Ethnobotanical Studies and Antimicrobial Activity of Chirchita (*Achyranthes aspera* Linn.) Extracts

Abdullah, Abdul Latif,
S.H. Afaq and Asad Ullah Khan
Department of Ilmul Advia,
Faculty of Unani Medicine,
Aligarh Muslim University,
Aligarh-202002, U.P.,
India.

The ethnobotanical studies were carried out to know about the phonological data and the medicinal uses by the local villagers, tribal peoples in the four districts of western Uttar Pradesh that were surveyed. The antimicrobial activity was tested using Agar well method of aqueous and alcoholic extract of *Chirchita* (*Achyranthes aspera* Linn.) at different concentration gradient. Zone of inhibition was compared with standard drugs (Ampicillin for Gram Positive bacteria and Gentamicin for Gram Negative bacteria). Against Gram Positive bacteria (i.e., *Bacillus cereus* [MTCC 430]) and Gram Negative bacteria (*Klebsiella pneumoniae* [MTCC 109]), *Proteus vulgaris* [MTCC 426] and *Escherichia coli* (clinical isolate) for antimicrobial activity. Chemical screening revealed the presence of alkaloids, glycosides, tannin, oleonolic acid based saponins, amino acids, essential oil, steroids and resins as major compounds and the antimicrobial activity may be attributed to any of these compounds.

Keywords: Ethnobotany, Gram positive bacteria, Gram negative bacteria, Clinical isolate, *Achyranthes aspera* Linn.

Introduction

Chirchita (*Achyranthes aspera* Linn.) Fig. 1, belongs to plant family Amaranthaceae, *Achyranthes* is a small genus of some 15 species of stiff herbs[1]. It is also known as *Latjira; Chirchita; Apang* (Hindi), *Chirchita* (Urdu), Rough chaff tree and Prickly chaff-flower (English). It is a stiff herb found throughout India and Ceylon, Tropical Asia, Africa, Australia and America[2]. The drug is sold in market under the name of *Aghada Bija* as whole fruit[5]. Literature reveals that the root of *Achyranthes aspera* Linn. contains ecdysterone (polyp dine A), two oleonolic acid based mucilages from fruits, ecdysone from roots[3] and two new saponins viz. C and D isolated from fruits[4]. Fruits also contain a large percentage of alkaline ash containing Potash[5, 6]. Lenoleic (49.4%), oleic (22.6%), palmative (18.6%), stearic (4.4%), behemic (1.8%), arachidic (1.6%), myristic (1.2%) and lauric (0.4%) acids are present in seed oil[7], a new aliphatic dihydroxyketone from shoots has also been reported and is characterized as 36, 47 dihydroxyhepentacontan 47 one, 17 Pentatriacontanol is isolated from shoots[8]. Essential oil from the shoots showed antifungal activity against *Aspergillus carneus.* The fruits easily stick into the skin of animals or clothes of human being and get dispersed[9]. In present study the ethnobotanical information of the plant has been collected and antimicrobial activity of alcoholic and aqueous extract of different dilution has been studied.

Material and Methods

Ethnobotanical Study

A survey of four districts (Aligarh, Mathura, Agra and Ghaziabad) of Western Uttar Pradesh was made during

the three seasons and the plants were collected and deposited in the museum of the Department of Ilmul Advia (Voucher No. SC 0114/09). The whole herb was collected in bulk from the locality of Aligarh Muslim University Campus, Aligarh. It was washed thoroughly with distilled water to avoid earthy materials, dried in shade and grounded to coarse powder. Villagers and local healers of differents districts were consulted for its medicinal or economic use. The statements were cross checked and recorded.

The visits in the fields were made at the different intervals to record the phonological data. Dried drug was subjected to physico-chemical standardization i.e. Ash values, successive extraction in different solvents, moisture content and loss on drying. Plants were also analysed for active compounds[10] and the quantitative estimation of alkaloids[11], carbohydrates[12] and phenols[13] were made. Water and Alcoholic extracts were made separately and subjected to antimicrobial studies. As expected antimicrobial activity may be attributed to alkaloids and/or phenols.

Preparation of the Extract

50 gm of crude drug powder was extracted by refluxing it for consequently six hours with 250 ml of 95% alcohol and DDW (Double distilled water) separately for alcoholic and aqueous extracts respectively and then were subjected to dryness in lypholizer. Different concentrations of alcoholic and aqueous extracts were prepared separately from the dried extract using their respective solvent viz. 2.5 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml and further dried and suspended in an inert and sterilized solvent (DDW).

