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Research Article

ANTIBACTERIAL ACTIVITY OF LIANOID AND ARBOREAL SPECIES OF *SCHEFFLERA* FROM SOUTHERN INDIA

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ABSTRACT

Objective: The genus *Schefflera* belongs to the family Araliaceae. The secondary metabolites of *Schefflera* include triterpene glycosides, oleanolic acid, and benzyl glycosides with proven biological activities. The objective of this study was to determine the antibacterial potentials of lianoid and arboreal *Schefflera* spp., from southern India.

Methods: *Schefflera venulosa*, *Schefflera stellata*, and *Schefflera racemosa* were collected from the natural forests of Kodagu and Mysore regions of southern Karnataka. The plant parts such as stem bark, leaves, and inflorescence were dried, powdered and known quantity was subjected to Soxhlet extraction based on the solvent polarity. The solvent extracts from each of the species were subjected to preliminary antibacterial screening against five test bacterial strains, namely, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae* by agar disc diffusion method and the inhibition zones were measured. The minimum inhibitory concentrations (MIC's) were calculated for the positive extracts and represented.

Results: Of the 30 solvent extracts tested, six solvent extracts showed antibacterial activity. The ethanol and ethyl acetate solvent extracts of all three plants showed positive results for antibacterial activity. The zone of inhibition against the bacterial test pathogens ranged from 6.00±0.00 to 15.00±0.00 mm against *B. subtilis*, *E. aerogenes*, *S. pyogenes*, and *K. pneumoniae.* The highest zones of inhibition were observed for *S. racemosa* leaf ethanol extract against *B. subtilis*, *E. aerogenes,* and *S. pyogenes* (10.00 mm to 15.00±0.00 mm). The MIC values of the positive extracts were 5.0 mg/ml in the leaf, flower ethanol, and stem bark ethyl acetate extracts of *S. venulosa*, leaf ethyl acetate and ethanol extracts of *S. stellata* and the leaf ethanol extract from *S. racemosa*, respectively.

Conclusion: *Schefflera* spp. from southern India possesses antibacterial potentials, which can be exploited pharmaceutically for potential health benefits against bacterial infections.

Keywords: *Schefflera*, Antibacterial, Minimum inhibitory concentrations, Phytochemicals, Solvent extracts.

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INTRODUCTION

World Health Organization has warned about the multidrug-resistant bacteria that are emerging worldwide as a huge challenge to healthcare as antibiotics may lose their power to cure diseases [1]. *Enterobacter aerogenes* and *Klebsiella pneumoniae* are Gram-negative bacteria responsible for a wide variety of diseases in humans. *E. aerogenes* is widely distributed in the soil, water, dairy products, and in the intestine of animals as well as humans. Antimicrobial resistance has been found in pathogenic and non-pathogenic strains of *E. aerogenes* [2]. *K. pneumoniae* is an important pathogen in nosocomial infections, which have been well documented in the US and India [3]. They are found to be the primary cause of respiratory tract infections and are also commonly involved in acute pyelonephritis in pregnant women with urinary tract abnormalities [4]. *Staphylococcus aureus* and *Streptococcus pyogenes* are Gram-positive, non-spore forming facultative anaerobic bacteria that are able to invade through the broken skin or mucous membrane. Infections caused by *S. pyogenes* include pharyngitis, localized skin infections, rheumatic heart disease, rheumatic fever, and streptococcal toxic shock syndrome [5], while *S. aureus* causes skin lesions such as boils, furuncles, and more serious infections such as pneumonia, phlebitis, meningitis, and urinary tract infections. *Bacillus subtilis* is a Gram-positive, mesophylic, endospore-forming saprophyte, which has been listed among foodborne pathogens involved in the outbreaks from contaminated food and water. These organisms have been recognized with the ability to develop resistance to antibiotics [6].

