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Comparative phytochemical and microbicidal properties of *Amaranthus viridis* L. and *Achranthes aspera* L.

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Abstract
Amaranthus viridis L. and *Achranthes aspera* L. are members of family Amaranthaceae and possesses various active secondary metabolites. The phytochemical constituents present in selected plants have known anti-microbial properties. Keeping this view present study was designed for to study the phytochemical and microbicidal properties of *Amaranthus viridis* L. and *Achranthes aspera* L. Preliminary phytochemical analysis and anti-bacterial potential of root, stem and leaves were examined by prescribed methods. The present study indicating presence and absence of various groups of phytochemical constituents and also showed does dependant anti-microbial potential. *Amaranthus viridis* L. And *Achranthes aspera* L. both possesses anti-bacterial activity against *Escherichia coli, Salmonella Typhi and Bacillus substils*. Separation and fractionation of active phytochemical are suggested.

Keywords: Secondary metabolites, anti-bacterial, *Escherichia coli, Salmonella Typhi, Bacillus substils*, etc

Introduction

Species belonging to Family Amaranthaceae are most important family of phanerogams (coated seeded plants). This is the family of true seeded and flowering plant well known as angiosperms; having more than 800 species belonging to 65 genus spread throughout the world. India has more than 50 species of Amaranthaceae especially possessing ornamental, edible and medicinal properties (Dey *et al*, 2020) [4]. *Achyranthes, Aerva, Alternanthera, Amaranthus, Blutaparon, Celosia, Chamissoa, Charpentiera, Cyathula, Deeringia, Digera, Froelichia, Gomphrena, Gossypianthus, Iresine, Nototrichium, Tidestromia* and *Psilotrichum* are the important genera having economic importance in various aspects (Basu *et al*, 2014).

Genus Amaranthus is one of the largest genera of the family Amaranthaceae consisting more than 300 known species all around the world which are perennial, short living herb, shrub or tree. Genus Amaranthus consisting of mostly erect plants showing colour variation in its stem and leaves (Temel *et al*, 2023). Medicinally valuable phytochemical components like zinc, linolic acid, beta-carotene, sugars, phenolic acids; etc isolated from leaves and glycosides, saponins, oxalic acids, catetonoids; etc isolated from root plays vital role in different ailments (Nawghare *et al*; 2024) [10]. *Amaranthus viridis* L. performed different activities like Antioxidant, antimicrobial, cardio-protective, Anti-inflammatory, anti-diabetic, Antihelmintic and antipyretic properties with traditional uses like laxative, antifungal and nutritional potential (Ali *et al*, 2023) [7].

Genus Achyranthes also known as genus of Chaff Flower mostly includes plant with annual or perennial life growing around the ridges of forest, river or barren land. Achyranthes aspera L. is one of the important member and is endemic to India (He *et al*, 2017) [12]. Phytochemical constituents like flavonoids, tannins, terpenoids, saponins, phytosterols and phenolic acid acts as an active ingredient in plants and isolated for its biological potential to quench free radicals, inhibit inflammation, scavenge hazardous microbes and even sugars and lipids during diseased condition. In India food poisoning is considered as a particular reason for the death of 1.7 million people during 2023 caused due the food bourne microorganisms like gram positive bacteria *viz*. Bacillus, Staphylococcus, *Escherichiai*, Pseudomonas and Salmonella (Alvarez Martinez *et al*, 2021) [5]. Leaves stem and roots of *Amaranthus viridis* L. and Achyranthes aspera L. are the plant species have proven anti microbial potential (Muhammad *et al*, 2021; Mai *et al*, 2024; Samantha *et al*, 2010). Keeping this view present study was designed for to study the phytochemical and microbicidal properties of *Amaranthus viridis* L. and *Achranthes aspera* L.

Fig 1: *Amaranthus viridis* L.

Fig 2: *Achranthes aspera* L.

Materials and methods

Collection of plant material: Whole plantlets of *Amaranthus viridis* L. and *Achranthes aspera* L. plant species were collected from the study area. Root, stem and leaves of the plantlets were separated using razor-sharp blade. Separated plant parts were chopped in small pieces and allow for drying in hot air oven at 45ºC for 15 hours. Well dried plant parts of *Amaranthus viridis* L. and *Achranthes aspera* L. were pulverized in fine powder using mixer grinder. After pulverization all the materials were sieved from sieves of mesh size of 315µm. The finely ground plan powders were used for the further experiments.

