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Original Article

Bioassay-Guided Isolation and Characterization of the Antibacterial Compound from *Sonneratia apetala* Buch. Ham Leaves Collected from the Maharashtra Coast of India

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Abstract

Background and Aim: *Sonneratia apetala* plant has been used in traditional medicine in south Asian countries for treating diarrhea, hepatitis, inflammation, wounds and ulcers. The leaf extract of this plant has shown promising antibacterial activity and it is essential to isolate that antibacterial compound in pure form.
Materials and Methods: In this study, a bioassay-guided isolation and characterization approach was taken to isolate and characterize the antibacterial compound from the *S. apetala* Buch. Ham leaf. Petroleum ether, acetone and water extracts were prepared and tested against eight human pathogenic bacteria. Water and acetone extracts have shown the inhibition of bacteria but the more promising acetone extract was carried forward for further study. The compound was isolated using the preparative column chromatography method. The isolated compound was studied for antibacterial activity using TLC-bioautography. Further characterization was done using the UV-Vis spectroscopy and FT-IR spectroscopy.
Results: The findings of the present study showed the presence of various valuable phytochemical constituents.
Conclusion: The presence of an antibacterial compound highlights the importance of this plant as a source of phytochemicals with medicinal properties. This study indicated that the geographical location of the plant is one of the factors which determines the antibacterial potency of the leaf extract.
Keywords: Phytochemicals, Antibacterial agents, Phytotherapy, Plant extracts, Ethnobotany

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Introduction

Medical scientists and researchers have been working on challenges such as malaria (1), chronic kidney disease (2), non-alcoholic fatty liver disease (3), polycystic ovary syndrome (PCOS) (4), drug-resistant TB (5), cancer incidence and deaths (6) since the last few decades. There are gender-specific silent medical challenges such as fibroids (7), and country-specific neglected challenges like human rabies (8) which need more attention. Anti-microbial resistance to the known compounds (9) and newly emerging diseases makes it essential to discover, design and engineer new promising classes of antibiotics (10). Screening the plants with ethnomedicinal significance for phytoconstituents has shown compounds with diverse uses such as medicinal compounds (11,12), oils (13), food (14,15), and fish meal supplements (16).

Plants have been used in traditional medicinal practices since prehistoric times. They are valuable and indispensable sources of natural products with great potential for producing new drugs for human beings (17). To date, the number of phytochemicals with potential biological activity has been identified. In certain cases, the phytochemicals of some folklore medicinal plants and their pharmacological actions have remained unexplored. It has been estimated that only 20% of the plant flora has been screened for drugs (18). Consequently, their potential to be used as a new drug has remained locked.

Many species of mangroves have traditionally been used to treat diseases such as rheumatism, smallpox, ulcers, hepatitis, leprosy, asthma, snake bites, toothache, and constipation (19). Mangroves have the ability to grow under stress factors such as high salt concentrations, tidal flooding, strong wind, solar radiation, and heat. This adaptability is based on morphological and physiological adaptations like stilt and air roots, salt excretion systems, and a high abundance of plant secondary metabolites (20).

The medicinal potential of mangrove plants can be evaluated by the presence of their bioactive phytochemical constituents enabling them to exhibit anti-inflammatory, anticancer, antiviral, antimalarial, antidiabetic and anti - hypersensitive activities (21). However, since a single plant contains diverse phytochemicals, effects of using a whole plant as a medicine are uncertain.

S. apetala (1023 – Herbarium JCB, India) is a fast-growing (22), woody evergreen tree species of the mangrove (23). The *S. apetala* plant is commonly called as mangrove apple or Kandel. The plant belongs to the family-Lythraceae, (24). This tree species is native to Bangladesh, India, Sri Lanka, Malaysia and Australia (25). In local languages, it is known by various names such as *kalinga* in Telugu, *keruan* in Oriya, *marama maram* in Tamil, and *keora* in Bengali.