Historically, plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have made large contributions to human health and well-being [7]. Approximately 25–50% of current pharmaceuticals are derived from plants. Several studies indicate that medicinal plants contain chemical compounds such as peptides, unsaturated long-chain fatty acids, aldehydes, alkaloids, essential oils, tannins, flavonoids, saponins, glycosides, and phenols as water or ethanol-soluble compounds which are significant in therapeutic applications against the pathogens such as bacteria, fungi, and viruses [8]. The antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world [9-11]. Several works have demonstrated in laboratory trials that different plant tissues such as roots, leaves, seeds, and flowers possess inhibitory properties against bacteria, fungi, and insects [12].

Schefflera comprises lianoids or arboreal genera in the family Araliaceae [13]. *Schefflera venulosa* (W.&A.) Harms., commonly called *Schefflera* vine, is a large climbing shrub with digitate compound leaves. The phytoconstituents *S. venulosa* are mainly caffeoyl acids, quercetin glycoside, and oleanolic acid glycoside which help in blood circulation and prevents cardiac and cerebral vascular diseases [14]. *Schefflera stellata* is a small tree distributed in peninsular India and Sri Lanka [15]. Ethnomedicinal uses of *S. stellata* revealed that it is used to cure neurological weakness [16] and a belief that the bark ash is used to control evil spirits [17]. The antimicrobial activity of *S. stellata* from volatile oils of root, stem, and leaves was determined [18]. *Schefflera racemosa* is a medium-sized tree with compound leaves and flowers

in lateral panicled racemes and is endemic to the Western Ghats. The phytochemicals, phenolic content, and antioxidant activities in the plant parts of *S. venulosa*, *S. stellata,* and *S. racemosa* are reported [19,20]. This study explores the antibacterial activity of solvent extracts from three *Schefflera* species against Gram-positive and Gram-negative bacteria.

METHODS

Collection of plant material

Schefflera species, namely, *S. venulosa* (W.&A.) Harms. and *S. racemosa* (Wight) Harms., were collected from the natural forests of Kodagu (N12°20′14.97″and E75°48′24.86″), in the Western Ghats, while *S. stellata* (Gaertn.) Harms. was collected from Chamundi Hills (N 12°16′21.1368" and E 76°40′14.3364") of Mysore, Karnataka, during May to September 2011 (Fig. 1). Each species considered for the study was photographed. The plant part such as leaves, stem bark, and inflorescence was excised with a plier and placed in zip lock polythene bags, labeled, brought to the laboratory, and processed for further use. A herbarium specimen of each plant species was prepared and submitted to the herbarium collection of the Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore.

Preparation of solvent extracts

The collected plant parts were thoroughly washed to remove dust and debris, and further dried under shade. Drying was completed at 40°C in a hot air oven (Neolab, Maag Industries, Mumbai) before blending to remove the water content and powdered. The powdered materials weighing approximately 500 g were placed in polyethylene zip lock covers for further use. Fifty gram of the powdered material was filled tightly into a thimble and was placed in the extraction tube of the Soxhlet apparatus (Borosilicate glass, Padmashree Scientific®). Five solvents were used for the extraction based on their polarity, namely, hexane (non-polar), chloroform (non-polar), ethyl acetate (semi-polar), ethanol (polar), and methanol (polar). Three hundred fifty milliliter of each solvent was used for the extraction process. After extraction, the distillate was collected and poured into Petri plates for drying. A similar process for other solvents was carried out. The extracts were scraped and transferred into pre-weighed Eppendorf tubes, labeled, and stored for further use.

Test strains and culture media

Strains of bacteria and fungi were obtained from MTCC (Microbial Type Culture Collection, Chandigarh, India). The antimicrobial activity of 30 extracts against *B. subtilis* (MTCC 121), *S. aureus* (MTCC 7443), *S. pyogenes* (MTCC 1925), *E. aerogenes* (MTCC 7325), and *K. pneumoniae* (MTCC 7407) were studied. The bacteria were grown in Mueller–Hinton Agar (MHA) (Merck) and Trypticase Soya Broth (Merck). The concentrations of bacterial suspensions were adjusted to 10^8 cells/ml.

