Original Article

In vitro Evaluation of the Antimicrobial Activity of Leaf Extracts of *Litsea iteodaphne* Against a Selected Group of Bacteria Including Methicillin-Resistant *Staphylococcus aureus*

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Abstract

Background: The quest for scientific endorsement of new drugs from plants continues due to the rising antibiotic resistance against pathogenic bacteria. *Litsea iteodaphne* is used in Sri Lanka in the treatment of infectious diseases. Therefore, *in vitro* antibacterial activity of *L. iteodaphne* plant extracts were evaluated against selected human pathogenic bacteria. **Materials and Methods:** Antibacterial activity of 400, 40, and 4 mg/ml concentrations of hexane, ethanol, and aqueous leaf extracts of *L. iteodaphne* were evaluated against *Staphylococcus aureus*, *Psedomonas aeruginosa*, *Klebsiella pneumoniae, Escherichia coli,* and methicillin-resistant *S. aureus* (MRSA) clinical isolates using disc diffusion method. Minimum inhibitory concentration (MIC) was identified, and phytochemical screening was carried out. **Results:** Significant zones of inhibition ranging from 5.7 mm to 8.1 mm, 7.1 mm to 8.0 mm, and 7.6 mm were obtained for ethanol, hexane, and aqueous extracts at 400 mg/ml, respectively, against above four bacteria. For MRSA clinical isolates, zones of inhibition ranging from 6.1 mm to 10.9 mm, 6.7 mm to 10.8 mm, and 6.4 mm to 8.6 mm were obtained for ethanol, hexane, and aqueous extracts at 400 mg/ml, respectively. Ethanol extract of *L. iteodaphne* showed the lowest MIC value (0.0256 mg/ml). Phytochemical screening revealed the presence of tannins, cardiac glycosides, reducing sugars, phenolic compounds, saponins, and flavonoids. **Conclusions:** *L. iteodaphne* crude leaf extracts showed promising antibacterial activity against Gram-positive and Gram-negative bacteria and clinical isolates of MRSA. Further investigations toward fractionation and the identification of an active compound will enhance the antimicrobial potential of *L. iteodaphne*.

Keywords: *Antibacterial efficacy, crude extracts, ethanol extract, Litsea iteodaphne, minimum inhibitory concentration*

Introduction

Unrestricted access and indiscriminate use of existing antibiotics in the treatment of infectious diseases has led to drug resistance worldwide.[1] Thus, scientists are in search of antimicrobial agents that are effective against pathogens which are resistant to currently available antibiotics.[2] Previous studies have shown that 80% of the world population currently use plant-based traditional medicine for their health care needs.[3] In addition to the mechanisms of action identified in known antibiotics, natural antimicrobial components in plants can also inhibit the growth of bacteria by unknown mechanisms.[1] The medicinal value of these plants may originate from the chemically active substances that produce a marked physiological action in the human body, such as inhibition of bacterial protein biosynthesis, cell wall biosynthesis, DNA replication and repair, cell membrane destruction, and inhibition of a metabolic pathway.[4,5] Consequently, it is of great interest to search for new antimicrobials from plants to validate their use in traditional medicine and to expose the active principles by isolation and the characterization of active constituents.[6]

Staphylococcus aureus has long been recognized as one of the most important bacteria that causes disease in humans. It is the leading cause of skin and soft-tissue infections such as abscesses, furuncles, impetigo, and cellulitis. The emergence of antimicrobial resistance

