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ANTIMICROBIAL ASSAY AND PHYTOCHEMICAL ANALYSIS OF *MENTHA VIRIDIS* (L.)L., FROM DIBRUGARH, ASSAM

Junali Chetia* and L. R. Saikia

Department of life sciences, Dibrugarh University, Dibrugarh, Assam, India

**Corresponding author: junali.chetia@yahoo.com*

ABSTRACT

The study was conducted to determine the antimicrobial, antioxidant and phytochemical properties of *Mentha viridis* (L.)L. collected from Dibrugarh, Assam. All the parts tested recorded presence of phytochemicals like- tannins, flavonoids, terpenoids, steroids, glycosides, cardiac glycosides, saponins, carotenoids, alkaloids, reducing sugar and phenol. Methanol extract of mature leaves recorded highest total phenol and flavonoid content (12.02±0.00 mgCE/g extract and 9.59±0.00 mgQE/g extract respectively). Methanol extract of young leaves recorded highest antioxidant inhibition against ABTS (81.62±1.43%). Solvent extract of young leaves recorded antibacterial inhibition against both gram positive and gram negative bacteria like-*Bacillus subtilis, B. cereus, Staphylloccus aureus* and *Escherichia coli.* Methanol extract of mature leaves recorded inhibition against *Candida albicans* (10±2mm) which is near to the inhibition recorded by Clotrimazole (10mcg)(11±2mm) against *C. albicans*. From the present study, it can be concluded that the methanol extract is more potent in extracting phytochemicals from the plant which are responsible for the antioxidant and antimicrobial activity of the plant.

Keywords: Antimicrobial, Antioxidant, Phytochemical

1. INTRODUCTION

Mentha viridis (L.)L. is locally known as bon pudina. A small perennial prostrate herb having aromatic leaves cultivated to extract spearmint oil used in confectionary, flavouring candies, toothpaste, chewing gums and dried plant is used medicinally as stimulant, carminative and in fever, bronchitis [1]. It is mostly cultivated, but also grows wild in some moist soils. It is used traditionally as a relaxant, antispasmodic, and soothing agent in nausea and vomiting, allays colics in children, fever, and stomach disorder, wound healer, anti-infectious, anti-flatulence and anti-inflammatory, viral hepatitis, colitis, gastric acidity, aerophagia and also stimulates the digestion antiemetic, carminative, restorative and stimulant itching skin conditions [2-8]. Various authors have carried out various experiments on various aspects of the plant [9-12].

Owing to the importance of the plant of Lamiaceae family, the present study proposes in-vitro screening of the plants selected on the basis of its ethno-medicinal importance. The main aim of the work is to find out the pharmaceutical prospective of the selected species with the following objectives:

To perform qualitative and quantitative phytochemical analysis, antioxidant activity and antimicrobial assay of the selected plants.

2. MATERIALS AND METHODS

2.1. Collection of plant sample

The collected flowering branches were brought to the laboratory. Different parts 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 allowed to sun dried 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 was kept in air tight bottles for further analysis.

2.2. 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 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.

2.3. 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 [13]; Wagner *et al.* [14]; Siddiqui and Ali [15]; Trease and Evans [16]; Edeoga *et al*. [17]; Egwaikhide and Gimba [18]; Chitravadivu [19]; Majaw and Moirangthem [20]; Aja *et al*. [21]; De *et al*. [22]; Ajayi *et al.* [23]; Ajiboye *et al.* [24] as laid down below:

2.3.1. Tests for Tannins

a) About 0.5 gm of the air dried powder was boiled in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for green or blue black colouration which indicates the presence of tannin.

b) 5 ml of water filtrate + 10% lead acetate solution, showed white precipitate, which indicates the presence of tannin.

2.3.2. Test for Phlobatannins (HCL test)

a) Deposition of a red precipitate when an aqueous filtrate was boiled with 10% aqueous HCl, was taken as evidence for the presence of phlobatannin.

b) The water filtrate was boiled with 2% HCl Solution. Red precipitation showed the presence of phlobatannin.

