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A COMPARATIVE STUDY OF ANTIFUNGAL ACTIVITY OF *ALSTONIA VENENATA* R. BR

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ABSTRACT: *Alstonia venenata* a member of the family Apocynaceae, finds a prominent place in Ayurveda system of medicine and also among different tribal groups in India for a variety of diseases such as fever, epilepsy, malaria and a variety of skin diseases. *A.venenata* was studied to investigate the bioactive properties. Various plant parts like leaves, stem-bark, root-bark, flowers and fruits were extracted with a variety of solvents ranging from non-polar to polar and screened for bioactivity. Butanol and methanol extracts of all the parts alone were selected for antifungal testing. The tested organisms included human pathogens and laboratory contaminants/opportunistic fungi .They were *Pencillium marniffi*, *Cyrptococcus* sp., *Candida* sp., *Epidermophyton* sp., *Microsporum* sp., *Fusarium* sp., *Pencillium* sp., *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus* sp. Different concentrations of the extracts dissolved in DMSO were incorporated in Sabouraud Dextrose Agar (SDA) and a final concentration of 50mg, 100mg and 125mg per ml of SDA was obtained for testing. The drug control used was Imidazole at a concentration of 100µg/ml of SDA. Butanol extracts of stem and root-bark inhibited all the tested strains of fungi at a concentration of 50mg and 100mg respectively. Methanol extract of stem-bark and root-bark either showed complete or partial inhibition at a concentration of 125mg/ml. Butanol and methanol extracts of fruits, flowers and leaves showed complete or partial inhibition of most of the fungi tested at 125mg/ ml concentration. Antifungal activity of the fruits and flowers of *Alstonia venenata* are reported for the first time.

INTRODUCTION: The family Apocynaceae is known for high biological activity. They are broadly distributed mainly in the tropical and subtropical regions with an estimated 375 genera and 5100 species[1, 2]. In modern medicine, a number of genera are employed for a diverse array of uses such as controlling tumour growth in treating cancer[3], as antiplasmodial agents in parasitic infections[4], as muscle relaxants during surgery[5] and as an appetite suppressant in controlling obesity[6].

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Around 400 compounds from *Alstonia* spp. have been isolated and characterized [7, 8]. *Rauwolfia serpentina, Alstonia scholaris* and *Alstonia venenata* are some of the well-known plants in this family for their medicinal properties. *Alstonia venenata* is widely distributed in the western Himalayas, Western Ghats, common in Deccan peninsula and in the southern region of India. It is known to the local 'Kani' tribe as 'analivegam' used as a powerful antidote for poisonous snake bites.

The plant is known by different vernacular names as 'Addasarpa' in Kannada, 'Analivegam' 'Theeppala' in Malayalam, 'Anadana' 'Rajaadana' 'Visaghni' in Sanskrit, 'Palamunnipalai' and 'Sinnappalai' in Tamil [9]. Stem and root bark, fruits and leaves are medicinally important. The plant is

an active ingredient in Tri Health Ayurvedic formulations and is used for treating brain and nerve functions, neurological disorders, gastrointestinal ailments, joint pain, for chronic and acute skin disorders. It also has laxative and anti-inflammatory properties. Twenty new indole alkaloids belonging to the class of Yohimbine, Aspirdofractinine and Vincadifformine have been reported from this plant[10]. Venenatine, Isovenenatine, Alstovenine, Reserpin, Venoxidine, Kopsinine have been identified from stem bark [11] and 16-Epivenenatine and 16-Epialstovenine 5-methoxy-1-oxo-tetrahydro-β-carboline,Venenatine, Alstovenine, Venoxidine and Dioxokopsane were identified from root bark[12, 13]. Antifungal activity from the plant indole alkaloid Venenatine[14] and quaternary alkaloid Δ^3-alstovenine [15] and have been studied. Venoterpene and Echitoserpidine [16] fruit alkaloids have been identified. Alkaloids of the leaf are Alstolenine, 19, 20-dihydropolyneuridine, deacetylakaummiline, polyneuridine and raucaffrinoline [17].

Antifungal activities have been reported earlier from the stem bark alkaloids of *A.venenata* against plant pathogens and saprophytic fungi. The objective of the present investigation was to understand the antifungal activity if any from other plant parts like root-bark, leaves, flowers and fruits of *A.venenata* in addition to stem-bark, to compare the activity of extracts obtained and to identify the most active plant part. The efficacy of the extracts against some human pathogenic fungi was also tested.

