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Antimicrobial, Antioxidant and Anticancer Activities of *Turnera ulmifolia* (Yellow Alder) Callus

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Authors' contributions

This study was carried out in collaboration between all authors. Author KK designed the study and corrected the manuscript. Author RP wrote the first draft of the manuscript. Author VC analysis the different activities of the callus. Author PS carried out callus induction experiment and author YSJ collected the literature and identified the species of plant. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The current work aims to study the effect of ethanolic and methanolic extracts of *Turnera ulmifolia* callus on antimicrobial, antioxidant and anticancer activities.
Study Design: The Five grams of leaf derived 30 days old callus powder was soaked in 10 ml of ethanol and methanol overnight and then filtered through Whatman filter paper No. 41 along with 2 gms sodium sulphate to remove the sediments and traces of water in the filtrate. Before filing the filter paper along with sodium sulphate is wetted with ethanol. The filtrate was then concentrated by bubbling nitrogen gas in to solution and reduces the volume to one ml.
Place and Duration of Study: Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, Tamil Nadu, India.

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Methodology: Ethanolic and methanolic callus extracts were evaluated for antimicrobial, antioxidant (DPPH & FRAP) and anticancer activities (MTT assay).
Results: The ethanolic and methanolic callus extracts of *Turnera ulmifolia* showed rich sources of antimicrobial, antioxidants, anticancer and can be incorporated into the drug formulations.
Conclusion: This study conformed the use of callus instead of wild plant for herbal medicine. Further studies are needed for isolation and identification of compounds.

Keywords: Antimicrobial; antioxidant; anticancer; Turnera ulmifolia; breast cancer cell.

1. INTRODUCTION

India is in a unique position to be a major global player in medicine field because of our rich heritage of flora and centuries of experience in the use of traditional system. Due to the side effect and higher cost of allopathic medicine, the plant based medicines has been over wheming in the recent times. Increasing the usage of herbal medicine, the Global market for medicinal plants is also increasing rapidly. The plant based drugs have the advantage of being simple, effective and broad spectrum of activity. Rapid increase in the demand poses a threat to their population in the natural habitats. In this regard, *in vitro* culture can provide alternative source of supply that will reduce the pressure of collecting from the wild.

Turnera ulmifolia Linn. or Damiana (Turneraceae) it 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 [1,2]. The plant has an antioxidant, antimicrobial activity [3]. It also has phytoconstituents like toosendanin, phenylmethanone phytfluene etc [4]. Researchers have detected flavonoids, alkaloids, tannins, and phenolic compounds in preparations from this plant [5,6,4].

T. ulmifolia is used as tonic, against indigestion and bronchitis in Mexico (Martinez. 1969). It is also used as a remedy against swellings, pains, fever, respiratory and pulmonary disorders, laxative, carminative, tonic expectorant, abortive agent and fertility related applications [7-11]. *Turnera ulmifolia* is used as expectorant, antidiabetic, anticancer agent [12-14].

To the best of our knowledge the antioxidant antimicrobial and anticancer properties of leaf callus extract of this plant is not yet reported. Hence to reduce the pressure on collection of wild plant it was worth to evaluate the antioxidants, antimicrobial and anticancerous potential of callus of this plant.

2. MATERIALS AND METHODS

2.1 Source of Plant Materials

Plants of *Turnera ulmifolia* L. var. elegans (Otto) Urb. (= *Turnera subulata* Sm.) were collected from garden and being identified by Botanical Survey of India (BSI) Coimbatore (Reference No: BSI/SRC/5/23/2013-14/Tech/408) and maintained in earthen pots in shade house at Government Arts College, Coimbatore.

2.2 Extraction from *in vitro* Callus

The leaf derived 30 days old callus collected from our laboratory was air dried and powdered and stored in room temperature. Five grams of powder was soaked in 10 ml of ethanol and methanol overnight and then filtered through Whatman filter paper No. 41 along with 2 gms sodium sulphate to remove the sediments and traces of water in the filtrate. Before filing the filter paper along with sodium sulphate is wetted with ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution and reduces the volume to one ml [15,16].

2.3 Antimicrobial Activity

2.3.1 Test microorganisms

The test organisms used were clinical isolates *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae.* The human fungal pathogens like *Candida albicans and Trichoderma viride,* which were obtained from Department of Microbiology, Hindusthan College of Arts and Science Coimbatore. The bacterial and the fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively.

2.3.2 Growth and maintenance of test microorganism for antimicrobial studies

The bacterial cultures were maintained on nutrient broth (NB) at 37℃ and fungus was

maintained on Potato dextrose agar (PDA) at 28℃.