2.3.3. Test for Flavonoides

a) 0.5 gm of powder was heated with 10 ml of ethyl acetate over a water bath for 3 mins. The mixture was filtered. 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed.

b) Alkaline reagent test: Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

2.3.4. Test for Saponins (Frothing test)

a) Small quantity of water filtrate was shaken vigorously, formation of one centimetre layer of foam, which was stable for 10 mins indicates the presence of saponins.

b) 0.5 gm of powder was shaken with water in a test tube and warmed in a water bath, persistent of froth indicates the presence of saponins.

2.3.5. Test for Alkaloids

0.5 gm of powdered sample was boiled separately with 5ml of water and 5 ml of HCl on water bath and filtered. The P^H of the filtrate was adjusted with ammonia in between 6-7. A very small quantity of following reagents were added separately 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.

2.3.6. Test for Glycosides

a) Liebermann's test: Crude extract was mixed with 2ml of chloroform and 2ml of glacial acetic acid. The mixture was cooled in ice. Concentrated H_2SO_4 was added carefully. A colour change from violet to blue to green indicated the presence of glycoside.

b) 2 g of powdered sample was mixed with 10 ml of distilled water and heated for 10 min on heater. The mixture was filtered and Fehling solution (A+B) was added until it turns alkaline and heated over heater. A brick red precipitate confirmed the presence of glycoside.

2.3.7. Test for Cardiac Glycosides

Keller killani test: 5 ml water filtrate was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of conc. H_2SO_4. A brown ring at the interface indicates the presence of cardiac glycoside. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout this layer.

2.3.8. Test for Terpenoids

Salkowski test: 5 ml of water filtrate was mixed with 2 ml of chloroform and conc. H_2SO_4 of about 3 ml was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

2.3.9. Test for Steroids

The water filtrate was treated with chloroform and a few drops of conc. H_2SO_4 was mixed, shake well the solution and allow the solution standing for some time. Red colour appeared at the lower layer indicate the presence of steroids.

2.3.10. Test for Anthraquinone

0.5 gm powdered sample was shaken with 5 ml of benzene and filtered. 0.5 ml of 25% ammonia solution was added to the filtrate and the mixture was shaken well. Presence of the violet colour in the layer phase indicate the presence of anthraquinone.

2.3.11. Test for Free Anthraquinone

0.5 gm powdered sample was mixed with 5 ml of chloroform, shaken for 5 mins and filtered. The filtrate was shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicates the presence of free anthraquinone.

2.3.12. Test for Phenol

5ml of the water filtrate was mixed with 10ml of distilled water, 2ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol and left for 30min. Bluish green colour was developed in the solution which indicates the presence of phenol.

2.3.13. Test for Carotenoids

0.5 gm of powdered sample was mixed with 5 ml of chloroform in a test tube with vigorous shaking. Then the mixture was filtered and 3ml of 98% H_2SO_4 was added. A blue colour at the interface showed the presence of carotenoids.

2.3.14. Test for reducing sugar

0.5 gm of the powdered sample was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling solution (A+B) for few minutes. An orange red precipitate indicates the presence of reducing sugar.

2.4. Quantitative Phytochemical Analysis

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

2.4.1. Determination of Total Phenol Content (TPC)

Total phenol content (TPC) of the sample extract was estimated following the method described by Malik and Singh [25]. 0.2 ml of the extract solution at 1mg/ml of concentration were taken in a 10 ml test tube and made up to a volume of 3ml with distilled water. Then 0.5 ml Folin-ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. The tubes with solution were warmed for 1 min. and then cooled. A blue colour was developed and absorbancy was measured at 760 nm.

2.4.1.1. Preparation of standard curve

A standard calibration plot was generated at 760 nm using known concentrations of Catechol. The total phenol content in extracts was expressed in terms of Catechol Equivalent (mg CE/g extract).

2.4.1.2. Preparation of standard sample

10mg catechol was dissolved in 100ml distilled water. 0.2, 0.4, 0.6, 0.8 and 1 ml of the standard sample in triplicate were pipetted out in test tubes. The volume was made upto 3 ml in all the test tubes using distilled water. A tube with 3ml of distilled water served as the blank. 0.5 ml of Folin-ciocalteau reagent (1:1 with water) was added to each test tube including the blank and shaken. After 30 mins, 2ml Na_2CO_3 (20%) was added. After 1min of warming, absorbency was taken at 760nm. A standard graph was drown. The concentrations of phenols in the test sample were calculated from the calibration plot and expressed as mg catechol equivalent /g dry extract (standard plot: $Y=0.318X$, $R^2=0.995$).