S. apetala has immense medicinal and economic values. Various parts of this plant such as its bark, root, leaves and fruits have been used in folk medicine in the South Asian countries for treating diarrhea, hepatitis, inflammation, wounds, and ulcers (26). The leaves of the plant are mainly used to treat hepatitis (27), dysentery, sprains and bruises, and eye troubles and for open sores in children's ears (28).

Few systematic studies have been carried out to evaluate the anti-microbial potential of *S. apetala* plant parts like its leaves (29,30), bark (31) and fruits (32). Scientific literature shows studies carried out for the isolation and characterization of phytochemicals from *S. apetala* leaves which reported the presence of alkaloids, terpenoids, phenols (33), phytosterols, and quinones (34) from *S. apetala* leaves. However, these attempts have not covered the antibacterial analysis of the isolated compounds.

It is necessary to isolate and characterize certain phytochemicals with antibacterial potential from the *S. apetala* plant leaves. A typical protocol to isolate a pure chemical agent from natural origin is bio-assay guided fractionation, meaning step-by-step separation of extracted components based on differences in their physicochemical properties, and assessing the biological activity, followed by separation and assaying (35). With the objective of isolating the compound(s) with antibacterial potential from *S. apetala* leaves, the present study was designed using a bio-assay-guided isolation and characterization procedure.

Materials and Methods

Collection and Processing of Plant Materials

Fresh, young and tender leaves of *S. apetala* Buch. - Ham (Lytheraceae) were collected from mangrove-growing area of Ghodbunder Road, Thane, Maharashtra coast, India (Fig. 1). The sampling was done in November at 10 am. The identity of the plant material was confirmed by an expert taxonomist of University of Mumbai, Mumbai.

The leaves were dried in the shade at room temperature, and the dried leaves were ground to a fine powder in a

Figure 1. Leaves of *S. apetala* Buch. Ham Photo: Wikimedia Commons.

Jankel and Kunkel model A10 mill. 10 g of the dried leaves powder was taken into cellulose thimble, single thickness supplied by Whattman's International Ltd., Maidstone, England and carefully placed in the central tube of the extractor of the Soxhlet apparatus. The extraction was carried out using different polar and non-polar solvents (Petroleum Ether, Acetone and Water). The extract was evaporated on water bath to a final volume of 20 ml to achieve the 50% (W/V) concentration. Later on, the extract was cooled and stored in airtight glass bottle at -4 ^{o}C in the refrigerator (36).

Bioactivity Evaluation of the Extract

Extraction of the *S. apetala* leaves was carried out using non-polar to polar solvents such as petroleum ether, acetone and water. The *in vitro* antibacterial activity of the leaf extracts of *S. apetala* was carried out against eight human pathogenic bacteria. The bacterial species used included four Gram-positive (*Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis*) and four Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella aerogenes*). They are clinical isolates from Haffkine's Laboratory, (Mumbai) India. The antibacterial evaluation of the extract(s), MIC and bioactivity of pure compound was carried out against the above-mentioned bacteria by the Agar Well Diffusion Method (37).

Among petroleum ether, acetone and water, the petroleum ether extract did not show any antibacterial activity. Water and acetone extract exhibited the inhibition of bacteria, but acetone extract was carried forward for the MIC. Compared with acetone (56 ^{0}C), water (100 ^{0}C) has a higher boiling point. Consequently, it is not easy to prepare perfectly dried samples for MIC from water extract as compared with acetone extract. There is the possibility that higher temperatures will destroy some of the compounds of interest. At the same time, acetone has the ability to dissolve both polar and non-polar substances, while other solvents can only dissolve one or the other (38). Considering all these reasons, the acetone extract of *S. apetala* leaves was selected for the MIC study. For the MIC test, acetone extract was evaporated to dry residue, and different concentrations (0.5, 1, 5, 10 mg/ml) of crude extracts were prepared using the fresh solvent.

