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Screening of Methanol and Acetone Extracts of Fourteen Indian Medicinal Plants for Antimicrobial Activity

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Abstract: The methanol and acetone extracts of 14 plants belonging to different families were evaluated for antimicrobial activity against five Gram-positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus flavus*; seven Gram-negative bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella typhimurium, Citrobacter freundii;* and three fungi: *Candida tropicalis*, *Cryptococcus luteolus* and *Candida albicans.* The *in vitro* antimicrobial activity was performed by agar disc diffusion method. The extractive yield was more in methanol than in acetone. The methanol extracts showed more antibacterial activity than acetone extracts. The most susceptible bacterium was *K. pneumoniae* and the most resistant were *P. vulgaris*, *S. typhimurium*, *P. aeruginosa* and *E. coli*. Preliminary phytochemical analysis revealed the presence of tannins, cardiac glycosides, steroids and saponins. Among the plant species screened, the best antimicrobial activity was shown by *Aristolochia indica*; hence, this plant can be further subjected to isolation of the therapeutic antimicrobials and to further pharmacological evaluation.

Key Words: Antimicrobial activity, methanol extracts, acetone extracts, agar disc diffusion method, medicinal plants

Ondört Hindistan Tıbbi Bitkisininin Metanol ve Aseton Özütünün Antimikrobiyal Aktivitelerinin Görüntülenmesi

Özet: Farklı familyalara ait 14 bitkinin metanol ve aseton özütünün beş Gram pozitif bakteri; *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Micrococcus flavus*; yedi Gram negatif bakteri; *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Salmonella typhimurium, Citrobacter freundii* ve üç fungus; *Candida tropicalis, Cryptococcus luteolus, Candida albicans* üzerlerine antimikrobiyal aktiviteleri çalışılmıştır. *In vitro* antimikrobiyal aktivite agar disk diffüzyon yöntemi ile yapılmıştır. Ekstre edilen ürün aseton da metanolden daha fazla olmuştur. En çok etkilenen bakteri *K. pneumoniae*, en dirençli bakteriler ise *P. vulgaris, S. typhimurium, P. aeruginosa* ve *E. coli* olmuştur. Ön fitokimyasal analizler tanin, kardiyak glikosid, steroid ve saponinlerin varlığını göstermiştir. İncelenen bitki türleri içinde en iyi antimiktobial aktiviteyi *Aristolochia indica* göstermiştir. Bu nedenle *A. indica* bitkisi ile ileri çalışmalarda terapatik antimikrobiyal izolasyon ve ileri farmakolojik incelemeler yapılmalıdır.

Anahtar Sözcükler: Antimikrobiyal aktivite, metanol özütü, aseton özütü, agar disk diffüzyon yöntemi, tıbbi bitki

Introduction

Infectious diseases are a major cause of morbidity and mortality worldwide (1). The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidermidis of human immunodeficiency virus (HIV) infections (2,3). This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants (4). Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections (5).

Plant extracts have been used for centuries as a popular method for treating several health disorders. Numerous studies have been carried out on various natural products screening their antimicrobial activity (6-11). In the past two decades, antibacterial properties of various plants and plant parts like root, stem, leaves,

seeds, and flowers have been well documented for some of the medicinal plants (12,13).

In the present work, 14 different medicinal plants belonging to different families were evaluated for their antibacterial properties.

Materials and Methods

Plant material

Fresh plant/plant parts were collected randomly from Gujarat region, India. The details of the plant/plant parts screened - their families, voucher number, vernacular names and their therapeutic uses are given in Table 1 (14,15). They were identified by Dr. P. S. Nagar, Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. Fresh plant materials were washed in tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preliminary phytochemical analysis

Qualitative phytochemical analysis of the crude powder of the 14 plants collected was performed (16,17).

Table 1. Ethnobotanical information of some traditionally used Indian plant species selected for antibacterial activity.

