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# POTENTIAL ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *ACHYRANTHES ASPERA* L.

Ramesh Londonkar*, Chinnappa Reddy V and Abhay Kumar K

*Department of Biotechnology, Gulbarga University, Gulbarga-585106, Karnataka, India*

## Abstract

Petroleum ether, Chloroform and Methanol extract of dried leaves of *Achyranthes aspera Family: Amaranthaceae* were obtained by infusion and maceration were screened for their antibacterial and antifungal activities. The extracts were tested against 5 different species of human pathogenic bacteria and 17 fungal strains by the agar-solid diffusion method. Most of the extracts were devoid of antifungal and antibacterial activities, except the methanolic ectracts of leaves of *Achyranthes aspera* obtained by infusion, which has showed a strong inhibitory activity against the Gram-positive bacteria *Staphlyococcus aureus* with a minimal inhibitory concentration (MIC) of 5000 µl $ml^{-1}$. The minimal inhibitory concentration values to dermatophyte strains were 2500 µl $ml^{-1}$ against *Trichophyton rubrum* (LM-09, LM-13) and *Microsporum canis*. In conclusion, it appears that *Achyranthes aspera* has non-specific antimicrobial activity.

Keywords: Antibacterial activity, Antifungal activity, *Achyranthes Aspera*. *Trichophyton rubru*, Minimal inhibition concentration

## Introduction

The plant kingdom has been the best source of remedies for curing a verity of disease and pain. This is why medicinal plant has played a key role in the world wide maintenance of health. Current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring leads from Ayurveda the traditional system of medicine in India.

*Achyranthes aspera* L. (Amaranthaceae) is one of the plant used for medicinal purposes. It is an erect, annual herb, distributed in the hilly districts of India(1). The plant is used in indigenous system of medicine as emenagogue, antiarthritic, antifertility, laxative, ecbolic, abentifacient, anti-helminthic, aphrodisiac, antiviral, anti-plasmodic, antihypertensive, anti-coagulant, diuretic and anti-tumor(2, 3). It is also useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal, infection, chronic malaria, impotence, fever, asthma, piles and snake bites(4). The root is astringent, diuretic and antispasmodic(5). It is used in the treatment of dropsy, rheumatism, stomach problems, cholera, skin diseases and rabies (5, 6). The juice extracted from the root of this plant, mixed along with the root extracts of *Urena lobata* and the bark of *Psidium guajava*, are used in the treatment of diarrhoea and dysentery (6). The plant is astringent, digestive, diuretic, laxative, purgative and stomachic. The juice of the plant is used in the treatment of boils, diarrhoea, dysentery, haemorrhoids, rheumatic pains, itches and skin eruptions. The ash from the burnt plant, often mixed with mustard oil and a pinch of salt, and is used as a tooth powder for cleaning teeth. It is believed to relieve pyorrhea and toothache. The leaf is emetic and a decoction is used in the treatment of diarrhoea and dysentery. A paste of the leaves is applied in the treatment of rabies, nervous disorders, hysteria, insect and snake bites.

## Material and Methods

### Collection of plant materials

*Achyranthes aspera* (*Family: Amaranthaceae)* were collected from the Gulbarga university region of Gulbarga GPS point (17°18'44.13"N. 76°52'19.59"E.) during the month of June 2010 for the study. Leafs are separated form the plants, washed with sterile water and dried at room temperature

### Preparation of extracts

Two extracts were obtained by infusion and maceration from 150g of the plant material. The material was weighed chopped and extracted with solvents. The infusion was prepared with 50gm of dried leaves in 2 X 200 ml of increasing polarity solvent (petroleum ether, chloroform and methanol) respective to their temperature and remove solid matter by filtration. After this preliminary step, the same plant material was extracted in boiling distilled water at the same conditions, and the maceration was obtained following the aforementioned process at room temperature ($28^0$C) overnight. The solvents were removed by rotary evaporation. The yields (w/w) of the infusion and the maceration were, respectively, petroleum ether (0.67 and 0.71%), Chloroform (0.38

* Corresponding Author, *Email*: londonkarramesh53@rediffmail.com

and 0.41%) and methanol (l 3.78 and 1.78%) in terms of newly collected plant material (7 8).

### Preliminary phytochemical analysis

The methanolic, Chloroform and petroleum ether extracts of leaves of *Achyranthes aspera* obtained by infusion were analyzed by qualitative method (thin layer chromatography on silica gel/ UV detection at 365 nm) for the presence of alkaloids saponins, carbohydrates, Flavonoids, phenols, tannins, terpenoides and sterols (9,10).

