**In vitro antioxidant activity of leaves of Mentha Arvensis linn.**

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**ABSTRACT**

The aim of the study is to investigate the antioxidant activity of ethanol extract of leaves of *Mentha arvensis* through TBARS (Thiobarbituric acid reactive substance), hydroxyl radical scavenging assay, DPPH (1,1-diphenyl-1-picryl hydrazyl radical) radical scavenging assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay and phosphomolybdonum method. Ethanol extract of leaves of *Mentha arvensis* showed significant dose dependent antioxidant activity, with a direct relationship between activity and concentration of extract. The extract showed an important free radical scavenging activity towards the lipid peroxidation inhibition, hydroxyl radical, DPPH, nitric oxide, superoxide radicals, with IC50 values of 64, 52, 46, 36 and 38 µg/mL respectively. Total antioxidant capacity was found to be 50 and 100 µg/mL of antioxidant activity, with a direct relationship between activity and concentration of extract. The extract showed an important free radical scavenging activity.

**Key words:** Mentha arvensis, DPPH, TBARS, superoxide, nitric oxide and hydroxyl radical scavenging activity.

**INTRODUCTION**

Free radicals are reactive oxygen species (ROS) or reactive nitrogen species (RNS) generated in the body during normal metabolic activities or by environmental conditions. The most common ROS include superoxide anion (O2•−), hydrogen peroxide (H2O2), peroxyl radicals (ROO•) and reactive hydroxyl (OH•) radicals. RNS includes nitric oxide (NO) and peroxynitrite anion (ONOO−). These species causes cellular damage by reacting with the various bio molecules of body such as membrane lipids, nucleic acid, proteins and enzymes. Excess production of these free radicals leads to a condition called as oxidative stress. This damage is the major contributor of many disorders like Cancer, Hepatopathy, Cardiovascular diseases, Inflammation, Diabetes mellitus, renal failure and Brain dysfunction.

Antioxidants have been reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers. Plant and plant products are being used as a source of medicine since a long time. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, less or no side effects and economic viability.

Flavonoids and phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc. In this view, we have selected one of such herb called as Mentha arvensis.

*Mentha arvensis* Linn. (Family: Lamiaceae) known as pudina in Hindi, Sanskrit and Kannada. 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, cardiotoxic, diuretic, dentifrice, jaundice, hepatalgia, inflammation of liver, peptic ulcer, diarrhea, bronchitis and skin diseases.

Phytochemical review reveals that the presence of major constituents of the essentials oil is carvone; other constituents include L-limonene, dihydro carvone, carvomenthone, iso-menthone, menthone, piperitone, a-pinene, fur-fural, camphene, Caryophyllene, d-3-ocanotol, d-sesquiterpene alcohol and dihydrocarvylelacate. Leaves contain the flavonoids diometin-7-glucoside, diosmin, diosmetin-7-o-beta-D-glucuronide and luteolin-3-o-beta-D-glucuronidé. The literature survey revealed that so far no scientific studies carried out on its in vitro antioxidant activity of the leaves of *Mentha arvensis*. Hence, in the present study, we focused to evaluate the in vitro antioxidant activity and free radical scavenging activity of the ethanol extract of *Mentha arvensis* leaves.

**MATERIAL AND METHODS**

**Plant material collection and extraction**

The fresh leaves of *Mentha arvensis* were collected from Harapanahalli, Davanagere (District), Karnataka, India. The air dried leaves of *Mentha arvensis* coarsely powdered and extracted with ethanol in a Soxhlet extractor for 18-20 hours and solution was evaporated to dryness under reduced pressure and controlled temperature by using rotavapor. The extract was stored in a refrigerator at 4°C until further use. The extract was subjected for preliminary qualitative phytochemical studies.

**Preliminary Phytochemical**

Phytochemical studies of ethanol extract of *Mentha arvensis* performed for the presence of alkaloids, carbohydrates, glycosides, flavonoids, triterpenoids and tannins.

**Antioxidant activity**

**Antioxidant activity by TBARS method:**

Antioxidant activity was determined by TBARS...
This is based on the formation malondialdehyde (MDA), when lipid is subjected to peroxidation. The MDA formed in the presence or absence of antioxidant was measured at 535 nm. The reaction mixture contained linolenic acid micelles (12 µL/mL of linolenic acid in hexane) in Tris Buffer saline (10 mM, pH-7.4), FeSO₄: ascorbate (10:100 µmole) and various concentrations of extract (10–125 µg) in a final volume of 0.5 mL. The reaction mixture were incubated at 37 ºC for 1 hour. The treatment of oxidized lipid with 1 mL of 1% TBA and incubated in hot water bath for 15 minutes, results in the formation of colored complex, cooled and 2 mL of acetone was added to stabilize the color which was measured at 535 nm. Lower the absorbance indicates higher the inhibition of lipid peroxidation. BHA was taken as standard antioxidant.

**Hydroxyl Radical Scavenging Activity:**

The scavenging capacity for hydroxyl radical was determined according to the modified method. The assay was performed by adding EDTA, ferric chloride, hydrogen peroxide, deoxyribose and M. arvensis extract (10-120 µg/mL) in phosphate buffer (10 mM, pH-7.4), reaction was initiated by adding ascorbic acid. Samples were added in the same sequence. The mixture was then incubated at 37°C for 1 hour and 1.0 ml the reaction mixture with 10% TCA and 1.0 ml of 1% TBA to develop the pink chromogen and measured at 532 nm. Percentage inhibition was calculated against 100% oxidation. BHA (400 µM) was taken as standard antioxidant. Reaction mixture without antioxidant is considered as the 100% oxidation.

