

PHYTOCHEMICAL INVESTIGATION AND ANTI-MICROBIAL ACTIVITY OF TECOMA STANS

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ABSTRACT

Tecoma stans Linn is also known as yellow bells, yellow elder, trumpet flower etc., belonging to the family: *Bignoniaceae*. *Tecoma stans* is widely distributed all over the world specially in countries like south east asia, south Africa and some oceanic islands with warm climate conditions. Pharmacologically *tecoma stans* flower is traditionally used for many ailments including cancer, diabetes and arthritis. It also possess anti-spasmodic, anti-nociceptive and anti-oxidant property. In present studies, Phytochemical investigations were done on powdered flower extracts based on the chemical constituents present. Anti-microbial activity was also performed on leaf and flower extracts of *tecoma stans* against different bacteria by using disc

diffusion method. The zones of inhibition of the extracts were determined. The various extracts (Methanol, 1µg/ml and Water) of flower were found to be effective anti-microbial activity against various gram positive and gram negative bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* using a standard as Amoxicillin (1µg/ml) compared to leaf extracts..

INTRODUCTION

PLANT PROFILE

Tecoma stans also known as *yellow bells*, belonging to the family *Bignoniaceae*. It is an ornamental plant. *Tecoma stans* grows best in full sunlight in disturbed areas such as roadside and it will grow in most well-drained soils, including calcareous fill, infertile sands, acidic soil, Ultisols, and volcanic regolith in areas receiving from 700 to 1800 mm of rainfall. The species is described as a water spender that is able to convert to water saved. It requires minimal competition and nearly full sunlight to survive (shade intolerant). Roadsides and

disturbed areas are the most common habitats. Given a start in secondary forest, it is usually present for only 10 to 20 years following disturbance"

Yellow elder has been used for a variety of purposes in herbal medicine. Extracts from *Tecoma stans* leaves have been found to inhibit the growth of the yeast infection. Yellow elder also contains several compounds noted for their catnip-like effects on felines. The leaves and roots of the plant contain bioactive compounds, especially monoterpenes, which may have medicinal uses; Honey bees are attracted to it, but-unlike most flowering plants-the honey produced from Yellow Trumpetbush's nectar/pollen is poisonous.

DESCRIPTION OF *Tecoma stans*

A large shrub or small tree, much branched, growing upto 1.5-5m tall, but grows occasionally upto 10m in height. twigs tan or reddish tan, smooth, scarcely 4-sided; leaves opposite, pinnately compound, leaflets 1-9, usually 3-7, ovate-lanceolate, apex acuminate, base acute or obliquely acute, very shortly petiolate or all but subsessile, slightly hirsute on midrib and in vein axils beneath, margins irregularly serrate, leaves quite variable, rachis and petiole slender, glabrous; inflorescence an axillary or terminal raceme, pedicels short, irregularly curved or twisted, bracts reduced to minute scales, flowers rather few, calyx narrowly cylindric-campanulate, 5-7 cm long, with 5 sub-equal acuminate teeth, glabrous; stamens 4, attached at summit of tube, in 2 unequal pairs, included, filaments pilose at base, curved above, anthers versatile, linear, yellow, pilose, 6 mm long; sterile fifth stamen much reduced; pistil about equaling stamens, ovary narrowly cylindric, about equaling calyx, style filiform, glabrous, stigma flat, elliptic; capsule linear, compressed, 10-20 cm long, 7-8 mm wide, brown when ripe, with raised line or suture lengthwise on each flat side, tardily dehiscent along suture, septum parallel with flat sides, firm, seeds flat, oblong, 7-8 x 4 mm, with a membranous transparent wing on each end, ends of wing erose, seeds entire including wing about 20 x 6 mm".

VERNACULAR NAMES

Table-1

S.NO.	LANGUAGES	NAMES
1.	Telugu	Suvarna ganneru, Pacha ganneru, Pachagotla.
2.	Hindi	Ganer, Trumpet flower.

SCIENTIFIC NAME: *Tecoma stans*(L) kunth.

