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Phenolic Profile, Essential Oil Composition and Bioactivity of *Lasia spinosa* (L.) Thwaites

Atiqur Rahman[1,*]
https://orcid.org/0000-0003-4228-1149

Shah Alam Siddiqui[1]
https://orcid.org/0000-0002-1707-6111

Feyza Oke-Altuntas[2]
https://orcid.org/0000-0001-6696-9090

Sezer OKAY[3,4]
https://orcid.org/0000-0003-0355-6672

Fatih GÜL[5]
https://orcid.org/0000-0002-4297-786X

Ibrahim Demirtas[5,*]
https://orcid.org/0000-0001-8946-647X

[1]Islamic University, Department of Applied Chemistry and Chemical Engineering, Kushtia, Bangladesh; [2]Gazi University, Faculty of Science, Department of Biology, Ankara, Turkey; [3]Cankiri Karatekin University, Faculty of Science, Department of Biology, Cankiri, Turkey; [4]Hacettepe University, Vaccine Institute, Ankara, Turkey; [5]Cankiri Karatekin University, Faculty of Science, Department of Chemistry, Cankiri, Turkey.

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***** Correspondence: marahman12@yahoo.com and ibdemirtas@gmail.com; Tel.: ++88-071-74910-20 Ext: 2266

HIGHLIGHTS

- *Lasia spinosa* has been investigated in detailed for phytochemicals.
- It showed broad spectrum of antioxidant properties.
- It showed significant antimicrobial properties.
- *Lasia spinosa* as a potential source of biological active compounds.

Abstract: *Lasia spinosa* (L.) Thwaites is a widely used ethnomedicinal plant in Bangladesh. In this study, we investigated phenolic contents, volatile compounds and fatty acids, and essential oil components of extracts prepared from aerial parts of the plant. The main volatile compounds were methyl ester of oleic acid, palmitic acid and stearic acid as

determined by GC/MS. Phenolic contents of the extracts were determined qualitatively and quantitatively by HPLC/TOF-MS. Six phenolic compounds (syringic acid, morin, gentistic acid, 4-hydroxybenzoic acid, cinnamic acid, and apigenin) were found in the extracts. GC/MS analysis of steam distilled essential oil showed camphor, α-pinene and δ-3-carene as the main constituents. In DPPH radical scavenging assay, the highest free radical scavenging activity was observed for the methanol extract with an IC_{50} value of 0.48 ± 0.04 mg/mL, whereas, in metal chelating activity on ferrous ions (Fe2+) assay, the highest chelating activity was observed for hexane extract (IC_{50} = 0.55 ± 0.08 mg/mL). The extracts and essential oil were tested against five severe human pathogenic bacteria using disc diffusion assay and subsequent MIC values were also determined. All the extracts (except methanol extract) and the essential oil were found to possess potential antimicrobial activity with corresponding inhibition zone and minimum inhibitory concentration (MIC) ranging from 9–23 mm and 62.5-500 µg/mL. This study has been explored the plant *Lasia spinosa* can be seen as a potential source of biologically active compounds.
Keywords: GC/MS; HPLC/TOF-MS; DPPH radical scavenging activity; Metal chelating; MIC.

INTRODUCTION

The chemical agent which can resist oxidation process either by inhibiting free ions or reducing oxidative stress is known as antioxidants [1]. Oxidative stress refers to a state when oxidation balance has been diminished by presence of excess amount of oxidizing agent (e.g., reactive oxygen and/or nitrogen species) and different biomolecules get oxidized. Oxidative stress plays very important role in developing different diseases e.g. coronary heart diseases, cancer and aging [2]. Synthetic antioxidants are being used for decades, but recently, different studies have already shown that the use of these synthetic antioxidants is not safe due to their long run adverse effect e.g. carcinogenesis [3]. So the quest for safer natural antioxidants is increasing day by day.

Again, it has already been well established that the pathogenic bacteria play a vital role in causing different diseases to animals. In every year, a numerous effort has been made to develop new antibiotics but the target bacteria species also develop their resistance to all those new antibacterial shortly. The increasing resistance to existing available antibiotics is now a global concern[4]. The wide spread evolution of multidrug resistant bacteria globally, increasing treatment failure of several fatal diseases [5]. Subsequently, it has become urgent to discover new more effective antimicrobial agent. As plant kingdom is a great source of effective therapeutic agents, so, the careful study of phytochemicals can led to discover some active compounds which can be used as a lead or as a template for synthesizing new antibacterial agent[6].

Plant derived bioactive compounds generally possess great medicinal values as most of them are evolved as chemical protection against various infections [7]. These bioactive compounds are known as secondary metabolites and further classified into different groups e.g., alkaloids, flavonoids, phenols, tannins, terpenes and terpenoids etc. All these bioactive compounds take the vital part to establish plant based ethno-medicinal system in Bangladesh and Southeast Asia as well. *Lasia spinosa* belongs to the Araceae family, is playing a very important role in this traditional medicinal system. Different parts of the plant are used as therapeutic agent in treating different ailments. In various districts of Bangladesh, the plant is used in treating colic, rheumatic pain or inflammation and mal-absorption problems. Corm is found to use in treating larynx infection whereas corm and leaves are applied in the treatment piles[8]. On the other hand, tuber is taken as a remedy of rheumatoid arthritis, constipation, and to purify blood[9]. Although the plant *Lasia spinosa* is extensively used as ethnomedicine, but the scientific investigation to reveal the potential behind it's notable biological properties to public is scarce. Although the antioxidant[10], anti-bacterial and cytotoxicity, antinociceptive, and anticestodal properties of the methanolic leave extract [11] were reported but it is deserved more detailed investigation using

sophisticated research methodology and equipment. This is the first attempt where we have investigated different extracts and essential oil of the aerial parts for their antioxidant properties and examined their inhibitory properties against five severe multi drug resistant human pathogenic bacteria. Moreover, in this study, we have chemically characterized the extracts and essential oil to understand the relationship between the chemical constituents and biological properties of the plants.

