International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 5(3): May-June 2015

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences

Research Article……!!!

Received: 08-06-2015; Revised: 10-06-2015; Accepted: 11-06-2015

STUDIES ON ANTIMICROBIAL ACTIVITY OF *ACHYRANTHES ASPERA* ALONG WITH PRILIMINARY PHYTOCHEMICAL SCREENING

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Keywords:

Achyranthes aspera, Agar Disc Diffusion Method, Phytochemicals, Antimicrobial Properties

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ABSTRACT

The aim of the present study was to investigate the antibacterial properties and phytochemical evaluation of *Achyranthes aspera*. The organic solvent (Ethanol, Methanol, Hexane) and water extracts from the whole plant of *Achyranthus aspera* (Amaranthaceae) were tested against gram negative bacteria like *Salmonella typhimurium*, *Proteus vulagaris*, *Shigella dysenteriae* and a fungal pathogen *Candida albicans* by Agar disc diffusion method. The results showed prominent antimicrobial activity against the tested microbial pathogens. Of all those, Ethanol extract was found to give a strong antimicrobial effect when compared to the other extracts (Methanol, Hexane and water). Phytochemicals like tannins, flavonoids, alkaloids, steroids and terpenoids are found. The Anthraquinones were found to be absent in plant material observed.

INTRODUCTION

Achyranthus aspera (Amaranthaceae) is a shrub distributed throughout India and other topical regions of the world. The various parts of the plant (leaves, roots, seeds and seed and seed oil) are widely used in variety of ailments in traditional system of medicine such as Ayurveda and Siddha. The aim of present research is, to determine the preliminary phytochemical constituents, antimicrobial activity of various extracts of the leaves and stems of *Achyranthus aspera.*

Achyranthes aspera (common name: prickly chaff flower, Devil's horsewhip, Sanskrit: Apamarga) is a species of plant in the Amaranthaceae family. It is distributed throughout the tropical world. It is one of the 21 leaves used in the Ganesh Patra Pooja done regularly on Ganesh Chaturthi day.

Plants are small, much branched, monoecious perennial sub shrub up to 0.8–1×0.8 m. Rootstockstout, woody. Stems somewhat succulent at first, ribbed, becoming basally woody with age, densely covered in appressed hairs. Leaves opposite, densely clustered toward branch tips 40–50×25–30 mm, spreading to decurved, mostly broadly ovate, ovate-orbicular or elliptic; apex blunt to abruptly sub acute, sometimes very shortly apiculate; base attenuate; lamina some what fleshy, purple-grey, veins often purple, abaxial and adaxial surfaces silky canescent, margins crenulate to crenate. Petioles 5–10mm long, pink,fleshy, velutinous, basal abscis-sion zone present. Inflorescence a terminal erect spike, 150–200mm long; peduncle 15mm long, fleshy, white-villous; spike rachis fleshy, white-vil-lous to purple-villous; flowers bisexual, retrorse, sessile, 180–200 per spike, these spaced initially at 10-mm intervals along rachis, diminishing rapidly to <1-mm intervals toward inflorescence apex. Bract persistent on rachis, ovate to lanceolate 3–3.5×0.5–1mm, strongly retrose, weakly keeled near apex only, pale white, margins entire, apex acute, sometimes with a small, 0.1–0.2-mm-long pale yellow mucro. Bracteoles 2; abscissing with senescent flowers; broadly ovate, 0.2–1mm long, hyaline, lustrous, pale caramel; margins entire; strongly keeled, keel extending well beyond bract as a hardened, strongly recurved, falcate spine 4–5mm long. Perianth segments (sepals) 5, lanceolate, central portion pale caramel-brown but distinctly pink-tinged, margins pale yellow or off-white opaque, hyaline; segments sub equal, 4.5–6mm, channelled. Stamens 4, connate at base, the filaments 0.5–1mm, alternating with 4 narrowly spathulate, 0.4×0.6 mm, white-hyaline, petaloid, fimbriate-argined pseudo staminodes; anthers 0.4–0.6mm, yellow, bilocular, dehiscing via longitudinal slits; pollen

yellow. Style 0.6–1mm, pink to pale orange, aris-ing from a fleshy papillate style base 0.8mm diam.; stigma brown, truncate. Utricle 2–2.5mm long, darkbrown, turbinate, chartaceous, surmounted by the dry, somewhat woody, style base. Seed 1.2–1.8×0.9–1.2mm, ovoidto ellipsoid.[1]

