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ORIGINAL ARTICLE

Extraction and Volatile Compounds Profiling of the Bioactive Fraction of *Monochoria hastata* (L.) Solms

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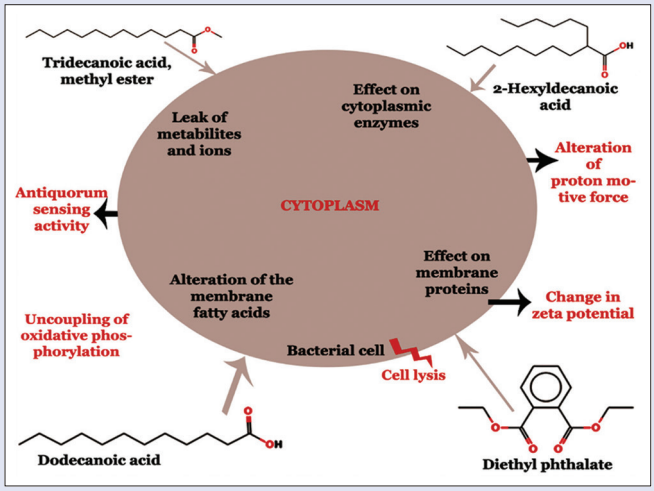
ABSTRACT

Background: An aquatic macrophyte *Monochoria hastata* (L.) Solms has ethnomedicinal application in various ailments and experimentally is proved to have antibacterial property. **Objectives:** To evaluate the optimized extraction methods and solvents for antibacterial activity and their characterization by gas chromatography-mass spectrometry (GC-MS). **Materials and Methods:** Extractions from the aerial parts of the plant in solvents with different polarities and various techniques, viz., microwave, infusion, Soxhlet, and maceration, were used to evaluate the best antibacterial efficacy by agar well diffusion method. The phytochemical constituents present in the bioactive extract were analyzed using standard phytochemical screening methods and characterized by GC-MS analysis. **Results:** Ethyl acetate extract derived by the Soxhlet method showed the highest antibacterial activity against all the test bacterial strains, and Gram-positive strains were more susceptible than Gram-negative strains. The crude extracts showed antibacterial activity which ranged from 7.0 ± 0.3 to 16.5 ± 0.8 mm at 100 mg/ml. Fifty percent of methanol had the highest extractive value (21.3%) in the Soxhlet method. Phytochemical tests showed the presence of alkaloids, phenols, flavonoids, terpenoids, glycosides, and fats in the bioactive ethyl acetate extract. GC-MS analysis showed the presence of different fatty acids and their saturated esters as the principal components. The major compounds were tridecanoic acid, methyl ester, 2-hexyldecanoic acid, dodecanoic acid, and diethyl phthalate. **Conclusion:** The results suggest that the ethyl acetate extract of *M. hastata* (L.) Solms possesses some bioactive volatile compounds including fatty acids and their esters, which have antibacterial potentiality. This is the first report of such antibacterial assessment from *M. hastata* (L.) Solms.

Key words: Antibacterial activity, extractive value, fatty acid esters, *Monochoria hastata* (L.) Solms, phytochemical constituents, volatile compounds

SUMMARY

- *Monochoria hastata* (L.) Solms is an ethnomedicinally important aquatic macrophyte, which has been used in various ailments to treat toothache, sore-throat, wounds and boils, gastropathy, hepatopathy, etc. However, its antimicrobial efficacy against the pathogenic bacteria remained unexplored. The GC-MS characterization of the phytochemical constituents of the ethyl acetate extract showed that the plant is the key repository of tridecanoic acid methyl ester, 2-hexyldecanoic acid, dodecanoic acid, and diethyl phthalate. The extract had a broad-spectrum antibacterial activity against the challenged pathogenic Gram-positive and Gram-negative strains. Therefore, mass-scale exploitation of these active fractions might contribute in human endeavor to treat such pathogenic bacteria. This is the first report on the characterization of potent antibacterial principles from *M. hastata* (L.) Solms.



