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Antimicrobial activity of *Achyranthes aspera* and *Aerva lanata* leaf and callus extracts

S.H.K.R. PRASAD[1]*, N.L. SWAPNA[2],K. ANTHONAMMA[4], MADANPRASAD[3] and D. RAJASEKHAR[4]

[1]SIMS College of Pharmacy, Guntur - 522 002 (India).
[2]Hindu College of Pharmacy, Guntur - 522 002 (India).
[3]Department of Biotechnology, R.P.S.College,Magadh University, Bodh Gaya - 824 234 (India).
[4]St. Ann's College for Women, Gorantla, Guntur - 522 035 (India).

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ABSTRACT

The *in vitro* antimicrobial activity of *Achyranthes aspera* and *Aerva lanata* leaf and callus extracts were studied against selected pathogenic fungi and bacteria, following broth dilution assay. Leaves and calli were extracted using absolute alcohol, benzene, chloroform, methanol and petroleum ether. Among the five solvents used, leaf and callus extracted in chloroform of both the plants were found to be more effective against pathogenic bacteria and fungi, where the minimum inhibitory concentration (MIC) ranged between 0.25 to 6 mg/ml. Absolute alcohol extracts showed MIC of 0.25 to 4 mg/ml for bacteria, whereas for fungi it ranged from 0.25 to 100 mg/ml. Extracts of benzene and petroleum ether were ineffective in inhibiting the bacterial and fungal growth or showed poor inhibition. Methanol extract showed MIC of 0.25 to 100 mg/ml against bacterial pathogens and 0.5 to 100 mg/ml against fungal pathogens. The antimicrobial activities of these two indigenous medicinal plants were discussed in the present paper.

Key words: *Achyranthes aspera*, *Aerva lanata*, leaf, callus, extract, antimicrobial assay.

INTRODUCTION

Use of plants as a source of medicine has been inherited and is an important component of the health care system. The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant pathogenic bacteria and fungi. A special feature of higher angiosperm plants is their capacity to produce a large number of organic chemicals of high structural diversity. The so-called secondary metabolites (Evans et al., 1986), which are divided into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial (Purohit & Mathur,1999). The antimicrobial activity of Amaranthaceae members were well documented in the literature. The plants of this family contribute to food products. Some are noxious weeds. A few plants are grown as ornamentals in the gardens. *Amaranthus tricolor* (Sharma & Behari, 1991), *Achyranthus aspera* (Misra and Singh, 1992), *Gamphrena martiana* and *G. boliviana* (*Pamilio* and Buschi, 1992), *Celosia argentea* (Shah & Patel, 1993). *Alternanthera basilian* (Lagrota and Wigg, 1994), P*upalia lappacea* (Muralidhar Rao, 1995), *A. caudatus,* (Last & Llewellyn, 1997), *Achyranthes bidentata* (Sangameswaran and Balakrishnan, 2003).The leaf and callus extracts of the herbaceous plant,

Achyranthes aspera (Amaranthaceae), is used for treatment of reproduction related disorders, dentalproblems, diarrhea, bonefractures, cuts and boils(Aminuddin and Khan,1992).It is also used as Antispasmodic, Astringent, Diuretic, treatment of dropsy, rheumatism, stomach problems, cholera, skin diseases and rabies (Lassak. E. V. and McCarthy. T, 1982**;** Manandhar.N.P, 2002).*Aerva lanata* belongs to the same family, a woody, prostrate or succulent, perennial herb grown as weed in crop fields. It is used as diuretic and demulcent, anthelmintic, treating sore-throat, to cure diarrhea, very effective in the treatment of urethral discharges and Gonorrhoea . Leaves and flower extracts relieve the pains in the lower part of the back. The root extract used in a snake-bite treatment and leaf-sap is used for eye-complaints (A B Aluka, 2008).The principle aim of the present work was to study the antimicrobial activity of *Achyranthes aspera* and *Aerva lanata* leaf and callus extracts in different solvents like, absolute alcohol, benzene, chloroform, methanol and petroleum ether against both human and plant pathogenic bacteria including *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas solanacearum*, *Xanthomonas axonopodis* pv. *malvacearum, Xanthomonas vesicatoria* and fungi like *Aspergillus ochreaceous*, *Aspergillus flavipes*, *Fusarium verticilloides*and *Penicillium* sp.

EXPERIMENTAL

Plant material

The fresh matured leaves of the *Achyranthes aspera* and *Aerva lanata* were collected randomly during the month of January-February, from the outskirts of Gorantla,Guntur, Andhra Pradesh, India. The voucher specimen was deposited at Department of Pharmacognosy, Hindu College, Guntur for future reference.

