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Screening for Bioactive Properties and Phytochemicals in Stem, Bark Extract of *Alstonia venenata* R.Br.

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Stem bark extract of *Alstonia venenata* R.Br was subjected to antibacterial and antifungal studies by *in vitro* methods against pathogenic and industrially important strains of bacteria and fungus. Crude extract showed significant results. On chromatographic fractionation of crude extract, ten compounds obtained; among them three compounds coded as c_1,c_2,c_3, which showed effective bioactive properties comparable to standards.

Key words: *Alstonia venenata*, Antibacterial & Antifungal activity, Phytochemical screening.

Alstonia venenata R.Br. is a small sized evergreen tree, growing in interior parts of hill stations. Stem bark greyish in colour, rough, hard, lenticellate, exudes latex when injured. Leaves simple, 6 to 8 inches long, and 3 to 6 in whorls. Flowers regular, bisexual, white in colour, borne in racemes. Fruit follicle, seeds hairy, numerous[1]. Stem bark, root bark and fruits used as astringent tonic, for skin diseases, snake poison[2] syphillis, leprosy etc.[3]. Major compounds isolated are alstovenine, venalstonine, venalstomidine[4,5,6] venenatin[7].

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MATERIAL AND METHODS

Fresh stem bark was collected from a 15 to 18 years old tree in the interior parts of Ponmudi hills, Kerala; identified systematically[1]. belongs to the family Apocynaceae and the sample has been documented in the Department of botany, University College, Trivandrum. Collected materials were weighed, oven dried at 39°C,weighed and powdered.50 g powder was extracted in methanol for 9 hours continuously to obtain bioactive principles from the bark[8].Crude extract was concentrated, weighed and made up to100ml. It is kept in sterilized bottle under refrigerated condition until use (Table 1).

Bioactivity assays by in vitro methods

Antibacterial activity

Crude stem bark extract was subjected to detect their antibacterial property against pathogenic and industrially important strains of bacteria by disc diffusion method with proper control[9,10,11]. Concentration of disc is 2.94mg/disc. Bacteria were provided from microbiology lab medical college, Trivandrum.

Antifungal activity

Crude extract was studied for antifungal property by invitro method. It is done by incorporating crude extract(concentration is 14.7mg/ml) in the media S.D.A (Sabouraud Dextrose Agar)[12] with proper controls againist phytopathogenic and industrially important strains of fungi procured from Agricultural College Vellayani,Trivandrum.

Phytochemical screening

Thin layer chromatography

Qualitative chemical analysis of crude extract was performed by Thin layer chromatography by different solvent systems indicated the presence of sterols, flavanoids, phenolic compounds, terpens, alkaloids etc[13-15].

Chromatographic fractionation

On chromatographic fractionation several fractions of 20 ml each were collected and their homogeneity were monitored by using T.L.C behaviour[16]. Identical fractions were pooled together to get single compound or mixtures[8]. The solvents from different elutes were evaporated and final compounds were collected[16] Ten compounds obtained each of them washed with respective solvents and alcohol; Final products were allowed to crystallize. All the compounds were subjected to antibacterial activity against Bacillus *subtilis* (concentration 1mg/disc) and antifungal activity *Rhizopus sps*. (concentration 5mg/ml). Effective bioactive compounds only were selected and coded as c_1,c_2,c_3. Bioactivity and chemical analysis of these compounds were performed[17] bioactivity property of these ccompounds were studied against microbes and these compounds were sunjected to several chemical tests like Liebermann Test, Liberamann and Burchord Test, Molish Test, Wagner's Test, Dragen droff's Test etc[16,14].

RESULTS

Antibacterial, antifungal activity of crude extract shown in Table 3 and 4, compounds c_1, c_2, c_3 were shown in Table 2. On chemical analysis compounds c_1 and c_2 showed positive results for the tests for sterols and c_3 expressed positive result for alkaloid detecting tests.

