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Research Article

Antagonistic, Antioxidant and Phytochemical Screening of *Amaranthus viridis* Against Bacteria Causing Urinary Infections

Divya P. V.[1*], K. Sukesh[2]

[1]*Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.*

[2]*Department of Microbiology, Malankara Catholic College, Mariagiri, Tamil Nadu, India.*

**Corresponding author: divyashivan2014@gmail.com*

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ABSTRACT

The present study was designed to determine the antagonistic activity exhibited by the medicinal plant *Amaranthus viridis*against bacteria causing urinary infections and to screen the antioxidant potentials and the phytochemical constituents possessed by them. Various solvent extracts of the leaves of *A. viridis* were prepared and evaluated for their antagonistic activities against urinary tract infection-causing bacteria by agar well and disc diffusion methods. The phytochemical screening of the extracts was done qualitatively and the antioxidant potentials were determined by DPPH assay. The results of the antagonistic activity of *A. viridis* assessed by agar well and disc diffusion methods revealed that the plant extracts possess remarkable antagonistic properties against bacteria causing urinary infections. The phytochemical analysis revealed the presence of secondary metabolites such as phenols, flavonoids, saponins, steroids, glycosides, quinones, tannins, and coumarins. However, the extracts were found to possess low levels of antioxidants. From the above study, the leaves of *A. viridis* can be considered as a candidate to develop novel drugs against bacteria causing urinary infections.

Keywords: Antagonistic activity, phytochemical screening, antioxidant activity, Leaves, *Amaranthus viridis*.

INTRODUCTION

Urinary tract infection or UTI was a serious health issue encountered globally with frequent incidence among females. The situation became more threatening due to the emergence of multi-drug resistance among pathogens, which has led to the necessity to explore alternative compounds having antimicrobial properties.According to the World Health Organization (2000),the greatest resource to obtain drugs is medicinal plants. Medicinal plants as in crude form have been found to have potential effect against urinary tract infections.[1-3]

Owing to this, the present study was designed to evaluate the antagonistic activities, antioxidant potentials, and phytochemical constituents of leaf extracts of the medicinal plant *Amaranthus viridis* against bacteria causing UTI.

A. viridis, commonly known as slender amaranth or Chowlai belongs to the family *Amaranthaceae* and is a native of Africa. It is found widely distributed in grasslands, cultivated and uncultivated fields in Asia, Africa, Latin America, Mexico, California and Chiapas. The other names of *A. viridis* are Jungali Chaulayi in Hindi, Kuppaikkirai in Tamil and Kuppacheera in Malayalam.[4,5]

It is an erect, annual or perennial herb, growing up to 100 cms high. The roots are whitish, cylindrical with rootlets, and grow horizontally downwards. The stems are light green, slender, and branched, consisting of alternate leaves of 10 cm long petiolate (Fig. 1). It consists of sub-sessile, small; unisexual, green flowers and also has axillary or terminal, frequently paniculated spikes. They have sub-globose fruits with dark brown to black shiny seeds.[5,6]

Traditionally various parts of this plant were used for treating eczema, psoriasis, leprosy, venereal diseases, anaemia, vermifuge, laxative, constipation, antiemetic, antiulcer, antipyretic, analgesic, anticholesterolemic, antirheumatic, antihepatotoxic, bronchitis, asthma, antidiabetic, anticancer, diuretic and inflammation of urinary tract.[5-7]

For this research, *A. viridis* wass chosen to investigateitsr antagonistic, antioxidant properties and phytochemical constituents, an approach toidentifying a candidate for developing ap drug to treat urinary tract infections.

MATERIAL AND METHODS

Isolation and Identification of Test Organisms

Pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were isolated from infected urine samples and confirmed by gram's staining, culturing on selective media and by biochemical tests such as IMViC test series, catalase test, etc. After the identification of bacterial isolates, the pure cultures were stored in nutrient agar slants at 4°C to determine the antagonistic activity of selected medicinal plants.

