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Antibacterial activity of some plants of Karnataka, India

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Abstract
In the present study, we determined antibacterial activity of 20 plants (belonging to 19 genera and 9 families) collected from different places of Karnataka, India by agar well diffusion assay. Plants were extracted using methanol by maceration process. All plants were capable of inhibiting test bacteria but to a varied extent. Among selected plants, *Conyza stricta*, *Striga gesnerioides*, *Syzygium leatum* and *Swertia lawii* displayed marked antibacterial activity as evidenced by wider zone of inhibition when compared with other plants. Among orchids, *Coelogyne nervosa* exhibited marked antibacterial activity. Among bacteria, *Staphylococcus aureus* was inhibited to higher extent followed by *Escherichia coli* and *Pseudomonas aeruginosa*. It is evident that the extracts possess antibacterial principles which are to be isolated, characterized and subjected for bioactivity determinations.

Keywords: Plants, Maceration, Agar well diffusion, Zone of inhibition

1. Introduction
All over the world, a large number of plants have been used traditionally for the treatment of various ailments such as infectious diseases, body pain, cough and inflammation. Medicinal plants have been an integral part of traditional medicine. It is estimated that nearly 80% of world's population rely on traditional medicine to meet the primary healthcare. Plant based medicines have been used in various parts of the world especially in developing and under developing countries especially people from rural areas. Herbal healers use whole plants or parts of the plants such as roots and leaves for preparing therapeutic formulations for human and veterinary use. Plants are an important component of medicinal systems such as Ayurveda, Unani and Sidda. The medicinal values of plants are attributed to the characteristic secondary metabolites such as alkaloids, polyphenolic compounds including flavonoids, saponins, terpenes and glycosides. Plants are the lead sources of compounds for the development of many drugs. Besides, most drugs such as vincristine, vinblastine, morphine, quinine, reserpine, digoxin, taxol and artemisinin are of plant origin. In recent years, much interest has been triggered on antimicrobial activity of plants due to certain drawbacks associated with antibiotics. Studies have shown that botanicals exhibit antimicrobial activity against a wide range of pathogens including drug resistant strains [1-12]. The present study was carried out to determine antibacterial activity of 20 plants (belonging to 19 genera and 9 families) collected from different places of Karnataka, India.

2. Materials and Methods
2.1 Collection and identification of plants
A total of 20 plants belonging to 19 genera and 9 families (Table 1) were collected from different places of Karnataka such as Guddekeri, Lakkavalli, Varadahalli, Sagara, Mullayanagiri, Bababudangiri and Thirthahalli during 2015-2016. The plants were identified by referring standard flora and with the help of taxonomists [13-15].

2.2 Extraction
The plants were washed well, dried under shade, powdered in a blender and extracted using methanol by maceration process. A known quantity (10g) of each of the plant materials was transferred into separate conical flasks containing 100ml methanol. The flasks were shaken well and left for 48 hours during which the flasks were stirred occasionally. Later, the contents of the flasks were filtered through muslin cloth followed by Whatmann no. 1 filter paper. The filtrates were evaporated to dryness and stored in refrigerator until use [10].

Table 1: Plants used in the study

Sl. No.	Plant	Family	Part used
1	*Crotalaria filipes* Benth.	Leguminosae	Whole plant
2	*Swertia lawii* Burkill	Gentianaceae	Whole plant
3	*Conyza stricta* Willd	Compositae	Whole plant
4	*Syzygium laetum* (Buch.-Ham.) Gandhi	Myrtaceae	Leaf
5	*Blepharis asperrima* Nees	Acanthaceae	Whole plant
6	*Emilia sanchifolia* DC. ex DC.	Compositae	Whole plant
7	*Smithia sensitiva* Aiton	Leguminosae	Whole plant
8	*Striga gesnerioides* (Willd.) Vatke	Orobanchaceae	Whole plant
9	*Calceolaria mexicana* Benth.	Calceolariaceae	Whole plant
10	*Ziziphus xylopyrus* (Retz.) Willd	Rhamnaceae	Leaf
11	*Eria mysorensis* Lindl.	Orchidaceae	Whole plant
12	*Bulbophyllum fischeri* Seidenf.	Orchidaceae	Whole plant
13	*B. neilgherrense* Wight	Orchidaceae	Whole plant
14	*Acampe praemorsa* (Roxb.) Blatter & McCann	Orchidaceae	Whole plant
15	*Dendrobium herbaceum* Lindl.	Orchidaceae	Whole plant
16	*Oberonia brunoniana* Wight	Orchidaceae	Whole plant
17	*Luisia macrantha* Blatt. McCann.	Orchidaceae	Whole plant
18	*Pholidota imbriacata* Lindl.	Orchidaceae	Whole plant
19	*Coelogyne nervosa* A. Rich.	Orchidaceae	Whole plant
20	*Vanda roxburghii* R.Br.	Orchidaceae	Whole plant

