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Antimicrobial and Antioxidant Activities of Ethanolic Crude Extracts of *Turnera ulmifolia* L.

K. Kalimuthu, R. Prabakaran*, V. Preetha

Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, Tamil Nadu, India

ABSTRACT

The present study was carried out to study the antimicrobial and antioxidant potential of ethanolic leaf extract of *Turnera ulmifolia*. The antimicrobial activity was tested against five bacteria. Among the five different bacteria used, in the case of *S. typhi* the zone of inhibition is higher (30.76 mm) in 40µg/ml concentration followed by 60µg/ml concentration (24.88 mm). In the case of *E. coli* the zone of inhibition of is higher in 20µg/ml concentration (31.2 mm) In *P. aeruginosa* also the maximum inhibition zone (20.70 mm) was observed in 20µg/ml concentration followed by 40µg/ml (17.28 mm). At 20µg/ml of concentration the zone of inhibition is higher (18.30 mm) in *K. pneumonia* .Where as in *S. aureus* the percentage of inhibition is 10.16 mm in 40µg/ml concentration. The extract was effective on all the five bacteria. The higher percentage of activity in DPPH was observed in 1000µg/ml (94.59%) followed by 800µg/ml (94.28%). More absorbance (0.174) was observed in the concentration of 1000µg/ml followed by 800µg/ml concentration (0.165) in FRAP activity. Antioxidant activity and reducing power of solvent extracts was found to be dose dependent manner.

Keywords: Antimicrobial, *Turnera ulmifolia,* Concentration, DPPH, FRAP and Inhibition.

INTRODUCTION

Herbs have been used as food and for medicinal purposes for centuries. Research interest has been focused on various herbs that possess hypolipidemic, antiplatelet, antitumor, or immune-stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer. [1] Herbal medicine is now in great demand in the developing world for primary health care not because they are inexpensive but because of their better acceptability, better compatibility with the human body and minimal side effects. [2]

The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care which has compounds derived from medicinal plants. [3] Therefore, such plants should be investigated to better understand their properties, safety and efficiency. [4] In the last few years, a number of studies have been conducted in different countries to prove such efficiency. [5-6] Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant.

Natural antioxidants have been studied extensively for decades in order to find compounds protecting against a number of diseases related to oxidative stress and free radical-induced damage. Oxidative stress occurs when the production of harmful molecules called free

***Corresponding author: Dr. K. Kalimuthu,**
Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, Tamil Nadu, India;
E-mail: k_kalimuthu@rediffmail.com
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radicals is beyond the protective capability of the antioxidant defenses. On the other hand, free radicals are known to be the major cause of various chronic and degenerative diseases. Oxidative stress is associated with pathogenic mechanisms of many diseases as well as aging processes. Antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. [7-8] To date, many plants have been claimed to pose beneficial health effects such as antioxidant properties. [9-10]

Turnera ulmifolia L. (Turneraceae), a small annual herb, can be found in the north and northeast Brazilian regions, where it is considered a weed. [11] It grows preferentially in sandy soils and on hill slopes. *T. ulmifolia* L. is already known to be of medicinal value, being used popularly as an anti-inflammatory, as an expectorant, and in the treatment of several problems [12] and antibacterial. [13] Flavonoids, alkaloids, tannins and phenolic compounds were detected from this plant. [14-15] Aminoglycosides are potent bactericidal antibiotics targeting the bacterial ribosome, and the increase in cases of bacterial resistance to aminoglycosides is widely recognized as a serious health threat. [16] The main mechanisms of resistance to aminoglycosides are active efflux and enzymatic inactivation. [17] Therefore, the present study has been designed to evaluate antimicrobial and antioxidant potential of ethanol extracts of *T. ulmifolia* traditional medicinal.

MATERIALS AND METHODS

Collection of Plant materials

The fresh leaves of *Turnera ulmifolia* L. var. elegans (Otto) Urb. (= *Turnera subulata* Sm.) were collected form garden and being identified by Botanical survey of India (BSI) Coimbatore Reference No **BSI/SRC/5/23/2013-14/Tech/408.** Voucher specimen was deposited in Botany Department of Botany, Government Arts College, Coimbatore.

Preparation of extracts for antimicrobial activity

A quantity of twenty five grams of the shade dried plant leaves were weighed and then grinded with ethanol. The leaves were then soaked into 50 ml ethanol (98%) for 72 hours. Then the ethanol was allowed to evaporate in water bath. The concentrated ethanolic extracts were weighed and preserved for further use. [18]

Test Microorganisms

The microorganisms used in this study includes *Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus* obtained from the Department of Microbiology, Hindustan College of Arts and Science, Coimbatore. The bacterial strains were cultured on respective selective media and stored at 20 ± 2°C.

