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PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND ANTIOXIDANT POTENTIAL OF EXTRACTS OF *MYRICA ESCULENTA* LEAVES IN DIFFERENT SOLVENTS

B. Manners [*1] and P. J. Handique [2]

Department of Biotechnology [1], St. Edmund's College, Shillong - 793003, Sikkim, Gangktok, India.
Department of Biotechnology [2], Gauhati University, Guwahati - 781014, Assam, India.

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Correspondence to Author:
B. MANNERS

Department of Biotechnology, St. Edmund's College, Shillong - 793003, Sikkim, Gangktok, India.

E-mail: baiamanners@gmail.com

ABSTRACT: *Myrica esculenta* Buch - Ham. ex D. Don has been used worldwide for the treatment of various ailments. The present work is an attempt to relate the presence of phytochemicals in leaves of *M. esculenta* with its antibacterial potential using seven different solvents for preparation of extracts. The preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, carbohydrates, tannins, vitamin C, fats and oils, terpenoids, and phenols. The total phenolic and flavonoid content in the examined plant extract was found to be 97.66 ± 0.16 mg GA/g and 51.3 ± 0.32 mg RE/g of leaf extract, respectively. The maximum zone of inhibition was recorded by aqueous extract at 22.66 mm against *Clostridium perfringens* followed by 15.66 mm against *Shigella boydii*. On comparing the antibacterial effect of various extracts, the methanolic and ethanolic extracts are not significantly different from each other in terms of inhibitory effect against the microbes under study. All the bacterial strains were found to be sensitive to at least two extracts of the plant, but the ethanol and methanol extracts were comparatively more effective than the others. Antioxidant activity was measured by four in vitro antioxidant assays, that is, free radical scavenging ability by using ABTS radical cation (ABTS assay), DPPH radical cation (DPPH assay) ,FRAP assay, and detection of reducing power. The leaf extract of *M. esculenta* showed significant scavenging and reducing power when compared to the standard synthetic antioxidants.

INTRODUCTION: The medicinal value of plants is contributed by their bioactive phytochemical constituents [1]. Phytochemicals are called secondary metabolites as they are often less used by the plants that manufacture them. They are synthesized naturally by all parts of the plant body, such as leaves, stems, roots, bark, flowers, and seeds, *etc.*

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However, the quantity and quality of phytochemicals present in the plant parts may differ from one part to another [2]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [3].

Secondary metabolites have shown curative activity against several ailments in man, which thus explains the use of a wide variety of plants in traditional medicines [4]. This may be due to synergistic interactions of several secondary metabolites rather than single compounds [5]. Phyto chemical screening is used to detect the various bioactive compounds and thus may lead to its

further isolation, purification and characterization. Such leads may eventually benefit the pharmaceutical industry in the designing of novel drugs for the treatment of various human ailments.

The type of solvent used in the extraction procedure also determines the success of phyto-chemical screening. For the extraction of phyto-chemicals from different parts of plants, solvents of varying polarity can be chosen. This is because solvent having similar polarity to the solute of interest will dissolve the solute appropriately [6]. Myrica comprises about 97 species ranging from trees to shrubs belonging to the family Myricaceae. They are distributed in both temperate and subtropical regions globally [7]. *Myrica esculenta* Buch Ham ex D. Don commonly known as “Kaphal" is a popular, wild edible, and high potential fruit species [8, 9]. *M. esculenta* is a woody, evergreen, dioecious tree, medium to large in size, and is found in the Indian Himalayas [10]. The people of Meghalaya, even at present, still have faith in local medical practitioners and their herbal remedies [11]. Like many other medicinal plants, different parts of *M. esculenta* are utilized traditionally by the local medical practitioners, particularly for ailments such as dysentery, fever, diarrhoea, asthma , bronchitis, lung infections, and skin diseases [12, 13]. An estimated quantity of 2,554 kg raw drug is being consumed through Folk Healers in Meghalaya annually [14]. Studies on its biochemical constituents, particularly polyphenolic compounds from fruits, have been reported [15].

