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ANTIBACTERIAL EVALUATION OF MEDICINAL PLANTS USED BY KORKUS IN MELGHAT FOREST AGAINST GASTROINTESTINAL INFECTIONS

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ABSTRACT

Use of plants as a source of traditional healing systems around the world that utilize herbal remedies is an important source for the discovery of new antimicrobials against resistant strains of bacteria. Medicinal plants have been a major source of therapeutic agents in Korkus from Melghat since time immemorial. Incredible knowledge of phytomedicine is acquired in non-coded form by these tribals and rural community is clear from evidences related to folklore medicines. *Acacia leucopholia* (Bark), *Butea monosperma* (Seed, Flowers), *Woodfordia fruticosa* (Root, Flowers), *Sphaeranthus indicus* (Fruits, Whole plant), *Maytenus emerginata* (Root, Leaves), *Acacia arabica* (Leaves), *Caesalpinia bonducella* (Seeds), *Gardenia gummifera* (Resin) were selected. Dried powders of plant parts were extracted in different solvents like water, ethanol, methanol and acetone. Antibacterial activity was tested by disc diffusion method against standard cultures of *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi, Salmonella typhimurium, Klebsiella pneumoniae, Shigella flexneri*. Methanol extract of *Woodfordia fruticosa, Acacia leucopholia,* ethanol extracts of *Sphaeranthus indicus, Butea monosperma, Maytenus emerginata* were active against the test pathogens. Phytochemical analysis of the extracts showed presence of cardiac glycosides, anthraquinone, flavonoids, tannins phenolics in the methanol extracts of *Woodfordia fruticosa, Acacia leucopholia*.

INTRODUCTION: The large Melghat tract In Maharashtra State is a hilly terrain of Satpura ranges, which is highly dense forest and occupies an area of about 4000 Sq. km. Melghat with dominant population of Korkus and Gond tribes still depends on knowledge of local "vaidus" and "bhagats" for primary health care. These local doctors depend on seasonally available local flora for treating human diseases and store herbal material available in hilly areas as dried roots, rhizomes, fruits, seeds, etc [1].

Charaka and Sushruta had recommended sweetened decoction of flowers of *Woodfordia fruticosa* for fever, haemothermia, persistent dysentery. Dried flowers are sprinkled over ulcers and wounds, for diminishing their discharge. Conflictions of flowers are used as stimulant and astringent, given in dysentery to check hemorrhages and leucorrhoea. Kumaraswamy *et al.,* [2] has studied extracts of *Woodfordia fruticosa* in various solvents and concluded that petroleum ether followed by methanol extract were highly potent antibacterial agent. Bark of *Acacia leucopholea* is used for cholera and diarrhea [3]. Flowers of *Butea monosperma* are boiled in water to obtain a dye and also used as an antiseptic, against infections of round worms, thread worms and Giardiasis Badhe and Pande [4].

Roots of *Maytenus emerginata* are used in gastrointestinal troubles, especially dysentery [5, 6]. Pulverized leaves are given in milk to children as a vermifuge. Leaf ash with ghee is applied on sores as ointment [7]. The juice of *Sphaeranthus indicus* an aromatic herb, the plant is styptic and useful in liver and gastric disorders [8]. Sticks of *Acacia arabica* are well known remedies for dental caries [4]. Dwivedi *et al* [9] studied antibacterial, antimalarial activity of Seed of *Caesalpinia bonducella*. Resinous exudation of leaf buds and shoots of *Gardenia gummifera* is used to stop diarrhea due to dentition [3].

Traditional medicines are cheaper, with minimum side reactions and safe. To ensure the safety of its products and practices standardization is of vital importance. The knowledge of medicinal plants came from our ancient literature i.e. Vedas, with time and fast developmental changes had lead pathogens to gain resistance against new generation antibiotics. Hence search for new antibacterial sources that are of green origin has multiple advantages. In the Indian system of medicine, most practitioners formulate and dispense their own recipes; this requires proper documentation and research.

MATERIALS AND METHODS: Frequent field visits were conducted in study area throughout year March to October for collection of specimen samples at flowering and fruiting stages. Specimen samples were identified by Mr. R.B. Giri, Ranger forest officer, Maharashtra forest Rangers college, Chikhaldara (**Table 1**). Selected parts of plants were collected, cleaned and disinfected with water and mercuric chloride (0.5%), dried in shadow and ground to powder in mixer grinder. For preparation of extracts, 10 g of powder was soaked in100 ml of solvents (water, ethanol, methanol, and acetone) refluxed after 24 h in Soxhlet apparatus, filtered and filtrate was evaporated in controlled conditions of temperatures to avoid destruction of dissolved phytochemicals.