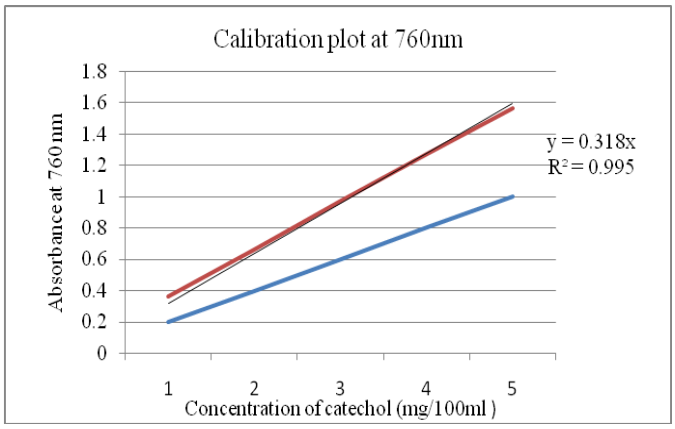

Fig. 1: Total phenol content calibration curve of Catechol

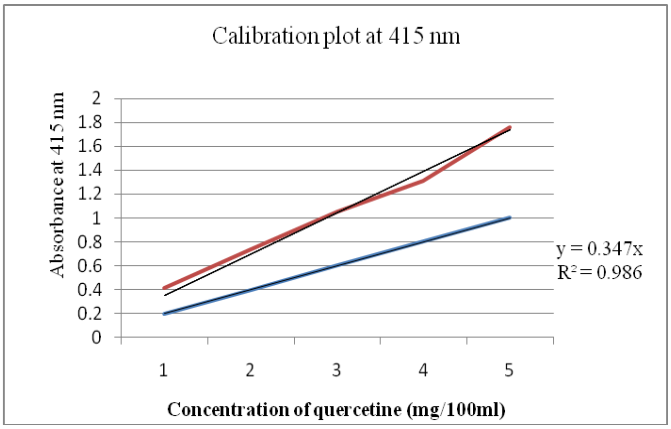

Fig. 2: Total flavonoid content calibration curve of Quercetin

2.4.2. 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 [26]. 0.2 ml of extract solutions were taken in test tubes and made up the volume 3 ml with methanol. Then 0.1 ml $AlCl_3$ (10%), 0.1 ml sodium potassium tartarate and 2.8 ml distilled water was added and shaken vigorously. Absorbancy at 415 nm was recorded after 30 mins of incubation.

2.4.2.1. Preparation of standard curve

A standard calibration plot was generated at 415 nm using known concentration of Quercetin. The total flavonoid content in extracts was expressed in terms of Quercetin Equivalent (mg CE/g extract).

2.4.2.2. Preparation of standard sample

10mg of Quercetin was mixed with 10ml of methanol. 0.2, 0.4, 0.6, 0.8 and 1 ml of the standard sample were pipette out in triplicate in test tubes. The volume was made 3 ml in all the test tubes using methanol. A tube with 3ml methanol served as the blank. 0.1 ml of $AlCl_3$ to each tube including the blank was added and shaken. 0.1 ml of sodium potassium tartarate to each tube including the blank was added and shakeden. 2.8 ml of distilled water was added to each tube including the blank and shaken. After 30 mins of incubation absorbency was taken at 415nm. A standard graph was drawn and counteraction of flavonoid in the test sample were calculated from the calibration plot and expressed as mg quercetin equivalent/g dry extract (Standard Plot: Y=0.347X, R^2=0.986).

2.5. 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.

2.5.1. 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.*[27] Antioxidants react with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical and convert it to 1,1-diphenyl-2-picryl hydrazine. The degree of change in colour from purple to yellow can be used as a measure of the scavenging potential of the extracts. 0.5ml of extract solutions (1mg/ml) were taken and made up the volume to 3ml with methanol. 0.15ml of freshly prepared DPPH solution was added, stirred and left to stand at room temperature for 30 minutes in dark. The control contains only DPPH solution in methanol instead of sample while methanol served as the blank (negative control). Absorbancy was recorded at 517 nm using UV-Vis spectrophotometer. A constant amount (0.5ml) of sample was treated using 0.5 ml of methanol against DPPH.

