

## Full Length Research Paper

# Phytochemical analysis and antibacterial efficacy of *Amaranthus tricolor* (L) methanolic leaf extract against clinical isolates of urinary tract pathogens

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Urinary tract infections (UTI) are the most common form of bacterial infections, affecting people throughout their lifespan. The present study was designed to evaluate the antibacterial activity of *Amaranthus tricolor* leaf extract and its phytoconstituents against clinical isolates of urinary tract infections. In the present study, the leaf extract of *A. tricolor* was prepared by cold maceration using methanol. The preliminary phytochemical screening performed indicated the presence of carbohydrates, aminoacids, proteins, steroids, alkaloids, glycosides, flavonoids and tannins. Clinical isolates of urinary tract pathogens such as *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris* were used for the study. The antibacterial property was determined by agar well diffusion method and minimum inhibitory concentration (MIC) were determined for crude *A. tricolor* leaf methanolic extract by resazurin microtitre plate assay method. The results indicate that the methanolic leaf extract of *A. tricolor* has a notable antibacterial activity against tested microorganisms. The maximum antibacterial activity was observed against *E. coli* (17.7±0.57 mm). A moderate activity was observed against *P. vulgaris* (16.6±0.57 mm) and minimum activity against *E. faecalis* (13.3±1.15mm) with respect to that of zone diameter exhibited by the organisms. The minimum inhibitory concentration was ranged from 5.0 to 0.36 mg/ml. The *A. tricolor* leaf extract was found to contain some bioactive compounds with pronounced antibacterial activity, however further phytochemical studies and their characterization will be needed to isolate the active constituents and evaluate the antimicrobial activities against a wider range of microbial pathogens.

**Key words:** *Amaranthus tricolor*, urinary tract infection (UTI), clinical isolates, antibacterial activity.

## INTRODUCTION

Bacterial infections are the most serious global issue regarding premature deaths. Recently, drug resistance to

human pathogenic bacteria has been reported worldwide. Each year, over 150 million people are diagnosed with

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urinary tract infections (UTI) and it is the second most common infectious disease in community practice worldwide (Gonzalez and Schaeffer, 1999). The occurrence of UTI has increased in recent years. Urinary tract system comprises kidneys, ureters, bladder and urethra. Its function is to collect, store and release urine. UTIs are caused by microorganisms capable of attacking anywhere in the urinary tract. In general, the infection may involve either lower tract or both upper and lower tracts. It is a common infection particularly among young and sexually active women. The incidence of UTI is more common in women than men. In women, it occurs in all age groups but the incidence and prevalence increase with age. Moreover it is the second most common bacterial infection that occurs during pregnancy (Sampson and Gravett, 1999). Around 80-85% of UTIs are caused by *Escherichia coli* and 5-10% by *Staphylococcus saprophyticus* (Nicolle, 2008). The UTIs may also have viral or fungal origin (Amdekar et al., 2011). The other bacteria known to cause UTI infections are *Enterobacter*, *Klebsiella*, *Proteus* and *Pseudomonas*.

*Amaranthus tricolor* L. belonging to the family Amaranthaceae, is an ornamental plant known as Tandalijo or Tandalja bhaji in India. The synonym of *A. tricolor* is *A. gangeticus*. In Andhra, it is commonly known as "Perugu thotakura". The leaves are highly nutritious. The nutrients present in the leaves are carbohydrates, protein, vitamin A, vitamin C, riboflavin (Vitamin B<sub>2</sub>), thiamin (Vitamin B<sub>1</sub>), niacin and minerals like calcium and iron. It is also a source of fiber. Because of its high nutritional value, *A. tricolor* is consumed more compared to other leafy vegetables.

The whole plant is traditionally used as an astringent (Chopra et al., 1956). The root decoction of *A. tricolor* along with *Cucurbita moschata* is used to control haemorrhage following abortion (Duke and Ayensu, 1985). The plant decoction is taken internally to strengthen the liver and to improve vision. Scientific study on the plant suggests that it may inhibit calcium retention (Larsen et al., 2007).