2.3.3 Preparation of inoculum

The gram positive bacteria *Streptococcus pyogenes*, *Staphylococcus aureus* and gram negative bacteria *E. coli* and *Klebsiella pneumoniae* were pre-cultured in nutrient broth overnight in a rotary shaker at 37℃, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610}nm). The fungal inoculums *Candida albicans*, *Trichoderma viride* were prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595}nm) to obtain a final concentration of approximately 10^5 spores/ml.

2.3.4 Antibacterial activity [17]

The *in vitro* callus extracts of *T. ulmifolia* were tested against bacteria by the well diffusion method. Different concentration of the extracts (20, 40, and 60 µg/ml) was prepared by reconstituting with ethanol and methanol. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells/ml) with the 24 h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Streptomycin (10 µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37℃ for 24 hrs. The diameters of the inhibition zones were measured in mm.

2.3.5 Antifungal activity [18]

The antifungal activities of ethanol and methanol *in vitro* callus extracts were tested by well diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 20, 40 and 60 µg/ml concentrations of the extracts were placed on test organism-seeded plates. Streptomycin (10 µg) used as positive control. The activity was determined after 72 hrs of incubation at 28℃. The diameters of the inhibition zones were measured in mm.

2.4 Antioxidant Activity

2.4.1 DPPH radical scavenging activity [19]

Various concentrations of ethanol and methanol extracts of the sample (0.5-2.5 mg/ml) were mixed with 1.0 ml of ethanolic and methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture were shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as control. The percentage of inhibition in DPPH radical scavenging activity was calculated as follows;

% Inhibition = A0- A1/A0 X 100

2.4.2 FRAP assay [20]

The stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ and 0.3 M acetate buffer (pH 3.6) was prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution and 25 ml acetate buffer. It was freshly prepared and warmed to 37℃. Then, 900 µl FRAP reagent was mixed with 90 µl water and 30 µl test sample/ ethanol/ methanol/ distilled water/ standard antioxidant solution. The reaction mixture was then incubated at 37℃ for 30 minutes and the absorbance was recorded at 593 nm. An intense blue colored complex were formed when ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex were reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded. The calibration was plotted with absorbance at 593 nm vs concentration of ferrous sulphate in the range 0.1 mM both aqueous and ethanol solutions. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidants L-ascorbic acid.

2.5 *In vitro* Cytotoxicity Assay

2.5.1 Methodology

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37℃, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

2.5.2 Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted by tryphan blue exclusion assay using a hemocytometer. The cell suspension was diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37℃, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dispersed in phosphate buffered saline (PBS) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following drug addiction the plates were incubated for an additional 48 h at 37℃, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

2.5.3 MTT assay

3-[4,5-dimethylthiazol-2-yl]2, 5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15 µl of MTT (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37℃ for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC_{50} was determined using Graph Pad Prism software.

3. RESULTS

3.1 Antimicrobial

The antimicrobial activity of *T. ulmifolia* ethanolic and methanolic callus extracts against various microbial strains and standard Streptomycin with respect to various concentrations (20, 40 and 60 µl) were presented in Tables 1 & 2. From the Table values, it was reported that the test extracts and standard Streptomycin have significant antimicrobial action against all the microbial stains *Escherichia coli, Streptococcus pyogenes*, *Staphylococcus aureus, Klebsiella pneumonia* and *Trichoderma viride.*

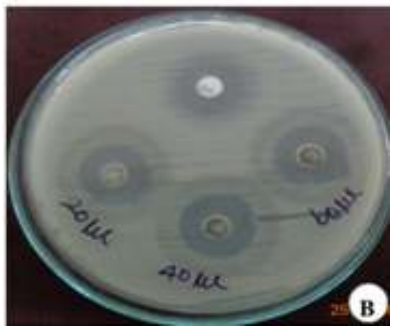
The zone of inhibition of test concentration was compared with standard Streptomycin (10 µg/ml). Figs.-1 A, B, C, D, E and F shows the zone of inhibition of different concentrations of ethanolic extract along with Streptomycin. Among the four different bacterial strains used, higher zone of inhibition (18 mm) was observed in 60 µl concentration of ethanol in *S. aureus* against the control (8 mm) followed by 20 µl and 40 µl concentrations (16 mm and 15.5 mm respectively). In the case of *E. coli, S. pyogenes* and *K. pneumoniae,* the zone of inhibition is higher in 60 µl concentration (12 mm, 11.5 mm and 10.5 mm respectively) against control (6, 8 and 8 mm) respectively. But the effect of ethanol extracts on fungal stains was less (8 and 6 mm) when compared to standard (10 and 8 mm).

