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Antibacterial activity of crude extracts of selected plants

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Abstract
Plants are considered as important resources such as food, medicine and dyes. The present study aimed at evaluating antibacterial activity of five plants viz. *Kigelia africana* (Lam.) Benth., *Clerodendrum philippinum* Schauer, *Ixora brachiata* Roxb., *Azima tetracantha* Lam. and *Phyllanthus reticulatus* Poir. collected from different places of Shivamogga district, Karnataka, India. Extraction of powdered plant materials was done by maceration technique using methanol. Antibacterial activity of extracts was performed against three Gram positive bacteria and three Gram negative bacteria by agar well diffusion technique. All extracts were effective in causing inhibition of bacteria as revealed by the presence of zone of inhibition around wells. Overall, *Staphylococcus epidermidis* and *Escherichia coli* were inhibited to highest and least extent respectively by extracts. Extracts displayed highest activity against Gram positive bacteria. Extract of *P. reticulatus* was more effective against test bacteria while flower extracts of *K. africana* and *C. philippinum* were least effective. The plants can be exploited for developing formulations and therapeutic agents that can be used to treat infectious diseases.

Keywords: Plants, antibacterial, maceration, agar well diffusion, zone of inhibition

Introduction
Plants are an integral part of human life. Humans depend on plants for various purposes such as food, shelter, cloth, construction, dyes and medicine. Traditional medicinal practitioners from various parts of the world use plants for treating several diseases and disorders. It is estimated that about 2/3rd of population of world depends on traditional medicine to meet the primary healthcare. Plants are used singly or in certain formulations. Traditional medicinal practices have been widespread in various countries. Plants play a crucial role in traditional medicinal practices such as Ayurveda, Unani and Siddha. Therapeutic potential of plants are ascribed to the presence of various secondary metabolites such as alkaloids, polyphenolic compounds and terpenes. Extracts and purified constituents of plants have shown to exhibit a wide array of biological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer, larvicidal and analgesic activity. Natural products including plants provide lead compounds for synthesis of therapeutic agents by pharmaceutical companies. Compounds such as morphine, quinine, taxol, vincristine, nicotine, vinblastine and digoxin are of plant origin [1-8]. Interest in plants with antibacterial activity arose significantly because of the emergence of antibiotic resistant pathogens (due to overuse and abuse of antibiotics) and the adverse effects associated with the use of antibiotics. It is shown that extracts and purified metabolites from plants exhibit lethal effect against pathogenic bacteria including antibiotic resistant strains of pathogens [8, 9-12]. The present study aimed at investigating antibacterial potential of 5 plants viz. *Kigelia africana* (Lam.) Benth, *Clerodendrum philippinum* Schauer, *Ixora brachiata* Roxb., *Azima tetracantha* Lam. and *Phyllanthus reticulatus* Poir.

Materials and Methods
Chemicals and media
Chemicals viz. dimethyl sufoxide (DMSO), methanol and chloramphenicol and culture media viz. nutrient broth and nutrient agar were procured from HiMedia laboratories, Mumbai, India.

Collection and identification of plants
The plants selected for this study (Table 1) were collected from different parts of Shivamogga district, Karnataka, India during January and February 2017. The authentication of plants was made by referring standard flora [13, 14] and with the help of taxonomists Dr. Vinayaka K.S and Prof. D. Rudrappa.

Table 1: Plants selected for this study

Name of the plant	Family	Habit	Part used	Place of collection
Kigelia africana (Lam.) Benth.	Bignoniaceae	Medium sized tree	Leaf and flower	Shikaripura
Clerodendrum philippinum Schauer	Lamiaceae	Shrub	Leaf and flower	Shikaripura
Ixora brachiata Roxb.	Rubiaceae	Small tree	Leaf	Shiralakoppa
Azima tetracantha Lam.	Salvadoraceae	Shrub	Leaf	Matturu
Phyllanthus reticulatus Poir.	Phyllanthaceae	Shrub	Leaf	Malalakoppa

Extraction

The plant materials were dried under shade and powdered in a blender. In stoppered containers, the powdered plant materials (10g) were extracted over a period of 48 hours by maceration process using methanol as solvent (100ml). The contents were filtered and the filtrates were evaporated to dryness at 40 ºC [15]. The crude extracts were used for antibacterial study.

