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ANTIMICROBIAL SCREENING AND ANTIOXIDANT POTENTIAL OF MENTHA PIPERITA OF DODDABALLAPUR TALUK, BENGALURU RURAL DISTRICT KARNATAKA

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ABSTRACT

Leaves of plant extract from *Mentha piperita* (commonly known as mint) are studied for its therapeutic activity. The sample (Mint extract) was tested in duplicates for their MIC property against organisms *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus mutans***,** the crude ethanol leaf extracts showed significant antibacterial activity. The antioxidant property was conducted by invitro method of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was conducted on the crude leaves ethanolic extract. DPPH assay showed 49% radical scavenging activity at 500 µg/ml. The antioxidant activity of the samples was compared to the gallicacid as a standard. This study revealed that the ethanolic extract of the plant is good source of antioxidants. It could explain and justify some of their uses in traditional medicine and they need further exploration for their use in modern system of medicines.

KEYWORDS: *Mentha piperita*, gallic acid, DPPH radical scavenging, MIC.

INTRODUCTION

The Lamiaceae or Labiatae are a family of flowering plants commonly known as the mint or deadnettle or sage family. Many of the plants are aromatic in all parts and widely usedculinary herbs like basil, mint, rosemary, sage, savory, marjoram, oregano, hyssop, thyme, lavender, and perilla, as well as other medicinal herbs such as catnip, salvia, bee balm, and oriental motherwort. Some species are shrubs, trees (such as teak), or, rarely, vines. Many members of the family are widely cultivated, not only for their aromatic qualities, but also their ease of cultivation, since they are readily propagated by stem cuttings. Besides those grown for their edible leaves, some are grown for decorative foliage.

Mint is a perennial herb with very fragrant, toothed leaves and tiny purple, pink, or white flowers. There are many varieties of mint—all fragrant, whether shiny or fuzzy, smooth or crinkled, bright green or variegated. However, you can always tell a member of the mint family by its square stem. Rolling it between fingers, we can notice a pungent scent and think of candy, sweet teas, or maybe even mint juleps. As well as kitchen companions, mints are used as garden accents, ground covers, air fresheners, and herbal medicines.[7,8]

Figure 1: *Mentha piperita.*

Classification of mint

Kingdom: Plantae
Order: Lamiales
Family: Lamiaceae
Subfamily: Nepetoideae
Genus: Mentha
Species: *Menthaspicata*
Binomialname*: Mentha piperita*

MATERIALS AND METHODS

Collection and Identification of Plant The plant was collected from the different region of Doddaballapur taluk, Bengaluru district, Karnataka, India (from their natural habitat with acceptable bio-conservation methods) has been identified and authenticated by

Botany Department, Nrupathunga University Bengaluru as *Mentha piperita* belongs to the family Lamiaceae.

Plant Material The leaves of *Mentha piperita* was washed thoroughly under running tap water followed by distilled water and then air dried under shade at room temperature for one week. The dried leaves were powdered with the help of porcelain mortar and pestle to increase the surface area for absorption of the solvents (Harborne 1973), stored in separate containers in moisture free environment and used for further analysis.

Preparation of Extract Four solvents were selected for the process of extraction on account of their polarity, namely Methanol, Ethanol, Carbon tetrachloride, and Water. 30 gm of dried leaves powder of each plant were taken separately and process of extraction using Soxhlet apparatus was performed out in a 250 mL of each solvent separately. The extraction process was time framed for complete 48 hrs after which the solvent mixture was concentrated at a temperature not exceeding 40C using a rotary evaporator and stored at 40C. The Preliminary Phytochemical Screening Different solvent extract of each plant were subjected to chemical test for different phytochemicals viz. Alkaloids, Flavonoids, Steroids, Terpenoids, Anthraquinones, Phenols, Saponins, Tanins, Carbohydrates and Oil by using standard procedures.

Phytochemicals investigation for mint

Phytochemical screening of the extracts of the plant was carried out in order to know the class of organic compounds present in the different extracts selected for the study, which further facilities for the identification of active constituents and their isolation.

The extract of mint were subjected to standard chemical tests to determine the presence or absence of steroids &triterpenoids, alkaloids, tannins, flavonoids, glycosides, carbohydrates, proteins and amino acids. Ethanol extract revealed presence of large number of phytoconstituents compared to other solvents used during the extraction.[5]

RESULTS AND DISCUSSION

The different phytochemical constituents present in the extract were identified by the color reaction with different reagents. The main objective of phytochemicals screening was to identify the different groups of chemical constitutes present in the plant extract. Results of the phytochemical screening are summarized below:

Table 1: Phytochemical testing of extract (Ethanol).