Qualitative phytochemical screening: Identification of different groups of secondary metabolites present in aqueous extracts of plant materials of selected plants was done by using preliminary phytochemical analysis.

Preparation of aqueous extracts: 10g of powdered plant material of *Amaranthus viridis* L. and *Achranthes aspera* L. root stem and leaves were extracted with 100ml of distilled water by boiling the powdered material. Boiled plant material first filtered with 8 layers of muslin cloth and then filtered using Whatman No. 1 filter paper.

Phytochemical screening: Preliminary qualitative screening of phytochemicals *viz*. Alkaloids, Tannins, Saponins, Steroids, Flavonoids, Terpenoids and Glycosides were performed using prescribed methods of Harborn (1973) [8]. The tests for alkaloids were performed by Mayer's test and Dragendorff's Test. Mayer's test was performed by addition of 1-2 ml of extracts with 2 drops of Mayer's reagent (potassium mercuric iodide). The confirmation was done by observation of precipitate. While performing the Dragendorff's Test reddish brown precipitation indicates the positive for alkaloids. Ferric Chloride Test was used as a confirmatory test for tannins in which blue-black colour of solution indicates the presence of tannins. The presence of saponins were done by foam test; while performing this test 1ml of plant extract were mixed with 10 ml of distilled water in test tube and test tube were shaken; if the foam is stable for more than 10 min indicates the presence of saponins. Salkowski Test was done for presence of steroids in this test 1 ml of plant extract was mixed with 2 ml of chloroform and 2 ml of sulphuric acid carefully. The red ring developed at the edge of solution indicates that the steroids are present. Tests for Flavonoids, Terpenoids and Glycosides were performed using Shinoda Test, Salkowski Test and Legal's Test respectively. The change in colour indicates the presence of respective secondary metabolites.

Antimicrobial assay

Preparation of plant extracts for Anti-microbial potential: Fresh weight of plant materials (10gm) were extracted in 100ml of methanol. The mixture was centrifuged at 7000 rpm for 5 min to sepearte the cell debris from the mixture. Then different concentration of sample extract (50mg/ml, 100mg/ml, 150ml) was prepared by diluting in 1ml methanol each.

Preparation of media: Antimicrobial assay of extracts of different plants was performed by agar well diffusion method in Mueller Hinton Agar (MHA) plates. MHA media plates were prepared by adding MHA (38 gm in 1000 ml) distilled water and sterilized by autoclaving media at 15 psi for 15 min. The media is poured into plates and kept for solidification.

Test organism: The test organisms (*Escherichia coli*, *Salmonella Typhi* and *Bacillus substils*) were used for the analysis of antimicrobial assay.

Antibacterial test: The test organisms (*Escherichia coli*, *Bacillus*, *Salmonella Typhi* and *Bacillus substils*) were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5 x 108 CFU/ml. MHA plate was lawn cultured with standardized microbial culture broth. Plant extracts of 50 mg/ml concentration were prepared in methanol. Wells of 6 mm were bored in the inoculated media with the help of a sterile cork-borer (6 mm). Each well was filled with 50 µl extracts from different plants: positive control (azithromycin) 1mg /ml for bacteria. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm.

Results and discussion

Preliminary phytochemical analysis of *Amaranthus viridis* L. and *Achranthes aspera* L. root, stem and leaves aqueous extracts were performed by prescribed methods of harborn (1973) [8] and the results expressed in Table 01. The results indicates the presence of alkaloids, tannins, saponins and flavonoids in aqueous extracts of root, stem and leaves of *Amaranthus viridis* L. and *Achranthes aspera* L. in low, moderate and high level. While steroids, terpenoids and glycosides were seems to be absent.

Table 1: Preliminary phytochemical analysis of *Amaranthus viridis* L. and *Achranthes aspera* L. root, stem and leaves aqueous extract.