Abbreviations used: AM: Arithmetic mean; GC-MS: Gas chromatography-mass spectrometry; MCC: Microbial culture collection; MRSA: Methicillin-resistant *Staphylococcus aureus*; MTCC: Microbial type culture collection; NB: Nutrient broth; SD: Standard deviation

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INTRODUCTION

Herbs have been used as sources of nutrients and medicines since ancient ages. They contain different pharmacologically active compounds that exhibit antibacterial properties and thus provide the scientific basis for the traditional use of these herbs as vegetables and condiments in the treatment of bacterial infections.[1] Plants can synthesize various secondary metabolites, and many of them possess antimicrobial activities.[2] Vegetables and medicinal plants are considered an economic, effective, and safe containing none or less toxic effects.[3] The World Health Organization estimated that 80% of the world population relies on traditional medicine.[4] Seventy percent of the Indian population use herbs as a remedy for various diseases and ailments based on their traditional and indigenous knowledge.[5] The proportion of plants used

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in different Indian systems of medicine includes 2000 in Ayurveda, 1300 in Siddha, 1000 in Unani, 800 in Homeopathy, 500 in Tibetan, and 200 in Modern and 4500 number of plants in folk medicine. In India, around 25,000 plant-based formulations are used as effective traditional and folk medicine.[6]

Naturally, plants protect themselves by producing some compounds called secondary metabolites including terpenes, phenolics, nitrogenous, and sulfur-containing compounds, which help them to defend against a variety of herbivores and other animals, such as nematodes, mites, and insects, as well as pathogenic micro-organisms such as bacteria, viruses, and fungi, as well as various kinds of abiotic stresses.[7] Several researchers have reported biological activities such as antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, hepatoprotective, and antidiabetic of different metabolites of pharmacological importance, such as terpenoids, phenols, glycosides, flavonoids, tannins, and alkaloids.[8] Fatty acids and their methyl esters are also potential bioactive compounds with multiple uses, such as antibacterial, antifungal, and antioxidant.[9,10] Saturated fatty acids have neither a double bond or a triple bond in their molecule and are represented by the chemical formula $C_nH_{2n+1}COOH$, and the unsaturated fatty acids have minimum one double bond or a triple bond. The study also supports that saturated and unsaturated fatty acids produced by lipid oxidation may signal for the induction of defense mechanisms.[11]

Many studies have been carried out on terrestrial plants, but the aquatic system remains neglected; in this regard, as the aquatic plants are often results of eutrophication, they are considered as weeds of the wetlands. However, a large number of wetland plants are useful to humankind as they develop many secondary metabolites.[12] Evidence and studies have established that defense mechanism occurs in all aquatic habitats, including marine and freshwater systems, and all primary producing organisms, including producing and releasing active compounds, which target multiple physiological processes through multiple biotic and abiotic factors determining the strength of the interactions.[13] Sophisticated analytical methods such as mass spectrometry (MS), including gas chromatography-MS (GC-MS), have enabled the profiling of metabolites with high throughput and high precision.[14] The solvent used for extraction influences on the isolation of a defined nature of antibacterial compounds present in the plant material. Therefore, to investigate potent antibacterial compounds in large quantities, the optimization of solvents and methods is essential.[15]

An emergent hydrophyte *Monochoria hastata* (L.) Solms belonging to the family *Pontederiaceae* which grows in wide geographical range is conventionally used by certain ethnic communities in Asia, especially in Indian subcontinent as remedy of several ailments such as wounds and boils, gastropathy, and hepatopathy, and leaf of this plant has antibacterial efficacy, but the characterization of active principles has not been done so far.[16] The present work aims to assess the best extraction methods with greater antimicrobial activity from the aerial part extracts of the aquatic vegetable *M. hastata* (L.) Solms and to determine the phytochemical constituents present in the most bioactive extract.

MATERIALS AND METHODS

Extraction and determination of extractive values

The aerial parts of *M. hastata* (L.) Solms were collected and prepared for extraction as per the method applied in our previous study.[17] Powder sample in different solvents such as absolute n-hexane, diethyl ether, dichloromethane, chloroform, ethyl acetate, methanol, 90% methanol in water, 50% methanol in water, and water in a ratio of 1:10 (w/v) was extracted according to the ascending polarity indices applying various extraction techniques. In the infusion extraction technique, the sample was taken in capped conical flasks and soaked with various solvents separately being agitated in an orbital shaker at 100 rpm for 24 h. Maceration was done by heating the flasks loaded with sample soaked with different solvents in the same manner as the infusion method on a heater at boiling temperatures for 15 min for each case. For the microwave technique, solvent-soaked sample was applied in a microwave oven at 80°C for 1 min. Similarly, Soxhlet extraction was done in a Soxhlet apparatus at 40°C for 48 h. The extracts were then passed through the Whatman No. 1 filter papers, and filtrates were made pigment-free by passing through an activated charcoal column. The filtrates were collected and concentrated by a rotary vacuum evaporator (Superfit Rotary Vacuum Evaporator, R-150, Mumbai, Maharashtra, India). After obtaining the filtered extract, it was then transferred into weighed Petri plates, and extracts were concentrated to dryness by keeping filtrates for complete evaporation of the solvent. The extractive values of ethyl acetate and 50% methanol extract using different extraction techniques were determined using the following formula:

Extractive value (%) = $W_e/W_p \times 100$, where W_e = weight of dried extract and W_p = weight of plant powder sample.