Plant species	Family (Voucher no.)	Common name	Part used	Therapeutic use
Abutilon glaucum (Cav.) Sweet	Malvaceae (PSN42)	Balbij	Leaf + stem	Seeds used as demulcent, diuretic
Agave vera Cruz Mill.	Agavaceae	Agave	Leaf	-
Alpinia speciosa L.	Zingiberaceae (PSN718)	Sthulagranthi	Leaf + stem	Diuretic and to control hypertension
Argyreia speciosa Sweet.	Convolvulaceae (PSN492)	Samudra Shokha	Leaf + stem	Tonic
Aristolochia bracteolata Lam.	Aristolochiaceae (PSN662)	Kidamari	Whole plant	In constipation, inflammation, foul ulcers, boils, syphilis, gonorrhea, eczema and intermittent fevers
Aristolochia indica L.	Aristolochiaceae	Isharmul	Leaf + stem	In venomous insect bites and internally in intermittent fevers, blood complaints
Asphodelus tenuifolius Cav.	Liliaceae (PSN726)	Dungaro	Leaf + stem	Diuretic, for inflammation, ulcers
Baliospermum montanum Mull. Arq.	Euphorbiaceae	Danti	Leaf + stem	-
Carissa carandas L.	Apocynaceae (PSN440)	Karamda	Leaf + stem	Fruit: antiscorbutic, stomachic, refrigerant, digestive. Unripe fruit: astringent. Root: anthelmintic
Cassia occidentalis L.	Caesalpiniaceae (PSN254)	Kasundro	Leaf + stem	Roots- inflammation, diabetes, strangury, elephantiasis. Seeds- leprosy, ulcers, constipation, hiccup and fever
Clerodendrum phlomides L.	Verbenaceae (PSN602)	Arni	Leaf + stem	Root: convalescence of measles. Leaf juice: neglected syphilitic complaints. Plant: in obesity, diarrhea, worms
Leptadenia reticulata (Retz.) Wight & Arn.	Asclepiadaceae (PSN454)	Dodi	Leaf + stem	Restorative
Murraya paniculata (L.) Jack	Rutaceae (PSN110)	Kamini	Leaf + stem	Regulation of fertility
Vitex negundo L.	Verbenaceae (PSN611)	Nagod	Leaf + stem	Catarrhal dengue, puerperal fever, splenic enlargement, irritable bladder, rheumatism, dyspepsia, colic worms, diarrhea, liver disease and hemoptysis

Preparation of extracts

10 g of air-dried powder was taken in 100 ml of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, the supernatant was discarded and petroleum ether was evaporated from the powder. This dry powder was then taken in 100 ml of solvent (methanol or acetone) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, the extracts were centrifuged at 5000 g for 10 min, the supernatant was collected, solvents were evaporated, and the dry extract was stored at 4 °C in airtight bottles. The extraction was done at least three times for each plant and the mean values are presented.

Microorganisms

The investigated microorganisms consisted of five Gram-positive bacteria: *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228, *Bacillus cereus* ATCC11778, *Bacillus subtilis* TCC6633, *Micrococcus flavus* ATCC10240; seven Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* NCIM2719, *Proteus mirabilis* NCIM2241, *Proteus vulgaris* NCTC8313, *Salmonella typhimurium* ATCC23564, *Citrobacter freundii* ATCC10787; and three fungi: *Candida albicans* ATCC2091, *Candida tropicalis* ATCC4563, *Cryptococcus luteolus* ATCC32044. Microorganisms were obtained from the National Chemical Laboratory (NCL), Pune, India. Microorganisms were maintained at 4 °C on nutrient agar slants.

Antimicrobial assay

The antimicrobial assay was performed by agar disc diffusion method (18,19). The molten Mueller Hinton agar (HiMedia) was inoculated with 200 µl of the inoculum (1 x 10^8 Cfu) and poured into the sterile Petri plates (Hi-media). The disc (0.7 cm) (Hi-Media) was saturated with 20 µl of the extract, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37 ºC. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls was maintained where pure solvent (DMSO) was used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented. The results were compared with the standard antibiotics amikacin (30 µg/disc), piperacillin (100 µg/disc), fluconazole (10 µg/disc) and amphotericin-B (100 units/disc).

