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Research Article

Chemical Constituents and *in vitro* Antibacterial Activity of Fixed Oils from Different Parts of *Bridelia stipularis* (L.) Blume

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Abstract

Background and Objective: Fixed oils used in traditional therapies also called volatile oils are generally aromatic oils obtained by the steam or hydrodistillation of plants. Different parts of plants have been used to obtain fixed oils. This study estimates the chemical constituents and *in vitro* antibacterial activity of fixed oils extracted by petroleum ether from the leaves, roots, stems and fruit part of *Bridelia stipularis* (L.). **Materials and Methods:** The natural fatty acids were extracted from different parts of *B. stipularis* by using petroleum ether. The fixed oils were studied by gas chromatography-mass spectrophotometry. The antibacterial test was carried out by the agar disc diffusion method. A Student's t-test was computed for the statistical significance of the results. **Results:** It showed 10 compounds from the leaf and 5 compounds from the stem. In both cases, the major components were methyl decanoate 93.56 and 74.98%, respectively. From the root parts, 6 compounds were identified in which the major compound was methyl linolelaidate (36.86%). Two compounds were identified from the fruit part and the major portion was methyl pentadecanoate (98.20%). The *in vitro* antibacterial potentials of the oils were tested against four pathogenic bacteria. Among the four fixed oils, the stem, leaf and root showed the strongest activity against *E. coli* (30, 21 and 15 mm). On the other hand, fruit fixed oil showed the highest zone of inhibition against *Bacillus cereus* (25 mm). **Conclusion:** The fixed oils of *B. stipularis* plant have the potential to be applied as an antibacterial agent, which can be selected for further analysis and can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals that address unmet therapeutic needs.

Key words: *Bridelia stipularis*, petroleum ether, fixed oil, GC-MS, antibacterial activity, zone of inhibition

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant-based natural products are the active ingredients of the ancient arts of healing practiced around the world. These can be obtained by extracting the leaves, barks, flowers, or any other such part of the medicinal herbs¹. The active ingredients of novel pharmaceuticals and health-care products in the world market have been discovered mainly from the medicinal plants². Due to the current changes in world climate and economy, various medicinal herbs in the world face the threat of losing genetic diversity or extinction³. Ayurveda, Chinese medicine and other ancient practices in different parts of the world make use of the therapeutic values of plants for many different diseases. Plants in the Phyllanthaceae family produce naphthoquinones, pyrrolizidines, phenols, flavonoids and many other pharmacologically active compound classes. The herb has been used as a medication⁴, to treat leucoderma, stranguria, constipation, diarrhea, amoebic dysentery and chest pain^{5,6}. Additionally, a number of pharmaceutically significant components, including proteins, carbohydrates, phytochemicals, vitamins, alkaloids, flavonoids, steroids and phenolic molecules^{3,7} have been extracted from various portions of this plant. The different parts of these plants possess a significant number of pharmacological and biological activities. The biological activities of the components indicated anti-inflammatory, antitumor, antiplatelet, antiviral, cardiogenic, prostaglandin, contraceptive and wound healing properties⁸.

A woody, climbing shrub with the common name "Pat Khoi" in Bangladesh, *B. stipularis* (L.) Blume (Family: Phyllanthaceae) is found widely throughout South Asian nations such as Bangladesh, India, China, Malaysia, Indonesia, Nepal, Myanmar, Vietnam and Sri Lanka⁹. It is a fruit-producing plant. Fruits are little (8 mm in length and 5-8 mm in diameter); they turn black when ripe and taste like currants. Coughs, fevers and jaundice are all treated using a decoction made from leaves and bark¹⁰. In addition to being well-known providers of nutrients, such as dietary fiber, vitamins and minerals, wild and underused fruits are also rich in bioactive compounds¹¹.

Bridelia stipularis (L.) has previously been the subject of some phytochemical studies in nations like Pakistan, India, Thailand, etc., with an emphasis on the fruit component. However, to date, no thorough phytochemical screening of extracts from various plant components has been carried out in the Bangladesh region. After all, Bangladesh differs from other nations both physically and geologically. This plant

produces non-identical plant metabolites in different geographic locations, which can be related to physiological variations in plants brought on by changes in geographic, climatic and environmental conditions. The chemical compositions and the antibacterial properties of fixed oils from various plant parts were investigated in light of these findings and therapeutic significance.

MATERIALS AND METHODS

Study area: The study was carried out at Chemical Research Division, BCSIR, Dhaka, Bangladesh since May, 2021 until the data were fully collected.