Assessment of antibacterial activity

Bacterial strains of *Bacillus cereus* Microbial Type Culture Collection and Gene Bank (MTCC) 1272, *Enterococcus faecalis* Microbial Culture Collection (MCC) 2041 T, *Escherichia coli* MTCC 571, *Salmonella enterica* var Typhimurium MTCC 98, *Staphylococcus aureus* MTCC 96, *Streptococcus mutans* MTCC 497, *Bacillus paraflexus* MTCC 9831T, *Staphylococcus epidermidis* MTCC 3086, and *Vibrio parahaemolyticus* MTCC 451 for antibacterial assessment were procured from the MTCC, Chandigarh, India, and the MCC, National Centre for Cell Science, Pune, India. The strains were maintained on nutrient agar slants at 4°C and activated at 37°C for 24 h on 1.3% nutrient broth in 2% agar (HiMedia, Mumbai, Maharashtra, India) before any susceptibility test. The antibacterial bioactivities of different extracts were performed by the agar well diffusion method and microdilution method. Ciprofloxacin (50 µg/ml) and respective solvents were used as a positive and negative control, respectively. The whole experiment was worked out in our laboratory equipped by Category 2 microbiological bio-safety level. The degree of antibacterial activity was expressed as the "activity index" using the following formula.[18]

Activity index = $(x - y)/z$, where x = total area of inhibition of test sample (plant extract), y = total area of inhibition of the negative control (extraction solvent), and z = total area of inhibition of positive control (50 µg/ml ciprofloxacin antibiotic).

Qualitative and chromatographic analysis of volatile compounds

Phytochemical constituents present in the bioactive ethyl acetate extract were assessed through the phytochemical screening tests following the standard protocol with some minor modification in the reactions which have been mentioned in detail in the "Volatile compounds profiling" subsection of the "Results" section of this article. The phytochemical profile of volatile compounds present in the most bioactive ethyl acetate extract was assessed by GC-MS analysis applying the same methodology, experimental condition, and instrument, which were applied in our previous experiment.[17]

Validation of data and statistical analysis

Results of triplicate experiments were taken as statistical data. Arithmetic mean (AM) and standard deviation (SD) of the results were calculated in MS-Excel 2007. The data were statistically validated as AM ± SD.

RESULTS

Evaluation of antibacterial efficacy of the solvent extracts

The antibacterial activity of different solvent extracts of the aquatic vegetable was assayed *in vitro* against some bacterial species as shown in Table 1. It showed the antibacterial activities against both Gram-positive and Gram-negative bacteria. Among all the solvents used in extraction, the ethyl acetate extract was found active against all the test bacterial strains as shown in Figure 1. It showed maximum inhibitory activity against *S. mutans* while minimum activity against *V. parahaemolyticus*. The n-hexane and 50% methanol extract showed antibacterial activity selectively against a very less number of bacteria.

Evaluation of extractive values of different extracts

As the ethyl acetate showed the best performance regarding antibacterial activity and the 50% methanol extract showed the best performance regarding extraction efficiency, the extractive values of the plant sample in these two solvents were further evaluated against different extraction techniques which are shown in Figure 2. The Soxhlet method was most efficient over other extraction methods in the case of both solvents ethyl acetate and 50% methanol.