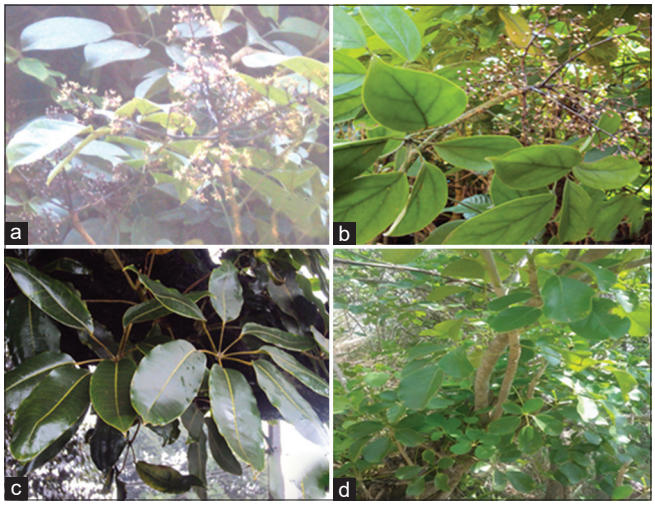

Fig. 1: Habit of *Schefflera* species collected from southern India. (a) *Schefflera venulosa* (W.&A.) Harms. habit with digitate leaves and panicle inflorescence. (b) A close up of *S. venulosa* leaves with prominent main nerves. (c) *Schefflera racemosa* (Wight) Harms. with digitate leaflets collected from the natural forests of Kodagu, Western Ghats. (d) *Schefflera stellata* (Gaertn.) Harms., with obovate, rounded leaflets collected from Chamundi Hills, Mysore

Antibacterial assay

Sensitivity testing of various solvent extracts was done using the agar well diffusion method as well as the agar disc diffusion method, as previously described with minor modifications [21]. Approximately 20 ml of molten, sterilized MHA media were poured into sterilized Petri plates and was allowed to solidify. A hundred microliter of the inoculum was used to prepare the bacterial lawn. In the agar well diffusion method, wells were bored into agar medium using sterile 6 mm cork borer. Ten microliter (100 mg/ml) of the plant extract was aseptically added to the well. In the agar disc diffusion method, 10 µl (100 mg/ml) of the plant extract was infused onto a sterile paper disc. Positive control (Ciprofloxacin 10 µl, 1 mg/ml) and negative control (respective solvent) (10 µl, 1 mg/ml) were maintained for both well diffusion and disc diffusion methods. The plates were then allowed to stand for 20 min to allow proper diffusion of the solution into the medium before incubation. They were then incubated at 28°C for 24 h. The antibacterial activity was evaluated by measuring the zones of inhibition against the test organisms. The experiment was replicated 2 times and zone of inhibition was recorded as mean±SD.

Determination of minimum inhibitory concentration (MIC)

MIC was determined by following the Clinical and Laboratory Standards Institute guidelines [22]. Cell suspension prepared from bacterial cultures grown on Trypticase Soya Broth adjusted to 1–2 × 10^5 cells/ml using turbidity equivalent to a 0.5 McFarland standard. Ciprofloxacin, 2-128 µg/ml (Twofold dilutions) in Mueller–Hinton Broth/Tryptone broth were used as standard. Mueller–Hinton broth inoculated with culture and without drug was used as control. Ninety microliter of the solvent extract of different concentrations was mixed with 10 µl of inoculum in a 96 – well microtiter plate (Tarsons, Mumbai) in triplicate. For the control, 90 µl Mueller–Hinton Broth without solvent extract was mixed with 10 µl of inoculum. The treated bacterial cultures were incubated at 25°C and 35°C, respectively. The bacterial test plates were observed after 24–48 h and OD at 600 nm were measure in Tecan plate reader (Tarsons, Mumbai). The MIC was determined as the minimum concentration of drug giving 50% inhibition of OD as compared with control using the formula

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{sample})/Abs_{control}) \times 100$$

Statistical analysis

All the experiments were done in triplicates. Statistical analysis was done using SPSS program (version 16.0). One-way ANOVA and *post hoc* tests were conducted and probability (p) value <0.05 was considered as significantly different.