Sl. No	Phytochemicals tests	Inference
1	Salkowski test	_
2	Leibermann-Burchard test	_
3	Mayer's test	_
4	Wagner's test	+
5	Gelatin test	_
6	Lead acetate test	_
7	Alkaline reagent test	+
8	Keller killiani test	+
9	Sodium hydroxide test	+
10	Molisch's test	+
11	Fehling's test	+
12	Conc.H2SO4 test	+
13	Ninhydrin test	+
14	Xanthoproteic test	+

Table 2: Phytochemical screening of extract.

Sl. no	Phytochemicals	Ethanol extract
1	Alkaloids	+
2	Carbohydrates	+
3	Amino acids	+
4	Glycosides	+
5	Flavonoids	+
6	Tannins	+
7	Steroids	+

(+) = presence & (-) = absence

A preliminary study has reported that the leaves extract contained large number of bioactive secondary molecules like alkaloids, glycosides, carbohydrates, flavonoids, amino acids (table 2). The presence of these components in this species is an indication that it may have some medicinal potential.

Antimicrobial screening

Well diffusion method to check the Minimum Inhibition Concentration (MIC)

The sample (Mint extract) was tested in duplicates for their MIC property against organisms **(*Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus mutans*)**

Culture Media Preparation for Bacteria

Luria Bertani (LB) broth (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Distilled water 1000mL) 30mL was prepared in 4 Erlenmeyer flasks by adding Tryptone 0.3g, Sodium chloride 0.3g, Yeast extract

0.18g, Distilled water 30mL and autoclaved at 121°C for 15 minutes. Later, ***Pseudomonas aeruginosa*** **strain** (MTCC 2453), ***Salmonella typhi*** **strain** (MTCC735), ***Staphylococcus aureus***(MTCC 96) and ***Streptococcus mutants*** **strain** (MTCC 497) was inoculated respectively in 30mL of sterilized LB broth and incubated at 37˚ C for 24h.

Bacterial Culture preparation

Cultured organisms **(*P.aeruginosa, S.typhi, S.aureus* and *S.mutans*)** were centrifuged at 6000rpm for 10 minutes respectively, supernatant was discarded and the pellets were dissolved in 1% ($^{w}/_{v}$) Sodium chloride and adjusted to absorbance 1.000 at 600nm under UV spectrophotometer (Genesys 10S UV-VIS Spectrophotometer).

Sample preparation

The sample 10mg (Mint extract) and Control 10mg (Tetracycline) was dissolved in 1mL Dimethyl sulfoxide (DMSO) respectively. Different aliquots of the sample and control was prepared by pipetting 10µL (100µg), 20µL (200µg), 30µL (300µg) and 40µL (400µg) and the final volume was made upto 50µL by adding DMSO.

Media preparation for MIC Luria Bertani (LB) agar media (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Agar 20g, Distilled water 1000mL) 500mL was prepared by adding Tryptone 5g, Sodium chloride 5g, Yeast extract 3g, Agar 10g, Distilled water 500mL in Erlenmeyer flask and autoclaved at 121°C for 15 mins.

Platting for MIC against organisms

Approximately 25mL of LB agar was poured into the sterilized petriplates and allowed it to solidify. 200µL prepared inoculums **(*P.aeruginosa, S.typhi, S.aureus*and *S.mutans*)** was poured in to the agar plates respectively and spread thoroughly using a plate spreader. Five wells measuring 0.6 cm was made in each plates using the borer and 50µL of prepared sample and control (Tetracycline) containing 100µg, 200µg, 300µg, 400µg were loaded into the respective wells and 50µL of DMSO was loaded in the middle well as control blank.

The bacterial plates incubated at 37°C for 24h. Later, zone of inhibition was recorded in mm (Millimeter).[2,3,4]

Table 3: MIC of Control (Tetracycline) against Organisms.

Organisms	Zone of inhibition (in mm) of Control			
	100 µg	200 µg	300 µg	400 µg
P.aeruginosa	22	24	26	29
S.typhi	20	21	23	25
S.aureus	20	22	24	26
S.mutans	23	25	27	29

Table 4: MIC of Sample Mint extract against Organisms.

Organisms	Zone of inhibition (in mm) of Sample							
	100 µg		200 µg		300 µg		400 µg	
Plates	1	2	1	2	1	2	1	2
P.aeruginosa	-	-	-	-	-	-	-	-
S.typhi	-	-	10	11	12	12	13	14
S.aureus	-	-	-	-	-	-	-	-
S.mutans	-	10	10	10	11	11	12	11

