

## SHORT COMMUNICATION

### Evaluation of antimicrobial activity of chemical constituents of *Achyranthes aspera* L. roots against human pathogens

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Received 6 February 2014; Accepted 1 August 2014

Antimicrobial activity of roots of *Achyranthes aspera* L. (Family-Amaranthaceae) was studied against seven pathogenic bacterial and three fungal strains by agar well diffusion method. Antimicrobial activity was recorded for hexane, chloroform, methanol, ethanol and aqueous extracts. Alcohol (ethanol and methanol) extracts exhibited higher degree of antimicrobial activity compared to aqueous, chloroform and hexane extracts. *Klebsiella pneumoniae* turned out to be the most susceptible bacterium to the crude chemical constituents of roots using the standard drugs, Tetracycline and Clotrimazole. Minimum inhibition concentration values of chloroform, methanol, ethanol and aqueous extracts were determined by the agar dilution method ranged between 62.5 and 1000 µg. The study suggests that the root extracts possess bioactive compounds with antimicrobial activity against the tested bacteria and fungi revealing a significant scope to develop a novel broad spectrum antimicrobial drug formulation from *A. aspera*.

**Keywords:** *Achyranthes aspera*, Alcohol extract, Antimicrobial activity, MIC, Human pathogenic bacteria.

**IPC code; Int. cl. (2014.01)**–A61K 36/00, A61P 31/04

#### Introduction

*Achyranthes aspera* L. (Telugu-Uttareni) of family Amaranthaceae is an annual erect winter herb found common as a weed throughout India, tropical Asia and other parts of world. It is a popular folk remedy in traditional system of medicine and it has been used as diuretic in the treatment of dropsy in Ayurvedic medicine<sup>1</sup>. It is also useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal infection, chronic malaria, impotence, fever, asthma, piles and snake bites<sup>2</sup>. Roots are reported to have application in infantile diarrhoea and cold<sup>3</sup> and used as astringent to wounds, in abdominal tumor and stomach pain<sup>4</sup>. To terminate pregnancy, the decoction of the fresh roots

is administered after menstruation<sup>5</sup>. The roots of *A. aspera* are reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids<sup>5,6</sup>. Besides the species has been reported as a potent antibacterial agent of its leaves, stem, roots, flowers, seeds and whole plant<sup>7-19</sup>. In order to demonstrate its efficacy, tests were conducted against human pathogenic bacteria and fungi.

#### Materials and Methods

##### Chemicals, media and antibiotics

The organic solvents such as hexane, chloroform, methanol, ethanol and Dimethyl sulphoxide (DMSO) were obtained from RANKEM Company, India. Nutrient broth, Nutrient agar and Sabouraud dextrose agar were obtained from Hi-media Laboratories Pvt. Ltd., Mumbai, India. The antibiotic drug Ciprofloxacin was obtained from Axiom Laboratories Ltd., India.

##### Root collection

The roots of *A. aspera* L. were collected from Sudikonda forest, East Godavari district, Andhra Pradesh. The specimen was authenticated by Prof. Vatsavaya S. Raju, Plant Systematics Lab, Kakatiya University, Warangal and voucher specimen (M. Krishna Rao - 2248) was deposited in the Herbarium of Botany Department (BDH), Andhra University, Visakhapatnam.

##### Root extract

The dugout roots were cleaned and dried in shade (25-28 °C) for a month. The dried roots were ground using a mechanical grinder. Sequential extraction of it was carried out using the solvents hexane, chloroform, methanol and finally ethanol. The filtrates were concentrated by removing the solvents under reduced pressure at 40 °C using a rotary evaporator. The concentrated crude extracts were labelled and stored at 4 °C<sup>20</sup>.

Simultaneously, the aqueous extract of the root was prepared by adding boiled water to the powder in a beaker on water bath with occasional stirring for 4 h. The aqueous extract was then filtered and reduced under pressure. At the time of testing known quantity of crude extract (100 mg/mL) was dissolved in DMSO.

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### Microbial strains and growth conditions

Seven bacterial strains namely *Bacillus subtilis* (MTCC 2763), *Escherichia coli* (MTCC 2960), *Klebsiella pneumoniae* (MTCC 4032), *Pseudomonas aeruginosa* (MTCC 6642), *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 7443), *Streptomyces pneumoniae* (MTCC 1935) and three fungal strains *Aspergillus niger* (MTCC 4360), *Candida albicans* (MTCC 4748) and *Saccharomyces cerevisiae* (MTCC 4742) were procured from Institute of Microbial Technology, Chandigarh, India. Broth and agar were prepared according to manufacturer's instructions.

