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SCREENING OF ANTIBACTERIAL POTENTIALS OF SOME MEDICINAL PLANTS FROM MELGHAT FOREST IN INDIA

Tambekar, D.H., B. S. Khante, B.R.Chandak, A.S.TItare, S.S.Boralkar, and S.N.Aghadte

P.G. Department of Microbiology
S.G.B. Amravati University,
Amravati 444 602 (India)
E-mail: diliptambekar@yahoo.com

Abstract

Cyperus rotundus, *Caesalpinia bonducella, Tinospora cordifolia, Gardenia gummifera, Ailanthus excelsa, Acacia arabica, Embelia ribes and Ventilago maderspatana* from Melghat forest were screened for their antibacterial potential against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi, Shigella flexneri, Salmonella paratyphi, Salmonella typhimurium, Pseudomonas aeruginosa, Enterobacter aerogenes* by disc diffusion method. Out of these medicinal plants *Caesalpinia bonducella, Gardenia gummifera* and *Acacia arabica* showed remarkable antibacterial potential. The phytochemical analysis had showed the presence of Cardiac glycosides in all extracts (aqueous, acetone, ethanol and methanol) of *Acacia arabica, Gardenia gummifera* and ethanol, methanol extracts of *Caesalpinia bonducella*. Flavonoids were present in *Gardenia gummifera, Ailanthus excelsa* and acetone, methanol extracts of *Acacia Arabica*. Tannins and phenolic were present in *Cyperus rotundus, Embelia ribes*, and organic extracts of *Ventilago maderspatana*.

Key words: Antibacterial activity, Melghat, Medicinal Plants, Phytochemical

Introduction:

Melghat forest is part of Amravati district of Maharashtra State (India) and it preserves innumerable valuable medicinal plants. The knowledge of these medicinal plants was passed traditionally from one generation to other without documentation (according to Mr. R. B. Giri, 1983). Korkus or Bhumka or Bhagats traditionally used plants for the treatments of diarrhea, dysentery, stomachache, and any other enteric disorder but their antibacterial potential were not documented (Badhe and Pande, 1988; Tambekar and Saratkar, 2005). Almas (2001) demonstrated antibacterial potential of Babul. Lavhale and Mishra, (2007) claimed antitumor, antileukemic antifeedant activities of quassinoids in *Ailanthus excelsa*; Rani and Khullar (2004) showed moderate antibacterial activity of aqueous and methanol extracts of *Embelia ribes, Caesalpinia bonducella.* Dwivedi, et al. (2006) studied antibacterial, antimalerial activity of *Caesalpinia bonducella* seeds. The aqueous, ethanol and methanol extracts of Nut grass were studied by Jha et al. (2006) while Shivkumar et al. (2007) demonstrated its anticonvalscent activity. Loizzo et al. (2006), Dell'Agli et al. (2008) and Shemali et al. (2001) studied the antihypertensive, antimalerial and antibacterial (ethyl acetate extract, 6mg/disc) properties of bark of *Ailanthus excelsa.* Jain et al. (2007) studied the antimicrobial activities of *Embelia ribes* in *Piper longum.* Diarex, an herbal formulation against non-specific diarrhea, containing *Tinospora cordifolia* is an effective drug (Irfan *et al*., 2001).

Despite the numerous advances in medicine, the prevalence of infectious diseases continues to rise due to emergence of antibiotic resistant pathogens, which are attributed to the widespread use of antibiotics. Search for new antibacterial agents from plants has now a day gained an importance. The interest primarily has arisen from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that can have adverse effects. Therefore, the objective of this study was to screen medicinal plants from Melghat forest for antibacterial potential against different enteric pathogens by scientific experimentation.

Materials and methods:

Selection of Medicinal plants and preparation of extracts

With help of traditional herbal healer (Korkus or Bhumka or Bhagats of Melghat forest), we identified 8 medicinal plants, Acacia *arabica* (leaves), *Caesalpinia bonducella* (seeds*), Cyperus rotundus* (rhizomes*), Embelia ribes* (seeds), *Gardenia gummifera* (resinous exudation of leaf buds and shoots*), Tinospora cordifolia* (stem*)* and *Ventilago maderspatana* (stem, bark) from Melghat forest (Table 1), which are used by these people against diarrhoeal or abdominal discomforts or intestinal infections. R. B. Giri, Range Forest Officer, Maharashtra Forest Rangers College, Chikhaldara identified these plants. Selected parts of plants were collected, cleaned and disinfected with water and mercuric chlorides (0.5%), dried in shadow and ground to powder in grinder mixer. A 10 g of powder was soaked in 100 mL of solvent (water, ethanol, methanol, and acetone), refluxed in soxlet apparatus, filtered and filtrate was evaporated in controlled conditions of temperature to avoid destruction of dissolved phytochemicals.