Micro-organisms

Clinical isolates of Gram Positive and Gram Negative bacteria were obtained from the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital and Department of Biotechnology, Interdisciplinary Unit, Aligarh Muslim University, Aligarh. The clinical bacterial species used were *Staphylococcus aureus, Streptococcus mutans, Bacillus cereus* and *Escherichia coli.* Bacterial control strains were procured from the Institute of Microbial Technology, Chandigarh, India. Bacterial control strains used were *Bacillus cereus* [MTCC 430]; *Klebsiella pneuomoniae* [MTCC 109]; *Pseudomonas aeruginosa* [MTCC 424] and *Proteus vulgaris* [MTCC 426]. Bacterial strains were grown on nutrient agar or Mac Conkey agar (M0085 Hi media) or Brain Heart Infusion Agar (BHI-Hi media) plates at 37°C and maintained on nutrient agar slants at 37°C.

Antimicrobial Assay

Antibacterial tests was performed as per National Committee for Clinical Laboratory Standards (NCCLS; now CLSI) 2000. Mueller Hinton Agar No. 2 (M1084 Hi media Labs, India) was used for Antibacterial susceptibility testing. An inoculum size of 10^6 cfu/ml of bacteria was used for inoculating the susceptibility plates. Corkborer was used to make well of equivalent size and 40 µl extract of each dilution were poured into the separate wells for susceptibility testing. Ampicillin discs (SD007 Hi media) 30 mcg were used as standard drug for Gram positive bacteria, while Gentamicin (SD170 Hi media) 30 mcg for Gram negative bacteria, the solvent used for making different dilution was taken as Control on same experimental plate. For different strains different plates were used. All the plates were incubated at 37°C overnight. All experiments were performed in triplicate.

Results and Discussion

Ethnobotanical Information

Achyranthes aspera Linn., is an erect, much branched herb or some time shrub, 60-90 cm tall. Stem is angular, ribbed, swollen at nodes, leaves are oval-obovate, 4.5-7.5 × 2.0-3.5 cm younger one distinctly hairy, older one sub-glabrous. Flowers are terminal or on axillary spikes, usually with incurved apex. Bracts are acuminate, 2-3 mm long, spreading after anthesis, silvery white, Bracteoles 2, appressed to the base of Parianth, spine-scent. Often pink-

purple tinged. Parianth lobes are 5, ovate-lanceolate, scarious margin, 3-nerved, yellowish-green, 4-6 mm long. Stamens are 5, filament cannate at base, pseudo-stamen with long fringed scale. Utricle thick walled, 2.0-2.5 mm long, 1-seeded.

Flowering: July to October, February to April.

Fruiting: November to early December.

Locality: Common plant, abundantly found in waste places and roadsides.

Part used: Whole plant, root and seed.

Uses: The plants are used as strong purgative, resolvent[14, 15], demulcent[16], diuretic[1, 17, 18, 19], digestive, antidote for scorpion sting[20], astringent[21] used in renal dropsy and piles[18, 22]. Its powder is used in cough[9] as expectorant, blood purifier[18, 19, 22] in malarial fever[18] and in toothache[17, 23]. Widely used as anti-inflammatory[24, 16] for rheumatic problems, bladder stone[9], pneumonia[21, 22] and for contraceptive action[25] as pungent and for skin eruption[21, 22, 26]. Root is used in pyorrhoea[20, 22]. It is used as laxative to improve appetite and also in treatment of heart diseases[27].

Physicochemical Assay

Alcoholic and Aqueous extracts have more extractive values as compared to Petroleum ether (60-80°C), Diethyl ether, Chloroform and Benzene. The chemical analysis of the whole herb of *Achyranthes aspera* Linn., showed the presence of alkaloids, amino acids, carbohydrates, glycosides, tannins/phenols, saponins, steroids/terpenes and resins. The total alkaloid percentage is 1.67 in whole plant. The other physicochemical standard features are mentioned in Table 1.

TABLE 1
Physico-chemical Analysis of *Achyranthes aspera* Linn.

S.No.	Physico-chemical Parameters	Percentage
Ash Value		
1.	Total Ash	5.50±0.10
2.	Acid Insoluble ash	5.13±0.05
3.	Water Soluble ash	3.82±0.05
Soluble Part		
1.	Petroleum ether	1.28±0.11
2.	Diethyl ether	1.50±0.05
3.	Chloroform	3.50±0.14
4.	Alcohol	22.00±0.28
5.	Aqueous	33.00±0.28
Successive Extraction		
1.	Petroleum ether	1.26±0.05
2.	Diethyl ether	3.75±0.13
3.	Chloroform	1.75±0.05
4.	Benzene	0.25±0.05
5.	Alcohol	12.16±0.05
6.	Aqueous	14.26±0.53
Moisture content		9.76±0.15
Loss on drying		6.78±0.05
Total Alkaloid content		0.77± 0.05

Antibacterial Activity

All the alcoholic extracts showed a wide range of antibacterial activity against Gram Positive and Gram Negative bacteria. Best results were given at the concentration of 25 mg/ml against *Bacillus cereus* among Gram Positive bacteria while among the Gram Negative bacteria *Proteus vulgaris, Pseudomonas aeruginosa,* and *Klebsiella pneuomoniae*. The antibacterial activity was compared to the Standard drug used and the Control (Tables 2 and 3) and (Figs. 2 and 3). The aqueous extract did not show activity at any concentration.