Crushed plant is boiled in water and is used in pneumonia. Infusion of the root is a mild astringent in bowel complaints.The plant is used in asthma and cough. It is pungent, antiphlegmatic, antiperiodic, diuretic, purgative and laxative, useful in oedema, dropsy and piles, boils and eruptions of skin etc. The flowering spikes or seeds, ground and made into a paste with water, are used as external application for bites of poisonous snakes and reptiles, used in night blindness and cutaneous diseases[2].The plant is used in dropsy, piles, skin eruptions, colic, as diuretic, astringent and purgative[3-5], as an antidote to snake bite[6], in fractured bones, whooping cough, respiratory troubles, in asthma laxative8and in leucoderma. The leaves are used in wounds, injuries, in intermittent fever, as an anti-asthmatic, for urination, dog bite and in typhoid. The seeds are employed as an emetic, purgative, and cathartic, in gonorrhoea, for insect bite and in hydrophobia,The inflorescence is used in cough and in hydrophobia. Fruit is used in hydrophobia, cough including whooping cough, as an anti-asthmatic. The root is used in whooping cough, cough and hydrophobia, as an antiasthmatic, diuretic, diaphoretic, and antisyphilitic, tonsillitis and Hemorrhage.

Traditional medicines derived from medicinal plants are used by about 60% of the world's population. Though there are various approaches to control diseases and their secondary complications, herbal formulations are preferred due to lesser side effects and low cost. The use of and search for drugs and dietary supplements derived from plants has been increased in recent years. Botanists, Ethno pharmacologists, microbiologists, and chemists are combing the earth for phytochemicals and drugs which could be developed for treatment of highly infectious diseases in a natural way.. While 30 to 50% of current pharmaceuticals are derived from plants, only few of them are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as Terpenoids, Tannins, Alkaloids, Flavonoids, saponins and Anthraquinones which have been found in vitro to have antimicrobial properties.

This work attempts to find out the anti microbial properties of *Achyranthus aspera* , against select list of microbes and extraction, isolation and characterization of compounds that give these properties to these plants.

MATERIALS AND METHODS

Achyranthus aspera plants were collected from various places in and around the areas of Kurnool. Whole Plants of both the species were collected from mature plants and identified by comparing with herbarium specimens. The plants were air- dried and powdered. The dry powder was extracted by reflexed in 100 mL methanol for 24 h, using a Soxhlet apparatus (Khan *et al*., 1988).. The extract was filtered using Whatman filter paper, No. 1. The filtrate was then evaporated using rotatory evaporator and dried at 55°C. Ethanol, methanol, hexane and distilled water extracts are obtained and all the extracts are preserved. Dried extract was stored at 20°C in labeled, sterile capped bottles. Stock cultures of microbes are maintained at a temperature of 4 degrees centigrade, active cultures are prepared by growing in tubes of Muller-Hinton (MHB) / Potato dextrose agar (PDA) for bacteria and Sabouraud dextrose broth (SDB) for fungi.

Microorganisms:

The bacterial colonies were isolated from hospital samples at Kurnool, their pure cultures were maintained in nutrient agar and stored at 4°C.Three gram negative bacterial species were grown, namely *Salmonella typhimurium*, *Proteus vulagaris*, *Shigella dysenteria* and the fungus *Candida albicans*.

Antimicrobial assay:

Sensitivity tests were performed by disc diffusion with standard antibiotics, following Kirby-Bauer method (Bauer *et al*., 1966). The assessment of antimicrobial activity was done based on measurements of the diameter of inhibition zones (NCCLS, 1998) .Of the four extracts ,ethanolic extract has given interesting results and the aqueous extract showed no response.

Phytochemical screening:

Phytochemical testing is done for the methanolic extracts as it has shown the interesting activity. The details of the tests are as follows:

1. Braemer's test for Tannins : To a 2–3 ml of methanolic extract, 10% alcoholic ferric chloride solution was added. (Dark blue or greenish grey coloration of the solution indicate the presence of tannins in the drug).
2. Liebermann-burchardt test for Steroids : To 1 ml of methanolic extract of drug, 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (Dark green coloration of the solution indicate the presence of Steroids)
3. Liebermann-burchardt test for Terpinoids: To 1 ml of methanolic extract of drug, 1 ml of

chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (dark pink or red coloration of the solution indicate the presence of terpenoids).

a) Salkowski Test for Terpenoids: The extract was mixed with 2ml of chloroform and concentrate H2SO4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terprnoids.