Bioassay of the pure compound was done at the concentration 250 µg/ml and compared with ten standard antibiotics (ampicillin, chloramphenicol, cefazolin, tetracycline, minocyclin, vancomycin, sparfloxacin, erythromycin, tetracycline hydrochloride and benzylpenicillin sodium) against *E. coli* and *B. subtilis* (Agar Well Diffusion Method).

Development of the Solvent System/s

The solvent system/s was/were developed using either an individual solvent or a mixture of solvents to obtain a range of polarity and appropriate phases for the better separation of the compound(s). The solvents used were petroleum ether, toluene, diethyl ether, chloroform, ethyl acetate, acetone, isopropanol, methanol and acetonitrile. In the individual solvent system, diethyl ether, chloroform, ethyl acetate, and isopropanol exhibited band(s) of separation. In combined solvents, ten different combinations showed band(s) of separation.

Thin-Layer Chromatography-Bioautography

TLC of potent crude extract(s) and fraction(s) isolated by preparative column chromatography was carried out using the dipping method (39). A single drop of the sample was applied on the TLC plate, about 2 cm from the edge with the help of capillary. The loaded TLC plate was allowed to develop in the selected solvent system(s). Since many compounds separated by TLC are colorless, detection of the separated bands on chromatoplates was carried out by exposing them to iodine vapor or placing in UV cabinet at 254 and 336 nm.

The TLC-bioautography method introduced by Fisher and Lauther (40) and Nicolaus *et al*. (41) was used for the bioassay of the chromatoplates showing well-separated band under UV. This method is also called contact bioautography where anti-microbial agents diffuse from a TLC plate or paper to an inoculated agar plate. The chromatogram is placed face down onto the inoculated agar layer and left for a few hours to enable diffusion. Then, the chromatogram is removed and the agar layer is incubated. The inhibition zones are observed on the agar surface in the places where the spots of anti-microbial agents are stuck to the agar.

The contact bioautography of all the chromatoplates showing bands was carried out. Only one solvent

system {Petroleum ether: methanol (7:3)} which showed one band was found to be active against *E. Coli* and *B. subtilis*. Hence, this particular solvent system was used for the preparative column chromatographic separation.

Isolation of the Compound by Preparative Column Chromatography

The glass column of length 75 cm and mouth with 2 cm diameter was rinsed with the help of petroleum ether and allowed to dry. Subsequently, the column was packed with double the amount of alumina than the slurry. The residue of the acetone fraction obtained by evaporating the entire solvent was then added in alumina by continuous and vigorous stirring with the help of glass rod in a beaker till the free-flowing slurry was produced. Free-flowing and thoroughly mixed slurry was placed on the top of the alumina. The cotton was placed on top of the slurry to avoid scattering of slurry/alumina in the solvent system. The solvent system {Petroleum ether: methanol (70:30)} was allowed to continuously pour on the column till the complete isolation of the compound was achieved. The purity of the compound was checked as a single TLC spot.

Characterization of the Pure Compound

Characterization of this organic compound was performed for solubility, detection of elements, and melting point with standard laboratory tests.

UV Spectroscopy

The dry, powdered compound was dissolved in methanol to make the concentration 0.02 mg/ml, and the spectrum was recorded on Shimadzu UV-visible recording spectrophotometer-UV-2100, supplied by Shimadzu Corporation, Japan).

FT-IR Spectroscopy

To detect functional groups in the pure compound isolated from *S. apetala* leaf, FT-IR spectroscopy analysis was carried out in the Department of Chemistry, University of Mumbai (Shimadzu FTIR-4200 spectrometer, Shimadzu Corporation, Japan).

Statistics

In this study, wherever applicable, all the tests were performed in triplicates and the data were expressed as mean ± standard deviation.

Results and Discussion

Screening of Antibacterial Properties

Petroleum ether, acetone and water extracts were tested against Gram-positive as well as Gram- negative bacteria. Petroleum ether extract did not show activity against any bacteria. Water extract and acetone extract showed zones of inhibition against the Gram-positive as well as Gram-negative bacteria. Water and acetone extracts exhibited zones of inhibition ranging from 16-21mm. The water extract has shown antibacterial activity against four bacteria, i.e. *S. typhi, B. subtilis, P. aeruginosa* and *E. Coli*, whereas the acetone extract showed zones of inhibition against all the bacteria except *Klebsiella aerogenes* (Table 1).