MATERIALS AND METHODS:

Collection of plant material and authentication:

Plant was collected from interior parts of Ponmudi hills, Kerala and was identified and authenticated at the Herbarium Department of Botany, University of Kerala, Thiruvanathapuram and a voucher specimen has been deposited (Voucher specimen accession No. KUBH 5847).

Preparation of plant extracts:

Different plant parts viz. the leaves, stem-bark, root-bark, flowers, and fruits were separated, washed several times with water, air dried in shade, powdered and stored in dry polythene bags. Quantitative analysis, in terms of fresh weight, dry weight, the weight of the powder and moisture content were estimated. The powdered material was extracted successively with hexane, butanol, methanol and water for 12 hours using a Soxhlet apparatus. The excess solvent in the extracts was removed by distillation and then concentrated at temperature not exceeding 40^0 C in incubators. All the extracts were then kept in screw capped bottles and stored at room temperature except water extract which was refrigerated at 4°C.

Microorganisms tested.

Pathogenic fungi such as *Pencillium marniffi*, *Cryptococcus* sp., *Candida* sp. *Epidermophyton* sp.**,** *Microsporum* sp.**,** *Fusarium* sp.**,** laboratory contaminants or opportunistic fungi like *Pencillium* sp., *Aspergillus flavus, Aspergillus niger, and Rhizopus* sp. were obtained from Centre for Health sciences, University of Calicut, Kerala.

Screening for Antifungal activity:

The crude hexane, butanol, methanol and water extracts from all the plant parts were initially screened for antifungal activity against all the fungi at an arbitrary concentration of 125mg/ml of SDA. Butanol and methanol fractions showed maximum activity and were selected for further evaluation with different concentrations.

The concentrations tested were 50mg, 100mg and 125 mg per ml of SDA. 500, 1000 and 1250mg each of the different concentration of the extracts were initially dissolved in 1ml DMSO and 250 µl from each of the extracts were added to 2.5 ml of Sabouraud Dextrose Agar (SDA) at 60ºC, mixed well and allowed to solidify. Imidazole 100µg/ml of SDA was used as drug control. Tubes with the extracts, drug control and the media control were then inoculated with fresh cultures of the test fungi on SDA slants. The tubes inoculated with yeast culture were incubated at 37^0C for 24-72hrs. Other tubes were incubated at room temperature for 7 to 10 days and presence or absence of growth was noted.

RESULTS:

The activitiy of the butanol and methanol extracts of different parts of *Alstonia venenata* against ten different fungal strains are given in Table[1]. Butanol extract of the stem-bark at a concentration of

50mg/ml and root-bark butanol at a concentration of 100mg/ml of SDA completely inhibited all the tested fungi. Butanol extract of fruit at 125 mg/ml completely inhibited the growth of *Cryptococcus* sp., *Microsporum* sp.**,** *Fusarium* sp.**,** *A. flavus* and *Rhizopus* sp. Methanol extract of stem bark and root bark showed complete or partial inhibition at a concentrate on of 125mg/ml against the tested fungi. Butanol and methanol extracts of flowers and methanol extract of the fruits also showed complete or partial inhibition of all fungi except *A.niger* and *Rizopus* sp. at a concentration of 125mg/ml which indicates that, most of the extracts may completely inhibit the fungi at higher concentrations.

TABLE 1: ANTIFUNGAL ACTIVITY OF BUTANOL AND METHANOL EXTRACTS OF *ALSTONIA VENENATA*

Fungi	Root-bark		Stem-bark		Leaf		Fruit		Flower	
mg/ml	B 100	M 125	B 50	M 125	B 125	M 125	B 125	M 125	B 125	M 125
P. marniffi	-	±	-	-	±	+	±	±	±	±
Cryptococcus sp.	-	±	-	-	±	+	-	±	±	±
Candida sp.	-	±	-	±	±	+	±	±	±	±
Penicillium sp.-	-	±	-	±	+	±	±	±	±	±
Epidermophyton sp.	-	-	-	-	±	+	±	±	-	±
Microsporum sp.	-	±	-	±	±	+	-	±	±	±
Fusarium sp.	-	±	-	±	±	+	-	±	±	±
A. flavus	-	±	-	±	+	+	-	±	±	±
A. niger	-	±	-	±	+	+	+	+	+	+
Rhizopus sp.	-	±	-	±	+	+	-	+	+	+

(-) no growth of fungus (+) growth of fungus (±) weak growth; B- Butanol extract, M- Methanol extract.