Collection of plant material: Fully matured fresh fruits, roots, stems and leaves of *B. stipularis* (L.) Blume's were collected from the university's botanical garden in Jahangir Nagar, Dhaka. A voucher specimen (No. = 47695) of the species was placed at the Bangladesh National Herbarium in Dhaka and was obtained in Bangladesh in the month of April, 2021.

Preparation of sample: The mature leaves, roots, stems and fruits of *B. stipularis* were gathered and properly cleaned under running water to remove dirt and also other dust. Then, to make it suitable for grinding, it was dried in an oven at a lower temperature less than 45°C. After screening, the powder was designated for subsequent investigations and kept in an inaccessible container (about 20 mesh).

Extraction of fatty acids and preparation of methyl esters

(FAMES): The fruits, roots, stem and leaves of *B. stipularis* were used to isolate the natural fatty acids. Petroleum ether (bp 40-60°C) was combined with 100 g of the powdered plant samples from each plant part and the mixture was then placed in a Soxhlet device (ABTD250, Henan, China) for 72 hrs. The extracts were first concentrated under reduced pressure in a rotary evaporator (EYELA N-1000, Japan) and after that, they were filtered through Whatman No. 1 filter paper. Finally, vacuum distillation was performed on the extracts to completely remove the solvent. The extracts from *B. stipularis* (L.) leaves (9.92 g), roots (0.56 g), stems (1.50 g) and fruits (5.50 g) were stored in a refrigerator with a nitrogen environment. The fatty acids in the extract were first converted into fatty acid methyl esters (FAMES), which were then analyzed by GC-MS (Model QP 2010, Shimadzu, Japan)

according to Griffin's method^{12,13}. By analyzing the methyl esters it contained, the fatty acid composition was investigated. According to the AOAC method¹⁴, the esterification reaction using the BF₃-MeOH complex produced the fatty acid methyl esters (FAMES). In a screw-capped glass tube, separate 10 mg extracts of the leaves, roots, stems and fruits of *B. stipularis* were taken. After diluting the BF₃-MeOH complex to 1 mL, the liquid was heated in a water bath (HH-S1, Jiangsu, China) at 100°C for an hour. It was then given 2 mL of hexane and 1 mL of deionized water after cooling to room temperature. After properly vortexing the glass tube, it was centrifuged for 2 min at a low RPM. The top layer was removed using a syringe and it was then placed in a fairly airtight glass vial and kept in the refrigerator. After that, the produced FAMES were then prepared for analysis.

Gas chromatograph-mass spectrum analysis: Gas chromatograph-mass spectrum analysis of the fixed oil from different plant parts was performed using an Agilent 7890A system with a split-less injection system and a mass spectrometer detector. A capillary column model HP-5MS was installed in the GC (30×0.25 mm, film thickness: 0.25 μm). The following were the components of the temperature program: Initial oven temperature was 70°C, rose to 150°C for 5 min at a rate of 10°C per minute, then 200°C for 15 min at a rate of 12°C per minute and lastly 220°C for 15 min. The injector temperature was set at 260°C. As the carrier gas, helium was used at a flow rate of 0.6 mL min⁻¹ at a pressure of 17.69 psi. A 1 μL aliquot of the sample's methanol-dissolved solution was automatically injected. The MS was in the scanning mode. Electrons were ionized during the ionization. Between 50 and 550 m/z was chosen as the mass range. In NIST libraries, fatty acid compositions were identified using MS spectra of separated components.

Antibacterial activity assay: The antibacterial test was carried out by Agar Disc Diffusion method¹⁵ using 100 mL of standardized inoculum suspension containing 10⁷ CFU mL⁻¹ of bacteria. The Institute of Food Science and Technology (IFST), BCSIR, Dhaka, Bangladesh, provided all microorganisms as pure cultures. The fixed oil was diluted 1:5 (v/v) with methanol and aliquots of 5 μL were spotted onto the filter paper discs, while 10 μL of 30 μg μL⁻¹ of each organic extract (300 μg/disc) was applied on the filter paper discs (6 mm diameter) and placed on the inoculated LB agar. Negative controls were prepared using the same solvents employed to

dissolve the samples. Streptomycin (Sigma-Aldrich Co., St. Louis, Missouri, USA) was used as Standard Reference antibiotics for the tested bacteria. The plates were incubated at 37°C for 24 hrs. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested bacteria.

Statistical analysis: Each experiment was run in triplicate and mean values were calculated. A Student's t-test was computed for the statistical significance of the results.