Although the presence of phytoconstituents such as flavonoids, terpenoids in the leaves of *Myrica esculenta* has been reported [16, 17]. The aim of the present study was to extract active phyto-constituents of *M. esculenta* leaves obtained from the state of Meghalaya, to screen for the presence of phyto-constituents, and to evaluate their antibacterial potential. Seven different solvents of varying polarity *viz.* acetone, chloroform, ethanol, hexane, methanol, petroleum ether, and water were used for the extraction. Thus the best solvent responsible for the extraction of maximum active phytoconstituents was determined, and the antibacterial efficacy of *M. esculenta* leaves was also established. Hence in the present study, an effort was made to understand the relationship between the plant extract obtained using different solvents and their corresponding antimicrobial and also their antioxidant activities. Reports on the antimicrobial and antioxidant activity of leaf extracts have been reported; however, this study was limited to three different leaf extracts prepared [18]. Therefore the aim of this study was to use a wide range of solvents for extract preparation having varied polarities and thus establish the antibacterial activity of these extracts on seven different pathogenic bacteria. The antioxidant activity and the reducing power of the leaf extracts were also studied.

MATERIALS AND METHODS:

Collection of Plant Material: Fresh leaves of *Myrica esculenta* were collected from the East Khasi Hills district of Meghalaya, and specimen samples were identified and authenticated at Botanical Survey of India, Eastern Regional Circle Shillong, Meghalaya vide. Certificate No. BSI/ ERC/Tech//Identification/2017/79.

Sample Preparation: The leaves were washed and air-dried under shade for 8-10 days. After drying, the samples were cut into smaller pieces, ground into a fine powder, and stored in airtight bottles before analysis.

Preparation of Plant Extract: About 10 gm of the dried leaf powder was extracted with 100 ml of hot solvents of increasing polarity such as ethanol, acetone, hexane, methanol, petroleum ether, chloroform, and water for 9-12 h with each solvent, using the Soxhlet apparatus at a temperature of 30 to 35 °C. Each time before extracting with the next solvent, the powdered material was air-dried below 50 °C and then subjected to further extraction. All the solvent-extracted fractions were subjected to desiccation at 40 °C in a rotary vacuum evaporator in vacuo so as to remove any traces of solvents and to yield residues. The concentrate was further evaporated to dryness using a water bath at 50 °C. For the aqueous extraction, 50 g of the plant powder was weighed, and to this was added 400 ml of distilled water and heated. The mixture was stirred at regular intervals (3-5 min) for one hour after which it was filtered using Whatman filter paper. The filtrate was then filtered sterilized using a membrane filter of pore size 0.45 cm diameter (millipore corp, England). The extracts were concentrated in a hot water bath at 80 ºC for 5 h

during which 0.5 g charcoal was added to decolorize it. All the extracts were stored in sterile glass bottles at 4 °C until screened.

Preliminary Phytochemical Screening: The condensed extracts were used for preliminary screening of phytochemicals such as carbohydrate, alkaloid, flavonoids, tannins, cardiac glycosides, anthraquinone glycosides, terpenoids, phenols, amino acids, fats and oils, and vitamin C as described by Harborne 1998 [19].

Screening for Antibacterial Activity (Agar Well Diffusion Assay): *In-vitro* antibacterial activity of all seven extracts was performed by standard agar well diffusion method [20]. The microbial strains used were *Staphylococcus aureus*, *Clostridium tetani*, *Clostridium perfringens*, *Bacillus cereus*, *Klebsiella pneumonia*, *E. coli,* and *Shigella boydii* obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial isolates were maintained on nutrient agar slants at 4 °C and sub-cultured onto nutrient broth for 24 h prior to the activity screening.

The respective bacterial strains were adjusted to a turbidity equivalent to 0.5 McFarland standards. (0.2 ml culture of the organism was dispensed into 20ml sterile nutrient broth and incubated for 24 h and standardized at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm. The Mueller Hinton agar was inoculated with 100 µl of the inoculum (10^6 CFU/ml) using the spread plate technique. Wells were prepared on the plates with the help of a cork-borer. Different extracts were dissolved in 100% DMSO at a concentration of 20 mg/ml, from this 50 µl of different extracts were added into the sterile 6 mm diameter well. The plates were incubated overnight at 37 ºC. For each bacterial strain, Gentamicin and Kanamycin for Gram-positive and Gram-negative bacteria respectively, were used as a positive control. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well in millimeters.

Statistical Analysis: The experiment was done in triplicate, and the values were expressed as average. The data were subjected to analysis of variance (ANOVA) using SPSS software. Means of three observations were compared with Duncan's Multiple Range Test (DMRT) at p 0 <05 for determining the statistical significance.

Total Phenolic: The desired concentration of methanolic extract (0.5 mL) was mixed with 0.5 mL of 10% Folin Ciocalteu reagent. To this, 2.5 mL of 7.5% Na_2CO_3 was added. The mixture was stored at room temperature for 45 min, and absorbance was measured at 765 nm on a UV spectrophotometer (Eppendorf). The assay was performed in triplicates. Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid. The phenolic content was expressed as a milligram equivalent of gallic acid per gram dry weight of sample [21].

