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Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity

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Abstract: Twelve medicinal plants were screened, namely *Abrus precatorius* L., *Caesalpinia pulcherrima* Swartz., *Cardiospermum halicacabum* L., *Casuarina equisetifolia* L., *Cynodon dactylon* (L.) Pers., *Delonix regia* L., *Euphorbia hirta* L., *Euphorbia tirucalli* L., *Ficus benghalensis* L., *Gmelina asiatica* L., *Santalum album* L., and *Tecomella undulata* (Sm.) Seem, for potential antibacterial activity against 5 medically important bacterial strains, namely *Bacillus subtilis* ATCC6633, *Staphylococcus epidermidis* ATCC12228, *Pseudomonas pseudoalcaligenes* ATCC17440, *Proteus vulgaris* NCTC8313 and *Salmonella typhimurium* ATCC23564. The antibacterial activity of aqueous and methanol extracts was determined by agar disk diffusion and agar well diffusion method. The methanol extracts were more active than the aqueous extracts for all 12 plants studied. The plant extracts were more active against Gram-positive bacteria than against Gram-negative bacteria. The most susceptible bacteria were *B. subtilis*, followed by *S. epidermidis*, while the most resistant bacteria were *P. vulgaris*, followed by *S. typhimurium*. From the screening experiment, *Caesalpinia pulcherrima* Swartz. showed the best antibacterial activity; hence this plant can be further subjected to isolation of the therapeutic antimicrobials and further pharmacological evaluation.

Key Words: medicinal plants, antibacterial activity, aqueous extracts, methanol extracts.

Introduction

Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times.

Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. However, the last few years have seen a major increase in their use in the developed world.

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (1,2). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions (3). This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance (4), there is a constant need for new and effective therapeutic agents (5). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (6,7). Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (8-11).

Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (12). Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (13).

All plants containing active compounds are important. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. These compounds are more complex and specific and are found in certain taxa such as family, genus and species, but heterogeneity of secondary compounds is found in wild species (14). The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (15). The plant's secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. Therefore, random screening of plants for active chemicals is as important as the screening of ethno-botanically targeted species (16).

In the present work 12 different medicinal plants were evaluated for their antibacterial properties.

Materials and Methods

Plant collection

Fresh plants/plant parts were collected in September 2004 from Rajkot and Jamnagar, Gujarat, India. Dr. N.K. Thakrar, Department of Biosciences, Saurashtra University, Rajkot, and Yogendra Vacharaja, Gujarat Ayurveda University, Jamnagar, identified these plants. Fresh plant material was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles. Ethno-botanical information of all the plants screened is given in Figures 1-3.

Crude Extraction

Aqueous extraction

Ten grams of dried plant material was extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume (17). The extract was then autoclaved at 121 °C and 15 lbs pressure, and stored at 4 °C.

Solvent extraction

Ten grams of dried plant material was extracted with 100 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume (17). It was stored at 4 °C in airtight bottles for further studies.

Microorganisms

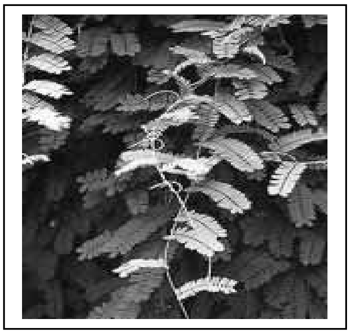
The microbial strains are identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The bacterial strains studied are *Bacillus subtilis* ATCC6633, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas pseudoalcaligenes* ATCC17440, *Proteus vulgaris* NCTC 8313 and *Salmonella typhimurium* ATCC23564.

