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EXPERIMENTAL PAPER

GC-MS analysis and evaluation of antibacterial activity of *Sida cordifolia* leaf extracts from Jaffna, Sri Lanka

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Summary

Introduction: Antimicrobials are becoming less effective due to growing antimicrobial resistance; thus, finding alternatives is crucial. Medicinal plants provide valuable sources of antimicrobial compounds. In traditional medicine, *Sida cordifolia* is employed to treat inflammation, wounds, and skin infections.

Objective: To identify the bioactive compounds in *S. cordifolia* leaf extracts and evaluate their antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.

Methods: Methanol, ethyl acetate, and petroleum ether leaf extracts were prepared using a maceration process. Antimicrobial activity was assessed using the agar well diffusion method and the minimum inhibitory concentration (MIC) method. Phytochemical analysis of the methanolic leaf extract was performed using GC-MS.

Results: Methanolic extracts showed the highest antimicrobial activity against *S. aureus* and *E. coli.* The MICs of methanol, ethyl acetate, and petroleum ether extracts were 50 mg/ml, 100 mg/ml, and 100 mg/ml, respectively. The GC-MS analysis results revealed that long-chain fatty acids are the major compounds.

Conclusion: The findings support the traditional use of *S. cordifolia* as an antimicrobial agent.

Keywords: ***antibacterial activity, Sida cordifolia, Staphylococcus aureus, Escherichia coli***

Słowa kluczowe: ***działanie przeciwbakteryjne, Sida cordifolia, Staphylococcus aureus, Escherichia coli***

INTRODUCTION

Superficial skin infections are the most common clinical conditions encountered worldwide and are primarily caused by opportunistic microorganisms that colonise the skin. An infected sebaceous cyst represents a localized superficial skin infection that develops when a previously benign cyst becomes secondarily infected. Sebaceous cysts arise due to obstruction or damage to hair follicles or the epidermis. It leads to the accumulation of keratinous material within a cystic cavity lined by stratified squamous epithelium. While these cysts are usually asymptomatic, rupture or microbial invasion can result in inflammation, pain, erythema, swelling, and purulent or foul-smelling discharge [1]. The most frequently implicated pathogens include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and anaerobic bacteria, though fungal organisms may be involved in rare cases.

The rise in the prevalence of antimicrobial resistance is a significant global health concern, as it diminishes the efficacy of conventional antimicrobial medications and increases morbidity and mortality [2]. This alarming situation has intensified the search for alternative and effective antimicrobial therapies, particularly those derived from natural sources. Medicinal plants, which have been used for centuries in traditional systems of medicine, are widely recognised as valuable reservoirs of bioactive compounds with significant therapeutic potential [3].

Sida cordifolia, which belongs to the family *Malvaceae*, is commonly known as country mallow. It is a well-known medicinal plant widely distributed throughout tropical and subtropical regions, including Sri Lanka and India. In terms of botany, it is a small, erect, branched shrub that is adorned with stellate hairs. The leaves are heart-shaped and serrated, the flowers are small and yellow, and the fruits are approximately the size of a bean.

In traditional medicine, *S. cordifolia* has been extensively used for the treatment of fever, inflammation, wounds, coughs, and skin infections. Previous studies have reported that *S. cordifolia* possesses a wide range of pharmacological properties such as anti-inflammatory, antipyretic, analgesic, antioxidant, wound-healing, central nervous system depressant, hypoglycaemic, and hypotensive activities [4]. Phytochemical investigations have revealed the presence of several bioactive constituents such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, and glycosides. Many of these phytochemicals are known to exhibit antimicrobial activity against bacterial and fungal pathogens [5].

Despite its extensive traditional use, scientific validation of the antimicrobial potential of *S. cordifolia* remains limited. Although antimicrobial activity has been reported in studies conducted in other countries, there are currently no published scientific reports evaluating the antimicrobial activity of *S. cordifolia* leaves sourced from Sri Lanka. Therefore, it is essential to scientifically investigate the antimicrobial efficacy of extracts from *S. cordifolia* leaves collected in Sri Lanka.