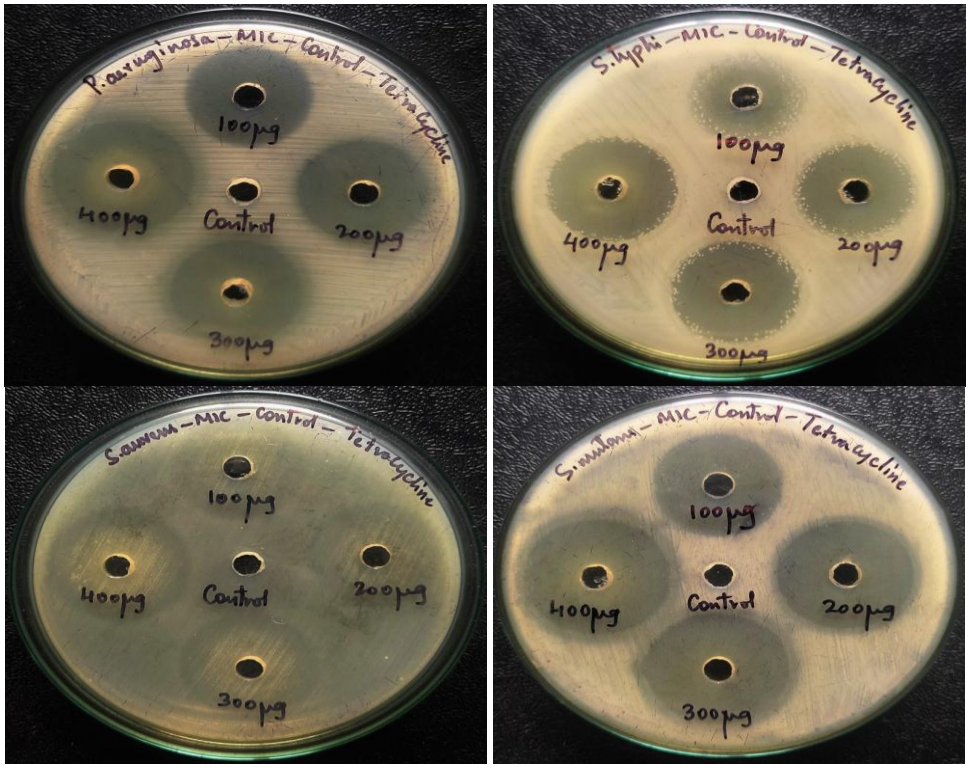


Figure 2: MIC of control (Tetracycline) against organisms.