TABLE 2
Effect of Ethanolic Extract on Gram Positive Bacteria (Zone of Inhibition)

S.No.	Bacteria tested	*Achyranthes aspera* Ethanolic extract at different concentration gradient				Plane Control	Ampicillin
		2.5 mg/ml	6.25 mg/ml	12.5 mg/ml	25 mg/ml	Solvent	30 µgm
1.	*Staphylococcus aureus*	–	–	–	10 mm	Solvent	22 mm
2.	*Streptococcus mutans*	–	–	–	–	Solvent	22 mm
3.	*Bacillus cereus*	11 mm	15 mm	17 mm	22 mm	Solvent	21 mm
4.	*Bacillus cereus* [MTCC 430]	–	9 mm	10 mm	11 mm	Solvent	22 mm

TABLE 3
Effect of Ethanolic Extract on Gram Negative Bacteria (Zone of Inhibition)

S.No.	Bacteria tested	*Achyranthes aspera* Ethanolic extract at different concentration gradient				Plane Control	Gentamicin
		2.5 mg/ml	6.25 mg/ml	12.5 mg/ml	25 mg/ml	Solvent	30 µgm
1.	*Escherichia coli*	–	–	–	10 mm	Solvent	14 mm
2.	*Klebseilla pneuomoniae* [MTCC 109]	–	–	14 mm	22 mm	Solvent	25 mm
3.	*Pseudomonas aeruginosa* [MTCC 424]	–	–	–	–	Solvent	25 mm
4.	*Proteus vulgaris* [MTCC 426]	11 mm	12 mm	14 mm	16 mm	Solvent	14 mm

Conclusion

As a whole the ethanolic extract of entire herb of *Achyranthes aspera* Linn. at 25 mg/ml dilution exhibited remarkable antibacterial activity against clinical and standard strains and thus could be used to derive antimicrobial agents (active constituents) to fight against the number of infections and diseases.

Fig. 1
Plant and parts of *Achyranthes aspera* Linn.