### Antimicrobial Assay

### Microorganism tested

Strains of human pathogen microorganisms used in this study were as follows. Three Gram negative bacterial *Escherichia coli* (LM-209), *Pseudomonas aeruginosa* (LM-B6) and *Klebsiella pneumoniae* (LM-49); two Gram positive bacteria, *Staphylococcus aureus* (ATTCC-6538) and *Staphylococcus epidermidis* (ATCC-12228); 17 yeasts of fungi including 9 potentially pathogenic yeasts, *Candida albicans* (ATCC-76615, ICB -12, FCF-243), *Cryptococcus neoformans* (FFCF- 19), *Cryptococcus guillermondii* (LM-1), *Cryptococcus krusei* (FCF-1161), *Cryptococcus stellatoidea* (LM-19), *Cryptococcus parapsilosis* (CM-1), *Trichosporon inkin* (LM-267), and filamentous fungi, *Trichophyton rubrum* (M-09,LM13), *Trichophyton mentagrophytes* (LM-16), *Microsporum canis* (LM -828), *Aspergillus flavus* (LM-127), *Pencullium sp* (LM -131),, *Geotrichum candidum* (LM-23) and *Fusarium sp* (LM-135). The microorganisms were originally obtained from the National Chemical Laboratory, Pune, India.

### Antimicrobial activity

Antimicrobial activity of the various solvents extracts form leaves of *Achyranthes aspera* obtained by infusion and maceration was determined by agar solid diffusion method (11-14). Bacteria strains were cultured overnight at 37$^0$C in Muller-Hinton agar, the filamentous and leveduriform yeasts in Sabouraud dextrose agar at 25$^0$C overnight and at room temperature for a period of 14 days. The agar-solid diffusion method was used to determine antibacterial and antifungal activities. The extracts, in concentration 500 and 625µg ml-1, were dissolved in dimethylsulfoxide (DMSO) for a final concentration of 4%.

### Agar-solid diffusion method

Suspension of microorganism (1 ml) with an optical density of McFarland 0.5 was prepared in physiological saline solution (0.9%) and it was adjusted to 90% of transmittance (530 nm) in a spectrophotometer. The antimicrobial spectrum of the extracts was determined qualitatively for the bacterial and fungal species in terms of zone sizes around wheels, cut in plates of agar Sabouraud and Muller-Hilton (supplemented as neccessary) surface-inoculate with approximately 106 CFU of various microbial species containing 50µl of the tested material dissolved in DMSO (equivalent to 5 mg of the dried extracts). The agar was melted (50$^0$C) and the microorganism cultures were then added aseptically to the agar medium at 45$^0$C in plates and poured into sterile Petri dishes to give a solid plate. All these experiments were performed in duplicate. The plates were incubated for 24-28 h, at 37$^0$C for bacteria. And 10-14 days, at 30$^0$C for filamentous fungi.

The inhibition zones produced by the plant extracts were compared with the inhibition zones produced by commercial standard antibiotic: chloramphenicol (30 µg) for bacteria and ketoconazole (500µg) for fungi. They were used as positive control and the solvent DMSO as negative control.

The minimal inhibitory concentration (MIC) was applied to the aqueous extract that had proved to be highly effective against microorganisms by the agar-diffusion method. The strains were designated arbitrarily as sensitive or resistant and the zones were measured at the end of the incubation time. An inhibition zone of 10mm or greater was considered to indicate good antibacterial and antifungal activities.

## Results

### Preliminary phytochemical analysis

The results of the above experimental assay on the methanolic, chloroform and petroleum ether extracts of leaves of *Achyranthus aspera* obtained by infusion are shown in Table 1. The methanolic extracts showed the presence of carbohydrates, glycoproteins, sterols, Triterpenes, flavanoids and coumarins. The chloroform and petroleum ether extract have shown the presence of triterpenes, sterols, azulene derivatives and they were negative for alkaloids, flavonids, phenylpropanoid and glycosides.

Table 1. Phytochemical screening of exreact of *Achyranthus aspera*

| Test material | Yeid (%) | Positive tests for | Negative tests for |
|---|---|---|---|
| Methnolic extract | 4.20 | Carbohydrates, glycoproteins, akaloids , sterols, triterpenes, flavonoids | Iridoids and soponins |
| Chloroform extract | 0.67 | Triterpenes , sterol and azulene | Alkaloids,polar flavanoids |
| Petroleum ether | 0.39 | Triterpenes, sterol and azulene | Alkaloids,polar flavanoids |