Results and Discussion

Traditional healers use primarily water as solvent, but in our earlier studies we found that the plant extracts extracted in organic solvents showed profoundly distinct antibacterial activity from aqueous extract (20). Hence, in the present work, methanol and acetone were used for the extraction. The extractive yield of all the plants is shown in Table 2. The extractive yield was considerably more in methanol than in acetone. The extractive yield in methanol ranged from 4.4% to 16.7% while in acetone the range was from 1.5% to 5.1%. Maximum methanolic extractive yield was in *M. paniculata* (16.7%) followed by *B. montanum* (13%). Minimum methanolic extractive yield was in *C. phlomides* (4.4%) followed by *A. glaucum* (5.4%). Maximum acetone extractive yield was in *B. montanum* (5.1%), as in methanolic extract, while minimum yield was in *A. glaucum* and *A. indica.*

The antibacterial activity of all the extracts is shown in Table 2. The acetone extract of *L. reticulate*, *A. tenuifolius, A. vera, Al. speciosa, C. occidentalis* and *C. phlomidis* did not show any activity against the five Gram-positive bacteria investigated, while the methanol extract of *A. bracteolata* did not show any antibacterial activity against Gram-positive strains. The methanol extracts of the studied plants were more potent than the acetone extracts. There are reports in the literature that methanol is a better solvent for consistent extraction of antimicrobial substances from medicinal plants (21,22).

The most susceptible bacterium was *K. pneumoniae* followed by *P. mirabilis* amongst the Gram-negative bacteria, while amongst Gram-positive strains, the most susceptible was *B. cereus* followed by *S. aureus*. The most resistant Gram-negative bacteria were *P. vulgaris*, *S. typhimurium*, *P. aeruginosa* and *E. coli,* while *C. freundii* showed negligible activity. The most resistant Gram-positive bacterium was *S. epidermidis*. The maximum antibacterial activity was shown by acetone extract of *A. indica* against *S. aureus* followed by acetone extract of *A. tenuifolius* against *K. pneumoniae*. The activity of the plant against both Gram-positive and Gram-negative

Table 2. Antimicrobial activity of methanol and acetone extracts of the screened medicinal plants against different microbial strains.

