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EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND IN VITRO ANTIOXIDANT ACTIVITIES OF MENTHA ARVENSIS

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ABSTRACT

Mentha Arvensis (Lamiaceae) commonly cultivated in India for its therapeutic value. The goal of current study was to evaluate the phytochemical constituents and to explore the in vitro antioxidant properties of ethanolic extracts of leaves *M. Arvensis*. Preliminary phytochemical evaluations revealed the presence of steroids, tannins, glycosides, phenols, flavonoids and saponinsin the Leaves ethanolic extracts. Determinations of their in vitro antioxidant activity were carried out by using 2,2-diphenyl-1-picrylhydrazyl in an assay based on the method of Oyaizu. Hydroxyl radical scavenging activity was also measured in the same extracts. Results from this research project indicated that *M. Arvensis* Leaves are indeed rich in phytochemicals and had substantial antioxidant activities, implying that *M. Arvensis* Leaves can be used as a potential source of natural antioxidants.

Keywords: Mentha Arvensis, Medicinal plants, Phytochemical screening, Antioxidant, DPPH assay, Reactive oxygen species

1. INTRODUCTION

Medicinal plants have been utilized for thousands of years as major sources of cures for human diseases **[1]**. Isolation and characterization of pharmacologically active compounds from medicinal plants have gained vital importance in developing countries. More recently, drug discovery techniques have been applied to the standardization of herbal medicines, to elucidate analytical marker **[2]**. The major highlights of plant derived drugs are their

efficacy, low cost and selectively without or with little side effects.

Mentha arvensis Linn belonging to family Lamiaceaeis native to the temperate regions of Europe and western and central Asia, east to the Himalaya and eastern Siberia, and America. It is an herbaceous perennial plant growing to 10–60 cm (rarely to 100 cm) tall **[3-5]**. 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 **[3-7]**. Antioxidants are molecules that can safely interact with free radicals and terminate their chain reaction before vital molecules are damaged **[8]**. Lack of antioxidants facilitates the development of degenerative disorders, cardiovascular diseases and cancer. One possible solution to this problem may be provided by the use of natural antioxidant compounds present in plant sources **[9]**, which can be useful in protocols preventive medicine. Most synthetic antioxidants, such as Butylated Hydroxytoluene (BHT) and Butylated Hydroxyl Anisole (BHA), which are widely used in the food industry, may have more damaging than beneficial effects as they could be responsible for liver damage and carcinogenesis **[7-10]**.

For this reasons, interest in the use of natural antioxidants has increased now a days. The purpose of this study was to investigate and elucidate the phytochemical antioxidant and phenolic content in Leaves extracts of M. Arvensis.

2. MATERIALS AND METHODS

2.1. Reagents

Folin-Ciocalteu reagent and catechol were purchased from Merck Pvt. Ltd, India. 2,2-diphenylhydrazyl (DPPH), Thiobarbituric Acid (TBA), Trichloroacetic Acid (TCA), ascorbic acid and quercetin were obtained from Himedia lab, India. All other chemicals were of analytical grade.

2.2. Collection and preparation of plant material

M. Arvensis Leaves were collected from the agriculture center, India authenticated in the SVS University. Leaves were cleaned, chopped, shade dried and powdered. Dried powder (50 g) was soxhlet extracted separately with 400 ml of acetone and ethanol for 48 h. Both acetonic and ethanolic extracts were concentrated under reduced pressure using a rotary evaporator and then kept either refrigerated at 4 ºC, or at -20 ºC for long term storage.

2.3. Preliminary phytochemical screening

2.3.1 Detection of alkaloids

A drop of Mayer's reagent was added to a few milliliter of filtrate, by the side of the test tube. Formation of a white creamy precipitate indicated that the test was positive.

2.3.2. Detection for saponins

About 0.2 g of the extracts were shaken with 5 ml of distilled water and then heated to boil. The appearance of creamy miss of small bubbles (frothing) showed the presence of saponins.

2.3.3. Detection for tannins

Small quantities of extracts were mixed with water and heated in a water bath. The mixtures were filtered and ferric chloride was added to the filtrates. The formation of a dark green solution indicated the presence of tannins.

2.3.4. Detection for flavonoids

The extracts (0.5 ml) were dissolved in methanol. After the addition of a few fragments of. Magnesium ribbon to the extracts followed by concentrated hydrochloric acid, the appearance of pink color indicated the presence of flavonoids.

