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REGULAR ARTICLE

ANTIMICROBIAL, ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF *TECOMA STANS* (L.) JUSS. EX KUNTH

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SUMMARY

The ethanol, methanol and water extracts of *Tecoma stans* effective against tested bacteria *(Pseudomonas fluorescens*, *Clavibacter michiganensis* sub sp. *michiganensis*, *Xanthomonas axanopodis* pv. *malvacearum*, *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*) and fungi (all species of *Aspergillus* and *Alternaria*). Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, phenols, steroids, anthraquinones and tannins. The three extract fractions have showed highest total Phenolic content (177-216 mg gallic acid equivalent/g). These three solvent fractions possessed strong radical scavenging activity from FRAP and DPPH. It was ranged from 1433.75 to 3841.17 g/ml. The results indicate that this plant is a potential candidate to be used as an antimicrobial and antioxidant.

Key words: *Tecoma stans*, Antimicrobial, antioxidant, phytochemicals

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1. Introduction

The increase in prevalence of multiple drug resistance has showed down the development of new synthetic antimicrobial, antioxidative drugs and the new drug is necessary to search for new antimicrobial, antioxidant and anti-inflammatory from alternative sources. Phytochemicals from medicinal plants showing antimicrobial and antioxidant activities have the potential of filling this need because of structures are different from those of the more studied and their of the more action may too very likely differ (Fabricant and Fanworth, 2001). In this growing interest, many of the Phytochemical bioactive compounds from a medicinal plants have shown many pharmacological activities (Prachayasittikul et al., 2008; Chen et al., 2008; Pesewu et al., 2008; Turker and Usta, 2008). Screening of various bioactive compounds from plants has lead to the discovery of new medicinal drug which have efficient protection and treatment roles in against various diseases (Kumar et al., 2004; Sheeja and Kuttan, 2007; Mukherjee et al., 2007). The rapid emergence of multiple drug resistance strains of pathogens to current antimicrobial agents has generated an urgent intensive for new antibiotics from medicinal plants. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide (Kaur and Arora, 2009; Mothana et al., 2009; Adedapo et al., 2009a). Free radicals which have one or more unpraired electrons (superoxide, hydroxyl, peroxyl) are produced in normal or pathological cell metabolism and the compounds that can scavenge free radicals have great potential in ameliorating the diseases and pathological cells (Halliwell, 1995; Squadriato and Peyor, 1998; Gulcin et al., 2001). Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. Free radicals or Reactive Oxygen Species

(ROS) are produced *in vivo* from various biochemical reactions and also from the respiratory chain as a result occasional challenges. These free radicals are the main culprits in lipid peroxidation. Plants congaing bioactive compounds have been reported to possess strong antioxidant properties.

Tecoma stans (Bignoniaceae) known as yellow elder is an erect shrub or small tree. The plant has been used for a variety of purposes in herbal medicine, treating diabetes and digestive problems. Extracts from *Tecoma stans* leaves have been found to be inhibit the growth of the yeast infection. Marzouk et al. (2006) have studied the anticancer activity of *Tecoma stans* and antioxidant constituents. Alanso-Castro et al. (2010) have reported that the *Tecoma stans* extracts exhibited antidiabetic activity. Senthilkumar et al. (2010) have reported that the extracts having antibacterial activity on human pathogenic bacteria.

In present study was aimed to examine the total Phenolic content and Phytochemical analysis of ethanol, methanol and water extract of *Tecoma stans* were screened for antimicrobial and antioxidant properties using standard methods. The findings from this work may add to the overall value of the medicinal potential of the plant.

2. Materials and Methods

The plant was collected in November 2009 from our college campus (Shridevi Institute of Engineering & Technology, Sira Road, Tumkur, Karnataka, India). The plant was identified by their vernacular names and later it was compared with the herbarium of Department of Studies in Botany, Manasa Gangothri, University of Mysore, Mysore and Government Ayurvedic College, Mysore, India.

Extract Preparation

Plant parts were air dried at room temperature for 4 weeks to get consistent weight. The dried parts were later ground to powder. 100 g of wet and dried samples were extracted with distilled water, ethanol and methanol (60^0-80^0C, 200ml) separately for 2 days in water both with a shaking attachment. The extract was lyophilized under 5 µm Hg pressure and stored at -20^0C. The experimental were carried out using an appropriate amount of lyophilized material.