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in *S. aureus* has resulted in limited treatment options against certain infections such as pneumonia, meningitis, osteomyelitis, and endocarditis.[7] Methicillin resistance is most commonly mediated by the mecA gene, which encodes for a single additional penicillin-binding protein, PBP2a, with low affinity for all β-lactam antibiotics.[8] Methicillin-resistant *S. aureus* (MRSA) is also a major cause of nosocomial infection globally. They account for 47%–62% of all hospital-acquired infections, causing skin and soft-tissue infection, and severe hemorrhagic pneumonia in children and young adults in Sri Lanka.[9] Recent studies indicated that MRSA strains account for 10%–40% of *S. aureus* isolated from some of the European hospitals.[10-12] In developed countries, fluoroquinolones are recommended for serious infections related to staphylococci, although resistance among MRSA has been documented in many instances.[13] Furthermore, in spite of recent reports of vancomycin-resistant MRSA strains in some parts of the world, vancomycin still remains the drug of choice for most MRSA-associated diseases.[14] Since medicinal plants have been used as remedies for infectious diseases in many tropical countries, they provide a rationale for investigating natural products for the treatment of MRSA infections. These medicinal plants may also provide new sources of therapeutic agents against multi-drug-resistant bacteria, including MRSA. Therefore, in this study, the antimicrobial activity of *Litsea iteodaphne* was assessed against both methicillin-sensitive and resistant *S. aureus*.

L. iteodaphne which is known in Sinhala as "Kalu Nika" is a plant growing mainly in India, Vietnam, Laos, and Sri Lanka. Plant is known to be a shrub to tree, with leaves alternate, variable, narrowly oval-lanceolate, rounded-to-acute base, subacute ape, and beneath paler. Trunk is rather rough, young parts finely silky and hairy. Flowers are greenish-white, and fruits are purplish red. They are found mainly in montane and rain forest sub-canopy and understory. It is rarely found in Sri Lanka and is known to be a source of several bioactive compounds and secondary metabolites. Over centuries, *L. iteodaphne* has been used in traditional Ayurveda medicine in the treatment of arthritis, boils, cough, ulcers, infections in the ear, and tuberculosis. People in Sri Lanka use the leaves [Figure 1], roots or the entire plant as folk medicine to combat different types of diseases and disorders.[15] However, systematic screening of antibacterial potential of *L. iteodaphne* extracts against multidrug-resistant Gram-positive and Gram-negative human bacterial pathogens was never carried out before. Therefore, the present investigation was designed to study the *in vitro* antibacterial potential of ethanol, hexane, and aqueous extracts of *L. iteodaphne* against human pathogenic bacteria and also to characterize the putative compounds responsible for this activity using phytochemical screening with the intention of evaluating the activity of the plant for its possible pharmaceutical applications in future.

Figure 1: Leaves of *Litsea iteodaphne*. Local name: "Kalu Nika"; Family: *Lauraceae*

Materials and Methods

Sample collection

L. iteodaphne plant leaves were collected from the Southern region of Sri Lanka. Plants were authenticated at the National Herbarium, Botanical Gardens, Peradeniya, Sri Lanka. The leaves were washed in water, air-dried in the oven at 40°C for 3–4 days, ground to a coarse powder, and stored in a sterile airtight container at 4°C until further use.

Antimicrobial assays

Extract preparation

Solvent extraction

Ethanol extract – Cold extraction was carried out. A total of 10 g of coarsely powdered leaves were soaked in 100 ml of ethanol for 72 h while shaking. The filtrate was concentrated using a rotary evaporator. The crude sample was dissolved in a minimum amount of 10% dimethyl sulfoxide (DMSO) to prepare 400, 40, and 4 mg/ml concentrations of ethanol extracts and extracts were stored at 4°C in the refrigerator.

Hexane extract – Cold extraction was carried out by soaking 10 g of coarsely powdered leaves in 100 ml of *n*-hexane for 72 h while shaking. The filtrate was concentrated using a rotary evaporator. The crude sample was dissolved in a minimum amount of 10% DMSO to prepare 400, 40, and 4 mg/ml concentrations of hexane extracts, and extracts were stored at 4°C in the refrigerator.