The present study aims to evaluate the *in vitro* antimicrobial activity of extracts of *S. cordifolia* leaves against selected pathogenic microorganisms associated with superficial skin infections, including infected sebaceous cysts. This study seeks to provide the first scientific evidence from Sri Lanka and to support the validation of the traditional medicinal use of *S. cordifolia* as a potential source of plant-based antimicrobial agents.

MATERIALS AND METHODS

Collection and authentication of plant material

Fresh leaves of *S. cordifolia* were collected from Mayiliddy, Jaffna, Sri Lanka, in January 2026. The collection site is located at approximately 9.80° N, 80.03° E. The area experiences a tropical climate with temperatures ranging from 27 °C to 32 °C. The soil was sandy loam and well-drained. The plant material was authenticated by a qualified botanist at the Department of Plant and Molecular Biology at the University of Kelaniya. The collected leaves were thoroughly washed with tap water to remove dust and debris, then shade-dried at room temperature until a constant weight was obtained. The dried

Figure 1.

Leaves of *S. cordifolia*

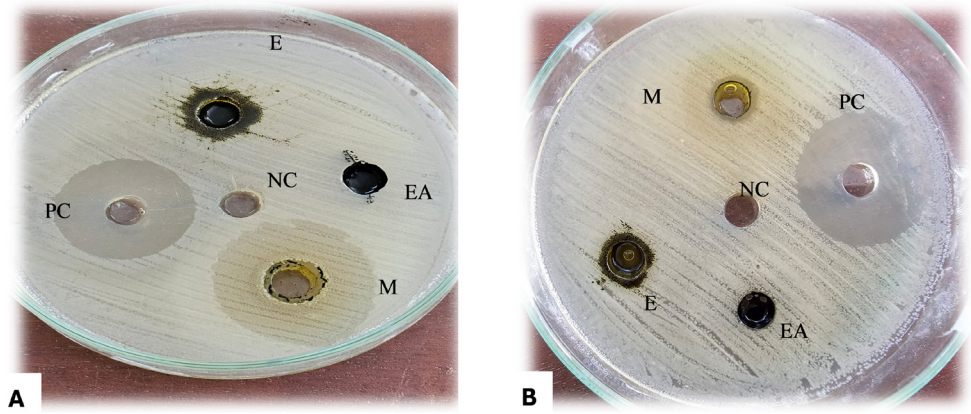

Figure 2.

Antimicrobial activity of *S. cordifolia* leaf extracts against (A) *S. aureus* (B) *E. coli*
PC – positive control, NC – negative control, EA – ethyl acetate, M – methanol, E – petroleum ether

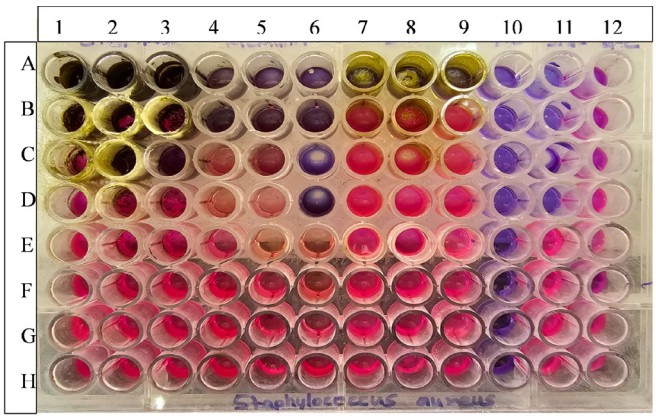

Figure 3.