Test bacteria

A total of 6 bacteria, that included three Gram positive bacteria (*Staphylococcus aureus* NCIM 5345, *Staphylococcus epidermidis* NCIM 2493 and *Bacillus cereus* NCIM 2016) and three Gram negative bacteria (*Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2200 and *Salmonella typhimurium* NCIM 2501), were used to assess their susceptibility to extracts of selected plants.

Antibacterial activity of extracts of selected plants

Broth cultures of test bacteria were prepared in sterile nutrient broth tubes by aseptically inoculating the pure cultures of test bacteria into broth tubes and incubating the tubes at 37 ºC for 24 hours. Antibacterial potential of extracts of selected plants was evaluated by Agar well diffusion method as described by Raghavendra *et al*. [16]. The bacteria were inoculated by swabbing broth cultures on the surface of sterile nutrient agar plates. Using a sterile cork-borer, wells of 8mm diameter were punched in the plates and the respective wells were filled with extracts (20mg extract/ml of DMSO), antibiotic (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO. Zones of inhibition formed around the wells were measured after an incubation period of 24 hours at 37 ºC.

Statistical analysis

The experiment was conducted in triplicates (n=3). The result of the study is presented as Mean ± Standard deviation (S.D) of three trials.

Results and Discussion

Methods such as disk diffusion, agar well diffusion and broth dilution have been widely used to evaluate antibacterial activity of various samples including plant extracts [11, 16, 17-20]. In the present study, we evaluated the potential of 5 selected plants to inhibit test bacteria by agar well diffusion assay and the result is shown in Table 2. Overall, *S. epidermidis* and *E. coli* were inhibited to highest and least extent respectively. In case of Gram positive bacteria, *S. epidermidis* and *S. aureus* recorded highest and least susceptibility respectively to extracts. In case of Gram negative bacteria, highest and least susceptibility to extracts was shown by *S. typhi* and *E. coli* respectively. In case of *K. africana* and *C. philippinum*, leaf extracts exhibited high inhibitory activity when compared to flower extracts. Overall, leaf extract of *P. reticulatus* exhibited highest inhibitory effect against test bacteria while flower extracts of *K. africana* and *C. philippinum* displayed least inhibitory activity against the test bacteria. Earlier studies have also shown the potential of *P. reticulatus* [4, 21], *I. brachiate* [1, 22], *K. Africana* [23, 24], *A. tetracantha* [25, 26] and *C. philippinum* [27, 28] to inhibit bacteria. In the present study, the extracts of selected plants displayed high activity against Gram positive bacteria. A possible reason for it could be the presence of an outer membrane in Gram negative bacteria which endows the bacteria an advantage in terms of acting as an additional barrier for the entry of materials such as antibiotics and extracts into the cell [29, 30].

Table 2: Antibacterial activity of methanol extract of selected plants

Name	Part	Zone of inhibition in cm (Mean±S.D; n=3)					
		S. aureus	***S. epidermidis***	***B. cereus***	***E. coli***	***S. typhimurium***	***P. aeruginosa***
K. africana	Leaf	1.80±0.00	2.00±0.10	1.80±0.10	1.56±0.05	1.60±0.00	1.50±0.00
	Flower	1.33±0.05	1.40±0.00	1.40±0.00	1.10±0.00	1.33±0.05	1.20±0.00
C. philippinum	Leaf	1.40±0.10	1.83±0.05	2.00±0.10	1.30±0.00	1.40±0.10	1.40±0.10
	Flower	1.20±0.00	1.40±0.00	1.60±0.00	1.10±0.10	1.20±0.00	1.10±0.00
I. brachiata	Leaf	1.66±0.05	1.80±0.00	1.76±0.05	1.20±0.00	1.40±0.00	1.40±0.10
K. reticulata	Leaf	2.10±0.00	2.80±0.10	2.10±0.00	1.56±0.05	1.70±0.00	1.60±0.00
A. tetracantha	Leaf	1.60±0.00	2.20±0.00	1.80±0.00	1.20±0.00	1.50±0.10	1.40±0.00
Antibiotic	-	2.66±0.05	3.10±0.10	2.80±0.00	2.10±0.00	2.30±0.00	2.33±0.05
DMSO	-	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Conclusions

The result of the present study indicates that the crude extracts of the plants screened possess bioactive principles which might have caused antibacterial activity. The plants can be exploited for developing antibacterial agents or formulations that can be used against disease caused by pathogenic bacteria. Further studies on purification of active principles, their nature and mode of action are to be carried out.

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Conflicts of Interest

None declared

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