Table 1. Anti-microbial activity of ethanol extract of leaf callus of *T. ulmifolia*

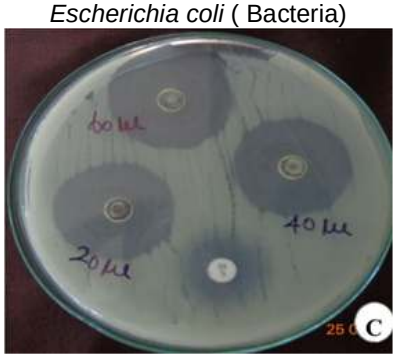
S.no	Pathogens	Ethanol extract zone of inhibition (mm)			Standard (streptomycin)
		20 µl	40 µl	60 µl	
1.	*Streptococcus pyogenes*	10	11	11.5	8
2.	*Staphylococcus aureus*	16	15.5	18	8
3.	*Escherichia coli*	8	12	12	6
4.	*Klebsiella pneumoniae*	8.5	10	10.5	8
5.	*Candida albicans*	4	5.5	8	10
6.	*Trichoderma viride*	5.5	5.5	6	8

The result of Table 2 revealed that all the four bacterial strains were found to be resistant to standard Streptomycin but the methanolic extract was very effective control in the growth of the four bacterial strains. Interestingly the zone of inhibition was found to be increased by 10.5 to 22 mm in the four strains of bacteria in 60 µl concentration against standard. In the present study the zone of inhibition (22 mm) is higher in *Klebsiella pneumoniae* at 60 µl concentration followed by 40 µl concentration (20 mm) and 20 µl concentration (18 mm) against control (8 mm) (Fig- 2 B). In the case of *S. aureus* the maximum zone of inhibition (20 mm) was observed in 60 µl followed by 40 µl concentration (18 mm) and 20 µl concentration (16 mm) against the value of standard Streptomycin(8 mm) (Fig- 2 C). In *E. coli* and *S. pyogenes* also the maximum inhibition zone (12, 10.5 mm respectively) was observed in 60 µl concentration followed by 40 µl concentration (8.5, 10 mm respectively) (Figs. - 2 A and D). Here the inhibition zone observed in Streptomycin was 6 mm. In all the four strains of bacteria the concentration increases the zone of inhibition also increases. Whereas, the effect of the methanol extract of callus against two fungal strains *C. albicans* and *T. viride* were equal (8, 10 mm) to the standard Streptomycin (8, 10 mm respectively) (Figs. 2 E and F).

Escherichia coli (Bacteria)



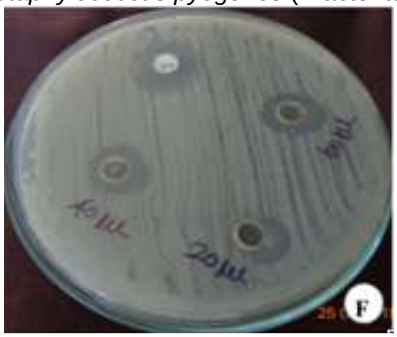
Klebsiella pneumoniae (Bacteria)



Staphylococcus aureus (Bacteria)

