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Antibacterial studies, GC/MS Analysis and Antioxidant activity of plant parts of *Achyranthes aspera*

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Abstract
Achyranthes aspera is the herb and one of the medicinal plant in India, belongs to the family Amaranthaceae which is used to treat various infections. *Achyranthes aspera* is called as Nayurivi in Tamil. The plant parts (leaf, stem and root) of *Achyranthes aspera* were collected and extracted with solvents such as ethanol, methanol and acetone. Antibacterial study was carried out by well diffusion method using ATCC pathogens, *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). Acetone extract of the leaf showed maximum inhibition of 25mm against *P. aeruginosa.* Stem extracts showed maximum inhibition of 16mm against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*. Ethanol root extracts of *Achyranthes aspera* showed the maximum inhibition of 17mm against *Pseudomonas aeruginosa* Among the 3 plant parts of *Achyranthes aspera*, acetone extracts of the leaf showed maximum inhibition of 25mm against *Pseudomonas aeruginosa.* The mass chromatogram of *Achyranthes aspera* leaf extract showed 48 different phytochemical compounds. Free radical scavenging activity exhibited by leaf extract of *Achyranthes aspera* using DPPH assay was 50%, where the positive control, ascorbic acid showed 87%.

Keywords: Nayurivi, Amaranthaceae, well diffusion, GC/MS, Antioxidant and ATCC pathogens

Introduction
Achyranthes aspera is a herb which can grows up to 1-2 meters height. In recent years there is a rapid progress in use of plant based health products. The medicinal plants are used for treatment of various diseases because of their safety and effectiveness (Saba Hasan, 2014) [14]. *Achyranthes aspera* is commonly found in tropical and warmer regions. It is found in tropical Asian, African countries, Balochistan, Srilanka, Australia and America (Praveen Kumar, 2014) [13]. There is growing demand for plant based medicines, pharmaceuticals, food supplements and cosmetics (Bhoomika *et al*., 2007) [5].

Plant parts like root, shoot and stem are used for medicinal purpose (Shinde Ganesh *et al*., 2021) [17]. *Achyranthes aspera* is used to treat cough, bronchitis, asthma, hypertension, diabetes, fistula, scrofula, skin rash, nasal infection, renal dropsy, piles and bites. It is also used to control vomiting, heart disease, abdominal pain and itching (Bhoomika *et al*., 2007) [5]. The plant can be used as anti –Parasitic (Zahir *et al*, 2009) [20], Hypoglycemic (Akhtar and Iqbal, 1991), hepatoprotective (Bafna *et al*., 2004) [4], Nephroprotective (Jeya Kumar *et al*., 2009), anticancer (Chakraborthy *et al*., 2009) and anti-inflammatory (Vijayakumar *et al*., 2009). It also possess antiperiodic, astringent, antiarthritic, laxative, antihelminthic, anticoagulant, diuretic and antitumour properties (Ratra *et al*., 1970) [19].

The plant parts are widely used for the treatment of upper respiratory tract infections, pneumonia, rheumatoid arthritis, urinary tract infections and sexually transmitted diseases (Lakshmi Naidu *et al*., 2006) [12]. *Achyranthes aspera* is used to cure oedema, dropsy, boils, piles and skin eruptions. Plant infusion is used to cure pneumonia and root infusion is used to cure bowel complaint. Boiled extract of plant inflorescence is used to treat jaundice. Paste of roots in water is used to treat ophthalmia (Srivastava *et al*., 2011). Fresh piece of the Root is used as tooth brush. *Achyranthes aspera* stem is used for the treatment of toothache. Decotion of powdered leaves with honey or sugar candy is useful to treat diarrhea and dysentery (Saurabh *et al*., 2011) [15].