Determination of MIC of *S. cordifolia* leaf extracts: Column A1-H1,A2-H2,A3-H3: Ethyl acetate extract, Column A4-H4,A5-H5,A6-H6: Methanolic extract, Column A7-H7,A8-H8,A9-H9 : Petroleum ether extract, Column A10-H10: Positive control, Column A11-D11: Sterility control, Column E11-H11: Growth control, A12-H12: Negative control

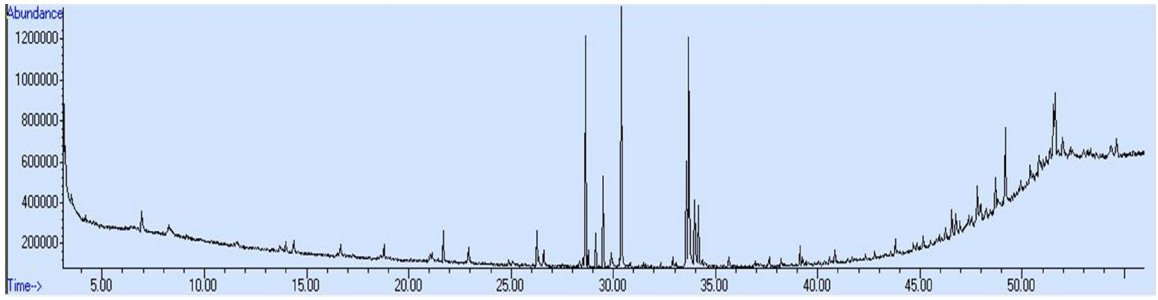

Figure 4.

GC-MS analysis of *S. cordifolia* methanolic leaf extract

leaves were ground into a fine powder using a mechanical grinder. The powdered material was stored in an airtight container in a refrigerator at 4 °C for further study.

Extraction of plant materials

Leaf extracts of *S. cordifolia* were prepared using the maceration method with different solvents. About 20 g of the powdered leaf material was separately soaked in 200 ml of hexane, 200 ml of ethyl acetate and 200 ml of methanol in clean, labelled conical flasks. The mixtures were kept at room temperature for 48 hours with occasional shaking. After the extraction period, the mixtures were filtered using Whatman No. 1 filter paper. The obtained filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C to remove the solvents. The resulting crude extracts were collected, transferred into sterile containers and stored in a refrigerator at 4 °C for further use [5].

Evaluation of antimicrobial activity

Preparation of stock solution

The concentration of 100 mg/ml of methanolic, ethyl acetate and petroleum ether extracts were prepared by dissolving in 10% dimethyl sulfoxide (DMSO) separately.

Tested organisms

Bacterial cultures, *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), were obtained from the Department of Botany, Faculty of Science, University of Jaffna. They were maintained on nutrient agar slants and stored at 4 °C to preserve viability during the study.

Preparation of bacterial inoculum

First, each bacterium was subcultured and incubated on Mueller-Hinton agar plates at 37 °C for 24 h. Bacterial inoculum for each test organism were prepared using sterile saline water in a universal bottle. The bacterial suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard (1×10^8 CFU/ml).

Agar well diffusion assay

The antimicrobial activity of *S. cordifolia* leaf extracts was evaluated by using the agar well diffusion method [6]. Mueller-Hinton agar plates were prepared. Bacterial suspensions were prepared equivalent to 0.5 McFarland, and they were streaked separately on prepared agar plates with sterile cotton swabs. Wells of 6 mm diameter were bored into the agar plate, and 100 μl; of methanolic, ethyl acetate and petroleum ether extracts of *S. cordifolia* leaf extracts (100 mg/ml) were added into each well. Gentamycin (0.2 mg/ml) was used as the positive control, while DMSO was used as the negative control. The plates were incubated at 37 °C for 24 hours. Then, the diameter of the zone of inhibition around each well was measured. Triplicates were carried out [7].