Plant Species	Plant part	Alkaloids	Tannins	Saponin	Steroids	Flavonoids	Terpenoids	Glycosides
Amaranthus viridis L.	Root	+	++	+++	-	++	-	-
	Stem	+	++	+++	-	++	-	-
	Leaves	++	++	++	-	+++	-	-
Achranthes aspera L.	Root	++	+++	+++	-	+	-	-
	Stem	+	+++	+++	-	++	-	-
	Leaves	++	++	++	-	++	-	-

$ Absent (-); Present in low concentration (+); Present in moderate concentration (++) and Present in higher concentration (+++)

Anti-bacterial potential of *Amaranthus viridis* L. and *Achranthes aspera* L. root, stem and leaves against *Escherichia coli*, *Salmonella Typhi* and *Bacillus substils* at various concentration was determined by agar well method and zone of inhibition (ZoI) were calculated denoted in Table. 02 and Table 03 as well as Zoi shown on Figure 03

and Figure 04. The results expressed in Table 02 and Figure 03 indicating the Zone of Inhibition (in mm) showed that root, stem and leaves of *Amaranthus viridis* L. At various concentration on *Escherichia coli*, *Salmonella Typhi* and *Bacillus substils.* Maximum ZoI was found by leaves with 5.2mm at 150mg/ml dose, whereas 3.2mm ZoI was on *E. Coli* by root extract at 50 mg/ ml. Maximum ZoI on *Salmonella Typhi* was observed to be 6.3mm by stem extract as well as minimum inhibition was observed by root extract (3.3mm) at 50mg/ml concentration. Most inhibition was found on *Bacillus substils* by *Amaranthus viridis* L. showing maximum inhibition (7.3mm) by leaves and minimum (3.9mm) by root extract.

Table 2: Anti-bacterial potential of *Amaranthus viridis* L. root, stem and leaves against *Escherichia coli*, *Salmonella Typhi* and *Bacillus substils* at various concentration

Plant part	Zone of Inhibition (in mm)									
	Control	*Escherichia coli*			*Salmonella Typhi*			*Bacillus substils*		
		50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml
Root	9mm	3.2mm	3.9mm	4.3mm	3.3mm	4.6mm	5.7mm	3.9mm	4.7mm	5.4mm
Stem	10mm	3.0mm	3.4mm	4.1mm	5mm	5.8mm	6.3mm	5.2mm	6.2mm	6.6mm
Leaves	10mm	4.1mm	4.6mm	5.2mm	4.1mm	4.9mm	5.5mm	5.6mm	6.8mm	7.3mm

While Table 03 and Figure 04 indicating the anti-bacterial potential of *Achranthes aspera* L. root, stem and leaves against *Escherichia coli*, *Salmonella Typhi* and *Bacillus substils* at various concentration (50 mg/ml, 100 mg/ml and 150 mg/ml). When considering inhibition of *Escherichia coli* dose dependent inhibition was found, indicating maximum (6.6mm) inhibition by leaves of *Achranthes aspera* L. and minimum (5.6mm) was found to be against root extract. Root, stem and leaves showed potential ZoI on *Salmonella Typhi* with inhibition of 5.7mm, 4.9mm and 6.9mm at the concentration of 150 mg/ml; respectively. Roots of *A. aspera* showed 6.0mm of inhibition by roots while 4.8mm and 4.9mm inhibition by stem and leaves respectively.

Table 3: Anti-bacterial potential of *Achranthes aspera* L. root, stem and leaves against *Escherichia coli*, *Salmonella Typhi* and *Bacillus substils* at various concentration.

Plant part	Zone of Inhibition (in mm)									
	Control	*Escherichia coli*			*Salmonella Typhi*			*Bacillus substils*		
		50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml
Root	9mm	3.6mm	4.5mm	5.6mm	3.2mm	4.8mm	5.7mm	4.1mm	4.9mm	6.0mm
Stem	10mm	3.8mm	4.9mm	5.9mm	3.0mm	4.1mm	4.9mm	3.5mm	4.1mm	4.8mm
Leaves	10mm	4.7mm	5.8mm	6.6mm	4.9mm	6.1mm	6.9mm	4.1mm	4.3mm	4.9mm

Fig 3: Anti-bacterial potential of *Amaranthus viridis* L. root, stem and leaves against *Escherichia coli*, *Salmonella Typhi* and *Bacillus substils* at various concentration