Plants	Extracts (Extractive yield in %)	Gram-positive bacteria					Gram-negative bacteria							Fungi		
		SA	SE	BC	BS	MF	PA	EC	KP	PM	PV	ST	CF	CA	CT	CL
A. glaucum	Methanol (5.4)	16	-	-	-	-	-	-	12	8	-	-	-	8	10	11
	Acetone (1.5)	-	-	9	-	-	-	-	12	-	-	-	-	-	-	15
A. vera	Methanol (9.4)	-	-	13	15	-	-	-	-	-	-	-	-	14	14	17
	Acetone (2.1)	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-
Al. speciosa	Methanol (7.9)	-	-	10	-	-	-	-	9	-	-	-	-	-	-	-
	Acetone (2.0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ar. speciosa	Methanol (5.8)	10	-	11	-	19	-	-	12	-	-	-	-	-	11	-
	Acetone (1.6)	-	-	12	-	11	-	-	13	9	-	-	-	-	-	-
A. bracteolata	Methanol (9.6)	-	-	-	-	-	-	-	16	11	-	-	-	-	11	-
	Acetone (2.0)	15	-	14	11	-	-	-	13	13	-	-	11	-	-	-
A. indica	Methanol (11.5)	10	11	15	11	-	-	-	13	11	-	-	-	-	10	-
	Acetone (1.5)	19	-	16	13	-	-	-	13	13	-	-	-	-	-	13
A. tenuifolius	Methanol (11.4)	9	-	13	-	-	-	-	-	-	-	-	10	-	11	-
	Acetone (3.6)	-	-	-	-	-	-	-	17	-	-	-	-	-	11	20
B. montanum	Methanol (13.0)	9	-	13	-	12	-	-	12	-	-	-	-	-	-	-
	Acetone (5.1)	-	-	12	-	12	-	-	14	10	-	-	-	-	-	-
C. carandas	Methanol (8.5)	10	-	12	12	-	-	-	11	10	-	-	-	-	13	15
	Acetone (4.1)	-	-	10	-	-	-	-	-	-	-	-	-	10	-	12
C. occidentalis	Methanol (8.8)	11	-	12	-	-	-	-	-	9	-	-	-	-	12	10
	Acetone (1.8)	-	-	-	-	-	-	-	11	-	-	-	-	-	11	-
C. phlomides	Methanol (4.4)	-	13	-	-	-	-	-	-	-	-	-	11	-	-	-
	Acetone (1.7)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. reticulata	Methanol (9.9)	9	11	-	-	-	-	-	13	12	-	-	-	-	9	-
	Acetone (2.5)	-	-	-	-	-	-	-	11	15	-	-	11	-	10	-
M. paniculata	Methanol (16.7)	-	-	10	-	-	-	-	12	-	-	-	-	9	9	12
	Acetone (4.7)	-	-	10	-	-	-	-	13	-	-	-	-	-	-	-
V. negundo	Methanol (9.3)	-	-	11	-	-	-	-	12	8	-	-	-	10	10	-
	Acetone (3.1)	-	-	12	-	-	-	-	10	9	-	-	-	-	-	-
Amikacin (30 µg/disc) -		14	22	19	32	24	21	15	24	23	14	15	12	-	-	-
Piperacillin (100 µg/disc) -		20	16	17	15	30	7	17	29	20	-	23	19	-	-	-
Fluconazole (10 µg/disc) -		-	-	-	-	-	-	-	-	-	-	-	-	-	-	29
Amphotericin B (100 units/disc) -		-	-	-	-	-	-	-	-	-	-	-	-	12	11	16

SA: *S. aureus*, SE: *S. epidermidis*, BC: *B. cereus*, BS: *B. subtilis*, MF: *M. flavus*, PA: *P. aeruginosa*, EC: *E. coli*, KP: *K. pneumoniae*, PM: *P. mirabilis*, PV: *P. vulgaris*, ST: *S. typhimurium*, CF: *C. freundii*, CA: *C. albicans*, CT: *C. tropicalis*, CL: *C. luteolus*. Inhibition zone is the mean of three replicates including the disc diameter (7 mm); (-): no activity, negative control did not show any activity.

bacteria may be indicative of the presence of broad spectrum antibiotic compounds in the plant. The antifungal activity was less when compared to antibacterial activity. The methanol extracts of three plants viz. *A. glaucum*, *M. paniculata* and *A. vera* showed activity against all three fungal strains while the other plants extracts inhibited either one or two fungal strains.

The results of the preliminary phytochemical analysis are shown in Table 3. Tannins were present in almost all the plants followed by cardiac glycosides and saponins; alkaloids and flavonoids were in lesser amounts and steroids were also present in tract amounts. The broad spectrum of antibacterial activity found in this study may be attributed to the presence of secondary metabolites of various chemical types present in the plants. Different plants possess different constituents and in different concentrations, which accounts for differential antimicrobial effects, as also suggested earlier (23). The antibacterial activity exhibited by the studied plants may be attributed to the various active constituents present in them either individually or in combination. It may also help in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of infectious diseases. The best antimicrobial activity was shown by *A. indica* and hence it can be selected for further studies. The discovery of a potent remedy from plant origin will be a great advancement in fungal and bacterial infection therapies.

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Table 3. Preliminary phytochemical analysis of the plants screened.

