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Preliminary Phytochemical Analysis, Antioxidant and Antibacterial Activities of *Tecoma Stan*s (L) Juss Ex Kunth Flowers

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ABSTRACT

Tecoma stans belongs to the family Bignoniaceae and it is a yellow bell plant. The present study deals with the phytochemical analysis, antioxidant and anti-bacterial activity of *Tecoma stans* flower. The study revealed the phytoconstituents of *Tecoma stans* flower in methanolic extract. The phytochemicals studies showed the presence of flavonoids, sterols, anthraquinone, proteins, carbohydrate, cardiac glycoside. The *Tecoma stans* flowers methanolic extract showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method. The anti-bacterial activity of *Tecoma stans* flowers showed the methanolic extract of Tecoma stans flowers showed the zone of inhibition in *staphylococcus aureus* and *E. coli* (16mm± 0.018 and 17mm ±0.063)

Keyword: *Tecoma stans*, phytochemical, antioxidant assay, antibacterial activity

1. Introduction

Medicinal plants are rich source of different types of medicines and produce various bioactive molecules. Herbal plant extracts are very useful and are the major sources of medicine which play vital role in controlling various types of pathogens and as growth promoters (Doss 2009). A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Among ancient civilizations, India has been known to be rich repository of medicinal plants (Prakash *et al*.,2020) . Antioxident assay is often used to measure the antioxidant capacity of foods, beverages and nutritional supplements containing polyphenols. The antioxidant activity was evaluated using three methods, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power. Lipoxygenase-inhibiting activity was assayed spectrophotometrically; the result was expressed as percent inhibition (Rasika *et al*., 2011). The use of antimicrobial testing methods for the in vitro investigation of extracts and pure drugs as potential antimicrobial agents. The crude extracts of all the species studied were found to show Antibacterial activities towards the Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa*), Gram-negative (*Escherichia coli*) and yeast *(Candida albicans)*. The extract of *Tabebuia spectabilis* was found to be the most active against the Gram-positive bacteria. *Tecoma stans* is a ornamental plants family Bignoniaceae. Twelve species are from the Americas, while the other two species are African. The generic name is derived from the Nahuatl word tecomaxochitl, which was applied by the indigenous peoples of Mexico to plants with tubular flowers. Fresh seeds germinate readily in sandy soil in the spring. Cuttings root easily under mist in the summerr (Archana *et al* 2011).

Scientific classification

***TECOMA STANS*(L.) Juss. ex kunth**

Class : Dicotyledons

Subclass : Gamopetalae

Order : Personales

Family : Bignoniaceae

Genus : Tecoma

Species : stans

2. Botanical description

Tecoma stans L.Juss.ex is an erect shrub commonly found in India. It Belongs to the family Bignoniaceae. The shrub has some common names in different native Indian languages. *Tecoma stans* is also known as yellow bells, yellow elder, trumpet flower in English and Piliya in hindi. *Tecoma stans* is an important medicinal plant.

Tecoma stans is a fast-growing plant in the trumpet vine family Bignoniaceae. It has sharp toothed, compound and imparipinnate with 2 to 5 pairs and a single terminal leaflet. Leaves are lanceolate which grows up to 10cm long with serrate margins and mid green coloured. *Tecoma stans* bears clusters of bright yellow flowers in the trumpet shaped with 5 rounded lobes, 6cm long. Pods are narrow, slightly flattened to pointed capsules ranges up to 20cm long

3. MATERIAL AND METHOD

3.1 Collections of Flowers

T. Stans flowers were collected in and from around Coimbatore district, Tamil Nadu in the month of August 2022.The flowers of *Tecoma stans* were collected and separated and are then dried under shade drying for 4-5days. Then the dried flowers powdered in mechanical grinder.

3.2 Process of Extraction

The dried *Tecoma stans* (20g) powder was placed in the conical flask and 75 ml of methanol was used for extraction procedure and the experiment was done separately. The solution was left to stand at room temperature for 24hrs with occasional shaking and was filtered with Whatsman 1 paper. The filtrate was used for the phytochemical screening using the following tests.