2.3.5. Detection of Steroids

About 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H_2SO4. Thecolor changed from violet to blue or green in some samples indicating the presence of steroids.

2.3.6. Detection of Glycosides

The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B for minutes. An orange red precipitate indicates the presence of reducing sugars.

2.3.7. Detection of Phenols

To 1 ml of aqueous solution of plant extract, 2 ml of distilled water followed by few drops of 10% aqueous $FeCl_3$ solution are added. Formation of blue or green precipitate indicates the presence of phenols.

2.4. Evaluation of in vitro antioxidant activity

The in vitro antioxidant activity of the acetone and ethanolic extracts of *M. Arvensis* Leaves was measured by the following assays:

2.4.1. Determination of total phenolic compounds in the extracts

The amount of total phenolic compounds in the extracts was determined using the Folin-Ciocalteu method **[11, 12, 13]**. Briefly, 0.5 ml of the sample was pipetted into a 10 ml volumetric flask containing 0.5 ml of Folin-Ciocalteu's reagent, 5 ml of distilled water and 1.5 ml of Na_2CO_3 solution (20% by weight), and the volume was made up to 10 ml with distilled water. During the oxidation of phenolic compounds, the phosphomolybdic and phosphotungstic acids contained in the

Folin-Ciocalteu's reagent were reduced to blue-colored molybdenum and tungsten oxides. After 2 h, the absorbance of the blue coloration was measured at 765 nm against a blank sample. Measurements were compared to a standard curve prepared with gallic acid solutions, and expressed per weight of dry extract. The total phenolic content was expressed as g gallic acid equivalent (GAE)/100 g dry weight. All samples were analyzed in triplicate.

2.4.2. Determination of total flavonoid content in the extracts

The total flavonoid content was determined colorimetrically by aluminum chloride method **[12, 13]**. Briefly, 0.5 ml solution of each plant extracts in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a Hitachi UV/Visible spectrophotometer (CA, USA). Total flavonoid contents were calculated as quercetin equivalently from a calibration curve. The calibration curve was determined by preparing quercetin solutions at concentrations 12.5 to 100 mg/ml in ethanol and expressed as milligrams of quercetin equivalents (QE/g of dry extract).

2.4.3. Evaluation of total antioxidant capacity

The total antioxidant capacity was measured by a spectrophotometric method **[14]**. At different concentration ranges, extract were dissolved in water and combined in eppendorf tubes with 1 ml of reagent solution (0.6 M H2SO4, 28 mM sodium phosphate, 4 mM ammonium molybdate). The tubes were incubated for 90 min at 95 ºC. The mixture was cooled to room temperature and the absorbance was read at 695 nm against a blank control. Ascorbic acid equivalents were calculated using standard graph prepared with various concentrations of ascorbic acid.

2.4.4. 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity

The antioxidant activity of the acetone and methanolic extracts of *M. Arvensis* Leaves was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Reduction of the DPPH radical was determined by the decrease in its absorbance at 517 nm **[15]**. Ascorbic acid was used as standard reference.

2.4.5. Determination of reducing power

As the antioxidant activity of the extracts would be also manifested through their reducing power, the Fe3+→ Fe2+ transformation assay was established as a measure of reducing capacity. The reducing power of the *M. Arvensis* extracts was determined by the method of Oyaizu as

previously described **[16]**. Extracts were first dissolved into 1 ml distilled water and then mixed with 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide [K3Fe (CN)6]. The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 10% TCA were added to the mixture, followed by centrifugation of 3000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml 0.1 % $FeC1_3$, then its absorbance was measured at 700 nm with a Hitachi UV/Visible spectrophotometer. Ascorbic acid was used as a reference antioxidant compound.

2.4.6. Assay of hydroxyl radical-scavenging activity

The inhibitory effect of the extracts to prevent the degradation of deoxyribose by Fe3+ ions in the presence of H_2O_2-EDTA-ascorbate was determined in hydroxyl radical scavenging assays **[17]**. To 0.5ml of plant extract was mixed with reaction mixture containing FeCl3 (100 μM), EDTA (104 μM), H_2O_2 (1 mM) and 2-deoxy-D-ribose (2.8 mM) in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and were incubated for 1 h at 37 ºC. The mixture was then heated at 95 ºC in water bath for 15 min, followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% butylhydroxyanisole (BHA)). Finally the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. The absorbance of the supernatant was measured at 532 nm. All readings were corrected for any interference from brown color of the extract or antioxidant by including appropriate controls. The negative control without any antioxidant was considered 100% deoxyribose oxidation. The percent hydroxyl radical scavenging activity of test sample was determined accordingly in comparison with the negative control. Ascorbic acid was taken as the positive control

2.4.7. Statistical analysis

For display of data, points on a graph represent a mean±standard deviation (SD; error bars) for an experimental group or observation.