Total Flavonoid: The total flavonoid content of the methanolic extract was determined by aluminium chloride colorimetric method with slight modification. In this assay, 1 mL of 10% (w/v) aluminium chloride was added to 1 ml of methanolic extract of *M. esculenta* The absorbance of the resulting reaction mixture was measured at 415 nm in a UV VIS spectrophotometer (Eppendorf) after incubation at room temperature for 1 h. A calibration curve was prepared with rutin as standard and the results were expressed as mg rutin equivalents (RE) per gram dry weight of sample [22].

Antioxidant activity assay: The antioxidant assay was carried out using four *in-vitro* assay techniques as described below.

ABTS Radical Cation Scavenging Assay: The ability of the test sample to scavenge ABTS radical cation was compared to standard. The working solution was prepared by mixing equal volumes of 7.4 mM ABTS and 2.6 mM potassium persulfate and allowing them to react at room temperature in the dark for 12 h. 1ml of this mixture was then diluted by mixing it with 60 ml methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm. This solution was always prepared fresh. 2850 µl of ABTS radical cation solution was mixed with 150 µl of the test sample (25-200 µg/ml), and the absorbance was measured at 734 nm after 2 h of incubation in the dark [23]. The percentage inhibition of absorbance was calculated and plotted as a

function of the concentration of DTE (1, 4-dithioerythritol) standard.

The inhibition percentage of ABTS was calculated using the following equation,

ABTS cation radical scavenging activity (%) = [(A blank - A] × 100

Where, A blank is an absorbance without extracts at 734 nm, and A 368 sample) / A sample blank is the absorbance of the test solution.

FRAP Assay: Ferric reducing antioxidant potential (FRAP) of the methanolic extract of *M. esculenta* and ascorbic acid standard was measured according to the method proposed by Benzie and Strain with slight modification [24]. FRAP reagent comprising of 25 mL acetate buffer (30 mM; pH 3.6), 2.5 mL TPTZ solution (10 mM), and 2.5 mL ferric chloride solution (20 mM) was prepared and incubated for 15 min at 37 °C before use.

To 2.85 mL FRAP reagent, 150 µL of the sample (0.1 mg/mL, in methanol) or standard was added. The mixture was incubated for 30 min, and its absorbance was measured at 593 nm. The blank contained an equal volume of methanol instead of the plant sample. The results were reported as µg of ascorbic acid equivalents (AAE) per mL. Ascorbic acid (vitamin C) was employed as a standard in this assay, and its calibration curve was obtained by using its concentrations ranging from 50 mg/L to 500 mg/L in water.

DPPH Radical Scavenging Activity: The DPPH free radical scavenging activity of methanolic, extracts of *M. esculenta* was determined according to the method reported by Karaman *et al.,* 2014 with slight modification [25]. The stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol. This solution was kept in a refrigerator until further use. The working solution was then prepared by diluting the DPPH stock solution with methanol to obtain an absorbance of about 0.98 ± 0.02 at 517 nm. For the assay, 3 mL DPPH working solution was mixed with 150 µL plant extract (1 mg/mL) or the standard solution. The absorbance was measured at 517 nm for a period of 30 min. The percent antioxidant or radical scavenging activity was calculated using the following formula:

% Antioxidant activity = [(Ac - As)/Ac] × 100

Reducing Power: The plant extract in varying concentrations was mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide. This mixture was kept at 50 ºC in a water bath for 20 min. Upon cooling 2.5 ml of 10% trichloro, acetic acid was added. The mixture was centrifuged at 2500 rpm for 15 min. To 1.5 ml of the supernatant, 2.5 ml of distilled water was added along with a freshly prepared 0.1% ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm [26]. The absorbance of the reaction mixture increased with increasing concentration which indicated an increased, reducing power.