Table 1: Plants selected for study

Botanical name	Local name	Vernacular name	Plants parts used	Medicinal use by Korkus
Acacia arabica (Mimosaceae)	Babul, Acacia	Babul	Leaves	Astringent, diarrhea, dysentery
Ailanthus excelsa (Simaroubaceae)	Maharukh	Tree of heaven	Leaves	Decoction of leaves in pectoral lesions, diarrhea
Caesalpinia bonducella (Caesalpiniaceae)	Sagargoti	Fever Nut, Nikkar nut, Bonduc nut,	Seeds	Digestive problems, dysentery, vomiting
Cyperus rotundus (Cyperaceae)	Nagarmotha	Nut grass	Rhizomes	Diarrhea, dysentery, indigestion
Embelia ribes (Myrsinaceae)	Vavdinga	Babreng, embelia	Seeds	Dyspepsia, colic pain, cough, asthma
Gardenia gummifera (Rubiaceae)	Dikamali	Gummy cape jasmine	Resinous exudates of leaf buds and shoots	Nervous disorders, diarrhea due to dentition
Tinospora cordifolia (menispermaceae)	Gulvel	Gulancha tinospora	Stem	Chronic fever, polyuria diabetes
Ventilago maderspatana (Rhamnaceae)	Raktavalli raktapapadi	--	Stem, bark	Stomachic, tonic and stimulant

Bacterial cultures

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study. The bacteria rejuvenated in Mueller-Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18hr and then stocked at 4^0C in Mueller-Hinton Agar. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 mL of sterile nutrient broth and incubated at 37^0C for 3hr. Turbidity of the culture was standardized to 10^5 CFU with the help of SPC and Nephlo-turbidometer.

Preparation of Disc for antibacterial activities

Sterile Whatman filter paper discs (10 mm) were soaked in the solution in such concentration that, the amount of solution absorbed by each disc contain 2, 4, 6, 8,10 mg of extract of each

aqueous and organic extracts of *Acacia arabica* (leaves), *Caesalpinia bonducella* (seeds*), Cyperus rotundus* (rhizomes*), Embelia ribes* (seed), *Gardenia gummifera* (resinous exudation of leaf buds and shoots*), Tinospora cordifolia* (stem*)* and *Ventilago maderspatana* (stem, bark). These prepared discs were dried in controlled temperature and used for the study.

Agar gel diffusion antibacterial activities

For antibacterial properties, 0.1 ml bacterial suspension of 10^5 CFU ml^{-1} was uniformly spread on Mueller-Hinton Agar (MHA) plate to form lawn cultures. The dried discs (dried at 37^0C overnight) were applied to the surface of MHA plates seeded with 3hr broth culture of the test bacterium. The plates were then incubated for 18hr at 37^0C. Antibiotic susceptibility discs, ampicillin 10µg, were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. The experiment was performed in duplicate and the mean of the diameter of the inhibition zones was calculated.

Phytochemical analysis

The presence of saponins, tannins, anthraquinones, alkaloids, triterpenes, flvonoids, glycosides, reduced sugar, and phlobatannins were detected by simple qualitative methods (Khandelwal, 2001).

Results and discussion

During the past decades, traditional systems of medicine have become increasingly important in view of their safety. A current estimate suggests that, in many developing countries, a large proportion of population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. The present study was conducted to investigate antibacterial properties of 8 selected plants from Melghat forest, which is less studied and used in Indian Folkloric Medicine. Herbal remedies play a fundamental role in traditional medicine in rural areas of India where the therapeutic treatment of choice as antiseptic, anti-inflammatory and in treatment of infectious diseases including diarrhea. In present study, attempt was made to correlate traditional herbal medicinal knowledge held by the Indian native people with modern scientific laboratory-based assay.

