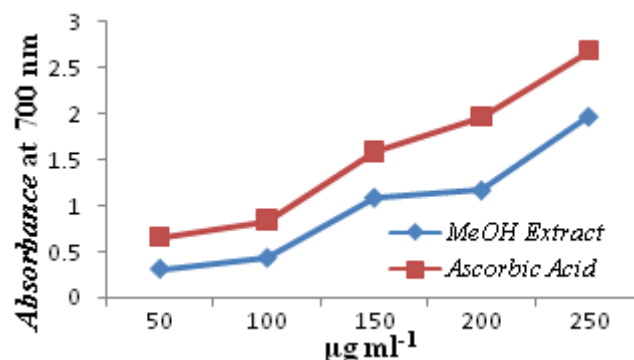


Fig. 2: Reducing power of methanol root extract of *Mentha arvensis* L.

Total Phenolic and Flavonoid content

As shown in (Table 2) below, the content of phenolic compounds (mg/g) in Gallic acid equivalent was found to be 489.31 mg/g in methanolic root extract of *Mentha arvensis* L. (Figure 3). The total Flavonoid content (mg/g) in Rutin equivalent was found to be 213.33 in methanolic root extract of *Mentha arvensis* L. (Figure 3).

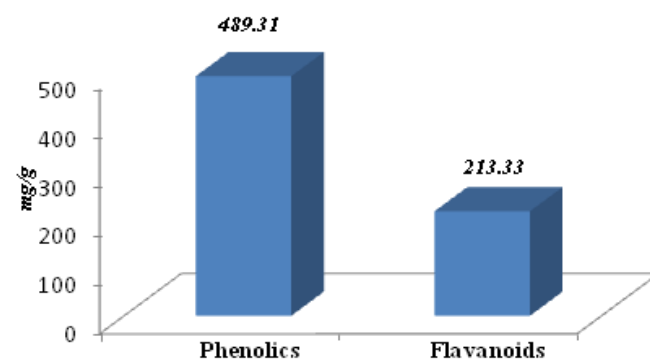


Fig. 3: Total phenolic content and Total flavonoid content of methanolic root extract of *Mentha arvensis* L.

Table. 2: Total amount of phenolic and flavonoid content of methanolic root extract of *Mentha arvensis* L., [Mean ± S.E.M. a]

Extract	Total phenolics mg/g plant extract (in GAE)	Total flavonoid mg/g plant extract (in RE)
Methanol	489.31 ± 6.82	213.33 ± 4.74

(A): average of three determinations

Nitric oxide radical inhibition assay

The ability of methanolic root extract of *Mentha arvensis* L. to scavenge Nitric oxide radical was determined by percentage inhibition which was found to be 71.28 at concentration 250 µg/ml, where as percentage inhibition of standard rutin at the same concentration were 98.74 (Figure 4).

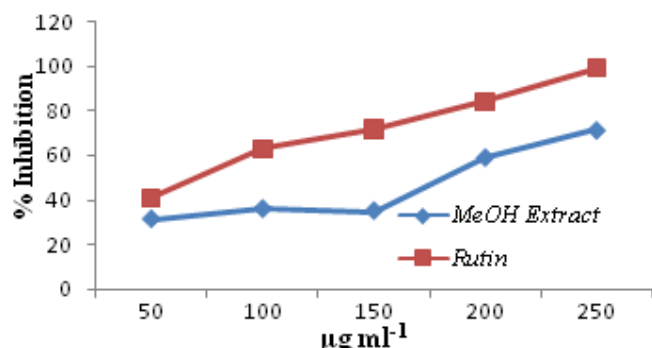


Fig. 4: Nitric oxide radical scavenging ability methanolic root extract of *Mentha arvensis* L.

Metal chelating ability

The metal chelating activities of methanolic root extract of *Mentha arvensis* L. were concentration dependent. The absorbance of Fe²⁺-ferrozine complex was linearly decreased with concentration dependently. The percentage of metal chelating capacity at the concentration of 500 µg/ml was found to be 79.31 and that for standard ascorbic acid was found to be 96 at same concentration (Figure 5).

