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Phytochemical Investigation and Anti-microbial Properties of Crude Flower Extract of *Tecoma stans* (L.) Juss. Ex Kunth

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ABSTRACT

Since plants are used as therapeutic agents, the present study is designed to evaluate the phytochemical profile and antimicrobial activities of flower of *Tecoma stans* (L.) ethanolic extract (TSEE) against selective Gram positive, Gram negative bacteria in-vitro. The dried flowers were extracted by cold maceration process using ethanol. Presence of phytoconstituents such as alkaloids, glycosides, saponins, carbohydrates, tannins, phenolics compounds, steroids and flavonoids were observed. The antimicrobial activity was carried against bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Bacillus megaterium*, *Streptococcus mutans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* by means of agar well diffusion method.

The TSEE extract exhibited moderate to high level of broad spectrum antimicrobial activities against these microorganisms. The diameters of zone of inhibition range from 20.33 ± 0.57 to 09.33 ± 1.52 mm; the activity was compared with standard tetracycline. MIC was performed by two-fold broth dilution method and the inhibition range was found to be 31.2 mg/mL to 125 mg/mL. The MBC values lies between 62.5 to 150 mg/mL. The results reported in the present work shows evidence that *Tecoma stans* flowers possess phytochemicals that exhibit broad spectrum antimicrobial activity against tested pathogenic bacteria.

Keywords: *Tecoma stans*, Anti-microbial activity, Minimum inhibitory concentration, Minimum bactericidal concentration

INTRODUCTION

The increase in incidence of infections and emergence of multidrug resistant pathogens paved the way in search of new drugs for the treatment of various ailments. As the microorganisms developing resistance to the existing antibiotics, the researchers are in search of new drugs from alternative sources. Traditionally plants have been used as curative agents for the treatment of numerous diseases. Phytochemicals from medicinal plants have the potential in filling this need. Hence it is essential to study medicinal plants with traditional reputation for proper use of phytochemicals and to find out their potential as a source of new drugs. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide. As a part of this, the present study was conducted to investigate the presence of phytochemicals and antimicrobial efficacy of flowers of *Tecoma stans*.

Tecoma stans is a flowering perennial shrub belongs to the family Bignoniaceae. The entire plant possess medicinal value and used for the treatment of various ailments. Traditionally the roots are used as diuretic, tonic, anti-syphilitic and vermifuge. The decoction of flowers and bark are used for stomach pains. Traditionally, in Mexico the plant is used in the treatment of diabetes [1,2]. The plant leaves have been found to inhibit the growth of yeast infection. The bark and flowers are used traditionally for antimicrobial activity and for the treatment of various cancers. It was reported that the leaves possess significant wound healing property [3], anti-oxidant and anti-microbial [4], anti-bacterial [5], anti-spasmodic [6] and anti-diabetic [7,8] properties.

MATERIALS AND METHODS

Plant materials

The flowers of *Tecoma stans* (L) were collected at our college premises and authenticated by P. Satyanaraya Raju, Plant Taxonomy Consultant, Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur. The collected flowers were shade dried and then ground to coarse powder.

Extraction

The *Tecoma stans* ethanol extract (TSEE) was prepared by cold maceration [9,10]. The dried flower powder (100 g) was macerated with ethanol (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 five Gram positive bacteria that are *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Enterococcus faecalis*, *Streptococcus mutans* and four Gram negative bacteria that are *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were employed.

Phytochemical screening

The phytochemical screening for TSEE was carried out by standard protocols. The presence of alkaloids, cardiac glycosides, saponins, carbohydrates, proteins, amino acids, flavonoids, steroids and phenolic compounds was analyzed [11,12].

Tests for carbohydrates

Molish's test (General test): To 2-3 ml aqueous extract, add few drops of alpha naphthol solution in alcohol shake and add concentrated sulphuric acid from sides of the test tube. Violet ring is formed at the junction of the two liquids.

Tests for the reducing sugars

Fehling's test: Mix 1 ml of Fehling's A and Fehling's B solution boil for 1 min. Add equal volume of test solution and heat on water bath for 5-10 minutes. First yellow, then brick red precipitate is observed.