A total of 32 extracts of 8 medicinal plants were tested for antibacterial activity. Out of these, 18 extracts were with antibacterial potential. *Escherichia coli, Salmonella typhi, Proteus vulgaris, Salmonella paratyphi, Salmonella typhimurium and Pseudomonas aeruginosa* were resistant to *Cyperus rotundus, Caesalpinia bonducella, Tinospora cordifolia, Ailanthus excelsa, Embelia ribes and Ventilago maderspatana* with 10mg/disc. *Proteus vulgaris* was sensitive to acetone extract (6mg/disc) of *Cyperus rotundus*. Methanol extract of *Caesalpinia bonducella* proved antibacterial to *S.aureus, S.flexneri,* and *E.aerogenes*. Organic extracts of *Gardenia gummifera* was active against *S.aureus, K. pneumoniae,* and *E.aerogenes. Ventilago maderspatana* was antibacterial against *S.aureus, K.pneumoniae, P.vulgaris, S. flexneri* and *E.aerogene.* (Parekh and Chanda, 2006) and Moon et al. (2006) also demonstrated similar antibacterial properties of these plants. *S. flexneri* a causative agent of bacterial dysentery was resistant to aqueous extracts of all plants but sensitive to methanol extract of *Caesalpinia bonducella* (2mg/disc), which was also observed by Jha et al. (2006). *Acacia arabica* proved its antibacterial against all test pathogens. *S. typhi* was inhibited by all three organic extracts of Babul while positive control was inefficient to inhibit the pathogen. Methanol extract of Babul showed maximum inhibition of *E.coli, S.aureus, S. typhi, K.pneumoniae, S. flexneri and E. aerogenes.* Almas, (2001) and bioassay studies of Dabur et al. (2007) also reported such antibacterial potentials (Table 2). Preliminary phytochemical analysis of the extracts of these plants showed presence of anthraquinones, flavonoids, cardiac glycosides, tannins, and phenolics (Table 3).

The difference in the antibacterial potentials of different extracts suggested that solubility of various phytochemical in various solvents made it different from the others. Acetone extract of *Ventilago maderspatana* proved antibacterial to *Klebsiella pneumoniae, Enterobacter aerogenes* (8mg/disc) and *Shigella flexneri* (10 mg/disc). Ethanol extract was sensitive to S*taphylococcus aureus, Shigella*

Table 2: Zone of inhibition (including the diameter of disc in mm) **of different extracts of selected plants with test pathogens**.