RESULTS AND DISCUSSION:

Phytochemical screening: In the present study, the phytochemical screening and antibacterial activities were performed with seven different extracts of the leaf of *M. esculenta* such as Acetone, aqueous, chloroform, ethanol, hexane, methanol, and petroleum ether. The phytochemical analysis of all the studied extracts and results are given in **Table 1**.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF THE VARIOUS EXTRACTS OF M. ESCULENTA

Phytochemical	Solvent Used						
	Acetone	Chloroform	Ethanol	Hexane	Methanol	Petroleum Ether	Aqueous
1.Alkaloid							
a. Dragendroff	+	-	+	+	+	+	-
b. Wagner	+	-	+	-	+	-	-
c. Hager	+	-	+	+	_	+	-
2.Flavonoids							
a. Lead Acetate	+	+	+	+	+	+	-
b. Alkaline Reagent	+	+	+	+	+	+	+
3.Carbohydrate							
a. Fehlings	-	+	-	-	+	+	+
b. Seliwan off	+	+	+	+	+	-	+

c. Molish	+	-	+	+	+	+	+
4.Tannins							
a. FeCl3	+	-	-	-	+	-	-
b. Gelatin	-	-	+	-	+	-	-
5.Amino Acids	-	-	-	-	-	-	-
6.Vitamin c	+	+	+	+	+	+	+
7.Fats and oils	+	+	+	+	+	+	+
8.Terpenoids	+	+	+	+	+	+	+
9.Phenols	+	+	+	+	+	+	+
10. Cardiac - glycoside	-	-	-	-	-	-	-
11.Anthraquinone glycosides	-	-	-	-	-	-	-

+ present; - absent

In the present study, the presence of Phenols, flavonoids, terpenoids, carbohydrate, fats, and oils, and vitamin C were observed in all seven extracts. Alkaloids were present in all extracts except in the aqueous extract.

Tannins were detected in acetone, ethanol, and methanol fractions but were absent in chloroform, hexane, petroleum ether, and aqueous. Cardiac glycoside, amino acids, and anthraquinone glycoside were not detected. Kabra *et al.,* reported the presence of flavonoids, steroids, volatile compounds, and terpenoids in leaves and fruits of *M. esculenta* [18], our study reveals the presence of various other phytochemicals such as alkaloids, phenols, Vitamin C, carbohydrate, tannins in 7 different extracts of the leaves of *M. esculenta*.

These secondary metabolites are reported to have many biological and therapeutic properties [27].To extract compounds for antibacterial activity using various solvents would depend on the polarity of the solvents. This is because different organic solvents can extract different phytoconstituents in diverse quantities, and hence there is a difference in inhibition of growth of microorganisms [28]. The variation in the antibacterial activity of the various solvents is due to the nature of the polarity of the solvents. Polar solvents are relatively better at extracting the antimicrobial constituents from plants [29].

Methanol has higher polarity in comparison to the other solvents and thus tends to dissolve different compounds from the plant materials soaked in them. This property of methanol thus explains the fact that out of 7 bacterial species tested, 6 were inhibited by Methanol. Similarly, ethanol exhibited a similar property in which it could inhibit the growth of 6 out of the 7 species tested. The use of ethanol and methanol for extraction of antibacterial compounds has been widely reported [24, 30].

Antibacterial Activity: The antibacterial activity of leaf extracts of *M. esculenta* in acetone, chloroform, ethanol, hexane methanol petroleum ether, and aqueous solvents were performed in vitro, and zones of inhibition were statistically evaluated using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). All the results are represented as means ± SE of three independent replications. P values > 0.05 were considered as significant **Table 2.**

TABLE 2: ANTIBACTERIAL ACTIVITY OF VARIOUS SOLVENT EXTRACTS OF M. ESCULENTA LEAVES (ZONE OF INHIBITION IN MM

	Solvents						
Microorganisms	**Acetone**	**Chloroform**	**Ethanol**	**Hexane**	**Methanol**	**Petroleum ether**	**Aqueous**
B. cereus	10.66 ^{a} ± 0.33	6.66 ^{a} ± 0.33	8.0 ^{c} ± 0.57	-	9.66 ^{c} ± 0.33	-	6.66 ^{bc} ± 0.33
S. aureus	12.66 ^{a} ± 0.33	9.66 ^{a} ± 0.33	12.33 ^{c} ± 0.33	10.33 ^{a} ± 0.33	11.33 ^{c} ± 0.33	6.66 ^{a} ± 0.33	-
C. tetani	-	-	-	-	11 ^{c} ± 0.00	9.66 ^{a} ± 0.33	-
E. coli	-	-	9.66 ^{c} ± 0.33	-	10.33 ^{c} ± 0.33	-	-
K. pneumoniae	10.66 ^{a} ±	12.0 ^{a} ± 0.57	11.66 ^{c} ± 0.33	11.33 ^{a}	-	-	-

	0.33			± 0.33			
C. perfringens	12.33 [a] ± 0.33	-	12 [c] ± 0.00	-	10.33 [c] ± 0.33	-	22.66 [b c] ± 0.88
S. boydii	15.0 [a] ± 0.57	-	10.66 [c] ± 0.33	-	13.66 [c] ± 0.33	-	15.66 [b c] ± 0.33

Values are the mean of three tests ± SEM a-c Mean values with the same superscript within a row do not differ significantly by DMRT (p>0.05)

Here, the maximum zone of inhibition was recorded by aqueous extract at 22.66 mm against Clostridium perfringens, followed by 15.66 mm against *Shigella boydii*, water being the most polar solvent. Based on the DMRT result obtained, the methanolic and ethanolic extracts are not significantly different from each other in terms of inhibitory effect against the microbes under study.