4. Dragendroff's reagent test for Alkaloids :A drop of methanolic extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Dragendorff's reagent. (Orange coloration of the spot indicates the presence of alkaloids)
5. Shinoda test for Flavanoids: To 2–3 ml of methanolic extract, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. (Pink red or red coloration of the solution indicate the presence of flavonoids in the drug).
6. Bornträger's test for anthraquinones: About 50 mg of methanolic extract was heated with 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. (Pink or deep red coloration of aqueous layer indicate the presence of anthraquinones).
7. Keller-Kilianii test for Cardiac glycosides: Methanol extract was obtained and the extract reduced to dryness. 50 mg of this was dissolved in 2 ml chloroform. H2SO4 was added to form a layer and the colour at interphase recorded. Brown ring at interphase is characteristic of deoxysugars in cardenolides.
8. Frothing test for saponins : A small amount of extract was shaken with water and observed for the formation of persistent foam.

Antimicrobial disc diffusion assay :

Antibacterial and antifungal activities of the four plant extracts were investigated by the disc diffusion method[7] . The MHA plates, containing an innoculum size of 106 colony-forming units (CFU)/mL of bacteria or 2x105 CFU/mL yeast cells on SDA were spread on the solid plates with a glass rod. Then discs (4.0-mm diam.) impregnated with 50 µL of each extract at a concentration of 100.0mg/mL were placed on the inoculated plates. Similarly, each plate carried a blank disk by adding solvent control alone in the centre, and antibiotic discs (6.0-mm diam.) of (20 µg/ml, Streptomycin sulphate for bacteria) and Nystatin (20 µg/ml, for fungal) were also used as a positive control. All of the plates were incubated at 37°C for 18

hours for bacteria and at 28°C for 48 hours for fungi. The zones of growth inhibition around the discs were measured after 18 hours of in incubation at 37°C for bacteria and 48 hours for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts was determined by measuring the sizes of inhibitory zones on the agar surface around the discs. In this experiment Streptomycin sulphate,Nystatin were used as positive controls.

RESULTS

The Aqueous and Hexane extracts *Achyranthus aspera* plant has shown negligible antimicrobial activity on tested pathogens, of which aquoes extarct inhibited only *Proteus vulgaris(*11.03),the bacterium which is resistant to all other exracts. whereas the Ethanol extract of plant has shown shown maximum inhibition on *Salmonella typhimurium (11.03±1.2).*and minimum on *Shigella dysenteriae(9.12±0.3*) and it has no effeect on *Proteus vulgaris*.Methanol extract of plant has shown maximum inhibition on *Salmonella typhimurium (9.7±0.8) and* minimum on *Candida albicans(8.14±0.8)* and it has no effect on *Proteus vulgaris* . Of all the exracts ethanolic extracts have shown maximum inhibition, so it is used for phytochemical screening of secondary metabolites.

The results of the phytochemical screening to test the presence of tannin, antharaquinone, alkaloid, saponin, phlobatannin, flavonoid, cardiac glycosides,terpenoids and steroids in the extracts from various parts of *Achyranthus aspera* are shown in Table I. The preliminary phytochemical screening study revealed that the leaf of *Achyranthus aspera* contains small amounts of tannins and rest of phytochemicals .The roots of *Achyranthus aspera* contain small amounts of flavonoid, saponins and terpenoids. Root also contains moderate amounts of tanins,cardiac glycosides and alkaloids.It has high quantities of steroids .The flowers contain high amounts of flavonoids saponins,tanins & alkaloids, moderate amounts of terpenoids and small amounts of cardiac glycosides .The stem contains small amounts of saponins, flavonoids,tanins and terpenoids and the stem has no cardiac glycosides,alkaloids and steroids. Antharaquinones were found to be absent in the entire plant.