2.3 Preparation of bacterial inoculum
Test bacteria namely *Staphylococcus aureus* MTCC-96, *Pseudomonas aeruginosa* MTCC-7296 and *Escherichia coli* MTCC-1610 were inoculated into sterile Nutrient broth tubes and incubated at 37 ^{o}C for 24 hours. Broth cultures thus obtained were used to determine their susceptibility to extracts of selected plants.

2.4 Antibacterial activity of extracts
Agar well diffusion assay was performed to evaluate antibacterial activity of selected plants. Under aseptic conditions, 24 hours old nutrient broth cultures of test bacteria were aseptically swab inoculated on sterile nutrient agar plates followed by punching wells of 8mm diameter using sterile cork borer. 100µl of extracts (20mg/ml of DMSO), standard antibiotic (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO were transferred into labeled wells. The plates were incubated in upright position for 24 hours at 37^{o}C. Diameter of zones of inhibition was recorded after incubation [10].

2.5 Statistical analysis
The antibacterial assay was done in triplicate. The result is presented as Mean±Standard deviation (S.D).

3. Results
The result of inhibitory effect of extracts of selected plants is shown in Table 2 and Figure 1. All extracts were effective in inhibiting the test bacteria but to a varying extent. Among bacteria, marked inhibition by extracts was observed in case of *S. aureus* while *P. aeruginosa* was susceptible to least extent. Among plants, high and least inhibitory effect was exhibited by *C. stricta* and *O. brunoniana* respectively. Overall, extract of *C. nervosa*, *S. leatum*, *C. stricta* and *S. lawii* exhibited marked antibacterial activity.

Table 2: Antibacterial activity of extract of selected plants

Sl. No.	Treatment	Zone of inhibition in cm (Mean±S.D)		
		S. aureus	***P. aeruginosa***	***E. coli***
1	*C. filipes*	1.26±0.05	1.0±0.00	1.20±0.00
2	*S. gesnerioides*	2.03±0.05	1.46±0.05	2.00±0.00
3	*C. stricta*	3.00±0.00	1.83±0.05	2.50±0.00
4	*S. laetum*	2.43±0.05	1.50±0.00	2.00±0.00
5	*B. asperrima*	1.66±0.05	1.43±0.05	1.60±0.00
6	*E. sanchifolia*	1.43±0.05	1.10±0.10	1.26±0.05
7	*S. sensitiva*	1.60±0.00	1.33±0.04	1.70±0.00
8	*S. lawii*	2.00±0.00	1.70±0.00	1.80±0.00
9	*C. mexicana*	1.56±0.05	1.20±0.00	1.66±0.05
10	*E. mysorensis*	1.50±0.00	1.06±0.11	1.26±0.05
11	*B. fischeri*	1.60±0.00	1.00±0.00	1.06±0.05
12	*B. neilgherrense*	1.66±0.05	1.06±0.11	1.33±0.05
13	*A. praemorsa*	1.40±0.00	1.06±0.11	1.20±0.00
14	*D. herbaceum*	1.43±0.05	1.26±0.05	1.20±0.00
15	*O. brunoniana*	1.00±0.00	1.00±0.00	1.00±0.00
16	*L. macrantha*	1.53±0.05	1.00±0.00	1.00±0.00
17	*P. imbriacata*	1.76±0.11	1.26±0.05	1.40±0.00
18	*C. nervosa*	2.16±0.11	1.80±0.00	1.80±0.00
19	*V. roxburghii*	1.26±0.05	1.00±0.00	1.06±0.05
20	*Z. xylopyrus*	1.50±0.00	1.33±0.05	1.56±0.00
21	Standard	3.33±0.23	2.83±0.05	3.06±0.11
22	DMSO	0.00±0.00	0.00±0.00	0.00±0.00