MATERIAL AND METHODS

Chemicals and Reagents

Analytical reagent and HPLC gradient grade, anhydrous sodium carbonate, *Folin-Ciocalteu*'s phenol reagent (mixture of phosphomolybdate and phosphotungstate used for the colorimetric in vitro assay of phenolic and polyphenolic antioxidants), methanol manufactured by Merck (Darmstadt, Germany) was used in the experiment. *EDTA* (2,2, 2, 2‴-(Ethane-1,2-diyldinitrilo) tetraacetic acid), *DPPH•* (2,2-Diphenyl-1-picrylhydrazyl), Ferrozine (Ferrozine Disodium Salt), iron (II) chloride ($FeCl_2$), gallic acid (3,4,5-trihydroxybenzoic acid), *BHT* (3,5-Di-tert-butyl-4-hydroxytoluene), dimethylsulphoxide*DMSO*) of Sigma–Aldrich, St. Louis, MO, USA were used in the study. For other chemicals, only the analytical grade chemicals (Sigma and Merck) were used.

Plant Materials

The aerial of *Lasia spinosa* (L.) Thwaites were collected from the local area of Gopalganj, Bangladesh, in March-June and July 2015. The taxonomic identification of the plant was confirmed by Prof. Dr. *M. Oliur Rahman*, Department of Botany, University of Dhaka, Bangladesh and a voucher specimen has been deposited at the Bangladesh National Herbarium (*DACB* 972941). After drying of the fresh aerial parts at room temperature, those were converted into powdered form using grinding mill. After grinding, the samples were sieved using a mechanical sieve shaker to obtain less than 100 µm size of aerial powder and this fine powder was used for further extractions.

Extraction of Essential Oil

Then the essential oil was extracted by steam distillation for 3 hours from (500g) powdered plant material. The yielding percentage was 0.62%(w/w) and the density at 25°C was 0.96 g/ml and the oil was dried over anhydrous Na_2SO4 and preserved in refrigerator in a lightproof bottle. For biological evaluation, essential oil was dissolved in 10% v/v dimethyl sulfoxide (DMSO).

Preparation of Plant Organic Extracts

Powdered aerial parts (300 gram) were used separately for extraction with hexane, chloroform, ethyl acetate and methanol (500 mL × 3 for each) at room temperature for 7 days. Then the solutions were filtered using *Whatman* filter paper No. 1. After filtration the solvents were evaporated using vacuum rotary evaporator at 50°C. These solvent extractions yielded 5.17 g (1.72%) of hexane extract, 7.53 g (2.51%) of chloroform extract, 8.27g (2.76%) of ethyl acetate extract and 11.56 g (3.85%) of methanol extract.

Analyses of Essential Oil by Gas Chromatography-Mass Spectroscopy

The analysis of the essential was carried out using *GC-MS* (Model QP 2010, Shimadzu, Japan) equipped with capillary column of fused silica (*ZB*-1 *MS*).The equipment also had anionization chamber of 70 eV. During analysis, the helium gas was controlled at a rate of 1 mL/min. 220°C and 290°C temperature were maintained at injector and 'transfer line' respectively. Oven temperature was increased at a rate of 3°C/min to obtain 150°C from 50°C. Then the oven temperature was kept controlled at 150°C for next 10 mins. Then the temperature again increased at a rate of 10 °C/min to reach to 250°C. After dilution with methanol (1/100 v/v), 1.0 µl of the samples was inserted manually. The relative proportions of the oil components were shown as percentage by peak area normalization. The oil

components were identified by comparing with the retention indices of n alkane (C8-C20) compounds, computerized comparison with the Wiley 6.0 libraries and comparing with mass spectra patterns available in literature[12].

Volatile Compounds and Fatty Acids Determination by Gas Chromatography/Mass Spectrometry (*GC/MS*)

Volatile compound and volatile fatty acids of hexane extracts were determined by *GC/MS (GC*: Agilent Technologies 7890A and MS: 5975C with Triple-Axis Detector) equipped with a built-in- Autosampler formed with the usage of HP-5 capillary column (30 m × 0.25 mm, 0.25 µm). The equipment also had an ionization chamber of 70 eV. The helium gas was controlled at a rate of 1 mL/min during analysis. Oven temperature was kept controlled at 100°C for 10 minutes and then the temperature was increased at a rate of 3°C/min to achieve 200°C and this temperature was maintained for next 10 mins. Then the temperature was again increased at a rate of at a rate 25°C/min to obtain 270°C and this temperature was kept controlled for next 50 min.

For the analysis, a solution of 15 mg of hexane extract of the plant was mixed in 3 mL of 2 M KOH in MeOH and vortexed rigorously for 3 mins. Then, the separated and esterified fatty acids in hexane phase was dried with sodium sulfate and filtered with a 0.45 µm syringe filter (ChromTech) and injected into GC.

Quantitative Analysis of Phenolics by *HPLC-TOF/MS*

The phenolic quantities of different solvent extracts (chloroform extracts and ethyl acetate extracts) were determined by Infinity High-Performance Liquid Chromatography (Agilent 1260 Infinity) equipped with Time-of-Flight Mass Spectrometry detector (Agilent 6210) and *SB*-C18 column (Agilent Zorbax) (100 mm × 4.6 mm, 3.5 µm). The mobile phase A and B were water with 0.1% formic acid and acetonitrile. The flow rate was 0.6 mL/ min and the column temperature was 35°C. The injection volume was 5µL[13]. The gradient program was as follows: 0–1 min, 10% B; 1–20 mins, 50% B; 20–23 mins, 80% B; 23–25 mins, 10% B; 25–30 mins, 10% B. Total time of evaluation was 30 mins. The Time-of-Flight Mass Spectrometry (*TOF*) was operated in positive ion mode while drying gas was flowed at 10mL/min at 325°C and fragmentor was maintained at 175 V. The analyses were carried out in triplicate.

