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Antimicrobial Activity Assay and Phytochemical Study of Different Aerial Parts of *Mentha Arvensis* L. Collected from Dibrugarh, Assam

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Abstract: **Owing to the ethno-medicinal importance of *Mentha arvensis* L. the present study proposes in-vitro screening of different aerial parts of the plant. Instead of using whole plant, different parts of a plant can also be used which helps in sustainable management of medicinal plants. Phytochemical, antioxidant and antimicrobial activity study of young and mature leaves, inflorescence and stem of the plant was carried out using standard laboratory methods to determine their efficiency against some bacterial and fungal strains. Inflorescence recorded better extraction of phenol and flavonoid content.**

Index Terms:

I. INTRODUCTION

Mentha arvensis L. is commonly known as Pudina. It is an erect aromatic herb with suckers, cylindrical stem, simple and opposite leaves. It is a small perennial aromatic plant and leaves and stem are used for flavouring chutney, food and beverages (Sharma and Jocob, 2001; Zakaria *et al*., 2008). It is used as a contraceptive, antispasmodic, anti-peptic ulcer agent, treat indigestion, skin diseases, coughs and colds, refrigerant, stimulant, carminative, rheumatism and indigestion, stomach cramps, menstrual cramps, flatulence, nausea, vomiting and colic in children, relieves pain, reduces sensitivity, caries prevention (Kanjanapothi *et al*., 1981; Dutta, 1985; Budavari *et al*., 1989; Gupta *et al.*, 1991; Chopra and Chopra, 1994; Sola, 1995; Benavente-Garcia *et al.*, 1997; Kiritikar and Basu, 1998; Ahmad *et al.*, 1998; Ganesh and Manjeshwar, 2002; IMR,HMRC, 2002; Verma *et al*., 2003; Khare, 2004; Edeoga *et al.*, 2005; Carounanidy *et al*., 2007; Nair and Chanda, 2007; Khan and Khatoon, 2008; Coutinho *et al*., 2009; Londonkar and Poddar, 2009; Shah *et al*., 2009; Vivek *et al.*, 2009; Nascimento *et al*., 2010; Akhtar *et al*., 2011; Akram *et al.*, 2011; Towseef *et al*., 2012; Malik *et al.*, 2012; Kowti *et al*., 2013; Rajesh, 2013). The plant have antimicrobial, antiviral and insecticidal activity (Franzias *et al*., 1997; Sartoratto *et al.*, 2004; Abu- Shanab *et al.*, 2006; Yadegarinia *et al.*, 2006; Kumar *et al.*, 2007; Jazani *et al*., 2009). Various compounds were separated and identified from the plant by various workers (Chopra *et al.*, 1956; CSIR, 1972; Satyavati *et al.*, 1987; IMR,HMRC, 2002; Ghani, 2003; Oinonen *et al.*, 2006; Verma *et al.*, 2010; Rastogi *et al.*, 2010; Rajesh *et al.*, 2013). Various experiments were carried out on phytochemical, antioxidant, antimicrobial and other aspects of the plant by various workers (Gupta *et al.*, 2010; Akram *et al.*, 2011; Hanifa and Santhi, 2012; Garg *et al.*, 2012; Pidugu and Arun, (2012); Malik *et al*., 2012; Dwivedi *et al.*, 2012; Biswas *et al.*, 2014).

Owing to the importance of the plant, the present study proposes in-vitro screening of the plant. The main aim of the work is to find out the most active part of the plant which can be used in medicinal practices, instead of using the whole plant. The use of the active parts also helps in sustainable management of these kinds of medicinal plants.

II. MATERIAL AND METHODS

A. Sample collection

For the study cultivation of the plant was done. Flowering branches were collected and brought to the laboratory. Different parts (young and mature leaves, inflorescence and stem) were

separated and cleaned properly and washed under running water to remove dust and other debris. The materials were air dried at room temperature. The stems were sliced before allowed to dry. After removal of surface water, the materials were wrapped with brown paper and allow sun dry for complete dryness (less than 1-2% moisture content). The materials were grounded to fine powder using mortar and pestle and then in electric grinder. The fine powder were kept in air tight bottles for further analysis.