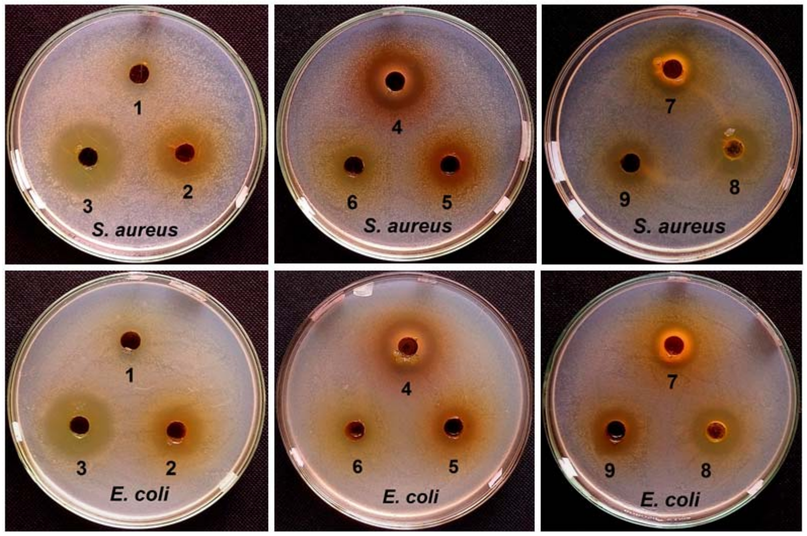

Fig 1: Inhibition of *S. aureus* and *E. coli* by selected extracts (1-*C.filipes*; 2-*S.gesnerioides*; 3-*C.stricta*; 4-*S.laetum*; 5-*B.asperrima*; 6-*E.sanchifolia*; 7-*S.sensitiva*; 8-*S.lawii*; 9-*C.mexicana*)

4. Discussion

In medicine, the discovery of antibiotics is one of the greatest discoveries as its use resulted in prevention of huge number of deaths, especially in II world war. However, development of resistance has been observed in several pathogens to many antibiotics. These resistant bacteria challenge the therapy as the most commonly used antibiotics are no longer effective against these resistant pathogens. Moreover, the use of antibiotics also suffers from other drawbacks such as high cost, side effects and effect on non-target microbes. This stimulated immense research on medicinal values of plants in recent years. Throughout world, in particular Africa and Asia, plants have been considered as richest source of raw materials for development of traditional medicine. The medicinal importance of a large number of plants remains to be explored. It has been shown that extracts and purified compounds from plants exhibit antibacterial activity against a wider range of pathogens including drug resistant bacteria [6-8, 16-18]. In the present study, we determined antibacterial activity of 20 plants by agar well diffusion method. The presence of zone of inhibition around wells is considered positive for inhibitory activity while the absence of zone is an indication of no activity. In case of *S. aureus*, the zone of inhibition ranged from 1.0cm to 3.0. *S. aureus* was inhibited to high extent by extract of *C. stricta* (zone of inhibition 3cm) followed by *S. leatum* (2.4cm), *C. nervosa* (2.1cm) and others. Extract of *C. stricta* and *C. nervosa* inhibited *P. aeruginosa* to high extent (zone of inhibition 1.8cm). Least inhibitory effect against *P. aeruginosa* was observed in case of extract of *C. filipes*, *E. mysorensis*, *B. fischeri*, *B. neilgherrense*, *A. praemorsa*, *L. macrantha* and *V. roxburghii* (zone of inhibition 1.0cm). None of the extracts revealed the inhibition of *P. aeruginosa* with zone of inhibition 2cm or higher. Inhibition of *E. coli* was marked in case of *C. stricta* (zone of inhibition 2.5cm) while least in case of *O. brunoniana* and *L. macrantha* (zone of inhibition 1.0cm). Three plants namely *S. gesnerioides*, *C. stricta* and *S. laetum* inhibited *E. coli* with zone of inhibition 2cm and higher. Among Gram negative bacteria, *E. coli* was found to be susceptible to more extent than *P. aeruginosa*. Reference antibiotic displayed marked inhibition of test bacteria when compared to control. There was no inhibitory activity in case of DMSO.

