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Research article

EVALUATION OF *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *MENTHA SPICATA* L. LEAVES

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ABSTRACT: Different extracts of *Mentha spicata L.* leaves were investigated for their *in vitro* antioxidant and antimicrobial activities. *In vitro* antioxidant activity was evaluated for chloroform, ethanol and aqueous extracts for scavenging of DPPH, Superoxide and FRAP radicals. Antimicrobial activity of the extracts was evaluated against five selected bacterial strains by using cylinder plate assay. Concentration dependent antioxidant activity was observed for all the tested extracts. All the tested extracts of *Mentha spicata* showed considerable zone of inhibition against the tested bacterial strains. All the extracts showed significant zone of inhibition at a dose of 500µg/ml. Of the three extracts tested the highest zone of inhibition was shown by ethanolic extract against *B.subtilis* at the dose of 500µg/ml. All extracts exhibited antioxidant and antimicrobial activity in a dose dependent manner.

Key Words: *Mentha spicata*, leaves, *in vitro* antioxidant, antimicrobial activity.

INTRODUCTION

Mentha spicata L. is commonly known as spear mint belongs to family Lamiaceae. Several reports indicate that *Mentha spicata* contained several phytochemical compounds like lupeol, geraniol, citronellal, carvone, R-(-)-2-amino-1-propanol, 1,2-epoxy-5,9-cyclododecadiene, 3,7,11,15-tetramethyl-2- hexadecen-1-ol, 9,12,15-octadecatreinoic acid, methyl ester, (Z,Z,Z)- 9,12-octadecadienoic cid, methyl ester, 9,12,15-octadecatrein-1-ol, benzophenone, caryophyllene oxide, 2-cyclohexene,1-ol, 2-methyl-5 (1-methylethenyl), 2-cyclohexene,1-one, 3,5,5 trimethyl-4 (3-oxo-1-butenyl)-, 2-pentadecanone β-sitosterol, daucosterol, stigmasterol. (Ramesh Naidu *et al.,* 2012). *Mentha spicata* has been reported to possess several medicinal properties. The medicinal properties of this plant is due to some of the bioactive compounds it contained like alkaloids, tannins and phenolic compounds (Edeoga *et al.,* 2005). In recent times antibiotic resistance of pathogens has become a growing problem worldwide (Gardam 2000 & Ali *et al.,* 2001). This has led to the search for new, safe and effective antimicrobial agents from natural products. Plants with medicinal properties offer the possibility for the development of new antimicrobial agents effective against infections currently difficult to treat (Iwu *et al.,* 1999). The present study was aimed to assess and evaluate the *in vitro* antioxidant and antimicrobial activity of *Mentha spicata* leaf extracts.

MATERIALS AND METHODS

Preparation of extracts from leaves of Mentha spicata L.

The leaves of *Mentha spicata.* were collected locally, shade dried, powdered and extracted in three different solvents viz., hexane, chloroform and methanol, with a Soxhlet apparatus for 6 hrs subsequently dried under vacuum at temperature of 45°C by using rotary evaporator (Buchi, Switzerland). Completely dried samples are stored in desiccators for further analysis.

In-vitro Antioxidant Activity

The three extracts of *Mentha spicata* at different concentrations were screened for free radical scavenging activity against DPPH, Superoxide and FRAP radicals. The Percentage Inhibition was calculated. All experiments were performed thrice and the results were averaged.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was measured according to the method of Braca *et al.,* 2001. An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed and incubated at 37˚C for 30 min. and absorbance of the test mixture was read at 517 nm.

Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity of the extract was measured according to Mc Cord and Fridovich method, 1969. The reaction depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. All the solutions were prepared in phosphate buffer (pH 7.8). The optical density was measured at 560nm.

Ferric reducing or antioxidant power assay (FRAP):

FRAP working reagent (3 ml) was taken in a test tube and added 100µl of plant extract , this is vortex mixed, and the absorbance was read at 593nm against a reagent blank at a predetermined time after sample–reagent mixture. The results are expressed as ascorbic acid equivalents (µ moles/ml) or FRAP units (Benzie and Strain 1996).

Calculation of Percentage Inhibition

The percentage inhibition of superoxide production by the extract was calculated using the following formula:

$$\text{Inhibitory ratio} = \frac{(A_0 - A_1) \times 100}{A_0}$$

Where, A_0 is the absorbance of control; A_1 is the absorbance with addition of plant extract/ ascorbic acid.

Test organisms

The microorganisms used in the experiment were procured from MTCC, IMTECH-Chandigarh. Of the five bacterial strains tested two are Gram-positive *Staphylococcus aureus* and *Bacillus subtilis* strains and three Gram-negative *Escherichia coli, Klebsiella pneumonia* and *Proteus vulgaris* strains.

Evaluation of *in vitro* antimicrobial activity

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test extracts. A sterile borer was used to prepare the cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculums. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicates antimicrobial activity (Pharmacopoeia of india 1996).