RESULTS

The antibacterial activities of various solvent extracts on the test organisms were assessed in terms of diameters of inhibition zones and are represented in Tables 1-3. The zones of inhibition (clear zones on agar) for all the organisms tested were measured in mm. Of the 30 solvent extracts tested, six solvent extracts showed antibacterial activity. The ethyl acetate, ethanol, and methanol solvent extracts of all three plants showed good results, while the non-polar extracts did not show positive results for antibacterial activity. The ethanol leaf and flower extract of *S. venulosa* showed an inhibition zone of 6.00±0.00 mm and 9.00±0.25 mm, respectively, against *B. subtilis*, while the ethyl acetate stem bark extract showed an inhibition zone of 8.00±0.50 mm and 8.00±0.25 mm against *E. aerogenes* and *K. pneumoniae*, respectively (Table 1). The ethanol leaf extract of *S. racemosa* showed potent antibacterial activity against *B. subtilis* (10.00±0.00 mm), *S. pyogenes* (13.00±0.50 mm), and *E. aerogenes* (15.00±0.00 mm) (Table 3). The ethyl acetate and the ethanol leaf extracts of *S. stellata* showed an

inhibition zone of 10.00±0.00 mm and 9.00±0.50 mm, respectively, against *S. pyogenes* (Table 3).

The MIC was also determined for the extracts showing positive results in the antibacterial activity. Even though eleven solvent extracts showed positive results for the antibacterial activity, their MIC was much greater when compared with the MIC of ciprofloxacin which ranged from 0.0012 to 0.0195 mg/ml. Most of the extracts showed MIC values >10.00 mg/ml which is not appreciable (Table 4).

DISCUSSION

Infectious diseases resulting from the presence of resistant microbial agents, including bacteria, fungi, and viruses, have become a major healthcare problem in the current century. The solution for antibiotic resistance is the development of new drugs natural sources because of minimal side effects. The healing power in plants is usually due to the presence of secondary metabolites. Plant extracts and large number of phytochemicals exhibited a strong inhibiting effect on a broad spectrum of microorganisms [9].

Table 1: Inhibitory activity of *Schefflera venulosa* solvent extracts against the test organisms

Extracts (10 µl, 100 mg/ml)	*B. subtilis*	*S. aureus*	*S. pyogenes*	*E. aerogenes*	*K. pneumonia*
Leaf					
Hexane	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	0.00	0.00	0.00	0.00
Ethyl acetate	0.00	0.00	0.00	0.00	0.00
Ethanol	6.00±0.00	0.00	0.00	0.00	0.00
Methanol	0.00	0.00	0.00	0.00	0.00
Ciprofloxacin (10 µl, 1 µg/ml)	40.00±1.00	32.00±0.50	40.00±0.00	40.00±1.00	30.00±0.00
Flower					
Hexane	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	0.00	0.00	0.00	0.00
Ethyl acetate	0.00	0.00	0.00	0.00	0.00
Ethanol	9.00±0.25	0.00	0.00	0.00	0.00
Methanol	0.00	0.00	0.00	0.00	0.00
Ciprofloxacin (10 µl, 1 µg/ml)	40.00±1.00	32.00±0.50	40.00±0.00	40.00±1.00	30.00±0.00
Stem bark					
Hexane	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	0.00	0.00	0.00	0.00
Ethyl acetate	0.00	0.00	0.00	8.00±0.50	8.00±0.25
Ethanol	0.00	0.00	0.00	0.00	0.00
Methanol	0.00	0.00	0.00	0.00	0.00
Ciprofloxacin (10 µl, 1 µg/ml)	40.00±1.00	32.00±0.50	40.00±0.00	40.00±1.00	30.00±0.00

*All the values are expressed as mean±standard error of mean (n=3). *B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes, E. aerogenes: Enterobacter aerogenes, K. pneumonia: Klebsiella pneumoniae*

Table 2: Inhibitory activity of *Schefflera racemosa* leaf extracts against the test bacterial organisms