The standards (phenolic compounds) used were obtained from Sigma. Methanol and formic acid were obtained from Merck (Darmstadt, Germany). For sample analysis, dried crude extracts (200 ppm) were dissolved in methanol at room temperature. Samples were filtered passing through a PTFE (0.45 µm) filter by an injector to remove particulates. Phenolic compound quantification was achieved by the absorbance recorded in the chromatograms relative to external standards, with detection for gentisic acid, 4-hydroxybenzoic acid, syringic acid, morin, cinnamic acid and apigenin. The other compounds were quantified as lower than detection limit.

Determination of *DPPH•* Radical Scavenging Activity Assay

DPPH radical scavenging activity was determined using the method described by *Blois*[14] wherein the bleaching rate of a stable free radical (*DPPH•*) is monitored at a characteristic wavelength in the presence of samples. Briefly, 1 mL of *DPPH•* in methanol 0.004% (w/v) solution was mixed with 1 mL of different concentrations (0.5-2.5 mg/mL) of the extracts (hexane extracts, chloroform extracts, ethyl acetate extracts and methanol extracts) and synthetic antioxidant (BHT). Then the mixtures were shaken vigorously and incubated for 30 minutes at normal room temperature. At 517 nm, the absorbance was measured using a blank. A decrease in absorbance of the *DPPH•* solution indicates an increase in *DPPH•* radical-scavenging activity. To calculate the inhibition percentage (I%), the equation was used as follows:

$I\% = \{(A_{blank} - A_{sample})/ A_{blank} \}\times 100$

Here, A_{blank} denotes the absorbance by the blank solution used and where A_{sample} refers to absorbance by sample solution. The *DPPH•* scavenging ability is expressed as IC_{50} (the

extract concentration required to inhibit 50% of the *DPPH•* in the assay medium). IC_{50} values were calculated based on regression equation, obtained from extract concentrations. Commercially available butylated hydroxytoluene (*BHT*) was taken as positive control in our experiment and the experiment was performed thrice for all of the samples.

Metal Chelating Activity on Ferrous Ions (Fe^{2+})

Metal chelating activity on ferrous ions (Fe^{2+}) was measured according to a slightly modified method described by *Decker and Welch*[15]. Briefly, 2 mM $FeCl_2$ solution (50 µL) was added to 500 µL of different concentrations (0.25-1.25 mg/mL) of the extracts (hexane extracts, chloroform extracts, ethyl acetate extracts and methanol extracts). Then, the reaction was initiated by adding 5 mM ferrozine solution (100 µL). The solution was kept 10 minutes at room temperature and then solution was passed through spectrophotometric measurement at 562 nm. A reaction mixture containing ethanol (500 µL) instead of substance solution served as a control. *EDTA* was used as the chelating standard. Chelating activity was expressed as IC_{50}, the concentration that chelates 50% of Fe^{2+} ions.

Determination of Total Phenolic Content

Total phenolic contents of the different solvent extracts were estimated by a colorimetric assay based on the method described by *Singleton and Rossi*[16], with some modification. In our experiment, gallic acid was taken as standard[17]. 0.1 mL of the extracts (hexane extracts, chloroform extract, ethyl acetate extract, and methanol extract; 1 mg/mL concentration) were added to 0.2 mL of 50% *Folin-Ciocalteu* reagent and it was left for 3 minutes to complete the reaction and then after adding 1 mL aqueous solution of 2% Na_2CO_3, the mixture was incubated over 45 minutes at room temperature. Then solution was passed through spectrophotometric measurement at 760 nm. Gallic acid was tested following same procedures. A standard curve was prepared using 0–100 µg/mLsolutions of gallic acid in ethanol ($y = 0.681x + 0.0096$, $R^2 = 0.9968$). Gallic acid equivalents µg per mg of the sample was used in expressing the total phenolic contents.

Bacterial Strains

Beta-lactamase-producing *Escherichia coli* ATCC 35218 (*EC*), methicillin-resistant *Staphylococcus aureus* (isolate) (*MRSA*), *Klebsiella pneumoniae* ATCC 700603 (*KP*), *Pseudomonas aeruginosa* ATCC 27853 (*PA*) and *Enterococcus faecalis* ATCC 291212 (*EF*) were used in this study. These bacteria were cultured using Nurtient Broth (NB) (Lab M Limited, UK) and Bacteriological Agar No.1 (Lab M Limited, UK) at 37ºC for 24 hours.

Disc Diffusion Assay

Disc diffusion assay was carried out following the method described by *Murray et al*[18], using 100 µL of standardized inoculums suspension containing 10^7*CFU*/mL of bacteria. Test samples i.e. essential oil and solvent extracts were dissolved in 10% v/v dimethyl sulfoxide (DMSO) and extracting solvents respectively, in ratio of 1:5 (v/v) to obtain final concentration of 30 mg/mL and filtered by Millipore filter (0.22µm) for sterilization. Discs (6 mm diameter) made of sterilized *Whatman* No.1 filter paper were impregnated with 10 µL of the sterilized solution containing test samples and applied on the inoculated LB agar medium. Only the specific solvents (used in dissolving samples, for example, DMSO for dissolving oil, methanol for dissolving *MEE*, hexane for dissolving *HEE*, chloroform for dissolving *CEE*, ethyl acetate for dissolving *EAE*) were taken as negative control (/blind control). Tetracycline (30 µg/disc); streptomycin (30 µg/disc); erythromycine (15 µg/disc) sourced from Sigma-Aldrich Co., St. Louis, MO, USA) were employed in the experiment as positive control. Then the micro aerobic incubation of the plates were done at 37ºC over 24 hours. The diameter of inhibition zone was considered as the antibacterial activity of the tested specimens. All the assays were repeated thrice.