Preparation of inoculum

Exactly 18 hour broth culture of the test bacteria isolates was suspended into sterile nutrient broth and were standardized according to National Committee for Clinical Laboratory Standards (NCCLS) by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 CFU/ml.

Antimicrobial assay – Well diffusion method

The modified agar well diffusion method was employed to determine the antibacterial activities**.** About 0.2 ml of the standardized 24 hour old broth culture of the test organisms were spread onto sterile Muller Hinton Agar plates. These were then allowed to set. With the aid of a sterile cork borer, wells of about 6 mm in diameter were bored on the plates. Different concentrations (20µl, 40µl and 60µl) of ethanolic extracts were dispensed into the wells and then allowed to stand for about 15 minutes for pre diffusion of the extracts to occur. The plates were then incubated at 37°C for 24 hours. At the end of the incubation period, inhibition zones formed on the agar plates were observed, measured and tabulated for various bacterial strains used.

Preparation of plant samples for antioxidant activity

The plant leaves were cleaned and cut into small pieces. Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged at 10,000 rpm for 15 min and the supernatant was preserved for estimation of various parameters. The residue was re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residue was dissolved in 5ml of distilled water and stored at 4-8°C in a refrigerator for further analysis. [19]

DPPH radical scavenging activity

Scavenging activity on DPPH free radicals by the test compound was assessed according to Blois (1958). [20] Different concentrations (200, 400,600, 800, and 1000µg/ml) of the test sample was dissolved in DMSO was mixed individually with 1 mL of 0.1 mM DPPH in ethanol solution and 450µL of 50 mM Tris-HCl buffer (pH 7.4) was added. The solution was incubated at 37^{o}C for 30 min and reduction of DPPH free radicals was measured by reading the absorbance at 517 nm **(**Shimadzu, 1601). This activity is given as % DPPH scavenging and calculated according to the following equation:

% Inhibition = $[(A_B - A_A)/A_B] \times 100$,

Where A_B, absorption of blank sample, A_A, absorption of test sample.

Reducing power activity (FRAP)

The reducing power of the test compound was determined by the method of Yildirim *et. al*, 2001. [21] Different concentration (200, 400, 600, 800, 1000µg/ml) of the test compound was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixture was incubated at 50^{o}C for 30 min. 2.5 mL of 10% trichloroacetic acid (TCA) was added to the above mixture and centrifuged for 10 min at 3000 rpm. 2.5 mL of supernatant solution was mixed with 2.5 mL of

distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm.

Table 1: Antibacterial activity of ethanolic extract of *T. ulmifolia*.

Type of strain	Zone of inhibition (mm) with respect to Conc. of the ethanolic extract			
	20µg/ml	40µg/ml	60µg/ml	Control 30µg/ml
Salmonella typhi	21.20±0.61	30.76±0.79	24.88±0.49	32.36±0.67
Klebsiella pneumonia	18.30±0.38	14.56±0.32	15.36±0.61	20.71±0.83
Pseudomonas aeroginosa	20.70±0.91	17.28±0.63	16.18±0.75	37.06±1.09
Escherichia coli	31.2±0.92	28.81±0.79	25.53±0.90	42.18±0.70
Staphylococcus aureus	9.31±0.79	10.16±0.92	8.73±0.64	10.30±0.77

Table 2: DPPH activity of different concentrations of ethanolic extract of *T. ulmifolia*

S. No	Sample Name	Concentration (µg/ml)	% of inhibition
1	*Turnera ulmifolia*	200	93.16
		400	93.85
		600	94.09
		800	94.28
		1000	94.59

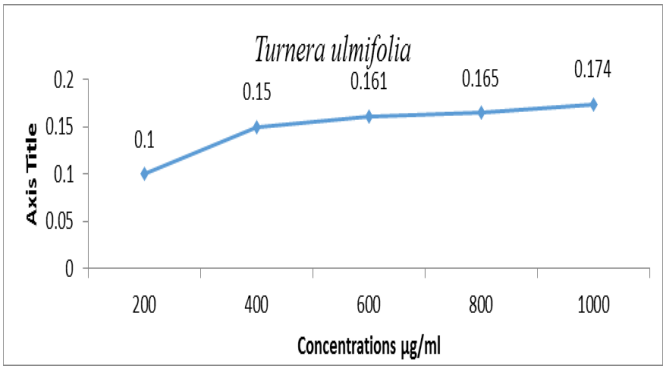


Fig. 1: FRAP Activity of different concentrations of ethanolic extract of *T. ulmifolia*

RESULTS

In the present study, the preliminary phytochemical screening of alcoholic extracts showed the presence of alkaloids, flavonoids glycosides, tannins, and phenol (unpublished data).

