Antimicrobial Properties of Achyranthes aspera

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ABSTRACT

Chloroform and methanol root and shoot extracts of A. aspera showed good amount of antibacterial activity against Klebsiella sp. While pet. Ether (60-80°C) root extract showed the activity against B. Subtilis only antifungal activity of roots was found in extracts with pet ether, chloroform and methanol against fusarium sp. only. Methanol and Aqueous shoot extracts were weakly active against Pencillium, Phytophthora and Scleroum sp. Results suggest that extract has significant antibacterial and antifungal activities against tested microorganisms. The present study justified the claimed uses of A. aspera in the traditional system of medicine to treat various infectious diseases.

INTRODUCTION

Micro-organisms and medicinal plants have been used for several decades as rich sources of agriculturally, industrially, biotechnology and medically important idiolites, hence are potential sources of useful products. These commercial products valued in terms of billions of dollars world wide and are large part of biotechnological industry. The discovery and isolation of leading structure of the active antimicrobial metabolite have provide a unique starting point for chemical modification in an attempt to improve their spectrum, pharamakoknetics and human safety. Achyranthes aspera has valuable medicinal properties as its effective in asthma, skin eruptions, piles, boils and snake bite and leaves are used in hydrophobia.

MATERIAL AND METHODS

Preparation of plant extracts.

Shade dried crude power (100g) of each root and shoot of Achyranthes aspera was extracted in soxhlet apparatus separately. Sequential extraction done and the order of solvent used was petroleum ether (60-80°C), chloroform, methanol and distilled water (cold) for about 18 hours with each solvent. The extracts were evaporated to dryness under vacuum using a rotary evaporator below 40°C and stored at 4°C. The residue obtained was used for determination of antimicrobial activities after making different concentration in dimethyl sulfoxide (DMSO, W/V), 100 μl of each extracted sample at different concentrations (50-200 μg/100μl) was used for the estimation of antimicrobial activities by well plate assay method.

Plate assay method

Lawn of each indicatore test bacteria and fungi made with the help of sterile cotton swabs on nutrient and potato dextrose agar
plates. Wells (8 mm) punched on the plate with the help of sterile creak borer. Plates incubated at 37°C and 28 ±2°C for 24 h -72 h respectively, after application of 100 μl of each concentration of plant extracts in the well plates observed for clear zone formation around the well. Activities were expressed in terms of mm diameter of clear zone produced around the well (8 mm) at 37°C for antibacterial and at 28 ± 2°C for antifungal activities after 24 h and 24.72h of incubation, respectively.

RESULTS AND DISCUSSION

Antibacterial activity

Petroleum ether extracts of root of Achyranthes aspera (Plate 1) were antagonistic against Gram positive Bacillus subtilis while chloroform and methanol root extracts showed good amount of antibacterial activity against Gram negative Klebsiella sp. In the range of 11-14 mm and also weak activity against E.Coli and shigella sp. chloroform and methanol shoot extracts were also found to active against Klebsiella (9-16mm). Aqueous extracts of both root and shoot did not show any antibacterial effect (Table 1).

Different concentrations (50-200 μg / 100 μl) of Petroleum ether, chloroform and methanolic extracts of A. aspera showed antifungal effect (Table 2 & plate 2) against Fusarium species (9-13mm) only. While pet ether and methanol root extracts showed antifungal effect against pythium and Alternaria (plate 3) species. Only methanolic shoot extract of A. aspera was found to show antifungal effect against Fusarium and Heterobardion sp. While aqueous shoot extract was weakly positive against penicillium. Photophthora and Sclerotium, Pet. Ether shoot extract also showed weak inhibiting effect of phytophthora sp only.

Statistically (Table 1-3) all the extracts of both plants (root and shoot both) and all the concentrations tested were significantly different from each other. Interactions between extracts and concentrations were significant. Statistically chloroform and methanol extracts of root of A. aspera showed, best antifungal activity against Fusarium (13.33 mm) and chloroform shoot extract showed best antibacterial activity against Klebsiella sp.