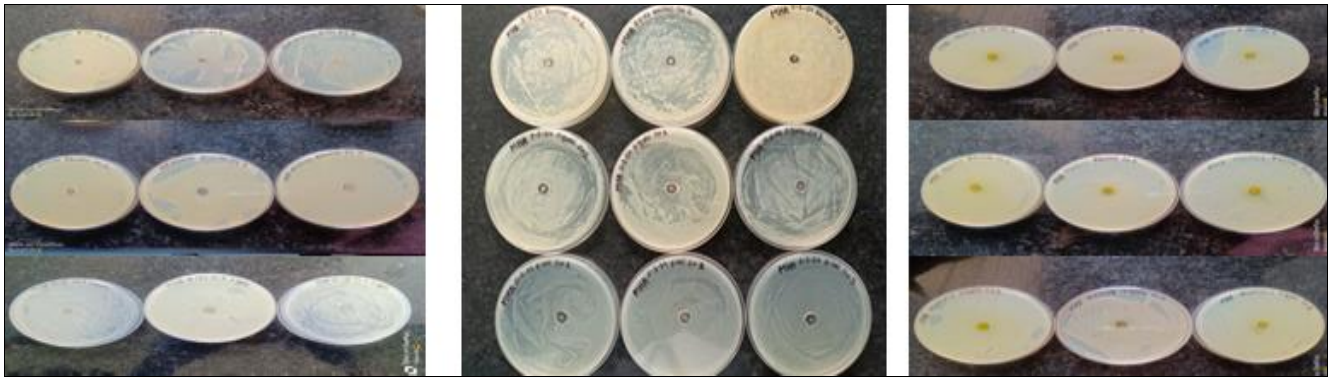

Fig 4: Anti-bacterial potential of *Achranthes aspera* L. root, stem and leaves against *Escherichia coli*, *Salmonella Typhi* and *Bacillus substils* at various concentration

Conclusion

Phytochemical constituents isolated from plants especially wild edible vegetables are known to serve as a potent source of anti-microbial agent. Present study supports this view and proves that the chemical components isolated from plant species *Amaranthus viridis* L. And *Achranthes aspera* L. can be used as anti-bacterial agents. Other members of the family Amaranthaceae also have an ability to possess anti-microbial properties. Future study will be focus on chromatographic separation of active biomolecules from *Amaranthus viridis* L. And *Achranthes aspera* L. and its characterization which can be useful for researchers.

References:

1. AI-Anh H, Latifah A, Aldawsari M, Bharathi D, Lee J. Evaluation of phytochemical, antibacterial, thrombolytic, anti-inflammatory, and cytotoxicity profile of Achyranthes aspera aerial part extracts. Environmental Research,2024:243(8):272-277.
2. Samanta A, Jena A, Sinha C, Ghosh S, Mandal M. Antibacterial and Pharmacological valuation of Root Extract of Plant Achyranthes aspera (Amaranthaceae). Research Journal of Pharmacy and Technology,2010:3(3):910-914.
3. Saikat B, Peiman Z, Ratnabali S, Cetzal W. Amaranthaceae, The pigweed family. Encyclopedia of Earth, 2014.
4. Dey P, Basumatary M, Rajkumari N. Medicinal values of selected species of family Amaranthaceae of India, 2020.
5. Alvarez-Martínez F, Barrajon-Catalan E, Herranz-Lopez M, Micol V. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. Phytomedicine,2021:90(1):142-149.
6. Fouad M, Ghareeb M, Hamed A. Exploring the antioxidant, anticancer and antimicrobial potential of *Amaranthus viridis* L. collected from Fayoum depression: Phytochemical, and biological aspects. South African Journal of Botany,2024:166(1):78-83.
7. Ali H, Muhammad I, Urooj F. Pharmaceutical Activity of Medicinal Plant *Amaranthus viridis* Linn. Due to Its Chemical Constituents: A Review Bioeduscience,2023:7(1):143-148.
8. Harborn J. Phytochemical methods: A guide to modern techniques of plant analysis, Second Edition; Chapman and Hall publication, London; New York, 1973.
9. Muhammad I. Mahmood H. Farooq A. Amer J. Antioxidant and antimicrobial activities of Chowlai (*Amaranthus viridis* L.) leaf and seed extracts. Journal of medicinal plant research,2012:6(1):4450-4455.
10. Nawghare S. Shrirao A. Chandewar A. Pharmacological Evaluation of *Amaranthus viridis* Linn leaves Extract for Anti Alzheimer's Activity in Rat Models; Research Journal of Pharmacology and Pharmacodynamics,2024:16(3):161-167.
11. Suleyman T, Bilal K. Amaranthus (Amaranthus spp.), alternative forage crops-I, Iksad Publications, Turkey, 2023.
12. He X, Wang X, Fang J, Chang Y, Ning N, Guo H. The genus Achyranthes: A review on traditional uses, phytochemistry, and pharmacological activities, Journal of Ethnopharmacology,2017:203(1):260-278.