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ANTIMICROBIAL INVESTIGATION OF SOME ARID ZONE PLANTS OF RAJASTHAN

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ABSTRACT: In the present study, the inhibitory effect of crude ethanolic extracts from four plants *viz. Bauhinia racemosa* Lamk (stem bark and roots), *Holoptelea integrifolia* (Roxb.) Planch. (roots), *Kirganelia reticulata* (Poir) Baill. (Stem bark) and *Phyllanthus maderaspatensis* Linn. (whole plant) were undertaken against some test bacteria and test fungi using Disc Diffusion Method. The screening data indicated that the antibacterial activity of the ethanolic extracts of *B. racemosa* (stem bark and root) inhibited the growth of *S. aureus* and *P. vulgaris* and *H. integrifolia* (roots) was found active against *S. paratyphi* B. The other two plants were totally inactive in this respect. A comparative study of the fungicidal activity of the four plant species indicated that although all of them exhibited activity against the select test fungi out of which the activity against *A. flavus* was most significant. Roots of *B. racemosa* were significantly active against *A. niger, F. moniliforme* and stem bark was active against *R. bataticola*. These could be attributed to the presence of racemosol and its derivatives possessing tetracyclic structure, the fungi toxicity of the roots of *H. integrifolia* was also exhibit good activity against *F. moniliforme*.

INTRODUCTION: Plants have been used for centuries as remedies for human diseases. Medicinal plants are used basically in two different ways: (i) as complex mixtures containing a broad range of constituents (infusions, essential oils, tinctures, extracts); (ii) as pure, chemically defined active principles. Pure compounds are generally employed when the active principles of a medicinal plant exhibit strong, specific activity and/or have a small therapeutic index, requiring accurate and reproducible dosage.

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On the other hand, the use of extracts is appropriate for plants exhibiting weaker and/or less specific pharmacological activities and if the active principles of the plant are not yet known. The efficacy of a number of phytopharmacological preparations such as ginkgo, garlic or valerian has been demonstrated by studies that applied the same scientific standard as for synthetic drugs [1-3]. Major pharmaceutical companies are, therefore, showing increased interest in higher plants as a source of new lead structure.

During the course of our studies four medicinally important plants were selected [4]. A glance at the literature of these plants indicated some bioactivity studies. The alcoholic extract of stem bark of *B. racemosa* exhibited anticancer activity against human epidermal carcinoma of the nasopharynx in tissue culture [5].

A methanolic extract of the stem and bark are also used as an anti-inflammatory, analgesic and antipyretic [6, 7]. The crude leaf sap of *H. integrifolia* was found to be mildly active against bean common mosaic virus [8], and anti-inflammatory and antifungal activity of leaf extract of this plant is also reported [9-12]. Ethanolic extract of aerial parts of *K. reticulata* showed antiviral effect against Ranikhet disease virus [5] whereas the bark extract completely inhibited the multiplication of potato virus X [13] and stem juice is used for eye washing in Ghana [14]. Anti-Arthritic activities of leaf extracts from Kirganelia reticulate are also reported [15]. *P. maderaspatensis* (whole plant) revealed anticancer activity against Walker carcinosarcoma 256 in the rat and Lewis lung carcinoma in the mice [5]. Anti microbial activity of Keeping these observations in view, the following bioactivity studies of the ethanolic extract of *Bauhinia racemosa* (stem bark and roots), *Holoptelea integrifolia* (roots), *Kirganelia reticulata* (stem bark) and *Phyllanthus maderaspatensis* (whole plant) were undertaken.

MATERIALS AND METHODS:

Plant Materials: The roots of *Bahunia racemosa* Lamk and *Holoptelea integrifolia* (Roxb.) Planch. were collected from the Rajasthan University Campus, Jaipur, the whole plant of *Phyllanthus maderaspatensis* Linn. was collected from the outskirt of Alwar (Rajasthan) and the stem bark of *Kirganelia reticulata* (Poir) Baill was collected from the field of Secretariat, Jaipur. All the plant materials were identified for authenticity in the Department of Botany, University of Rajasthan, Jaipur (Herbarium sheet No. RUBL 8211, 4334, 4262 and 7549 respectively).