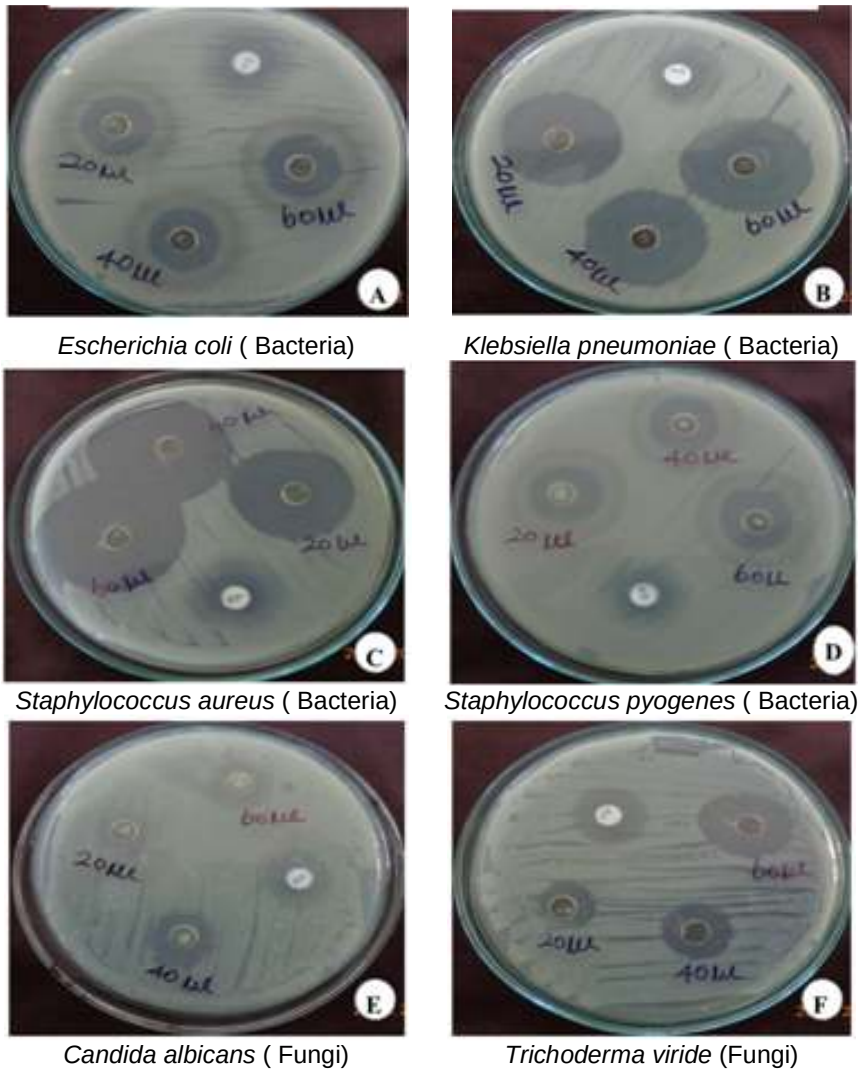
Staphylococcus pyogenes (Bacteria)

Candida albicans (Fungi)



Trichoderma viride (Fungi)

Fig. 1. Antimicrobial activity of ethanolic leaf callus extract of *Turnera ulmifolia*



Escherichia coli (Bacteria) *Klebsiella pneumoniae* (Bacteria)

Staphylococcus aureus (Bacteria) *Staphylococcus pyogenes* (Bacteria)

Candida albicans (Fungi) *Trichoderma viride* (Fungi)

Fig. 2. Antimicrobial activity of methanolic leaf callus extract of *Turnera ulmifolia*

Table 2. Anti-microbial activity of methanol extract of leaf callus of *T. ulmifolia*

S.no	Pathogens	Methanol extract zone of inhibition (mm)			Standard (streptomycin)
		20 µl	40 µl	60 µl	
1.	*Streptococcus pyogenes*	10	10	10.5	6
2.	*Staphylococcus aureus*	16	18	20	8
3.	*Escherichia coli*	8.5	8.5	12	6
4.	*Klebsiella pneumoniae*	18	20	22	8
5.	*Candida albicans*	5.5	8	8	8
6.	*Trichoderma viride*	6	8	10	10

3.2 Antioxidant

3.2.1 DPPH scavenging activity

The antioxidant activities of *T. ulmifolia* ethanolic and methanolic callus extracts were assessed by using 2,2-diphenylpicryl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power assay (FRAP). The DPPH activity of different concentration of solvent extracts (1 mg/ml to 5 mg/ml) along with standard ascorbic acid is presented in the Table 3. Among the five

different concentration of extracts tested, the higher percentage of inhibition (56.29±0.94) was observed in 5 mg/ml of methanolic extract followed by ethanolic extract (53.27±0.99), against the standard ascorbic acid (74.25±0.56) (Fig. 3). The minimum DPPH activity 15.25±1.02 and 12.56±0.94 was noticed in 1 mg/ml concentration of ethanolic and methanolic extract respectively. The dose titration curves allowed determination of IC_{50} for the ethanolic and methanolic callus extracts towards DPPH scavenging activity. The extracts demonstrated dose dependent DPPH scavenging activity effects with IC_{50} values 4.36 mg/ml, 4.50 mg/ml and 3.10 mg/ml in methanolic, ethanolic extracts and standard ascorbic acid respectively. The result showed that the both ethanolic and methanolic extracts possess almost similar potent scavenging activity of the stable face radical DPPH.