The variation in antagonistic activity is due to the difference in the chemical nature of cell walls cell membranes of each bacteria. The effectiveness of A. aspera to control infections in skin eruptions, piles and boils etc, is due to presence of unknown antimicrobial secondary metabolites. The information regarding antimicrobial and pharmacological activity about this plant is very scanty. Only few preliminary investigations showed that shoots of A. aspera possessed essential oil and new long chain alcohol and their essential oil exhibited antifungal activity against aspergillus corneus. Raman et al., 7, have shown that antibacterial effect of alcoholic extracts against B. subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Shigella dysentriae is due to presence of alkaloids AM-I and AM- II. Bisht et al5 have also shown the antibacterial effect of alcohol root extract of achyranthes bidentata against E.Coli, S. aureus and Pseudomonas sp. But have not purified and identified the effective component. In our studies antibacterial and antifungal effect of different extract may also be due to presence of alkaloids, long chain of alcohol or essential oil or may be due to some another unidentified bioactive secondary metabolites with antagonistic activities. But the plant
still requires detailed investigation about the characterization of these secondary metabolites. Present finding support the applicability of A. aspera in traditional system for it’s claimed users in skin eruptions boils, snake bite and asthma etc. Further work is necessary to isolate and purification of compound in root and shoot extracts which will allow the scientific communities to recommend the utilization as an accessible alternative to synthetic antibiotics.

Table 1.
Antibacterial activity of root & shoot extract of Achyranthes Aspera by well plate method.

| Plant  | Extract | Indicator Test bacteria | Antibacterial activity (μg/100μl) | Marginal mean |
|--------|---------|-------------------------|-----------------------------------|--------------|
|        |         |                         | 50  | 100  | 150  | 200  |               |
| A.aspera |         |                          |     |      |      |      |               |
| Root    | Pet ether | Bacillus                | 9.0 | 9.0  | 10.33| 10.00| 10.00         |
|         | Chloroform | Klebsiella              | 11.67| 12.67| 10.50| 12.67| 12.50         |
| Shoot   | Methanol  | Klebsiella              | 12.33| 14.33| 13.33| 14.00| 13.50         |
|         | Chloroform | Klebsiella              | 9.33 | 11.33| 13.67| 16.33| 12.66         |
|         | Methanol  | Klebsiella              | 10.67| 12.67| 14.67| 14.67| 13.08         |
|         | Marginal mean |               | 10.60| 12.00| 12.93| 13.86|               |

CD=0.05; Conc. O.16; Indicator test bacteria 0.18; Conc. X Indicator test bacteria 0.26
Antibacterial activity expressed in terms of mm, diameter of clear zone produced around the well (8 mm) by 100 ml of plant extract at 37oC for 24 h.

Table 2.
Antibacterial activity of root & shoot extract of Achyranthes Aspera by well plate method.

| Plant  | Extract  | Indicator Test bacteria | Antibacterial activity (μg/100μl) | Marginal mean |
|--------|----------|-------------------------|-----------------------------------|--------------|
|        |          |                         | 50  | 100  | 150  | 200  |               |
| A.aspera |         |                          |     |      |      |      |               |
| Root    | Pet ether | Fusarium sp             | 9.66| 11.33| 11.67| 12.00| 11.16         |

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| Shoot | Chloroform | Methanol | Chloroform | Methanol | Methanol | Marginal mean |
|-------|------------|----------|------------|----------|----------|---------------|
|       | Fusarium sp | Alternaria sp | Fusarium | Fusarium | Heterobaridon sp. |  |
|       | 9.00 | 12.00 | 11.60 | 12.00 | 11.67 | 11.00 |
|       | 9.67 | 12.33 | 12.00 | 12.00 | 11.67 | 9.67 |
|       | 10.33 | 12.33 | 13.00 | 13.00 | 12.00 | 12.33 |
|       | 13.33 | 13.33 | 13.33 | 12.00 | 11.55 | 13.00 |
|       | 16.58 | 12.41 | 12.50 | 12.17 | 11.75 | 12.78 |

**Effect**

**CD0.05**

- Concentration = 0.19
- Indicator test bacteria = 0.23
- Conc. X Indicator test bacteria = 0.46

Antifungal activity expressed in terms of mm, diameter of clear zone produced around the well (8mm) by 100μl of plant extract at 28 ± 2°C for 24-72 h.
Antifungal activity of chloroform root extracts (50-200 ug / 100ul) against Fusarium sp.

Antifungal activity of methanol root extracts (50-200 ug / 100ul) against Alternaria sp.

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