Bacterial inoculums preparation: The standard pathogenic bacterial strains were procured from IMTECH, Chandigarh, India. The bacteria were rejuvenated in nutrient broth medium. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 ml of sterile nutrient broth and incubated at 37^0 C for 3h turbidity of the culture was standardized to 10^5 CFU with the help of SPC and Nephlo-turbidometer.

TABLE 1: PLANT PARTS SCREENED FOR ANTIBACTERIAL STUDY

Botanical name	Local name	Vernacular name	Plants parts used	Medicinal use by Korkus
Acacia leucopholea (Mimosaceae)	Safed Kikar	White Babul	Bark	Astringent, Diarrhea, Cholera
Butea monosperma (Papilionaceae)	Tesu, Palasha	Flame of the forest	Flowers, Seeds	Antiseptic, Depurative, Diuretic, Antihelmintic
Maytenus emerginata (Celastraceae)	Bharatti	Maytenus emerginata	Leaves, Root	Gastrointestinal troubles, vermifuge
Woodfordia fruticosa (Lythraceae)	Dhayati	Fire flame bush	Flowers, Root	Astringent, dysentery, damage of mucous membrane
Sphaeranthus indicus (Asteraceae)	Gorakhmundi	East India globe thistle	Whole plant, Fruits	Styptic, antihelmintic, depurative, bowel complaints
Acacia arabica (Mimosaceae)	Babul, Acacia	Babul	Leaves	Astringent, Diarrhea, dysentery
Caesalpinia bonducella (Caesalpiniaceae)	Sagargota	Fever nut	Seeds	Digestive problems, dysentery, vomiting
Gardenia gummifera (Rubiaceae)	Dikamali	Gummy cape jasmine	Resinous exudation of leaf buds and shoots	Nervous disorders, diarrhea due to dentition

Preparation of disc for antibacterial activities: Sterile blotting paper disc (10mm) were soaked in the solution in such concentration that the amount of solution absorbed by each disc was 2, 4, 6, 8, 10 mg of each extracts of *Acacia leucopholia* (Bark), *Butea monosperma* (Seed and Flowers), *Woodfordia fruticosa* (Root, Flowers), *Maytenus emerginata* (Leaves and Root), *Sphaeranthus indicus* (Fruits and Whole plant), *Acacia arabica* (Leaves), *Caesalpinia bonducella* (Seed), *Gardenia gummifera* (Resins). The prepared discs were dried in controlled temperature and used for study.

Agar disc diffusion for antibacterial activities: For antibacterial properties, 0.1mL 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 over night) were applied to the surface of MHA plates seeded with 3h broth culture of test bacterium. The plates were incubated for 18 h at 37^0C. Antibiotic susceptibility discs, Ciproflxacin10 µg, 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 for 10mg/disc and diameter of inhibition zones were measured.

Phytochemical analysis: The presence of saponins, tannins, anthraquinones, alkaloids, triterpenes, flavonoids, glycosides, reduced sugar and phenols were detected by qualitative methods given by Khandelwal [10]. Antibacterial response of the medicinal plants against various pathogens was calculated as:

$$\text{Antibacterial sensitivity index for plant} = \frac{\text{Total score of antimicrobial sensitivity of bacteria}}{\text{Number of antimicrobial agents tested X No. of bacterial pathogens}}$$

Results and Discussion: The present study was conducted to investigate antibacterial properties of selected plants from Melghat forest and used in Indian Folkloric Medicine. Total 11 parts of eight plants were used in 44 extract preparations (**Table 2**). Leaves of *Acacia arabica* proved strongest antibacterial among the selected plants for study, followed by flowers of *Woodfordia fruticosa, Butea monosperma,* whole plant of *Sphaeranthus indicus* and so on (**fig. 1**). Methanol extract of flowers of *Woodfordia fruticosa* and bark of *Acacia leucopholia* were effective against all the test pathogens followed by ethanol extracts. *S. aureus* was most sensitive test pathogen. It was sensitive to ethanol extracts of *Acacia arabica*, *Acacia leucopholia, Sphaeranthus indicus, Caesalpinia bonducella, Gardenia gummifera* and *Woodfordia fruticosa.* Strong sensitivity was observed in *S. aureus, E. aerogenes, K. pneumoniae, Pr. vulgaris, Sh. flexneri, E. coli, S. typhimurium, S. typhi, Ps. aeruginosa* and *S. paratyphi* in decreasing order (**fig. 2**).

