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Antimicrobial and Antioxidant Actitivies of the Seeds of *Achyranthes Aspera*

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Research Article

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Abstract

The aim of this study was to investigate the antimicrobial and antioxidant activities of the seeds of *Achyranthes aspera*. The plant part was extracted with methanol to yield the crude extract. The antimicrobial activity test of the methanol extract of the seeds of *Achranthes aspera* was done using disc diffusion method. Standard antibiotic discs of Kanamycin (30 µg/disc) were used as standard. The crude extract was used at a concentration of 500 µg/disc. All the microorganisms were susceptible in various degrees to the extract. The methanol extract was found to be moderately active against *Staphylococus aereus, Bacillus cereus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa*. The zone of inhibition was found from 12 mm to 19 mm. The crude methanol extract was again studied for investigating free radical scavenging potentiality and was subjected to this study with 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). The methanol extract of the bark of the plant exhibited the potential free radical scavenging activity (antioxidant effect) having IC50 value of 54.405 µg/ml. Preliminary phytochemical analysis of the seed extracts revealed that the antibacterial and the radical scavenging activities are mainly due to the presence of the phenolic compounds especially alkaloids. The results obtained suggest that *A. aspera* could be exploited in the infections management of various diseases.

Key words: *Achyranthes aspera*, phenolic compounds and infections management, phytochemical analysis.

INTRODUCTION

Free- radical reactions have been implicated in the pathology of many human diseases/disease conditions like atherosclerosis, ischemic heart disease, aging process, aging process, inflammation, diabetes, immuno- suppression, neurodegenerative disease etc., [1- 3] Radicals and other reactive oxygen species are formed constantly in the human body and are removed by the enzymatic and non-enzymatic antioxidant defense [4]. The disturbance in 'redox homeostasis' occurring when antioxidant defenses are inadequate can damage lipids, proteins, carbohydrates and DNA. Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing tissue injury [5]. In recent years, many studies evidenced that plants containing high content of antioxidant phytochemical can provide protection against various diseases [6]. The free radical scavenging activity of these phytochemical is predominantly determined by their structures [7]. The antioxidant and antimicrobial properties of various plants have been reported by several studies [8-10]. In recent years the popularity of complementary medicine has increased. Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India [11]. The World Health Organization has also recommended the evaluation of the plants effective in conditions where safe modern drugs are lacking [12]. Recently an intensive search for novel types of antioxidants has been carried out from numerous plant materials.

Achyranthes aspera (*A. aspera*) is an important medicinal herb which is found as a weed throughout India. Every parts of the plant had been used in traditional systems of medicines. Seeds, roots and shoots are the most important parts which are used medicinally. Wide numbers of phytochemical constituents have been isolated from the plant which possesses activities like antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, anti-allergic and various other important medicinal properties. The crushed plant is used in pneumonia and infusion of the root is used as mild astringent in bowel complaints. Decoction of powdered seeds

with honey or sugar candy is useful in early stages of diarrhoea and dysentery. For the last few decades or so, extensive research work has been done to prove its biological activities and pharmacology of its extracts. Saponins, oleonolic acid, dihydroxy ketones, alkaloids, long chain compounds and many other chemical constituents have been isolated [13]. (Raji R, 2013)[2]

MATERIAL & METHODS

Plant materials:

The seeds of *Achyranthes aspera* were purchased from the local herbal market, Hyderabad, Andhra Pradesh, India

Processing of plant materials: The seeds were washed and air-dried over a period of 2-3 days, then the dried samples were milled into fine power by pounding manually with a clean and sterile mortar and stored in sterile cellophane bags in a cool dry place till further use.

Extraction of plant materials:

Dried ground seeds of 50grams were extracted in Soxhlet sequentially in 300ml of Chloroform, Petroleum ether, Acetone, methanol and Aqueous. The process was run for 48 hrs after which the sample was concentrated using rotary evaporator and freeze dried to powdered form. The dried extracts were weighed and kept in labeled sterile specimen bottles.

Preliminary phytochemical investigations:

The major secondary metabolites classes such as Tannins, Saponins, Terpenoids, Steroids, Flavonoids, Alkaloids and Glycosides were screened according to the common phytochemical methods [14].

Microbial strains:

The Microbial strains used are *Enterobacter aerogenes; Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC 15380, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella paratyphi; Bacillus cureus; Bacillus subtilis* MTCC 441 and *Staphylococcus aureus* ATCC 25923. The bacterial isolates were cultured on nutrient agar and incubated at 37°C for 24 hrs and the microorganisms were repeatedly sub-cultured in order to obtain pure isolation. Morphological and biochemical reactions were carried to ascertain proper identification. They were inoculated into nutrient agar slants and stored at 4°C. Overnight broth culture of the respective bacteria strains were adjusted to turbidity equivalent to 0.5 McFarland standards. (0.2 ml culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 24 hrs and standardized at 1.5×106 CFU/ml by adjusting the optical density to 0.1at 600nm (PERKIN-ELMER UV-spectrophotometer) [15].