Before testing, the bacterial suspension was transferred to nutrient broth and cultured at 37 °C. Inoculates were prepared by adjusting the turbidity of the medium to match the 0.5 Mc Farland standards. The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100 mL of sterile normal saline and then suspension was stored in refrigerator till used.

### Determination of antimicrobial activity

Antibacterial and antifungal activities of root extracts of *A. aspera* were determined using agar well diffusion method<sup>21</sup>. For susceptibility test, 100 µL of inoculum, equivalent to 10 CFU was mixed with 6 mL of nutrient agar (to ensure even distribution of bacteria) and poured immediately into the sterile petri-plates. The petri-plates were left to solidify for 10 minutes. A sterilized 6 mm borer was used to make wells in the centre of the divided areas. About 50 µL of each extract was then pipette into the wells. Petri-plates with bacteria and test extracts was incubated at 37 °C for 16-18 h after which the inhibition zone (IZ) was measured using an antibiotic zone reader scale (HiAntibiotic Zonescale-C).

For the antifungal activity, the same method as for bacteria was adopted of nutrient agar, Sabouraud dextrose

agar was used. The inoculated medium was incubated at 25 °C for two days for the *C. albicans*, *S. cerevisiae* and three days for *A. niger*. About 500 µg of Clotrimazole was dissolved in 1 mL of sterile de-ionized water. About 10 µL of 0.5 mg/mL Clotrimazole (equivalent to 5 µg dose) and 10 µL of DMSO were pipetted into wells. For bacteria, multi-drug antibiotic disc was used (Axiom Laboratories Ltd., India). The experiments were conducted in triplicates and the diameter of the inhibitory zone (IZ) surrounding each well was measured.

Each extract which exhibited IZ was subjected to minimum inhibition concentration (MIC) assay by using serial two-fold dilution<sup>22</sup>. A quantity of 0.6 g of each extract was dissolved in 300 mL sterile nutrient broth which yielded initial concentration of 2000 µg/mL. Subsequently, two-fold serial dilution was made from the stock to obtain 1000, 500, 250, 125, and 62.5 µg/mL concentrations. One mL of standardized inoculum of each test organism was introduced into each extract nutrient broth mixture and then incubated at 37 °C. The lowest concentration inhibiting growth was regarded as the MIC of the extracts. For the fungi, the inoculated medium was incubated at 25 °C for two (*C. albicans*, *S. cerevisiae*) to three (*A. niger*) days.

### Statistical analysis

The experimental data from the triplicates were subjected to one way ANOVA using Minitab version 15. A significant level of 0.05 was used for all statistical analyses.

### Results and Discussion

Of the five different solvent extracts of *A. aspera* tested, the ethanol extract had significant activity against all the tested microorganisms followed by methanol extract. While the aqueous and chloroform extracts evinced moderate activity, and the hexane extract showed least activity (Table 1). The results of the present study are significant at level of P > 0.05.

Table 1— Inhibition zone values of extracts of *Achyranthes aspera* roots

Extracts/Antibiotic	BS	EC	KP	PA	PV	SA	SP	AN	CA	SC
Hexane	12±0.02	12±0.16	16±0.75	13±0.43	12±0.62	14±0.45	12±0.14	-	13±0.34	13±0.08
Chloroform	14±0.45	15±0.46	18±0.14	15±0.25	16±0.23	16±0.12	15±0.46	16±0.08	16±0.70	16±0.25
Methanol	20±0.56	22±0.54	24±0.16	20±0.45	22±0.08	22±0.36	20±0.20	20±0.04	20±0.25	22±0.09
Ethanol	24±0.49	22±0.12	27±0.26	21±0.12	25±0.02	24±0.46	24±0.22	21±0.18	22±0.04	24±0.12
Aqueous	20±0.19	16±0.08	16±0.34	16±0.18	20±0.32	20±0.50	18±0.13	18±0.26	20±0.20	20±0.45
Tetracycline	24±0.25	21±0.48	19±0.16	24±0.60	23±0.13	23±0.43	24±0.42	-	-	-
Clotrimazole	-	-	-	-	-	-	-	18±0.25	21±0.24	24±0.24
DMSO	-	-	-	-	-	-	-	-	-	-

Note: The values represent the Mean±SE of triplicates and significant at level of P > 0.05.

