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Free Radical Scavenging Potential of *Mentha arvensis* of South Gujarat: Evidence from *In-vitro* Assay

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ABSTRACT

Present work was undertaken to evaluate efficacy of aqueous and methanol extracts *Mentha arvensis leaves* as free radical scavenger. The free radical scavenging activity was evaluated by nitric oxide scavenging method, hydrogen peroxide scavenging method and ferric thiocyanate method. The result of the studies was compared with the standard solution of Ascorbic Acid (Vitamin C) treated with same reagent. The results of all the studies showed significant free radical scavenging activity in both aqueous and methanolic extracts of *Mentha arvensis* and methanolic extract showed the highest free radical scavenging potential than aqueous extract.

Keywords: Free radical, *Mentha arvensis,* Nitric oxide, Hydrogen peroxide, ferric thiocyanate

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INTRODUCTION

Medicinal plants have significant potentials for human societies and consumed by people across the entire world. Although most of their health benefits have not investigated yet, their medical activities can be considered in the treatment of present or future diseases[1].

Various chronic diseases are mainly due to oxidative stress. Free radicals and reactive oxygen species (ROS) are leading various sicknesses such as cancer, diabetes, inflammatory diseases, asthama, Alzheimer disease etc. ROS are also said to be responsible for the aging[2, 3,4].

An antioxidant playing important role to inhibit oxidative stress[5]. The important characteristic of an antioxidant is its ability to scavenge free radicals. Antioxidant compounds like flavonoids and polyphenolic compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit degenerative diseases due to the oxidative[6]. Plants are considered as good antioxidant since ancient times.

Mentha arvensis Linn is important medicinal plat of family Lamiaceae. It is traditionally used in hypertension and in patients with ischemic heart disease. it is used as a food seasoner, household remedy, and industrial purposes. Leafy juice is given in diarrhea and dysentery. The leaves are used for stomach problems and allergy. It is also used for the treatment of liver and spleen disease, asthma and jaundice. The infusion of these leaves is used in indigestion, rheumatic pains, arthritis, and as remedy for inflamed joints. Menthol derived from its essential oil is used in pharmaceutical, perfumery, and food industries. Menthol is antiseptic, carminative, refrigerant, stimulant and diuretic in properties and is used against skin infections[7, 8, 9].

The plant has been reported with antibacterial[10], hepatoprotective and antioxidant activity[11], antifertility action[12], anti – inflammatory and sedative – hypnotic activity[13]. The plant consist essential oils of monoterpenes like menthol, menthone, carvone and pulegone major constituents. This plant also possesses anti-Candida[14] and also radio protective activity against gamma radiation[15].

It has been reported to possess diverse medicinal properties, and hence there is a need to explore its medicinal properties to support the traditional claim.

MATERIALS AND METHODS

The leaves of Mentha arvensis was collected from the South Gujarat. Plant specimen was authentified by Dr. Minoo Parabia, Department of Bioscience, VNSGU, Surat. The herbarium was deposited at Department of Pharmacognosy, Rofel, Shri G.M. Bilakhia College of Pharmacy, Vapi.

2.1 Preparation of extract: -

Leaves of *Mentha arvensis* were air dried and powdered. 50 gm of powdered leaves were macerated with methanol and water respectively for 7 days. Solvents were filtered and concentrated by rotary vacuum evaporator. The yield of methanolic extract was found to be 20.00% and the yield of aqueous extract was found to be 27.14%. The extracts were stored at 4°C until use.

Preliminary phytochemical investigation

The primary metabolites like; proteins, carbohydrates and fixed oils and fats, were analyzed for their presence as per the standard procedures. Similarly, the secondary metabolites like, alkaloids, flavonoids, saponins, phenolics, tannins volatile oils, terpenoids and glycosides were also assessed in the leaf extracts of *Mentha arvensis*[16].

Evaluation of Free radical scavenging potential

Free radical scavenging potency of methanolic extract and aqueous extract of leaves of *Mentha arvensis* was carried out by using following methods.

A. Nitric Oxide Scavenging Method.
B. Hydrogen peroxide Scavenging Method.
C. Ferric Thiocyanate Scavenging Method.

Nitric Oxide Scavenging Method.