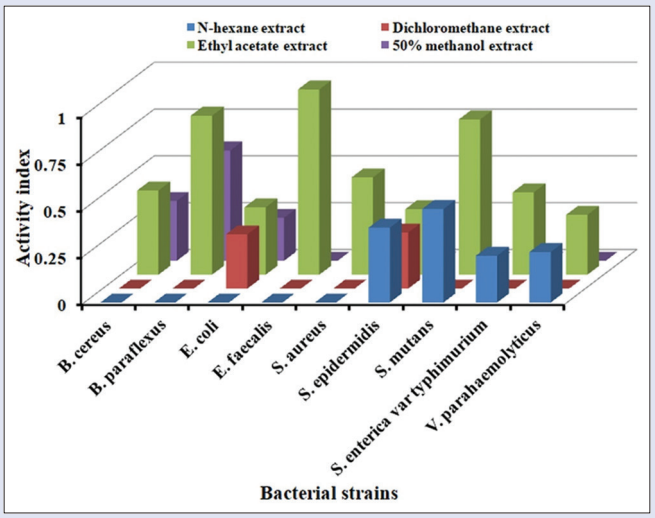

Figure 1: Antibacterial activity index of different solvent extracts. Here, the *x*-coordinate represents the bacterial strains and the *y*-coordinate represents the activity index

Volatile compounds profiling through phytochemical screening tests and gas chromatography-mass spectrometry analysis

The results of the qualitative phytochemical analysis showed the presence of different active principles of pharmacognostic importance that showed reactivity with the phytochemical screening reagents which are shown in Table 2. The GC-MS chromatogram of ethyl acetate extract exhibited several compound peaks, which is shown in Figure 3. The NIST library search results of the GC-MS data also confirmed the presence of various secondary as well as primary metabolites such as 3 alkaloids, 1 phenol, 2 terpenoids, 1 flavonoid, 2 glycosides, 1 monoacylglycerol, 1 fatty alcohol, and an array of carboxylic acids and their esters, most of which were different allies of fatty acids (63.3% of total moles) as shown in Table 3.

DISCUSSION

Extractive values and antibacterial activities of different solvents

Different extracts (100 mg/ml) of *M. hastata* (L.) Solms aerial parts in various solvents showed varied antimicrobial activity against the test

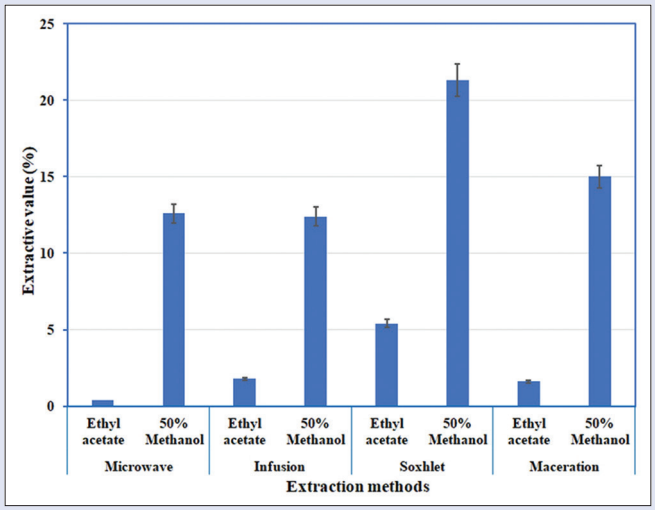

Figure 2: Extractive values of ethyl acetate and 50% methanol in different extraction techniques. Here, the *x*-coordinate represents the different extraction methods and solvents and the *y*-coordinate represents the extractive values (%); the values represent the average of triplicate trials with the standard deviation values

Table 1: Bacterial zone inhibition in different solvent extract

Bacteria strain	The diameter of zone inhibition (mm) of the extracts (100 mg/ml, each)							
	N-hexane extract	Dichloromethane extract	Ethyl acetate extract	50% methanol extract	Diethyl ether extracts	Chloroform extracts	90% methanol extracts	Ciprofloxacin (positive control)
Bacillus cereus (Gram+)	-	-	8.5±0.4	6.0±0.3	-	-	-	19.0±0.95
Bacillus flexus (Gram+)	-	-	14.5±0.7	10.0±0.5	4.0±0.2	-	3.1±0.15	17±0.8
Escherichia coli (Gram−)	-	8.0±0.4	10.0±0.5	6.5±0.3	-	-	-	28.0±1.4
Enterococcus faecalis (Gram+)	-	-	14.8±0.74	-		5.0±0.25		15.0±0.75
Staphylococcus aureus (Gram+)	-	-	12.0±0.6	-	5.2±0.26	-	-	23.0±1.15
Staphylococcus epidermidis (Gram+)	10.0±0.5	7.5±0.375	8.75±0.438	-	-	-	-	25.0±1.25
Streptococcus mutans (Gram+)	10.0±0.5	-	16.5±0.8	-	3.2±0.16	3.1±0.15	5.5±0.28	20.0±1.0
Salmonella typhimurium (Gram−)	6.0±0.3	-	10.5±0.5	-	-	-	-	24.0±1.2
Vibrio parahaemolyticus (Gram−)	6.0±0.3	-	7.0±0.3	-	-	-	-	22.0±1.1