Disc Diffusion Method:

The freeze dried extract was reconstituted with DMSO to obtain a stock solution of, 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. Nutrient agar (Hi Media Laboratories Pvt. Ltd. Mumbai) plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. Discs of 6 mm were punched from Whatmann No.1 filter paper. Up to 10µl of each concentration of the extract were respectively introduced in the discs using sterile automatic pipettes. The discs were allowed to dry at room temperature for 2 hrs and were placed at equidistance in each of the plates using a sterile forceps. The plates were incubated to 37°C for 24hrs. The control antibiotic Kanamycin (30µg) (Hi Media Laboratories Pvt. Ltd. Mumbai) was used. Diameters of the inhibition zones were measured. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract.

Antioxidants assay with 1, 1- diphenyl- 2-picrylhydrazyl (DPPH) Radical:

DPPH is a free radical which when dissolved in ethanol has a blue-violet color. When it reacts with the reducing agent, the solution loses color which depends upon the number of electrons taken up. Hence, the loss of color indicates radical scavenging activity of test material. DPPH was measured [16]. Three ml of 60 µM DPPH in ethanol was added to 1 ml of *A.aspera* seed extract and then incubated at room temperature for 15 minutes. Absorbance was read at 517 nm using a Spectrophotometer (PERKIN-ELMER UV- spectrophotometer). The Antioxidant activity was calculated as inhibition (%) of DPPH radical formation.

Inhibition (%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}}$$

RESULTS

Phytochemical analysis:

Phytochemical analysis of various solvent extracts revealed the presence of alkaloids, tannins, saponins, steroids, glycosides and flavonoids (**Table 1**). In particular, the acetone and methanol extracts showed highly positive effects for the alkaloids but it was negative for chloroform and benzene fraction. Saponin was detected only in the aqueous extract that was negative for the presence of tannins and steroids. Methanol extracts showed only presence of alkaloids.

Antibacterial activity:

The antibacterial activity of different solvent fractions of *A.aspera* extract was evaluated (**Table 2; Fig 1**). The *A.aspera* extract was screened for the activity against *E. coli* (10.3mm), *K. pneumoniae* (11.2mm), *P. vulgaris* (12.1mm), *P. aeruginosa* (13.2mm), *B. cereus* (16.9mm), *B. subtilis* (12.5mm), *S. aureus* (16.8mm) and *S. faecalis* (13.8 mm) by agar

TABLE 1: Phytochemical Screeening Of Extracts Of Achyrnathes Aspera Seeds

Sample	Alakloids	Tannins	Saponins	Steroids	Glycosides	Flavonoids
Chloroform	–	+	–	–	+	–
Benzene	–	+	–	–	–	–
Acetone	++	–	+	–	+	++
Methanol	+++	+	+	–	++	++
Aqueous		–	+++	–		

Phytochemical test: - Nil, + Minimal presence, ++ Higher presence and +++ Maximum presence

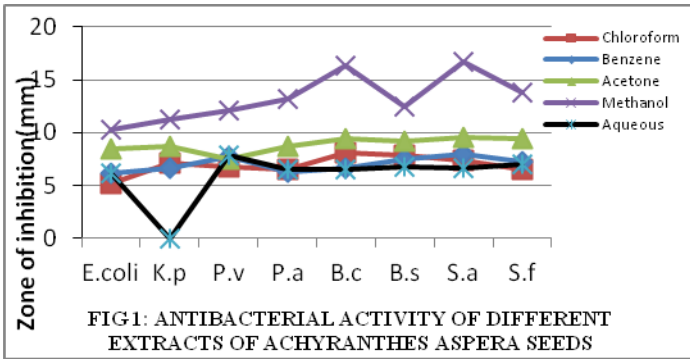

FIG1: ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF ACHYRANTHES ASPERA SEEDS

disc diffusion method. Results were compared with the drug such as Streptomycin and Kanamycin. *A.aspera* extract at the concentration of 1000 µg/ml showed appreciable zone of activity against all the bacterial pathogens tested. Among the various solvent extracts of *A.aspera,* methanol and acetone fractions showed higher zone of inhibition against bacteria which are comparable to that of standard drugs. No appreciable zone of inhibition was observed with other solvent fractions.