Benedict's test: Mix equal volume of Benedict's reagent and tests solution in test tube. Heat in boiling water for 5 min. Solution appears green yellow or red depending on amount of reducing sugars present in test solution.

Test for proteins

Biuret test (general test): To 3 ml test solution add 4% NaOH few drops of CuSO_4 solution, violet or pink color appears.

Millions test: Mix 3 ml test solution with 5 ml reagent white precipitate is formed and turns brick red after heating.

Xanthoproteic test: Mix 3 ml test solution with 1 ml Concentrated sulphuric acid. White precipitate is formed and turns yellow after boiling. Add ammonium hydroxide precipitate turns orange.

Test for amino acids

Ninhydrin test: Heat 3ml test solution and 3 drops 5 % Ninhydrin solution in boiling water bath for 10 min. purple or bluish color appears.

Test for alkaloids

Dragendorff's test: Alkaloids gives reddish brown precipitate with this reagent (mercuric iodide solution)

Mayer's test: Alkaloids give cream color precipitate with Mayer's reagent (Potassium mercuric iodide).

Wagner's test: Alkaloids give reddish brown precipitate (Iodine potassium iodide solution)

Hager's test: Alkaloids give yellow precipitate (saturated solution of picric acid).

Test for glycosides**Test for cardiac glycosides**

Baljet's test: To aqueous or alcoholic extract, add 1 ml pyridine and 1 ml sodium nitroprusside. Pink to red color appears.

Keller Killiani test: To 2 ml extract add glacial acetic acid and one drop of 5% ferric chloride and concentrated sulphuric acid. Reddish brown color appears at the junction of the two liquid layers and upper layer appears bluish green.

Liebermann's test: Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops of concentrated sulphuric acid from the side of the test tube. First red, then blue and finally green color appears.

Test for anthraquinone glycosides

Borntreger's test: To 3 ml extract add 5 ml 5% ferric chloride and 5 ml dilute HCl. Heat for 5 min in boiling water bath. Cool and add benzene or any other organic solvent. Shake well. Separate organic layer. Add equal vol. dil. ammonia. Ammonical layer turns pinkish red color.

Modified Borntreger's test: To 5 ml extracts add 5 ml 5% ferric chloride and 5 ml dilute HCl. Heat for 5 min in boiling water bath. Cool and add benzene or any other organic solvent. Shake well. Separate organic layer. Add equal cold dil. ammonia. Ammonical layer turns pinkish red color.

Test for saponin glycosides

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

Test for phenolic compounds

Ferric chloride test: Treat the extract with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Test for chlorogenic acid: Treat the test solution with Aqueous Ammonia and exposure to air gradually green color is developed.

Potassium dichromate: To the test solution add potassium dichromate red precipitate appears.

Bromine water: To the test solution add bromine water, discoloration of bromine water.

Test for flavonoids

Shinoda test: To dry powder or extract, add 5 ml 95% ethanol, few drops concentrated HCl and 0.5g magnesium turnings. Pink colored observed.

Lead acetate test: To small quantity of residue, add lead acetate solution. Yellow precipitate is observed.

Test for steroids and triterpenoids: To 2 ml extract, add 2 ml chloroform and 2 ml concentrated sulphuric acid and shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Liebermann Buchard reaction: Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and add 2 drops of concentrated sulphuric acid from the side of test tube. First red, then blue and finally green color appears.

Sulphur powder test: Add small amount of sulphur powder to the test solution, it sinks at the bottom.

Anti-microbial assay: The antimicrobial efficacy of TSEE was determined by agar well diffusion method [13,14]. The present study was designed to determine the susceptibility pattern of TSEE against a broad spectrum of pathogenic bacteria. To check the susceptibility pattern of TSEE against selected bacteria nutrient agar medium was inoculated with specific organisms. The wells of 6 mm diameter were made equidistantly in the agar plate with sterile borer. The extracts were dissolved in dimethyl sulphoxide to get a final concentration of 10 mg/mL. The wells were filled with 50 μ l (500 μ g) and 100 μ l (1000 μ g) of drug respectively. The activity was compared with standard drug tetracycline at a concentration of 30 μ g. The plates were incubated in upright position in an incubator at 37°C for 24 h. The zone of inhibition was measured with ruler.