- – No zone inhibition. Here, the values represent the average of triplicate trials with the standard error values

Table 2: Phytochemical constituents of ethyl acetate extract

Phytochemical screening tests	Reactants	Expected outcomes	Observed outcomes
Tannins (Braymer's test)	1 ml extract + 1 ml H_2O + 1 drop 5% ferric chloride ($FeCl_3$)	Green precipitation	–
Phenols (ferric chloride test)	1 ml extract + few drops of 1% $FeCl_3$ (5%)	Deep blue coloration	+
Flavonoids (lead acetate test)	1 ml extract + 1 ml lead acetate (10%)	Yellow coloration	+
Terpenoids (Salkowski test)	1 ml extract + 1 ml chloroform ($CHCl_3$) + 2 ml concentrated sulfuric acid (H_2SO_4)	Reddish brown color at inter-phase	+
Saponins (foam test)	1 ml extract + 1 ml H_2O + Heat	Froth appears	–
Steroids (Salkowski Test)	1 ml extract + 1 ml $CHCl_3$ + 1 ml concentrated H_2SO_4	Reddish brown ring at the junction	–
Phlobatannins (Precipitate Test)	1 ml extract + 1 ml HCl (1%) + heat	Red precipitation	–
Carbohydrates (Molisch's test)	1 ml extract + 5 ml H_2O + 2 drops ethanolic α-naphthol (20% α-naphthol in ethanol) + 2 ml concentration H_2SO_4	Reddish violet ring at the junction	–
Glycosides (Liebermann's test)	1 ml extract + 1 ml $CHCl_3$ + 1 ml CH_3COOH (acetic acid)	Violet to blue to green coloration	+
Coumarins (sodium hydroxide test)	1 ml extract + 1 ml NaOH (10%)	Yellow coloration	–
Alkaloids (Wagner's test)	2 ml extract + 1 ml iodine in potassium iodide	Brown/reddish precipitation	+
Proteins (xanthoproteic test)	1 ml extract + few drops of concentration HNO_3	Yellow coloration	–
Emodins (ammonium hydroxide test)	1 ml extract + 1 ml NH_4OH + 2 ml Benzene	Red coloration	–
Anthocyanins (ammoniacal test)	1 ml extract + 1 ml 2 N HCl + NH_3	Pinkish red to bluish violet coloration	–
Leucoanthocyanin turns (isoamyl alcohol)	1 ml extract + 1 ml isoamyl alcohol	Red coloration in organic layer	–
Anthraquinones (Borntrager's test)	1 ml extract + 1 ml benzene + 2 ml 10% NH_3	Ammoniacal layer turns pink, violet, or red	–
Fats (saponification test)	1 ml extract + few drops of 0.5 N alcoholic KOH + 1 drop of phenolphthalein	Formation of soap or partial neutralization of alkali on heating in water bath for 1.2 h	+