The capacity of scavenging free radicals was calculated as 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/standard.

Ascorbic acid was used as standard sample and the solution was prepared by mixing 10mg ascorbic acid

in 10 ml methanol. Then 0.5ml of this solution was taken in test tube and made up the volume to 3ml with methanol. 0.15ml freshly prepared DPPH solution was added to it, stirred, and left to stand at room temperature for 30mins in dark. The control contains only DPPH solution in methanol instead of sample, while methanol served as the blank (negative control). Absorbancy was recorded at 517 nm using UV-Vis spectrophotometer.

2.5.2. 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*.[28] The stock solution included 7 mM ABTS solution and 2.4 mM potassium persulfate solution and mixed them in equal proportion, then allowed to react for 12 hrs at room temperature in dark. Mixed 1 ml solution with 60 ml methanol to obtain an absorbancy of 0.706 + 0.001 units at 734 nm using the UV-Vis spectrophotometer. The ABTS solution was prepared fresh 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.

Ascorbic acid was used as standard sample and the solution was prepared by mixing 10mg ascorbic acid in 10 ml methanol. 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.

2.6. 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.* [29] using 6mm borer. The intensity of the activity was determined by measuring the diameter of the zone of inhibition (ZOI).

Gram positive and gram negative bacterial strains and fungal strains were 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.

For bacterial strains standard antibiotics viz, Chloramphenicol (C) 30mcg, Tobramycin (TOB) 10mcg, Clotrimazole (CC) 10mcg, Ampicillin (AP) 10mcg, Streptomycitin (ST) 10mcg, Imipenem (IPM) 10mcg, Ciprofloxacin (CI) 30mcg, Streptomycin (S) 25mcg, Gentamycin (GEN) 30mcg, Erythromycin (E) 15mcg, Co-trimaxazole (COT) 25mcg and for fungal strains, Nystatin (NS) 50mcg, Clotrimazole (CC) 10mcg, Ampicillin (AP) 10mcg were employed as standard for comparision of ZOI with sample.

2.6.1. 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 poured onto 100mm petriplates (25-30ml/plate) while still molten.

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

2.6.2. Working Procedure

Petri-plates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains.

Wells were cut using 6mm of borer and 20 µl of the sample extracts were added. The samples were allowed to diffuse out into the medium on the petri-plates and interact freshly seeded with the test organisms. 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) [30].

The resulting 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).

2.6.3. Test for fungal strains

The fungicidal effect of the sample extracts can be assessed by the inhibition of mycelial growth of the fungus by the extracts which is generally recorded and as a zone of inhibition near the disc or the wells.

2.6.3.1. Preparation of media

The commercially available potato dextrose agar medium (39gm) was suspended in 1000ml of distilled water. The medium was dissolved completely by boiling and was then autoclaved at 15 lbs pressure (121°C) for 15 minutes.

2.6.3.2. Working procedure

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 of the plates. The antifungal effect was seen as crescent shaped zones of inhibition [31].

2.7. Statistical analysis

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

3. RESULTS AND DISCUSSION

Table 1 presents the results of qualitative phytochemical tests of different parts of *M. viridis*. Tannins, flavonoids, terpenoids, steroids, glycosides, cardiac glycosides, saponins, carotenoids, alkaloids, reducing sugar, phenols are recorded in the plant parts. Singh *et al*. [32]; Naidu *et al*. [33]; Soni and Sora [34]; Aziz *et al*. [35]; Zaidi and Dahiya [36] also showed the presence of various phytochemicals in the plant collected from various places. In the present study, it is recorded that some of the phytochemicals are absent in some parts of the plants. Variation in phytochemicals in different parts of plant is may be due to the microclimate and soil condition of habitat area of the plants.