Antibacterial assay

The antibacterial activity of different plant species was evaluated by agar disk diffusion method (18,19) for aqueous extract and agar well diffusion (20,21) for solvent extract using Mueller Hinton Agar No. 2 medium for the assay. The microorganism was activated by inoculating a loopful of the strain in the nutrient broth (25 ml) and incubated at room temperature on a rotary shaker. Then 0.2 ml of inoculum (inoculum size was 10^8 cells/ml as per McFarland standard) was inoculated into the molten Mueller Hinton agar media and after proper homogenization it was poured into 100 mm petri dishes (Hi-Media). For the agar disk diffusion, the test compound was introduced onto the disk (7 mm) (Hi-Media) and then allowed to dry. Thereafter, the disk was impregnated on the seeded agar plate. For the agar well diffusion, a well was made in the seeded plates with the help of a cup-borer (8.5 mm). The test compound was introduced into the well and all the plates were incubated at 37 °C for 24 h. The experiment was performed 3 times under strict aseptic conditions. Microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented (Table 1).

***Abrus precatorius* L.**

Vernacular name : Chanothi
Family : Fabaceae
Parts used : Leaf and stem
Habit : Climber
Traditional uses : Root, Leaf, Seeds: Purgative, emetic aphrodisiac; used in nervous disorders.



***Caesalpinia pulcherrima* Swartz.**

Vernacular name : Galtoro
Family : Fabaceae
Parts used : Aerial parts
Habit : Shrub or Tree
Traditional uses : Reclamation plant

***Cardiospermum halicacabum* L.**

Vernacular name : Karodiyo
Family : Sapindaceae
Parts used : Leaf and stem
Habit : Climber
Traditional uses : Plant: in rheumatism, stiffness of limbs and snake-bite, fever, nervous disorders and piles. Root: diaphoretic and diuretic, Leaf juice: to cure earache.

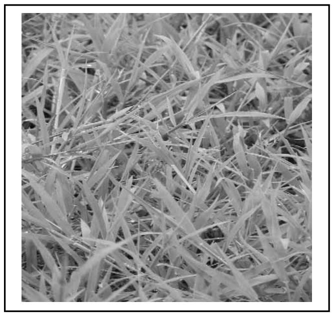
***Casuarina equisetifolia* L.**

Vernacular name : Saru
Family : Casuarinaceae
Parts used : Leaf and stem
Habit : Tree
Traditional uses : Diarrhea, dysentery, beriberi, headache, fever and dropsy.

Figure 1. Ethno-botanical data of *Abrus precatorius* L., *Caesalpinia pulcherrima* Swartz., *Cardiospermum halicacabum* L. and *Casuarina equisetifolia* L.

Cynodon dactylon **(L.) Pers.**

Vernacular name : Dhrokhad
Family : Poaceae
Parts used : Whole
Habit : Tree
Traditional uses : Decoction of root: Diuretic, in dropsy, in secondary syphilis. Infusion of roots for stopping bleeding from piles. Juice of plant: used in wound infection.



Delonix regia **L.**

Vernacular name : Gulmohor
Family : Fabaceae
Parts used : Pod
Habit : Tree
Traditional uses : Not recommended

Euphorbia hirta **L.**

Vernacular name : Rati dudheli
Family : Euphorbiaceae
Parts used : Whole
Habit : Shrub
Traditional uses : Used in diseases of children, in worms, bowel complaints, cough. Juice of plant: in colic and dysentery.

Euphorbia tirucalli **L.**

Vernacular name : Dandilyo thor
Family : Euphorbiaceae
Parts used : Stem
Habit : Tree
Traditional uses : Bark: in infections of spleen, colic and blood complaints, whooping cough and asthma.

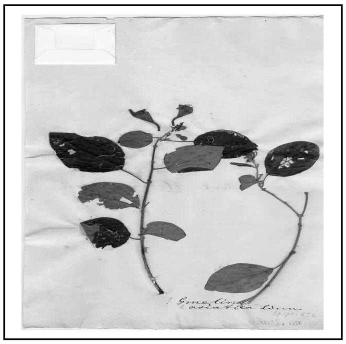
Figure 2. Ethno-botanical data of *Cynodon dactylon* (L.) Pers., *Delonix regia* L., *Euphorbia hirta* L. and *Euphorbia tirucalli* L.

***Ficus benghalensis* L.**

Vernecular name : Vad
Family : Moraceae
Parts used : Branching root
Habit : Tree
Traditional uses : Panchang: useful in vomiting, leucorrhoea, burning sensation, diarrhea, dysentery, diabetes and skin diseases.