Measurement of nitric oxide generated by sodium nitroprusside with the help of Griess reagent is as described previously. At physiological pH in aqueous solution sodium nitroprusside impulsively generates nitric oxide. Griess reagent plays an important role in the measurement of nitric ions formed by the coupling of nitric oxide and oxygen. Nitric oxide scavengers comet with oxygen radical result in production of nitric oxide

Preparations

Preparation of Sodium Nitroprusside Solution

An accurately weighed 0.148 gm of sodium Nitroprusside as transferred in 100 ml volumetric flask, it was dissolved in standard phosphate buffer and volume was up to 100 ml with phosphate buffer.

Preparation of Griess reagent

An accurately weighed sulfanilamide (0.5gm) and N-(1-naphthyl) ethylene diamine were added to it. This was then dissolved in standard phosphate buffer, finally make up the volume.

Preparation of standard phosphate buffer (saline)

An accurately weighed 2.5 gm potassium hydrogen phosphate and 2.523 gm of anhydrous disodium phosphate and 8.2 gm of sodium chloride dissolve in sufficient water to produce 1000ml.

Preparation of stock test extracts solution.

An accurately weighed quantity of alcoholic and aqueous extract (50mg) was dissolved in respective solvents.

Sodium nitroprusside (10mm) in phosphate buffer saline was mixed with different conc. of each extract (50,200,500µg /ml) dissolved in respective solvent incubated at 25°C for 150 min. The same reaction mixture without extract but equivalent amount of ethanol served as control. At interval, sample (1.5 ml) of the incubated solution were removed & diluted with (1.5 ml) Griess Reagent (1% Sulphanilamide, 2% H_3PO_4 & 0.1 % Naphthyl ethylene diamine dihydrochloridre). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide subsequent coupling with NEDA was read at 564 nm. Curcumin was use as positive control.[17,18]

% Inhibition = [1-Abs. of Sample/Abs. of Control] x 100

Hydrogen peroxide scavenging method

Measurement of free radical that is hydroxyl ion (OH^-) generated by hydrogen peroxide activity is carried using phosphate buffer (pH-7.4). At physiological pH the body generates free radical from hydrogen peroxide and drug possessing antioxidant activity neutralizes the hydroxyl ion radical by converting it into a molecule of water and oxygen.

Preparation

Preparation of 43mM hydrogen peroxide solution

An accurately measured volume 0.1462 ml of hydrogen peroxide (1M) is dissolved in 100ml water.

Preparation of standard phosphate buffer (pH-7.4)

An accurately weighed 2.38 gm of anhydrous disodium phosphate, 0.19 gm potassium hydrogen phosphate and 8.0 gm of sodium chloride dissolve in sufficient water to produce 1000ml.

Preparation of stock test extracts solution.

An accurately weighed quantity of alcoholic and aqueous extract (50mg) was dissolved in respective solvents.

All the different solvent extracts of were evaluated for their hydrogen peroxide radical scavenging potential by reported method. An aliquot of 0.6 mL of hydrogen peroxide (43 mM) and 1.0 ml of different concentrations of extracts ranging from (50-300 µg/ml) was mixed, then 2.4 ml of 0.1M phosphate buffer (pH 7.4) was added. The resulting solution was kept for 10 min and the absorbance was recorded at 230 nm using uv spectrophotometer (Shimadzu UV-1401 Pharma Spec Japan) against a blank (without hydrogen peroxide). All the readings were taken in triplicate and their mean value was taken into consideration. Ascorbic acid was used as a reference standard[19]. The percentage scavenging of hydrogen peroxide was calculated using the following formula:

$$\text{Scavenging activity (\%)} = [(V_c - V_t)/V_c] \times 100$$

Where

V_c is absorbance of control and V_t is absorbance of test sample.

Ferric thiocyanate Scavenging method

This Chloride reagent is based on the method of Zall, Fisher and Garner. When chloride is mixed with a solution of undissociated mercuric thiocyanate, the chloride preferentially combines with the mercury to form mercuric chloride. The thiocyanate that is released combines with

ferric ions present in the reagent to form ferric thiocyanate which can be measured spectrophotometrically. The procedure is very sensitive and needs to be reduced for routine clinical applications by the addition of mercuric nitrate. The mercuric nitrate binds a fixed amount of chloride ions and therefore makes them unavailable for reaction with mercuric thiocyanate. Only the chloride present in excess of that bound by the mercury from mercuric nitrate will be able to react with mercuric thiocyanate to produce the red ferric thiocyanate.

Preparation

Preparation of phosphate buffer (0.05M, pH-7)

An accurately weighed 0.5 gm of anhydrous disodium phosphate and 0.301gm potassium hydrogen phosphate dissolve in sufficient water to produce 1000ml.