The MIC values for the acetone leaf extract (0.5, 1, 5 and 10 mg/ml) exhibited inhibition against *S. typhi, B. Subtilis, P. aeruginosa* and *E. Coli* (Table 2).

Table 1: The Antibacterial Potential of Non-Polar to Polar Solvent Extracts in Leaves of the *S. apetala* Plant. (--): No zone of inhibition was observed.

Bacterial Strains	The Antibacterial Potential of the Extracts of *S. apetala* Leaves (Zones of Inhibition in mm)	
	Petroleum Ether	Acetone
S. pyogenes	--	20 (±0.1)
S. aureus	--	21 (±0.2)
B. subtilis	--	16 (±0.1)
S. epidermidis	--	16 (±0.3)
E. coli	--	18 (±0.1)
P. aeruginosa	--	19 (±0.2)
S. typhi	--	17 (±0.3)
K. aerogenes	--	--

Table 2: MIC of the acetone extract of *S. apetala* leaves (zones of inhibition in mm): (--): No zone of inhibition was observed.

Bacterial Strains	10 mg/ml	5 mg/ml	1 mg/ml	0.5 mg/ml
S. typhi	16 (±0.1)	15 (±0.2)	12 (±0.3)	10 (±0.3)
B. subtilis	15 (±0.1)	14 (±0.1)	12 (±0.2)	10 (±0.3)
P. aeruginosa	15 (±0.4)	13 (±0.1)	--	--
E. coli	15 (±0.3)	13 (±0.3)	11 (±0.1)	10 (±0.1)

To obtain an appropriate solvent system for the separation and isolation of bioactive principles, column chromatography was done using either individual or a mixture of the solvents (non-polar and polar). In a single solvent system, among all the solvents used (petroleum ether to methanol), diethyl ether and ethyl acetate showed clear separation of bands (Table 3a).

Diethyl ether and ethyl acetate showed three bands, whereas chloroform exhibited four bands. The remaining solvents showed either trailing or no clear cut separation of any band. The petroleum ether:ethyl acetate (70:30) resulted in five bands followed by acetone:chloroform (50:50) and petroleum ether:isopropanol (80:20) showing three bands. The solvent systems acetone:chloroform (80:20) and petroleum ether:methanol (70:30) separated only one band (Table 3b).

Bioautography of all the chromatoplates showing clear-cut separation of the bands was carried out to observe active principles among the separated bands. A single band obtained by the solvent system petroleum ether: methanol (70:30) was found to be active against *E. coli* and *B. subtilis* (Fig. 2).

None of the other bands observed on the chromatoplates developed by various solvent systems showed antibacterial activity. Based on bioautography results, the petroleum ether: methanol (70:30) solvent system was selected to isolate the bioactive compound by preparative column chromatography.

Purity of the isolated compound was checked by TLC using 100% methanol as a solvent system where the prominent single spot was centrally developed on a TLC plate. The bioactivity of the isolated compounds along with standard antibiotics was studied against test microorganisms *E. Coli* and *B. subtilis* (Table 4). The isolated compound showed the zones of inhibition 10 mm and 9 mm respectively against test bacteria which were better than ampicillin and Cefazolin. Similarly, vancomycin, erythromycin and benzyl penicillium sodium showed zones of inhibitions comparable to that of the isolated fraction which ranged between 12-14 mm.

Table 3: (**a)**: Column chromatography for the separation and isolation of bioactive compounds using the single solvent system: No. of bands/trailing for the separation of the compound was observed. Other solvents had not shown bands. (b): Column chromatography using a mixture of polar and non-polar solvents.