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

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

The quantitative analysis was performed for total phenol and flavonoid content of different parts of *M. viridis* and the results are presented in Table 2. Water and methanol showed better extraction of phenol and flavonoid content than ethanol, acetone and petroleum ether. Water and methanol extract of mature leaves (4.57±0.98 mgCE/g extract and 12.02±0.00 mgCE/g extract respectively) recorded higher amount of phenol content. Water and methanol extract of mature leaves (2.98±0.07mgQE/g extract and 9.59±0.00 mgQE/g extract) and acetone extract of young leaves (3.49±0.07 mgQE/g extract and 3.80±0.00 mgQE/g extract respectively) recorded higher flavonoid content than other extracts of the plant. Zaidi and Dahiya [36] showed that 5mg of oil contain 9.41±0.594 µg Gallic Acid Equivalent of phenol content. Methanolic leaf extracts contains 18.41% of phenolic content [34]. Extraction efficiency of phenol and flavonoid content by different solvents is may be due to the phytochemicals present in the plant samples. It is necessary to select different solvents for extraction of phytochemicals and other active compounds which are responsible for antioxidant and antimicrobial activities of a plant.

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

Sample (mg/ml) ↓	Total phenol content (mg catechol equivalent/gm dry extract)					Total flavonoid content (mg quercetin equivalent/gm dry extract)				
	WE	ME	EE	AE	PEE	WE	ME	EE	AE	PEE
Young leaf	2.00±0.00	2.10±0.00	1.15±0.00	1.86±0.00	1.93±0.00	1.45±0.00	2.59±0.00	1.26±0.00	3.49±0.07	2.34±0.00
Mature leaf	4.57±0.98	12.02±0.00	1.13±0.12	1.44±0.06	1.35±0.25	2.98±0.07	9.59±0.00	1.03±0.00	3.80±0.00	1.15±0.00
Stem	2.70±0.00	2.86±0.00	2.00±0.00	1.49±0.00	1.00±0.35	1.00 ±0.00	1.32±0.00	1.00±0.98	1.09±0.00	1.45±0.00

Table 3: Anti-oxidant activity study of sample extracts of different parts of *Mentha viridis* L.

Sample (500µl) ↓	DPPH radical scavenging activity (% inhibition in mg/ml)					ABTS radical scavenging activity (% inhibition in mg/ml)				
	WE	ME	EE	AE	PEE	WE	ME	EE	AE	PEE
Young Leaf	50.00±0.00	48.13±0.00	41.67±0.00	66.56±0.00	43.17±0.00	76.76±0.00	81.62±1.43	79.96±0.20	79.98±0.00	77.23±0.79
Mature Leaf	50.18±0.01	36.18±0.00	42.39±0.00	68.91±0.01	39.12±0.00	75.34±0.00	76.22±2.44	66.85±0.00	69.98±0.00	52.16±2.03
Stem	43.00±0.00	34.45±0.03	38.98±0.01	38.00±0.00	30.01±0.09	65.00±0.01	70.19±0.00	65.23±0.04	71.01±0.09	61.03±0.23
Ascorbic acid	90.28 ±0.02					89.00 ±0.00				

Table 4: Antimicrobial activity study of sample extracts of different parts of *M. viridis* 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	-	-	-	-	-	-	-	-	-
	Methanol Extract	11±2	10±1	-	-	12±2	-	-	-	-
	Ethanol Extract	8±2	-	-	-	-	-	14±2	-	-
	Acetone Extract	12±0	8±2	10±0	10±1	8±1	8±1	-	-	-
	Petroleum Ether Extract	10±0	10±4	18.4±0.4	-	12±0	-	-	-	-
	Hot Petroleum Ether Extract	8±1	8±2	10±0	-	-	-	-	-	-
Mature Leaf	Water Extracts	-	-	-	-	-	-	-	-	-
	Methanol Extract	-	9±2	8±2		-			10±2	-
	Ethanol Extract	-	-	-	-	12±0	10.6±1.6	8±2	-	-
	Acetone Extract	-	8±0	8±2	-	8±0	8±1	-	-	-
	Petroleum Ether Extract	-	-	-	-	-	-	-	-	-
	Hot Petroleum Ether Extract	-	-	-	-	-	-	-	-	-
Stem	Water Extracts	9±2	8±1	-	-	-	-	-	-	-
	Methanol Extract	-	8±1	8±0	8±2	-	-	-	-	-
	Ethanol Extract	8±1	8±1	11.9±1.2	8±0	8±0	-	-	-	-
	Acetone Extract	8±0	10±0	10±2	10±1	8±0	8±0	8±0	-	-
	Petroleum Ether Extract	-	-	-	-	-	-	-	-	-
	Hot Petroleum Ether Extract	-	-	-	-	-	-	-	-	-