Similarly, acetone, chloroform, and hexane extracts are also not significantly different from each other. The non-polar solvent hexane showed antibacterial activity only against *S. aureus* and *K. pneumoniae*; similarly, petroleum ether showed antibacterial activity against *S. aureus* and *C. perfringens* as depicted in **Fig. 1**.

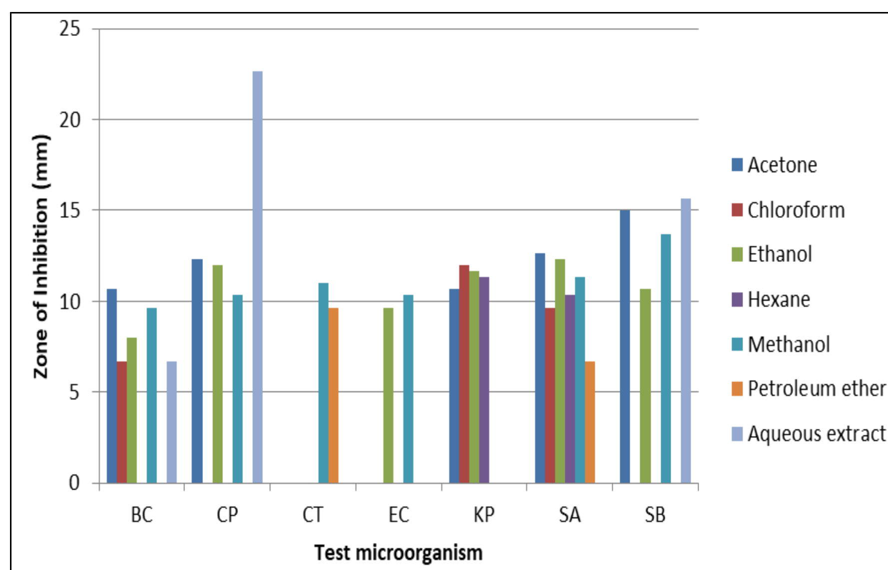

FIG. I: INHIBITION OF BACTERIAL GROWTH BY DIFFERENT SOLVENT EXTRACTS OF LEAVES OF M. ESCULENTA IN AGAR WELL DIFFUSION ASSAY. BC, *Bacillus cereus*; CP, *Clotridium perfringens*; CT, *Clostridium tetani*; EC, *Escherichia coli;* KP, *Klebsiella pneumonia*; SA, *Staphylococcus aureus*; SB, *Shigella boydii*

The ability to inhibit the growth of the bacterial species tested was also shown by Acetone whereby it could prevent the growth of 5 out of the 7 organisms.

All the bacterial strains were found to be sensitive to at least two extracts of the plant, but the ethanol and methanol extracts were comparatively more effective than the others.

To extract compounds for antibacterial activity using various solvents would depend on the polarity of the solvents. This is because different organic solvents can extract different phyto-constituents in diverse quantities, and hence there is a difference in inhibition of growth of microorganisms.

The use of several extracts to study the antimicrobial activity of plants has been a focus of many researchers [31, 32]. Antimicrobial molecules are abundantly found in medicinal plants, and due to this reason, plant extracts are used to treat various diseases.

Reports suggest that alkaloids and flavonoids are responsible for showing antifungal properties in higher plants. In addition, secondary metabolites such as tannins and other phenolic compounds are also termed as antimicrobial compounds [33]. The bioactive molecules responsible for the antimicrobial activity have been screened and used for the production of herbal medicines [34, 35, 36, 37].