B. Preparation of extracts

Extracts were prepared in five solvents viz- water, methanol, ethanol, acetone and petroleum ether by cold maceration methods and are known as cold extracts. The solvents were selected on the basis of polarity level and their extraction ability. Extracted 10 g air dried powder by soaking it in 500ml of solvent (except water) for 72 hours with intermittent shaking. The extracts were filtered through whatman no. 1 filter paper into pre-weighed beakers. The filtrate was dried on water bathe to obtain a dried mass. The water extract was prepared by soaking 10 gms of powder in 500 ml distilled water for 48 hrs with intermittent shaking. The soaking for 72 hours cause fungal infection. The solution was filtered through whatman no. 1 filter paper. The filtrate was dried to sticky mass using water bath. The extracts were kept in air tight glass bottles at 5° C for further analysis. Hot petroleum ether extract was also prepared using soxhlet extractor and antimicrobial activity of the extract was done to observe the difference in activities of both cold and hot petroleum ether extract.

The dried extracts were dissolved in DMSO (Dimethyl Sulfoxide) to obtain sample solution at 1mg/ml of concentration. Aqueous extracts were dissolved in distilled water at 1mg/ml of concentration.

C. Qualitative phytochemical analysis

Qualitative analysis for detection of tannins, phlobatannins, flavonoids, saponins, alkaloids, cardiac glycosides, terpenoids, steroids, anthraquinone, free anthraquinone, carotenoids and reducing sugar were performed using standard laboratory methods after Iyengar, (1995), Wagner *et al.*, (1996), Siddiqui and Ali, (1997), Trease and Evans, (2002), Edeoga *et al*., (2005), Egwaikhide and Gimba, (2007), Chitravadivu, (2009), Majaw and Moirangthem, (2009), Aja *et al*., (2010), De *et al*., (2010), Ajayi *et al.*, (2011), Ajiboye *et al.*, (2013) as laid down below:

D. Tests for tannins

a) Boiling of 0.5 gm of powdered sample in 10 ml of water and addition of few drops of 0.1% ferric chloride results green or blue black colouration. The colouration indicates the presence of tannin in the sample.

b) Small amount water filtrate of the sample was added to 10% lead acetate solution. The presence of white precipitate, confirmed the presence of tannin in the sample.

E. Test for phlobatannins (HCL test)

a) Aqueous filtrate of the sample was boiled with 10% and 2 % HCl and red precipitation indicates the presence of phlobatannin in the sample.

F. Test for flavonoides

a) 0.5 gm of powdered sample was heated with 10 ml of ethyl acetate and filtrate was taken and shaken with 1 ml of dilute ammonia solution. Appearance of yellow colouration indicates the presence of flavonoids.

b) Small amount of crude extract was mixed with 2ml of 2% solution of NaOH. Formation of yellow colour confirms the presence of flavonoids. This yellow colouration becomes colourless on addition of few drops of diluted hydrochloric acid which confirmed the presence of flavonoids in the test sample.

G. Test for saponins (Frothing test)

a) Water filtrate of the powdered sample was shaken vigorously to form a layer of foam, which was stable for 10 mins. This indicates the presence of saponins in the test samples.

b) Small amount of powdered sample was shaken with distilled water and warmed. The persistance of froth layer confirmed the presence of saponins in the test samples.

H. Test for alkaloids

1 gm of powdered sample was boiled separately with 10ml of distilled water and 5 ml of HCl and filtered. The P^H of the filtrate was adjusted between 6-7 with ammonia. Below mentioned reagents were added to 0.5 ml of the filtrate in different test tubes and observed.

i. Addition of Picric Acid = Colour precipitate or turbidity indicates the presence of alkaloids.

ii. Addition of Mayer's reagent = Colour precipitate or turbidity indicates the presence of alkaloids.

iii. Addition of Dragendroff reagent = Colour precipitate or turbidity indicates the presence of alkaloids.

Addition of three different reagents to the sample filtrate confirmed the presence alkaloids in the sample.

I. Test for glycosides

a) Liebermann's test: a mixture was prepared by adding small amount of crude extract with 1ml of chloroform and glacial acetic acid respectively and than cooled in ice. Addition of concentrated H_2SO_4 was done to the mixture very carefully. Changing of colour from violet to blue to green indicated the presence of glycoside in the test samples.

b) A mixture was prepared by adding 1 g of powdered sample with 10 ml of distilled water. The mixture was heated for 10 min. Then the mixture was filtered. Fehling solution (A+B) was added to the filtrate and heated over heater. Appearance of brick red precipitate confirmed the presence of glycoside in the test samples.

