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STUDY ON THE PHYTOCHEMICAL AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF *ACHYRANTHUS ASPERA* LEAVES

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Abstract:

The petroleum ether, hexane, chloroform, ethyl acetate and methanol extracts of the dried leaves of *Achyranthus aspera*(Willd.) was evaluated for their phytochemical constituents, antibacterial and antifungal activity against twelve pathogenic microorganisms. The methanol, petroleum ether and hexane extracts were found to possess antimicrobial activity, particularly against *Aspergillus niger*, *Bacillus cereus*, *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa* compared with their MIC values.

Keywords: *Achyranthus aspera*; Antibacterial activity; Antifungal activity.

Plant: *Achyranthus aspera* (AMARATHACEAE) leaves were collected from the shola of the semi-evergreen forest in Nallamalis of Eastern Ghats, India during August 2010. Specimen was deposited for the further reference.

Medicinal Uses: According to the ayurveda it is used as laxative, useful in heartdiseases, blood purifier and other chronic diseases.[1-3]

Tested material: Petroleum ether, benzene, chloroform, ethyl acetate and methanol extracts (yields: 0.17, 0.12, 0.36, 2.02 and 2.18% respectively) obtained in succession by maceration of dried leaves. Pytochemical screenings were done according to standard methods [4-6].

Experimental study:

Preparation of discs: 6mm discs were prepared by sung whatmanfilter paper.

Antimicrobial susceptibility test:

Different successive extracts were taken and prepared discs as per the procedures by Kirbey method.

Studied activity. Antibacterial and antifungal activity by disc diffusion method and determination of minimum inhibitory concentration (MIC) [7-10].

Used microorganisms. Listed in Table 2.

Results: Reported in Table 1 (phytochemical screening) and Table 2 (antibacterial and antifungal activity). For all the extracts of *A.aspera* measured minimum inhibitory concentrations. *Bacillus cereus* and *Psuedomonas* are very interesting. The chloroform and ethyl acetate extracts were also significantly inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. Results obtained supports the local traditional uses of the plant against diarrhoea, skin and urinary infections.

Table-1: Phytochemical screening of *Achyranthus aspera* leaf extracts^a

Constituents	P	H	C	E	M
Alkaloids	++	++	+	++	+++
Carboxylic acids	-	-	-	-	-
Coumarins	-	-	-	-	-
Fixed oils	-	-	-	-	-
Flavonoids	-	-	-	++	+++
Phenols	-	-	+	++	+++
Quinones	+	-	-	-	-
Resins	-	-	-	-	-
Saponins	-	-	++	+++	+++
Steroids	-	-	-	-	+
Tannins	+	+	+	++	++
Glycosides	+++	++	+++	+++	+++

^aP, petroleum ether extract, H, Hexane extract, C, Chloroform extract, E, Ethyl acetate extract, M, Methanol extract, -, absent, +, low concentration; ++, high concentration; +++, very high concentration.

According to Ayurveda, it is pungent, heating, laxative, stomachic, carminative and useful in treatment of bronchitis, heart disease, piles, itching abdominal pains, ascites, dyspepsia, dysentery, blood diseases etc

Table-2: Antibacterial and antifungal activity of *Achyranthusaspera* leaf extracts*

Organism	Minimum inhibitory concentration (mg/mL)					Zone of inhibition (mm)					Standard [#]
	P	H	C	E	M	P	H	C	E	M	
<i>Aspergillus niger</i> MTCC 1344	3	2	-	-	-	20	7	-	-	-	10
<i>Bacillus cereus</i> MTCC 430	-	-	-	8	12	-	-	-	34	32	27
<i>Candida albicans</i> MTCC 183	10	4	-	-	-	20	2	-	-	-	6
<i>Candida tropicalis</i> MTCC 184	8	8	7	-	-	14	9	3	-	-	12
<i>Escherichia coli</i> MTCC 1687	3	3	2	-	10	5	12	13	-	14	9
<i>Klebsiella pneumoniae</i> MTCC 109	-	-	-	-	-	-	-	-	-	-	n.t.
<i>Micrococcus luteus</i> MTCC 1541	4	-	-	2	2	14	-	-	8	6	12
<i>Micrococcus roseus</i> MTCC 2522	-	-	-	-	10	-	-	-	-	17	20
<i>Proteus mirabilis</i> MTCC 1429	-	-	-	-	22	-	-	-	-	3	10
<i>Proteus vulgaris</i> MTCC 1771	-	12	-	-	12	-	6	-	-	10	11
<i>Pseudomonas aeruginosa</i> MTCC 1688	-	-	-	1	1	-	-	-	14	12	14
<i>Staphylococcus aureus</i> MTCC 737	-	-	-	-	2	-	-	-	-	12	25

* Values (zone of inhibition in mm) are the mean of three replicates.

[#] Clidamycin (2mg/ml) for *Staph. aureus*; gentamycin (2mg/ml) for *Ps. aeruginosa*, *Pr. vulgaris* and *Pr. mirabilis*; tetracyclin (3mg/ml) for *B. cereus* and *E. coli*; clotrimazole (5mg/ml) for *C. albicans* and *C. tropicalis*; Nystatin (10mg/ml) for *A. niger*; Chloramphenicol (100µg/ml) for *M. luteus* and *M. roseus*. P, petroleum ether extract; H, hexane extract; C, chloroform extract; E, ethyl acetate extract; M, methanol extract (all tested at 25mg/ml); - no inhibition; n.t. - not tested.

Conclusions: *Achyranthus aspera* leaf extracts were active against all the pathogenic microorganisms tested except *Klebsiella pneumoniae*. The methanol, petroleum ether and hexane extracts were found to inhibit the growth of eight, five and five of the twelve used microorganisms, respectively. On the basis of the determined MICs, the effects of methanol extract against *Bacillus cereus* and *Pseudomonas aeruginosa* and of petroleum ether and hexane extracts against *Aspergillus niger*, *Candida albicans* and *Escherichia coli* were the most interesting.

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