The plant has been scientifically reported to possess antioxidant (Samsul et al., 2013), hepatoprotective (Simran et al., 2013), antinociceptive and anti-inflammatory activities (Gopal et al., 2013). It is also reported for *in vitro* and *in vivo* anti-cancer effects (Sani et al., 2009). The leaves are reported for hepatoprotective properties (Mohammed S. Al-Dosari, 2010) and *in-vitro* antioxidant, anti-amylase, anti-arthritis and cytotoxic activity (Vivek Kumar et al., 2011). The presence of betalain pigments such as amaranthin, betaxanthin, methyl derivative of arginine betaxanthin and betalamic acid has been reported in leaves (Mousumi et al., 2013). The present work was undertaken to explore the antibacterial activity of methanolic leaf extract of *A. tricolor* against urinary tract pathogens of clinical origin.

## MATERIALS AND METHODS

### Plant material

The plant *Amaranthus tricolor* L was collected in and around Guntur, Andhra Pradesh, India, identified and authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. The collected leaves were shade dried and then ground to coarse powder.

### Extraction

The powdered leaf material was extracted by cold maceration with methanol. The leaf powder (100 g) was macerated with methanol (1000 ml) by occasional shaking for two days. The extract was collected by filtering through 5 layers of muslin cloth and concentrated at low temperature. The prepared extract was preserved in a desiccator for further study.

### Test bacteria

In this study, the two Gram positive bacteria that is *S. saprophyticus*, *E. faecalis* and four Gram negative bacteria that is *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *Proteus vulgaris* employed were isolated from subjects suffering with urinary tract infections (UTI).

### Phytochemical screening

The phytochemical screening for the crude methanolic extract of *A. tricolor* was carried out by standard protocols (Evans and Trease, 2005; Kokate et al., 2005). The presence of alkaloids, cardiac glycosides, saponins, carbohydrates, proteins, aminoacids, flavonoids, steroids, tannins was analyzed.

### Isolation and Identification of UTI bacteria

The microorganisms present in urine samples of UTI infected patients were cultured in the nutrient broth and were differentiated using the gram staining procedure into gram positive and gram negative organisms.

The organisms were transferred to cystine lactose electrolyte deficient (CLED) agar medium for further differentiation of urinary organisms. Six biochemical tests were performed for each organism. Catalase activity, indole production test, citrate utilization test, urease test, methyl red and voges proskauer's test were done.

### Antibacterial activity

The antibacterial efficacy of methanolic leaf extract of *Amaranthus tricolor* was tested by agar well diffusion method (Bauer et al., 1966). The collected clinical isolates were grown in Muller Hinton broth (Himedia, Mumbai, India) at 37°C for 24 h, with constant agitation in a shaker. The cultures from broth were aseptically swabbed on sterile Muller Hinton agar (Himedia, Mumbai) plates using sterile cotton swabs. The wells of 6 mm were punched in the inoculated plates using a sterile borer. Aliquots of 100 µl of methanolic leaf extract (50 mg/ml in dimethyl sulphoxide) was transferred into labeled wells. The wells were also filled with 50 µl positive (Amikacin, 10 mg/ml in dimethyl sulphoxide) and 50 µl negative (dimethyl sulphoxide only) controls. The plates were incubated at 37°C for 24 h in upright position and the zones of

**Table 1.** Biochemical tests of recovered clinical isolates.

S/N	Catalase	Indole	MR	VP	Citrate	Urease	Organism confirmed
1	+	+	+	-	-	-	<i>E. coli</i>
2	+	+	-	-	+	+	<i>Pseudomonas</i>
3	+	+	-	-	+	+	<i>Klebsiella</i>
4	+	+	+	-	+	+	<i>Proteus</i>
5	+	+	+	-	-	-	<i>Enterococcus</i>
6	+	-	-	-	+	+	<i>Staphylococcus</i>

'+' indicates positive, '-' indicates negative.

inhibition were recorded. The activity assays were conducted in triplicate.