Oxidation is the normal physiological and metabolic process in the cell. Nearly 5%oxygen gets reduced to the oxygen based free radicals including hydrogen peroxide, superoxide, hydroxyl and nitric oxide radicals. They are said to be Reactive Oxygen Species (ROS). Free radicals formed during metabolism react with nucleic acid, enzymes, proteins and lead to damage (Halliwell, 1996) [8]. Damage induced by ROS include DNA mutation, protein oxidation and lipid peroxidation leads to the development of cancer, diabetes, atherosclerosis inflammation and premature ageing (Finkel *et al*., 2000) [7] Herbs contain secondary metabolites like polyphenols, flavonoids, triterpenoids which have significant antioxidant and antibacterial properties.

Phytochemicals are responsible for medicinal activity of plants and these biochemicals are naturally occurring in plants that have defence mechanism and protect from various diseases (Hasler and Blumberg, 1999) [9]. Wide number of phytochemical constituents have been isolated from *Achyranthes aspera* which possess activities like antiperiods, diuretic, laxative, antiasthmatic, hepatoprotective, antiallergic and other medicinal properties (Saurabh Srivastav *et al*.,) [15].

Methodology

Collection and processing of plant parts of *Achyranthes aspera*

Achyranthes aspera (Leaf, Stem and Root) were collected from Cuddalore and thoroughly washed with water to free from dust particles and shade dried for 10-15 days at room temperature. Then the dried parts of plants were crushed and stored in a sterile container for further use.

Preparation of plant extracts

For solvent extraction 15g of ground plant parts were added in 150ml of different solvents such as methanol, ethanol and acetone separately. The extraction was carried out using Soxhlet Apparatus at 35-40 ºC. The individual extracts were concentrated in Rotary evaporator. The extracts were stored and used for further studies. The stock solution of plant parts extract were prepared in 100mg/ml concentration and was loaded in the well for antimicrobial studies against ATCC pathogens.

ATCC cultures used for Antibacterial studies

ATCC cultures such as *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922) were used for antibacterial studies.

Antibacterial activity of plant parts extract of *Achyranthes aspera*

Antimicrobial activity of plant extracts were determined using well diffusion method. Ethanol, Methanol and Acetone extracts of leaf, stem and root of *Achyranthes aspera* were prepared and were tested against 3 ATCC pathogens. These cultures were inoculated in tryptic soy broth and were adjusted to 0.5 Mac Farland standard and inoculated in Muller Hinton agar plates and were allowed to solidify. The bacterial culture were swabbed over agar surface aseptically. Then plant part extract in different concentration (25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml) were loaded in the well. Then the plates were incubated at 37ºC for 24 hours and observed for zone of inhibition in mm. Control plates containing solvents and a standard broad spectrum antibiotic Ciprofloxacin were are also kept and observed for zone of inhibition.

GC-MS analysis

Rxi-5SiL MS column (fused silica) cross bond with 1, 4-bis (dimethyl silica) phenylene dimethyl polysiloxane was used. Sample elution using 50:1 helium was used. Column temperature 40 ^{0}C for 2 minutes to 300 ^{0}C. Time taken for chromatography per sample is 40 minutes.

Analysis of the phytocomponents in *Achyranthes aspera* leaf using GC- MS technique

GC-MS analysis was carried out in Run VIKA Research Remedies, Chennai. One micro litre of the filtrate was injected into the GC-column. Then the sample get evaporated and carried away by the carrier gas, helium and it got segregated into individual fraction. The sample fraction coming out of the column was let into the mass detector and the mass spectrum of each compound was recorded. The mass spectrum of the unknown compound was compared with the spectrum was accomplished using data base dictionaries.

Identification of component

The database in the WILEY online library has been used for the interpretation on GC-MS. The spectrum of the unknown component was compared with the spectrum of the known component stored in the WILEY online library. Then the molecular formula and molecular weight of component were identified accordingly.

Antioxidant activity

DPPH radical scavenging activity of extract was determined according to the method reported by Blois (1958). An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula. % of inhibition = absorbance of control – absorbance of sample / absorbance of control ×100.