Aqueous extract

A total of 2.5 g of coarsely powdered leaves were refluxed in 30 ml of distilled water for 3 h. The filtrate was concentrated using an oven at 40°C. The crude sample was dissolved in a minimum amount of 10% DMSO to prepare 400, 40, and 4 mg/ml concentrations of aqueous extracts, and extracts were stored at 4°C in the refrigerator.

Tested organisms

The extracts were tested against standard bacterial cultures of *S. aureus* (ATCC 25923), *Psedomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), and 20 methicillin-resistant *S. aureus* (MRSA) clinical isolates obtained from pus and wound swabs at the Department of Microbiology, Faculty of Medicine, University of Ruhuna.

McFarland standard preparation

McFarland number 0.5 standard was prepared by mixing 9.95 ml of 1% H_2SO_4 in distilled water and 0.05 ml of 1% $BaCl_2$ in distilled water.

Disc diffusion assay

Initial antibacterial activity was screened using the disc diffusion method. Different concentrations (crude extract, ten-fold dilution, and hundred-fold dilution) of plant extracts were prepared in 10% DMSO. The bacterial colonies were dissolved in normal saline, and cell suspensions were adjusted to 0.5 McFarland turbidity standards. About 1.5×10^8 CFU/ml of test organism was spread on Mueller-Hinton agar plate. Sterilized Whatman No. 1 filter paper discs were impregnated with 10 µl of different concentrations of plant extracts and solvent blank (10% DMSO) and placed on the inoculated plates with cefotaxime (30 µg) as the positive control for the above four bacteria and vancomycin (30 µg) as the positive control for MRSA pathogenic bacteria. The plates were incubated at 35°C ± 2°C in the incubator for 18 h. Diameters of the zones of inhibition were measured using a Vernier caliper to determine the antibacterial activities.

Determination of minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) of the plant extracts were determined by broth microdilution assay using sterile 96-well microtiter plates adhering to Clinical and Laboratory Standards Institute, M07-A8.[16] The plant extracts were serially diluted 5 fold to produce a concentration series from 400 mg/ml to 0.0256 mg/ml using 10% DMSO. Cefotaxime IV was used as the positive control for the above four bacteria and vancomycin IV was used as the positive control for MRSA pathogenic bacteria. About 10% DMSO and plant extract without bacterial suspension was used as negative control. Inoculum was prepared in Mueller–Hinton broth, and the turbidity was adjusted approximately to 0.5 McFarland turbidity standard to prepare 1.5×10^8 CFU/ml. A volume of 30 µl of inoculum was added into each well containing 90 µl plant extract in the dilution series, except negative controls and mixed. The plates were incubated at (35 ± 2) °C for 24 h in an ambient air incubator. The absorbance of the plates was measured at 630 nm, and MIC was determined.

Phytochemical assays

Preparation of plant extracts

L. iteodaphne plant extracts were prepared according to the relevant methods used for the analysis of phytochemicals. Unless stated otherwise, all aqueous extracts were prepared by refluxing 2.6 g of powdered dried plant material in 30 ml of distilled water for 1 h and then concentrating the extract to a final volume of 20 ml.[17]

Phytochemical analysis

Qualitative phytochemical tests to detect the presence of tannins, cardiac glycosides, reducing sugars, alkaloids, phenolic compounds, cyanogenic glycosides, saponins, and flavonoids were carried out using the standard procedures.[18,19] All procedures were optimized with positive control, and necessary precautions were taken to remove the interference from chlorophyll.

