problem of large-scale propagation for conservation of this threatened tree species.

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Antibacterial activity of some important medicinal plants

The micro-organisms induced diseases are responsible for over 50% deaths and are still a key concern to public health especially in developing countries[1]. Synthetic antimicrobial drugs are expensive and relatively unavailable in developing and under-developed countries[2]. Again, the safety aspect of synthetic drugs is a major concern since they may be responsible for causing cancers and other lethal diseases when used for a long time. This is attributed to the presence of many carcinogenic and teratogenic agents[3]. Emergence of anti-microbial resistance in pathogenic bacteria against antimicrobial drugs is another concern[4,5].

Medicinal plants are considered valuable sources and called nature's 'chemical factories' for providing secondary metabolites that contain anti-microbial properties[6–9]. Plant-based drugs are considered safe for health. Moreover, compounds derived from plants are found more active when compared to well-established antibiotics[10]. For these reasons, scientists are diverting their attention towards the discovery of plant-based anti-microbial drugs[11]. In this communication, we study the anti-bacterial activity of the methanolic extracts of *Swertia chirata*, *Berberis aristata*, *Paris polyphylla*, *Digitalis purpurea*, *Digitalis lanata*, and essential oil of *Cymbopogon flexuosus* against *Citrobacter freundii*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Proteus vulgaris*.

Root/rhizome samples of *S. chirata*, *B. aristata*, *P. polyphylla*, *D. purpurea*, *D. lanta* and fresh leaves of *C. flexuosus* were collected from the herbal garden of Herbal Research and Development Institute, Mandal (1550 m amsl (above mean sea level)). The roots were washed with running tap water and then cut into small pieces. These pieces were then dried at 25°C over glass plate, and were ground through a pulverizer. The root powder passed through 0.35 mm sieve was taken for extraction and anti-bacterial analysis. Exactly 100 mg powdered rhizomes/roots of each species were extracted in 20 ml of methanol on a water bath at 70°C for 6 h and replicated three times. The solvent of the combined extracts was removed to dryness under vacuum. The extract was then dissolved in 10 ml methanol to obtain 10 mg ml^{-1} solution. Syringe filter (0.45 µm) was used to filter the extract used for analysis. The fresh aerial part of *C. flexuosus* was cut into small pieces and put into Clevenger apparatus for hydro-distillation for 5 h. The method described in *British Pharmacopoeia* was followed for isolation of essential oil[12]. Anhydrous sodium sulphate was used to dry the extracted essential oil and was kept in a brown glass jar and stored at 4°C for further analysis.

Six bacterial strains obtained from Microbial Type Culture Collection and Gene Bank, CSIR-Institute of Microbial Technology (IMTECH), Chandigarh, selected as representative of the class of Gram-positive (*E. faecalis* and *S. aureus*) and Gram-negative (*S. typhimurium*, *E. coli*, *C. freundii* and *P. vulgaris*) were used for assay. Well diffusion method was adopted for screening of anti-bacterial activity of tested extracts/oil following Deshmukh *et al.*[13]. The bacterial strains were maintained on nutrient agar No. 2 (Himedia, India) at 37°C and were pre-cultured in nutrient broth. Sterile saline water (0.85% NaCl) was used to dilute the stock culture suspension. The Mueller Hinton Agar (Himedia, India) was poured in sterile petri plates and kept for solidification. To attend confluent growth of test micro-organism, 0.1 ml of diluted inoculums was spread over in the plates using sterile L spreader. Wells of 6 mm were then bored into the agar using a sterile cork borer which was then filled with exactly 100 µl of different concentrations of crude extracts of different species. The petri plates were incubated in a refrigerator for 2 h to permit diffusion of crude extract in the medium. To obtain colonies of test bacteria, plates were then incubated for

Table 1. Antimicrobial activity of methanol extract of *Swertia chirata*

Pathogen microorganism		Concentration of the extract (mg ml^{-1})							
		100	50	25	12.5	6.25	3.12	1.56	0.78
S. typhimurium	ZOI (mm)	–	–	–	–	–	–	–	–
E. coli	ZOI (mm)	17	13	12	10	08	–	–	–
C. freundii	ZOI (mm)	31	20	18	14	13	08	–	–
P. vulgaris	ZOI (mm)	–	–	–	–	–	–	–	–
E. faecalis	ZOI (mm)	22	18	10	07	–	–	–	–
S. aureus	ZOI (mm)	–	–	–	–	–	–	–	–

Table 2. Antimicrobial activity of methanol extract of *Berberis aristata*

Pathogen microorganism		Concentration of the extract (mg ml^{-1})							
		100	50	25	12.5	6.25	3.12	1.56	0.78
S. typhimurium	ZOI (mm)	–	–	–	–	–	–	–	–
E. coli	ZOI (mm)	–	–	–	–	–	–	–	–
C. freundii	ZOI (mm)	22	20	16	10	08	–	–	–
P. vulgaris	ZOI (mm)	–	–	–	–	–	–	–	–
E. faecalis	ZOI (mm)	24	20	17	10	–	–	–	–
S. aureus	ZOI (mm)	29	18	14	10	–	–	–	–

24 h at 37°C in an incubator. For controls, the solvent used to dissolve extracts was used. All samples were tested in triplicate and the results shown are the average. These plates were examined for minimum inhibitory concentration (MIC) and zones of inhibition (ZOI). The MIC value is the lowest concentration of crude extract which completely inhibited the growth of test micro-organism[14]. The anti-bacterial activities of studied plants were compared with those of Ciprofloxacin at a concentration of 100 µg ml^{-1}. Antibiotic zone scale was used to measure zone of inhibition.