3.2.2 FRAP activity

FRAP activity of ethanolic and methanolic extracts of *T. ulmifolia* callus were assayed by using five different concentrations (1 mg/ml to 5 mg/ml). The result of reducing power of solvent extracts is presented in Table 4 and Fig. 4. The absorbance was increased with the increasing concentrations of both methanolic and ethanolic callus extracts. In this study more absorbance (0.62±0.02 and 0.58±0.02) was observed in the concentration of 5 mg/ml of methanolic and ethanolic extracts respectively. It is followed by 4 mg/ml concentration with 0.50±0.01 and 0.45±0.01 absorbance in methanolic and ethanolic extracts respectively, where as in standard ascorbic acid the absorbance was 0.71±0.02 in 5 mg/ml concentration and 0.65±0.01 in 4 mg/ml concentration (Table 4, Fig. 4). The absorbance value of methanolic and ethanolic callus extract was almost equal.

3.3 Anticancerous Activity

To evaluate the cytotoxic activity, the extracts were tested with various doses on the human breast cancer cell line (MCF 7) and normal cells. After 48 hours, cell viability was analyzed using end point MTT assay. Some cells were beginning to detach from the plate and become rounded after 48 hours of treatment (18.75-300 µg/ml). All the samples used exhibition different levels of cytotoxicity like cell rounding, shrinkage, aggregation and cell death depending upon the concentration of the extracts (Figs. 5 and 6). At the concentration of 300 µg/ml the tested samples produced more cytotoxic effect as evidenced by the increased number of dead cells (47.00 and 33.64, methanolic and ethanolic extracts respectively). The callus extracts exhibited greater cytotoxicity on human breast cancer cells with IC_{50}> 300 mg/ml. The methanolic and ethanolic callus extracts induced cell cytotoxicity in a concentration dependent manner (Figs. 5 and 6).

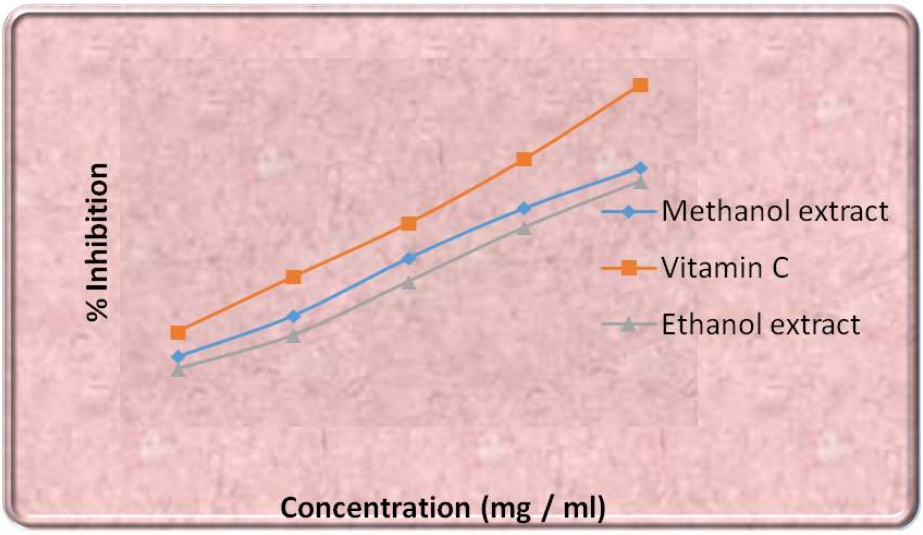


Fig. 3. Antioxidant (DPPH assay) activity of ethanolic and methanolic extracts of leaf callus of *Turnera ulmifolia*

Table 3. Antioxidant (DPPH scavenging) activity of ethanolic and methanolic leaf callus extracts of *T. ulmifolia*

S. no.		% inhibition				
		1 (mg/ml)	2 (mg/ml)	3 (mg/ml)	4 (mg/ml)	5 (mg/ml)
1	Methanol extract	15.25±1.02	24.15±0.47	36.67±0.77	47.54±0.86	56.29±0.94
2	Ethanol extract	12.56±0.94	19.88±0. 76	31.52±0.59	43.26±0.46	53.27±0.99
3	Ascorbic acid	20.66±0.49	32.58±0.80	44.25±0.76	58.16±0.83	74.25±0.96

The experiment was conducted in triplicates (n=3)

Table 4. Antioxidant (FRAP assay) activity of ethanolic and methanolic leaf callus extracts of *T. ulmifolia*