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using a broth microdilution assay in sterile, 96-well microtiter plates [8]. Two-fold serial dilutions of each extract were prepared with concentrations ranging from 100 mg/ml to 0.78125 mg/ml. In a microtiter plate, each well was filled with 50 μl of nutrient broth. Fifty microlitres of the stock solutions were added separately to the first well of each column and serially diluted twofold across

eight wells. At the end, 50 μl of the mixer from the last well of each column was discarded. To each well, 30 μl of nutrient broth were added again. Finally, 10 μl of fresh bacterial suspension (1×10^8 CFU/ml) was pipetted into each well. The same procedure was done to prepare the plate for each bacterial strain. Then the plates were covered with parafilm and incubated for 18 hours at 37 °C. Each plate had a set of controls. Column 10 was added with a broad-spectrum antibiotic, gentamycin (initial concentration of 0.2 mg/ml), which served as the positive control. It was serially diluted in the same way. Column 11 was added with 10% DMSO, which served as the negative control. The test for each plant extract was done in triplicate, and all the procedures were done aseptically. After incubation, 10 μl of resazurin solution (0.015% w/v) was added to each well, followed by an additional two-hour incubation. MIC was determined to be the lowest concentration that retained the blue colour of resazurin, indicating no microbial growth.

GC-MS characterization of plant extracts

GC-MS analysis of methanolic extracts of *S. cordifolia* was performed for the identification of volatile and semi-volatile compounds. The plant extracts were freeze-dried and diluted in methanol. The diluted samples were filtered using a 0.22 μm PTFE syringe filter to remove particulates and protect the GC column and injector from contamination. A sample solution of 1 mg/ml concentration was prepared for analysis. Analysis was carried out using GC–MS (Agilent 6890 GC coupled with 5971 MSD). Helium gas (99.995% purity) was used as the carrier gas. 1 μl of sample was injected into the column with a flow rate of 1.00 ml/min, linear velocity of 36.7 cm/s, total flow of 14.0 ml/min, and purge flow of 3.0 ml/min. The injector temperature is 260 °C; the ion source temperature is 200 °C. The initial oven temperature was maintained at 70 °C for 5 minutes, then increased gradually to 310 °C at a rate of 5 °C/min, with a final hold time of 10 minutes. Phytoconstituents were identified based on relative retention time and peak area. Mass spectra obtained were compared with the National Institute of Standards and Technology (NIST) library database and available literature. Only peaks showing a ≥85% similarity match were considered significant. The compound name, molecular weight, peak area and retention time were recorded [9].

RESULTS

Antimicrobial activity

Methanolic extracts of *S. cordifolia* exhibited antimicrobial activity against *S. aureus* and *E. coli* with zones of inhibition of 11.7±0.95 mm and 11.5±0.55 mm, respectively. While ethyl acetate extracts and petroleum ether extracts didn't exhibit any detectable zone of inhibition (Table 1). This study revealed that methanolic extracts are potent. MIC values of methanolic, ethyl acetate and petroleum ether extracts of *S. cordifolia* against *S. aureus* and *E. coli* were 50 mg/ml, 100 mg/ml and 100 mg/ml, respectively (Table 2).

GC-MS analysis

GC-MS analysis of the methanolic extract of *S. cordifolia* revealed the presence of 75 chromatographic peaks, indicating multiple bioactive compounds. The major identified bioactive compounds with their retention time (RT), molecular formula (MF), molecular weight (MW), peak area (PA) and properties are presented in Table 3.

Table 1.

Antimicrobial activity of leaf extracts of *S. cordifolia* at 100 mg/ml concentration

Microorganism	Mean diameter of zone of inhibition [mm ±SD]				
	Positive control (gentamycin – 0.2 mg/ml)	Ethyl acetate extract	Methanolic extract	Petroleum ether extract	Negative control (10% DMSO)
S. aureus	30±0.00	NZ	11.7±0.95	NZ	NZ
E. coli	26±0.00	NZ	11.5±0.55	NZ	NZ

Values are expressed as mean ±SD, SD – standard deviation, NZ – no zone of inhibition

Table 2.

MIC value of leaf extracts of *S. cordifolia* extracts

Microorganism	MIC [mg/ml]		
	Methanolic extract	Ethyl acetate extract	Petroleum ether extract
S. aureus	50	100	100
E. coli	50	100	100

Table 3.