Among 20 plants, highest inhibition of test bacteria was observed in case of *C. stricta*. In an earlier study, Shah *et al*. [19] reported dose dependent inhibition of test bacteria by *Conyza sumatrensis*. Extract of *S. laetum* was shown to exhibit good antibacterial activity in this study. Similarly, the study of John *et al*. [20] revealed the inhibitory activity of *S. laetum* against diarrhoeal pathogens. Extract of *S. gesnerioides* was found to inhibit test bacteria to an appreciable extent when compared to most of the extracts in this study. Earlier, the study of Koua *et al*. [21] revealed varied antimicrobial activity of *S. hermonthica*. *Swertia lawii* was also shown to exhibit good antibacterial activity in this study. Recently, various extracts from different parts of *S. cordata* and *S. chirayita* were shown to display antibacterial activity [22]. Among orchids, marked and least inhibitory activity was observed in case of *C. nervosa* and *O. brunoniana* respectively. In an earlier study, Rashmi *et al*. [23] showed antibacterial efficacy of four orchids namely *Coelogyne breviscapa*, *Dendrobium nutantiflorum*, *Luisia zeylanica* and *Pholidota pallida* against Gram positive and Gram negative bacteria. Extract of *C. breviscapa* exhibited stronger inhibition of test bacteria when compared to other orchids. In an earlier study, Marasini and Joshi [24] found inhibition of bacteria and fungi by extracts of orchids such as species of *Coelogyne*, *Pholidota*, *Bulbophyllum*, *Dendrobium* and *Eria*. In similar studies, Hoque *et al*. [25], Radhika *et al*. [6], Paul *et al*. [7], Sandrasagaran *et al*. [26] found antimicrobial activity of extracts of orchids. In the present study, *S. aureus* was inhibited to higher extent when compared to *E. coli* and *P. aeruginosa* i.e., inhibitory effect of extracts was more against Gram positive bacterium when compared to Gram negative bacteria. Similar results i.e., higher inhibition of Gram positive bacteria than Gram negative bacteria by plant extracts was observed in some earlier studies [27, 28]. The lower susceptibility of Gram negative bacteria could be ascribed to the presence of outer membrane which acts as an additional barrier to the entry of extracts and other compounds [29-31].

5. Conclusion

In the present study, we observed antibacterial activity of 20 plants against Gram positive and Gram negative bacteria. The plants selected were shown to exhibit antibacterial activity. Plants namely *C. stricta*, *S. leatum*, *S. lawii* and *C. nervosa* exhibited good antibacterial activity. Hence these plants may be useful in the treatment of diseases caused by pathogenic bacteria. The study of the chemical constituents in plants is essential because they serve as lead compounds for development of many modern medicines. It is evident from the study that plants possess antibacterial constituents which are to be isolated, characterized and subjected for inhibitory activity determination.