The antimicrobial activity of *T. ulmifolia* leaf ethanolic extract against various microbial strains with respect to various concentrations (µg/ml) was presented in the table 1. The zone of inhibition of test concentrations was compared with standard concentration of control (Rifampicin 30µg/ml). Plate 1A, B, C, D and E showed significant result of different concentration of extract and the control. Among the five different bacteria used, in the case of *S. typhi* the zone of inhibition in higher (30.76 mm) in 40µg/ml concentration (Plate 1 A) against the control (32.36 mm), followed by 60µg/ml concentration (24.88 mm). In the case of *E. coli* the zone of inhibition of is higher in 20µg/ml concentration (31.2 mm) against its control (42.18 mm) (Plate 1E) followed by 40µg/ml concentration (28.81 mm). In *P. aeruginosa* also the maximum inhibition zone (20.70 mm) was observed in 20µg/ml concentration followed by 40µg/ml (17.28 mm) (Plate 1C) against the control (37.06 mm). At 20µg/ml of concentration the zone of inhibition is higher (18.30 mm) in *K. pneumonia* against control (20.71 mm).Where as in *S. aureus* the percentage of inhibition (10.16 mm) is almost equal to the control (10.30 mm) in 40µg/ml concentration.

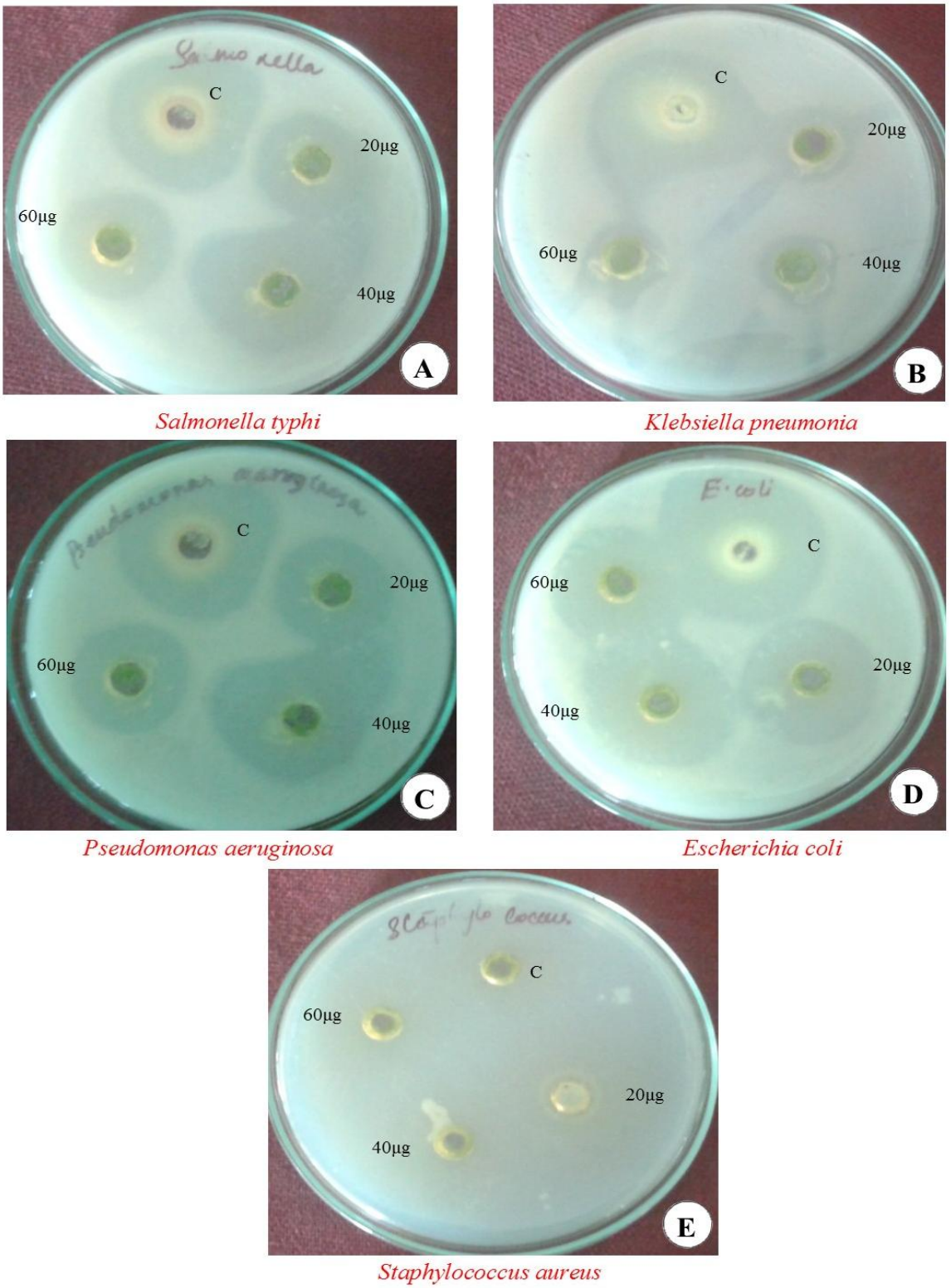
The antioxidant activity of *T. ulmifolia* leaf ethanolic extracts were assessed using DPPH and FRAP activity. The antioxidant activity of different concentration of solvent extract of DPPH showed in table 2. Among the five different concentration of extracts, the higher percentage of activity was observed in 1000 µg/ml (94.59%) followed by 800µg/ml (94.28%) and 600µg/ml (94.09%). The minimum DPPH activity is noticed in 200µg/ml (93.16%) concentration. The result of reducing power of solvent extract is presented in Fig 1. The result showed that the ethanolic extract possess potent scavenging activity of the stable free radical DPPH. In this study more absorbance (0.174) was observed in the concentration of 1000µg/ml followed by 800µg/ml concentration (0.165). In 200µg/ml concentration the absorbance is less (0.10).