Table 2— Minimum inhibition values of extracts of *Achyranthes aspera* roots

Microorganisms	Chloroform	Methanol	Ethanol	Aqueous
<i>Bacillus subtilis</i>	>1000	250	125	125
<i>Escherichia coli</i>	1000	250	250	500
<i>Klebsiella pneumoniae</i>	500	62.5	62.5	125
<i>Pseudomonas aeruginosa</i>	1000	250	250	500
<i>Proteus vulgaris</i>	500	125	62.5	250
<i>Staphylococcus aureus</i>	500	250	125	250
<i>Streptomyces pneumoniae</i>	>1000	250	125	500
<i>Aspergillus niger</i>	>1000	250	250	500
<i>Candida albicans</i>	500	250	250	500
<i>Saccharomyces cerevisiae</i>	500	125	62.5	125

Ethanol extract exhibited the maximum inhibitory effect against *K. pneumoniae* followed by *P. vulgaris*, *B. subtilis*, *S. aureus*, *S. pneumoniae*, *S. cerevisiae* whereas the methanol extract exhibited maximum inhibitory effect against *K. pneumoniae* followed by *E. coli*, *P. vulgaris*, *S. aureus* and *S. cerevisiae*. The aqueous extract showed maximum IZ against *B. subtilis*, *S. aureus*, *S. cerevisiae* and considerable inhibitory activity against *S. pneumoniae*, *A. niger* and *C. albicans* while chloroform extract had the high IZ against *K. pneumoniae* followed by *P. vulgaris*, *S. aureus*, *A. niger*, *C. albicans*, *S. cerevisiae*. The hexane extract exhibited high IZ against *K. pneumoniae* followed by *S. aureus* and *S. pneumoniae*. While the ethanol extract evinced significant antimicrobial activity with standard antibiotics Tetracycline and Clotrimazole, the methanol extract showed activity more or less on par with standard values.

From the MIC values (Table 2), it is obvious that *K. pneumoniae*, *P. vulgaris* and *S. cerevisiae* are least sensitive to the ethanol extract of concentration 62.5 µg/mL. Alcohol extract of *A. aspera* roots produced consistent level of inhibition of microbial growth and it was significantly high. The results indicated that most of the active constituents responsible for exerting antimicrobial action are expected to be soluble in alcohol. The preliminary phytochemical investigation revealed the presence of tannins, saponins, flavonoids and alkaloids<sup>6</sup>. The combined activities of the secondary metabolites such as alkaloids, steroids, triterpenes and flavonoids produce a synergic effect thus increasing the antimicrobial potency of alcohol extracts of *A. aspera*.

Hexane and chloroform extracts have low antimicrobial effect on the tested organisms.

It was reported that ethanol and chloroform extracts of seeds show mild to moderate activity against *B. subtilis*, *E. coli* and *P. aeruginosa*<sup>17</sup>. Significant activity by ethyl acetate extract of leaves against *K. pneumoniae* and *Salmonella* sp.<sup>7</sup>, alcohol extracts of stem and against *E. coli*<sup>13</sup> and methanol extracts of leaf infusion against *Staphylococcus aureus*, *Trichophyton rubrum* and *Microsporum canis*<sup>11</sup> were also reported. Alcohol extract with triterpenoid saponin was dose dependent against *S. aureus*<sup>16</sup> while the methanol extracts of root showed activity against *B. subtilis*, *Klebsiella* sp. and *Fusarium* species<sup>15</sup>.

## Conclusion

In the present study the root extracts exhibited high degree of antimicrobial activity against all tested bacterial and fungal strains. The study also suggests that root possess bioactive compounds responsible for exerting antimicrobial action against infectious diseases caused by bacteria and fungi in human beings. Conversely, the alcohol extracts of root of *A. aspera* brings to light the scope of developing a novel broad spectrum of antimicrobial drug formulation.

## Acknowledgements

We are grateful to UGC-SERO, Hyderabad for providing FDP teacher fellowship to one of us (MKR) and Prof. Vatsavaya S. Raju, Department of Botany, Kakatiya University, Warangal, for constructive suggestions.

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