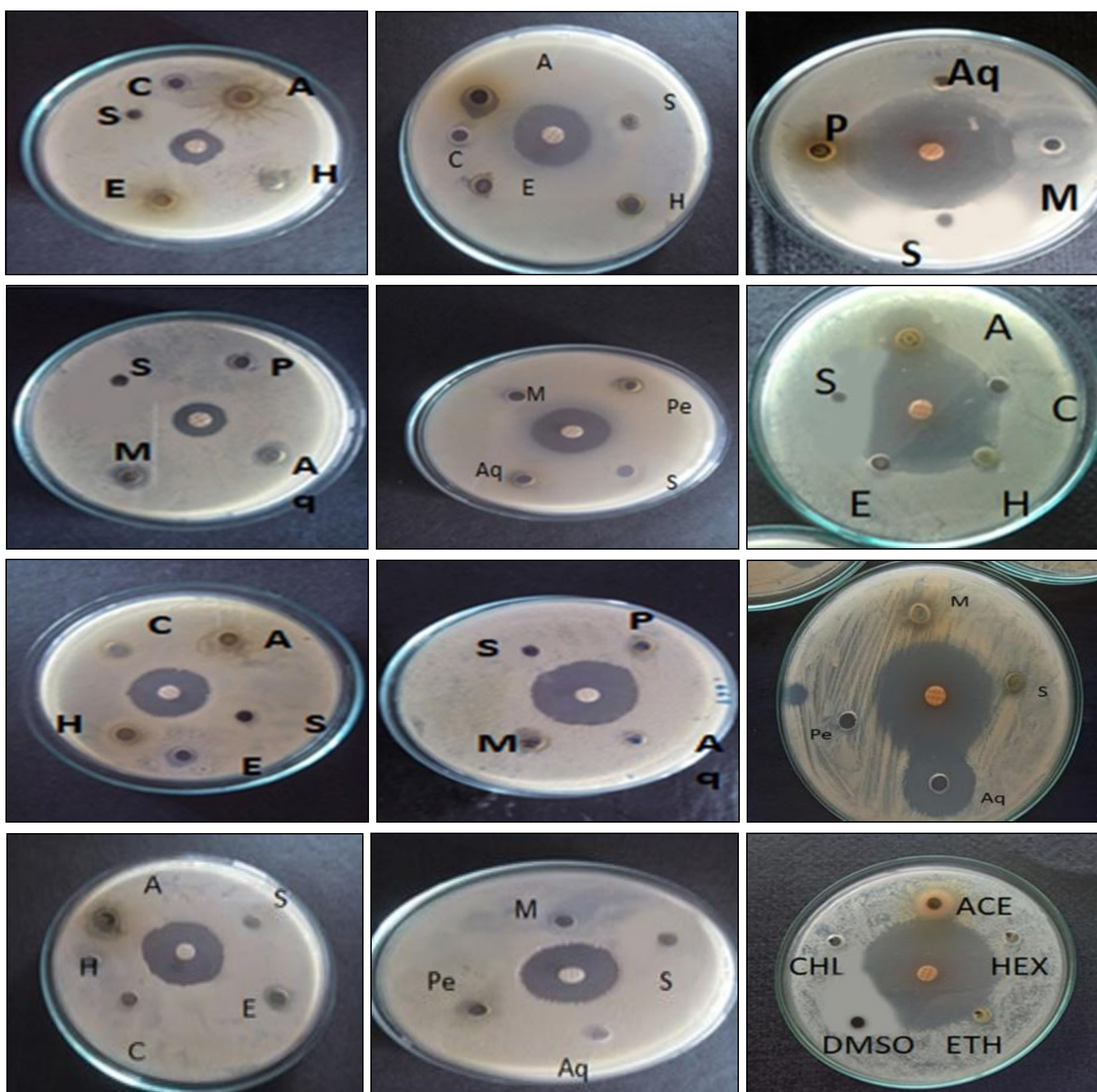

FIG. 2: ZONE OF INHIBITION OF VARIOUS EXTRACTS OF *M. ESCULENTA*

Leaf:

Phenolic and Flavonoid Content: The phenolic content of methanolic extract of *M. esculenta* using Folin Ciocalteau's reagent is expressed in terms of gallic acid equivalent. The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract. The total phenolic content in the examined plant extract was found to be 97.66 ± 0.16 mg GA/g of the extract. The concentration of flavonoids in plant extract of *M. esculenta* was determined using a spectrophotometric method with aluminium chloride. The content of flavonoids was expressed in terms of rutin equivalent, mg of RU/g of extract. The total flavonoid content in the examined plant extract was 51.3 ± 0.32 mg RE/g of leaf extract. Kabra *et. al*. 2019, in their findings, reported the total phenolic and flavonoid content in the methanolic extract were 88.94 ± 0.24 mg /GAE and 67.44 ± 0.14 mg /g QE respectively [18]. For the estimation of phenolic content, during reaction, a phenol loses an H^+ ion to produce a phenolate ion, which reduces Folic-Ciocalteu reagent. This change was detected spectrophotometrically. The estimation of phenolic and flavonoid content was carried out in methanolic extract of leaf of *M. esculenta*, as many flavonoids as well as phenolics contain polar phenolic hydroxyl groups, which could be easily extracted in methanol. It was also observed that phenolic content was higher than flavonoid in the extract, which justifies the fact that most flavonoids are phenolic compounds [38].