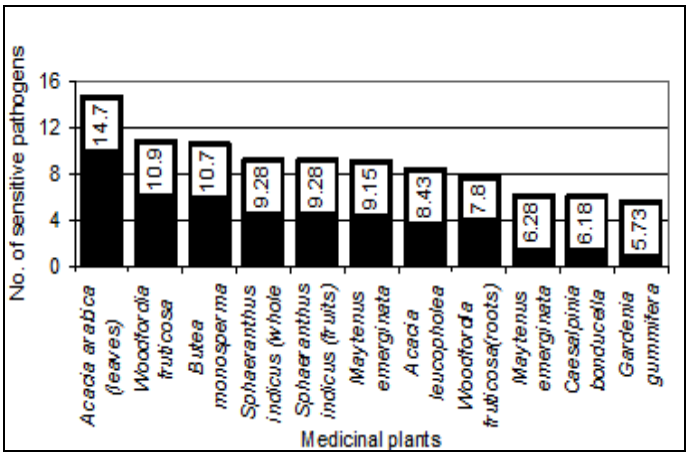

FIG. 1: COMPARISON OF ANTIBACTERIAL RESPONSE IN VARIOUS EXTRACTS OF SELECTED MEDICINAL PLANTS

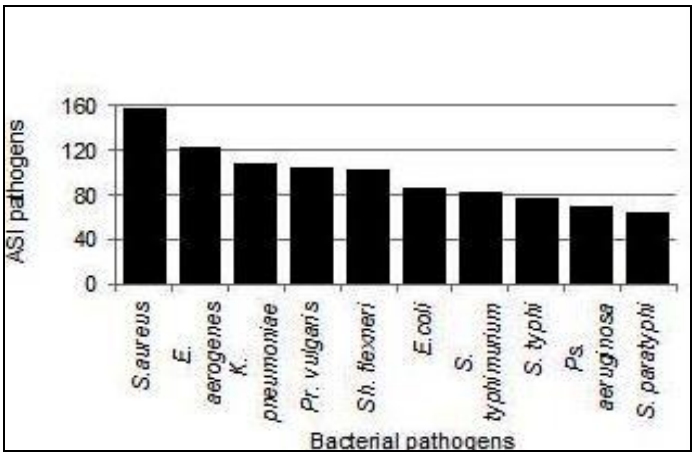

FIG. 2: COMPARITIVE ANTIBACTERIAL SENSTIVITY OF PATHOGENS AGAINST VARIOUS MEDICINAL PLANTS

TABLE 2: ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS (DIAMETER OF ZONE OF INHIBITION MEASURED IN mm. (ZOI)