Phytochemical analysis

Phytochemical analysis was carried out for saponins, flavonoids, steroids, phenol, anthroquinone, alkaloids (Obdoni and Ochuko, 2001) and tannins (Kaur and Arora, 2009) were performed as described by the authors. Wagner's and Heger's reagents was used for alkaloid foam test for saponins, Mg-HCl and Zn-HCl for flavonoids, acetic anhydride and sulphuric acid for steroids, chloride and gelatin for tannins, ferric chloride for phenol, hexane and diluted ammonia for anthraquinones test. All these experiments were carried out for distilled water, ethanol and methanol extracts individually.

Determination of total Phenolic content

Total Phenolic Content (TPC) in extracts was determined by Folin-Ciocalteu's colorimetric method as described by Adedapo et al. (2009b). Each solvent extracted solution (0.3ml in triplicate) was mixed with 1.5 ml of 10% Folin-Ciocalteu's reagent and 1.2 ml of 7.5% (W/V) sodium carbonate. The mixture was kept in the dark for 30 min and absorbance was measured at 765 nm. Quantification was done on the basis of a standard curve of gallic acid. The results were expressed as gallic acid equivalent (GAE) i.e. mg gallic acid/100ml. All tests were performed in triplicate.

Determination of antimicrobial activity
Antimicrobial assay

Pseudomonas fluorescens, *Clavibacter michiganensis* sub sp. *michiganensis*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axanopodis* pv. *malvacearum* and strains of *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacteria were obtained from stock cultures presented at -80°C at Department of Studies in Applied Botany, Seed pathology and Biotechnology, University of Mysore, Manasa Gangothri, Mysore, Karnataka, India and Department of Studies in Biotechnology and Microbiology, Bangalore University, Gnana Bharathi,

Bangalore, India respectively. Two Gram positive bacteria tested were *Clavibacter michiganensis* sub sp. *michiganensis*, *Staphylococcus aureus* and six Gram negative bacterias tested were *Pseudomonas fluorescens*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axanopodis* pv. *malvacearum*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. All bacteria were grown on nutrient agar media.

Fungi (*Aspergillus flavus, Aspergillus niger, Alternaria carthami, Alternaria helianthi, Cercospora carthami, Fusarium solani, Fusarium oxysporum, Fusarium verticilloides* and *Nigrospora oryzae* were obtained from Department of Studies in Applied Botany, Seed Pathology and Biotechnology, University of Mysore, Manasa Gangothri, Mysore, Karnataka, India and Department of Studies in Microbiology, Bangalore University, Gnana Bharathi, Bangalore, India respectively. All fungi were grown on potato dextrose agar medium.

Paper disc method

Diameter of zone of inhibition was determined using the paper disc diffusion method as described by Lai et al. (2009) and Adedapo et al. (2008). A swab of the bacteria or fungi suspension containing $1x10^8$ CFU/ml was spread on to Petri plates containing nutrient agar media separately. Each solvent extracts were dissolved in each solvent to final concentration of 10mg/ml. Sterile filter paper discs (6mm in diameter) impregnated with 1mg of plant extracts were placed on culture plates separately for bacteria and fungi. The plates were incubated at 37°C for 24h. The standard chloromphenicol (10µg) for bacteria and carbendazim for fungi discs were used as positive controls. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs. The assay was repeated thrice and mean of three experiments was recorded.

Determination of antioxidant activity

In order to investigate the antioxidant properties of the examined extracts, ferric ion reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

FRAP assay

FRAP reagents was freshly prepared by mixing 25 mL acetate buffer (300 mM, pH 3.6), 2.5 mL 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) solution (10 mM TPTZ in 40 mM/L HCl) and 2.5mL $FeCl_3$ (20 mM) water solution. Each sample (150 µL) (0.5 mg/mL) dissolved in methanol was added in 4.5 mL of freshly prepared FRAP reagent and stirred and after 5 min, absorbance was measured at 593nm, using FRAP working solution as blank (Szollosi and Szollosi Varga, 2002; Tomic et al., 2009). A calibration curve of ferrous sulfate (100-1000 µmol/L) was used and results were expressed in µmol Fe^{2+}/mg dry weight extract. The relative activity of the samples was compared to L-ascorbic acid and BHT. The assay was repeated thrice for each solvent extracts.