J. Test for cardiac glycosides

Keller killani test: a solution was prepared by mixing 5 ml water filtrate with 2 ml of glacial acetic acid which contains 1 drop of ferric chloride solution. 1 ml of conc. H_2SO_4 was added to the solution and a brown ring was formed at the interface which indicated the presence of cardiac glycoside in the test samples. Below the brown ring, a violet ring may appear. On the other hand in the acetic acid layer, a greenish ring may form, which confirmed the presence of cardiac glycosides in the test samples.

K. Test for terpenoids

Salkowski test: a solution was made by adding 5 ml of water filtrate with 2 ml of chloroform and 3ml of conc. H_2SO_4 was added carefully to the solution to form a layer. A reddish brown colouration at interface indicates the presence of terpenoids in the test samples.

L. Test for steroids

Small amount of water filtrate was mixed with chloroform and a few drops of conc. H_2SO_4 was added and shaken. Appearance of red colour in the lower layer of the solution indicates the presence of steroids in the test samples.

M. Test for anthraquinone

Filtrate was taken after mixing 0.5 gm powdered sample with 5 ml of benzene. To the filtrate 0.5 ml of 25% ammonia solution was added and the obtained mixture was shaken properly. Presence of the violet colour in the layer phase indicates the presence of anthraquinone in the test samples.

N. Test for free anthraquinone

Filtrate was taken after mixing 0.5 gm powdered sample with 5 ml of chloroform and shaking for 5 mins. Equal volume of 10% ammonia solution was added to the filtrate. A bright pink colour in the aqueous layer indicates the presence of free anthraquinone in the test samples.

O. Test for phenol

A mixture was prepared by adding 5ml of the water filtrate with 10ml of distilled water, 2ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol sequentially and the mixture was left for 30min. Formation of blue green colouration in the solution indicates the presence of phenol in the test samples.

P. Test for carotenoids

Filtrate was obtained after mixing 0.5 gm of powdered sample with 5 ml of chloroform in a test tube with vigorous shaking. 3ml of 98% H_2SO_4 was added to the filtrate and a blue colour observed at the interface which indicates the presence of carotenoids in the test samples.

Q. Test for reducing sugar

Filtrate was obtained after mixing 0.5 gm of the powdered sample with distilled water. Fehling solution (A+B) was added to the filtrate and boiled for few minutes. An orange red precipitation indicates the presence of reducing sugar in the test samples.

R. Quantitative phytochemical analysis

Quantitative estimation for total phenol content (TPC) and total flavonoid content (TFC) were performed following standard methods noted below:

S. Determination of total phenol content (TPC)

Total phenol content (TPC) of the sample extract was estimated following the method described by Malik and Singh,(1980). For determination of Total phenol content a extract solution was prepared by mixing the extracts with DMSO at a concentration of 1mg/ml. 0.2 ml of the extract solution was taken in 10 ml test tube and made up to a volume of 3ml by adding distilled water. Then sequential addition of 0.5 ml Folin-ciocalteau reagent (1:1 with water) and 2 ml Na_2Co_3 (20%) was done. The solution were warmed for 1 min. and then cooled. Development of blue colour indicates the presence of phenol. Absorbancy of the solution was measured at 760 nm and phenol content was determined using the standard curve of Catechol. The total phenol content in extracts was expressed in terms of Catechol Equivalent (mg CE/g extract).

T. Determination of total flavonoid content (TFC)

The Aluminium chloride method was used for determination of total flavonoid content of the sample extracts as described by Mervat and Hanan, (2009). The extracts were mixed with DMSO to form a solution having concentration of 1mg/ml. 0.2 ml of extract solutions were taken in test tubes in triplicate form and volume was made to 3 ml by adding methanol. 0.1 ml $AlCl_3$ (10%), 0.1 ml sodium potassium tartarate and 2.8 ml distilled water were added sequentially to the solution and shaken vigorously and carefully. After 30 mins of incubation absorbancy of the solutions were taken at 415 nm using spectrophotometer and flavonoid content was determined using the standard curve of Quercetin. The total flavonoid content in extracts was expressed in terms of Quercetin Equivalent (mg QE/g extract).

U. Antioxidant activity assay of the sample extracts

DPPH radical scavenging activity and ABTS radical scavenging activity tests were performed for determination of antioxidant activity of the crude extracts of different parts of the plants.