(a)

Solvents	No. of bands
Diethyl ether	3
Chloroform	4
Ethyl acetate	3

(b)

Solvents	Solvent proportion	No. of bands
Petroleum ether: Ethyl acetate	70:30	5
Acetone: Chloroform	50:50	3
Acetone: Chloroform	80:20	1
Petroleum ether: Methanol	70:30	1
Petroleum ether: Isopropanol	80:20	3

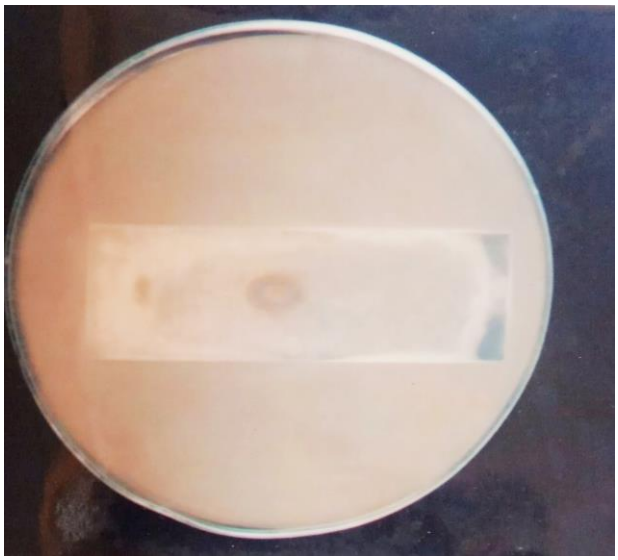

Figure 2. The petri plate showing the bioautography of the column chromatography separated active fraction against *E. coli*.

Table 4: Comparison of the activity of the isolated compounds with standard antibiotics.

Standard Antibiotics	*E. coli*	*B. subtilis*
Ampicillin	8	8
Cefazolin	10	8
Vancomycin	13	13
Erythromycin	12	14
Benzyl Penicilium sodium	12	14

Characterization and Analysis of the Bioactive Compound

Preliminary observations of the active compound isolated by column chromatography such as appearance, flame test and heating in a test tube were made and shown in **Table 5**. The compound was found to be solid, white or colorless, odorless crystals and completely soluble in water. A sooty flame was observed in the porcelain piece test while burning of the compound on copper wire exhibited a green flame. Besides, when strongly heated in a test tube instead of melting, the compound was found to be charred.

Detection of Elements

When an organic compound is fused with metallic sodium, the elements, 'N', 'S' and halogen are converted to corresponding sodium salts. On the basis of this sodium fusion or Lassaigne's test, elements such as 'N', 'S' and halogen were detected (Table-5). Nitrogen and sulfur gave negative tests while the halogen test was found to be positive with respect to chlorine.

Melting Point: The uncorrected melting point of the compound was about 300 ^{0}C.

UV-Vis Spectroscopy: The isolated compound showed a single absorption band at 208.5 nm (λmax), and the value for the absorbance of the compound was 0.740. This shows the compound comes in the UV spectrum. Consequently, it is a colorless compound.

Infra-Red Spectroscopy: FT-IR spectrum of the isolated compound was studied under two regions, i.e. the functional group region (4000-1300 cm^{-1} / 2.5-7.7 µm) and the fingerprint region (1300-900 cm^{-1} / 7.7-11.0 µm). The spectrum showed the presence of two absorption peaks at 1384 cm^{-1} and 1636 cm^{-1} regions. Moreover, a broad hump was observed at 3436 cm^{-1} regions. The absorption in the region between 1950-1550 cm^{-1} characterized the 'carbonyl' stretching vibration. Absorption peaks located in the 1690- 1600 cm-1 range arise from C=C and C=N stretching vibrations (Fig. 3 and Table 6).

Table 5: Preliminary tests of the isolated compound.