3.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical analysis was carried out for all the extracts according to the standard methods Harbone (1984)methods.

3.3.1 TEST FOR ALKALOIDS

Small quantities (2ml) of various extracts were separately boiled with 5 ml of 2% hydrochloric acid on a water bath for minutes. The mixture was allowed to cool and filtered. The filtrates were used for the following test.

Wagner's test: Filtrates were treated with few drops of Wagner's reagent. Formation of brown reddish precipitate indicates the presence of alkaloids

Dragendroff's test. Filtrates were treated with Dragendroff's reagent, presence of alkaloids confirmed by the formation of red precipitate.

3.3.2 TEST FOR FLAVONOIDS

A small quantity of various extracts was heated with 10 ml of ethyl acetate in water bath for 3 minutes. The mixture is filed differently and the filtrates are used for the ammonium and aluminum chloride test.

NaOH Tests: To 2-3 ml of extract, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicated the presence of flavonoids

Shinoda Tests: To 2-3 ml extract, few fragments of magnesium metal were added in a test tube, followed by dropwise addition of concentrate HCl. Formation of magenta colour indicated the presence of flavonoids

Lead acetate test : 1 ml of extracts was treated with few drops of 10% lead acetate solution. Formation of precipitate indicated the presence of steroids.

3.3.3 TEST FOR STEROIDS

Lieberman-burchard's test: 2ml of the solvent extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mind. The change of red color through blue in green indicated the presence of steroids.

3.3.4 TEST FOR TERPENOIDS:

Liebermann test: 1ml of extract was treated with chloroform, acetic anhydride and few drops of H_2SO_4 was added and observed the formation of dark green color indicates of the presence of terpenoids.

3.3.5 TEST FOR ANTHOCYANIN:

HCl test: 2ml of different extracts was treated with 2mlof hcl. No any other colour change the presence of anthocyanin.

3.3.6 TEST FOR PROTEIN

Biuret test : A quantity each of extracts was put in a test-tube and treated with 5 drops of 1% hydrated copper sulphate. To 2ml of 40% sodium hydroxide was also added and the test-tube shaken vigorously to mix the contents. A blue color showed presence of proteins.

Ninhydrin test: 2**ml** of plant extract then added few drops of ninhydrin solution in 2%.The test tube must be kept in a warm water bath for approximately 5 minutes. Presence of purple colour

Xanthoproteic test: 2ml of plant extract few drops of concentration nitric acid mixed well and warm to the lamp change in yellow colour presence of protein.

3.3.7 PHENOLIC COMPONENT

Ferric chloride test: Extract aqueous solution add a neutral solution of ferric chloride slowly dropwise. change in colour of bluish green coloured solution the presence of phenol.

Gelatin test : Plant extract is dissolved in 5mL distilled water and 1% gelatin solution then mixed 10% NaCl A white precipitate. The presence of phonol.

Ellagic Acid Test : Plant extract aqueous solution then 5% glacial acetic acid few drop of 5% sodium nitrite solution. Solution turns niger brown precipitate presence of phenol.

3.3.8 TEST FOR CARBOHYDRATE

Fehling's Test: Equal amount of Fehling A and Fehling B reagents were mixed and 2ml of it was added to the plant extract and then gently heated the sample. Appearance of brick red precipitate indicated the presence of reducing sugars.

Molisch's Test: 2ml of Molisch's reagent was added to 0.5 ml of crude extract and the mixture was shaken properly. Appearance of a violet ring at the interface indicated the presence of carbohydrate.

3.3.8 TEST FOR TANNIN

Gelatin test : Plant extract is dissolved in 5mL distilled water and 1%gelatin solution add 10% NaCl other than white precipitate is presence

Braymer's test : 1ml extract and 3ml distilled water mixed well then 3 drops 10% Ferric chloride solution. The extract is change in the colour of blue green is called presence of tannin.

3.3.9 TEST FOR SAPONINS

Frothing test: Small quantities of different extracts were diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed presence of saponin.