+ – Same as expected outcome; – – Not same as expected outcome

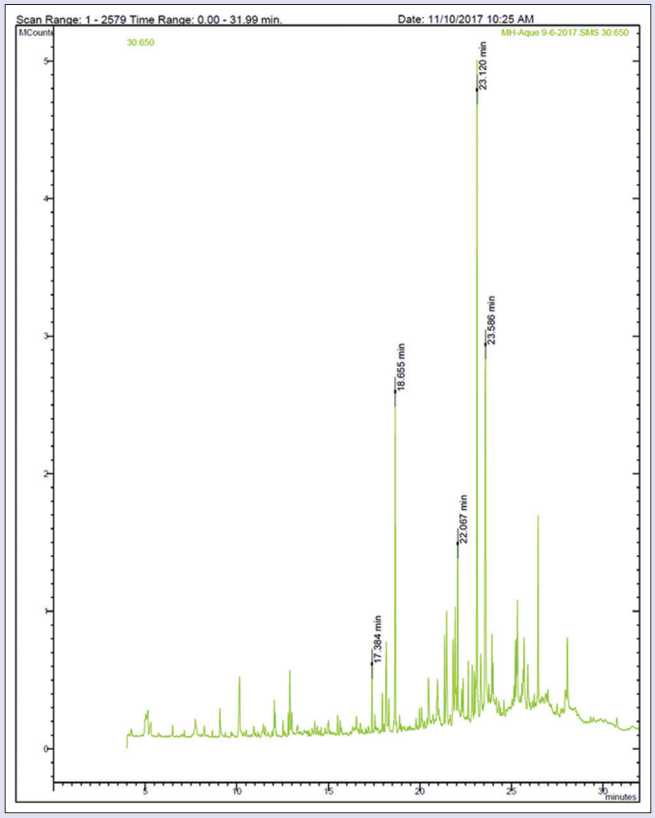

Figure 3: Gas chromatography-mass spectrometry chromatogram of ethyl acetate extract. Here, the *x*-coordinate or abscissa represents the scan time (min) and the *y*-coordinate or ordinate represents the relative abundance in ppm

bacterial strains. The ethyl acetate extract exhibited grater antibacterial potential against a range of Gram-positive and Gram-negative bacterial growth, while the other active fractions were species dependent. Although both types of bacterial strains were susceptible, the Gram-positive bacteria were more susceptible. The average zone inhibition diameter of Gram-positive strains was 12.8 mm, while it was 9.1 mm against Gram-negative strains. Although the 50% methanol had the highest extractive value (21.3) which is four times higher than the ethyl acetate extract in the Soxhlet method, the later showed the maximum antibacterial activity. The polarity index of methanol (5.1) is higher than that of ethyl acetate (4.4). Hence, methanol extracted a mixture of compounds of which the proportion of antibacterial compound was low. On the other hand, in course of successive extraction, according to the ascending polarity, the ethyl acetate extracted the proportionately high amount of less polar fatty acids and their esters, which showed the antibacterial activity. The more potential antibacterial activity in ethyl acetate indicates that the polarity factor might be the key determining parameter in accessing the target site of antibacterial activity in the Gram-positive and Gram-negative strains. Thus, the antibacterial chemical constituents of the plant extracts might act on the physical disruption of the membrane and loss of the proton motive force or inhibition of membrane-associated enzyme activity.[19]

Phytochemical constituents of the extract

Phytochemical screening showed that several metabolites such as phenols, flavonoids, alkaloids, terpenoids, glycosides, and fats, which were present in *M. hastata* (L) Solms, are the potent bioactive compounds with active antimicrobial moiety. Phenolic compounds act possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins of micro-organisms. Flavonoids disrupt mainly the bacterial membranes as they are lacking hydroxyl groups on their β-rings and are more active against micro-organisms than other compounds, such as alkaloids which can intercalate with DNA.[20] Fatty acids inhibit bacterial enoyl–acyl carrier protein reductase (FabI), an essential component of bacterial fatty acid synthesis, which has served as a promising target for antibacterial activity.[21] Nair[22] reported that the phytochemical constituents detected in another member of the same plant genus, i.e., *Monochoria vaginalis* (Burm. F) C. Presl, were alkaloids, phenols, carbohydrates, flavonoids, tannins, and glycosides.

Table 3: Compounds profile of ethyl acetate extract

Chemical name	Class of the compounds	Chemical formula	Peak number	Retention time (min)	NIST Match Factor: Normal-Forward	Molecular weight (g/mol)	Mole (%)	Total mole (%)
2-Pyrrolidinone, 1-methyl	Alkaloids	C_5H_9NO	1	9.081	14051 in replib	99.13	1.1	5.1
Azelaic dihydrazide		$C_9H_{20}N_4O_2$	12	21.931	25396 in replib	216.28	2.6	
Benzothiophene-3-carbonitrile, 4,5,6,7-tetrahydro		C_9H_9NS	18	22.975	124674 in mainlib	162.23	1.4	
Phenol, 2,5-bis (1,1-dimethylethyl)-	Phenol	$C_{14}H_{22}O$	3	17.384	157188 in mainlib	206.32	1.9	1.9
Phytol	Terpenoids	$C_{20}H_{40}O$	26	25.330	8579 in replib	296.53	2.7	7.6
3,7,11,15-Tetramethyl-2-hexadecen-1-ol		$C_{20}H_{40}O$	29	26.457	45842 in mainlib	296.53	4.9	
2 (4H)-Benzofuranone, 5,6,7,7a-tetrahydro	Flavonoid	$C_8H_{10}O_2$	4	17.954	79232 in mainlib	138.16	1.3	1.3
2-Pentadecanone, 6,10,14-trimethyl	Glycosides	$C_{18}H_{36}O$	13	22.067	2305 in replib	268.48	4.9	7.2
2H-Pyran-2-one, tetrahydro-6-octyl-		$C_{13}H_{24}O_2$	30	28.058	65567 in mainlib	212.33	2.3	
1,2,3-Propanetriol, 1-acetate	Monoacyl glycerol	$C_5H_{10}O_4$	2	12.905	10218 in mainlib	134.13	2.2	2.2
Z, E-3,13-Octadecadien-1-ol	Fatty alcohol	$C_{18}H_{34}O$	27	25.693	18110 in mainlib	266.46	1.0	1.0
Diethyl phthalate	Other carboxylic	$C_{12}H_{14}O_4$	6	18.655	47 in tutorial	222.23	8.9	10.1
Phthalic acid, 6-ethyl-3-octyl isobutyl	acids and its esters	$C_{22}H_{34}O_4$	15	22.363	122531 in mainlib	362.50	1.2	