S. no		Absorbance at 593 nm				
		1 (mg/ml)	2 (mg/ml)	3 (mg/ml)	4 (mg/ml)	5 (mg/ml)
1	Methanol extract	0.19±0.01	0.29±0.02	0.38±0.009	0.50±0.01	0.62±0.02
2	Ethanol extract	0.14±0.02	0.22±0.01	0.34±0.03	0.45±0.01	0.58±0.02
3	Ascorbic acid	0.38±0.012	0.45±0.01	0.53±0.02	0.65±0. 01	0.71±0.02

The experiment was conducted in triplicates (n=3)

Table 5. Anticancer activity of ethanol extracts of leaf callus of *T. ulmifolia*

Concentration	18.75 µg	37.5 µg	75 µg	150 µg	300 µg	Control
Absorbance	0.311	0.31	0.293	0.24	0.215	0.313
	0.314	0.313	0.304	0.249	0.209	0.324
	0.319	0.305	0.307	0.241	0.207	0.314
Average	0.314	0.309	0.301	0.243	0.210	0.317
Cell Inhibition %	0.73	2.41	4.94	23.23	33.64	-

IC50 value >300 µg/ml

Table 6. Anticancer activity of methanol extracts of leaf callus of *T. ulmifolia*

Concentration	18.75 µg	37.5 µg	75 µg	150 µg	300 µg	Control
Absorbance	0.319	0.304	0.268	0.200	0.175	0.313
	0.315	0.309	0.261	0.208	0.168	0.324
	0.313	0.302	0.259	0.194	0.161	0.314
Average	0.315	0.305	0.262	0.200	0.168	0.317
Cell Inhibition %	0.42	3.78	17.13	36.69	47.0	-

IC50 value >300 µg/ml

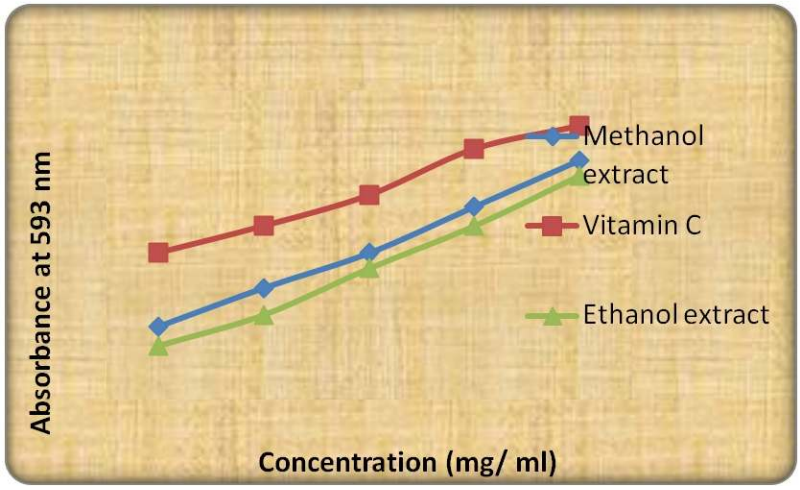


Fig. 4. Anti-oxidant (FRAP assay) activity of ethanolic and methanolic extracts of leaf callus of *Turnera ulmifolia*