Plants		*Cyperus rotundus*		*Caesalpinia bonducella*					*Tinospora cordifolia*	*Gardenia gummifera*					*Ailanthus excelsa*	*Acacia arabica*					*Embelia ribes*	*Ventilago madraspatana*		Amoxicillin	Negative Control
Extract mg /disc →		8	10	2	4	6	8	10	10	2	4	6	8	10	10	2	4	6	8	10	10	8	10	10	-
Escherichia coli (MTCC443)	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-
	Acetone	13	15	16	17	19	20	21	13	16	17	18	19	20	-	-	15	16	18	21	-	-	-		11
	Ethanol	13	16	18	18	19	19	20	12	15	18	19	19	21	-	-	-	14	15	17	-	-	-		11
	Methanol	-	-	-	-	-	-	-	15	-	-	-	-	-	-	-	17	18	20	22	-	-	-		-
Staphylococcus aureus (MTCC96)	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	40	-
	Acetone	13	17	-	-	-	-	13	16	18	18	19	20	20	-	14	16	20	22	24	-	-	14		-
	Ethanol	14	18	-	-	-	13	14	13	15	17	17	18	19	-	-	14	15	16	17	14	14	16		12
	Methanol	14	15	25	27	31	33	34	12	14	18	19	21	22	-	14	20	20	21	25	-	13	16		-
Enterobacter aerogenes (MTCC111)	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	38	-
	Acetone	-	-	-	-	-	-	16	-	-	-	16	18	20	-	-	14	15	16	18	13	16	19		11
	Ethanol	13	14	-	-	-	16	18	13	-	14	15	17	20	-	-	-	14	16	18	14	14	16		12
	Methanol	14	15	-	15	24	28	33	-	-	12	14	16	20	-	-	-	13	18	22	-	14	16		-
Pseudomonas aeruginosa (MTCC424)	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	12
	Acetone	14	14	15	15	17	18	19	18	12	15	17	18	20	-	-	-	-	14	15	-	-	-		13
	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-		-
	Methanol	13	14	16	18	19	20	22	14	16	18	18	19	20	-	-	-	-	16	18	-	-	-		-
Salmonella typhi (MTCC734)	Aqueous	13	16	18	18	19	20	20	13	14	16	17	18	19	-	-	-	-	-	-	-	-	20	30	-
	Acetone	14	14	16	16	17	18	19	18	12	15	17	18	18	-	15	16	18	20	23	-	-	17		-
	Ethanol	15	17	15	17	18	19	19	19	19	21	15	17	19	-	15	17	19	19	21	-	-	19		13
	Methanol	17	17	12	14	16	17	19	18	19	26	17	17	18	-	17	17	18	19	26	-	-	23		11
Salmonella typhimurium (MTCC98)	Aqueous	15	17	14	16	19	19	20	19	19	21	15	17	19	-	-	-	-	-	-	-	-	-	32	12
	Acetone	14	14	16	16	17	19	19	18	12	15	17	18	18	-	-	-	-	13	15	-	-	-		11
	Ethanol	13	14	13	15	17	17	20	17	15	17	19	19	22	-	-	-	-	12	15	-	-	-		11
	Methanol	12	13	13	15	17	18	19	18	12	15	16	18	20	-	-	-	-	14	15	-	-	-		-
Proteus vulgaris (MTCC426)	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55	11
	Acetone	17	23	14	19	20	21	22	-	14	15	19	20	21	-	11	14	16	16	21	-	-	13		11
	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	16	19	20	20	-	-	15		12
	Methanol	-	14	14	16	18	20	22	-	13	14	16	18	20	-	-	13	18	20	23	-	-	13		11
Klebsiella pneumoniae (MTCC2653)	Aqueous	-	-	-	-	-	-	-	13	14	16	18	20	21	-	-	-	-	-	-	-	-	-	35	-
	Acetone	12	13	13	14	16	18	20	-	-	-	16	18	20	-	-	-	-	15	16	14	15	16		12
	Ethanol	12	13	12	16	18	20	21	-	-	14	17	21	22	-	-	-	-	14	16	16	-	14		11
	Methanol	13	15	14	16	18	20	21	-	-	14	18	20	21	-	-	-	-	15	17	-	-	16		12
Salmonella paratyphi (MTCC735)	Aqueous	-	-	-	-	-	-	-	13	-	-	-	-	14	14	-	-	-	-	14	-	-	-	32	-
	Acetone	-	-	-	-	-	-	16	13	-	-	-	-	-	-	-	-	-	13	15	-	-	-		11
	Ethanol	-	14	15	16	18	22	22	14	14	15	19	20	21	14	-	17	21	23	25	-	-	13		12
	Methanol	13	13	13	16	18	18	22	13	14	16	18	20	21	15	-	14	16	18	20	-	-	14		-
Shigella flexineri (MTCC1457)	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	-
	Acetone	14	16	14	16	17	18	20	19	-	12	14	16	18	-	14	15	19	20	21	-	-	15		11
	Ethanol	13	16	16	17	19	20	22	14	18	14	18	19	20	-	-	17	18	20	25	-	-	15		11
	Methanol	-	-	16	17	19	19	22	14	18	18	21	21	22	-	15	16	18	22	24	-	-	-		

Table 3: Phytochemical analysis of the plant's extracts

Constituent	*Cyperus rotundus*				*Caesalpinia bonducella*				*Tinospora cordeopholia*				*Gardenia gumiphera*				*Ailanthus excelsa*				*Acacia arabica*				*Embelia ribes*				*Ventilago madraspatna*			
	Aqueous	Acetone	Ethanol	Methanol	Aqueous	Acetone	Ethanol	Methanol	Aqueous	Acetone	Ethanol	Methanol	Aqueous	Acetone	Ethanol	Methanol	Aqueous	Acetone	Ethanol	Methanol	Aqueous	Acetone	Ethanol	Methanol	Aqueous	Acetone	Ethanol	Methanol	Aqueous	Acetone	Ethanol	Methanol
Reducing Sugars	+	-	+	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Gums	+	-	+	+	+	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Proteins	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-
Fats & Oils	-	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	+	+	-	+	-	-	-	+	-	-	-	-
Steroids	+	+	+	+	-	-	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	+	+
Cardiac Glycosides	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Anthraquinones	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Alkaloids	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Tannins & Phenolics	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+

flexneri, and Enterobacter aerogenes (10mg/disc) and the aqueous extract had antibacterial effect on *Enterobacter aerogenes* only. Basu et al. (2005) also observed similar antibacterial activity in chloroform and ethanol extracts. *Tinospora cordifolia* did not posses bactericidal activity (Thatte et al. 1992); which is reestablished in present study. The aqueous and organic solvents extracts of *Cyperus rotundus* proved mild antibacterial (Grewal, 2000; Jha et al. 2006).

Conclusions:

The extracts possessing high antibacterial effects should be further studied for their therapeutic use. The present study suggests that these plants extracts were antibacterial against bacterial pathogens thus supporting their folkloric usage.

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