Gas chromatography-mass spectrometry analysis of volatile compounds

The GC-MS results confirmed the presence of secondary metabolites, such as phenol (1.9%), flavonoid (1.3%), alkaloids (5.1%), terpenoids (7.6%), and glycosides (7.2% of total moles). Monoacylglycerol (2.2%) and fatty alcohol (1.0% of total moles) were also detected. Carboxylic acids and their esters were present in a very high proportion (73.4% of total moles) than other molecules. Among which 63.3% of molecules were different fatty acids and their esters and 10.1% were another carboxylic acid (i.e., phthalic acid) and its ester. Total saturated fatty acid content including its esters was 53.2% of total moles, which is five times higher than the total content of unsaturated fatty acids, including its esters. Therefore, the bioactivity exhibited by the ethyl acetate extract was a combinatorial effect of the unsaturated fatty acids and its esters. The compounds with the highest proportions were tridecanoic acid, methyl ester (18.6%); 2-hexyldecanoic acid (16.6%); dodecanoic acid (6.2%); and diethyl phthalate (8.9%) among which the first three are saturated fatty acids and its esters cumulatively occupying 41.4% of the total extractable compounds. The fourth compound is a product of oxidation of fatty acids, i.e., diethylester of phthalic acid or benzene dicarboxylic acid, with 50.1% of total moles. Molecular structures of these four compounds are shown in Figure 4. Similar compounds such as hexadecanoic acid, methyl ester; 9,12-octadecadienoic acid (Z, Z)-, methyl ester; and didodecyl phthalate were also present in *M. vaginalis* (Burm. F) C. Presl as reported by Varadharajan and Palani.[23] Besides, the well-known antimicrobial methyl esters of fatty acids including tridecanoic acid, methyl ester, and 2-hexyldecanoic acid, which is an dialkylated branched saturated fatty acid, may be employed as an antibacterial, antimycotic, or antiviral active ingredient to combat seborrheic phenomena, particularly such as dandruff on impure skin, mild forms of acne, or deodorant-preventing deterioration of organic matter, in particular, to cosmetic and dermatological preparations and the infections with Gram-positive and Gram-negative bacteria, fungi, *Mycoplasma*, and viruses.[24] Dodecanoic acid or lauric acid, which is the main fatty acid in coconut oil and palm kernel oil, is a saturated fatty acid with a 12-carbon atom chain, thus having many properties of medium-chain fatty acids, and it is a potential antimicrobial substance, also suitable for the external application. In combination with existing antimicrobial agent gentamicin, it showed synergistic activity against methicillin-resistant *Staphylococcus aureus*.[25] Phthalic acid esters such as diethyl phthalate which is a plasticizer compound and phthalic acid, 6-ethyl-3-octyl isobutyl, also could be used as a potential antimicrobial agent.[26] Most of the other fatty acids and their esters detected in the MS such as tetradecanoic acid or myristic acid; pentadecanoic acid or palmitic acid; methyl 9-methyl-tetra decanoate;[27] and fatty amines such as 2-aminocaprylic acid, n-propoxycarbonyl-, and heptyl ester[28] also have potential antimicrobial activities [Table 4].