Antioxidant Activity Assay:

ABTS Radical Cation Scavenging Assay: Methanolic extract of Myrica showed a concentration-dependent rise in the scavenging of the ABTS free radicals. The extract showed the highest scavenging percentage at 175 µg/ml and is comparable with the standard DTE that was used for the assay **Fig. 3**. The IC_{50} value of methanolic Myrica extract was 91.6 µg/ml and that of DTE was 94.2 µg/ml.

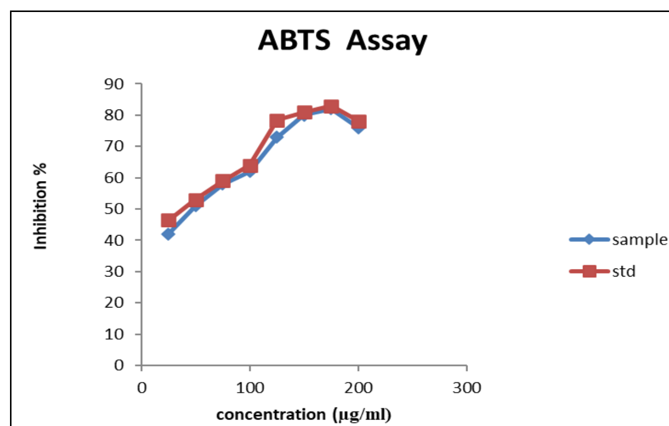

FIG. 3: FREE RADICAL SCAVENGING ACTIVITY OF METHANOLIC EXTRACT OF *M. ESCULENTA* IN COMPARISON WITH THE STANDARD 1, 4-DITHIOERYTHRITOL (DTE)

The ABTS• + chromophore is produced when ABTS reacts with potassium per sulfate. This radical cation is blue in color and absorbs light at 734 nm. ABTS• + is reactive towards most anti-oxidants including phenols, thiols, and vitamin C. The scavenging ability of methanolic extract of Myrica has a significant value of 83% for ABTS which corresponds to high phenolic content. This high scavenging activity of the extract has also been reported by Kabra *et al.,* 2019 [18].

FRAP Assay: FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [F^{e3+} - TPTZ] complex and produces a colored ferrous tripyridyltriazine [Fe^{2+} - TPTZ]. The antioxidants in the sample act as a reductant in the redox-linked colorimetric reaction. **Fig. 4**.

Shows the trend for ferric ion reducing activities of extract of Myrica and abscorbic acid standard. The absorbance of Myrica increased due to the formation of Fe^{2+}-TPTZ complex with increasing concentration. This increasing absorbance indicates an increase in the reductive ability of sample [39].

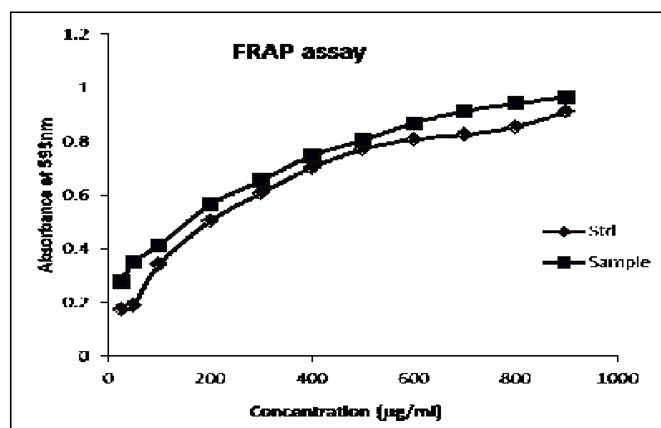

FIG. 4: INCREASE IN ABSORBANCE OF SAMPLE (METHANOLIC EXTRACT OF M.ESCULENTA IN COMPARISON TO STANDARD (ASCORBIC ACID) WITH INCREASE IN CONCENTRATION

DPPH Radical Scavenging Activity: The reactivity of methanolic extract of *Myrica esculenta* was analysed with DPPH, a stable free radical. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases, and the resulting discoloration from purple to yellow is stoichiometrically related to the number of electrons gained. The lower the absorbance of the reaction mixture, the higher was the free radical scavenging activity [40]. The reduction of the DPPH was indicated by the decrease in absorbance at 517 nm. The methanolic extract of *M. esculenta* significantly reduced the DPPH. The values of percent scavenging of DPPH are presented in **Fig. 5**. It was observed that scavenging activity increased with increasing concentration of the methanolic fraction in the assay.