Extracts (10 µl, 100 mg/ml)	*B. subtilis*	*S. aureus*	*S. pyogenes*	*E. aerogenes*	*K. pneumoniae*
Hexane	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	0.00	0.00	0.00	0.00
Ethyl acetate	0.00	0.00	0.00	0.00	0.00
Ethanol	10.00±0.00	0.00	13.00±0.00	15.00±0.00	0.00
Methanol	0.00	0.00	0.00	0.00	0.00
Ciprofloxacin (10 µl, 1 µg/ml)	40.00±1.00	32.00±0.50	40.00±0.00	40.00±1.00	30.00±0.00

*All the values are expressed as mean±standard error of mean (n=3). *B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes, E. aerogenes: Enterobacter aerogenes, K. pneumonia: Klebsiella pneumoniae*

Table 3: Inhibitory activity of *Schefflera stellata* extracts against the test organisms

Extracts (10 µl, 100 mg/ml)	*B. subtilis*	*S. aureus*	*S. pyogenes*	*E. aerogenes*	*K. pneumoniae*
Leaf					
Hexane	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	0.00	0.00	0.00	0.00
Ethyl acetate	0.00	0.00	10.00±0.00	0.00	0.00
Ethanol	0.00	0.00	9.00±0.50	0.00	0.00
Methanol	0.00	0.00	0.00	0.00	0.00
Ciprofloxacin (10 µl, 1 µg/ml)	40.00±1.00	32.00±0.50	40.00±0.00	40.00±1.00	30.00±0.00
Stem bark					
Hexane	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	0.00	0.00	0.00	0.00
Ethyl acetate	0.00	0.00	0.00	0.00	0.00
Ethanol	0.00	0.00	0.00	0.00	0.00
Methanol	0.00	0.00	0.00	0.00	0.00
Ciprofloxacin (10 µl, 1 µg/ml)	40.00±1.00	32.00±0.50	40.00±0.00	40.00±1.00	30.00±0.00

*All the values are expressed as mean±standard error of mean (n=3). *B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes, E. aerogenes: Enterobacter aerogenes, K. pneumonia: Klebsiella pneumoniae*

Table 4: MIC of different solvent extracts from *Schefflera* species

Extracts of *Schefflera* species	MIC values of extracts (mg/ml)				
	B. subtilis	***S. aureus***	***S. pyogenes***	***E. aerogenes***	***K. pneumoniae***
S. venulosa leaf ethanol extract	5.0	>10.0	>10.0	>10.0	>10.0
S. venulosa flower ethanol extract	5.0	>10.0	>10.0	>10.0	>10.0
S. venulosa stem bark ethyl acetate extract	>10.0	>10.0	>10.0	5.0	5.0
S. racemosa leaf ethanol extract	5.0	>10.0	5.0	>10.0	>10.0
S. stellata leaf ethyl acetate extract	>10.0	>10.0	5.0	>10.0	>10.0
S. stellata leaf ethanol extract	>10.0	>10.0	5.0	>10.0	>10.0
Ciprofloxacin	0.0012	0.0024	0.0049	0.0036	0.0195

*All the values are expressed as mean ± standard error of mean (n=3). MIC: Minimum inhibitory concentration, *B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes, E. aerogenes: Enterobacter aerogenes, K. pneumoniae: Klebsiella pneumoniae, S. venulosa: Schefflera venulosa, S. racemosa: Schefflera racemosa, S. stellate: Schefflera stellata*

The present study resulted in the antibacterial screening of 30 solvent extracts from four species of *Schefflera*, namely, *S. venulosa*, *S. racemosa*, and *S. stellata*. All three species studied showed positive results for antibacterial activity. Among the three plant species, *S. venulosa*, *S. racemosa* showed antibacterial activity against *B. subtilis* which is an encapsulated, Gram-positive, endospore-forming obligate aerobe, which causes food poisoning in immune-compromised patients. Of the various solvent extracts, the ethanol leaf and flower extract of *S. venulosa*, ethanol leaf extract of *S. racemosa* showed positive results against *B. subtilis*. The ethyl acetate stembark extract of *S. venulosa*, and ethanol leaf extract of *S. racemosa* showed positive results against *E. aerogenes*. *E. aerogenes* is a Gram-negative, rod-shaped bacteria which causes urinary infections and endocarditis among immune-compromised patients. Similarly, the ethyl acetate stem bark extract of *S. venulosa* showed positive results for *K. pneumoniae* a, facultative anaerobe which causes most of the nosocomial infections leading to morbidity and mortality. The ethyl acetate and ethanol leaf extract of *S. stellata* and ethanol leaf extract of *S. racemosa* showed potent activity against *S. pyogenes*.