Results

Antimicrobial assay

From the three extracts used in the study, *L. iteodaphne* ethanol extract showed significant antimicrobial activity against all four pathogenic bacteria. For the ethanol extract at 400 mg/ml, significant antibacterial activity was observed with zones of inhibitions of 8.1, 6.3, 6.2, and 5.7 mm [Figure 2] against *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* pathogenic bacteria, respectively. Hexane extract of *L. iteodaphne,* at 400 mg/ml showed positive results for *S. aureus* [Figure 2] where the zone of inhibition was 8.0 mm and for *K. pneumonia*, where the zone of inhibition was 7.1 mm. For the aqueous extract, at 400 mg/ml a zone of inhibition of 7.6 mm was obtained only for *S. aureus.* In the disc diffusion method, antimicrobial activity was not shown at 40 and 4 mg/ml of all three extracts for the above pathogenic bacteria [Table 1]. According to the above results, ethanol, hexane, and aqueous extracts had a significant ability of inhibiting *S. aureus* with zones of inhibition ranging from 7.6 mm to 8.1 mm compared to *P. aeruginosa*, *K. pneumoniae* and *E. coli.* From the three extracts, the highest zone of inhibition (8.1 mm) was obtained for *S. aureus* [Figure 2] for 400 mg/ml ethanol extract. The lowest zone of inhibition was obtained for *E. coli* where zone of inhibition was 5.7 mm for the ethanol extract of *L. iteodaphne* [Table 1]. Out of the three extracts tested against the above four organisms, ethanol extract of *L. iteodaphne* showed the highest antimicrobial activity followed by hexane and aqueous extracts [Table 1].

According to results, a significant antimicrobial activity of *L. iteodaphne* was shown against *S. aureus* which is a Gram-positive bacterium. Hence, a further study was carried out to find the antimicrobial activity of ethanol, hexane, and aqueous extracts of *L. iteodaphne* against MRSA pathogenic bacteria.

The most significant inhibition zones for the MRSA strains were given by ethanol extract followed by hexane and water extracts [Table 2]. Out of the three extracts tested, 400 mg/ml concentration of both ethanol and hexane extracts were sensitive against all the MRSA strains. Except few, the majority of the organisms were sensitive for the same concentration of the aqueous extract. Further, few organisms were sensitive for the 40 mg/ml concentration of both ethanol and hexane extract, whereas only one strain was sensitive for the 4 mg/ml of the hexane extract. However, none of the organisms were sensitive for the 40 mg/ml and 4 mg/ml concentrations of the aqueous extract. Zones of inhibition ranging from 6.1 mm to 10.9 mm were obtained for 400 mg/ml of ethanol extract, while zones of inhibition ranging from 6.7 mm to 10.8 mm were obtained for the same concentration of the hexane extract [Figure 2]. For the 400 mg/ml concentration of the aqueous extract, zones of inhibitions were ranged from 6.4 mm to 8.6 mm [Table 2].