Callus culture

Leaves from axenic plants were cut aseptically and placed on Murashige and Skoog's medium (Murashige and Skoog, 1962) containing 30 g/l sucrose and supplemented with 3.0 mg/l BAP (Benzyl Amino purine) + 1.0 mg/ml NAA (α-Naphthalene Acetic Acid) was used as standard medium for *Achyranthes aspera* and *Aerva lanata*. Both are cultured at 22 ± 2°C with 16 h light and 8 h darkness. After 3 weeks, calli were subcultured on MS Basal medium and harvested after 4 weeks.

Preparation of extracts

Fresh leaf material were washed thoroughly under running tap water, shade dried and used for extraction. 4 week-old-calli, derived from the leaf were collected and dried in an oven at 50±1°C for 60-72 h. Both dried leaf and calli were homogenized to a fine powder and stored in airtight bottles. 25 g of leaf/calli powder were extracted with 150 ml of solvent (absolute alcohol, benzene, chloroform, and methanol and petroleum ether) for 24 h by using Soxhlet apparatus. The extract was dried in a flash evaporator for 30 min and the left over powder was considered 100%. Different concentrations; 0.25, 0.5, 1, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 80 and 100 mg/ml were prepared by redissolving the extracted powder in the same solvent which was used in the extraction

Test microorganisms

Selected pathogenic bacteria; *Bacillus subtilis, Escherichia coli, Pseudomonas solanacearum, Xanthomonas axonopodis* pv. *malvacearum, Xanthomonas vesicatoria* and fungi like *Aspergillus ochreaceous, Aspergillus flavipes, Fusarium verticilloides* and *Penicillium* sp. were obtained from culture collection of the Department of Pharmaceutical microbiology and Biotechnology, Hindu college of pharmacy, Guntur, A.P, India. All the test bacterial species were maintained on nutrient agar media. 36 h old bacterial culture were inoculated into nutrient broth and incubated at 35±2°C on a rotary shaker at 100 rpm. After 36 h incubation, the bacterial suspension was centrifuged at 10,000 rpm for 15 min. The pellet was resuspended in sterile distilled water and the concentration was adjusted to 1 x 108 cfu/ml using UV-visible spectrophotometer by reading the OD of the solution to 0.45 (A610 nm) and used for further studies. Fungal colonies were harvested from 9 - 10 day old cultures, which were maintained on Potato dextrose agar. The spores were suspended in sterile distilled water and the spore suspensions were adjusted to 1 x 108 spores/ml.

Antimicrobial assay

Antimicrobial assay was performed in microtiter plates, based on broth microdilution assay,

which is an automated colorimetric method, uses the absorbance (optical density) of cultures in a microtiter plate (Ali and Reddy, 2000). Each well of microtiter plate was filled with 200 µl of nutrient broth/potato dextrose broth, 1 µl of test organism and 15 ìl different concentrations leaf/callus extracts. For bacteria and fungi the microtiter plates were incubated at 35±2°C for 24 h. After the incubation period the plates were read at 465 nm. Minimum inhibitory concentration (MIC), which was determined as the lowest concentration of plant extracts inhibiting the growth of the organism, was determined based on the readings.

RESULTS AND CONCLUSION

The ethnobotanical screening tests of leaf and calli extracts of *Achyranthes aspera* and *Aerva lanata* in different solvents against both human and plant pathogenic bacteria and fungi using microdilution technique are depicted in Tables 1 & 2. The extracts are found to be more effective against bacteria rather than fungi. Both benzene and petroleum ether extracts were found to be ineffective or showed poor inhibition of bacterial and fungal growth. The leaf extract of *Achyranthes aspera* in Absolute alcohol showed MIC of 2.0 mg/ml against all tested bacteria except *X. vesicatoria* (4.0 mg/ml). Chloroform leaf extract showed MIC of 4.0 mg/ml against *B. subtilis*, *E. coli* and *X. vesicatoria*, whereas MIC of 1.0 and 2.0 mg/ml were found against *P. solanacearum* and *X. axonopodis* pv. *malvacearum*, respectively (Table 1). The MIC of 1.0 mg/ml was found against *E. coli*, *P. solanacearum* and *X. axonopodis* pv. *malvacearum* when absolute alcohol extract of *Achyranthes*

Table 1: Minimum inhibitory concentration (MIC) of *Achyranthes aspera* and *Aerva lanata* for antibacterial activity