V. Determination of antioxidant activity assay of the sample extract by DPPH method

DPPH radical scavenging activity was determined by the method described by Anti-Stanojevic *et al.,* (2009). In this method, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical was converted to 1,1-diphenyl-2-picryl hydrazine by the reaction of the radicals present in the sample. The scavenging capacity of the sample was determined through the degree of change in colour from purple to yellow of the sample solution. 0.5ml of extract solutions (1mg/ml) were taken in test tubes in triplicate form and the volume of the solution were made to 3ml with methanol. Test tubes with 3ml of methanol in triplicate from were used as blank. 0.15ml of freshly prepared DPPH solution was added to each of the test tubes. The solutions were then shaken and left to stand at room temperature for 30 minutes in dark. A control solution was prepared by mixing DPPH solution in methanol. Absorbancy was recorded at 517 nm using UV-Vis spectrophotometer. The capacity of scavenging free radicals by the sample extracts was calculated using the following formula

DPPH radical scavenging activity (%) = $[(Abs_{control}-Abs_{sample}/Abs_{control}.] \times 100$

Where,

$Abs_{control}$ is the absorbance of DPPH radical + methanol

Abs_{sample} is the absorbance of DPPH radical + sample extract

W. Determination of antioxidant activity assay of the sample extracts by ABTS method

The ABTS assay was carried out following the method of Re *et al*., (1999). A stock solution was prepared by mixing equal proportion of 7 mM ABTS solution and 2.4 mM potassium persulfate solution and kept for 12 hrs at room temperature in dark. 1 ml of the solution was mixed with 60 ml methanol to obtain an absorbancy of 0.706 + 0.001 units at 734 nm using the UV-Vis spectrophotometer. Freshly prepared ABTS solution was used for each assay. 1 ml extract solution (1mg/ml) was allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the UV-Vis spectrophotometer. The ABTS scavenging capacity of the extract was compared with standard ascorbic acid and calculated the percentage of inhibition.

ABTS radical scavenging activity (%) = $[(Abs_{control}-Abs_{sample})/Abs_{control}] \times 100$

Where,

$Abs_{control}$ is the absorbance of ABTS radical + methanol;

Abs_{sample} is the absorbance of ABTS radical + sample extract/standard.

X. Antimicrobial activity assay of the sample extracts

Antimicrobial activity of the bacterial strains was carried out by agar well diffusion method described by Nair *et al.,* (2005) using 6mm borer.

Y. Test organisms

Gram positive and gram negative bacterial strains and fungal starins are used in this experiment to know the antimicrobial activity of the sample extracts.

a) Gram positive bacterial strains- *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744).

b) Gram negative bacterial strains- *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439).

c) Fungal strains- *Candida albicans* (MTCC 3017) and *Penicillium chrysogenum* (MTCC 947).

Strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference of bacterial strains were maintained on nutrient agar slants and fungal strains on PDA slants and stored in freeze. Strains were regularly sub-cultured using nutrient broth for bacterial strains and Potato Dextrose Broth for fungal strains.

Z. Test for bacterial strains

The Muller Hinton Agar Medium was prepared by dissolving 33.9 gm of the commercially available Muller Hinton Agar Medium (Hi-Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and 20 ml was poured in molten form onto each petriplates.

Nutrient broth was prepared by dissolving 13 gm of commercially available nutrient medium (Hi-Media) in 1000ml of distilled water and boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

The Petri-plates containing 20 ml Muller Hinton medium were seeded with 24 hr cultured bacterial strains. Wells were prepared using 6 mm of borer and 20 µl of the sample extracts were added into the wells. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993).

The resulting Zone of Inhibition (ZOI) will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres (mm).

AA. Test for fungal strains

The fungicidal effect of the sample extracts was determined by the inhibition of mycelial growth of the fungus by the extracts which are generally recorded as a zone of inhibition near the wells.

39 gm of the commercially available potato dextrose agar medium (39gm) was dissolved in 1000 ml of distilled water and boiled to dissolve completely. The medium was autoclaved at 15 lbs pressure (121ºC) for 15 minutes to get the sterile media.

20ml of Potato Dextrose Agar medium was poured on to the petri-plates. A fungal plug was placed in the centre of the plate. Sample extracts were also placed in the well (6mm) of the plates. The antifungal effect was seen as crescent shaped Zones Of Inhibition (ZOI). The ZOI was measurd in millimeter (mm) (Schlumbaum *et al*., 1986).