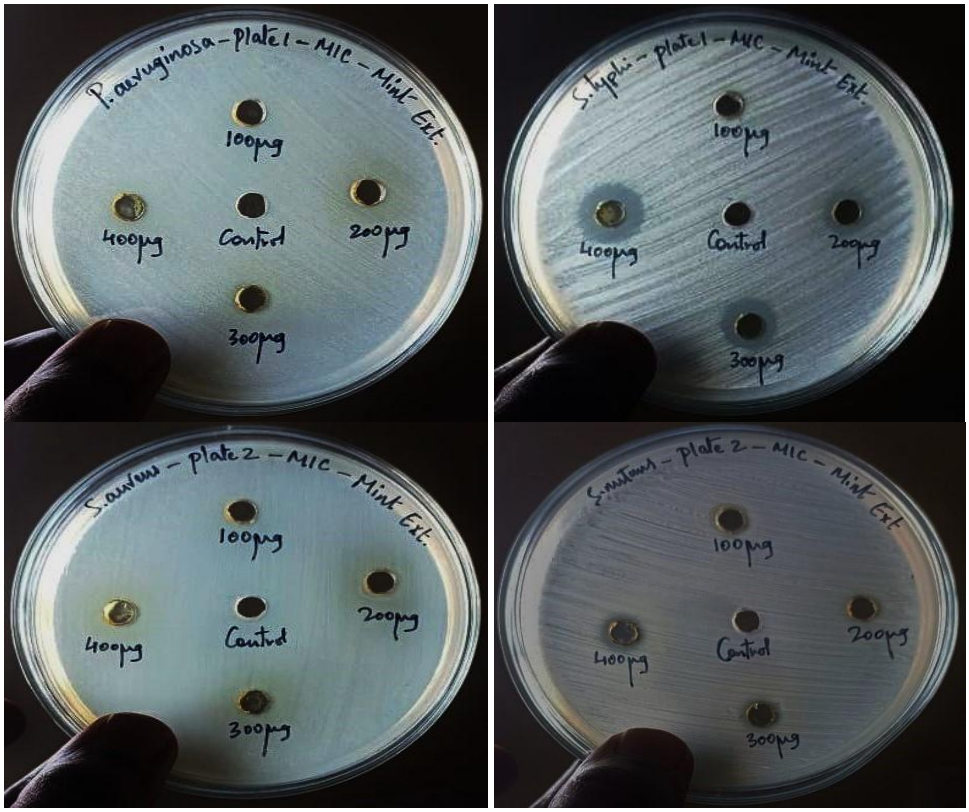


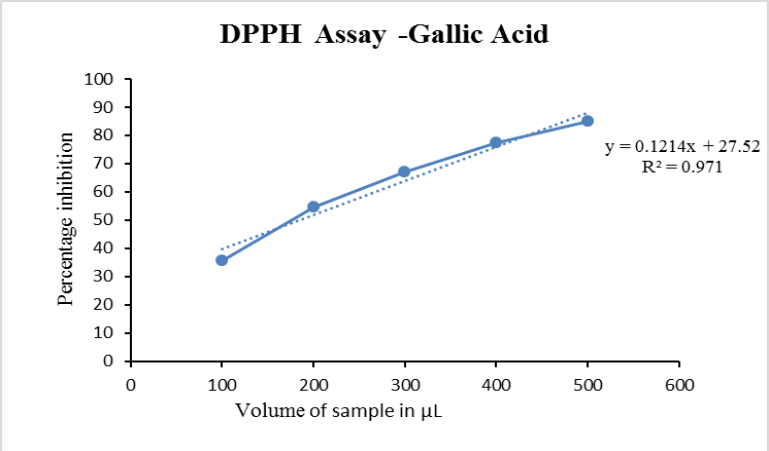
Figure 3: MICS of sample mint extract against organisms.

Antioxidant analysis (DPPH scavenging assay)

Free radical scavenging capacities of the extracts from different samples were estimated using the stable DPPH radical. Different concentrations (0.1 – 0.5mg) of the samples were taken in the test tubes and the volume in each test tube was made up to 0.1mL with methanol. To all the tubes, 3mL of DPPH solution (whose absorbance was pre-set to 1) was added and incubated in dark condition for 15minutes. After incubation, the absorbance was read at 517nm spectrophotometrically with methanol as a blank. Percentage inhibition was calculated using the formula.[1]

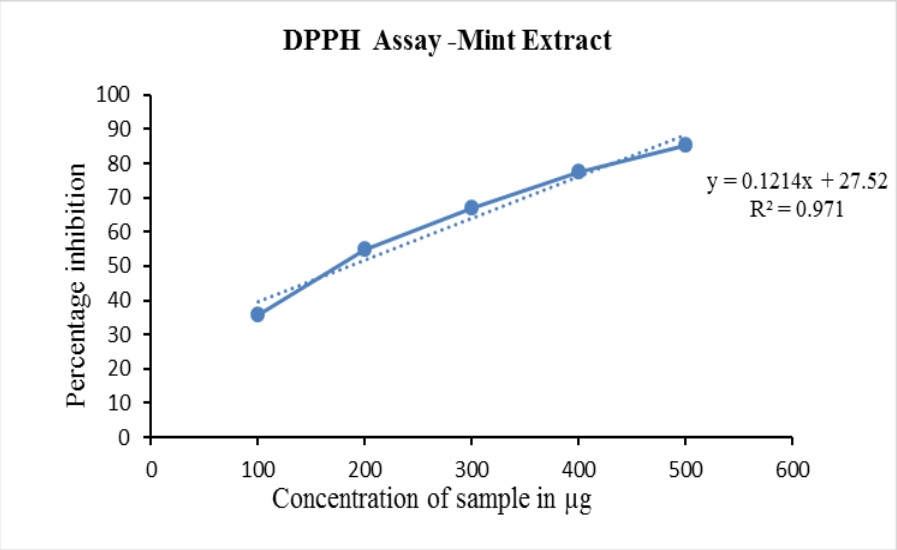
Percentage inhibition = [Abs of control – Abs of sample/ abs of control] * 100

Conc of sample in µg	Gallic Acid	
	Absorbance at 517 nm	Percentage Inhibition
100	0.914	8.6
200	0.815	18.5
300	0.695	30.5
400	0.622	37.8
500	0.510	49.0



Graph 1: DPPH assay (Gallic acid).

Conc of sample in µg	MINT EXTRACT	
	Absorbance at 517 nm	Percentage Inhibition
100	0.914	8.6
200	0.815	18.5
300	0.695	30.5
400	0.622	37.8
500	0.510	49.0



Graph 2: DPPH assay (Mint extract).

RESULT

Sample	IC50 value (mg)
Gallic acid	0.511
Mint Extract	0.185

CONCLUSION

Herbal medicine is known as botanical medicine or phytomedicine. It refers to use of plant seeds, roots, berries, leaves, bark and flowers for medicinal purposes. Recently, the World Health Organization estimated that 80% of people worldwide relay on herbal medicines for some part of their primary health care. Herbal medicines have been the oldest forms of health care. In this study *Mentha piperita* plant extracts has been investigated for antibacterial and antioxidant properties. Plant extracts showed appreciable antibacterial properties when treated as crude extracts. DPPH scavenging assay has been performed to study the antioxidant activity of the plant extracts. Ethanolic extracts of leaves of the plant showed radical scavenging activity of 49% at 500 µg/ml. All extracts under consideration showed good antioxidant property.

Conflict of Interest Authors declare no conflicts of interest.

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