Minimum Inhibitory Concentration (MIC)

The method described by *Chandrasekaran and Venkatesalu*[19], was followed in determining *MIC* values of the samples against the tested bacteria. In this assay, a loopful of cells was transferred from the stock culture into NB followed by incubation at 37°C for 24 hours. The samples were mixed with LB medium in a way that different concentrations ranging from 0 to 1000 µg/mL can easily be obtained. The experiment was done by transferring 20 µL inoculums of bacteria strain (10^7*CFU*/mL) to the tube and the final test volume was 2 mL. The same procedure was followed for each type of samples and bacterial strains. On the other hand, for control tube, the test was run using only bacterial strain without samples. After incubation of the culture tubes at 37°C for 24 hours, the growth of the bacterium was observed by microscopic evaluation and the lowest concentration of the sample, which completely inhibited the growth of the bacterium was considered as *MIC* value and denoted in µg/mL.

RESULTS

Analyses of Volatile Organic Compounds and Fatty Acid

The GC-MS chromatograms of volatile compounds and fatty acidsof hexane extract *(HEE)* are shown in the Figure 1 and the corresponding compounds are recorded in (Table 1), in accordance with elution order through *HP*-5 column. The *HEE* was found to comprise of saturated and unsaturated fatty acids, and their ester derivatives with a trace amount of terpenes, polyphenols, and steroids. In total, 40 compounds were identified and the major volatile compounds and fatty acids were methyl ester of oleic acid (49.98%), palmitic acid (26.71%) and stearic acid (10.15%) (Table 1, Fig. 1).

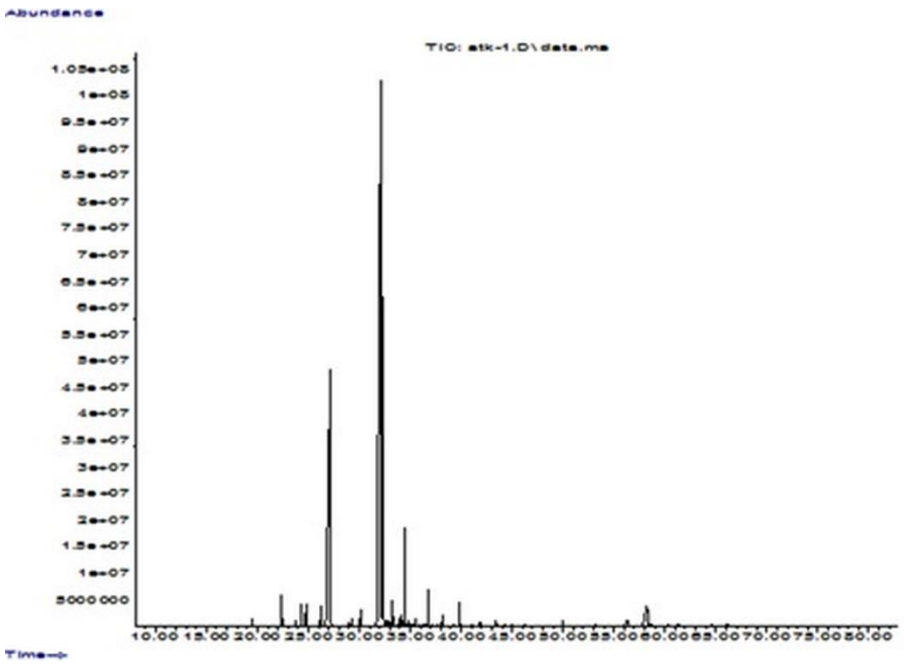

Figure 1: GC-MS chromatogram of volatile compounds and fatty acids of *Lasia spinosa* (L.) Thwaites.

Oleic acid, palmitic acid and stearic acids were found as major fatty acid components in plants of Araceae family and our reports are congruent to those results reported earlier [20]. This study reveals that the unsaturated fatty acids comprised the major portion of the tested extract (Table 1). From literature, it has been apparent that unsaturated fatty acids have a range of beneficial effects on human health [21]. Therefore, unsaturated fatty acids (monounsaturated fatty acids and polyunsaturated fatty acids) as a part of dietary intake

play very important role in maintaining good health. These could help to explain the rationality of ethno-medicinal use of the plant *L. spinosa.*