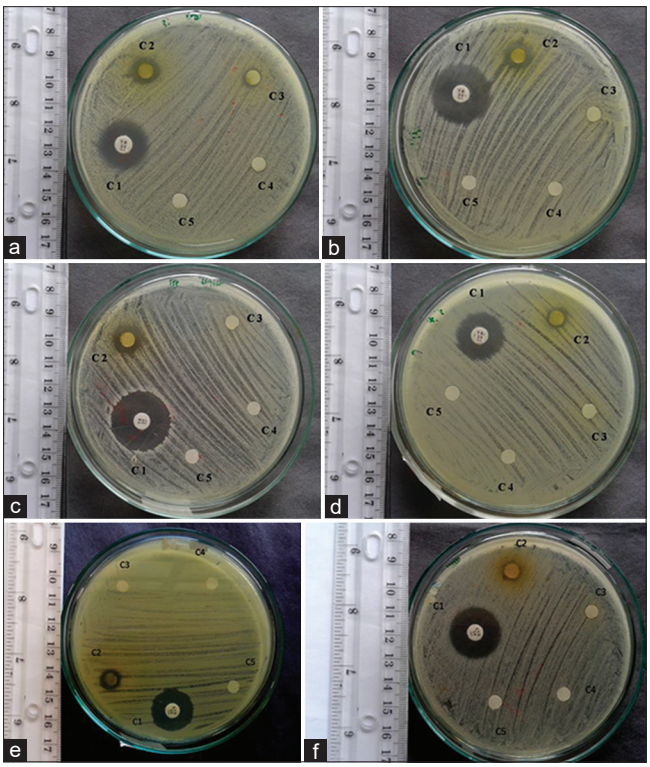

Figure 2: Disc diffusion assay. (a) Ethanol extract against MRSA (methicillin resistant *Staphylococcus aureus*) 14 (b) ethanol extract against MRSA 4 (c) ethanol extract against MRSA 8 (d) ethanol extract against *S. aureus* (e) hexane extract against MRSA 5 (f) hexane extract against *S. aureus* C1: Positive control; C2: Crude extract; C3: 10-fold dilution; C4: 100-fold dilution; C5: Negative control. MRSA: Methicillin resistant *Staphylococcus aureus*

Minimum inhibitory concentration determination

MIC of the ethanol extracts for *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* pathogenic bacteria ranged between 0.64 mg/ml and 3.2 mg/ml. The lowest MIC for ethanol extract was obtained for *S. aureus*, *P. aeruginosa* and *K. pneumoniae* [Table 3]. The MIC of hexane extract was 0.64 mg/ml for *K. pneumoniae* and 3.2 mg/ml for *S. aureus*. Therefore, the lowest MIC for hexane extract was obtained for *K. pneumoniae* [Table 3]. For the aqueous extract, MIC of 0.64 mg/ml was obtained for *S. aureus* [Table 3]. From the three extracts tested against the above four organisms, ethanol extract of *L. iteodaphne* showed the highest antimicrobial activity [Table 3].

Against MRSA, MIC values of the ethanol extract ranged from 0.64 mg/ml to 0.0256 mg/ml. MIC values of both hexane and water extract ranged from 3.2 mg/ml to 0.0256 mg/ml. Among the three extracts, majority of the organism were inhibited by the 0.0256 mg/ml concentration of the ethanol extract followed by hexane and aqueous extracts. MIC values could not be calculated for a few organisms for the water extract for the concentration range tested in this study. Therefore, from the three extracts tested against the twenty MRSA strains used in the study, ethanol extract of *L. iteodaphne* showed the highest antimicrobial activity [Table 3].

Phytochemical analysis

Out of the main phytochemicals tested only tannins, cardiac glycosides, reducing sugars, phenolic compounds, saponins, and flavonoids were present in *L. iteodaphne* leaf extracts [Table 4].

Discussion

In this study, the leaves of *L. iteodaphne* were screened *in vitro* for antibacterial activity against human pathogenic bacteria. In general, Gram-positive bacteria are considered as more sensitive than Gram-negative bacteria toward different antimicrobial compounds because of the variation in the structure of their cell walls, but *L. iteodaphne* showed positive results against both Gram-positive and Gram-negative bacteria.[20] The active components present in the plant extracts may interfere with the growth and

Table 1: Diameters of zones of inhibition of *Litsea iteodaphne* leaf extracts against clinical isolates of ATCC reference strains (mm)

Organism	Positive control	Ethanol extract	Hexane extract	Aqueous extract
Escherichia coli (ATCC 25922)	31.43	5.7	-	-
Staphylococcus aureus (ATCC 25923)	31.07	8.1	8.0	7.6
Pseudomonas aeruginosa (ATCC 27853)	32.33	6.3	-	-
Klebsiella pneumoniae (ATCC 700603)	21.93	6.2	7.1	-

ATCC: American Type Culture Collection

Table 2: Diameters of zones of inhibition of *Litsea iteodaphne* leaf extracts against clinical isolates of methicillin-resistant *Staphylococcus aureus* (mm)