RESULTS

Antioxidant activity

The results of *in-vitro* antioxidant activity of methanolic leaf extract of *Mentha spicata* clearly indicate the presence of free radical scavenging activity and it produced dose dependent inhibition of free radical generation of DPPH, Superoxide and FRAP radicals. Graphs were plotted from the observed values to find the percentage inhibition (Fig 1, Fig 2 & Fig 3) of the leaf extracts of *Mentha spicata*. Evaluation of antimicrobial activity. All the extracts of *Mentha spicata* leaves had produced a minimum zone of inhibition against *P.vulgaris* species. The three extracts of *Mentha spicata* had produced a minimum zone of inhibition against the tested bacterial strains at the highest dose of 500µg/ml. However, aqueous extract did not induce any significant zone of inhibition against any of the bacterial strains. Among all the tested extracts, ethanol extract has shown significant antimicrobial activity when compared to that of chloroform and aqueous extracts. The highest zone of inhibition was exerted by ethanolic extract against *B.subtilis* at the dose of 500µg/ml. The results are summarized and presented in Table 4.

Table 1: ***In-vitro* concentration dependent percentage inhibition of DPPH radical by leaf extracts of *M. spicata.***

Conc. of plant extract	% inhibition of DPPH radical		
	Aqueous extract	Chloroform extract	Ethanol extract
100µg	23.132±0.15	26.910±0.14	30.511±0.14
250 µg	27.683±0.15	30.042±0.18	37.013±0.12
500 µg	30.723±0.18	33.887±0.17	44.612±0.14
1000 µg	31.832±0.12	37.818±0.12	47.188±0.14

Values are means ± SD (n=3)

Table 2: ***In-vitro*** **concentration dependent percentage inhibition of Superoxide radical by leaf extracts of *M. spicata.***

Conc. of plant extract	% inhibition of Superoxide radical		
	Aqueous extract	Chloroform extract	Ethanol extract
100µg	26.190±0.05	49.127±0.04	51.790±0.04
250 µg	48.88±0.07	69.579±0.05	70.324±0.06
500 µg	64.705±0.05	80.300±0.08	81.533±0.07
1000 µg	78.571±0.04	87.179±0.05	90.146±0.04

Values are means ± SD (n=3)

Table 3: Total Antioxidant Assay-FRAP Method

Plant Name	FRAP units in µM
Mentha spicata	430±0.04

Values are means ± SD (n=3).

Table 4: Antimicrobial activity of *Mentha spicata L.* leaf extracts

Organism	Diameters of zones of inhibition in mm			
	(E)	(C)	(A)	Rifampicin (50 µg/ml)
E.coli	10	18	2	24
B.subtilis	20	10	2	22
S.aureus	7	17	1	24
K. pneumoniae	14	10	2	25
P.vulgaris	13	16	3	23

A:Aqueous extract; C- Chloroform extract; E- Ethanol extract of *M. spicata.*

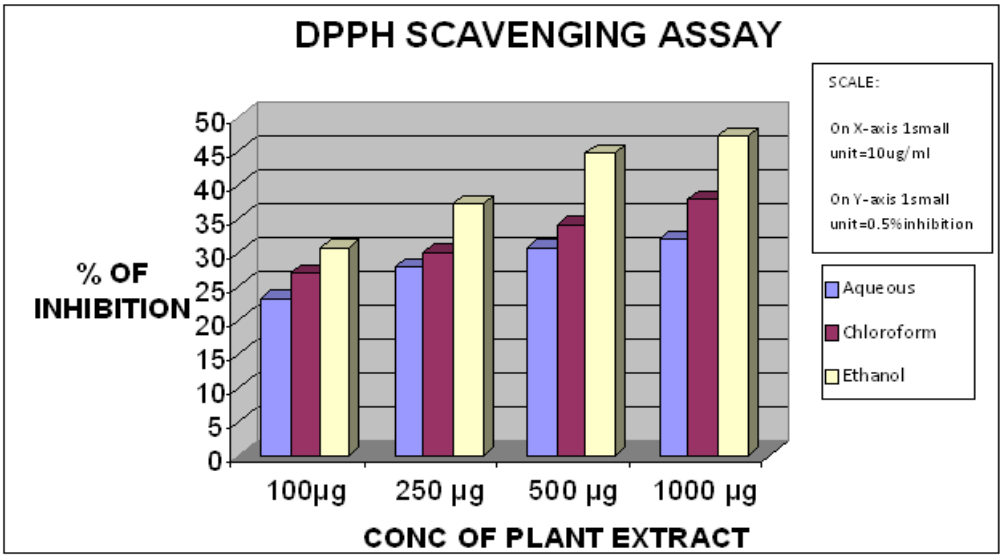

Fig 1: ***In-vitro*** **concentration dependent percentage inhibition of DPPH radical by leaf extracts of *M. spicata.***