III. RESULTS AND DISCUSSION

Table 1 presents the results of qualitative phytochemical tests of different aerial parts of *M. arvensis*. Presence of various phytochemicals in all the parts of the plant indicates their equal importance in medicinal practices. But, according to Malik *et al*., (2012) from Punjab, leaves are rich source of secondary metabolites than the roots and stem. Alkaloids, flavonoids, polyphenols, tannins, glycosides, cardiac glycosides, steroids, sugar, reducing sugar, gum, saponins, carbohydrate, phlobatannins, terpenoids, phenolic compounds are recorded in different parts of the plant from different places like-Bangladesh, Brazil, Delhi, Tamilnadu and Kashmir (Gupta *et al.,* 2010; Londonkar and Poddar, 2009; Nascimento *et al.,* 2009; Sohail *et al.,* 2016; Hanifa and Santhi, 2012; Pidugu and Arun, 2012; Dar *et al.,* 2014; Palshikar *et al.,* 2015; Sagadevan *et al.,* 2014; Sharma and Patel, 2017). The presence and absence of phytochemicals in the plant may be due to the method of extraction, age of the plant, location and season of collection of the plant samples. According to Kochhar, (1981) the concentration of active compounds of plants is more in storage organs like- leaves, roots, seeds, bark.

The quantitative analysis was performed for total phenol and flavonoid content of different parts on *M. arvensis* and the results are presented in Table 2. The extraction of phenol and flavonoid by the solvents from inflorescence is better than leaves and stem. Malik *et al*., (2012) recorded 3.51±0.1% phenol and 22.86±0.3% flavonoids in leaves and 1.55±0.1% phenol and 19.9±0.16% flavonoids in stem of the plant. Garg *et al.,* (2012) from Maharashtra recorded total phenolic and flavonoid content of methanolic extract (5.13±0.25mg/g and 1.57±0.13mg/g) and water extracts (3.13±0.25mg/g and 1.94±0.33mg/g) respectively. Dar *et al.,* (2014) showed that the plant have significant quantity of phenol and flavonoids content. Ramasubramania, (2015) recorded total flavonoid and phenol content in hydro-alcoholic extract as 479±66 % and 936±71% respectively. The experiment suggested that extraction efficiency of the solvents is may be due to the phytochemicals present in the plants. In the present study, the better extraction from inflorescence is may be due to the essential oils present in inflorescence of the plant.

Table 3 presents the results of antioxidant activity study of the sample extracts of *M. arvensis*. The antioxidant inhibition of leaf extract is more than the extracts from inflorescence and stem. Biswas *et al*., (2014) showed IC_{50}=41µg/ml of extract which was compared to ascorbic acid having IC_{50}=19 µg/ml. Garg *et al.,* (2012) recorded antioxidant activities of methanolic and aqueous extracts of leaves of the plant as 16.4±0.37 mg/g and 1.42±0.69mg/g against DPPH respectively. Kowti *et al.,* (2010) recorded that ethanol extract have inhibition of around 82% at 100µg/ml of concentration against DPPH. IC_{50} value was recorded as 46 µg/ml as compared to standard gallic acid having 90% of inhibition at 10 µg/ml of concentration. Ramasubramania (2015), recorded antioxidant potential of the plant extracts against DPPH as 92.8%. The more antioxidant activity recorded by leaves may be due to the more phytochemicals present in the leaves.

The results of antimicrobial activity study of the sample extracts of *M. arvensis* are presented in Table 4. Ethanol extract of mature leaves recorded highest (18.2±2.4mm) activity against *S. aureus* than the other extracts of the plant. Acetone extract of young and mature leaves recorded inhibition against fungi *P. crysogenum*. All the extracts from inflorescence and stem did not recorded activity against *E. coli*. Water extract of inflorescence and stem did not recorded activity against all the tested organisms. Extracts from all the parts did not have potential antimicrobial activity against *P. vulgaris* and the fungal strain *C. albicans.* Kumbalwar *et al*., (2014) examined the antimicrobial activity of various extracts of leaves against some medically important gram negative bacteria collected from Nagpur, Maharashtra. Agar well diffusion method showed that aqueous extract showed 6mm of ZOI against *E. coli* which was lower than other solvent extracts and strains. Biswas *et al.,* (2014) showed that extracts did not show any activity against *E. coli*, but its inhibition against *S. aureus* were 8mm and 17mm at the dose of 250 µg/disc and 500 µg/disc respectively which was compared to the standard dry Kanamycin, having ZOI of 22mm at the dose of 30 µg/disc. Nascimento *et al.,* (2009) also showed antimicrobial activity of the ethanolic leaf extracts at different concentrations (%) like- 10, 5, 2.5, 1.25, 0.6 and 0.3 and found Zone of inhibition as 12±0.1mm, 11±0.3mm, 10±0.7mm, 9±0.8mm, 8±0.1mm, 7±0.1mm against *E. coli* and 21±0.7mm,