Results and Discussion

Antibacterial activity of *Achyranthes aspera* leaf, stem and root extracts against *Pseudomonas aeruginosa*

Ethanol leaf extract of *Achyranthes aspera* showed the maximum inhibition of 15mm against *Pseudomonas aeruginosa.* Methanol leaf extract showed maximum inhibition of 19mm and acetone extract showed maximum inhibition of 25mm in 100mg/ml concentration of extract. Among the three solvents used, acetone extracts of the leaf showed maximum inhibition of 25mm against *Pseudomonas aeruginosa.*

Ethanol and methanol stem extract of *Achyranthes aspera* showed the maximum inhibition of 16mm against *Pseudomonas aeruginosa.* and acetone extract showed 15mm in 100mg/ml concentration. Among the three solvents used, ethanol and methanol extracts of the stem showed maximum inhibition of 16mm against *Pseudomonas aeruginosa*.

Ethanol root extracts of *Achyranthes aspera* showed the maximum inhibition of 17mm against *Pseudomonas aeruginosa*. Methanol and acetone root extract showed maximum inhibition of 15mm in 100mg/ml concentration. Among the three solvents used, ethanol extract of the root

showed maximum inhibition of 17mm against *Pseudomonas aeruginosa*.

Antibacterial activity of *Achyranthes aspera* leaf stem and root extracts against *Staphylococcus aureus*

Methanol leaf extract showed maximum inhibition of 19mm against *Staphylococcus aureus.* Ethanol leaf extract showed the maximum inhibition of 18mm against *Staphylococcus aureus* and acetone extract showed 16mm in 100mg/ml concentration. Among the three solvents used, methanol extract of the leaf showed maximum inhibition of 19mm against *Staphylococcus aureus*.

Methanol stem extract showed maximum inhibition of 16mm against *Staphylococcus aureus.* Ethanol stem extracts of *Achyranthes aspera* showed the maximum inhibition of 15mm and acetone extract showed 15mm in 100 mg/ml concentration. Among the three solvents used, methanol extract of the stem showed maximum inhibition of 16mm against *Staphylococcus aureus*.

Methanol root extract showed maximum inhibition of 15mm against *Staphylococcus aureus.* Ethanol root extracts of *Achyranthes aspera* showed the maximum inhibition of 14mm and acetone extract showed 13mm in 100mg/ml concentration. Among the three solvents used methanol extract of the root showed maximum inhibition of 15mm against *Staphylococcus aureus*.

Antibacterial activity of *Achyranthes aspera* leaf stem and root extracts against *E. coli*

Ethanol and acetone leaf extracts of *Achyranthes aspera* showed the maximum inhibition of 16mm against *E. coli*. Methanol extract showed maximum inhibition of 15mm in 100mg/ml concentration. Among the three solvents used, ethanol and acetone extract of the leaf showed maximum inhibition of 16mm against *E. coli.*

Methanol stem extracts of *Achyranthes aspera* showed the maximum inhibition of 16 mm against *E. coli.* Ethanol stem extract showed maximum inhibition of 13mm and acetone extract showed 15mm in 100mg/ml concentration. Among the three solvents used, methanol extracts of the stem showed maximum inhibition of 16mm against *E. coli*.

Methanol and acetone root extracts of *Achyranthes aspera* showed the maximum inhibition of 14mm against *E. coli*. Ethanol root extract showed maximum inhibition of 13mm in 100mg/ ml concentration. Among the three solvents used, methanol and acetone extracts of the root showed maximum inhibition of 14mm against *E. coli.*

Among the 3 plant parts of *Achyranthes aspera*, acetone extracts of the leaf showed maximum inhibition of 25mm against *Pseudomonas aeruginosa.*

The broad spectrum antibiotic Ciprofloxacin showed a zone of inhibition of 37mm against *S. aureus*, 35mm against *P. aeruginosa* and 42 mm against *E. coli*. The solvents did not showed any inhibitory activity.