DISCUSSION

Methanol fraction of the stem bark possess antibacterial property zone of inhibition even at 2.94 mg/ disc MIC (minimum inhibitory concentration) and total inhibition of fungal growth at concentration 14.70 mg/ml. isolated compounds showed remarkable results.

Reports regarding bioactive properties in several members of the family Apocynaceae strongly support present study[18,19] isolation, structure and bioactivities of several compounds from different members of the family[20]. Most of the compounds generally possess characteristic bioactive properties and many of them are used for medicinal purpose and leads to the development of new synthetic drugs.

Table 1. Quantitative data

Plant	Fresh weight(g)	Dry weight (g)	Powder weight(g)	Wt/10ml crude (mg)	Concentration mg/disc	Concentration mg/ml
Alstonia venenata R.Br. Sterm bark	150	49.97	50	2940	2.94	14.70

Table 2. Quantitative chemical analysis

Eluent	Fraction	Volume(ml)	Colour nature	Yield (mg)	Bioactivity*	
					1‘	2
Hexane	F_1-F_{10}	220	Light yellow sticky	Very less quantity	Not enough for further work	
Hexane: benzene (70:30)	F_{11}-F_{22}	240	Light yellow solution	67	(-)	(-)
Hexane : Benzene (30:70)	F_{23}-F_{34}	240	Slight yellow	Very less quantity	Not enough to do further work	
Benzene	F_{35}-F_{49}	300	Pale white crystals	97	(+)	(+)
Benzene : Chloroform(70:30)	F_{50}-F_{63}	260	Pale orange solution	Very less	Not enough to do further work	
Benzene : Chloroform(30:70)	F_{64}-F_{78}	300	Light orange pasty	79	Less active	(-)
Chloroform	F_{79}-F_{95}	340	Pale white pasty	99	(+)	(+)
Chloroform: Ethyl acetate(70:30)	F_{96}-F_{108}	260	Light brown solution	Less quantity	Not enough to do further work	
Chloroform : Ethyl acetate(30:70)	F_{109}-F_{122}	280	Brown solution	Very less	Not enough to do further work	
Ethyl acetate	F_{123}-F_{136}	280	Brown wax like	91	(-)	(-)
Ethyl acetate: Methanol (70:30)	F_{137}-F_{148}	240	Greenish brown solution	Very less	Not enough to do further work	
Ethyl acetate : Methanol (30:70)	F_{149}-F_{160}	240	Pale white granules	93	(+)	(-)
Methanol	F_{161}-F_{175}	300	White granules	96	(+)	(+)

*Bioactivity
1- againist *Bacillus circulans* 2- aginist *Rhizopus nigricans*
(+)- active
(-) – not active

Table 3. Anti bacterial activity

Test organism	Zone of inhibition (mm)					
	Stem bark concentration 2.4mg/disc	c_1	c_2	c_3	control	Standard*
Bacillus circulans	17	15	13	17	-	27
Bacillus coagulans	16	15	14	17	-	25
Bacillus licheniformis	16	16	15	19	-	27
Proteus species	13	14	10	16	-	18
Pseudomonas aeruginosa	16	18	13	19	-	24

Values are zone of inhibition
(-) no inhibition
*standard- Tetracyclin

Table 4. Antifungal activity

Test organism	Stem bark concentration 14.70 mg	$c_1$5mg	$c_2$5mg	c_3 5mg	control	Standard*
Candida albicans	+	+	+	+	-	+
Rhizopus nigricans	+	+	+	+	-	+
Phytophthora colocasiae	+	+	+	+	-	+
Saccharomyces cerevisiae	+	+	+	+	-	+
Penicillium digitatum	+	+	+	+	-	+

Standard* - Miconazole
+ nogrowth
- no inhibition
+/- partial growth

CONCLUSION

From the results of present study, stem bark extract and isolated compounds possess bioactive property against microbes. The use of plant extract provides a potential alternative to antibiotics against phytopathogens and the compoundc_1 and c_3 are as potent as standards standards and deserve further investigation.

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