#### Determination of MIC by Microtitre plate assay

The microtitre plate was prepared in aseptic conditions. A stock solution of test sample (10% w/v) was prepared in dimethyl sulphoxide. A volume of 100  $\mu$ l of test material was filled in first row of the plate. To all other wells 50  $\mu$ l of sterile nutrient broth was filled. The test material was transferred to the next well to attain serial dilutions. To each well, 30  $\mu$ l of resazurin indicator solution (0.02%) was added. Finally 10  $\mu$ l of bacterial suspension ( $1 \times 10^8$  CFU/ml) was added to each well. The plate was set with positive control and a column with all solutions except test compound and negative control; a column contains 10  $\mu$ l of sterile nutrient broth except test compound and bacterial suspension. The plates were prepared and incubated at 37°C for 24 h. The colour change from purple to pink indicated a positive response. The lowest concentration at which colour change was noted was the minimum inhibitory concentration (MIC) values for the test material and bacterial strain.

#### Statistical analysis

The values represented in the results are mean  $\pm$  standard deviation (SD) of each measurement.

## RESULTS AND DISCUSSION

The common triggering factors of urinary tract infections are pregnancy, diabetes, immunosuppression and other urologic disorders. To provide appropriate therapy for UTI, the current knowledge and antibiotic susceptibility of the organisms is essential. The clinical isolates of urinary tract infections were confirmed by biochemical tests and are reported in Table 1. The medicinal value of plants depends on the presence of phytoconstituents. The preliminary phytochemical screening revealed the presence of various phytoconstituents such as aminoacids, carbohydrates, proteins, cardiac glycosides, steroids, alkaloids, flavonoids and tannins. The results of phytoconstituents presence are reported in Table 2.

The results of inhibitory effect of methanolic leaf extract of *A. tricolor* are shown in Table 3. The results show that

**Table 2.** Preliminary phytochemical screening of leaves of *Amaranthus tricolor*.

S/N	Name of the test	Methanol Extract
1	Carbohydrates	+
2	Proteins	+
3	Aminoacids	+
4	Steroids	+
5	Cardiac glycosides	+
6	Flavonoids	+
7	Alkaloids	+
8	Tannins	+

'+' indicates positive.

**Table 3.** Antibacterial activity of methanolic *Amaranthus tricolor* against selected UTI pathogens.

S/N	Name of the Microorganism	Zone of inhibition in mm	
		Methanolic extract	Amikacin
1	<i>S. saprophyticus</i>	14.3 $\pm$ 0.57	19.3 $\pm$ 0.57
2	<i>E. faecalis</i>	13.3 $\pm$ 1.15	15.6 $\pm$ 0.57
3	<i>E. coli</i>	17.7 $\pm$ 0.57	22.3 $\pm$ 0.57
4	<i>P. aeruginosa</i>	15.3 $\pm$ 0.57	17.6 $\pm$ 0.57
5	<i>K. pneumoniae</i>	14.6 $\pm$ 1.15	18.6 $\pm$ 0.57
6	<i>P. vulgaris</i>	16.6 $\pm$ 0.57	20.6 $\pm$ 1.15

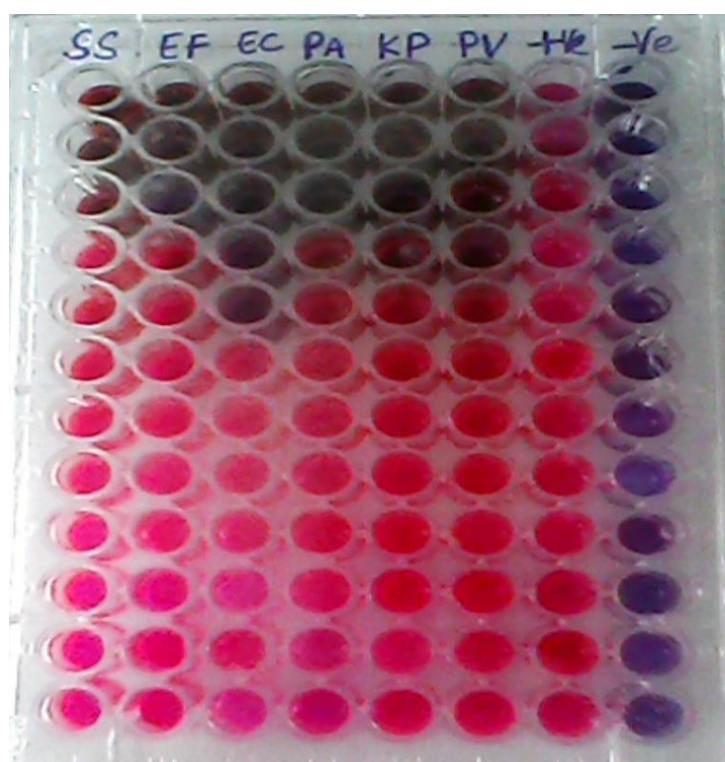
Values are mean  $\pm$  SD of triplicates.