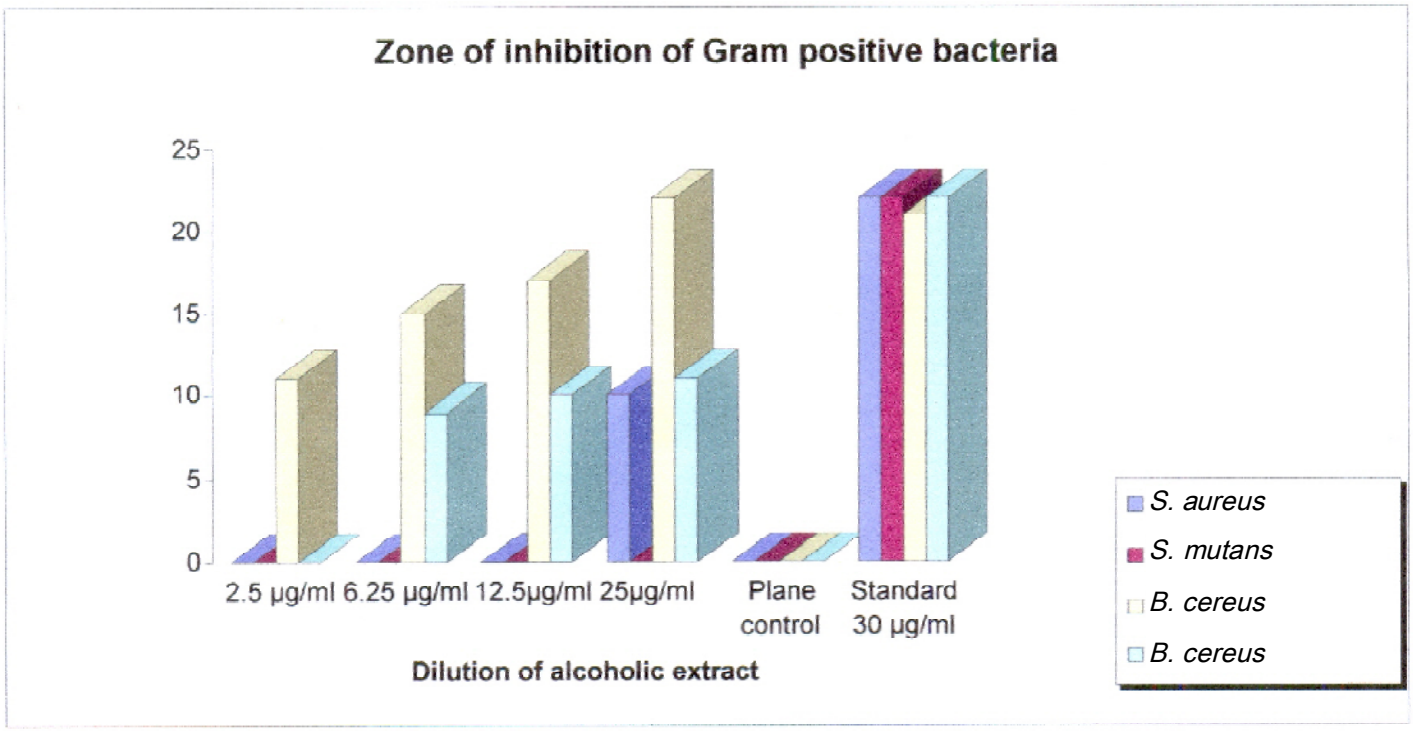

Fig. 2
Effect of different dilutions of ethanolic extract on Gram positive bacteria

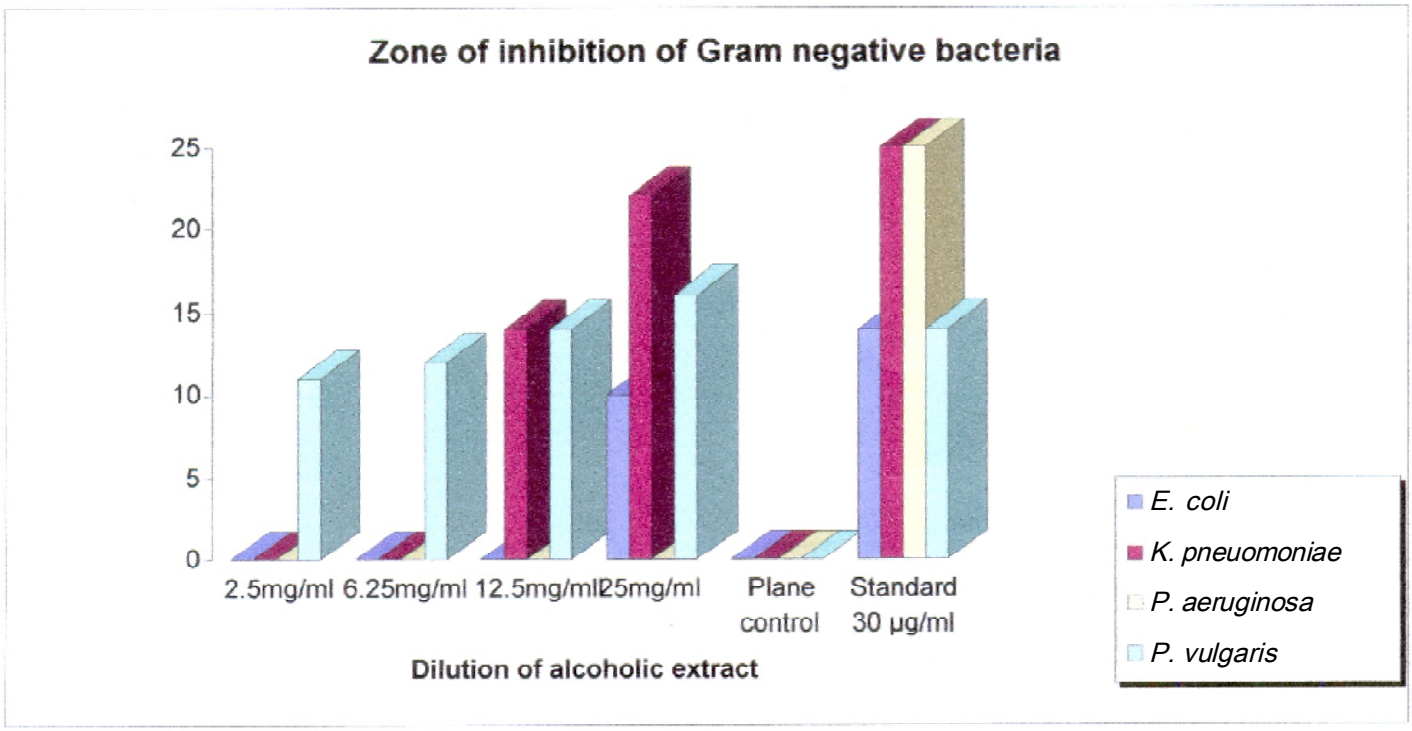

Fig. 3
Effect of different dilutions of ethanolic extract on Gram negative bacteria

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