19±0.8mm, 17±0.5mm, 13±0.4mm, 12±0.7mm, 9±0.7mm against *S. aureus*. The ZOI were compared to tetracycline (22±0.1mm) and chloramphenicol (18±0.5mm) against *S. aureus* and chloramphenicol (9±0.4mm) against *E. coli*. Palshikar *et al.*, (2015) recorded antibacterial inhibition of water, methanol and acetone extract against *S. aureus* and *E. coli* and found Zone of inhibition as, 8±0.22mm, 15±0.55mm, 17±0.22mm, 15±0.55mm and 16±0.22mm, 15±0.55mm. Sagadevan *et al.*, (2014) recorded antibacterial activity against *B. subtilis*, *E. coli*, *P. vulgaris* and *S. aureus* were 18±0.38mm, 13±0.03mm, 11±0.03mm and 19±0.06mm respectively in comparison with Ampicillin (18±0.57mm, 14±0.03mm, 12±0.89mm and 19±0.13mm respectively). The difference in antimicrobial activities of different solvent extracts is may be due to the phytochemicals responsible for the antimicrobial activities of the plant. The extraction method may be the another reason for these differences in antimicrobial activity.

Table 1: Qualitative phytochemical analysis of different parts of Mentha arvensis L.

Sample	Tannins	Phlobatannins	Flavonoids	Terpenoids	Steroids	Glycosides	Cardiac Glycosides	Saponins	Anthraquinones	Free Anthraquinones	Carotenoids	Alkaloids	Reducing Sugar	Phenols
Young Leaf	+	-	+	+	+	+	+	+	-	-	+	+	+	+
Mature Leaf	+	-	+	+	+	+	+	+	-	-	+	+	+	+
Inflorescence	+	-	+	+	+	+	+	+	-	-	+	+	+	+
Stem	+	-	+	+	+	+	+	+	-	-	+	+	+	+

Table 2. Quantitative estimation for total phenol and total flavonoid content of sample extracts of different parts of Mentha arvensis L.

Sample (mg/ml) ↓	Total phenol content (mg CE/gm dry extract)					Total flavonoid content(mg QE/gm dry extract)				
	Water Extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract	Water Extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract
Young leaf	1.18 ±0.01	1.27 ±0.14	1.17 ±0.00	2.09 ±0.00	1.22 ±0.09	1.00 ±0.00	1.50 ±0.53	1.33 ±0.01	2.01 ±0.00	1.31 ±0.00
Mature leaf	1.33 ±0.00	1.36 ±0.12	1.23 ±0.04	5.36 ±0.21	1.26 ±0.00	0.88 ±0.02	1.69 ±0.0	1.41 ±0.00	5.58 ±0.12	1.01 ±0.01
Inflorescence	2.98 ±0.01	3.19 ±0.23	3.01 ±0.03	4.00 ±0.00	2.01 ±0.02	1.98 ±0.89	1.34 ±0.00	1.34 ±0.00	2.04 ±0.44	1.00 ±0.77
Stem	1.22 ±0.50	1.10 ±0.00	1.88 ±0.00	1.23 ±0.01	1.01 ±0.00	0.99 ±0.00	1.99 ±0.07	1.00 ±0.00	1.40 ±0.09	0.96 ±0.00

Table 3. Antioxidant activity study of the sample extracts of different parts of Mentha arvensis L.