Organism	Positive control	Ethanol extract	Hexane extract	Aqueous extract
MRSA 1	16.67	8.1	7.2	6.4
MRSA 2	17.6	8.6	9.8	8.6
MRSA 3	16.9	8.4	8.3	7.1
MRSA 4	19.97	10.5	7.7	-
MRSA 5	21.3	7.2	10.8	-
MRSA 6	22.3	8.8	7.4	-
MRSA 7	21.5	10.1	8.4	-
MRSA 8	20.4	10.1	7.4	7.3
MRSA 9	22.2	9.5	7.6	7.4
MRSA 10	22.63	8.5	7.9	7.8
MRSA 11	19.1	8.1	8.9	6.8
MRSA 12	21.3	7.8	7.5	7.4
MRSA 13	20.2	9.1	10.1	8.1
MRSA 14	21.5	10.9	8.1	7.4
MRSA 15	22.53	8.8	7.4	-
MRSA 16	20.7	6.1	8.9	7.9
MRSA 17	21.57	9.4	8.6	-
MRSA 18	22.2	7.9	8.1	7.9
MRSA 19	20.23	9.7	9.1	7.8
MRSA 20	20.7	7.5	6.7	-

MRSA: Methicillin resistant *Staphylococcus aureus*, ATCC: American Type Culture Collection

Table 3: Minimum inhibitory concentration of the *Litsea iteodaphne* leaf extracts against clinical isolates of methicillin-resistant *Staphylococcus aureus* bacteria and ATCC reference strains (mg/ml)

Organism	Ethanol extract	Hexane extract	Aqueous extract
Escherichia coli (ATCC 25922)	3.2	-	-
Staphylococcus aureus (ATCC 25923)	0.64	3.2	0.64
Pseudomonas aeruginosa (ATCC 27853)	0.64	-	-
Klebsiella pneumoniae (ATCC 700603)	0.64	0.64	-
MRSA 1	0.0256	3.2	0.64
MRSA 2	0.64	3.2	3.2
MRSA 3	0.0256	3.2	3.2
MRSA 4	0.0256	0.0256	-
MRSA 5	0.64	0.0256	-
MRSA 6	0.0256	0.0256	-
MRSA 7	0.0256	0.64	-
MRSA 8	0.0256	0.0256	0.128
MRSA 9	0.0256	0.0256	0.64
MRSA 10	0.0256	0.0256	0.128
MRSA 11	0.0256	3.2	0.0256
MRSA 12	0.0256	0.0256	0.0256
MRSA 13	0.0256	3.2	0.128
MRSA 14	0.0256	3.2	0.0256
MRSA 15	0.0256	0.64	-
MRSA 16	0.0256	0.0256	0.0256
MRSA 17	0.64	3.2	-
MRSA 18	0.0256	0.0256	0.0256
MRSA 19	0.0256	3.2	0.0256
MRSA 20	0.0256	0.64	-

MRSA: Methicillin-resistant *Staphylococcus aureus*, ATCC: American Type Culture Collection

metabolism of microorganisms and prevent them from multiplication.

The antimicrobial activity of *L. iteodaphne* has never been investigated before. In this study, we tested the activities of the plant extracts against clinical isolates of MRSA and highlighted the potential of these extracts to be used against such multi antibiotic-resistant organisms.

According to the disc diffusion method performed against the Gram-positive and Gram-negative microorganisms, the highest diameter was observed for the ethanol extract of *L. iteodaphne* [Table 1]. Out of the three extracts tested against the above four organisms, ethanol extract of *L. iteodaphne* showed the highest antimicrobial activity with a MIC of 0.64 mg/ml [Table 3]. From the above four organisms, significant antimicrobial activity was shown against *S. aureus*. Therefore, a further study was carried out to find the antimicrobial activity of ethanol, hexane, and aqueous plant extracts against MRSA.

It was found that ethanol extract of *L. iteodaphne* showed the highest diameter against the twenty MRSA strains tested [Table 2]. Majority of MRSA strains were inhibited by 0.0256 mg/ml concentration of the ethanol extract which was the lowest MIC value observed from all the tests performed [Table 3]. Therefore, from the ethanol, hexane, and aqueous extracts tested against *S. aureus*, *P. aeruginosa*, *K. pneumonia, E. coli* and MRSA organisms, ethanol extract of *L. iteodaphne* showed the highest antimicrobial activity.