DDPH radical assay

The effect of different solvent extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). DPPH solution was freshly prepared by dissolve 24mg DPPH in 100ml of each solvent, stored at -20^{0}C before use. 150µl of sample (10µl sample + 140µl distilled water) is allowed to react with 2850µl of DPPH reagent (190µl reagent + 2660µl distilled water) for 24h in the dark condition. Absorbance was measured at 515nm. Standard curve is linear between 25 to 800µM ascorbic acid. Results expressed in µm AA/g fresh mass. Additional dilution needed if the DPPH value measured will over the linear range of the standard curve. Mix 10ml of stock solution in a solution of 45ml of solvents, to obtain an absorbance of 1.1±0.02 units at 517nm using spectrophotometer (Katalinic et al., 2006). All determinations were performed in triplicate. The percentage inhibition of DPPH radical by the samples was calculated according to formula of Yen and Duh (1994),

% inhibition= [{Abs $_{control}$- Abs $_{sample}$}/Abs $_{control}$] x 100,

Where Abs $_{control}$ is the absorbance of the DPPH radical+ ethanol, Abs $_{sample}$ is the absorbance of DPPH radical+ sample extract/standard.

Statistical analysis

Analysis of variance (ANOVA) was used to determine the significance of difference between treatment groups ($p<0.05$). Means between treatment groups were compared for significance using Duncan's new Multiple Range post test.

3. Results

Antimicrobial assay

The antimicrobial activities of methanol and ethanol extracts of *T. stans* gave different zones of inhibition on the organisms tested (Table 1). The methanolic and ethanolic extracts inhibited the growth of all most all the isolates of bacteria and fungi. The methanol and ethanol extract showed more potent against *E. coli*, *Xanthomonas axanopodis* pv. *malvacearum*, *Clavibacter michiganensis* sub sp. *michiganensis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluorescens* and moderate activity observed in *Xanthomonas oryzae* pv. *oryzae*. All the solvent extracts exhibited high activity on all species of *Aspergillus* and *Alternaria.* All the extracts did not showed any effect on species of *Fusarium* and *Nigrospora oryzae*.

Table 1. Zone of inhibition (in mm) of antimicrobial activity y disc diffusion method using different solvent extract of *Tecoma stans*

Microorganisms	Samples			
	Methanol	Ethanol	water	chloromphenicol
Bacterial pathogens				
Klebsiella pneumonia	10±1	9±1	7±1	18±
Escherichia coli	15±2	14±2	2±1	20±
Staphylococcus aureus	11±1	11±1	2±1	18±2
Pseudomonas aeruginosa	10±1	9±1	6±1	18±
Pseudomonas fluorescens	9±1	9±1	4±1	21±
Clavibacter michiganensis sub sp. *michiganensis*	13±2	13±2	6±1	16±
Xanthomonas oryzae pv. *oryzae*	6±1	5±1	2±1	16±
Xanthomonas axanopodis pv. *malvacearum*	15±2	16±2	6±1	22±
Fungal pathogens				Carbendazim
Aspergillus niger	16±2	14±2	5±1	21±
Aspergillus flavus	16±2	11±2	2±1	21±
Alternaria carthami	9±1	8±1	4±1	19±
Alternaria helianthi	6±1	6±1	2±1	21±
Cercospora carthami	8±1	6±1	4±1	21±
Fusarium solani	2±1	2±1	2±1	19±
Fusarium verticilloides	2±1	2±1	2±1	20±
Nigrospora oryzae	4±1	3±1	2±1	20±

+: Presence; -: absence, repeated the each experiments three times for each replicates

Phytochemical analysis

The phytochemical screening showed that the different solvent extracts of *T. stans*, the tannin, flavonoids, phenol, alkaloids, steroids, anthraquinones and saponins were present in all solvent extracts. The phytochemicals strongly present in the ethanol and methanol extracts. But the water extract yielded less quantity of phytochemicals (Table-2).

Table 2: Phytochemical analysis for the different solvent extracts of *T. stans*

Extracts	Alkaloids	Flavonoids	Saponins	Phenols	Steroids	anthroquinones	Tannins
Methanol	+++	+++	+++	+++	+++	+++	+++
Ethanol	+++	+++	+++	+++	+++	+++	+++
Water	+	+	+	+	+	+	+

+++ Strong; ++ medium; +poor Presence; -: absence, repeated the experiments three times for each replicates, Classification was based on observation of colour intensity and amount of precipitate

Table 3: Total Phenolic Content and total antioxidant activity from different solvent extract of *T. stans*

Extracts	FRAP (□mol/L)	TPC (mg gallic acid/g plant extract)
Methanol	3841.17±66.34[a]	216+16[a]
Ethanol	3416.24±62.71[b]	206+09[a]
Water	1433.75+14.22[d]	177+12[b]
Ascorbic acid	1648.52±17.46[c]	-
BHT	64.84±2.97[e]	-