**DPPH Radical Scavenging Activity:**

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. A 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of control (gallic acid) and test solutions at different concentrations (5-100 µg/ml). After 30 minutes incubation, the absorbance was measured at 517 nm. Equal volume of methanol served as control.

**Nitric Oxide Scavenging Activity:**

Nitric oxide scavenging activity was measured by the spectrophotometric method. Sodium nitroprusside (5 mM) in phosphate-buffer saline (10 mM) was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solutions at different concentrations (5-100 µg/ml) were dissolved in methanol and incubated at 25°C for 30 minutes. After 30 minutes, to 1.5 ml of the incubated solution was diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyethylene diamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylene diamine dihydrochloride was measured at 546 nm. Curcumin was taken as standard antioxidant.

**Superoxide Scavenging:**

Superoxide scavenging was carried out using the alkaline dimethyl sulfoxide (DMSO) method. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 hours and the solution was filtered immediately before use; the filtrate (200 µL) was added to 2.8 ml of an aqueous solution containing nitroblue tetrazolium (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 µM, pH 7.4). Test solutions at different concentrations (10-100 µg/mL) were added and absorbances were recorded at 560 nm against the control. Curcumin was taken as standard antioxidant. (10-100 µg/ml).

**Evaluation of antioxidant capacity by phosphomolybdenum method:**

The total antioxidant capacity of test sample and a-tocopherol were...
evaluated according to the method described by Prieto\(^4\). An aliquot of 0.1 mL of sample solution and α-tocopherol (0-600 µg/mL) was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In case of blank, 0.1 mL of ethanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90 minutes. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in spectrophotometer. Antioxidant capacity was expressed as equivalents of α-tocopherol.

**Statistical analysis**

Linear regression analysis was used to calculate the IC\(_{50}\) values of the extracts.

**RESULTS.**

**Inhibition Lipid peroxidation**

Amount of lipid peroxidation reaction was assay in TBARS. If any inhibition in the lipid peroxidation occurs, the colour development with TBA is decreased than 100 % oxidation. Effect of extract on lipid peroxidation was checked in different concentrations (Figure 1). At 100 µg/mL concentration, Mentha arvensis showed about 76 % inhibition of lipid peroxidation. IC\(_{50}\) value for Mentha arvensis extract was 36 µg/mL (Table 1). Standard curcumin showed 89 % inhibition at 10 µg concentration.

**Hydroxyl radical activity by Deoxy ribose method**

Effect of ethanol Mentha arvensis extract on Hydroxy radical was significant. The effect was also in dose dependent manner, where the % inhibition at 10–125 µg/mL of Mentha arvensis varied from 17–85 % (Figure 2). IC\(_{50}\) value was 52 µg/mL of Mentha arvensis extract (Table 1). Standard BHA showed 88 % inhibition at 400 µM concentration.

**Inhibition of DPPH radical.**

Potential decrease in the concentration of DPPH radical due to the scavenging property of Mentha arvensis extract showed around 82% at 100 µg/mL concentration (Figure 3). The IC\(_{50}\) value of Mentha arvensis was found to be 46 µg/mL (Table 1). Standard gallic acid showed 90% inhibition at 10 µg concentration.

**Nitric oxide scavenging activity**

Nitric oxide scavenging activity was assessed by Sodium nitroprisside method. It was checked in the range of 0–100 µg/mL concentration of Mentha arvensis. Maximum inhibition was about 86% (Figure 4). IC\(_{50}\) value of Mentha arvensis extract was 36 µg/mL (Table 1). Standard curcumin showed 79 % inhibition at 15 µg concentration.

**Total antioxidant activity of MA by Phosphomolybdate method.**

Total antioxidant activity was compared to the concentration of α-Tocopherol. Mentha arvensis extract was checked in 50 and 100 µg/mL concentration which showed 0.044 and 0.122 OD at 695 nm, where as standards showed 0.178 OD at 100 µg/mL. IC\(_{50}\) value of 36 µg/mL Mentha arvensis ethenolic extract was 38 µg/mL (Table 1). Standard curcumin showed 89% inhibition at 100 µg concentration.

**DISCUSSION**

There is a strong need for effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases which can have serious effects on human health, either through lipid peroxidation or vasoconstriction\(^2\). Synthetic antioxidants also have many side effects on human health at higher concentration. BHA and BHT are suspected to cause fore-stomach tumor in mice\(^3\).

Secondary metabolites such as polyphenols and flavinoids are not required for plant development and growth, but are involved in plant communication and defense mechanism\(^2\). Defense mechanism mainly depends on the antioxidant activity, as the generated ROS by UV, microbial attack, pesticides on plants are nullified by these antioxidants present in plants. Hence identification and isolation of these component from plants has been an usual trend these days as they are easily available, less toxic and less side effects to human health.

Ethenolic extract from Pudina showed a significant antioxidant activity and scavenged hydroxyl, superoxides, nitric oxides, DPPH radicals. It also inhibited lipid peroxidation.

**Phyto chemical analysis of Mentha arvensis** showed the presence of alkaloids, carbohydrates, glycosides, flavonoids, triterpenoids and tannins. The antioxidant activity may be due to collective effect of all these components.

To conclude, ethanolic leaves extract of Mentha arvensis, has shown a significant antioxidant and free radical scavenging activity in different invitro model systems. These activities due to the combined effect of all the phytochemicals present in Mentha arvensis. Further work to be carried out to purify the constituents and ensure their antioxidant activities individually.
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