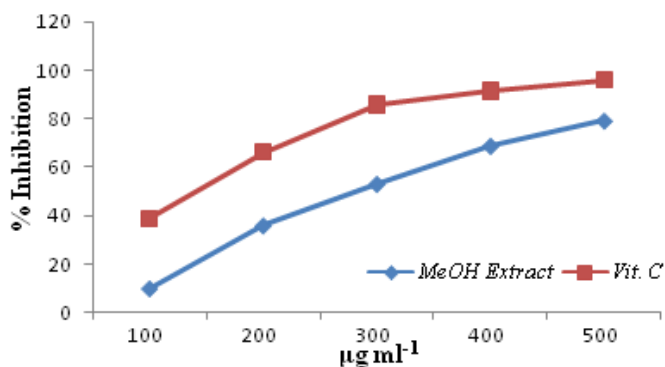


Fig. 5: Metal chelating ability of methanolic root extract of *Mentha arvensis* L.

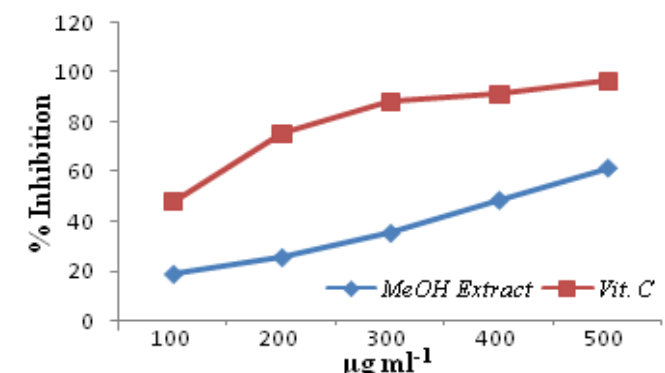


Fig. 6: Hydrogen peroxide scavenging ability of methanolic root extract of *Mentha arvensis* L.

Scavenging of Hydrogen Peroxide

The scavenging of hydrogen peroxide by methanolic root extract of *Mentha arvensis* L. was expressed as percentage scavenging. The extract showed 61.39 percentage inhibition at concentration 500 µg/ml as compared to standard ascorbic acid 96.43 at same concentration. (Figure 6).

DISCUSSION

From the results obtained from the preliminary phytochemical screening of root of *Mentha arvensis* L. (Table 1), it was observed that the methanolic extract contains phenolics in a moderate amount and flavonoids in high amount along with other phytoconstituents. This depicts that the crude drug may have antioxidant effect due to its flavanoidal and polyphenolic property which is to be further investigated. Polar solvents are able to extract out phenolic compounds in high concentrations (Mohsen and Ammar, 2008; Zhou and Yu, 2004). Phenols are very important plant constituents; they show high scavenging ability of free radicals due to their hydroxyl group. Therefore, the phenolic content of plants may contribute directly to their antioxidant action (Tosun *et al.*, 2009). The secondary plant metabolites flavonoids show significant antioxidant and chelating properties, which are based on the structure and substitution pattern of hydroxyl groups (Sharififar, 2008).