Table 3 presents the results of antioxidant activity study of the sample extracts of *M. viridis*. The extracts from all the parts are more active against ABTS than DPPH. Acetone extract of mature leaves and methanol extract of mature leaves recorded the highest (68.91±0.01% and 81.62±1.43% respectively) antioxidant activity against DPPH and ABTS, than the other extracts of the plant. In case of ABTS, water extract of young (76.76±0.00%) and mature leaves (75.34±0.00%) leaves; methanol

extract of mature leaves (76.22±2.44%) and stem (70.19±0.00%); ethanol extract of young leaves (79.96±0.20%); acetone extract of young leaves (79.98±0.00%) and petroleum ether extract of young leaves (77.23±0.79%) recorded higher inhibition at a constant (500µl) of sample at 1mg/ml of concentration. Soni and Sora [34] carried out antioxidant activity of methanolic extract of dried leaves of the plant. Antioxidant activity was determined by DPPH radical scavenging and reducing power assays. IC_{50} values obtained by DPPH activity for crude extract was found to be 170µg/ml and reducing power was found to be maximum (1.92) at 1mg/ml concentration. The more antioxidant activity recorded by leaves is may be due to the phytochemicals present in the leaves of the plant, which is considered as storage organ of the plant.

Table 5: Zone of Inhibition (ZOI) of standard antibiotics for antibacterial and antifungal inhibition

Standard ↓	Diameter of inhibition of zone (mm)								
	B.subtilis	*B.cereus*	*S.aureus*	*S.epidermis*	*P.vulgaris*	*E.faecalis*	*E.coli*	*C. albicans*	*P. aysogenum*
Chloramphenicol(C) 30mcg	15±2	-	-	30±0	-	8±0	-		
Tobramycin(TOB) 10mcg	44±2	24±0	32±0	-	40±4	42±2	35±5		
Clotrimazole (CC) 10mcg	20±0	10±1	14±0	20±0	-	-	26±0		
Ampicillin(AP) 10mcg	-	-	-	-	12±2	10±1	10±0		
Streptomycitin (ST) 10mcg	18±0	-	10±1	-	-	10±0	12±0		
Imipenem(IPM) 10mcg	66±0	-	-	-	32±2	-	30±1		
Ciprofloxacin(CI) 30mcg	44±0	32±2	40±4	-	40±4	36±3	22±1		
Streptomycin(S) 25mcg	-	32±0	28±2	-	22±2	60±2	28±2		
Gentamycin(GEN) 30mcg	40±0	32±3	30±4	-	-	-	24±2		
Erythromycin(E) 15mcg	32±2	30±1	28±0	30±0	12±2	48±6	12±2		
Co-trimaxazole(COT) 25mcg	46±1	-	-	-	30±4	-	24±0		
Nystatin (NS) 50mcg								-	24±2
Clotrimazole(CC) 10mcg								11±2	32±0
Ampicillin(AP) 10mcg								-	46±0

'-' indicates no inhibition

The results of antimicrobial activity study of various sample extracts of *M. viridis* are presented in the Table 4 and the results are compared with standard antibiotics presented in Table 5. Petroleum ether extract of young leaves showed highest inhibition against *S. aureus* (18.4±0.4mm) which is more than the standard antibiotic Clotrimazole (10mcg) and Sreptomycitin (10mcg) having inhibition of 14±0mm and 10±1mm respectively against *S. aureus*. Acetone extract from all the tested parts showed comparatively good result than other extracts. Petroleum ether extract (both cold and hot) of mature leaves and stem did not show any activity against tested bacterial and fungal strains. Methanol extracts of all the parts recorded inhibition against *B. cereus* as 10±1mm, 9±2mm and 8±1mm respectively. Methanol extract of mature leaves showed activity against *C. albicans* (10±2mm), but other extracts did not recorded inhibition against fungal strains. Water extracts of both young and mature leaves did not recorded inhibition against all the tested organisms. Zaidi and Dahiya [36] recorded good inhibition by acetone extract of all the parts of the plant. They recorded antibacterial activity against *S. aureus* and *E. coli* as 21±0.09 mm and 14±0.05mm respectively. These differences in antimicrobial activities by various extracts of the plant parts are may be due to the phytochemicals present in that part of the plant.

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