Table 2: Antibacterial Activities Of Different Extracts Of Achyrnathes Aspera Seeds

Name of the extract	Concn. of extract (ug)	Zone of the Inhibition							
		Gram Negative bacteria				Gram positive bacteria			
		E.coli	K.p	P.v	P.a	B.c	B.s	S.a	S.f
Chloroform	1000	5.2	7.1	6.8	6.5	8.1	7.8	7.4	6.5
	500	5.1	6	6.5	5.5	-	7.2	7.6	6
	250	-	5	6.1	4.3	-	5.1	4.8	5.1
Benzene	1000	6.2	6.6	7.7	6.3	6.6	7.5	8	7.3
	500	5.1	6	6.2	5.8	5.5	6.1	-	7.1
	250	5.2	-	-	5.2	4.8	-	5.8	-
Acetone	1000	8.5	8.7	7.5	8.7	9.4	9.2	9.5	9.4
	500	6.5	6.5	6.2	6.4	8.6	6.9	7.2	7.1
	250	5.8	7.4	5.4	6.1	6.6	7.4	7.3	5.3
Methanol	1000	10.3	11.2	12.1	13.2	16.9	12.5	16.8	13.8
	500	8.3	10.3	10.8	13.2	15.3	10.4	17.8	11.7
	250	8.4	7.8	8.8	12.5	13.2	14.6	13.7	10.1
Aqueous	1000	6.2	-	7.8	6.5	6.5	6.7	6.6	7.1
	500	5.1	-	6.2	5.8	5.6	5.1	5.2	6.2
	250	-	-	5.3	5.2	5.4	-	-	5.1
Antibiotic									
Kanamycin	30	21	18	15	19	23	21	23	15
Sterptomycin	30	19	18	16	19	21	20	21	16

E.a - Esherichia coli, K.p- Klebsiella pneumonia, P.v -proteus vulgaris; P.a- Pseudomonas aeruginosa;
B.c-Bacillus cereus; B.s-Bacillus subtilis; S.a-Staphylococcus aureus; S.f- Staphylococcus faecalis
Kanamyacin and Sterptomycin-control antibiotics. Each value represents the mean of triplicate analysis.

DISCUSSION

Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use for the past few decades, numbers of plants have been widely used for the treatment of various diseases due to their antioxidant properties [17]. Antioxidants can be defined as compounds of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction and by many other mechanisms and thus prevent disease [18-19]. Results from our phytochemical analysis revealed that ethanol, methanol and acetone fractions showed the presence of antibacterial activities. Chloroform, Hexane, Benzene, Dichloromethane and Petroleum ether fraction did not show remarkable antimicrobial activity. In the present study, initial antibacterial screening of the different extracts from the seeds of *A.aspera*showed varying degrees of antibacterial activity against human pathogenic

Antioxidant Activity:

The Antioxidant Activity reflected by the DPPH radical scavenging assay was clearly observed in various solvent extracts of plant (**Table 3**). Among the various solvent extracts of *Achyranthes aspera*, methanol had radical scavenging activity. Lower EC50 value indicates greater antioxidants activity. Only 50 ug/ml of methanol extract was required to reduce the DPPH radicals by 50% whereas the acetone and aqueous fraction needs 80mg/ml and 120mg/ml. This was significantly similar to the concentration needed for commercial antioxidants BHT. On the other hand, more than 100mg/ml was necessary for other extracts to achieve the same results.

bacteria such as *E. coli*, *B. cereus*, *B. subtilis*, *S. aureus* and *S. faecalis*. The variation in the effectiveness depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism. It has been suggested that the antibacterial activity is mainly due to the presence of essential oil, flavonoids and triterpenoids and other natural polyphenolic compounds or free hydroxyl groups[20]. The antioxidant activity reflected by the DPPH radical scavenging assay was clearly observed in *A.aspera* extracts in dose dependent manner. This suggests that the physicochemical nature of the individual phenolics in the extracts may be important in contributing to the antioxidant activity. The antioxidant property of the plant is well correlated with the concentration of the extracts which showed the presence of active principles in the extract.

TABLE 3: Free Radical Scavenging Activity Of Different Extracts Of Achyranthes Aspera Seeds

Sample	Concentration (ug/ml)	Scavenging activity (%) [a]
Chloroform	10	9.76
	50	17.38
	100	28.54
Benzene	10	3.74
	50	5.92
	100	7.63
Acetone	10	12.52
	50	27.34
	100	57.58
Methanol	10	27.34
	50	49.52
	100	74.02
Aqueous	10	12.39
	50	20.42
	100	34.72
Standard BHT	10	35.25
	50	67.32
	100	92.57

a-values are the mean of three replicates

CONCLUSION

Based on the results described, we may conclude that the ethanol, methanol and acetone extract of *Achyranthes aspera* posses significant free radical scavenging and antibacterial activities. We will conduct further research to isolate the antioxidant constituents of the plant with high activity which will pave the way for the production of bioactive prophylactic agents.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.