N.	Test	Observation
1	**Appearance**	
	Nature	Solid
	Color	White or colorless
	Odor	No particular smell
2	**Flame test**	
	Porcelain piece test	Sooty flame
	Copper wire/Beilstein test	Burns with green flame
3	**Heating in a test tube**	Does not melt but chars on strong heating
4	**Water solubility test**	Water soluble
5	**Detection of elements**	
	Detection of N	No greenish blue coloration or precipitate
	Detection of Sulfur	No black precipitate
	Detection of halogen	
	a. Test for halogen in absent of N and S	Thick white precipitate obtained (Halogen present)
	b. Detection of individual halogen	Lower layer was colorless (Chloride present)

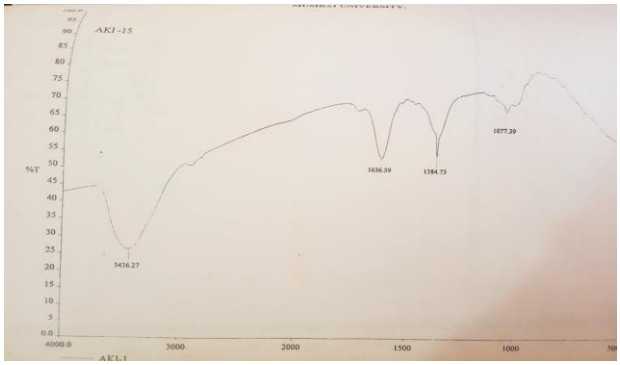

Figure 3. FT-IR Spectrum of the isolated compound.

Table 6: FT-IR functional group table of the isolated compound.

N	Peak Position	Group	Class	Peak Details
1	3436.27	O-H stretching	alcohol	Strong, broad
2	1636.59	C=C stretching	alkene	Medium
3	1384.73	C-H bending	aldehyde	Medium

The present study investigates the phytochemical profile of *S. apetala* leaf extracts obtained from the west coast of India in order to show the potent antibacterial activity of *S. apetala* leaves. The antibacterial property of *S. apetala* leaves has been investigated in some studies, but the present study is different with respect to the geographical location of the plant collection, bacterial strains used, and polarity of extraction solvents. In the present study, the bioassay-guided antibacterial fraction was isolated and characterized in contrast with earlier reports where primary screening for antibacterial activities was performed. This study, in line with earlier reports, validates the significance of *S. apetala* leaves as an important resource in traditional medicine for antibacterial remedies aimed at human pathogenic diseases (29,30).

This study showed better results for the plant than the study carried out by Patra *et al*., where plant leaves were collected from the state of Odisha (Eastern coast of India). It shows that the geographical location of the plant determines the antibacterial potency of the extract.

These results are in line with the study carried out to find the impact of geographical locations on the antibacterial activity of *Mentha spicata*. The *M. spicata* samples obtained from higher altitude regions and fertile cultivars were found to be more effective as far as the antibacterial activities are concerned. This signifies a positive impact of geographical location and richness of land with respect to nutrients (42). There are other studies which show geographical distributions consequences on phytochemical constituents of the plant extracts collected from different locations (43,44).

In order to isolate the bioactive components from the *S. apetala* leaves, solvent extraction was carried out by selecting the appropriate solvent system using column chromatography with both single/mixture of solvents having varying polarity. Then, the purity and bioactivity of the isolated compounds were validated by TLC and against standard test antibiotics on different Gram-positive and Gram-negative bacteria.

In the present study, the leaf extracts were tested against different human pathogenic bacterial species like *S. pyogenes, S. aureus, Bacillus subtilis, E. coli, P. aeruginosa,* and *S. typhi*. Moreover, a potent degree of antibacterial activity was observed against all these pathogenic species. MIC of crude extract(s) in the present study showed the ability to inhibit the growth of pathogenic Gram-positive and Gram-negative bacteria at lower concentrations (0.5mg/ml) in both acetone and water-soluble extracts as compared with the results of the study conducted by Patra *et al*., where the MIC was found to be 2.5mg/ml and no activity was established for pathogenic strains like *S. pyogenes* and *S. typhi* (30). This result further validates the potent antibacterial activity of S. *apetala leaf* extracts against a variety of pathogenic bacterial species and its potential as a traditional medicine for common bacterial diseases. These findings are in accordance with the study where the extracts from the leaves, stems, barks and roots of the same mangrove species exhibited positive results for anti-oxidant activity (45).