The present study has shown that ethanol extract of *L. iteodaphne* possesses the highest antibacterial property that supports their value in ayurvedic medicine for the treatment of infectious diseases. According to Rob *et al*., ethanol extract of stem bark of *Litsea glutinosa*, that belong to the same family as *L. iteodaphne,* exhibited significantly high inhibitory zones against *E. coli* (16.40 ± 0.55), *S. aureus* (15.20 ± 0.84) and *K. pneumoniae* (14.80 ± 1.30) for most of the clinical pathogens in comparison to other extracts. The MIC value of ethanol extract of stem bark ranged from 2.5–5 mg/ml for *E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*. This emphasized that the results of the present study showed a moderate activity of *L. iteodaphne* against the clinical isolates of MRSA organisms.[21]

The solvent extracts of leaves of *L. iteodaphne* showed the presence of phytochemicals such as tannins, cardiac

glycosides, reducing sugars, phenolic compounds, saponins, and flavonoids [Table 4]. These phytochemicals may be responsible for the significant activity observed against the bacterial strains used in the study. Several compounds, such as tannins found in plant cells, are potent inhibitors of hydrolytic enzymes used by pathogenic bacteria.[22] The phytochemicals like phenolic compounds present in the extract of this plant are powerful inhibitors of microbial growth, and the presence of glycosides are of importance and interest in pharmacology due to their ability of elimination of poisonous compounds from the body.[23] The presence of the above-mentioned phytochemical constituents was known to confer protection against different bacterial strains. Hence, the phytochemicals detected in the *L. iteodaphne* plant extracts may be responsible for its significant antimicrobial activity.

Since ethanol extract showed the highest antimicrobial activity, it can be suggested that the compounds extracted to water and hexane may not possess a significant antibacterial activity. These observations may be attributed to two reasons, such as the nature of the biologically active components which could have been enhanced in the presence of ethanol and the ability of ethanol to extract a greater number of active constituents responsible for antibacterial activity due to its higher polarity. In this study, more active compounds may have been extracted in a versatile solvent such as ethanol than in an organic solvent such as hexane and both have demonstrated higher significant antibacterial activity compared to those extracted to water. Polarity of the compounds extracted by each solvent and the capability of extracts to diffuse in different culture media may affect the results obtained. Out of the solvents used in the study, ethanol is more polar than hexane. As the crude extracts showed such antimicrobial properties, isolation of active constituents may lead to the development of a potential therapeutic agent.

In vitro investigation of *L. iteodaphne* showed that it has potential antimicrobial properties. Therefore, once the active compounds are isolated and identified, *L. iteodaphne* could be used in the preparation of herbal drugs with modern standards of safety and efficacy to meet the health care needs.

Table 4: Phytochemicals identified from *Litsea iteodaphne* leaves

Phytochemical	Presence
Tannins	+
Saponins	+
Cardiac glycosides	+
Reducing sugars	+
Alkaloids	−
Phenolic compound	+
Flavonoids	+
Cyanogenic glycosides	−

+: The presence of the phytochemical, −: The absence of the phytochemical

Conclusions

From the ethanol, hexane and aqueous extracts tested against *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *E. coli* and MRSA isolates, ethanol extract of *L. iteodaphne* showed the highest antimicrobial activity with a MIC of 0.0256 mg/ml. The presence of phytochemicals such as tannins, cardiac glycosides, reducing sugars, phenolic compounds, saponins, and flavonoids in the plant extract suggests that these phytochemicals may be responsible for the antibacterial activity. Further studies where activity guided fractionation and isolation of active compounds may warrant the development of potential antimicrobial agents from *L. iteodaphne* in the future.

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Conflicts of interest

There are no conflicts of interest.

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