DISCUSSION

Turnera ulmifolia leaf is considered to be a potent source of bioactive compounds. Many of the naturally occurring compounds present in plants have antimicrobial functions and could thus serve as a source of traditional drugs. [22] In the present study five different bacterial strains were used to study the effect of ethanolic extract of *T. ulmifolia*. Significantly results were observed in almost in all the cases when compared to the control. The maximum inhibition percentage was observed on 20µg/ml concentrations against *K. pneumonia* (18.3 mm) *P. aeruginosa* (20.70 mm) and *E. coli* (31.2 mm) when compared to the control (20.71 mm, 37.06 mm and 42.18 mm respectively), where as in *S. typhi* and *S. aureus* the maximum zone of inhibition (30.76 and 10.16 mm) was observed in the concentrations 40µg/ml against the control (32.36 mm and 10.30 mm). When compared to the control inhibition percentage of all the organisms is almost equal.

Only few articles were published focusing pharmacological activity of the genus *Turnera*. Some species of *Turnera* are widely used in folk medicine. [23] *T. ulmifolia* is used popularly as an anti-inflammatory and as an expectorant. [24] Another report the ethanolic extracts of *T. ulmifolia* against methicillin resistant *S. aureus* and confirmed the antibacterial activity. [23] As far as we know there is only one report on antibacterial activity of *T. ulmifolia.* [13]. In the present study we confirmed the antimicrobial activity of *T. ulmifolia* against five bacterial species. The present result in the case of *Pseudomonas aeruginosa* is confirmed by Sethi [13] in *P. fluorescens*. From the obtained results *T. ulmifolia* could serve as a source of plant derived natural products with antimicrobial resistance activity to be used against microbes.

PLATE – 1. Antibacterial activity of ethanolic extract of *T.ulmifolia*



Turnera ulmifolia ethanolic leaf extract significantly scavenged the DPPH radical and the results are given in the table 2. The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activity of several natural compounds such as extract of plants in a relative short time. [25] In the present study the extract have exhibited concentration dependent radical scavenging activity ie. higher the concentration more scavenging activity. DPPH, the natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of pro oxidant metals, quenches of singlet oxygen. [26] DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to

become stable diamagnetic molecule. [27] The present results confirmed the presence of scavenging activity for the *T. ulmifolia* leaf extract. Figure 1 represents the reducing power of ethanolic extracts *T. ulmifolia*. In this study, the absorbance was increased with the increasing concentration of ethanolic leaf extract. Which is due to the reducing power of the extract. An increasing in the absorbance revealed the reducing power of extract. The antioxidant activities have been reported to be the concomitant development of reducing power. [28] This positive results of our study on antioxidant activity are in justification with the medicinal importance of plants as naturally occurring antioxidants.

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