



Short Communication

Antibacterial Potential of *Achyranthus aspera* Linn Procured from Himachal Pradesh, Punjab and Haryana, India

Sharma Raj Neeta, Bala Jyoti, Singh Anjuvan and Kaur Prabhjot

Department of Biotechnology, Lovely School of Bio-Sciences, Lovely Professional University, Phagwara, INDIA

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Abstract

Achyranthus aspera, Family: *Amaranthaceae* was procured from Himachal Pradesh, Punjab and Haryana regions in the month of January-2010. Methanol extract of dried whole plants of *Achyranthus aspera* was evaluated against bacterial species viz., *Bacillus cereus*, *Escherchia coli*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The plants procured from Himachal Pradesh showed the best antimicrobial activity followed by Punjab and Haryana region respectively. *Escherchia coli* was maximally inhibited ($24\text{mm}\pm 0.5$) by the plants from Himachal Pradesh region followed by *Klebsiella pneumoniae* ($23\text{mm}\pm 0.7$) and *Bacillus cereus* ($20\text{mm}\pm 0.51$) at the concentration of $2000\mu\text{g}$. The zone of inhibition could not be detected at $500\mu\text{g}$ for *Proteus mirabilis*, *Pseudomonas aeruginosa*, from all the locations investigated and at $1000\mu\text{g}$ also, no inhibition was observed in plant samples collected from Haryana region. All the bacterial species were found susceptible to methanolic extract of whole plants of *Achyranthes aspera* however at high concentrations.

Keywords: Herbal antibiotic, *achyranthes aspera*, weed, phytochemicals.

Introduction

India is a land of rich biodiversity. The plants are potential source of medicines since ancient times. Medicinal plants play an important role in the development of potent therapeutic agents. *Achyranthes aspera* Linn belongs to the family *Amaranthaceae*. It is an annual, stiff erect herb, and found commonly as a weed throughout India and is one of the important medicinal plants having many therapeutical uses as odontalgic, Rheumatism, Bronchitis, skin disease and Rabies¹. Leaf decoction for cardiovascular toxicity has been reported and the methanol crude extract showed high antibacterial activity against *Escherchia coli* and *Bacillus cereus*. Root extract of the plant inhibited the growth of *Bacillus cereus* and *Staphylococcus aureus* bacterial strains. Seeds are rich in protein, cooked and eaten. Leaf extracts were reported to possess thyroid stimulating and anti peroxidative properties. Roots are also used as astringents to wounds, in abdominal tumor and stomach pain². The benzene extract of the stem bark showed abortifacient activity in the rat³. Leaf extracts were reported to possess thyroid stimulating and anti-peroxidative properties⁴. The aqueous and methyl alcohol extracts of the plant also decreased blood glucose levels in normal and alloxan diabetic rabbits⁵. A leaf extract from *Achyranthes aspera* collected in different areas of the United Arab Emirates was tested against Gram-positive bacteria. It showed inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli* and

Aspergillus terreus, respectively. The root extract was less active⁶.

The present investigation was carried out to study the potential of methanolic extract of dried whole plants of *Achyranthes aspera* procured from different locations such as Himachal Pradesh, Punjab and Haryana to screen the best strain of *Achyranthes aspera* in terms of high antimicrobial potential. The extract of dried whole plant was tested against *Bacillus cereus*, *Escherchia coli*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

Material and Methods

Fresh plants of *Achyranthes aspera* Linn. were collected from three locations namely Punjab, Himachal Pradesh and Haryana in the month of January 2010. The plants were identified taxonomically and authenticated in the Department of Biotechnology, Lovely Professional University, Phagwara. Fresh plants were washed thoroughly 3-4 times with running tap water then finally with sterile water followed by shade drying at room temperature for 15-20 days. The dried plant material was made into coarse powder and passed through sieve and then used for crude extraction. Fine powder (20gm) was extracted in 100 ml of methanol at $50-55^{\circ}\text{C}$ for 24 hrs in shaker. The extract was filtered through Whatman filter paper No.1 and then concentrated

by using a rotary evaporator at low temperature (40-50°C) and reduced pressure. The extraction value in methanol was 800 mg. Further, the dried residue was preserved in airtight container and kept at 4-5°C until further use. The extract was then subjected to preliminary qualitative analysis of phytochemicals namely carbohydrate, alkaloid, Terpenoid⁷, Protein (Biuret method), Flavonoids, Tannin⁸ and Saponin⁹. The selected microbes were procured from Christian Medical College, Ludhiana, Punjab. The organisms were *Escherichia coli*, *Bacillus cereus*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Salmonella typhi*. Nutrient agar media was poured in Petri plates and then inoculum was spread with the help of L- spreader¹⁰. A well was made in the plates with sterile borer. The extract was introduced into the well and plates were incubated at 37°C for 24 hrs in B.O.D incubator. The experiment was performed under strict aseptic conditions. The antimicrobial property of the plant was checked against the different strains of bacterial species. Microbial growth was determined by measuring the diameter of zone of inhibition.