In order to gain more information on the appropriate solvent system and active compound(s)/functional group(s) present in the *S. apetala* crude extract, multiple solvent systems and chromatographic characterizations were carried out in the present study.

For the selection of the solvent system, preparative column chromatography was performed with either individual or a mixture of the solvents (Polar to Non-Polar). For a single solvent system, diethyl ether (3 bands), ethyl acetate (3 bands), and chloroform (4 bands) showed clear separation and resolution of the bands.

In a solvent combination system, petroleum ether:ethyl acetate (70:30) showed 5 bands, while acetone: chloroform (50:50) and petroleum ether:isopropanol (80:20) showed 3 bands each, and petroleum ether:methanol (70:30) showed one band. Thus, this analysis indicated the efficacy and specificity of various solvent systems for isolation of the active compound(s) from crude extracts of *S. apetala* leaves.

The TLC-bioautography method was carried out using the plates showing well-separated bands. Here, the petroleum ether:methanol (70:30) solvent system showed one band active against *E. coli* and *B. subtilis* validating the potential of *S. apetala* leaves as a potent antibacterial agent. The isolation of this antibacterial compound was carried out using column chromatography and was confirmed as a single TLC spot. These results are in line with the results of another study conducted on Ajwa date (*Phoenix Dactylifera. L*) plants. A combination of the strong polar solvent and strong non-polar solvent gives potent anti-microbial compounds, which cannot be extracted using a single polar or non-polar solvent (46).

Characterization of this organic compound was carried out using tests for solubility, melting point, and elemental analysis via standard laboratory procedures. UV-visible spectroscopy of this compound showed a single absorption band at 208.5 nm (λ max) indicating the compound to be UV-absorbable in nature.

For obtaining further details about this compound, analysis via FT-IR spectroscopy was also performed (47). The FT-IR spectrum showed the presence of two absorption peaks at 1384 cm^{-1} and 1636 cm^{-1} regions. The absorption in the region between 1950-1550 cm^{-1} characterized the 'Carbonyl' stretching vibration. Absorption peaks located in the 1690- 1600 cm^{-1} range arise from C=C and C=N stretching vibrations.

The FT-IR analysis indicates the molecular absorption and transmission and it is possible that the potent antibacterial activity of *S. apetala* leaves may be due to the presence of aromatic and aliphatic groups (UV and FT-IR spectra), and detailed analysis with techniques like ^{1}H NMR spectroscopy will further elucidate the exact structure and profile of this chemical compound.

Based on all the above-mentioned investigations, it is clear that the acetone extract of *S. apetala* leaves shows potent antibacterial activity against pathogenic Gram-positive and Gram-negative bacterial strains at low concentrations. TLC-bioautography of the chromatoplates also showed potent antibacterial activity against both *E. coli* and *B. subtilis* in the petroleum ether:methanol (70:30) solvent system. The use of such bioactivity-guided characterization of the active constituent(s) contributes to their isolation and purification processes. Further *in vivo* tests should be carried out to evaluate whether the potent antibacterial activity found *in vitro* is translated into *in vivo* activity. The present study; therefore, elucidates the role of *S. apetela* leaves as a therapeutic agent in the treatment of bacterial infections and its use in traditional medicine.

Conclusion

The findings of the present study showed the presence of various valuable phytochemical constituents in the *S. apetala* Buch. Ham leaves. The isolated pure compound is responsible for antibacterial activity. The presence of an antibacterial compound highlights the importance of this plant as a source of phytochemicals with medicinal properties. This study also showed that the geographical location of the plant is one of the factors which determine the antibacterial potency of the extract.

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None.

Conflict of Interest

The authors declare that they have no conflict of interest.

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