At present, there is no evidence on the antimicrobial properties of *S. venulosa* and *S. racemosa*. However, the antimicrobial activity of *S. stellata* has been studied [18]. The volatile oils from the roots, stems, and leaves of *S. stellata* were responsible for the antifungal activity of leaf oil against *Candida albicans* and *Candida glabrata* by disc diffusion technique. Similarly, the methanolic leaf extracts of *S. stellata* showed a greater zone of inhibition (11 mm) when compared to all the tested organisms, followed by *Xanthomonas axonopodis* pv. *malvacearum* with a zone of 9 mm [23]. An inhibition zone of 7 mm was observed with *S. aureus*, *Xanthomonas oryzae* pv. *Oryzae*, and *Salmonella* Typhi strains. The antibacterial activity of *S. stellata* leaf extracts against six pathogenic bacteria was determined using a disc diffusion assay [24]. Different solvent extracts were used for the study, wherein the petroleum ether, chloroform, and the aqueous extract showed potent antibacterial activity against *Escherichia coli* (3.5 mm zone of inhibition), *Lactobacillus bacillus* (6.0 mm zone of inhibition), and *B. subtilis* (4.0 mm zone of inhibition), respectively.

Of the 30 solvent extracts evaluated for the antibacterial activity, six extracts exhibited antibacterial activity (Tables 1-3). Among various solvent extracts from leaves and flower of *S. venulosa*, the ethanol extract showed a zone of inhibition against *B. subtilis,* whereas the ethyl acetate stem bark extract showed antibacterial activity against *E. aerogenes* and *K. pneumoniae* (Table 1). The ethanol leaf extracts of *S. racemosa* showed positive results against *B. subtilis, S. pyogenes*, and *E. aerogenes* (Table 3). Inhibitory activity against *S. pyogenes* was observed in the ethyl acetate and ethanol leaf extract of *S. stellata* (Table 3). The inhibition zone of methanol extract varied from 9.00±0.00 mm to 15.00±1.00 mm against both Gram-positive and Gram-negative bacteria. The MIC result obtained for the positive extracts of three *Schefflera* spp. strongly suggests that the extracts could be good antimicrobial agents (Table 4). Phytochemical investigations on these species indicated the presence of saponins, tannins, terpenoids, flavonoids, alkaloids, steroids, cardiac glycosides in the ethyl acetate, and methanolic extracts. The phytochemicals present in the plant species may be responsible for the antimicrobial activity of the extracts.

The non-polar solvent extracts demonstrated poor activity in all the organisms since no zones of inhibition were seen on the agar disc diffusion method. This is an indication that non-polar solvents were not good solvents, probably because the compounds responsible for bioactivity were not soluble in non-polar solvents such as hexane and chloroform [5]. In the present study, 30 solvent extracts of all the three species of *Schefflera* were screened for antibacterial activity and among them six solvent extracts showed potent antibacterial activity. This is the first report on the antibacterial activity of *S. venulosa* and *S. racemosa* extracts.

CONCLUSION

The present work is addressed on the antibacterial activities of three *Schefflera* species. The methanol extracts of *Schefflera* spp. showed a broad spectrum of activity against all the test organisms, employed. This may be attributed to the phytochemicals present in the plant species. Further, studies on the fractionation of the methanolic extracts and characterization by spectroscopy techniques may reveal the compounds responsible for the antibacterial potentials.

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AUTHORS' CONTRIBUTIONS

The first author conducted the experimental parts involving solvent extractions of plant parts, screening for antibacterial activities, and the writing of the manuscript. The second author collected the plants *S. venulosa*, *S. racemosa*, and *S. stellata* from the Western Ghats and Chamundi Hills, respectively, and is involved in the overall presentation of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest were involved in this study.

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