Minimum Inhibitory Concentration (MIC): The broth dilution technique was used where the plant extract was prepared to the highest concentration of 250 mg/mL (stock concentration) in DMSO and serially diluted (two-fold) to a working concentration ranging from 0.97 mg/mL to 250 mg/mL using peptone broth. And the tubes were inoculated with 0.1ml suspension of the test organisms. Control was used with peptone broth, plant extract and without test organism. After 24hours of incubation at 37°C, the tubes were observed for turbidity. The least concentration where no turbidity was observed determined as MIC value [15,16].

Minimum Bactericidal Concentration (MBC): To determine the MBC, from each set of test tubes in the MIC reports, a loopful of inoculum from each tube was transferred into nutrient agar medium plates. The inoculated plates were incubated at 37°C for 24 hours. The lowest concentration of the plant extract has shown no bacterial growth. Then the results were recorded as the MBC Value [17].

RESULTS AND DISCUSSION

Medicinal plants have been used in the treatment of numerous human diseases since time immemorial. Traditionally they have been using in the treatment of various diseases such as wound healing, typhoid, dysentery, ulcers, cough, skin diseases and urinary tract infections. They have been used as curative agents because of their antimicrobial traits, which are due to compounds synthesized during secondary metabolism of the plant. The therapeutic index of the plants lies in the presence of phytochemicals. The ethanolic extract of *Tecoma stans* flowers showed the presence of carbohydrates, amino acids, proteins, alkaloids, glycosides, steroids, saponins, phenolics, flavonoids and tannins. The results of preliminary phytochemical screening were reported in Table 1. The leaves of *T. stans* have been found to contain chrysoeriol, luteolin and hyperoside (quercetin-3-O-beta-D-galactoside) an iridoid glucoside, 5-deoxystansioside [18,19] (Table 1 and Figure 1).

The present investigation has shown that the flowers possess active phytochemicals which are able to inhibit the growth of human pathogenic bacteria. The TSEE showed more potent antimicrobial efficacy against *B. subtilis* (20.33 \pm 0.57 mm), *S. aureus* (18.33 \pm 0.57) and moderate activity was observed in *K. pneumoniae* (17.0 \pm 1.0), *B. megaterium* (16.66 \pm 1.52), *E. faecalis* (15.66 \pm 1.15) and minimum activity in *S. mutans* (14.33 \pm 0.57), *E. coli* (14.33 \pm 1.52), *P. vulgaris* (13.66 \pm 1.52) and *P. aeruginosa* (12.66 \pm 1.52) (Table 2 and Figure 2). The MIC of TSEE is ranged from 31.2 mg/mL to 125 mg/mL (Table 3 and

Figure 3). The results of MIC indicated that *B. subtilis*, *S. aureus*, *K. pneumoniae* were the most sensitive bacteria to TSEE because of their lowest MIC 31.2 mg/mL. The MBC values are ranged from 62.5 to 150 mg/mL (Table 4 and Figures 4 and 5).

The results of the present study confirm the antimicrobial efficacy against the tested pathogenic bacteria. However, the activity differs with the type of bacteria used for the study. Further the plant can be explored to identify the phytochemicals responsible for the activity.

Kingdom	: Plantae	
Division	: Tracheophyta	
Subdivision	: Spermatophytina	
Class	: Magnoliopsida	
Order	: Lamiales	
Family	: Bignoneaceae	
Genus	: Tecoma	
Species	: Stans	
Binomial name	: <i>Tecoma stans</i> (L.) Juss. ex Kunth	
Common names	: Yellow trumpet, Yellow Bell, Yellow elder	

Figure 1: Scientific classification of *Tecoma stans* (L.) Juss. ex Kunth

Table 1: Scientific classification of *Tecoma stans* (L.) Juss. ex Kunth

S. No	Name of the test	TSEE
1.	Carbohydrates	+
2.	Amino acids	+
3.	Proteins	+
4.	Steroids	+
5.	Glycosides	+
6.	Saponins	+
7.	Alkaloids	+
8.	Flavonoids	+
9.	Phenolic compounds & Tannins	+