MIC (mg/ml) Source	Solvents	*B. subtilis*	*E. coli*	*P. solanacearum*	*X. axonopodis*	*X. vesicatoria*
Achyranthes aspera	Absolute alcohol	2.00	2.00	2.00	2.00	4.00
	Benzene	-	-	*	-	-
Leaf extract	Chloroform	4.00	4.00	1.00	2.00	4.00
	Methanol	-	*	-	-	-
	Petroleumether	-	-	-	-	*
Callus extract	Absolute alcohol	2.00	1.00	1.00	1.00	2.00
	Benzene	*	20.00	80.00	-	-
	Chloroform	2.00	4.00	6.00	4.00	4.00
	Methanol	1.00	2.00	1.00	1.00	2.00
	Petroleum ether	-	-	*	*	*
Aerva lanata	Absolute alcohol	2.00	4.00	2.00	2.00	4.00
Leaf extract	Benzene	-	-	*	-	-
	Chloroform	4.00	4.00	2.00	4.00	4.00
	Methanol	10.00	30.00	2.00	4.00	4.00
	Petroleum ether	-	-	*	-	*
Callus extract	Absolute alcohol	4.00	2.00	4.00	4.00	4.00
	Benzene	-	-	-	-	-
	Chloroform	*	-	4.00	4.00	-
	Methanol	*	10.00	2.00	*	10.00
	Petroleum ether	*	*	*	*	6.00
Chloramphenicol		4.00	10.00	6.00	4.00	4.00

Values are the average of at least three determinations.

-: Not active; *: shows poor inhibition of bacterial growth

aspera callus was used. The same concentration was found to be effective against *B. subtilis*, *P. solanacearum* and *X. axonopodis* pv. *malvacearum* when methanol extract of *Achyranthes aspera* callus was used (Table 1). The MIC of 2.0 and 4.0 mg/ml was found against all the tested bacteria when absolute alcohol and chloroform extracts of *Aerva lanata* leaf were used. Whereas absolute alcohol extract of *Aerva lanata* callus showed MIC of 4.0mg/ml against all the test bacteria except for *E. coli* (2.0 mg/ml) (Table 1). *Achyranthes aspera* leaf extract showed MIC of 2.0 and 4.0 mg/ml against all the tested fungi when extracted in absolute alcohol and chloroform, where the MIC ranged between 1.0 to 6.0 mg/ml when the *Achyranthes aspera* callus extracted with absolute alcohol, chloroform and methanol were used (Table 2). *Aerva lanata* leaf extracted with chloroform also showed MIC of 2.0 and 4.0 mg/ml against all the tested fungi. The callus extract of *Aerva lanata* with Chloroform showed MIC of 4 mg/ml against*A. ochreaceous*, *F. verticilloides* and *Penicillium* sp. (Table 2). Petroleum ether and benzene extracts of *Achyranthes aspera* and *Aerva lanata* (leaf and callus) were weakly active to inactive against all the tested bacteria and fungi (Tables 1 and 2). The extracts of higher plants can be very good source of antibiotics (Fridous et al., 1990) against various fungal and bacterial pathogens. Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Darokar *et al.* (1998) detected antibacterial activity of floral parts of 51 plants belong to different 21 families. Comparatively the plants belong to Amaranthaceae, Asteraceae and Rubiaceae showed higher activity.In conclusion chloroformic and absolute alcoholic leaf

Table 2: The Minimum inhibitory concentration (MIC) of *Achyranthes aspera* and *Aerva lanata* for antifungal activity

MIC (mg/ml) Source	Solvents	*A. ochreaceous*	*A. flavipes*	*F. verticilloides*	*Penicillium* sps.
Achyranthes aspera	Absolute alcohol	2.00	4.00	2.00	4.00
	Benzene	-	-	-	10.00
	Chloroform	4.00	4.00	2.00	2.00
Leaf extract	Methanol	*	*	*	2.00
	Petroleum ether	-	-	-	-
Callus extract	Absolute alcohol	1.00	4.00	4.00	2.00
	Benzene	-	*	-	*
	Chloroform	2.00	2.00	4.00	2.00
	Methanol	2.00	4.00	4.00	6.00
	Petroleum ether	-	-	-	-
Aerva lanata	Absolute alcohol	4.00	30.00	-	4.00
	Benzene	-	-	-	*
Leaf extract	Chloroform	2.00	2.00	4.00	4.00
	Methanol	6.00	10.00	2.00	20.00
	Petroleum ether	-	-	*	-
Callus extract	Absolute alcohol	-	10.00	20.00	4.00
	Benzene	-	*	-	*
	Chloroform	4.00	4.00	4.00	6.00
	Methanol	4.00	-	-	*
	Petroleum ether	-	-	-	*
	Fluconazole	4.00	6.00	4.00	2.00

Values are the average of at least three determinations.

-: Not active; *: shows poor inhibition of fungal growth.

and callus extracts of *Achyranthes aspera* and *Aerva lanata inhibited* bacterial and fungal growth. Further work is needed to isolate the active principle from the plant extracts and to carry out pharmaceutical studies.

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