Table 1. Volatile compounds and fatty acids of *Lasia spinosa* (L.) Thwaites

Sl#	*RT* [a]	Name	*RA* [b](%)
1	17.524	β-Elemene	0.04
2	19.47	Lauric acid, methyl ester	0.12
3	22.389	Myristic acid, methyl ester	0.55
4	23.765	13-Methyltetradec-9-enoic acid methyl ester	0.14
5	24.277	Pentadecanoic acid, methyl ester	0.45
6	24.763	Hexahydrofarnesyl acetone	0.53
7	26.248	Palmitoleic acid, methyl ester	0.88
8	27.171	Palmitic acid, methyl ester	26.71
9	28.907	Palmitic acid, ethyl ester	0.16
10	29.285	10-Heptadecenoic acid, methyl ester	0.31
11	30.141	Margaric acid methyl ester	0.71
12	32.12	Oleic acid, methyl ester	49.98
13	32.355	Stearic acid, methyl ester	10.15
14	32.556	Methyl octadec-6,9-dien-12-ynoate	0.16
15	32.682	Linoleic acid ethyl ester	0.15
16	32.909	Palmitic acid, butyl ester	0.11
17	32.993	Stearic acid, ethyl ester	0.05
18	33.077	Cyclopropaneoctanoic acid,2-octyl-,methyl ester	0.09
19	33.236	10-Nonadecenoic acid, methyl ester	0.31
20	33.353	Nonadecanoic acid, methyl ester	0.15
21	33.941	Epoxyoleic acid	0.18
22	34.117	10,13-Eicosadienoic acid, methyl ester	0.19
23	34.16	11-Eicosenoic acid, methyl ester	0.18
24	34.444	Arachidic acid methyl ester	1.57
25	34.796	Octadecanoic acid, 5-hydroxy-, δ-lactone	0.14
26	35.526	Heneicosanoic acid, methyl ester	0.12
27	36.331	Heptacosane	0.05
28	36.44	13-Docosenoic acid, methyl ester	0.04
29	36.524	α-Glyceryl linolenate	0.04
30	36.751	Behenic acid, methyl ester	0.67
31	38.135	Tricosanoic acid, methyl ester	0.25
32	39.821	Lignoceric acid methyl ester	0.60
33	41.91	Squalene	0.22
34	44.334	Cerotic acid methyl ester	0.07
35	46.28	Hexatriacontane	0.09
36	53.193	Crinosterol	0.10
37	56.271	Campesterol	0.52
38	58.217	Stigmasterol	2.31
39	61.212	γ-Sitosterol	0.18
40	66.103	Spinasterone	0.22
		Total	99.49

[a] Retention time (in min). [b] Relative area

Essential Oil Composition

The leaves of *L. spinosa* afforded yellowish oil and the hydro-distillation yielded 0.62% (w/w) moisture free oil. The compositions of the compounds identified by *GC-MS* analyses

of oil are listed in (Table 2) in accordance of their elution from *ZB*-1 Column. There were six compounds namely α-pinene (68.71%), camphene (3.76%), δ-3-carene (15.19%), caryophyllene (4.98%) limonene (2.13%) and α-selinene (3.23%) and all these six compounds comprised of 98.0% of essential oil (*EO*). α-pinene, camphene, caryophellene, limonene, α-selinene compounds are reported to have numerous pharmacological benefits to human health[22]. All those compounds present in aerial parts of *Lasia spinosa* (L.) Thwaites may have a good correlation to the bioactivities of the plants. The results of the some other studies showed that the terpenes are predominant in the plants of Araceae family[23] and our findings are in line with those reports. As there is no published data available in literature on the essential oil of *L. spinosa,* we are confident that this is the first report on essential oil composition of *L. spinosa*.

Table 2. Essential oil composition of *Lasia spinosa* (L.) Thwaites

Sl#	Compound	*RI* [a]	*RA* [b] (%)	Identification[c]
1	α-Pinene	909	68.71	RI, MS
2	Camphene	927	3.76	RI, MS
3	δ-3-Carene	986	15.19	RI, MS
4	Limonene	1004	2.13	RI, MS
5	Caryophyllene	1399	4.98	RI, MS
6	α-Selinene	1475	3.23	RI, MS

[a] Retention index relative to n-alkanes. [b] Relative area. [c] Identification: MS, comparison of mass spectra with MS libraries; RI, comparison of retention index with bibliography.

Quantitative Analysis of Phenolics and Total Phenolics

HPLC-TOF/MS studies showed that the natural products present in chloroform (*CHE*), and ethyl acetate extract (*EAE*) comprised of fair number of phenolics (Table 3 and 4). Chloroform and ethyl acetate solvent peaks of *L. spinosa* are shown in the chromatograms (Fig. 2).

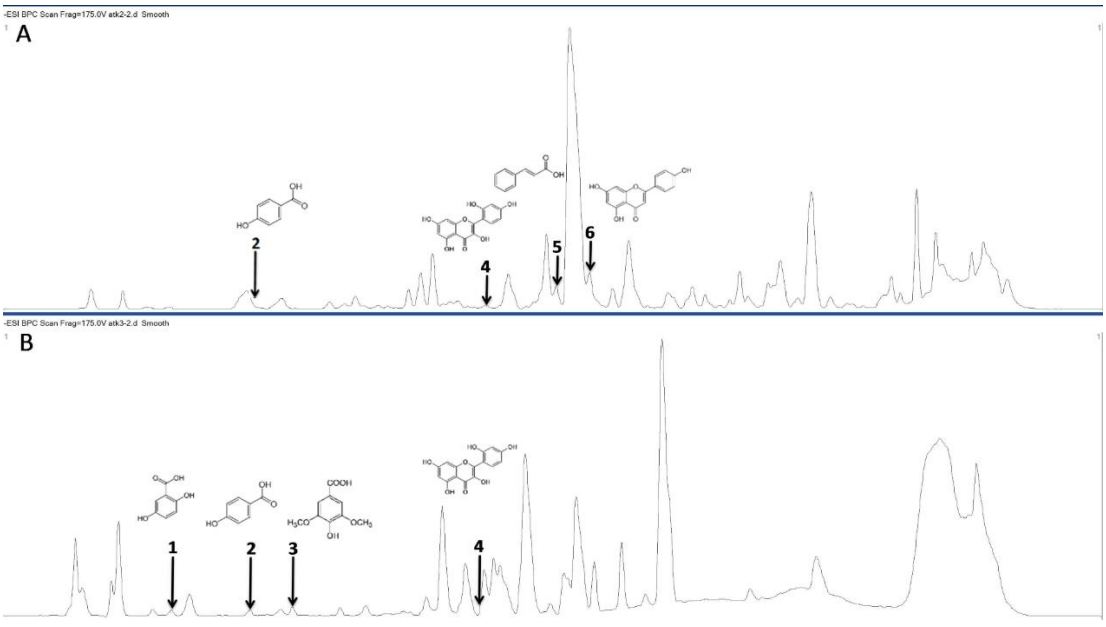

Figure 2: HPLC-TOF/MS chromatograms of phenolics in A) chloroform and B) ethyl acetate solvent extracts of *Lasia spinosa* (L.) Thwaites; the numbers representing the peaks corresponding to different compounds; 1: Gentisic acid; 2: 4-Hydroxybenzoic acid; 3: Syringic acid; 4: Morin; 5: Cinnamic acid; 6: Apigenin