Table 2: Antimicrobial efficacy of TSEE

S. No.	Name of the Microorganism	Zone of inhibition in mm			
		Concentration of TSEE & Tetracycline $\mu\text{g}/\text{well}$			
		500	1000	TET (30)	DMSO
1.	<i>S. aureus</i>	15.66 \pm 1.15	18.33 \pm 0.57	28.33 \pm 0.57	-
2.	<i>E. faecalis</i>	13.33 \pm 0.57	15.66 \pm 1.15	20.0 \pm 1.0	-
3.	<i>S. mutans</i>	11.0 \pm 1.0	14.33 \pm 0.57	25.33 \pm 0.57	-
4.	<i>B. subtilis</i>	16.66 \pm 1.52	20.33 \pm 0.57	26.66 \pm 1.15	-
5.	<i>B. megaterium</i>	13.33 \pm 0.57	16.66 \pm 1.52	21.33 \pm 0.57	-
6.	<i>E. coli</i>	10.0 \pm 1.0	14.33 \pm 1.52	27.66 \pm 1.15	-
7.	<i>K. pneumoniae</i>	13.66 \pm 1.52	17.0 \pm 1.0	25.66 \pm 0.57	-
8.	<i>P. aeruginosa</i>	09.33 \pm 1.52	12.66 \pm 1.52	21.33 \pm 0.57	-
9.	<i>P. vulgaris</i>	10.0 \pm 1.0	13.66 \pm 1.52	15.0 \pm 1.0	-

Note: Values are mean \pm SD of triplicates

**Figure 2:** Anti-microbial efficacy of TSEE and Tetracycline against *S. aureus*, *E. faecalis* & *S. mutans*

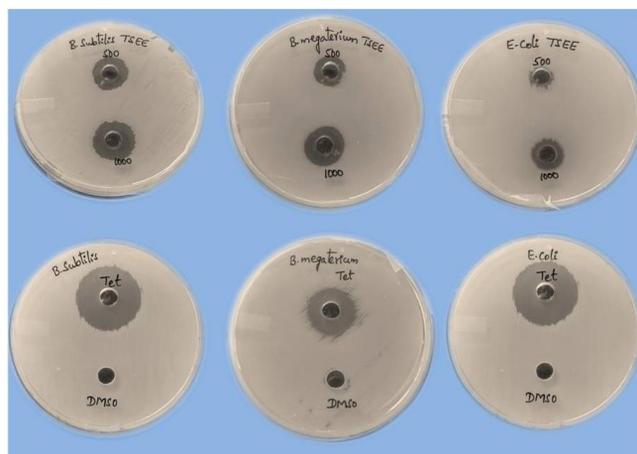


Figure 3: Anti-microbial efficacy of TSEE and Tetracycline against *B. subtilis*, *B. megaterium* and *E. coli*



Figure 4: Anti-microbial efficacy of TSEE and Tetracycline against *P. aeruginosa*, *K. pneumoniae* and *P. vulgaris*

Table 3: Minimum inhibitory concentration (MIC) of TSEE

Name of the organism	Extract concentration mg/mL								
	0.97	1.95	3.90	7.81	15.6	31.2	62.5	125	150
<i>S. aureus</i>	+	+	+	+	+	β	-	-	-
<i>E. faecalis</i>	+	+	+	+	+	+	β	-	-
<i>S. mutans</i>	+	+	+	+	+	+	+	β	-
<i>B. subtilis</i>	+	+	+	+	+	β	-	-	-

<i>B. megaterium</i>	+	+	+	+	+	+	β	-	-
<i>E. coli</i>	+	+	+	+	+	+	+	β	-
<i>P. aeruginosa</i>	+	+	+	+	+	+	+	β	-
<i>K. pneumoniae</i>	+	+	+	+	+	β	-	-	-
<i>P. vulgaris</i>	+	+	+	+	+	+	+	β	-
Note: += Turbidity observed, - = No turbidity observed, β= MIC value									