DISCUSSION

Tremendous research is going on to search new metabolites from this plant. From the stem of *A. aspera* isolation and characterization of pentatriacontane, 6-pentatriacontanone, hexatriacontane and tritriacontane[8] is also known. Presence of coumarin derivatives, carotenoids, triterpenoids, flavone aglycones, emodins, carotenoids, gallic tannins and antherancene glycosides anthocyanins and polyuronoids[9] as well alkoloidal content is

known.[10] The fatty acid composition such as oleic (55.4%), linoleic (25.0%) ,linolenic (12.8%) acids, Palmitic, arachidic, eicosenic, behenic and erucic in traces reported.[11]Two new bisdesmosidic triterpenoid saponins are isolated in addition to known three saponins, from the methanolic extract of the aerial parts of *A. aspera*[12]. Presence of Betain, betalaine and achyranthine,[13-17] flavonoids and alkaloids,[18] oleanolic acid[19-20] is known. Antifungal acivity essential oil extracted from *A. aspera* is well known.[21] Both antibacterial and antifungal activity of petroleum ether, chloroform and methanol extracts of dried leaves have been reported.[22] The antimicrobial,[23-27] antifungal[28-30] and antibacterial potential of this plant have been evaluated by many studies.Various types of Saponines such as oleanolic acid based saponins, ester of saponin A,[31]bisdesmosidic triterpenoid saponins[10], ecdysterone, saponins, sapogenin,cardiac glycosides are also known in this plant .

Treating Gram-negative bacterial infections can be difficult because of several unique features of these bacteria. For example, the unique nature of their cell wall makes them resistant to several classes of antibiotics. Infections have typically been treated with broad-spectrum antibiotics, such as beta-lactams followed by carbapenems. However, even these drugs have become ineffective against some bacteria, leaving researchers to go for natural resources ,which are medicinal plants.New drugs to combat Gram-negative bacterial infections are needed. In addition, researchers are unraveling the molecular mechanisms of drug resistance in Gram-negative bacteria to identify novel strategies to combat these pathogens. This paper helps in formulating natural principles to combat drug resistance of certain gram nagative bacteria.

Table.I. Antimicrobial activity of ***Achyranthus aspera.***

Solvent extracts	**µL**	**Zone of inhibition in mm**			
		Salmonella typhimurium	***Shigella dysenteriae***	***Proteus vulagaris***	***Candida albicans***
Aqueous	50	-N-	-N-	11.03	-N-
Methanol	50	9.7±0.8	8.6	-N-	*8.14±0.8*
Ethanol	50	11.03±1.2	9.6±*0.4*	-N-	9.12±*0.3*
Hexane	50	-N-	-N-	-N-	-N-
Streptomycin sulphate(µg/ml)	20	28±1.2	25±0.9	20±0.6	-
Nystatin(µg/ml)	20	-	-	-	17±0.9

-N- --No activity

Table II. Phytochemical Screening of Secondary Metabolites from *Achyranthus aspera* Methanolic extract

S.NO	Secondary metabolites	Name of the test	Leaf	Stem	Flower	Root
1.	Tannins	Braemer's test	+	+	3+	2+
2.	Flavonoids	Shinoda test	+	+	3+	1+
3.	Anthraquinone	Bornträger's test	--	--	--	--
4.	Saponins	Frothing test	+	+	3+	1+
5.	Cardiac glycosides	Keller-Kilianii test	+	--	1+	2+
6.	Alkaloid	Dragendorff test	+	--	3+	2+
7.	Steroids	Lieberman Burchardt test	+	--	--	3+
8.	Terpenoids	LiebermannBurchardt test	+	+	2+	1+
		Salkowski test	+	+	2+	1+

'+' Present , 2+ moderate, 3+high, '--' Absent

CONCLUSION

The present study revealed that the leaf and stem of the plant *Achyranthes aspera* possess pronounced anitmicrobial activity may be due to presence of polyacetylenes, sesquiterpene lactones, monoterpenes,alkaloids, and flavonoids. All the extracts investigated except Hexane possessed activity against at least one strain of bacteria and/or fungi. Further studies aimed at the isolation and identification of active substances from the ethanolic extracts of *Achyranthus aspera* could also evolve compounds with effective natural medicinal values for the cure of microbial disorders. The plant is said to be a source of many bioactive compounds acting against some human diseases. The present study helps in herbal formulation of *Achyranthus aspera* for its fight against infectious microbes.

ACKNOWLEDGEMENTS

Financial assistance from the University Grants Commission, SERO, Hyderabad to carry out this work is acknowledged. (MRP4819/14/SERO/UGC)

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