Botanical name	Solvent	*E. coli*	*S. aureus*	*E. aerogenes*	*Ps. aeruginosa*	*S. typhi*	*S. typhimurium*	*S. paratyphi*	*Pr. vulgaris*	*K. pneumoniae*	*Sh. flexneri*	Reducing sugars	Proteins	Fats and oil	Steroids	Cardiac glycosides	Anthraquinone	Flavonoids	Alkaloids	Tannins and phenolics
Acacia arabica (leaves)	aq	0	0	0	0	0	0	0	0	0	0	+	-	+	+	+	+	+	+	+
	e	17	17	18	13	21	15	25	20	16	25	+	-	-	+	+	+	+	+	+
	m	22	25	22	18	26	15	20	23	17	24	+	-	+	+	+	+	+	+	+
	ac	21	24	18	15	23	15	15	21	16	21	+	-	+	+	+	+	+	+	+
Acacia leucopholea (bark)	aq	0	0	0	0	0	0	0	0	0	0	+	-	-	-	-	-	+	+	+
	e	18	25	0	0	16	16	0	20	15	22	+	-	-	+	+	+	+	-	+
	m	20	24	21	21	17	20	22	22	16	22	+	-	-	+	+	+	+	-	+
	ac	0	0	0	0	0	0	0	0	0	0	+	-	-	+	+	+	+	-	+
Butea monosperma (flowers)	aq	0	14	15	20	15	0	0	0	0	0	-	-	-	-	-	-	+	-	+
	e	20	18	17	20	20	17	20	17	17	22	-	-	-	-	-	+	+	+	+
	m	16	14	14	14	0	0	0	14	15	0	-	-	-	-	-	+	+	+	+
	ac	14	14	0	0	14	17	0	15	15	0	-	-	-	-	-	+	+	+	+
Caesalpinia bonducella (seeds)	aq	0	0	0	0	0	0	0	0	0	0	-	+	+	-	-	+	-	+	-
	e	0	14	18	0	0	0	15	0	0	22	-	+	+	-	+	+	-	+	-
	m	0	34	33	0	0	0	16	0	0	22	-	+	+	-	+	+	-	+	-
	ac	0	13	16	0	0	0	14	0	14	16	+	+	+	-	+	+	-	+	-
Gardenia gummifera (Resins)	aq	0	0	14	0	0	0	14	0	16	0	-	-	+	+	+	+	+	+	+
	e	0	19	20	0	0	0	0	0	22	0	-	-	+	+	+	+	+	+	+
	m	0	22	20	0	0	0	0	0	21	0	-	-	+	+	+	+	+	+	+
	ac	0	21	20	0	0	0	0	0	20	0	-	-	+	+	+	+	+	+	+
Maytenus emerginata (leaves)	aq	0	0	0	0	0	0	0	0	0	0	+	-	+	-	-	+	+	+	+
	e	17	18	18	0	17	16	15	20	20	15	+	-	+	+	-	+	+	+	+
	m	0	16	0	0	0	0	0	0	0	0	+	-	+	+	-	+	+	+	+
	ac	15	18	15	0	0	15	0	0	0	16	+	-	+	+	-	+	+	+	+
Maytenus emerginata (roots)	aq	0	0	0	0	0	0	0	0	0	0	+	-	+	-	-	+	+	+	-
	e	14	17	17	20	16	17	0	30	15	14	+	-	+	+	+	+	+	+	-
	m	15	18	0	0	0	15	0	16	15	15	+	-	+	+	+	+	+	+	-
	ac	15	19	15	0	17	14	0	14	0	18	+	-	+	+	+	+	+	+	-
Sphaeranthus indicus	aq	0	0	15	0	0	14	0	14	0	0	+	-	+	+	-	-	+	+	-

(whole plant)	e	15	26	18	14	13	16	26	15	24	22	+	-	+	+	-	+	+	+	-
	m	14	0	14	0	0	0	0	16	0	0	+	-	+	+	-	+	+	+	-
	ac	0	0	15	0	0	0	0	14	14	0	+	-	+	+	-	+	+	+	-
Sphaeranthus indicus (fruits)	aq	0	17	0	0	0	0	0	0	0	0	+	-	+	-	-	+	+	+	+
	e	16	30	16	17	15	18	0	20	19	29	+	-	+	+	-	+	+	+	+
	m	15	27	15	14	17	16	0	16	16	25	+	-	+	+	-	+	+	+	+
	ac	0	0	0	0	0	0	0	0	13	0	+	-	+	-	-	+	+	+	+
Woodfordia fruticosa (flowers)	aq	0	15	20	15	0	0	0	15	17	0	+	-	+	+	+	-	+	+	+
	e	16	25	14	20	14	14	0	23	17	19	+	-	-	+	+	+	+	-	+
	m	14	22	17	24	0	24	18	23	16	21	+	-	-	+	+	+	+	-	+
	ac	0	0	0	0	0	0	0	0	13	0	+	-	-	+	+	+	+	-	+
Woodfordia fruticosa(roots)	aq	13	14	0	12	15	0	0	0	0	0	+	-	-	+	-	+	+	+	+
	e	15	21	15	18	17	16	21	19	15	19	+	-	-	+	+	+	+	+	+
	m	0	13	0	0	16	16	14	13	0	0	+	-	-	+	+	+	+	+	+
	ac	0	13	0	0	0	0	0	0	0	0	+	-	-	+	+	+	+	+	+
Ciprofloxacin 10 µg		15	25	16	17	16	18	17	15	17	18									

Note: Aqueous - aq, Ethanol - e, Methanol -m, Acetone –ac

Presence of steroids cardiac glycosides, anthraquinone, flavonoids and phenolics in both extracts suggested that solubility of different phytochemicals in different solvents must be responsible to show the difference in their antibacterial effects. Moderate sensitivity of *Acacia leucopholia* in ethanol and Methanol extracts in most of test pathogens must be due to its tannin contents found in Acacia species [11]. Methanol extracts when tested by Dabur *et al.,* [12], against clinical isolates and standard strains found that MIC was 300µg/ml for *E. coli, B. cereus* and *Klebsiella* sp.