***Gmelina asiatica* L.**

Vernacular name : Shivan
Family : Verbenaceae
Parts used : Leaf
Habit : Shrub
Traditional uses : Diabetes



***Santalum album* L.**

Vernacular name : Sukhad
Family : Santalaceae
Parts used : Leaf and stem
Habit : Tree
Traditional uses : Hard wood: In gastric irritability, dysentery, gonorrheal, urethral, bronchial and skin disorders.

***Tecomella undulata* (Sm.) Seem.**

Vernacular name : Ragat-rohido
Family : Bignonaceae
Parts used : Leaf and stem
Habit : Shrub / Tree
Traditional uses : Bark: Remedy for syphilis and spleen diseases.

Figure 3. Ethno-botanical data of *Ficus benghalensis* L., *Gmelina asiatica* L., *Santalum album* L. and *Tecomella undulata* (Sm.) Seem.

Table 1. Antibacterial activity of aqueous and methanol extracts of screened medicinal plants.

	*Zone of Inhibition (mm)				
Plant Extracts	*S. epidermidis*	*B. subtilis*	*P. pseudoalcaligenes*	*P. vulgaris*	*S. typhimurium*
Abrus precatorius L.					
Aq.	-	-	-	-	-
Me.	-	-	-	-	-
Caesalpinia pulcherrima Swartz.					
Aq.	13	9	11	9	10
Me.	22	12	22	14	17
Cardiospermum halicacabum L.					
Aq.	-	-	-	-	-
Me.	-	-	-	-	-
Casuarina equisetifolia L.					
Aq.	11	8	11	-	14
Me.	15	12	15	17	18
Cynodon dactylon (L.)Pers.					
Aq.	-	-	-	-	-
Me.	-	11	-	-	-
Delonix regia L.					
Aq.	-	-	-	-	-
Me.	11	12	-	-	-
Euphorbia hirta L.					
Aq.	-	-	12	8	11
Me.	14	11	23	24	24
Euphorbia tirucalli L.					
Aq.	-	-	13	-	-
Me.	12	10	19	13	16
Ficus benghalensis L.					
Aq.	-	-	-	-	-
Me.	11	11	-	-	-
Gmelina asiatica L.					
Aq.	-	-	11	-	-
Me.	-	11	-	-	-
Santalum album L.					
Aq.	-	-	-	-	-
Me.	-	13	-	-	-
Tecomella undulata (Sm.) Seem.					
Aq.	-	-	-	-	-
Me.	11	12	-	-	-

*: Inhibition zones including disk (7 mm) and cup borer (8.5 mm) diameter
Aq: Aqueous, Me: Methanol.

Results and Conclusions

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent but in our studies we found that plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The results of screening are presented in Table 1. The aqueous and methanol extracts of 12 plants belonging to 9 families were tested against 2 Gram-positive and 3 Gram-negative bacteria using agar disk and agar well diffusion.

Abrus precatorius L. and *Cardiospermum halicacabum* L. extracts were totally inactive against all the microorganisms tested. The plants that exhibited antibacterial activity to a certain degree were *Casuarina equisitifolia* Forest., *Euphorbia hirta* L. and *Euphorbia tirucalli* L. For *Casuarina equisetifolia* Forest. aqueous as well as methanol extracts were active against all bacterial strains with the exception that aqueous extract could not inhibit *P. vulgaris*. Aqueous extract of *Euphorbia hirta* L. was inactive against both the Gram-positive bacteria, i.e. *B. subtilis* and *S. epidermidis*, while that of *Euphorbia tirucalli* L. was active only against *P. pseudoalcaligenes*. The aqueous extracts of *Cynodon dactylon* (L.) Pers., *Ficus benghalensis* L. and *Tecomella undulata* (Sm.) Seem. were inactive against all the tested bacterial strains, while methanol extracts could inhibit only *S. epidermidis. B. subtilis* was the only bacteria inhibited by methanol extract of *Cynodon dactylon* (L.) Pers. and *Santalum album* L. Aqueous extract of *Gmelina asiatica* L. was active only against *P. pseudoalcaligenes*, while methanol extract could inhibit only *B. subtilis*.