The high correlation between the values of phenol concentration in plant extracts and antioxidant activity is a well documented study (Borneo *et al.*, 2008; Katalinic *et al.*, 2004). Methanolic extract from root of *M. arvensis* L. have high concentration of total phenols and flavonoids Table 2, which is in correlation with intense antioxidant activity of these extracts. The content of phenolic compounds (mg/g) in Gallic acid equivalent was found to be 489.31 mg/g and the total Flavonoid content (mg/g) in Rutin equivalent was found to be 213.33 in methanolic root extract of *Mentha arvensis* L. (Figure 3). The antioxidant activity of methanol extract from root of *M. arvensis* L. was determined using a methanol solution of DPPH reagent. DPPH is very stable free radical. Unlike *in vitro* generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution has a deep purple colour with an absorption maximum at 517 nm. The antioxidant compounds leads to fadeness of deep purple colour by quenching DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably *via* a free-radical attack on the DPPH molecule) and convert them into a colourless- /bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), which leads to decrease in absorbance and hence provides antioxidant potential (Amarowicz *et al.*, 2003). The ability of methanolic root extract of *Mentha arvensis* L. to scavenge DPPH free radical was found to be increased with concentration and was calculated as percentage inhibition which was found to be 70.08% at concentration 250 µg/ml, where as percentage inhibition of ascorbic acid at the same concentration was 99.16% (Figure 1).

As shown in (Figure 2), methanol extract and ascorbic acid had significant reducing power with increasing concentration in the range of 50-250 µg/ml. At the concentration of 50 µg/ml, the reducing power expressed by increase in absorbance of methanolic root extract and ascorbic acid was 0.31 and 0.66 respectively and increased to 1.98 and 2.69 at concentration of 250 µg/ml

respectively. The reducing power reflects the electron donating capacity of methanol extract, is associated with antioxidant activity. Antioxidant can be reductants and inactive of oxidants. The reducing capacity of plant extracts can be measured by the direct reduction of $\text{Fe}[(\text{CN})_6]_3$ to $\text{Fe}[(\text{CN})_6]_2$. Addition of free Fe^{3+} to the reduced product leads to the formation of intense Perl's Prussian blue complex, $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$, which shows a strong absorbance at 700 nm. The reductones have the ability to show reducing action. They show antioxidant potential by breaking them free radical chain by donating a hydrogen atom (Pin-Der, 1998). In this regarded, increase in Fe^{3+} to Fe^{2+} conversion in presence of extract implies that sample is electron donor and thus can cause reduction of the oxidized intermediates of lipid peroxidation process. In this assay, depending on the reducing power of antioxidant samples the yellow colour of the test solution changes to various shades of green and blue (Karimi *et al.*, 2010). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

Nitric oxide radical inhibition assay proved that methanolic root extract of *Mentha arvensis* L. is a potent scavenger of nitric oxide. In this assay sodium nitroprusside generates nitric oxide which form nitrite when reacts with oxygen. The methanolic root extract of *Mentha arvensis* L. inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Maccoci *et al.*, 1994). The ability of methanolic root extract of *Mentha arvensis* L. to scavenge Nitric oxide radical was determined by percentage inhibition which was concentration dependent and was found to be 71.28% at concentration 250 $\mu\text{g}/\text{ml}$, where as percentage inhibition of standard rutin at the same concentration were 98.74% (Figure 4). Ferrozine can quantitatively form complexes with Fe^{2+} but in the presence extracts able of ion chelation, the complex formation is disrupted resulting in a reduction in the red colour of the complex. The measurement of the rate of reduction of the colour, therefore allows estimation of the chelating activity of the coexisting chelator. The absorbance of Fe^{2+} - Ferrozine complex was linearly decreased in concentration dependent manner. The data obtained from results shows that the methanol extracts of root of *Mentha arvensis* L. demonstrate an effective capacity for iron binding, suggesting that its action as peroxidation protector may be related to its iron binding capacity. In this assay the methanolic root extract of *Mentha arvensis* L. and standard compound interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that it has chelating activity and possess the capacity to captures the ferrous ion before ferrozine. The ion chelating activity of the methanolic root extract of *Mentha arvensis* L. may be attributed may be due to the presence of endogenous chelating agents, mainly phenolics (Orech *et al.*, 2005; Senevirathne *et al.*, 2006). The metal chelating activities of methanolic root extract of *Mentha arvensis* L. were concentration dependent. The percentage of metal chelating capacity at the concentration of 100 $\mu\text{g}/\text{ml}$ was

found to be 9.93% for extract and that for standard ascorbic acid, it 39.0% and at 500 $\mu\text{g}/\text{ml}$ 79.31 and 96.0% respectively (Figure 5).