DISCUSSION: Stem and root bark of *A. venenata* are rich source of different alkaloids. Sing *et al* [14] reported antifungal activity of indole alkaloids venenatine from the stem bark of *Alstonia venenata* against ten different plant pathogen and saprophytic fungi. In their study 30-40µL of 0.5, 1, 1.5, 2 mg/L of venenatine were mixed with 200-300 fungal spores and percentage spore germination was observed.

Ustilago cynodontis and *Alternaria brassicicola* showed highest sensitivity to Venenatine [14]. The activity of Δ^3Alstovenine, another indole alkaloid from A. *venenata* against seventeen plant pathogens were also reported [15]. 250-1000 mg/L was used for testing and percentage spore germination was noticed as above. Among the fungi tested *Cercospora* sp. was the most sensitive followed by *Helminthosporium maydis, H. sativum, Erysiphe polygoni* at a higher concentration[15]. In the present study also stem bark extracts are more active than the extract obtained from other part of the plant.

Strong bioactivities have been reported for members of the family Apocynaceae. Butanol and ethyl acetate fractions from stem bark and leaf of *Alstonia scholaris* were reported to be active against different strains of *Candida* tested. Stem bark Butanol extract of *A.scholaris* showed the largest zone of inhibition (16mm) against *Candida krusei* [18]. The benzene, ethanol and aqueous extracts of stem bark of *A. Scholaris* at a concentration of 200µg/disc when tested against *Aspergillus niger, Alternaria alternata, Fusarium solani, Trichoderma virens* using agar disc diffusion method exhibited significant antifungal activity [19].

In a similar work *Alstonia macrophylla* showed a minimum inhibitory concentration ranging from 32–128 mg/ml in case of *Trichophyton rubrum*, *Trichophyton mentagrophytes* var. *mentagrophytes* and *Microsporum gypseum* [20]. Methanolic crude extract of the leaves were more active than the stem bark extract prepared in a similar manner. Methanolic stem extract at 64 mg and 32 mg/ml showed antifungal activity against *T. mentagrophytes* and *T. rubrum. Candida albicans* and *Cryptococcus neoformans* were resistant at 128 mg/ml concentration. Monoterpenoid indole alkaloids from the leaves of *Alstonia rupestris* also showed activity against two plant pathogens *Gibberella pulicaris* and *Cercospora nicotiana* [21]. Both leaf extract and stem bark extracts were very effective in other *Alstonia* sp tested for antifungal

activity. In our study stem bark and root bark extracts were more effective than leaf.

In a study by Wankhede *et al*., petroleum ether, methanol, ethyl acetate and water extracts obtained from the leaves of *Catharanthus roseus*, *Nerium oleander* and *Tabernemontana divaricata* belonging to Apocynacea family showed activity against *C. Albicans* ATCC 90028 [22]. Acetone and water extracts from aerial parts of *Catharanthus roseus* at 50 percent concentration were inactive on *Neurospora crassa* [23]. Hot water extract of dried leaves of *Catharanthus roseus* was active on *Trichophyton mentagrophytes* [24]. Efficacy of dried stem hot water extract of *Vinca rosea* was also reported active against *T. mentagrophytes* and *T. rubrum* [25]. There are several reports on the antibacterial, antifungal and anti-inflammatory properties of *A. venenata* and *A. scholaris* (Shirly *et al* [26], Williams SJ and Thankamani V [27], Sutha S e*t al* [28], Thankamani *et al* [29], Misra *et al* [30], Joel *et al* [31]). All the reports point out that most of the plants in the Apocynaceae family possess significant antifungal and bacterial property.

CONCLUSION: *A.venenata* extracts were effective against a variety of fungi tested. Butanol extracts of root-bark and stem-bark exhibited the highest activity which inhibited all the tested strains of fungi followed by butanol extract of fruits and flowers. The presence of Venenatine and other indole alkaloids in *A.venenata* may contribute to the activity. The present evaluation offers a scientific basis for the use of this plant by folklores/traditional medicines as suitable antifungal agent against a range of pathogens. An in depth study using purified compounds, toxicological testing, understanding the mechanisms of action and therapeutical investigations are further needed for developing a new bioactive molecules from the plant.

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