Preparation of 20mM ferrous chloride ($FeCl_2$)

An accurately weighed 0.324 gm of ferrous chloride is dissolve in sufficient water to produce 100ml.

Preparation of stock test extracts solution.

An accurately weighed quantity of alcoholic and aqueous extract (50mg) was dissolved in respective solvents.

Each extract [500 µg/ ml] & BHT (50 µg/ ml) was mixed with 4.0 ml of 95 % (v/v) ethanol,4.0 ml of linoleic acid (2.51% v/v) in 99.5% ethanol,8.0 ml 0.05M phosphate buffer pH 7.0 & 4.0 ml distilled water. The vials were incubated at 40°C for 5 days in dark with the sampling interval of 24 hr. To 0.1 ml of sample withdrawn, 9.7 ml of 75 % (v/v) ethanol & 0.1 ml of 30% (w/v) ammonium thiocyanate was added. Precisely after 3 min 0.1 ml of 20 mM ferrous chloride was added. The absorbance at 500 nm was measured. The percentage of inhibition of linoleic acid peroxidation[21, 22].

Scavenging Effect (%) = [1-Abs. of Sample/Abs. of Control] x 100

RESULT AND DISCUSSION

Preliminary Phytochemical Investigation

Qualitative Phytochemical evaluation illustrates the presence of Carbohydrates, Proteins, Amino acids and Glycosides.

Table 1: Qualitative Phytochemical evaluation of extract of leaves of *Mentha arvensis*

Sr. No.	Test	Methanol	Water
1	**Test for Carbohydrates**		
	1.Molish's test:	-	-
	2.. Test for Reducing sugars:		-
	Fehling's test:	-	-
	Benedict's test:	+	+
	3.Test for Monosaccharides		
	Barfoed's test:	+	+
	4.Test for Pentose Sugars:		
	Bial's Orcinol test:	-	-
	Aniline acetate test:	-	-
	5.Tests for Hexose Sugars:		
	Selwinoff's test:	-	-
	Tollen's phloroglucinol test	-	-
	Cobalt-chloride test:	-	-
	7.Test for non-reducing Polysaccharides(Starch):		
	Iodine test :	-	-
	Tannic acid test for starch :	-	-
	8. Test for gums	-	-
	9. Test for Mucilage :		
	Powdered drug material with ruthenium red.	-	-
	Powered drug	-	-
2.	**Test for proteins :**		
	Biuret test	-	-
	Million's test	+	+
	Xanthoprotein test	-	-
	Test for proteins containing sulphur :	-	-
	Precipitaion test :	-	-
3.	**Tests for Amino Acids**		
	Ninhydrin test	-	-
	Test for Tyrosine :	+	+
	Test for Tryptophan :	-	-
	Test for Cysteine:	-	-
4.	**Tests for Steroids**		
	Salkowski reaction:	-	-
	Liebermann-Burchard reaction:	-	-
	Liebermann's reaction:	-	-
5.	**Tests for Glycosides**		
	1.Cardiac Glycosides:	-	-
	Baljet's test:	-	-
	Legal's test:	-	-

	Keller-Killiani test:	-	-
	2.Anthraquinone Glycosides:	-	-
	Borntrager's test:	-	-
	Modified Borntrager's test:	+	+
6.	**Test for Alkaloids**		
	Dragendorff's test:	-	-
	Mayer's test:	-	-
	Hager's test :	-	-
	Wagner's test:	-	-
	Murexide test:	-	-
7.	**Test for Tannins and Phenolic Compounds.**		
	5% $FeCl_3$ solution:	-	-
	Lead acetate solution:	-	-
	Gelatin solution:	-	-
	Bromine water:	-	-
	Acetic acid solution:	-	-
	Potassium dichromate:	-	-
	Dilute iodine solution:	-	-
	Dilute HNO3:	-	-
	Dilute NH4OH and potassium ferricyanide solution	-	-
	Dil. Potassium permanganate solution:	-	-

3.2 Pharmacological evaluation

Evaluation of free radical scavenging potential of leaves of *Mentha arvensis* The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with Naphthyl ethylene diamine was read at 564 nm and referred to the absorbance of standard solution of ascorbic acid (Vit. C) treated in the same with Griess reagent. Table no. 2 illustrates significant decrease in concentration of extracts. IC_{50} value for aqueous and alcoholic extract were found to be 772.5 and 682.5 µg/ml respectively. IC_{50} value for ascorbic acid was found to be 594.82 µg/ml.