Extraction: Powdered roots and stem bark of *Bahunia racemosa,* roots of *Holoptelea integrifolia,* whole plant of *Phyllanthus maderaspatensis* and stem bark of *Kirganelia reticulata* were extracted on a steam bath for 8 X 3 hrs with ethanol separately. Later, each of these extract was filtered, the residue re-extracted (2 X) for complete exhaustion, the extracts were pooled individually and concentrated under reduced pressure and stored in dark colored bottle at 4 °C in a refrigerator.

Sources of Test Organisms:

Bacteria: Pure culture of all test organisms, namely *Escherichia coli, Klebsiella aerogenes*, *Proteus vulgaris* and *Salmonella paratyphi* B as Gram -ve and *Staphylococcus aureus* as Gram +ve bacteria, the human pathogens were obtained through the courtesy of SMS Medical College, Jaipur, which were maintained on Nutrient Broth Medium.

Fungi: The pure cultures of test fungi, namely *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme* and *Rhizoctonia bataticola* were obtained from the Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur, which were maintained on Potato Dextrose Agar (PDA) medium.

Culture of Test Microbes: For the cultivation of bacteria, Nutrient Broth Medium (NBM) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 15 Ibs for 25-30 min. The agar test plates were prepared by pouring -15 ml of NBM into the petri-dishes (10 mm) under aseptic conditions. The peptone saline solution was prepared (by mixing 3.56 g KH_2PO_4 + 7.23 g NaH_2PO_4 + 4.30 g NaCI + 1.00 g peptone in 1000ml distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37 °C for 24 hrs.

However, for the cultivation of fungi, Potato Dextrose Agar (PDA) medium was prepared by mixing 1000ml potato infusion prepared from 200 g potatoes, 20 g agar and 20 g glucose, followed by autoclaving; the test fungi were incubated at 27 °C for 48 hrs and the cultures were maintained on the same medium by regular sub-culturing.

To prepare the test plates, in both bacteria and fungi, 10-15ml of the respective medium was poured into the petri-dishes and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown agar slant, while for fungicidal efficacy; a uniform spread of the test fungi was made using sterile swab.

Bactericidal and Fungicidal assay: For both, bactericidal and fungicidal assays, disc diffusion method [16] was adopted, because of re-productivity and precision. The different test organisms were preceded separately using a sterile swab over previously sterilized culture medium plates and the

zone of inhibitions were measured around sterilized dried discs of What man No.1 paper (6mm in diameter), which were containing 500µg and 1000 µg of the test extracts and control amikacin (10 µg/ml for bacteria) and nystatin (100 units/disc for fungi) as reference drugs separately. Such treated discs were air-dried at room temperature, to remove any residual solvent which might interfere with the determination, sterilized and inoculated. Before incubation, these plates were placed at low temperature for 1 hr, so as to allow maximum diffusion of the compound from the test discs into the agar plate and later, incubated at 37 °C for 24 hrs in case of bacteria and 48 hrs for fungi, after which the zones of inhibition could be easily observed. Three replicates of each test extract were examined and the mean values were then referred.

RESULT AND DISCUSSION:

Bactericidal Activity: While evaluating the bactericidal activity in *B. racemosa*, the ethanolic extract of roots showed moderate activity against all the test bacteria with maximum inhibition against *S. aureus* (AI = 0.63, at both the concentrations). However the ethanolic extract of stem bark of *B. racemosa* exhibited a better spectrum of activity against all the selected bacteria except *E. coli*. The extract showed pronounced activity against *S. aureus* (AI = 0.78, 1000µg/disc) and significant activity against *P. vulgaris* (AI = 0.63, 1 000µg/disc). ln *K. aerogenes* the inhibition was shown only at high concentration (AI = 0.61, 1000µg/disc).

In *H. integrifolia*, the ethanolic extract of roots showed pronounced activity against *S. paratyphi* B at 1000µg/disc, in this case the activity was equal to that of standard (AI= 1:00), it also exhibited appreciated activity against *E. coli* at 1000µg/disc. The ethanolic extract of *K. reticulata* (stem bark) and *P. maderaspatensis* (whole plant) did not demonstrated significant activity against any of the test bacteria except trace activity against *S. aureus* (**Table 1**).