Table 3. Phenolics in chloroform extract of *Lasia spinosa* (L.) Thwaites determined by *HPLC-TOF/MS.*

Sl#.	*RT*[a]	Compound	mg phenolic kg-1 plant
1	6.96	4-Hydroxybenzoic acid	3.81
2	13.01	Morin	19.69
3	15.16	Cinnamic acid	14.37
4	15.64	Apigenin	1.78

[a]Retention time (in min)

Table 4. Phenolics in ethyl acetate extract of *Lasia spinosa* (L.) Thwaites determined by HPLC- TOF/MS.

Sl#	*RT*[a]	Compound	mg phenolic kg-1 plant
1	4.50	Gentisic aid	10.16
2	6.65	4-Hydroxybenzoic acid	3.31
3	8.04	Syringic acid	25.89
4	13.05	Morin	15.79

[a]Retention time (in min)

Both of the extracts found to afford four different compounds (Table 3 and 4) and these compounds were identified by library search and confirmed by their mass. Morin and 4-hydroxybenzoic acid were found in both of *CHE* and *EAE*. Morin was the highest in abundance in *CHE* (19.69%), on the other hand, syringic acid (25.89%) was the highest in abundance in *EAE* of *L. spinosa*. Two other compounds found in *CHE* extract were cinnamic acid and apeginin, whereas, genestic acid was also identified as one of the major components in *EAE*. The Total phenolic content of the extracts were determined and expressed in 'µg GAE/mg extract' (Table 5). The extraction yield of hexane (*HEE*), chloroform (*CHE*), ethyl acetate (*EAE*) and methanol extracts (*MEE*) were (17.23 g kg^{-1}), (25.1 g kg^{-1}), (27.56 g kg^{-1}) and (38.53 g kg^{-1}), respectively, whereas, their total phenolic contents were (12.1 µg GAE/mg extract), (34.4 µg GAE/mg extract), (24.1 µg GAE/mg extract) and (52.4 µg GAE/mg extract) respectively (Table 5).

The identified phenolic compounds in extracts are belongs to two classes of compounds i.e., the phenolic acid and flavonoids. The phenolic acids can be distinguished as derivatives of benzoic acid (4 hydroxy benzoic acid, syringic acid and gentistic acid) and derivatives of cinnamic acid. The hydroxybenzoic acids are components of complex structures[24] and found in small amount in edible plants[25]. The hydroxybenzoic acids, both free and esterified, have not been extensively studied for their bioactivities. On the other hand, the cinnamic acids are more available comparing to the hydroxybenzoic acids and are rarely found in the free form. The compounds morin and apiginin, are the most common flavonoids, frequently occurs in plants, fruits, grains and dietary supplements for human consumption reported to have a diverse range of biological functions e.g. anticancer, antimicrobial and antioxidant activities[26].

Antioxidant Activity

The *DPPH•* radical scavenging activities of the different extracts and standard synthetic antioxidant (BHT) are shown in (Table 5) and expressed in IC_{50} values. The lower IC_{50}value implies the higher scavenging ability. According to the IC_{50} values presented in (Table 5), the DPPH radical scavenging abilities of the extracts of *L. spinosa* were in the order of methanol

extract (MEE) > chloroform extract (CHE) > ethyl acetate extract (EAE)> hexane extract (HEE) ($p < 0.05$).

Table 5. Antioxidant activities of different organic extracts from *Lasia spinosa (L.)* Thwaites*

Sl#	Material	DPPH scavenging IC_{50} (mg/mL)	Metal chelating IC_{50} (mg/mL)	Total phenolic content (µg GAE/mg extract)
1	*HEE*	>2.5	0.55 ± 0.08^{a}	12.1 ± 0.4^{d}
2	*CHE*	1.47 ± 0.01^{c}	0.82 ± 0.02^{b}	34.4 ± 0.3^{b}
3	*EAE*	2.64 ± 0.02^{d}	1.06 ± 0.03^{c}	24.1 ± 2.0^{c}
4	*MEE*	0.48 ± 0.04^{b}	1.00 ± 0.01^{d}	52.4 ± 0.6^{a}
5	*BHT*	0.02 ± 0.00^{a}	NS	NS
6	EDTA	NS	< 0.25	NS

**HEE*, hexane extract; *CHE*, chloroform extract; *EAE*, ethyl acetate extract; *MEE*, methanol extract; *BHT, butylated hydroxytoluene;* Values represent averages ± standard deviations for triplicate experiments. Values in the same column with different superscripts are significantly ($p < 0.05$) different, NS: Not studied.

MEE extracts showed the highest activity among all the extracts with IC_{50} values of 0.48 ± 0.04 mg/mL, whereas, compared to the activity of synthetic antioxidant *BHT*, *MEE* had moderate lower scavenging activity. Figure 3, shows the dose-response curves representing the abilities of the extracts to scavenge *DPPH•* radicals. *DPPH•* radical scavenging abilities were increased with the increased concentration of the test samples. The *MEE* exhibited 81% scavenging activity at the concentration of 1 mg/mL and our result is consistent with that of Mahato *et al*.,[27],who investigated the methanol extract of *L. spinosa* for free radical scavenging activities and found moderate antioxidant activity.