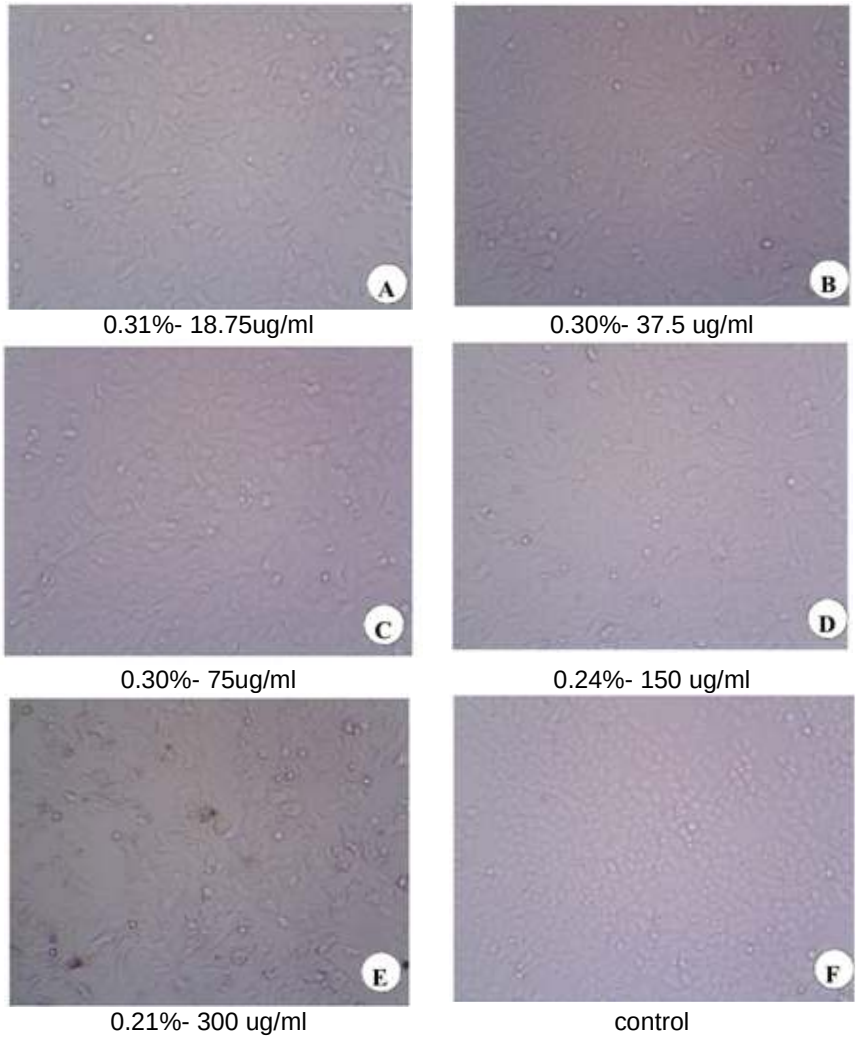


Fig. 5. MTT assay (cytotoxic activity) of the ethanolic callus extract of *Turnera ulmifolia*

4. 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. Naturally occurring compounds presents in plants many have antimicrobial functions and could thus serve as a source of traditional drugs [21]. Four different bacterial stains and two fungal pathogens were used in the present work to study the effect of ethanolic and methanolic callus extracts of *T. ulmifolia*. The test extracts and standard streptomycin have significant antimicrobial action against all the microbial strains.

Among the four different bacterial strains used, higher zone of inhibition (18 mm) was observed in 60 µl concentration of ethanol in *S. aureus* against the control (8 mm) followed by 20 µl and 40 µl concentrations (16 mm and 15.5 mm respectively). In the case of *E. coli, S. pyogenes* and *K. pneumoniae,* the zone of inhibition is higher in 60 µl concentration (12 mm, 11.5 mm and 10.5 mm respectively) against control (6, 8 and 8 mm) respectively. But the effect of ethanol extracts on fungal stains was less (8 and 6 mm) when compared to standard (10 and 8 mm).

Very few articles were published focusing on pharmacological activity of the genus *Turnera* [2,22,23,4]. In *T. ulmifolia* the effect of ethanolic extracts against methicillin resistant *Streptococcus aureus* conformed the