Fig.1: *Amaranthus viridis*

Collection of Plant Material

Fresh, healthy leaves of *A. viridis* were collected from Malayadi, Kanya Kumari District, Tamil Nadu. The leaves were thoroughly washed, shade dried at room temperature, then powdered using a mixer grinder and stored in airtight bottles for further study.

Preparation of Plant Extracts

10 gms of the powdered plant parts were taken separately in conical flasks and 100 ml of different solvents such as hexane, acetone, methanol, ethanol and water were added to it and placed under dark condition. After 3 days, the contents were stirred well and filtered using Whatman No.1 filter paper. After evaporation the filtrates were collected and stored in sterile glass beakers for further study.

Antagonistic Activity Assay by Well Diffusion Method

Antagonistic activity of various extracts of leaves of *A. viridis*was evaluated against the four bacterial isolates by agar well diffusion method.[8,9] Sterilized Mueller Hinton agar was poured in Petri plates. After solidification, the inoculum was swabbed on the entire surface of the agar medium. After some time, wells of 6 mm diameter were punched over the agar surface in each of the labeled plates for various plant extracts. 100 µl of respective plant extracts were added to each well with the help of micro-pipette. Then, the plates were kept for incubation at 37°C for 24 hours and observed for zones of inhibition after incubation. The results were recorded.

Antagonistic Activity Assay by Disc Diffusion Method

Antagonistic activity of various extracts of leaves of *A. viridis*was evaluated against the four bacterial isolates by agar disc diffusion method [8,10].SterilizeWhatman No: 1 filter paper discs of 6 mm diameter were impregnated with100 µl of various extracts of *A. viridis*. Then the prepared discs were placed on the surface of previously labeled Mueller Hinton agar plates swabbed with test organisms. The plates were then allowed for incubation at 37°C for 24 hrs. After the incubation period, the plates were detected for the presence of zones of inhibition, and the results were recorded.

Antioxidant Activity by DPPH Assay

Radical scavenging activity of the methanolic and petroleum ether extracts of leaves of *A. viridis* against stable 2,2- diphenyl 2-picryl hydrazyl hydrate (DPPH) was determined.[11] For DPPH assay, the ascorbic acid was used as reference standard. The ascorbic acid stock solution was prepared in distilled water (1 mg/ ml; w/v). A 60 µM solution of DPPH in methanol was freshly prepared, and 3.9 ml of this solution was mixed with 1 ml of test sample at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800 µg/ml). The tubes were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without adding any extract or ascorbic acid. 95% methanol was used as blank. Radical scavenging activity was calculated by the following formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Phytochemical Analysis of Active Plant Extracts

The plant extracts were screened qualitatively [12-15] for the presence or absence of secondary metabolites such as alkaloids, carbohydrates, amino acids, phenols, flavonoids, saponins, steroids, glycosides, terpenoids, tannins, quinones, reducing sugars, catechins, and coumarins.

RESULT AND DISCUSSION

Antagonistic Activity

The antagonistic activities of hexane, acetone, methanol, ethanol, and water extracts of leaves of *A. viridis* were evaluated against UTI bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by agar well and disc diffusion methods. All the plant extracts except hexane and water extracts revealed different ranges of antagonistic activities against the tested bacterial isolates, and the results are shown in Table 1.

The literature showing the antagonistic activity of *A. viridis* against UTI-causing bacteria was scanty. However, the findings from this study were in a fair correlation with the findings of Sarwar*et al*.[16] and Rashid *et al*.,[10] where both the studies revealed the antibacterial activities of *A. viridis* against tested pathogens.In this study, the extracts exhibited greater antagonistic activity against *E. coli* in disc diffusion method. The methanolic extracts were found to be highly antagonistic to all the bacteria tested in both methods.

Antioxidant Activity

The radical scavenging activity or antioxidant potentials of methanolic and petroleum ether extracts of *A. viridis* were determined by adopting the DPPH assay, and the results are displayed in Figure 2. The standard Ascorbic acid exhibited significantly higher DPPH radical scavenging activities than the plant extracts used in this study.