Table 2: Evaluation of free radical potency by Nitric Oxide Scavenging Method

Sr. No.	Drug/Extract	% Scavenging activity and Concentration (µg/ml)			
		50	200	500	IC_{50}
1	Water	8.46	27.79	32.36	772.5
2	Methanol	17.36	22.89	36.63	682.5
3	Ascorbic acid	20.19	29.13	42.03	594.82

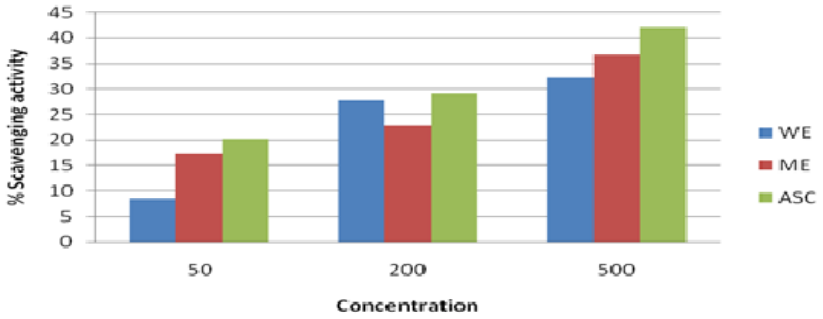

Figure 1: Evaluation of free radical potential by Nitric Oxide Scavenging Method

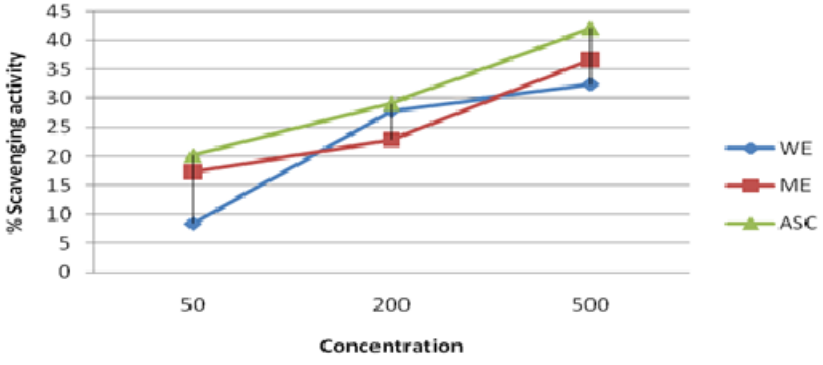

Figure 2: Evaluation of free radical potential by Nitric Oxide Scavenging Method

The absorbance of the chromophore group formed by the reaction with hydrogen peroxide was read at 230 nm and referred to the standard solution of ascorbic acid treated in the same way with hydrogen peroxide. Table no. 3 illustrates significant decrease in concentration of Hydroxyl radical as increase in concentration of extract.

Table 3: Evaluation of free radical potential by Hydrogen Peroxide Scavenging Method

Sr.No	Extract	% Scavenging activity and Concentration (µg/ml)				
		50	100	200	300	IC_{50}
1	Water	62.51	67.63	88.0	89.26	40
2	Methanol	37.51	47.67	70.65	82.24	105
3	Ascorbic acid	65.23	70.73	85.58	86.78	38

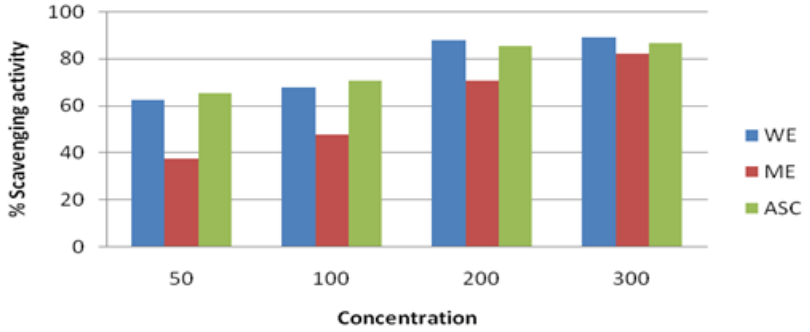

Figure 3: Evaluation of free radical potency by Hydrogen Peroxide Scavenging Method