different bacterial species exhibit different sensitivities towards the extract. The extract was found to be inhibitory to all bacterial isolates but with variable extent. The order of activity against selected bacteria was *E. coli* > *P. vulgaris* > *P. aeruginosa* > *K. pneumoniae* > *S. saprophyticus* > *E. faecalis*.

Different plant metabolites have shown effective antibacterial activity against uropathogens including drug resistant strains. For example the leaf and bark extracts of *Pimenta dioica* (Linn) Merrill (Myrtaceae) and *Anacardium occidentale* L. (Anacardiaceae) exhibited

**Table 4.** Minimum inhibitory concentration (MIC) of methanolic extract of *Amaranthus tricolor* against selected UTI pathogens.

S. No	Name of the Microorganism	MIC (mg/ml)
1	<i>S. saprophyticus</i>	5.0
2	<i>E. faecalis</i>	2.5
3	<i>E. coli</i>	0.36
4	<i>P. aeruginosa</i>	1.25
5	<i>K. pneumoniae</i>	0.62
6	<i>P. vulgaris</i>	0.62



**Figure 1.** Determination of minimum inhibitory concentration (MIC) by resazurin microtiter assay method (REMA). SS, *S. saprophyticus*; EF, *E. faecalis*; EC, *E. coli*; PA, *P. aeruginosa*; KP, *K. pneumoniae*; PV, *P. vulgaris*.

antibacterial efficacy against drug resistant clinical isolates of urinary tract infection (Manasa et al., 2013). Plants such as *Coccinia grandis* (Poovendran et al., 2011), *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferruginum* (Sachidananda Swamy et al., 2014) were reported for antibacterial efficacy against uropathogens. In the present study, the *A. tricolor* (L) leaf methanolic extract effectively inhibited all bacteria tested. The zone inhibition values of the extract against tested bacteria ranged from  $13.3 \pm 1.15$  to  $17.7 \pm 0.57$  mm. Amikacin showed inhibition zones that ranged from

$15.6 \pm 0.57$  to  $22.3 \pm 0.57$  mm. The methanolic extract exhibited maximum activity against *E. coli* ( $17.7 \pm 0.57$  mm) followed by *P. vulgaris* ( $16.6 \pm 0.57$  mm) and then against *P. aeruginosa* ( $15.3 \pm 0.57$  mm). The results in the present study indicate that the antibacterial activity varies according to type of bacteria used for the study. The least activity was exhibited by *E. faecalis* with the smallest zone ( $13.3 \pm 1.15$  mm) and inhibited at lowest concentration 1.25 (mg/ml) (Table 4).

The antibacterial activity of tested *A. tricolor* was compared with the standard drug amikacin. The cell growth

was evaluated using resazurin, an oxidation-reduction indicator. A change in colour from blue to pink indicated the growth of bacteria, and the minimal inhibitory concentration (MIC) was noted as lowest concentration of the test compound that prevented this change in colour (Figure 1). The MIC of methanolic leaf extract ranged from 5 to 0.62 mg/ml. The results from MIC indicated that *E. coli* was the most sensitive microbe to the *A. tricolor* leaf extract, being negatively affected at lowest concentration tested 0.36 (mg/ml).

## Conclusion

The results from the current study indicate that *A. tricolor* (L) leaves contained various types of compounds with potential pharmacological activity against bacterial pathogens associated with UTIs. Further research work involving more detailed *in vitro* and *in vivo* investigations is required to establish which components of the extract are biologically active in terms of antibacterial activity versus UTI causing pathogens. The isolation of bioactive components from this readily available natural resource and their utilization as potential natural antibacterial agents could be of high economic value.

## Conflict of interests

The author(s) did not declare any conflict of interest.

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