Methanol extracts of the East India globe thistle was potent antibacterial against *S. aureus* and *Sh. flexneri.* Deshpande *et al.,* [13] studied acetone and methanol extracts of fruit of *Sphaeranthus indicus* exhibited activity against Gram +ve and resistant to Gram –ve bacteria. Similar results were also observed in organic solvents extracts in discs with highest concentration of 10 mg in present work. Khare [3], stated that contents of Alkaloids, Tannins and volatile oil posse's antibacterial properties against *Vibrio cholera* and *Micrococcus* sp. Flavonoids are synthesized by the plants in response to microbial infections, in vitro they were effective antimicrobial substances against wide array of microorganisms [14]. Kumar *et al.,* [15] found that extracts of *Sphaeranthus indicus* were highly antibacterial. Antibacterial activity of *Woodfordia fruticosa flowers* was recorded [12]. The results were parallel to our findings for the highest contents in the discs i.e.10 mg. Sensitivity of *S. aureus, Pseudomonas* supported flower's use as an antiseptic by the Korkus. Parekh and Chanda[16] recorded that crude methanol extracts of *Woodfordia fruticosa* was good antibacterial at against all tested microorganisms as it is rich in tannins.

Ethanol extract of *Butea monosperma* was able to produce sensitivity in *S. typhi, E. coli, Pseudomonas* and *S. paratyphi*. *Shigella flexneri* was strongly inhibited by the same extract. Moderate sensitivity was observed in *S. aureus, E. aerogenes, S. typhimurium, Pr. vulgaris* and *Klebsiella sp.* Flowers of *Butea monosperma* were reported as active against worms, piles, colic pains. Tannins in *Butea monosperma* were active in diarrhea, dysentery when new formulations in herbal medicines were designed and studied for their antibacterial phytochemicals [3]. Ethanol extract of *Maytenus emerginata* (leaves) was antibacterial to *Klebsiella* sp. and *Pr. Vulgaris*

proved by Nair *et al.,* [17] and Tambekar and Khante [18]. *E. coli, S. aureus, E. aerogenes*, *S. typhi, S. typhimurium* and *Sh. flexneri* were mild sensitive. *Pr. vulgaris* was strongly sensitive to ethanol extract of root of *Maytenus emerginata*. *Pseudomonas* was also strongly inhibited but *Sh. flexneri; S. paratyphi* and *E. coli* were resistant to ethanol extract at highest conc. of 10 mg of the root. While others were mild sensitive to the same extract.

Methanol extract of seeds of *Caesalpinia bonducella* proved antibacterial against *S. aureus, Sh. flexneri* and *E. aerogenes* [19]. Organic extracts of *Gardenia gummifera* were active against *S. aureus, K. pneumoniae,* and *E. aerogenes. Sh. flexneri* a causative agent of bacterial dysentery was resistant to aqueous extracts of all plants but sensitive to methanol extract of *Caesalpinia bonducella* (2 mg/disc). Parekh and Chanda [20] and Moon *et al.,* [8], also demonstrated similar antibacterial properties of these plants. *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, Sh. flexneri and E. aerogenes.* Almas [21] and bioassay studies of Dabur *et al.,* [12] 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.

SUMMARY AND CONCLUSION: Further study of phytochemicals present in these medicinal plants may reveal some new antimicrobials. Alkaloids from *Butea monosperma*, essential oils in *Sphaeranthus indicus,* and tannins in *Acacia leucopholia, Acacia arabica* must be studied in detail. From the results we can conclude that the crude extracts of *Woodfordia fruticosa, Sphaeranthus indicus, Butea monosperma, Acacia leucopholia* and *Maytenus emerginata* exhibited significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases as broad spectrum antimicrobial agent. This probably explains the use of these plants by the indigenous people in Melghat against a number of enteric infections since generations.

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