Results and Discussion

Achyranthes aspera collected from Himachal Pradesh, Punjab and Haryana region were tested for the presence of carbohydrate, proteins, alkaloids, saponins, terpenoids, tannins and flavonoids and found positive for all the constituents table-1. The same has also already been reported earlier¹¹. Methanol extract of whole plant of *Achyranthes aspera* was selected to test antibacterial activity against eight different microorganisms using agar well diffusion method. The efficiency of methanolic extract was furnished at 500µg, 1000µg and 2000µg. Plants procured from Himachal Pradesh showed maximum inhibition at 2000µg concentration. *Escherichia coli* was inhibited maximally (24 ± 0.5), followed by *Klebsiella pneumoniae* (23 ± 0.7), *Bacillus cereus* (20 ± 0.51), *Acinetobacter baumannii* (19±0.15), *Pseudomonas aeruginosa* (19 ± 0.2), *Salmonella typhi* (18 ± 0.53), *Proteus mirabilis* (18 ± 0.62), *Staphylococcus aureus* (16 ± 0.67) Table-2. Plants collected from Punjab region showed better inhibition of *Klebsiella pneumoniae* (20 ± 0.65) at 2000µg.

Table-1
Phytochemical screening of *Achyranthes aspera* from Himachal Pradesh, Punjab and Haryana region

S.No.	Phytochemicals	Whole Plant Extract in methanol		
		Himachal Pradesh	Punjab	Haryana
1.	Sugars	+	+	+
2.	Proteins	+	+	+
3.	Alkaloids	+	+	+
4.	Tannins	+	+	+
5.	Saponins	+	+	+
6.	Terpenoids	+	+	+
7.	Flavonoids	+	+	+

Table -2
Zone of inhibition of different test organisms against methanol extract of *Achyranthes aspera* procured from three different locations

Test organism	Zone of inhibition								
	Himachal pradesh	Punjab	Haryana	Himachal pradesh	Punjab	Haryana	Himachal pradesh	Punjab	Haryana
	2000µg			1000µg			500µg ml ⁻¹		
<i>Bacillus cereus</i>	20±0.51	17±0.34	15±0.6	12±0.38	7.3±0.13	8.0±0.56	8.5±0.51	0	0
<i>Escherichia coli</i>	24±0.5	19±.55	19±0.18	12.2±0.41	8.5±0.27	9.0±0.4	10.3±0.41	6.2±0.8	7±0.22
<i>Acinetobacter baumannii</i>	19±0.15	17±0.41	15±0.45	9.5±0.8	10±.34	0	7.7±0.34	7.2±0.67	0
<i>Staphylococcus aureus</i>	16±0.67	19±0.7	17±0.8	9.7±0.23	12±0.27	10±0.56	0	9.4±0.3	8.1±0.9
<i>Klebsiella pneumoniae</i>	23±0.7	20±0.65	20±0.56	13.3±0.7	10±0.55	11.4±0.23	9.5±0.27	8.7±0.52	8.0±0.63
<i>Proteus mirabilis</i>	18±0.62	16±0.5	10±0.25	9.2±0.43	8.4±0.45	0	0	0	0
<i>Pseudomonas aeruginosa</i>	19±0.2	16±0.13	14±0.6	12.4±0.27	10.8±0.7	0	0	0	0
<i>Salmonella typhi</i>	18±0.53	12±0.3	16±0.3	11.5±0.56	7.8±0.15	7.2±0.18	8.3±0.92	0	0

Plants from Haryana region were found less effective as compared to Himachal Pradesh and Punjab region Table-2. Zone of inhibition was beyond detection limit at 1000µg in case of *Acinetobacter baumannii*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The results indicate significant impact of environmental conditions and allelopathy on the concentration of secondary metabolites in plants which might be attributed to potentiate antibacterial activity. Antibacterial and antifungal activity has also been tested in leaves of *Achyranthes aspera* collected from Gulbarga and reported that *Achyranthes aspera* has non-specific antimicrobial activity¹² which is in support of present investigation. At 500µg concentration, the sensitivity was reflected only by *Escherichia coli* and *Klebsiella pneumoniae* from all the regions investigated table-2.

Available 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 compounds¹³. Flavonoids have a number of nutritional functions and have been described as biological response modifiers; most act as an anti-oxidant and some have anti-inflammatory properties. Flavonoids have been shown to prevent or slows the development of some cancers¹⁴. Several investigators have reported that the methanolic extracts of leaves *Achyranthes aspera* has significant antimicrobial activity against the Gram-Positive (*S. aureus*, *Bacillus subtilis*), Gram-negative bacterial (*K. pneumoniae*, *E. coli*) and fungal species (*Aspergillus niger*, *C.albicans*)¹².

Conclusion

Although methanolic extract of whole plant is having antibacterial potential yet at higher concentration of constituents. It has also been observed that antimicrobial activity is not specific and dependent on the location of procurement which shows that there is somehow correlation between allelopathic effect and environmental conditions as well on antimicrobial potential of *Achyranthes aspera*. Further study is needed to identify such factors and the strain of *Achyranthes aspera* having high antimicrobial potential and its conservation.

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