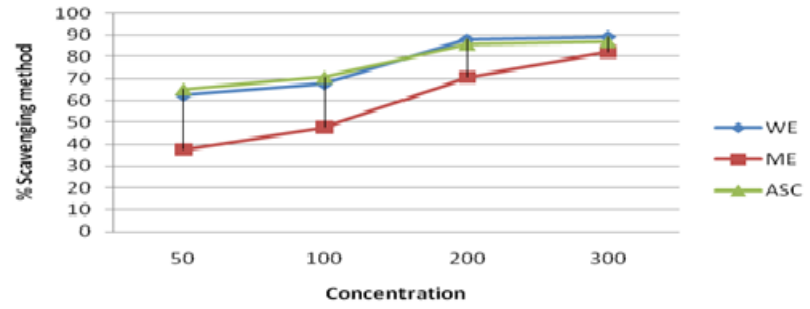

Figure 4: Evaluation of free radical potency by Hydrogen Peroxide Scavenging Method

Delay or prevention of Lipid peroxidation is important function of antioxidants. Table no.4 shows time coarse plot of antioxidants radical for different extract of leaves of *Mentha arvensis* and ascorbic acid using Ferric Thiocyanate Method. Methanolic extract has the highest antioxidant activity than aqueous extract.

Table 4: Evaluation of free radical potential by Ferric Thiocyanate Scavenging Method

Extract	**% Scavenging activity**				
	1	**2**	**3**	**4**	**5**
Water	46.33	50.59	15.46	50.6	79.65
Methanol	61.57	56.12	26.11	41.71	77.81
Ascorbic acid	68.61	87.78	24.46	52.18	81.95

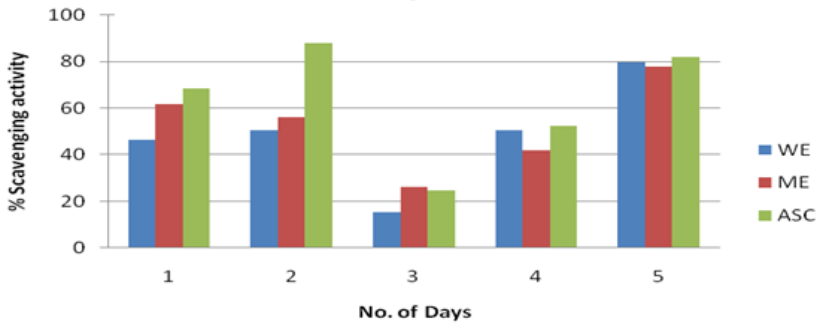

Figure 5: Evaluation of free radical potency by Ferric Thiocyanate Scavenging Method

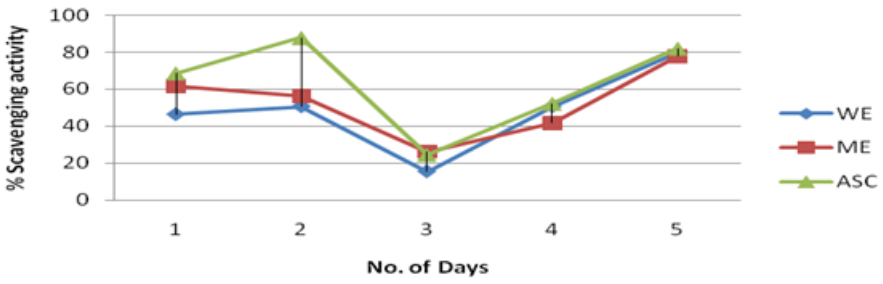

Figure 6: Evaluation of free radical potency by Ferric Thiocyanate Scavenging Method

CONCLUSION

Cancer, stroke, atherosclerosis, diabetes etc. are still unresolved clinical problems. Increased involvement of free radical in this complication make it serious problem now-a-days. Free radicals are also involved in cause of disease related to each organ of body. Long term use of Allopathic drug causes severe side-effects. Drug which has more than one action without side-effects is readily acceptable. Such a beneficial are only obtained from herbal drug. Present work was undertaken to evaluate efficacy of leaf extract of *Mentha arvensis* as free radical scavenger. *Mentha arvensis* have already being reported for its antibacterial, anti-inflammatory and anthelmentic activity, but no one satisfactorily evaluate it as antioxidant. The free radical scavenging activity evaluated by nitric oxide scavenging method, hydrogen peroxide scavenging method and ferric thiocyanate method. Result revealed that *Mentha arvensis* leaf have good antioxidant activity. Further, detail chemical investigation is needed.

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