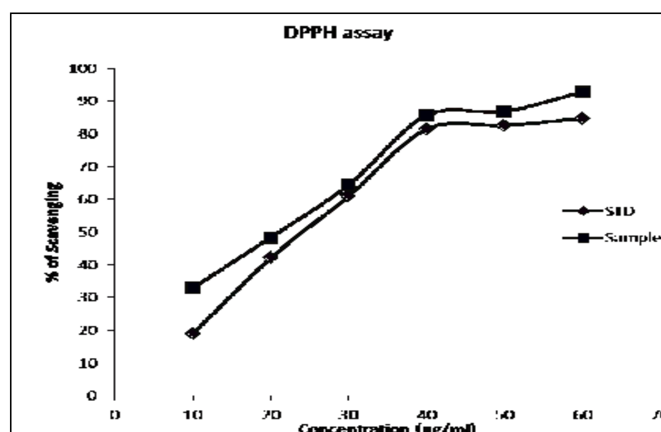

FIG. 5: PERCENTAGE OF FREE RADICAL SCAVENGING OF METHANOLIC EXTRACT OF *M. ESCULENTA* IN COMPARISON TO STANDARD ASCORBIC ACID AGAINST DPPH

The DPPH radical scavenging (%) activity of the Myrica extract exerted an inhibition of 92.8 %, and

that of Ascorbic acid was 84.7 at 60 µg/ml. The IC_{50} value is defined as the concentration of a substrate that causes 50% loss of the DPPH activity and is calculated by linear regression of plots of the percentage antiradical activity against the concentration of the tested compounds.

In this study, the IC_{50} of the extract was 28.4 µg/ml, while that of the ascorbic acid standard was 24.5 µg/ml.

Reducing Power: The reducing ability of leaf extract of *M. esculenta* in, methanol is shown in **Fig. 6**, which is comparable with that of standard ascorbic acid. The reducing power of the extract increased with increasing concentration which was also indicated by the change in colour of the solution from blue to green. Antioxidant activity measured by four *in-vitro* antioxidant assays, that is, free radical scavenging ability by using ABTS radical cation (ABTS assay), DPPH radical cation (DPPH assay), FRAP assay and detection of reducing power.

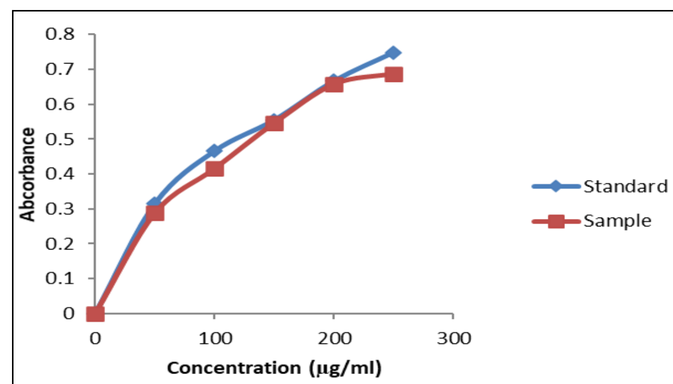

FIG. 6: REDUCING ABILITY OF ASCORBIC ACID AND METHANOLIC EXTRACT OF *M. ESCULENTA*

The leaf extract of *M. esculenta* showed a significant scavenging and reducing power when compared to the standard synthetic antioxidants. The presence of reductones is generally associated with reducing properties and thus serves as an antioxidant by donating hydrogen atoms and disrupting the action of free radicals [41].

CONCLUSION: The present findings revealed the presence of various phytochemicals in the 7 extracts of the leaves of *M. esculenta*, which justifies the use of different solvents for the extraction process. Thus it may be concluded that among the extracts studied, acetone, ethanol, methanol, and petroleum ether can be utilized for the extraction and subsequent screening of phytochemicals in the leaves of *M. esculenta*. In addition to this, the various extracts also showed antibacterial as well as antioxidant activity which may in turn be attributed to the presence of the natural products in the leaves. The present investigation quantified the total phenolic as well as flavonoids present in the methanol extract of the plant. The isolation and purification of the phytochemicals from the plant followed by a detailed study might result in the identification of novel antimicrobial compounds leading to the development of therapeutics and thus a potential cure for the diseases caused by these organisms.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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