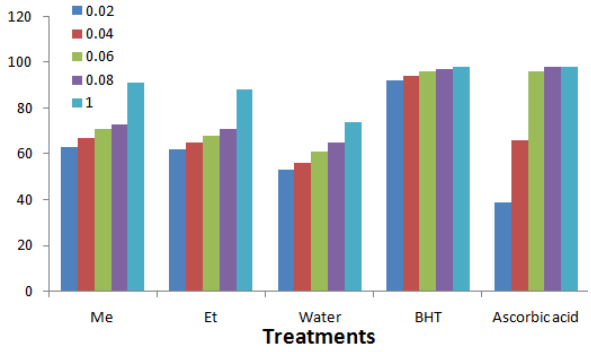
All the data is three replicates of each samples
Phenolic antioxidant coefficient calculated as the ratio FRAP (μM/L)

Total phenol contents and antioxidant activity

Total Phenolic Content (TPC) was determined using the Folin-Ciocalteau reagent and expressed in terms of mg gallic acid equivalent (GAE)/ g ml extract. The more TPC was observed in methanol (216) followed by ethanol (206) and water (177) extracts (Table 3).

The antioxidant activity of the ethanol and methanol crude extract and its various fractions, as measured by the ability to scavenge DPPH free radicals, was compared with the standards/ ascorbic acid and Butylated Hydroxy Toluene (BHT). It was observed that ethanol and methanol extracts of *T. trans* had higher activity than that of water extract. At a concentration of 0.1mg/ml, the scavenging activity of ethanol and methanol extracts reached 56.88% and 58.92% respectively while at the concentration, that of water was 39.23%. Though the DPPH radical scavenging abilities of the extract were less than those of ascorbic acid (98%) and BHT (97.8%) at 0.1 mg/ml, the study showed that the extracts have the proton donating ability and could serve as free radical inhibitors or scavenging, acting possibly as primary antioxidants (Figure-1).

Figure 1. DPPH scavenging activities of the different solvent extracts of *T. stans*



The reducing ability of the extract was in the range of 1433.75- 3841.17 μm Fe (II)/mg (Table 3). The antioxidant potentials of the ethanol and methanol extracts of *T. stans* were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). The

FRAP values for the ethanol, methanol and water extract of *T. stans* were significantly lower that of ascorbic acid but higher that of BHT.

4. Discussion

In recent years, the search for phytochemicals possessing antimicrobial and antioxidant properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging etc (Halliwell, 1996). Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal plants may offer an alternative source for antimicrobial agent with significant activity against pathogenic and infective microorganisms. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes (Berahou et al., 2007).

Results of our findings confirmed the use of *T. stans* as traditional medicine. We found strong antimicrobial and antioxidant activities specifically in the ethanolic and methanolic extracts of *T. stans*. High TPC values found in ethanolic and methanolic extracts (11.32 and 11.64 mg GAE/g extract) imply the role of phenolic compounds in contributing these activities. Plant phenolic compounds have been found to possess potent antioxidants (Adedapo et al., 2009b; Adesegun et al., 2009; Lai et al., 2010) and antimicrobial (Kaur and Arora, 2009; Alcaraz et al., 2000; Lai et al., 2010).

The flavonoids from plant extracts have been found to possess antimicrobial and antioxidants properties in various studies (Lin et al., 2008; Lopez-Lazaro, 2009; Yoshida et al., 2009; Amaral et al., 2009). The presence of alkaloids have shown as antimicrobial (Erdemoglu et al., 2007) and antioxidant (Maiza-Benabdesselam et al., 2007) activity. Plant based steroids also possess as antimicrobial (Shihabudeen et al., 2010) and antioxidant (Koduru et al., 2007) extracts. The anthraquinones have also shown as antimicrobial (Comini et al., 2011) and antioxidant **(**Gow-Chin et al., 2000**)** properties. Strong presence of tannins in all extracts may explain its potent bioactivities are known to possess potent antimicrobial activities (Kaur and Arora, 2009) and antioxidants (Zhang and Lin, 2008). The Saponins from plant extracts have already reported as potent antimicrobial (Mandal et al., 2005) and antioxidant activity (Gulcin et al., 2004).

The present investigation has shown that the ethanol and methanol extract of *T. stans* have active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. The ethanol and methanol extract fractions showed significantly antimicrobial activity against all Gram-positive and Gram-negative bacteria and different fungi tested. Strong antioxidant properties were confirmed in the ethanol and methanol extract fractions. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, alkaloids, steroids, phenols and Saponins. The antioxidant activity was comparable with standard ascorbic acid and BHT. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial and antioxidant agent from *T. stans* plant. This medicinal plant by *in vitro* results appear as interesting and promising and may be effective as potential sources of novel antimicrobial and antioxidant drugs.

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