TABLE 1: BACTERICIDAL ACTIVITY OF SELECTED PLANTS

Plant species	Part used	Dose µg/disc	*E. coli*		*K. aerogenes*		*P. vulgaris*		*S. paratyphi* B		*S. aureus*	
			IZ*	AI*	IZ	AI	IZ	AI	IZ	AI	IZ	AI
B. racemosa	Root	1000	9	0.27	12	0.46	12	0.54	9	0.54	12	0.63
		500	11	0.33	-	-	11	0.50	9	0.50	12	0.63
	Stem	1000	-	-	16	.61	14	0.63	8	0.44	15	0.78
	bark	500	-	-	-	-	11	0.50	10	0.55	14	0.73
H. integrifolia	Root	1000	13	0.39	-	-	-	-	18	1.00	-	-
		500	-	-	-	-	-	-	±	-	-	-
K. reticulata	Stem	1000	-	-	-	-	-	-	-	-	±	-
	bark	500	-	-	-	-	-	-	-	-	-	-
P. maderaspatensis	Whole	1000	-	-	-	-	-	-	-	-	±	-
	plant	500	-		-	-	-	-	-	-	±	-

IZ: inhibition zone (in mm) including the diameter of disc (6mm).
AI: activity index = (inhibition zone of sample/inhibition zone of standard.
Standard: Amikacin = 10µg/ml; (±) Trace activity: (-) No activity.

Fungicidal Activity: In *B. racemosa*, the ethanolic extract of roots showed maximum activity against *A. flavus* (AI = 0.63, 1000µg/disc) and appreciable activity against *A. niger* (AI = 0.58, 1000µg/disc) and *F. moniliforme* (AI = 0.57, 1000µg/disc). The extract of stem bark was also effective against *A. flavus* at both concentrations (AI = 0.68, 1000 µg/disc; AI = 0.63, 500µg/disc) and showed moderate activity against *R. bataticola* (AI = 0.57, 1000µg/disc; AI = 0.53, 500µg/disc). The ethanolic extract of roots of *H. integrifolia* demonstrated maximum inhibition against *A. fiavus* (AI = 0.78, 1000µg/disc) and significant activity (AI = 0.60, 1000µg/disc) against *F. moniliforme*. Likewise, in *K. reticulata* (stem bark), the ethanolic extract inhibited the growth of nearly all the selected fungi in general, but was found to be more effective against *A. flavus* (AI = 0.73, 1000µg/disc; AI = 0.63, 500µg/disc).

In *P. maderaspatensis* (whole plant), the ethanolic extract was found to inhibit the growth of all four selected fungi, but showed maximum activity against *A. flavus* (AI = 0.73, 1000µg/disc; AI = 0.52, 500µg/disc). The inhibition of *R. bataticola* was also significant, being the same at both concentrations (AI = 0.61, 1000µg/disc and 500 µg/disc) (**Table 2**).

TABLE 2: FUNGICIDAL ACTIVITY OF SELECTED PLANTS

Plant species	Part used	Dose µg/disc	*A. flavus*		*A. niger*		*F. moniliforme*		*R. bataticola*	
			IZ*	AI*	IZ	AI	IZ	AI	IZ	AI
B. racemosa	Root	1000	12	0.63	14	0.58	16	0.57	12	0.46
		500	10	0.52	8	0.33	14	0.50	10	0.38
	Stem	1000	13	0.68	12	0.50	14	0.50	15	0.57
	bark	500	12	0.63	10	0.41	12	0.42	14	0.53
H. integrifolia	Root	1000	15	0.78	10	0.41	17	0.60	14	0.53
		500	11	0.57	8	0.33	15	0.53	12	0.46
K. reticulata	Stem	1000	14	0.73	13	0.54	10	0.35	14	0.53
	bark	500	12	0.63	8	0.33	8	0.28	12	0.46
P. maderaspatensis	Whole	1000	14	0.73	12	0.50	14	0.50	16	0.61
	plant	500	10	0.52	10	0.41	12	0.42	16	0.61

IZ: inhibition zone (in mm) including the diameter of disc (6mm).
AI: activity index = (inhibition zone of sample/inhibition zone of standard; Standard: Nystatin = 100 units/disc.

CONCLUSION: The present investigation revealed that selected arid zone plants are potentially a good source of antimicrobial agents and certifies their traditional uses and they can be a natural, harmless replacement of expansive conventional medicines.

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CONFLICT OF INTEREST: No conflict of interest.

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