Plants	Alkaloids			Tannins	Cardiac glycosides	Steroids	Flavonoids	Saponins
	Dragen-dorff	Mayer	Wagner					
A. glaucum	+	-	+	-	+++	+	-	-
A. vera	+	-	+	++	-	-	-	+++
Al. speciosa	-	-	+	+++	-	-	+	-
Ar. speciosa	-	-	-	-	-	-	-	+
A. bracteolata	-	-	-	+++	++	++	-	+
A. indica	-	+	+	+	+	++	-	+
A. tenuifolius	-	-	+	-	-	-	-	-
B. montanum	+	+	-	+++	-	-	+	++
C. carandas	+	-	-	+++	-	+		+
C. occidentalis	+	-	-	++	+	+	+	-
C. phlomides	-	-	-	-	-	-	-	-
L. reticulata	-	-	-	+	+++	++	-	-
M. paniculata	-	-	+	++	++	-	-	+
V. negundo	-	-	+	+++	+++	-	-	+

(-) Not present, (+) Sparingly present, (++) Present, (+++) Highly present.

References

1. WHO (World Health Organization). The world health report. Changing history. Statistical annex. Death by cause, sex and mortality stratum in WHO regions, estimates for 2002. Geneva, Switzerland, WHO; 2004: pp. 120-121.
2. Dean DA, Burchard KW. Fungal infection in surgical patients. Am J Surg 171: 374-382, 1996.
3. Gonzalez CE, Venzon D, Lee S et al. Risk factors for fungemia in children infected with human immunodeficiency virus: a case-control study. Clin Infect Dis 23: 515-521, 1996.
4. Cordell GA. Biodiversity and drug discovery -- a symbiotic relationship. Phytochemistry 55: 463-480, 2000.
5. Sieradzki K, Wu SW, Tomasz A. Inactivation of the methicillin resistance gene mecA in vancomycin-resistant *Staphylococcus aureus*. Micro Drug Resist 5: 253-257, 1999.
6. Nita T, Arai T, Takamatsu H et al. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. J Health Sci 48: 273-276, 2002.
7. Ates DA, ErdoUrul OT. Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol 27: 157-162, 2003.
8. Bhattacharjee I, Chetterjee SK, Chetterjee SN et al. Antibacterial potentiality of *Argemone mexicana* solvent extracts against some pathogenic bacteria. Mem Ins Oswaldo Cruz 101: 645-648, 2006.
9. Parekh J, Chanda S. Screening of some Indian medicinal plants for antibacterial activity. Indian J Pharm Sci 68: 835-838, 2006.
10. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr J Biomed Res 10: 175-181, 2007.
11. Vaghasiya Y, Nair R, Baluja S et al. Antibacterial and preliminary phytochemical analysis of *Eucalyptus citriodora* Hk. Leaf. Nat Prod Res (In Press), 2007.
12. Leven M, Vanen Berghe DA, Mertens F. Medicinal plants and its importance in antimicrobial activity. J Planta Med 36: 311-321, 1979.
13. Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk J Biol 29: 203-210, 2005.
14. Anjaria J, Parabia M, Dwivedi S. 1st ed., Ethnovet Heritage Indian Ethnoveterinary Medicine - An Overview. Pathik Enterprise, Ahmedabad, India; 2002: pp. 1-160.
15. Sriram S, Patel MA, Patel KV et al. 1st ed., Compendium on Medicinal Plants. Gujarat Agricultural University, Ahmedabad, India; 2004: pp. 1-116.
16. Harbone JB. Phytochemical Methods. London, Chapman and Hill; 1973.
17. Parekh J, Karthia N, Chanda S. Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark. Afr J Biomed Res 9: 53-56, 2006.
18. Bauer AW, Kirby WMM, Sherris JC. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493-496, 1966.
19. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol 29: 41-47, 2005.
20. Parekh J, Chanda S. In vitro antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents. Afr J Biotech 6: 760-770, 2007.
21. Sengul M, Ogutcu H, Adiguzel A et al. Antimicrobial effects of *Verbascum georgicum* Bentham extract. Turk J Biol 29: 105-110, 2005.
22. Ozturk S, Ercisli S. The chemical composition of essential oil and in vitro antibacterial activities of essential oil and methanol extract of *Ziziphora persica* Bunge. J Ethnopharmacol 106: 372-376, 2006.
23. Parekh J, Chanda S. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol 31: 53-58, 2007.