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7. References

1. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives 2001; 109:69-75.
2. Idu M, Erhabor JO, Efijuemue HM. Documentation on medicinal plants sold in markets in Abeokuta, Nigeria. Tropical Journal of Pharmaceutical Research. 2009; 9(2):110-118.
3. Jain AK, Vairale MG, Singh R. Folklore claims on some medicinal plants used by *Bheel* tribe of Guna district Madhya Pradesh. Indian Journal of Traditional Knowledge. 2010; 9(1):105-107.
4. Kingston DGI. Modern natural products drug discovery and its relevance to biodiversity conservation†. Journal of Natural Products. 2011; 74(3):496-511.
5. Modi C, Mody S, Patel H, Dudhatra G, Kumar A, Awale M. Herbal antibacterials: A review. Journal of Intercultural Ethnopharmacology. 2012; 1(1):52-61.
6. Radhika B, Murthy JVVSN, Grace ND. Preliminary phytochemical analysis & antibacterial activity against clinical pathogens of medicinally important orchid *Cymbidium aloifolium* (L.) Sw. International Journal of Pharmaceutical Sciences and Research. 2013; 4(10):3925-3931.
7. Paul P, Chowdhury A, Nath D, Bhattacharjee MK. Antimicrobial efficacy of orchid extracts as potential inhibitors of antibiotic resistant strains of *Escherichia coli*. Asian Journal of Pharmaceutical and Clinical Research. 2013; 6(3):108-111.
8. Yusuf AZ, Zakir A, Shemau Z, Abdullahi M, Halima SA. Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* Linn. Journal of Pharmacognosy and Phytotherapy. 2014; 6(2):10-16.
9. Kumar S, Pandey AK. Pharmacological activities of some common Indian weeds: A review. Mintage Journal of Pharmaceutical and Medical Sciences. 2014; 3(S1):12-17.
10. Kekuda PTR, Akarsh S, Darshini SM, Prafulla D, Raghavendra HL. Antiradical and antimicrobial activity of *Atylosia lineata* Wt. and Arn. Science, Technology and Arts Research Journal. 2015; 4(3):180-183.
11. Khamkar AD, Motghare VM, Deshpande R. Ethnopharmacology- A novel approach for drug discovery. Indian Journal of Pharmacy and Pharmacology. 2015; 2(4):222-225.
12. Singh R. Chemotaxonomy: A tool for plant classification. Journal of Medicinal Plants Studies. 2016; 4(2):90-93.
13. Saldhana CJ, Nicolson DH. Flora of Hassan District, Karnataka, India. Amerind Publishing Co. Pvt. Ltd., New Delhi, 1978.
14. Yoganarasimhan SN, Subramanyam K, Razi BA. Flora of Chikmagalur district. International Book Distributors, Dehra Dun, 1981.
15. Bhat GK. Flora of South Kanara (Dakshina Kannada and Udupi Districts of Karnataka). Aakriti Prints, Mangalore, 2014.
16. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999; 12(4):564-582.
17. Shakeri A, Hazeri N, Vlizadeh J, Ghasemi A, Tavallaei FZ. Phytochemical screening, antimicrobial and antioxidant activities of *Anabasis aphylla* L. extracts. Kragujevac Journal of Science. 2012; 34:71-78.
18. Gupta C. Isolation and characterization of new 17-ketosteroid as antimicrobial agent from *Vanda tessellata* (Roxb.) hook. World Journal of Pharmaceutical Sciences 2016; 4(4):45-51.
19. Shah NZ, Khan MA, Sadia NM, Azeem Rauf A. Antimicrobial and phytotoxic profile of *Conyza sumatrensis*. Middle-East Journal of Medicinal Plants Research. 2012; 1(3):44-50.
20. John J, Sujana KA, Ragi PR, Kumar AN. Screening of antidiarrheal properties and phytochemicals of four rare plants used in traditional medicine. Journal of Biological and Scientific Opinion. 2015; 3(6):266-270.
21. Koua FHM, Babiker HA, Halfawi A, Ibrahim RO, Abbas FM, Elgaali EI *et al*. Phytochemical and biological study of *Striga hermonthica* (Del.) Benth callus and intact plant. Research in Pharmaceutical Biotechnology. 2011; 3(7):85-92.
22. Roy P, Abdulsalam FI, Pandey DK, Bhattacharjee A, Eruvaram NR, Malik T. Evaluation of antioxidant, antibacterial, and antidiabetic potential of two traditional medicinal plants of India: *Swertia cordata* and *Swertia chirayita*. Pharmacognosy Research. 2015; 7(S1):S57-S62.
23. Rashmi K, Shweta SD, Sudeshna CS, Vrushala PS, Kekuda PTR, Raghavendra HL. Antibacterial and radical scavenging activity of selected orchids of Karnataka, India. Science, Technology and Arts Research Journal. 2015; 4(1):160-164.
24. Marasini R, Joshi S. Antibacterial and antifungal activity of medicinal orchids growing in Nepal. J Nepal Chem Soc. 2012; 29:104-109.

25. Hoque MM, Khaleda L, Al-Forkan M. Evaluation of pharmaceutical properties on microbial activities of some important medicinal orchids of Bangladesh. Journal of Pharmacognosy and Phytochemistry. 2015; 4(4):265-269.
26. Sandrasagaran UM, Subramaniam S, Murugaiyah V. New perspective of *Dendrobium crumenatum* orchid for antimicrobial activity against selected pathogenic bacteria. Pakistan Journal of Botany. 2014; 46(2):719-724.
27. Nalubega R, Kabasa JD, Olila D, Kateregga J. Evaluation of antibacterial activity of selected ethnomedicinal plants for poultry in Masaka District, Uganda. Research Journal of Pharmacology. 2011; 5(2):18-21.
28. Anusha KS, Ganashree NK, Kruthika GN, Poojashree R, Sushmitha KK, Kekuda PTR. Antimicrobial and antioxidant activity of leaf and flower of *Hypericum mysorense*. Journal of Chemical and Pharmaceutical Research. 2015; 7(5):981-987.
29. Mann CM, Cox SD, Markham JL. The outer membrane of *Pseudomonas aeruginosa* NCTC6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). Letters in Applied Microbiology 2000; 30:294-297.
30. Inouye S, Takizawa T, Yamaguchi H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. Journal of Antimicrobial Chemotherapy 2001; 47(5):565-573.
31. Sung WS, Lee DG. Mechanism of decreased susceptibility for Gram-negative bacteria and synergistic effect with ampicillin of indole-3-carbinol. Biological and Pharmaceutical Bulletin 2008; 31(9):1798-1801.