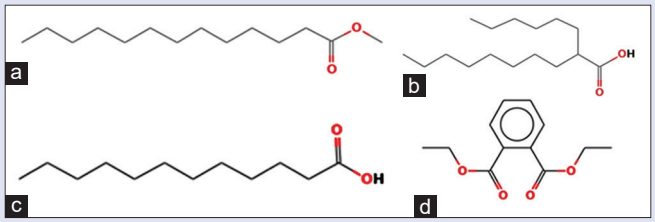

Figure 4: Molecular structures of major compounds. (a) Tridecanoic acid, methyl ester; (b) 2-hexyldecanoic acid; (c) dodecanoic acid; and (d) diethyl phthalate

In this study, ethyl acetate extract which was enriched in short-chain fatty acids and its esters exhibited more inhibition of Gram-positive bacteria than Gram-negative strains. Long-chain unsaturated fatty acids, including linoleic acid, are well known to inhibit Gram-negative bacteria, such as *Escherichia coli*. That kind of relation in the fatty acid sensitivities between Gram-positive and Gram-negative bacteria may be resulted due to hydrophobic interactions with the protein molecules and its interference with membrane permeability of the bacterial strain.[29] The presence of fatty acids and its esters in *M. hastata* (L) Solms is not only important from the pharmacological point of view, but it has also ecological implications for the plant itself. Previous studies suggested that fatty acids and their esters are potential allelochemicals.[30] Several endophytic fungi have been isolated from *M. hastata* (L) Solms as reported by Chowdhury *et al.*[31] Hence, fatty acids in the plant may act as the signal molecules against these endophytes.

CONCLUSION

Green leafy vegetables contain health-protective active principles that are important for free radical scavenging and antioxidant activities. Their antimicrobial efficacy may increase their nutritional and pharmaceutical values. The present study focuses on the scientific validation of the traditional use of aquatic leafy vegetable *M. hastata* (L.) Solms, indicating that it is a repository of antimicrobial compounds in treating bacterial infections. This study may serve as the source of providing valuable information and suitable standards, regarding the best suitable solvent

Table 4: Fatty acids and its esters of ethyl acetate extract

Name of the compound	Chemical formula	Peak number	Retention time (min)	NIST Match Factor: Normal-Forward	Molecular weight (g/mol)	Atom number and unsaturation	Mole (%)
Dodecanoic acid	$C_{12}H_{24}O_2$	5 and 11	18.168 and 21.819	7152 in replib and 9051 in replib	200.3178	C12:0	6.2
Tetradecanoic acid	$C_{14}H_{28}O_2$	7 and 8	20.468 and 20.966	37450 in mainlib and 9042 in replib	228.3709	C14:0	2.6
Methyl 9-methyltetradecanoate	$C_{16}H_{32}O_2$	9	21.345	40693 in mainlib	256.424	C16:0	2.3
2-Aminocaprylic acid, N-propoxycarbonyl-, heptyl ester	$C_8H_{17}NO_2$	10	21.456	162632 in mainlib	159.226	C8:0	3.1
Pentadecanoic acid	$C_{15}H_{30}O_2$	14	22.283	9038 in replib	242.3975	C15:0	0.8
Dodecanoic acid, 10-methyl-, methyl ester	$C_{14}H_{28}O_2$	16	22.646	40682 in mainlib	228.3709	C14:0	1.6
9-Hexadecenoic acid, methyl ester, (Z)	$C_{17}H_{32}O_2$	17 and 19	22.860 and 23.052	4701 in replib	268.43	C17:1	2.7
Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	20	23.120	9818 in replib	228.37	C14:0	18.6
cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	21	23.322	18940 in mainlib	310.522	C20:1	2.7
2-Hexyldecanoic acid	$C_{16}H_{32}O_2$	22	23.586	9295 in replib	256.43	C16:0	16.6
Dodecanoic acid, 2-octyl	$C_{20}H_{40}O_2$	23	23.940	2274 in mainlib	312.538	C20:0	1.4
Trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	24 and 25	25.213 and 25.279	18765 in mainlib	296.4879	C19:1	3.4
Oleic acid	$C_{18}H_{34}O_2$	28	25.897	4486 in replib	282.468	C18:1	1.3

and method components from the particular plant material for further investigations. This is the first reporting on the detection of fatty acids and the related compounds with large content in the ethyl acetate extract of *M. hastata* (L) Solms aerial parts, most of which have antibacterial activities. Therefore, successful isolation, purification, and molecular characterization of the lead molecules might be a crucial step toward the development of a potential broad-spectrum antibacterial drug in the future days.

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Conflicts of interest

There are no conflicts of interest.

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