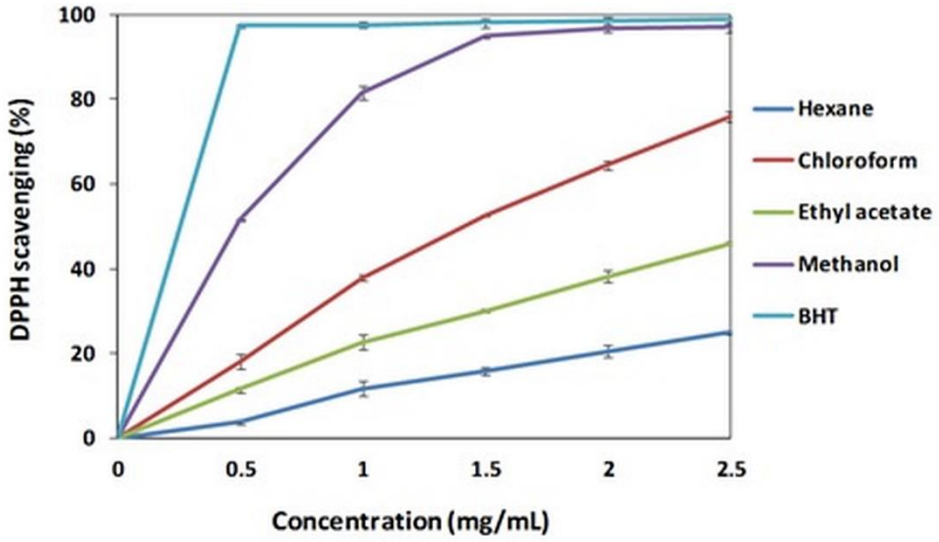

Figure 3: DPPH scavenging activity of *Lasia spinosa* (L.) Thwaites extracts at tested concentrations.

Phenolic compounds are reported as strong antioxidant compounds in different studies[28]. In this study, the tested extracts are found to afford 'total phenolic content' ranging from 12.1 to 52.4 µg GAE/mg (Table 5). In this study, a significant correlation ($p < 0.01$) was found between total phenolic content and *DPPH•* scavenging activity that indicating that phenolic compounds were primarily responsible for this activity. The results of our study is in good agreement to that of *Maisuthisakul et al*. [29], who observed a positive correlation between *DPPH•* activities and total phenolic contents of some Thai plants including *L. spinosa*.

The capacity of chelating transition metal ion e.g., Fe^{2+}generally denotes the antioxidant ability of an extract or compound and in this study, we investigated chelating ability of different extracts of *Lasia spinosa* (L.) Thwaites by ferrozine assay. In this experiment, ferrozine produces complexes in combination with Fe^{2+} quantitively and the existence of other chelating agents generally interrupt the complex producing process and thus diminish the intensity of red color of complexes. By measuring the reduction of the intensity of the red color, the chelating activity of the coexisting chelator can be estimated[30]. In our study, the highest chelating activity was observed for the hexane extract (IC_{50}= 0.55 ± 0.08 mg/mL). Generally, the unique phenolic structure and the number of hydroxyl groups of an antioxidant compound determine its free radical scavenging and metal ion chelating ability [31]. From phenolic compounds' lists identified in this study, it is apparent that phenolic compounds from *L. spinosa* contained dihydroxy groups, which have the ability to conjugate with transition metals, therefore inhibiting the production of the metal-induced free radicals.

Antibacterial Activity

The antibacterial activities of the essential oil (*EO*) and different extracts of *L. spinosa* are shown in (Table 6). The *EO* showed potent efficacy against all five human pathogens. The *EO* inhibited strongly the growth of *Escherichia coli* ATCC 35218 (*EC*), methicillin-resistant *Staphylococcus aureus* (*MRSA*), *Klebsiella pneumoniae* ATCC 700603 (*KP*) and *Pseudomonas aeruginosa* ATCC 27853 (*PA*) with the corresponding zones of inhibition ranging from 17–23 mm, while, moderate efficacy was noticed against *Enterococcus faecalis* ATCC 291212 (*EF*) and the respective inhibition zone was 13 mm. On the other hand, *EAE* exhibited promising antibacterial activities against all of the tested bacteria with inhibition zone ranging from 15-22 mm. *CHE* exhibited high to moderate efficacy against *EC*, *MRSA*, *KP*, *EF* (inhibition zones ranging from 16 to 14 mm), and somewhat weak activity against *PA* (inhibition zones 12 mm). *HEE* also showed moderately weak activity in inhibiting the growth of all the tested organisms except *PA*. *MEE* was found inactive against *EC, KP EF* and showed very weak activities against *MRSA* and *PA.* From the assays, it has been observed that the efficacy showed by *EO* and *EAE* against the tested organisms is similar and comparable to that of streptomycin (*ST*), whereas, the efficacy showed by tetracycline (*TE*) was stronger almost in every case (Table 6). On the other hand, the efficacy of erythromycin (*ET*) found higher only against *MRSA* and *PA* but was lower against all other tested pathogen comparing to the activities of *EO* and *EAE*. The negative controls (/blind controls) showed no activity against the tested bacteria.