RESULTS

Investigation of fatty acid by GC-MS: Fatty acids were extracted from the leaves, roots, stems and fruits of *B. stipularis* and GC-MS analysis of the fatty acids revealed the presence of 10 compounds for the leaves, 6 compounds for the root portion, 5 compounds for the stem portion and 2 compounds for the fruit component. The results of the GC analysis were provided in Table 1-4, which comprise the active components and their retention durations as well as the fatty acids extracted from the leaves, roots, stems and fruits of *B. stipularis* from petroleum ether extract. It showed that all contained both saturated and unsaturated fatty acids as shown in Fig. 1.

In vitro antibacterial activity assay: The *in vitro* antibacterial activity of the leaves, roots, stems and fruits of *B. stipularis* (L.) Blume fixed oil was done by four different bacteria (Fig. 2). Table 5 illustrated the antibacterial activity results, which plainly demonstrate that all of the fixed oil from various plant sections has antibacterial activity. The appearance or non-appearance of an inhibition zone was favored to appreciate the *in vitro* antibacterial resistivity of *B. stipularis* (L.) Blume roots, leaves, stems and fruits fixed oil against four pathogenic bacteria. Four pathogenic bacteria were used in this study, named, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus cereus* and *Escherichia coli*. The largest inhibitory zone (30 mm) was noticed for the case of stem fixed oil against *E. coli*. Subsequently, against *E. coli*, the leaf part showed a strong zone of inhibition (21 mm), followed by the root part showing a near-proper zone of inhibition (15 mm) against the gram-negative bacteria. In the case of fruit fixed oil, *Bacillus cereus* exhibited the maximum zone of inhibition (25 mm). With leaf-fixed oil, *Staphylococcus aureus* was subjected to the strongest zone of inhibition (20 mm).

Table 1: Fatty acid analysis using GC-MS on petroleum ether extract of *B. stipularis* (L.) Blume leaves

Retention time (min)	Name of the compound	Molecular weight	Molecular formula	Concentration (%)
7.67	Methyl decanoate	186.29	C ₁₂ H ₂₄ O ₂	93.56
13.85	Trans-9-elaidic acid methyl ester	282.5	C ₁₈ H ₃₄ O ₂	0.16
14.27	Linolelaidic acid methyl ester	294.5	C ₁₉ H ₃₄ O ₂	2.87
16.09	Methyl eicosanoate	326.6	C ₂₁ H ₄₂ O ₂	0.67
16.45	Cis-11,14-eicosadienoic acid ester	322.5	C ₂₁ H ₃₈ O ₂	0.47
17.39	Cis-8,11,14 eicosadienoic acid ester	320.51	C ₂₁ H ₃₆ O ₂	1.01
19.85	Cis-13,16-decanoate	350.6	C ₂₃ H ₄₂ O ₂	0.06
20.04	Methyl tricosanoate	368.6	C ₂₄ H ₄₂ O ₂	0.48
21.49	Methyl lignocerate	382.7	C ₂₅ H ₅₀ O ₂	0.19
21.98	Methyl nervonate	380.65	C ₂₅ H ₄₈ O ₂	0.52

Table 2: Fatty acid study using GC-MS of a petroleum ether extract of *B. stipularis* (L.) Blume roots

Retention time (min)	Name of the compound	Molecular weight	Molecular formula	Concentration (%)
11.06	Methyl palmitate	270.4	C ₁₇ H ₃₄ O ₂	8.56
13.85	Trans-9-elaidic acid methyl ester	282.5	C ₁₈ H ₃₄ O ₂	7.20
14.27	Methyl linolelaidate	294.5	C ₁₉ H ₃₄ O ₂	36.86
15.38	Methyl eicosanoate	326.6	C ₂₁ H ₄₂ O ₂	13.52
19.85	Cis-13,16-docosadienoic acid methyl ester	350.6	C ₂₃ H ₄₂ O ₂	5.45
20.04	Methyl tricosanoate	368.6	C ₂₄ H ₄₂ O ₂	28.48

Table 3: Fatty acid analysis using GC-MS of the stem of *B. stipularis* (L.) Blume's petroleum ether extract

Retention time (min)	Name of the compound	Molecular weight	Molecular formula	Concentration (%)
7.67	Methyl decanoate	186.4	C ₁₂ H ₂₄ O ₂	74.98
13.98	Trans-9-elaidic acid methyl ester	282.5	C ₁₈ H ₃₄ O ₂	2.37
14.28	Linolelaidic acid methyl ester	294.6	C ₁₉ H ₃₄ O ₂	17.69
19.85	Cis-13,16-docosadienoic acid methyl ester	350.6	C ₂₃ H ₄₂ O ₂	1.88
21.49	Methyl lignocerate	382.7	C ₂₅ H ₅₀ O ₂	3.08