3. RESULTS

3.1. Determination of plant yield

The percentage yield of *M. Arvensis* Leaves extract in ethanol and acetone were analyzed. Among this, ethanolic extract gave the highest yield (16.83%) while the yields were lower for the acetone (14.31%), methanol (9.25%), petroleum ether (4.14%), and water (6.35%) extracts, respectively. Since both the ethanolic and acetone extracts of *M. Arvensis* Leaves exhibited the highest yields, they were selected for the phytochemical screening.

3.2. Preliminary phytochemical analysis of *M. Arvensis*

The acetone and ethanolic extracts of *M. Arvensis* Leaves extract subjected for preliminary phytochemical screening revealed the presence of various phytochemical constituents as given in **Table 1**. It became evident from these results that ethanolic extracts (EMAL) contained all analyzed phytochemicals and appeared richer when compared with the acetonic Leaves extracts. These phytochemical compounds are known to support bioactive activities in medicinal plants and thus may be responsible for the biological activities of the *M. Arvensis* extracts used in this study.

3.3. Evaluation of in vitro antioxidant assay

3.3.1. DPPH radical scavenging activity

EMAL had substantial DPPH radical scavenging activity compared to ascorbic acid, and this activity increased in parallel to the increase in extract concentration, as shown in **Figure 1**. The IC50 value of the EMAL extract was 79.1µg/ml while that for reference ascorbic acid was 4.2µg/ml. These results signify that the extract had distinct free radical scavenging activity in comparison to ascorbic acid.

3.3.2. Hydroxyl radical scavenging activity

EMAL extracts had also detectable hydroxyl radical scavenging activity compared to quercetin, and this activity increased proportionately to the increase in concentration of the extract, as shown in **Figure 2**. The estimated IC50 values of EMAL and quercetin were 78.2µg/ml and 20.9 µg/ml respectively. An increase in the reaction mixtures absorbance indicates an increase in the reducing capacity of the sample.

3.3.3. The reducing power potentials of the extract

Figure 3 shows the reducing power potential of EMAL in comparison with standard ascorbic acid at 700 nm. The extract was found to have marked reducing capacity. The results illustrate that there was an increase in reducing power of the plant extract as the EMAL concentration increased in the assay.

3.3.4. Total phenolic contents, flavonoid contents and total antioxidants

Table 2 shows that the total phenolic content of *M. Arvensis* Leaves EMAL extract is 116.6±32.08 mg/g, whereas the total flavonoid content is 39.2±640 mg/g and the total antioxidant is 683±885 mg/g, which may specify with the previous studies **[16-18]**.

Table 1: Phytochemical screening of *M. Arvensis* Leaves extracts

Constituents	Acetone extract	Ethanolic extract
Steroids	+	+
Alkaloids	+	+
Tannins	-	+
Glycosides	-	+
Phenols	-	+
Flavonoids	-	+
Saponins	+	+