TEST FOR CARDIAC GLYCOSIDES

Killer killiani test: Glycoside is dissolved in a mixture of 1 % ferric sulphate solution in (5%) glacial acetic acid. Add one or two drop of concentrated sulphuric acid. A blue colour develops due to the presence of deoxy sugar.

Baljet test: Take a 2ml of extract and add sodium picrate reagent. If glycoside is present yellow to orange colour will be seen.

3.3.10 TEST FOR LIGNINS

Labat test: Gallic acid is added to the plant extract it results in the formation of olive green colour. The presence of lignin.

3.3.11 TEST FOR VOLATILE OIL

Fluorescence test: l0 ml of extract, filtered till saturation, exposed to UV light Bright pinkish fluorescence presence.

3.4 ANTIOXIDANT ACTIVITY

3.4.1 Preparation of DPPH:

DPPH and L-Ascorbic acid standard antioxidants were soluble in methanol. Fresh DPPH stock solution (0.2mg/ml) was prepared on each day of analysis.

3.4.2 Preparation of ascorbic acid:

stock solutions of ascorbic acid were prepared in methanol at a concentration of 0.1 mg/ml and stored at 25°C.

3.4.3 Procedure

The antioxidant capacity of the tested compounds was studied through their scavenging activity against 1,1-diphenyl-2-pycryl-hydrazyl (DPPH) radicals, using the method of Gerhauser et al. (2003). In brief, 190 µL of DPPH (100 µM in methanol), a stable free radical, were added to 10 µL of different concentrations of fruit extract, compounds 1, 2, 4, and 8 in a 96-well microplate and incubated at 37°C for 30 minutes. The bleaching of DPPH was monitored at an absorbance of 515 nm. The percentage of DPPH bleaching utilized for SC50 (half maximal scavenging concentration) was calculated as being 0% for the absorbance of DPPH and 100% for the absorbance of DPPH with 10 mm ascorbic acid, AA.

3.5 ANTIBACTERIAL ACTIVITY

3.5.1 Inoculum preparation

- At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as Nutrient broth.
- The broth culture is incubated at 35°C until it achieves or exceeds the turbidity (usually 2 to 6 hours)
- The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity. This results in a suspension containing approximately 1 to 2 x 108 CFU/ml for *E.coli* and *Staphylococccus aureus.*

3.5.2 Inoculation of Test Plates

- Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
- The dried surface of a Nutrient agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
- The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.
- The media was punctured by making a well of 6 mm in diameter and filled with 20 µl of a sample. Further the petriplates were placed inversely for complete diffusion and inhibition zones were examined by measuring the diameter (mm) formed around the well after 24 hrs incubation at 37°C. The zones were measured by using standard (Hi-Media) scale.

4. Results and discussion

4.1 Phytochemical analysis

Tecoma stans flowers methanol extract revealed the presence of flavonoids, sterols, Andraquinone, protein, carbohydrate, cardiac glycosides (Table 1)

Table 1 - phytochemical screening of *tecoma stans* flowers methanol extract

phytochemicals	***Tecoma stans* flowers methanolic extract**
Alkaloids	-
Flavonoids	+
Sterols	+
Terpenoids	-
Andraquinone	+
Anthocyanin	-
Protein	+

Phenolic compound	**-**
Quinones	**-**
Carbohydrates	**+**
Tannin	**-**
Saponins	**-**
Cardiac glycosides	**+**
Glycosides test	**-**
Lignin	**-**
Coumarins	**-**
Volatile oils	**-**

Key: + present, - absent

4.2 Antioxidant activity

The *Tecoma stans* flowers methanolic extract showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method . The result of this test activity was found to be higher for 750µg /ml of methanolic extract where the results obtained using the DPPH assay were 64.75% of inhibition(Table 2,fig 1) (Bijaya 2013)

Table 2-DPPH assay of standard: ascorbic acid

Concentration (µg/ml)	**% Inhibition**
3	**62.30 ± 0.021**
6	**70.49 ± 0.012**
9	**83.61± 0.031**
12	**90.16± 0.052**
15	**95.90± 0.071**

dpph assay of *tecoma stans* flower methanol extract

	% Inhibition
10	**48.82 ± 0.032**
50	**50.82 ± 0.027**
150	**51.64 ± 0.018**
250	**54.92. ± 0.015**
350	**59.02 ± 0.018**
500	**62.75 ± 0.022**
750	**64.75 ± 0.033**