The hydrogen peroxide scavenging ability of methanolic root extract of *M. arvensis* L. extract and ascorbic acid is shown in (Figure 6). Hydrogen peroxide scavenging activity of methanol extract at 500 $\mu\text{g}/\text{ml}$ was found to be 61.39 % and for ascorbic acid at the same concentration was 96.43 %. The methanol extract was capable of scavenging H_2O_2 in a concentration dependant manner. Hydrogen peroxide is a weak oxidizing agent that inhibits the oxidation of essential thiol (-SH) groups directly by few enzymes. Many of its toxic effects are because H_2O_2 has the ability to rapidly cross the cell membrane and once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals (Miller *et al.*, 1993). From the results, methanol extract was capable of scavenging H_2O_2 in a concentration dependant manner. These results suggest that methanol extract can be a better antioxidant for removing H_2O_2 and thus protecting living or food systems.

CONCLUSION

Based on the various results, it can be concluded that the methanolic root extract of *Mentha arvensis* L. posses string antioxidant activity, evidenced by the free radical scavenging property, iron chelating, reducing power property, Nitrous oxide scavenging and H_2O_2 scavenging, which may be due to the presence of phenolic and flavonid components in the extract. Overall, the methanolic root extract of *Mentha arvensis* L. is a source of natural antioxidant that can be important in disease prevention, health preservation and promotion of longevity promoter.

ACKNOWLEDGMENTS

Authors are grateful to Prof. M.Y Shah former Head of the Department, Department of Pharmaceutical Sciences, University of Kashmir for his full support and also to Prof. A.R Naqshi, former Head of Department, Department of Botany, University of Kashmir, who supported in collection and identification of the plant used in this research.

REFERENCES

- Akhtar HM, Anzar AK, Dar GH, Khan ZS. Ethnomedicinal uses of some plants in the Kashmir Himalaya. Indian Journal of Traditional Knowledge 2011; 10(2): 362-366.
- Amarowicz R, Pegg BR, Rahimi-Moghaddam P, Bar B, Weil JA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem 2003; 84: 551-562.
- Apel K, Hirt H. Reactive oxygen species: Metabolism, Oxidative Stress, and Signal Transduction. Annu. Rev. Plant Biol 2004; 55: 373-99.
- Borneo R, Leon EA, Aguirre A, Ribotta P, Cantero JJ. Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and there in vitro testing in model food system. Food Chem 2008; 112: 664-670.

- Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis*. *J Nat Prod* 2001; 64: 892-895.
- Brown JE, Rice-Evans CA. Luteolin-Rich Artichoke Extract Protects Low Density Lipoprotein from Oxidation in vitro. *Free Radical Research* 1998; 29: 247-255.
- Chopra RN, Chopra IC. 1994. Indigenous drugs of India, Calcutta, India: Academic Publishers.
- Coutinho HD, Costa JG, Lima EO, Falcao-Silva VS, Siqueira-Júnior JP. Potentiating effect of *Mentha arvensis* L. and chlorpromazine in the resistance to aminoglycosides of methicillin-resistant *Staphylococcus aureus*. *In Vivo*. 2009 Mar-Apr; 23(2):287-9.
- Dinis TC, Maderia VM, Almeida LM. Action of phenolic derivatives (acetomenophen, salicylate and 5- amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Arch Biochem Biophys* 1994; 315(1): 161-169.
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal Plant. *African J. Biotechnology* 2005; 4(7): 685- 688.
- Evans WC. 2002. Trease and Evan's Pharmacognosy. 5th ed., Harcourt Brace and Company.
- Freeman BA, Crapo JD. Laboratory Investigation. *Journal of Technical Methods and Pathology* 1982; 47(5): 412-426.
- Ganesh CJ, Manjeshwar SB. Influence of the leaf extract of *Mentha arvensis* L. Linn. (mint) on the survival of Mice exposed to different doses of Gamma Radiation. *Strahlenther Onkol* 2002; 178: 91-8.
- Garratt DC. The Quantitative analysis of Drugs. Chapman and Hall Ltd: Japan; 1964 .p. 456-458.
- Gil MI, Ferreres F, Tomas-Barberan FA. Effect of Postharvest Storage and Processing on the Antioxidant Constituents (Flavonoids and Vitamin C) of Fresh-Cut Spinach. *Journal of Agricultural and Food Chemistry* 1999; 47: 2213-2217.
- Halliwell B, Aeschbach R, Loliger J, Aruoma OI. The characterization of antioxidants. *Food Chemistry and Toxicology* 1995; 33: 601-617.
- Halliwell B. Establishing the significance and optimal intake of dietary antioxidants: The biomarker concept. *Nutr. Rev* 1999; 57(4): 104-113.
- Halliwell B. Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Research* 1996; 25: 1-32.
- Iyengar MA. 1995. Study of Crude Drugs. 8th ed., Manipal Power Press Manipal: India.
- Jayaprakash GK, Rao LJ. Phenolic constituents from lichen *Parmentaria stipitata*. *Food Control* 2000; 56: 1018-1022.
- Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. *Journal of Agricultural and Food Chemistry* 1999; 47: 3954-3962.
- Kanjanapothi D, Smitasiri Y, Pathong A, Taesotikul T, Rathanapanone V. Postcoital antifertility effect of *Mentha arvensis*. *Contraception*. 1981, 24:559-567.
- Karimi E, Oskoueian E, Hendra R, Jaafar HZ. Evaluation of *Crocus sativus* L. stigma phenolic and flavonoid compounds and its antioxidant activity. *Molecules* 2010; 15: 6244-6256.
- Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem* 2004; 94: 550-557.
- Khandelwal KR. 2003. A textbook of practical Pharmacognosy. 16th ed. Nirali Prakashan: Pune, India.
- Khare CP. 2004. Encyclopedia of Indian Medicinal plants, Springer-Verlag Berlin: Heidelberg.
- Kiritkar KR, Basu BD. 1998. Indian medicinal Plants. Allahabad, India: Lalit Mohan Basu.
- Kowti R, Vishwanath S, Shivakumar SI, Vedamurthy J, Abdul NK. Hepatoprotective and Antioxidant Activity of Ethanol Extract of *Mentha arvensis* L. Leaves against Carbon Tetrachloride Induced Hepatic Damage in Rats. *International Journal of PharmTech Research* 2013; 5(2): 426-430.
- Krishnaveni S, Theymoli B, Sadasivam S. Phenol Sulphuric acid method. *Food Chem* 1984; 15: 229.
- Liao KL, Yin MC. Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidyl choline liposome systems: Importance of the partition coefficient. *Journal of agricultural and food chemistry* 2000; 48: 2266-2270.
- Marcocci L, Packer L, Droy-Lefai MT, Sekaki A, Gardes-Albert M. Antioxidant action of Ginkgo biloba extracts EGB 761. *Methods Enzymol* 1994; 234: 462- 475.
- Marta C, Teixeira D, Glyn Mara F, et al. Anti-candida activity of Brazilian medicinal plants. *J Ethnopharmacol* 2005, 97; 305-311.
- Milan SS. Total phenolic content and antioxidant activity of *Marrubium peregrinum* L extract. *Kragujevac J. Sci* 2011; 63-72.
- Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL, Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. *J. Pharmacol. Exp Ther* 1993; 264:11-16.
- Mohsen MS, Ammar SMA. Total phenolic contents and antioxidant activity of corn tassel extracts. *Food Chem* 2008; 112: 595-598.
- Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. In vitro antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. *Pharmacognosy Magazine* 2009a; 4(18): 123-127.
- Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline* 2008a; 2: 560-567.
- Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Jafari M. Free radical scavenging activity and antioxidant capacity of *Eryngium caucasicum* Trautv and *Froripia subpinnata*. *Pharmacologyonline* 2008b; 3: 19- 25.
- Oguyemi AO, Sofowora A. Proceedings of a Conference on African Medicinal Plants, Ife-Ife. *Univ Ife* 1979; 20-22.
- Orech FO, Akenga T, Ochora J, Friis H, Aagaard-Hansen J. Potential Toxicity of some traditional leafy vegetables consumed in Nyang'oma division, Western Kenya. *Afri J Food Nutri Sci* 2005; 5(1): 1-13.
- Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Jap. J. Nutr* 1986; 44: 307- 315.
- Pin-Der X. Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free radical and active oxygen. *J. Amer. Oil Chem. Soc* 1998; 75: 455- 461.
- Prasanth T, Bimlesh K, Mandeep K, Gurpreet K, Harleen K. Phytochemical screening and Extraction: A Review. *International Pharmaceutica Sciencia* 2011; 1(1): 98-106.
- Quettier DC, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC, Cayin JC, Bailleul F, Trotin F. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *J. Ethnopharmacol* 2000; 72: 35-42.
- Senevirathne M, Kim S, Siriwardhana N, Ha J, Lee K, Jeon Y. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Sci Tech Int* 2006; 12(1): 27-38.
- Sharififar F, Nudeh-Dehghan G, Mirtajaldini M. Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chem* 2008; 112: 885-888.
- Siddiqui AA, Ali M. 1997. Practical Pharmaceutical chemistry. 1st ed., CBS Publishers and Distributors: New Delhi. India.
- Sies H. Strategies of antioxidant defense. *European Journal of Biochemistry* 1993; 215: 213-219.
- Singleton V, Rossi J. Colorimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagents. *Am J Enol Vitic* 1965; 16:144-158.

Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant Activities of some Extracts of *Thymus zygis*. *Free Radical Research* 1997; 26: 469-478.

Sola AV. 1995. Indian medicinal plants. Orient Longman: Private Ltd.

Tosun M, Ercisli S, Sengul M, Ozer H, Polat T. Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. *Biol. Res* 2009; 41: 175-181.

Towseef AB, Gaurav N, Masood M1. Study of Some Medicinal Plants of the Shopian District, Kashmir (India) With Emphasis on Their Traditional use by Gujjar and Bakerwal Tribes. *Asian Journal of Pharmaceutical and Clinical Research* 2012; 5(2).

Tseng TH, Kao ES, Chu CY, Chou FP, Lin Wu HW, Wang CJ. Protective Effects of Dried Flower Extracts of *Hibiscus sabdariffa* L. against Oxidative Stress in Rat Primary Hepatocytes. *Food Chemistry and Toxicology* 1997; 35:1159-1164.

Verma SM, Arora H, Dubey R. Anti-Inflammatory And Sedative –Hypnotic Activity of The Methanolic Extract of The Leaves of *Mentha Arvensis*. *Anc Sci Life*. 2003; 23(2): 95–99.

Vinson JA, Dabbagh YA, Serry MM, Jang J. Plant Flavonoids, Especially Tea Flavonols, Are Powerful Antioxidants Using an in vitro Oxidation Model for Heart Disease. *Journal of Agricultural and Food Chemistry* 1995; 43: 2800–2802.

Wu Y, Hong C, Lin S, Wu P, Shiao M. Increase of vitamin E content in LDL and reduction of atherosclerosis in cholesterol fed rabbits by a water-soluble antioxidant-rich fraction of *Salvia miltiorrhiza*. *Arterioscler. Thomb. Vasc. Biol* 1998; 18: 481-486.

Zhou K, Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT* 2004; 37: 717-721.

How to cite this article:

M. Akbar Dar, Mubashir H. Masoodi, Adil F. Wali, Mudasir A. Mir, Nida S. Shapoo. Antioxidant potential of methanol Root extract of *Mentha arvensis* L. from Kashmir Region. *J App Pharm Sci*, 2014; 4 (03): 050-057.