Out of 12 plant species, *Caesalpinia pulcherrima* Swartz. showed significant antibacterial activity and both the extracts (aqueous and methanol) were active against the investigated bacterial strains. The plant extracts were more active against the Gram-positive microorganisms than against the Gram-negative microorganisms. This is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria than against Gram-negative bacteria (22,23). *B. subtilis* was the most susceptible bacteria amongst all the bacterial strains investigated in the present work.

From our investigation of screening different plant species, the results obtained confirm the therapeutic potency of some plants used in traditional medicine. In addition, these results form a good basis for selection of candidate plant species for further phytochemical and pharmacological investigation. The results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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References

1. Davis J. Inactivation of the antibiotics and the dissemination of resistance genes. Science 264: 375-382, 1994.
2. Service RF, Antibiotics that resist resistance. Science 270: 724-727, 1995.
3. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 62: 183-193, 1998.
4. Monroe S, Polk R. Antimicrobial use and bacterial resistance. Curr Opin Microbiol 3: 496-501, 2000.
5. Bhavnani SM, Ballow CH. New agents for Gram-positive bacteria. Curr Opin Microbiol 3: 528-534, 2000.
6. Clark AM Natural products as resource for new drugs. Pharm Res 13: 1133-1141, 1996.
7. Cordell GA. Biodiversity and drug discovery a symbiotic relationship. Phytochemistry 55: 463-480, 2000.
8. Chung PY, Chung LY, Ngeow YF et al. Antimicrobial activities of Malaysian plant species. Pharm Biol 42: 292-300, 2004.
9. Nair R, Chanda SV. Antibacterial activity of some medicinal plants of Saurashtra region. J Tissue Res 4: 117-120, 2004.
10. De Boer HJ, Kool A, Broberg A et al. Antifungal and antibacterial activity of some herbal remedies from Tanzania. J Ethnopharmacol 96: 461-469, 2005.
11. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol 29: 41-47, 2005.
12. Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. J Ethnopharmacol 70: 343-349, 2000.
13. Iwu MW, Duncan AR, Okunji, CO. New antimicrobials of plant origin In: Janick, J. (Ed), Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA, pp. 457-462, 1999.

14. Balandrin MFJ, Kjocke A, Wurtele E et al. Natural plant chemicals: sources of industrial and medicinal materials. Science 228: 1154-1160, 1985.

15. Wink M. Introduction biochemistry, role and biotechnology of secondary products. In: M Wink, Ed, Biochemistry of Secondary Product Metabolism. CRC Press, Boca Raton, FL, pp. 1-16, 1999.

16. Principle PP. The economic significance of plants and their constituents as drugs. In: Economic and medicinal plant research, vol, 3. (Eds.) Wagnor H., Hikino, H. and Farnsworth, N. R. London: Academic Press, pp. 1-17, 1989.

17. Parekh J, Nair R, Chanda S. Preliminary screening of some folkloric plants from western India for potential antimicrobial activity. Indian J Pharmacol. (India) (In Press), 2005.

18. Bauer AW, Kirby WMM, Sherris JC et al. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493-496, 1966.

19. Salie F, Eagles PFK, Leng HMJ. Preliminary antimicrobial screening of four South African Asteraceae species. J Ethonopharmacol 52: 27-33, 1996.

20. Perez C, Paul M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Acta Biol Med Exp 15: 113-115, 1990.

21. Nair R, Chanda S. Anticandidal activity of Punica granatum exhibited in different solvents. Pharm Biol 43: 21-25. 2005.

22. Vlietinck AJ, van Hoof L, Totte J et al. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J Etyhnopharmacol 46: 31-47, 1995.

23. Rabe T, Van Staden J. Antibacterial activity of South African plants used for medicinal purposes. J Ethnopharmacol 56: 81-87, 1997.