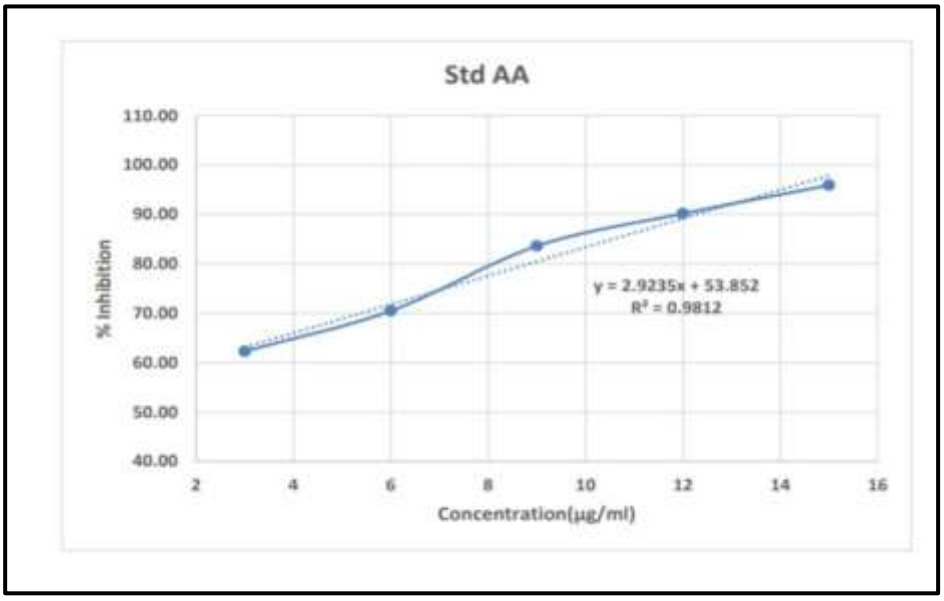
4.3 Antibacterial activity

Antibacterial activity is tested for *T. Stans* extract by disc diffusion method. In methanol extract of *T.stans* flowers showed a highest zone of inhibition recorded against *Staphylococcus,E. coli* as (16 ± 0.018), (17 ± 0.063) mm.

Table 3-antibacterial activity of *tecoma stans* flowers methanol extract

Test	**Zone of inhibition(mm)**	
	Staphylococcus aureus	**E.coli**
Standard(Rifampicin)	**25mm±0.042**	**20mm±0.032**
Negative control (DMSO)	**0mm**	**0mm**
***Tecoma stans* flowers**	**16mm± 0.018**	**17mm± 0.063**

Figure 1-antioxidant assay of *tecoma stans* flower methanol extract against standard ascorbic acid



Conclusion

Tecoma stans belongs to the family bignoniaceae and it is a yellow bell plant. The present study deals with the phytochemical analysis,antioxidant and anti bacterial activity of *Tecoma stans* flower.The study revealed the phytoconstituents of *Tecoma stans* flower in methanolic extract. The phytochemicals studies showed the presence of flavonoids,sterols,anthraquinone,proteins,carbohydrate,cardiac glycosides.The *Tecoma stans* flowers methanolic extract showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method .

The anti bacterial activity of *Tecoma stans* flowers showed the significant acivity against some tested bacterial strains and reveals thr presence of various bio active compounds responsible for various medicinal properties. The methanolic extract of Tecoma stans flowers showed the zone of inhibition in *staphylococcus aureus* and *E.coli* (16mm± 0.018 and 17mm ±0.063)

This study suggests the crude methanol extracts of *Tecoma stans* flowers are found to exhibit potent antioxidant and antibacterial activity that provides the partial scientific validation for using these flowers against infectious diseases. The results indicated that flowers are the good sources of secondary metabolites from which pure compound can be isolated and perform in-vivo biological activities with the mechanism of action to develop products for the herbal remedy against infectious diseases.

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