"+" and "–" indicate the presence or absence of the constituents indicated

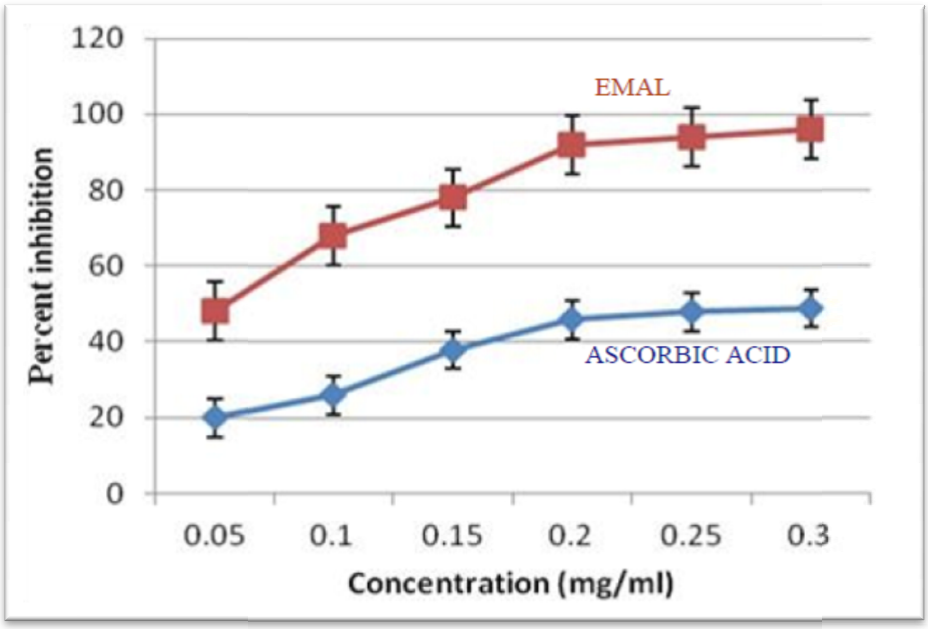

Figure 1: Percentage inhibitions of DPPH free radical activity of EMAL compared with standard antioxidant ascorbic acid
The data shown are the mean from three independent experiments. Each value is the mean±SD of three determinations

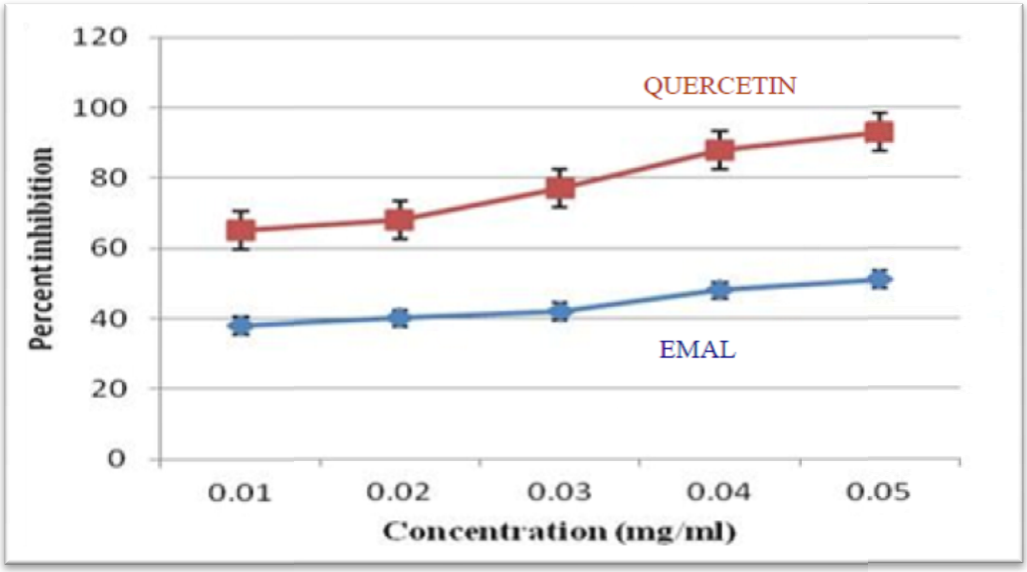

Figure 2: Percentage inhibition of hydroxyl radical scavenging activity of EMAL compared with quercetin as antioxidant standard.
The data shown are the mean from three independent experiments. Each value is the mean±SD of three determinations