GC-MS analysis for *Achyranthes aspera* leaf sample

The mass chromatogram of the *Achyranthes aspera* leaf extract showed 48 different phytochemical compounds. Major compounds were dimethooxymethyl silane, Cyclotetrasiloxane Octamethyl, Phytyl acetate, 1, 2-Bezenedicarboxylic acid, bis (2-methylpropyl) ester, Phthalic acid isobutyl 2- pentyl ester, Dibutyl phthalate, phthalic acid butyl 3- methyl butyl ester and Bis (2-ethyhexyl) phthalate. In the chromatogram the height of each peak is in proportion to the amount of particular compound present in the sample.

Antioxidant activity of *Achyranthes aspera* leaf extract

The leaf extract of *Achyranthes aspera* showed the 50% scavenging activity found out using DPPH assay, where the positive control ascorbic acid showed 87%.

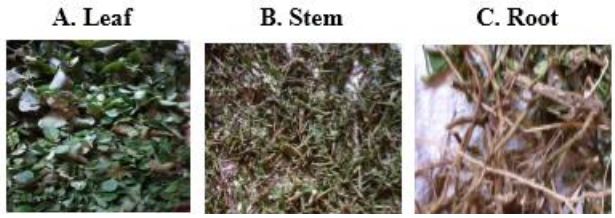

Fig 1: *Achyranthes aspera* plant parts

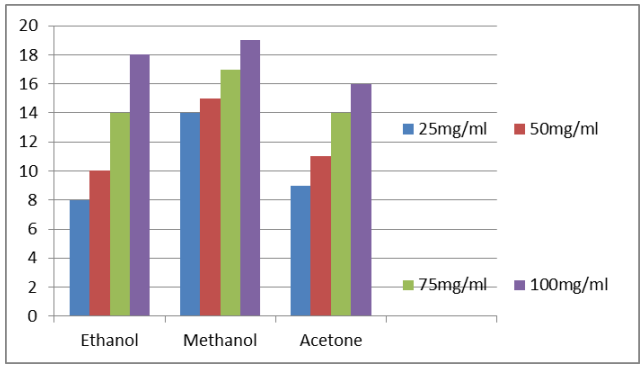

Fig 2: Antibacterial activity of *Achyranthes aspera* Methanol Root extracts against *Staphylococcus aureus*

Fig 3: Mass Chromatogram of *Achyranthes aspera*

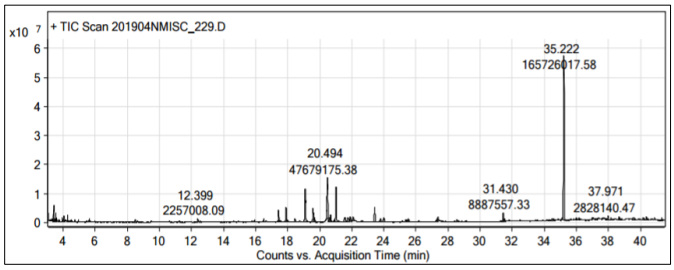

Fig 4: Mass Chromatogram of *Achyranthes aspera*

Table 1: Antioxidant activity of leaf extract of *Achyranthes aspera*

Tested concentrations µg/ml	OD at 517nm (triplicate values)		
25	0.794	0.782	0.788
50	0.726	0.73	0.728
100	0.686	0.694	0.690
250	0.552	0.565	0.559
500	0.436	0.429	0.433
Control	0.861	0.852	0.857

Tested concentrations µg/ml	% of scavenging (triplicate values)		
25	7.35	8.75	8.05
50	15.29	14.82	15.05
100	19.95	19.02	19.49
250	35.59	34.07	34.83
500	49.12	49.94	49.53

Table 2: Ascorbic acid (Positive control)

Tested concentrations µg/ml	OD at 517nm (triplicate values)		
5	0.741	0.731	0.734
10	0.663	0.681	0.677
20	0.501	0.529	0.517
40	0.142	0.153	0.147
50	0.098	0.114	0.108
Control	0.805	0.784	0.794

Tested concentrations µg/ml	% of scavenging (triplicate values)		
5	25.88	29.11	27.50
10	38.40	43.74	41.07
20	49.65	48.66	49.16
40	66.95	67.65	67.30
50	85.94	86.78	86.36

Lakshmi Naidu *et al.*, (2006) [12] carried out antimicrobial studies of *Achyranthes aspera* whole plant, root, leaf, stem and inflorescences against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi.* The solvents used for extraction were methanol, chloroform, N-hexane and acetonitrile. The methanol extract of *Achyranthes aspera* showed maximum inhibitory activity of 9mm.