In the present study, *S. chirata*, *B. aristata*, *P. polyphylla* and *C. flexuosus* exhibited anti-bacterial activity against one or more microbial strains, while *D. purpurea* and *D. lanata* were found ineffective. The plant extract of *S. chirata* exhibited high inhibitory activity against *C. freundii* with MIC of 3.12 mg ml^{-1} and ZOI of 8 mm. For *E. coli* and *E. faecalis*, MIC was 6.25 and 12.5 mg ml^{-1} respectively while ZOI was 8 mm for *E. coli* and 7 mm for *E. faecalis*. *S. chirata* did not show any activity against *S. typhimurium*, *P. vulgaris* and *S. aureus* (Table 1). Likewise, methanolic extract of *B. aristata* was found effective against *C. freundii* with MIC of 6.25 mg ml^{-1} followed by *E. faecalis* and *S. aureus* with MIC of 12.5 and 6.25 mg ml^{-1} respectively, while ZOI was 10 mm for both test pathogens (Table 2). *Paris polyphylla* was found effective only against *S. aureus* (MIC of 6.25 mg ml^{-1}) (Table 3). The essential oil of *C. flexeosus* exhibited strong antibacterial activity against *E. coli* (MIC of 0.78 mg ml^{-1}) followed by *C. freundii* and *E. faecalis* (MIC of 3.12 mg ml^{-1} for each) and *S. aureus* (MIC of 6.25 mg ml^{-1}) (Table 4). *S. typhimurium* and *P. vulgaris* were found resistant to all plant extracts and essential oil analysed. *Digitalis purpurea* and *Digitalis lanata* did not exhibit any activity against test micro-organism.

The study reveals that *S. chirata* exhibited antibacterial activity only against *E. coli*, *C. freundii* and *E. faecalis* and was found ineffective against *S. typhimurium*. The antibacterial activity of *S. chirata* was attributed to the presence of gentianine[15]. Aqueous extract of *B. aristata* was also found to show activity against *S. epidermidis*, *A. niger* and was attributed to the presence of berberine[16]. However, Sharma *et al.*[17] reported that acetonic extract of *B. aristata* showed maximum ZOI as compared to ethanol and methanolic extract. *P. polyphylla* exhibited antibacterial activity only against *S. aureus*. The peptide (PCP-1) present in *P. polyphylla* has been reported to have strong activity against *C. lunata* and *E. coli*[18]. Different compounds present in the root of *P. polyphylla* exhibited antimicrobial activity against different micro-organisms[18]. It is a well recognized fact that plants rich in polyphenols and flavanoid contents exhibit potent anti-microbial activities[19]. The oil of *C. flexeosus* showed strong antibacterial activity against *E. coli*, *C. freundii*, *E. faecalis*, and *S. aureus*. Marques *et al.*[5] reported that the major compounds present in the essential oil are responsible for anti-bacterial activity. The ZOI also differs as per the test micro-organism. High anti-bacterial activity of *S. chirata* and *B. aristata* against *C. freundii*, *P. polyphylla* against *S. aureus* and *C. flexuosus* against *E. coli* is an important finding in terms of providing opportunity to develop new antimicrobial drugs.

Due to changes in the epidemiology of invasive diseases, bacterial infections are considered as emerging diseases[20]. Thus, screening of medicinal plants for search of anti-bacterial activity and its mode of action is gaining substantial interest in the scientific community. The test species that show anti-bacterial activities against Gram-positive bacteria also exhibit similar activities against Gram-negative bacteria. It is observed that *S. aureus* reduces the colony-forming ability, when exposed to methanolic extract of *B. aristata*, *P. polyphylla* and essential oil of *C. flexuosus*. Thus the extract/oil of these species may be applied to surfaces and floors to prevent the colonization of *S. aureus*. The secondary metabolites

Table 3. Antimicrobial activity of methanol extract of *Paris polyphylla*

Pathogen microorganism		Concentration of the extract (mg ml^{-1})							
		100	50	25	12.5	6.25	3.12	1.56	0.78
S. typhimurium	ZOI (mm)	–	–	–	–	–	–	–	–
E. coli	ZOI (mm)	–	–	–	–	–	–	–	–
C. freundii	ZOI (mm)	–	–	–	–	–	–	–	–
P. vulgaris	ZOI (mm)	–	–	–	–	–	–	–	–
E. faecalis	ZOI (mm)	–	–	–	–	–	–	–	–
S. aureus	ZOI (mm)	25	20	16	11	09	–	–	–

Table 4. Antimicrobial activity of essential oil of *Cymbopogon flexuosus*

Pathogen microorganism		Concentration of the oil (mg ml^{-1})							
		50	25	12.5	6.25	3.12	1.56	0.78	0.39
S. typhimurium	ZOI (mm)	–	–	–	–	–	–	–	–
E. coli	ZOI (mm)	24	18	16	12	10	07	05	–
C. freundii	ZOI (mm)	38	19	13	10	07	–	–	–
P. vulgaris	ZOI (mm)	–	–	–	–	–	–	–	–
E. faecalis	ZOI (mm)	40	22	14	11	09	–	–	–
S. aureus	ZOI (mm)	21	17	12	10	–	–	–	–

present in the extract/essential oil have been known to possess these properties[6,7,16]. The mode of action of secondary metabolites against pathogen microorganisms is still not fully understood, but it is considered that they may disrupt the membrane through lipophilic products[21]. Further studies are required to understand whether the anti-bacterial activity is the result of a synergism between secondary metabolites or is the result of one compound[22].

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