Table 4: Minimum bactericidal concentration (MBC) of TSEE

Name of the organism	Extract concentration mg/mL								
	0.97	1.95	3.90	7.81	15.6	31.2	62.5	125	150
<i>S. aureus</i>	+	+	+	+	+	+	β	-	-
<i>E. faecalis</i>	+	+	+	+	+	+	+	β	-
<i>S. mutans</i>	+	+	+	+	+	+	+	β	-
<i>B. subtilis</i>	+	+	+	+	+	+	β	-	-
<i>B. megaterium</i>	+	+	+	+	+	+	+	β	-
<i>E. coli</i>	+	+	+	+	+	+	+	+	β
<i>P. aeruginosa</i>	+	+	+	+	+	+	+	+	β
<i>K. pneumoniae</i>	+	+	+	+	+	+	β	-	-
<i>P. vulgaris</i>	+	+	+	+	+	+	+	+	β
Note: += Growth observed, - = No growth observed, β= MBC value									

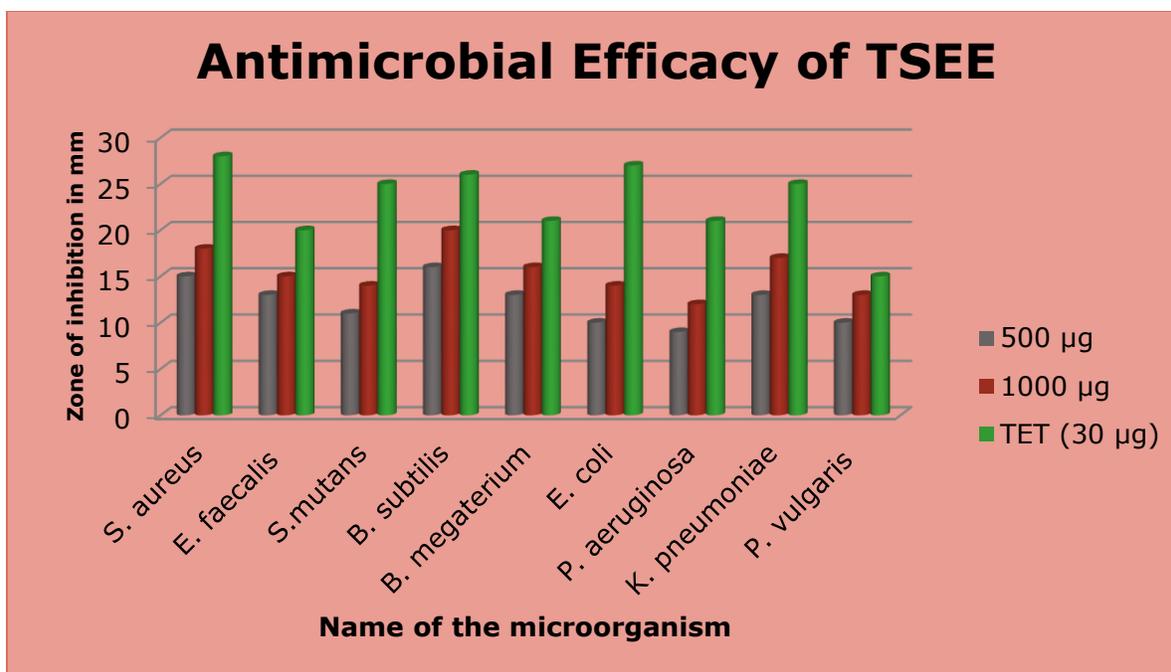


Figure 5: Anti-microbial efficacy of TSEE

CONCLUSION

The present study indicated that the flowers of *Tecoma stans* (L.) Juss. ex Kunth possess potent antimicrobial agents. The results of the study provide scientific evidence to support the traditional use of the plant medicinally. The TSEE showed significant antimicrobial activity against Gram positive and gram negative bacteria, but predominantly against Gram positive bacteria. The activity may be due to the presence of phytochemicals alkaloids, glycosides, polyphenols, saponins, flavonoids and tannins. This medicinal plant may be useful as potential source for the discovery of novel antimicrobial agents. However, further study is required to identify the active principles responsible for antimicrobial activity.

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