The major identified compounds in methanolic leaf extract of *S. cordifolia*

No	Compounds	RT	Quality	PA %	MW [g/mol]	MF	Nature of the compound	Properties
1.	Dodecanoic acid	21.6745	98	1.645	200.23	$C_{12}H_{24}O_2$	Saturated fatty acid	Antimicrobial, antioxidant; common in plant oils [18]
2.	Methyl tetradecanoate	26.2500	98	2.010	242.40	$C_{15}H_{30}O_2$	Saturated fatty acid	Antimicrobial, anti-inflammatory and lipid-related bioactivity [19]
3.	Hexadecanoic acid	30.3942	99	10.569	256.42	$C_{16}H_{32}O_2$	Fatty acid methyl ester	Antimicrobial, antioxidant, anti-inflammatory [19]
4.	9,12-Octadecadienoic acid	33.5707	99	4.672	280.45	$C_{18}H_{32}O_2$	Polyunsaturated fatty acid methyl ester	Antioxidant, antiinflammatory, antimicrobial [20]
5.	9,12,15-Octadecatrienoic acid	33.6932	99	10.809	278.43	$C_{18}H_{30}O_2$	Polyunsaturated fatty acid methyl ester	Antioxidant, cardio protective, anti-inflammatory [19]
6.	Octadecanoic acid	34.1595	99	2.747	284.48	$C_{18}H_{36}O_2$	Fatty acid methyl ester	Contributes to lipid profile, plant extract bioactivity [18]
7.	Phenol, 2,2'-methylenebis	39.1196	99	0.868	200.23	$C_{15}H_{24}O_2$	Phenolic	Free radical scavenger, endocrine disrupting activity, oestrogen receptor activity [21]
8.	Octacosanoic acid	49.1624	96	4.071	422.78	$C_{28}H_{56}O_2$	Long chain fatty acid methyl ester	Found in plant waxes, antiplatelet activity, cholesterol lowering minor bioactivity (22)

DISCUSSION

The present study aimed to evaluate the antibacterial activity of *S. cordifolia* against *S. aureus* and *E. coli.* For the extraction, the maceration method was used, as it is convenient, cost-effective and suitable to extract heat-sensitive compounds. Three solvents (methanol, ethyl acetate and petroleum ether) were utilised for the extraction of bioactive compounds from *S. cordifolia.* The selection of multiple solvents was used based on their varying polarity, which significantly influences the type and quantity of phytoconstituents extracted. Methanolic extracts are particularly effective in isolating polar compounds such as phenolics and flavonoids. Petroleum ether extracts, being non-polar, facilitate the extraction of lipophilic constituents such as fatty acids and terpenoids. Ethyl acetate extracts with intermediate polarity enable the extraction of alkaloids and other semi-polar compounds [10].

Antibacterial activity was evaluated using the agar well diffusion assay and the broth dilution method. Agar well diffusion is a qualitative method used to assess the ability of plant extracts to inhibit microbial growth. When the leaf extracts are added to wells in the agar, antimicrobial compounds diffuse into the surrounding medium, inhibiting the growth of the pathogens. Methanolic extracts exhibited antibacterial activity at 100 mg/ml, whereas ethyl acetate and petroleum ether extracts did not exhibit a noticeable zone of inhibition against *S. aureus* and *E. coli* at 100 mg/ml. The observed activity of the methanolic extract may be attributed to the ability of methanol to extract a broad spectrum of bioactive constituents. Methanol is a polar organic solvent that efficiently penetrates the cell wall and dissolves polar and nonpolar compounds, thereby yielding a higher concentration of bioactive constituents compared to other extracts. The absence of activity in ethyl acetate extract and petroleum ether extract may be due to the lower solubility of active compounds, insufficient concentration of active compounds, and poor diffusion of extracted compounds in the agar medium. Limited studies were carried out to evaluate the antimicrobial activity of *S. cordifolia* in other countries. A study conducted by Kumar *et al*. to evaluate antibacterial activity of methanolic, hexane, chloroform and aqueous extracts of *S. cordifolia* leaf, bark and root showed activity against *S. aureus, Bacillus subtilis* and *E. coli* at 1 mg/ml [5]. In a study carried out by Prabhakar *et al*. to evaluate antibacterial and antifungal activity, methanolic and chloroform *S. cordifolia* leaf and root extracts exhibited activity against *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* at 10 mg/ml [7]. Venkatachalam D *et al*. evaluated the antimicrobial activity of chloroform leaf extracts of *S. cordifolia*, which showed activity against *S. aureus* [11]. In another study, methanolic fractionation of roots of *S. cordifolia* identified two compounds, rosmarinic acid and its 4-O-β-D-glucoside derivative, which had potent activity against *S. aureus* and methicillin-resistant *Staphylococcus aureus* [12]. These previous findings align with our findings demonstrating antimicrobial activity; however, variations were observed in the concentrations.