## Antimicrobial activity of extracts of *Achyranthus aspera* leaves

The Methanolic, Choloroform and Petroleum ether of *Achyranthes aspera* leaves obtained by infusion and maceration in a concentration of 500 µg ml-1 were tested against 22 microorganisms (bacterial and fungal) by means of agar-solid diffusion method. From those extracts, only the methanol obtained by infusion method has variable degrees of antibacterial and antifungal activities against one or more of the tested organisms. The methanolic extracts of leaves of *Achyranthes aspera* obtained by infusion exhibited results against *S. aureus, T. rubrum* (LM-09, LM-13) and for *M. canis*, the above extract has not shown any activity. An inhibition zone of 10 or greater was considered as good antimicrobial activity. The methanolic extract by maceration did not shown any effect against both gram- negative and gram-positive bacteria and fungi. The solvents used as control exerted no effect against the microorganisms in broth medium.

The methanolic extract of leaves of *Achyranthes aspera* obtained by infusion has provided inhibitory activity against *S. aureus* in a concentration of 5000 µg ml-1 with an inhibition zone of 10 mm. The *T. rubrum* (LM-09, LM-13) has showed an activity which is more significant in a concentration of 2500 µg ml-1 with an inhibition zone from 12 to 14 mm, respectively, and *M. canis* at the same concentration with an inhibition zone of 12 mm. (Table-2)

Table 2. Values of inhibiton zone in (mm) by MIC evaluation of methanolic extracts of *Achyranthus aspera*. Obtained by infusion against bacterial and fungal activities

| Microorganisms | Methanolic extract (µgml-1) | | | | | Microorganism growth without antimicrobians | Chloramphenicol ( 30 µg) | Ketoconazol (50 µg) |
|---|---|---|---|---|---|---|---|---|
| | 10000 | 5000 | 2500 | 1250 | 625 | | | |
| *Staphyloccous aureus* | 12 | 10 | 7 | 0 | 0 | + | 20 | 0 |
| *Trichophyton rubrum* | 20 | 17 | 14 | 10 | 7 | + | 0 | 20 |
| *T. rubrum* | 18 | 15 | 12 | 8 | 0 | + | 0 | 20 |
| *Microsporum canis* | 17 | 15 | 12 | 8 | 0 | + | 0 | 20 |

## Discussion and conclusion

Recently the attention has been directed toward extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in convering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (15, 16).

The Phytochemical analysis of methanolic extract (alkaloids, sterols, triterpenes, Flavonoids, carbohydrates) Choloroform and Petroleum ether extract (Triterpenes, Sterol and Azulene derivatives) of *Achyranthes aspera* obtained by infusion revealed no antimicrobial effects. However, the methanolic extract obtained by infusion (carbohydrates, glycoproteins, cinnamic derivatices and leococyanidines) has showed the presence of biologically active compounds correlated to known substance that possess antimicrobial properties (17-18). The study provides strong circumstantial evidence that small protein or peptide present in the plant extract will play an important role in plant's antimicrobial defense system (19). The protein or peptide fractions from leaves of *Achyranthes aspera* were reported (19).

The methanolic extracts of leaves *Achyranthes aspera* has shown different activities against 22 microorganism (bacteria and fungal). The methanolic extracts *Achyranthes aspera* leaves by infusion has shown an activity which is more significant on dermatophyte *T. rubrum* (LM-09,LM-13) and *M. canis*. the methanolic extracts of leaves *Achyranthes aspera*

obtained by infusion has provided of 10 mm diameter (mmd-1) for S.aureus and a zone of inhibition of 14 mm d-1 and 12 mm d-1for T.rubrum (LM09, LM-13) and of 12 mm d-1for *M. canis* (filamentous fungal).

Several investigators have reported that the methanolic extracts of leaves *Achyranthes aspera* has significant antimicrobial activity against the Gram-Psitive (*S. aureus, Bacillus subtilis*), Gram-negative bacterial (*K. pneumoniae, E. coli*) and fungal species (*Aspergillus niger, C.albicans*).

The literature indicates that the antibacterial activity is due to different chemical agents present in the extract, Including essential oils (especially thymol), flavonoids and triterpenoids and other natural phenolic compounds or free hydroxyl groups. These are classified as active antimicrobial compounds (20).

Many plant extracts have been used as a source of medicinal agents to cure urinary tract infections, cervicitis vaginitis, gastrointestinal disorders, respiratory diseases, cutaneous affections, helmintic infections, parasitic protozoan diseases and inflammatory process (21-28).

From the present study we can draw the conclusion that the traditional use of the plant *Achyranthes aspera* for the treatment of infectious diseases is promising, mainly against bacteria and fungi. Purification of the bioactive components from the extracts understands the possible antimicrobial and antifungal activities.

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