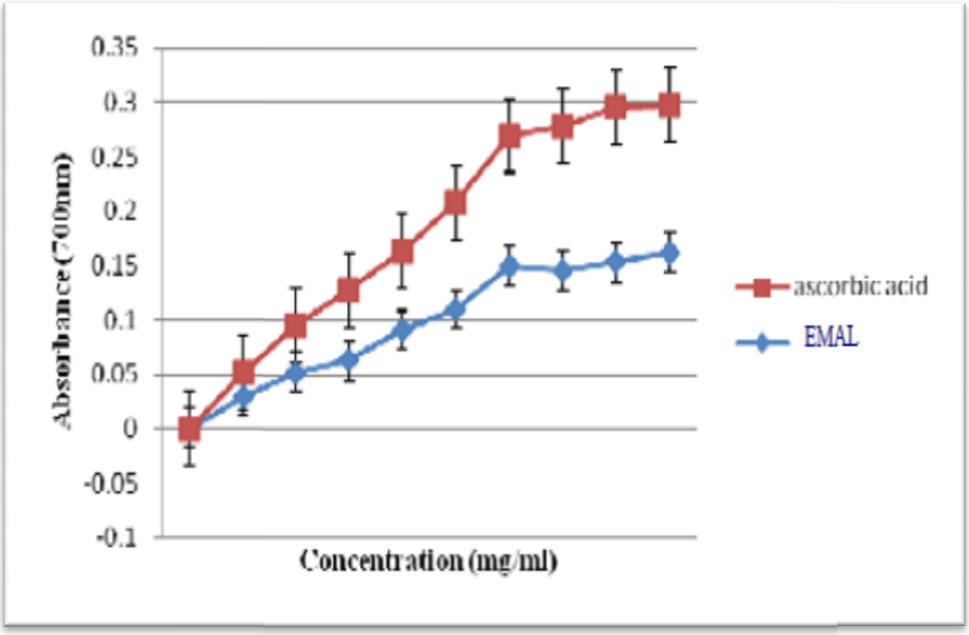

Figure 3: Reducing power of EMAL compared with ascorbic acid as antioxidant standard. The data shown are the mean from three independent experiments. Each value is the mean±SD of three determinations

Table 2: *In vitro* total phenolic and flavonoid contents and antioxidant activity of EMAL

Material	Total phenolic content (mg GAE/g dry extract)	Total flavonoid content (mg QE/g dry extract)	Total antioxidant (mg ascorbic acid/g dry extract)
EMAL	116.6±32.08	39.2±640	683.8±885

The data shown are the mean from three independent experiments. Each value is the mean±SD of three determinations.

4. DISCUSSION

Phytochemicals are defined as bioactive non-essential nutrients from plants, which possess a variety of human health effects such as possessing antioxidant and anti-carcinogenic activity **[19]**. Results on the percentage yield suggest that the methanolic extract of *M. Arvensis* Leaves (EMAL) was better than the acetonic extract. As a result EMAL was chosen for further studies.

The phytochemical analysis conducted on EMAL revealed the presence of alkaloids, flavonoids, saponins, glycosides and tannins. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity **[20]**, and their presence in this plant tend to increase the risk of poisoning by the plant. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane-bound enzymes, such as the ATPase and phospholipase A2 **[21]**, a property that may explain the mechanism of antioxidative action of EMAL. The presence of flavonoids in EMAL may be responsible for its antioxidant activity. The antioxidant capacity of flavones is attributed to the high

reactivity of the hydroxyl substituent, with the number of hydroxyl groups on the B-ring being correlated with reactive oxygen species (ROS) scavenging capability **[22, 23]**. Also, the plant extract was revealed to contain saponins known to produce cytotoxic effect. The presence of these phenolic and non-phenolic compounds in these plant extracts contributed to their antioxidant properties and thus their usefulness of these plants in herbal medicament.

The in vitro antioxidant activity of EMAL indicates that the plant is potently active. This suggests that the plant extracts contain compounds that are capable of donating hydrogen to a free radical in order to remove electrons responsible for the radicals reactivity. Though the DPPH radical scavenging activity of the EMAL extract was lower than that of the ascorbic acid reference, the study showed that EMAL have potent proton-donating ability and therefore could serve as free radical inhibitor. The reducing power of the EMAL extract was evaluated by the transformation of Fe3+ to Fe2+ through electron transfer ability, which serves as a significant indicator of its antioxidant activity. The reductive activity of the extract and that of the reference drug were augmented in parallel to their increasing concentrations, as confirmed by escalating absorbance values at 700 nm. The antioxidant activity of the EMAL extract was found to be substantial when compared with the standard ascorbic acid standard used in this study.

Findings from this study showed that the antioxidant activity observed with the EMAL extract agrees well with the amount of phenolic constituents detected in the extract. The present data suggest that EMAL can effectively scavenge ROS, including hydroxyl radical as well as other free radicals, under in vitro conditions, and can be a potential source of natural antioxidant that could be of great importance for the treatment of radical-related diseases.

5. CONCLUSION

At present, a desirable portion of the global plant population still persist unexplored, and it seems reasonable to suggest that they hold the potential of the innovation for safer and more active new drugs. It is well known that medicinal plants are rich in antioxidants and can be used in the treatment of wide variety of diseases. Our findings show that the antioxidant activity of *M. Arvensis* extracts can be attributed to the pharmacological actions of alkaloids, saponins, flavonoids, tannins, glycosides, phenols and steroids present in the crude extract. These data strongly support the possible utility of these extracts in disease prevention and treatment.

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