The percentage of inhibition of standard ascorbic acid at a concentration of 800 µg/mL is 93.2, while the percentage of inhibition exhibited by petroleum ether and methanolic extract of *A. viridis* is 34.41 and 32.81, respectively. The findings had some correlation with the findings of Rashid *et al*.[10] where the methanolic leaf extract of *A. viridis* exhibited 29.0 ± 0.2 % of free radical scavenging activity. From the results of this study, it could be understood that though the plant has some antioxidant potential but was not much greater than that of the standard ascorbic acid.

Table 1: Antagonistic Activity of leaf extracts by well diffusion method

Test Organisms	*Well diffusion*			*Disc diffusion*		
	A	*M*	*E*	*A*	*M*	*E*
	mm	*mm*	*mm*	*mm*	*mm*	*mm*
Escherichia coli	13	12	18	16	22	12
Klebsiella pneumoniae	13	23	-	11	17	10
Pseudomonas aeruginosa	-	18	14	11	16	10
Staphylococcus aureus	12	16	16	14	15	12

A-Acetone; M-Methanol; E-Ethanol; mm-millimeter

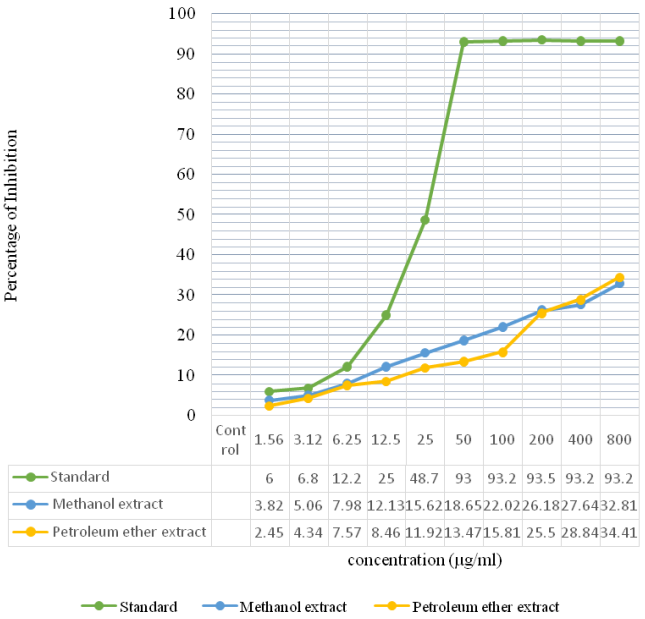

Figure 2: Antioxidant Activity of *Amaranthus viridis*

Table 2: Phytochemical analysis of various extracts

Phytochemical tests	*A. viridis*		
	A	*E*	*M*
Alkaloids	-	-	-
Carbohydrates	-	-	-
Amino acids	-	-	-
Phenols	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Glycosides	+	+	+
Terpenoids	-	-	-
Tannins	+	+	+
Quinones	+	+	+
Reducing sugars	-	-	-
Catechins	-	-	-
Coumarins	+	+	+

A-Acetone; E-Ethanol; M-Methanol; +- Presence; -- Absence

Phytochemical Screening

The phytochemical screening of the most active extracts, acetone, ethanol, and methanol extracts, was performed qualitatively, and the results are shown in Table 2.

The finding of this study goes in agreement with the review of Ferdous *et al*. [6] regarding the phytochemicals present in *A. viridis*, in which the leaves possess saponins, tannins, phenols, flavonoids, glycosides, and steroids. The results of this study showed that all three extracts of the plant possessed a consistent number of phytochemicals.

CONCLUSION

This study of screening for antagonistic activity of various extracts of leaves of *A. viridis* derived the conclusion that *A. viridis* possess strong antagonistic activity. Also, the plant possesses enormous number of phytochemicals in them. Hence, *A. viridis* can be considered as a potential agent in drug research against bacteria causing urinary infections.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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