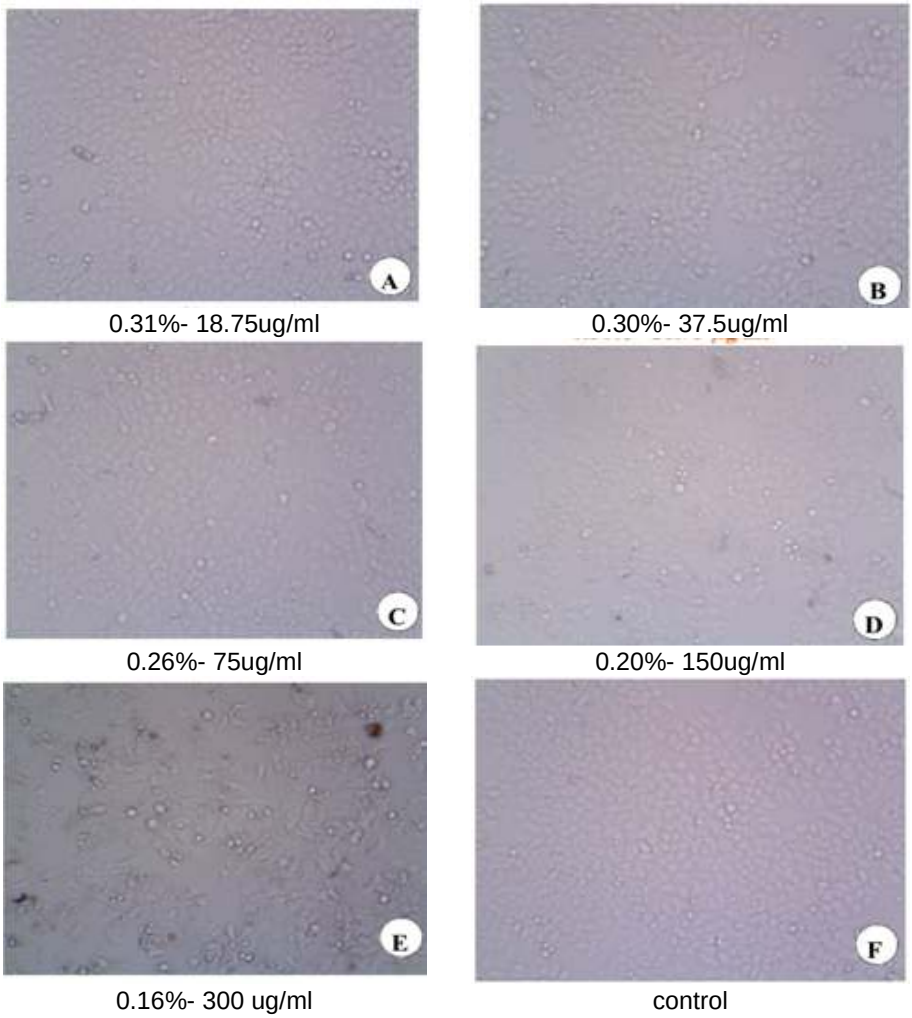


Fig. 6. MTT assay (Cytotoxic activity) of the methanolic callus extract of *Turnera ulmifolia*

antimicrobial activity [22]. Poonam Sethi [23] and Kalimuthu et al. [3] also reported the antibacterial activity of *T. ulmifolia*. As far as we know there is no report on antimicrobial activity of the callus of *T. ulmifolia*. All the reports are from *in vivo* plants extracts. In the present result confirmed the antimicrobial activity of *T. ulmifolia* callus extracts against 4 bacterial and 2 fungal strains. Among the two extracts (ethanol and methanol) tested against bacterial and fungal strains, both the extracts have almost similar effect except the strain *K. pneumoniae*. Our callus extracts results were comparable to that of earlier reports published by using *in vivo* plant extracts. From the obtained results this plant could serve as a source of plant derived natural products with antimicrobial activity to be used against microbes.

FRAP activity of ethanolic and methanolic leaf callus extracts of *T. ulmifolia,* the activity of these extracts to reduce Fe^{3+} to Fe^{2+} was determined and compared with that of standard ascorbic acid. Hence, the absorbance was increased with the increase concentrations of the extracts and decreased when compared to that of standard ascorbic acid. The present finding agrees well with the earlier report on antioxidant activity of ethanolic leaf extract of *T. ulmifolia* [3]. The present study confirmed that the *in vivo* leaf and leaf callus might have similar therapeutic properties because of the similar activities.

The value of medicinal plants lies in the potential access to extremely complex molecular structures that would be difficult to synthesize in the laboratory [26]. The limitations of the

available cancer management modalities create an urgent need to screen and generate novel molecules. Despite, well-documented illustrations of phytochemicals being used for prevention and treatment of cancer, their importance in modern medicine remains underestimated. Plants are the storehouse of "pre-synthesized" molecules that act as lead structure, which can be optimized for new drug development. In practice, a large number of cancer chemotherapeutic agents that are currently available in the market can be traced back to their plant resource [27]. Leaf derived callus ethanolic and methanolic extracts of *T. ulmifolia* were tested for their antiproliferative activity. *In vitro* antiproliferative property of the extracts of *T. ulmifolia* was minimum in the present results. Morphological analysis of the cells exhibited that the extract treatment had initiated apoptotic mechanism to trigger cell death. At 300 µg/ml concentration over 17%, 25% of the cell population had been rendered apoptotic by both the extracts respectively through flow cytotoxic observation. In general both the extracts showed very less inhibition percentage. So it is confirmed that the leaf derived callus was having very less anticancerous activity.

5. CONCLUSION

In this report the antimicrobial potential of ethanolic and methanolic callus extracts of *T. ulmifolia* were evaluated against pathogenic microbes and both the extracts were found active against the strain of bacteria and fungi. Therefore these extracts could be used for antimicrobial drug development. Further these extracts showed strong antioxidant activity in both DPPH & FRAP in dose depending manner. It also had profound cytotoxic activity against human breast cancer cell line. Together, our finding showed that the callus can be used for the isolation and evaluation of bioactive compounds. This will save the wild plant from collection for herbal remedy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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