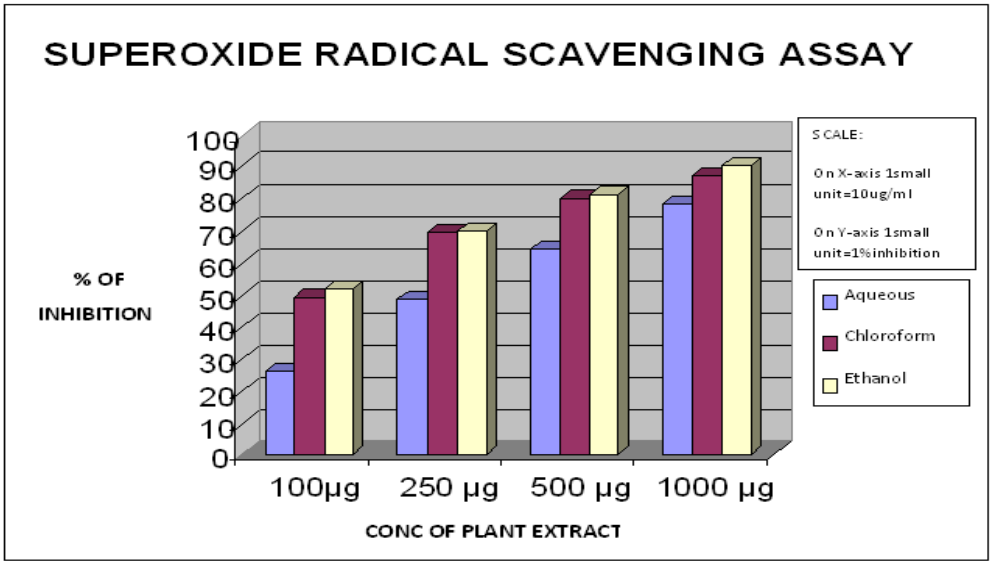

Fig 2: *In-vitro* concentration dependent percentage inhibition of Superoxide radical by leaf extracts of *M. spicata.*

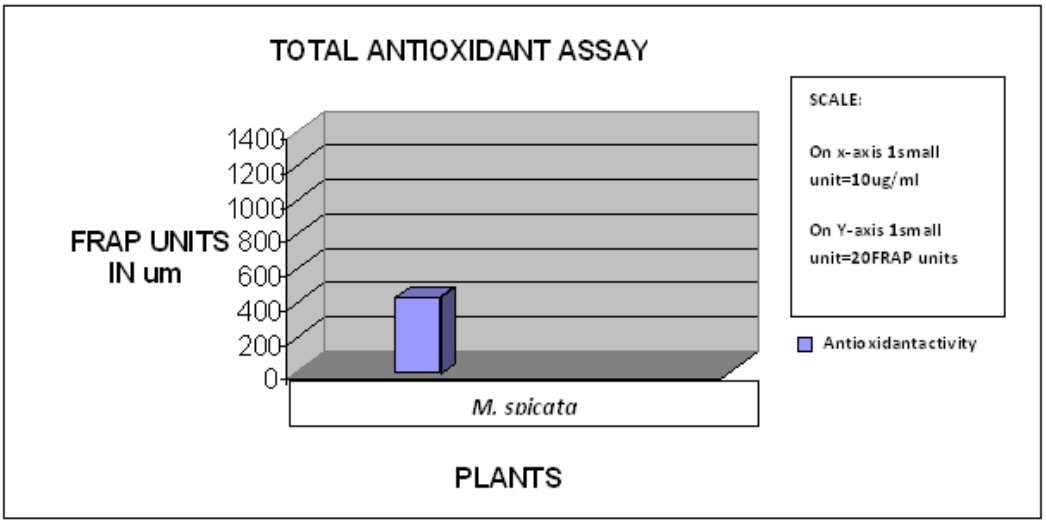

Fig 3: *In-vitro* total antioxidant assay (FRAP) by leaf extracts of *M. spicata.*

DISCUSSION

By and large, the three tested extracts showed good percentage of inhibition of DPPH, Superoxide and FRAP radicals. Of the three tested extracts ethanolic extract exerted highest activity followed by chloroform and aqueous extracts. The activity of all the three extracts was dose dependent. Of the three extracts tested for antibacterial activity, Chloroform extract induced considerable zone of inhibition on *E. coli*, *S.aureus* and *P.vulgaris* while ethanolic extract was very effective in inhibiting *B.subtilis.* Compared to the zone of inhibition generated by Rifampicin. aqueous extract did not show any significant effect at any concentration. The non-activity of aqueous extract on the tested bacterial strains could be attributable to presence of active compound(s) in insufficient quantities at the tested concentration. (Taylor *et al.,* 2001) or it could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents (Jigna Parekh and Sumitra Chanda 2007).

CONCLUSIONS

All the three tested extracts showed dose dependent antioxidant activity. However, the three different leaf extracts showed differential antimicrobial activity, aqueous extract recorded with lowest activity. On the whole, the three extracts recoded with a bit lower antibacterial activity compared to that of standard Rifampicin.

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