Sample (500µl)	DPPH radical scavenging activity (% inhibition in mg/ml)					ABTS radical scavenging activity (% inhibition in mg/ml)				
	Water Extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract	Water Extract	Methanol extract	Ethanol extract	Acetone extract	Petroleum ether extract
Young Leaf	72.76 ±1.87	72.78 ±0.00	71.37 ±0.17	69.09 ±0.00	50.00 ±0.00	81.74 ±0.51	79.00 ±0.00	77.00 ±0.00	70.01 ±0.00	59.93 ±3.57
Mature Leaf	78.61 ±2.11	78.27 ±0.00	74.01 ±0.00	75.58 ±0.94	57.72 ±3.04	85.93 ±0.42	49.19 ±5.67	55.62 ±0.15	62.93 ±1.11	77.69 ±3.69
Inflorescence	67.56 ±0.03	60.00 ±0.22	60.67 ±0.23	57.09 ±0.00	60.09 ±0.34	75.98 ±0.00	76.99 ±0.09	74.00 ±0.00	70.00 ±1.00	70.04 ±0.00

Stem	56.98 ±0.04	55.56 ±0.00	59.00 ±0.00	49.76 ±0.02	52.34 ±0.00	49.87 ±0.99	44.56 ±0.00	46.10 ±0.00	40.00 ±0.33	41.89 ±0.00
Ascorbic acid	90.28 ±0.02					89.00 ±0.00				

Table 4. Antimicrobial activity study of the sample extracts of different parts of Mentha arvensis L.

Sample	**Extracts (mg/ml)**	**Diameter of Zone of Inhibition (mm)**								
		Bacterial strains							**Fungal strains**	
		B. subtilis	***B. cereus***	***S. aureus***	***S. epidermis***	***E.coli***	***E. faecalis***	***P. vulgaris***	***C. albicans***	***P. crysogenum***
Young Leaf	Water Extracts	-	-	8±0	8±0	8±0	10±2	-	-	-
	Methanol Extract	16±1	8±0	8±0	8±0	8±1	8±1	-	-	-
	Ethanol Extract	8±1	8±0	-	-	8±1	8±1	-	-	-
	Acetone Extract	10±1	-	10±2	-	10±0	12±1	-	-	16±1
	Petroleum Ether Extract	12±2	8±0	11.6 ±2.1	-	10±0	14±2	8±0	-	-
	Hot Petroleum Ether extract	10±1	-	12±0	-	-	12±1	-	-	-
Mature Leaf	Water Extracts	8±0	-	10±0	8±1	8±1	8±1	-	-	-
	Methanol Extract	8±0	8±0	10±1	8±1	-	-	-	-	-
	Ethanol Extract	8±0	8±0	18.2 ±2.4	9±1	8±0	8±1	-	-	-
	Acetone Extract	-	-	-	-	-	-	-	-	10±2
	Petroleum Ether Extract	8±0	-	8±0	-	-	8±0	-	-	-
	Hot Petroleum Ether extract	-	-	12±0	-	-	-	-	-	-
Inflorescence	Water Extracts	-	-	-	-	-	-	-	-	-
	Methanol Extract	10±1	8±0	15±1	8±0	-	8±1	-	-	-
	Ethanol Extract	8±0	10±1	8±0	-	-	8±1	-	-	-
	Acetone Extract	-	8±0	10±0	-	-	8±1	-	-	-
	Petroleum Ether Extract	-	8±1	8±1	8±0	-	8±1	-	-	-
	Hot Petroleum Ether extract	10±0	12±2	-	-	-	-	8±0	-	-
Stem	Water Extracts	-	-	-	-	-	-	-	-	-
	Methanol Extract	-	8±1	9±1	-	-	8±0	-	-	-
	Ethanol Extract	-	-	-	-	-	-	-	-	-
	Acetone Extract	9.3 ±1.3	8±1	8±1	-	-	-	-	-	-
	Petroleum Ether Extract	-	-	8±1	-	-	-	-	-	-
	Hot Petroleum Ether extract	8±0	8±0	22±2	8±1	-	-	8±0	8±0	-

(-) signifies no inhibition

IV. STATISTICAL ANALYSIS

All the experiments were done in triplicate and mean and SD was calculated and are presented in ± form.

CONCLUSION:

The study revealed that some of the phytochemicals present in different parts of the plant is may be responsible for its

medicinal properties. Total phenol and flavonoid content, antioxidant and antimicrobial activity of the various extracts of different aerial parts of the plant signifies its potential use in traditional medicines. The study further indicates that instead of using whole plant together, different parts can be used separately in medicinal practices, for various ailments and the practice may be helpful in sustainable management of this valuable plant family.

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