Table 6. Antibacterial activity of essential oil and organic extracts of *Lasia spinosa* (L.) Thwaites

Bacteria	**Zone of growth inhibition (in mm)** [a]							
	Organic Extracts					**Standard antibiotics**		
	HE	***CHE***	***MEE***	***EAE***	***EO***	***TE***	***ST***	***ER***
EC	10 ± 1.1	15 ± 0.9	-	20 ± 0.7	17 ± 1.1	24 ± 0.7	16 ± 0.5	13 ± 0.9
MRSA	9 ± 0.9	14 ± 1.7	7± 0.5	22 ± 1.1	23 ± 0.9	29 ± 1.1	23 ± 0.5	25 ± 0.7
KP	11 ± 0.5	16 ± 0.3	-	15 ± 0.9	17 ± 0.5	13 ± 0.5	16 ± 0.9	10 ± 0.5
PA	-	12 ± 0.1	5± 0.1	15 ± 0.7	21 ± 1.2	32 ± 1.5	32 ± 1.2	25 ± 1.2
EF	10 ± 0.5	14 ± 1.5	-	18 ± 1.3	13 ± 0.5	12 ± 0.6	18 ± 1.1	12 ± 0.5

[a]Values are given as mean ± S.D of triplicate experiment. [a] Diameter of inhibition zones (mm) of the compounds around the discs (6 mm) impregnated with 10/15 µL/disc corresponding to 300 µg/disc. *HEE*, Hexane extract; *CHE*, Choloroform extract; *MEE*, Methanol extract; *EAE*, Ethyl acetate extract; *EO*, essential oil. Standard antibiotics: *TE*, Tetracycline (30 µg/disc); *ST*, streptomycin (30 µg/disc); *ER*, erythromycine (15 µg/disc). Bacteria: *EC*, *Escherichia coli* ATCC 35218; *MRSA*, methicillin-resistant *Staphylococcus aureus* (isolate); *KP*, *Klebsiella pneumoniae* ATCC 700603; *PA*, *Pseudomonas aeruginosa* ATCC 27853; *EF*, *Enterococcus faecalis* ATCC 291212.

From the minimum inhibitory concentration (*MIC*) assay, it had been observed that the *MRSA*, *KP*, and *PA* strain were very much susceptible to *EO* with lowest *MIC* (62.5–125

µg/mL) values (Table 7) whereas, *EC* and *EF* were found less susceptible to the *EO* with *MIC* values (250 µg/mL). Besides, the *MIC* values of *EAE* and *CHE* were found in the range of (62.5–250 µg/mL). The *MIC* values observed for *EAE* were lowest comparing to that of other extracts indicating stronger efficacy against the tested strains (Table 7). No significant MIC values were found for *MEE* against tested organisms. Excluding methanol extract, the antibacterial activity of the *HEE* was lowest among the tested specimen (extracts and essential oils) (Table 7).

From the assays it has been appeared that *EO* and solvent extracts of *L. spinosa* had very promising effect against the tested human pathogens. The antibacterial activity of *EO* may be associated with the contribution of the monoterpene, α-pinene, which was found to present as the predominant compound in essential oil comparing to other volatile compounds (camphene, δ-3-carene, caryophyllene), and is well known for antibacterial activity [32]. The antibacterial efficacy of some other individual components e.g. camphene, limonene, caryophyllene has already been established [22](22). The presence of all these compounds as major components may enable essential oil to show promising antibacterial activity. Moreover, other minor compounds may also take part directly in antibacterial activity or some short of synergistic effect among all components might also be involved [33].

Table 7. Minimum inhibitory concentration (*MIC*) values of essential oil and organic extracts of *Lasia spinosa* (L.) Thwaites

Bacteria	Minimum Inhibitory Concentration (*MIC*) Organic Extracts (µg/mL)				
	HEE	***CHE***	***MEE***	***EAE***	***EO***
EC	500	125	-	62.5	250
MRSA	500	125	1000	62.5	62.5
KP	250	125	-	250	125
PA	-	250	-	125	62.5
EF	500	250	-	125	250

HEE, Hexane; *CHE*, Choloroform; *EAE*, Ethyl acetate; *EO*, essential oil; Bacteria: *EC,Escherichia coli* ATCC 35218; *MRSA*, Methicillin-resistant *Staphylococcus aureus* (isolate); *KP*, *Klebsiella pneumoniae* ATCC 700603; *PA*, *Pseudomonas aeruginosa* ATCC 27853; *EF*, *Enterococcus faecalis* ATCC 291212

From the quantitative analysis, it has been noticed that the plant extracts were rich in phenolic compounds such as phenolic acids and flavonoids compounds. Phenolic compound possess potent antibacterial activity [34]. All the identified phenolic acids (Table 3 and 4) like hydroxy benzoic acid, syringic acid, cinnamic acid, gentistic acid were reported to have potent antimicrobial activities [35]. Again, several flavonoids including apigenin, flavones (morin) have been shown to possess potent antibacterial activity[36]. Moreover, polyphenols found to play very important role against some antibiotic resistant bacteria strains e.g., *Staphylococcus aureus* resistant to methicillin, *Enterococci* resistant to glycopeptide antibiotics and vancomycin, *Pneumococci* resistant to β-lactam and macrolides, and *Pseudomonas aeruginosa* with its defense mechanism against phagocytic activity of polymorphonuclear leucocytes [34]. The mode of action of polyphenols on cell membranes makes those compounds active against bacteria [37]. So we can say that the exhibited antibacterial activity of solvent extracts of *L. spinosa* is due to the presence of several types of compounds such as flavonoids and phenolic acids in those extracts.

CONCLUSION

Here we have investigated the phytochemicals of a widely used ethnomedicinal plant *L. spinosa* and their usefulness in curbing two global concern i.e., antibacterial activity and bane effect of synthetic antioxidant. Essential oil and different solvent extracts of *L. spinosa* showed important antibacterial activity against important human pathogens and prominent antioxidant effect due to the presence of potential phytochemicals as explored in this study.

Our results can also be seen as scientific support for the traditional and folkloric usage of *L. spinosa* in Bangladesh for the treatment of different ailment and provide opportunities to explore this plant as source bioactive compounds for biochemical and pharmaceutical industries.

Conflicts of Interest: The authors declare no conflict of interest.

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