Table 4: Fatty acid study using GC-MS on a petroleum ether extract of *B. stipularis* (L.) Blume's fruit

Retention time (min)	Name of the compound	Molecular weight	Molecular formula	Concentration (%)
10.73	Methyl pentadecanoate	186.29	C ₁₁ H ₂₂ O ₂	98.19
14.28	Linolelaidic acid ester	294.6	C ₁₉ H ₃₄ O ₂	1.9

Table 5: Circle of inhibition measurements of fatty oils from different plant parts of *B. stipularis* (L.) Blume

Name of bacteria	Leaf (a) (mm)	Root (b) (mm)	Fruit (c) (mm)	Stem (d) (mm)
<i>Bacillus cereus</i> (Gram +)	18	12	25	10
<i>Staphylococcus aureus</i> (Gram +)	20	14	15	8
<i>Escherichia coli</i> (Gram -)	21	15	5	30
<i>Salmonella typhi</i> (Gram -)	17	13	15	15

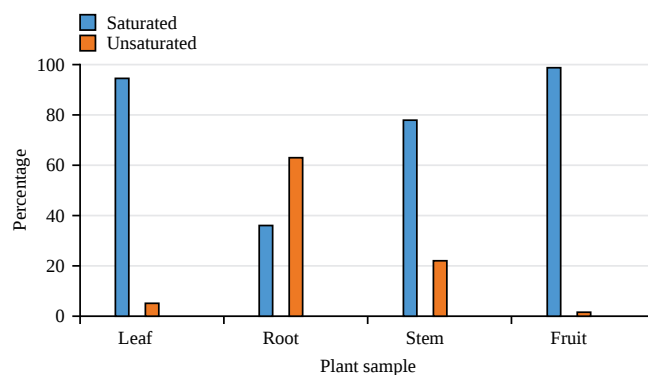


Fig. 1: Comparison of saturated and unsaturated fatty acid of *B. stipularis* (L.) Blume's various sections

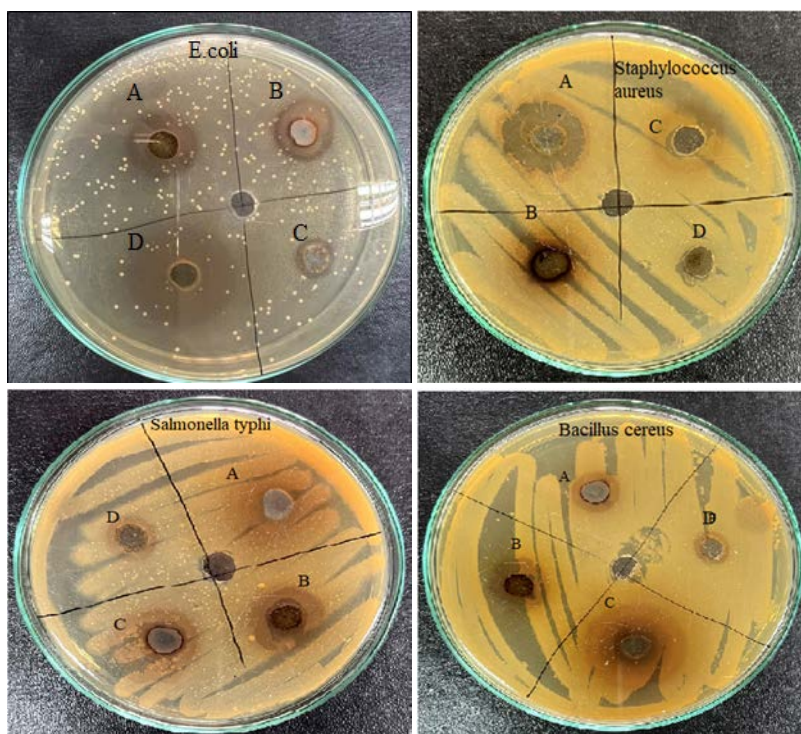


Fig. 2: *In vitro* antibacterial activity of fixed oil of leaves, roots, stems and fruits of *B. stipularis* (L.) Blume