Antimicrobial activity of root of *Achyranthes aspera* was studied against 7 bacterial and 3 fungal strains by agar well diffusion method. Antimicrobial activity was recorded for hexane, chloroform, methanol, ethanol and aqueous extracts. Ethanol and methanol extracts exhibited high degree of antimicrobial activity compared to others (Aniel Kumar *et al*., 2014) [3]. In the present study, Ethanol, Methanol and Acetone extracts of leaf, stem and root of *Achyranthes aspera* were tested against 3 ATCC pathogens. Among the 3 plant parts of *Achyranthes aspera*, acetone extracts of the leaf showed maximum inhibition of 25mm against *Pseudomonas auruginosa.*

GC-MS is the one of the best technique to identify the constituents of volatile matter long chain, branched chain hydrocarbons, alcohols, acids and esters. The GC-MS analysis of *C italic*a leaves revealed the presence of 17 compounds that could contribute the medicinal quality of the plant. The identification of phytochemical compounds was confirmed based on peak area, retention time and molecular formula. (Sermakkani and Thangapandian, 2012) [16].

Kumari Pushpa Rani and Doss (2017) [11] carried out phytochemical screening and GC-MS analysis of *Achyranthes aspera* leaves using Perkin Elmer Gas Chromatography Mass Spectrometry. The mass spectra of the compounds found in the extracts matched (NIST) library. The GC-MS analysis revealed the presence of Linoleic acid(12%), Palmitic acid (8.67%),2-Furaldehyde, 5- (Hydroxymethyl) (7.13%) and 3,5 – Dihydroxyl-6-Methyl-2,3-Dihydro-4H-pyran-4-one(2.64%).

In the present study, the mass chromatogram of the ethanol extract of *Achyranthes aspera* leaf showed 48 different phytochemical compounds. Major compounds were dimethooxymethyl silane, Cyclotetrasiloxane Octamethyl, Phytyl acetate, 1,2- Bezenedicarboxylic acid, bis (2-methylpropyl) ester, Phthalic acid isobutyl 2- pentyl ester, Dibutyl phthalate, phthalic acid butyl 3- methyl butyl ester and Bis (2-ethyhexyl) phthalate.

AbiBeaulah *et al.*, (2011) [1] found out the anti-oxidant activity of *Achyranthes aspera* root, stem, leaf and inflorescence by DPPH assay. Anti-oxidant activity of plants extracts were studied using different solvents such as hexane, chloroform, ethyl acetate and methanol. In methanolic extracts all the parts of the plants exhibited very high anti-oxidant activity which was closer to standard L-Ascorbic acid (94%). The activity of root was 90%, stem and inflorescences was 93% and leaf was 87%.

Anand *et al.*, (2014) [2] carried out anti-oxidant studies of *Achyranthes aspera* root extracts by DPPH method. The root extract showed potent anti-oxidant activities with the percentage of 96, which is greater than the standard, ascorbic acids. In the present study, ethanol leaf extracts of *Achyranthes aspera* was studied for anti-oxidant activity using DPPH method and it showed 50% where the standard ascorbic acid showed 87%.

4. Conclusion

Phytomedicine has promising activity against variety of infectious diseases. Microbial pathogens are known to gain resistance against antimicrobial agents. In the present study, the medicinal plant *Achyranthes aspera* leaf, stem and root were studied for its antibacterial activity against ATCC pathogens *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922).All the three plant parts showed inhibitory activity against the pathogens. The major phytochemicals present in the leaf extract and antioxidant potential were found out.

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