MIC was performed to determine the lowest concentration of each extract capable of inhibiting visible growth. In the present study, the MIC of methanolic extract, ethyl acetate extract and petroleum ether extract was 50 mg/ml, 100 mg/ml and 100 mg/ml, respectively. MIC determination allows direct and uniform contact between the microbial cells and the extract. This eliminates limitations related to diffusion and provides more accurate quantification of antimicrobial potency. The comparatively lower MIC was observed for the methanolic extract against the tested microorganisms. This suggests the methanolic leaf extract of *S. cordifolia* is more potent than other solvent extracts. Very limited studies have investigated the MIC values of *S. cordifolia* extracts. Ouédraogo *et al.* reported that bioactive compounds from *S. cordifolia,* exhibited significant anti-candidal activity with a low MIC ranging from 4 to 6 μg/ml when combined with nystatin and clotrimazole [13].

GC-MS is an analytic technique that combines gas chromatography (GC) and mass spectrometry (MS) to allow separation, identification and characterisation of volatile and semi-volatile bioactive constituents based on retention time and mass spectral fragmentation patterns. As the methanolic leaf extract of *S. cordifolia* is more potent, it was analysed. The chromatogram revealed multiple peaks indicating the presence of various bioactive constituents in the extract. The identified compounds mainly belonged to fatty acid esters and phenolic compounds. Hexadecanoic acid, 9,12,15-octadecatrienoic acid, 9,12-octadecadienoic acid and octacosanoic acid were identified as major compounds with the highest peak area as 10.569, 10.809, 4.672 and 4.070, respectively. These compounds have been previously reported to exert antimicrobial activities. The long-chain saturated and unsaturated fatty acids exert antibacterial activity by inserting into bacterial phospholipid

bilayers, increasing permeability and disrupting cell membranes [14, 15]. The predominance of the long-chain saturated and unsaturated fatty acids in GC-MS analysis supports the observed antibacterial potential of *S. cordifolia* extract, with the methanolic extract indicating stronger antimicrobial potency. Few studies were carried out to investigate the phytochemical composition of *S. cordifolia*. Khurana *et al.* revealed that *S. cordifolia* extracts possess alkaloids, flavonoids, glycosides, phenolic compounds, saponins and tannins [16]. Joseph *et al*. carried out a study on GC-MS analysis, which exhibited that the methanolic extract contains vasicinol, ephedrine, vasicinone and hypaphorine [17]. These GC-MS analysis findings are in contrast with our present study, which exhibited that the methanolic extract possesses polyunsaturated fatty acids as major compounds.

CONCLUSION

The present study demonstrated that the methanolic leaf extract of *S. cordifolia* possesses antibacterial activity against *S. aureus* and *E. coli*. GC-MS analysis showed that the methanolic leaf extract of *S. cordifolia* contains polyunsaturated fatty acids as a major compound. These findings support the traditional use of it in treating bacterial infections and highlight its potential as a source for developing novel antibacterial agents.

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Ethical approval
The research conducted is not related to either human or animal use.

Conflict of interest
Authors declare no conflict of interest.

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