A: Leaf, B: Root, C: Fruit and D: Stem

DISCUSSION

The evaluation of fatty acids by petroleum ether solvent extraction of leaves, roots, stems and fruits have been done. The leaf portions contain 94.7 and 5.29%, respectively, root 37.04 and 63.07%, respectively and stem 78.06 and 21.94%, respectively and fruit 1.9 and 98.95%, respectively. The role of impregnated fats in cardiovascular health is crucial. For calcium to be efficiently absorbed into bone, they are necessary. The liver is protected by saturated fatty acids from alcohol and pharmaceuticals, such as acetaminophen and other regularly used pain killers and anti-inflammatory drugs¹⁶. Some saturated fatty acids, particularly those found in plants; act as metabolic signaling messengers, carrying out vital functions like the proper release of insulin. White blood cells' capacity to identify and eradicate foreign invaders like viruses, bacteria and fungus is hampered by a decrease in adequate saturated fatty acids¹⁴. Additionally, the risk of getting diabetes is reduced by unsaturated fatty acids. Unsaturated fat facilitates the absorption of several fat-soluble vitamins, such as vitamins A, D, E and K, through the intestinal wall. They are the primary sources of dietary energy. Eating a lot of protein and unsaturated fat is good for the health of the brain.

The most significant originality of the study was the finding that methyl decanoate (93.59%) and methyl pentadecanoate (98.95%) are the main components in the petroleum ether extract of the leaf and stem, respectively, while methyl linolelaidate (36.86%) and methyl pentadecanoate are the main components of the root and fruit, respectively. A medium-chain saturated fatty acid called methyl decanoate is present in several species. It is widely used in food additives, soaps, cosmetics, vitamins and dietetics. Nuclear receptor PPAR-gamma, also known as the peroxisome proliferator-activated receptor that controls lipid and glucose metabolism, has been shown to directly bind to and partially activate decanoic acid, without triggering adipogenesis^{17,18}.

Methyl pentadecanoate and methyl linolelaidate are examples of polyunsaturated fatty acids (PUAFs). For perfect health, PUAFs are necessary. The amount of PUAFs found in serum and erythrocyte phospho-lipids, which is dependent on endogenous metabolism managed by genetic polymorphisms and dietary intake, is a crucial component in defining both health and disease. Although PUAFs have a reputation for being cardioprotective, they may also have anti-arrhythmic, anti-inflammatory, lipid-lowering and anti-hypersensitive properties¹⁹.

In general, communicable diseases are the main cause of bad news and transience. Currently, the ongoing battle against germs and fungi prevails thanks to the increasing confidence of the resistance. However, advancements in the medical sector result in more people suffering from severe, resistant illnesses, necessitating the constant creation of new antibiotics and antifungal medications. Therefore, the time is right to discover new microbicidal medications^{20,21}.

Among the four fixed oils from petroleum ether extract, the stem, leaf and root showed the highest zone of inhibition against *E. coli* (30, 21 and 15 mm), while fruit fixed oil showed the maximal zone of inhibition against *Bacillus cereus* (25 mm) at 300 µg mL⁻¹ concentration, respectively. The lowest zone of inhibition was found in the case of fruit fixed oil in a zone of inhibition of 5 mm against *E. coli*. While *B. stipularis* (L.) Blume was reported by Anjum *et al.*⁹, to exhibit mild to high activity in the region of inhibition between ranges of 11.9 and 23.0 mm. The most potent effect was shown by the methanolic extract of the stem bark, which had an inhibition zone measuring 23.0 mm against *S. aureus*.

The aforementioned discoveries make it clear that the fixed oils of the plant's leaf, root, stem and fruit may have antibacterial properties. In general, little is known about how microbes survive and how antimicrobial agents work and these topics are still up for debate. However, the chemical components of this fixed oil may play a causative part in the in vitro prevention of bacterial infections. However, this distinct scientific knowledge can act as a crucial foundation for the development of natural medicines that are both safe and efficient. Nowadays, natural medicines are an interesting subject for the therapeutic industry of important recuperation²².

CONCLUSION

The major findings of the inquiry are that fatty acid constituents of various parts of the plant have the ability to kill the bacteria. Also, the existence of an eminent amount of various fatty acids indicates the effectiveness of the use of the plant in traditional medicine. Because of the plant's diverse biological activity and limited research on *B. stipularis* (L.) Blume, more research is needed to isolate promising bioactive compounds from this plant.

SIGNIFICANCE STATEMENT

The research was accomplished to open a new era in improving traditional medicine with modern microbiology.

The encouraging outcome of this investigation may bring about the different parts of *B. stipularis* would be a prospective source of antibiotics. This is not to claim that the assays' results are conclusive; they may be the first stage in a considerable process that will ultimately lead the new researchers to the selection of more valuable substances in the plant.

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