TAXONOMY:

Kingdom	<u>Plantae</u> – plantes, Planta, Vegetal, plants
Subkingdom	<u>Viridaeplantae</u> – green plants
Infrakingdom	<u>Streptophyta</u> – land plants
Division	<u>Tracheophyta</u> – vascular plants, tracheophytes
Subdivision	<u>Spermatophytina</u> – spermatophytes, seed plants, phanérogames
Infradivision	<u>Angiospermae</u> – flowering plants, angiosperms, plantas com flor, angiosperma, plantes à fleurs, angiospermes, plantes à fruits
Class	<u>Magnoliopsida</u>
Superorder	<u>Asteranae</u>
Order	<u>Lamiales</u>
Family	<u>Bignoniaceae</u> – bignonias
Genus	<u>Tecoma</u> Juss. – trumpetbush
Species	<i>Tecoma stans</i> (L.) Juss. ex Kunth – yellow elder, yellow trumpet-flower, trumpetbush, trumpetflower.

DISTRIBUTION

Locations within which *Tecoma stans* is naturalised include Australia, south and south east Asia, South Africa, eastern Africa and some oceanic islands with warm climates.



Fig-11: Tecoma stans flowers



Fig-12: Tecoma stans plant

CHEMICAL CONSTITUENTS OF *Tecoma stans*

- 1) Alkaloids
- 2) Carbohydrates
- 3) Saponins
- 4) Tannins
- 5) Phenolic compounds.

LITERATURE REVIEW

Pharmacological review

- ❖ Mule SN.et.al. reported that the methanolic extract of *tecoma stans* flowers were found to have anti-nociceptive activity when assessed in swiss-albino mice using acetic acid induced writhing test.
- ❖ Aguilar-Sanlamaria et.al. reported that the aqueous extract of *tecoma stans* plant exhibited anti-diabetic , α -glucosidase and hypoglycemic activity when tested in type-2 diabetic male Sprague-Dawley rats.
- ❖ Gandhi MI et.al reported that antifungal and haemolytic activities are due to organic extracts of *Tecoma stans* (Bignoniaceae).
- ❖ Das C et.al. reported that the wound healing potential is due to methanolic leaf extract of *Tecoma stans* Linn.
- ❖ Torane RC, Kamble GS et.al reported antioxidant activity is due to aerial Parts of *Tecoma stans*.

Phytochemical review

- ❖ Marzouk et.al. reported that aqueous extract *tecoma stans* fruits with compounds isoacteoside exhibited potent growth inhibition of human breast carcinoma cells.
- ❖ Marzouk et.al. reported that the aqueous fruit extracts of *tecoma stans* with compounds 5-hydroxy-skytanthine hydrochloride and E/Z-acteoside exhibited cytotoxic activity against human hepatocarcinoma cells.
- ❖ Bianco A et.al. reported the presence of 5-deoxystansioside, and iridoid glucoside in *Tecoma stans*.
- ❖ Satya P. Kunapuli et.al reported the presence of indolic compounds in the leaves of *Tecoma stans*.

Reasons for taking up the present work

Research literature have revealed that *tecoma stans* is medicinally important. There were many research reports on phytochemical screening of leaves and other parts of *tecoma stans* and reports on anti-diabetic, anti-cancer, anti-nociceptive, anti-spasmodic activity etc. In view of the above claims and facts, the present investigation was undertaken to find out chemical constituents of flower extract and to explore possible anti-microbial activity of leaf and flower extracts of *tecoma stans*.

MATERIALS AND METHODS

1) COLLECTION OF PLANT MATERIAL

The Tecoma stans plant was collected in the month of February 2013 from Sri Devi engineering College, V.N.Pally, Gandipet. The plant was then identified by their vernacular names and later it was compared with the herbarium of the department by the botanist. The leaves and flowers of Tecoma stans were collected and separated and are then dried under shade drying for 4-5 days. Then the dried leaves and flowers were grinded sieved to get nearly fine amorphous powder.

2) Processing of Crude Drug

The leaves and flowers of Tecoma stans were collected and separated and are then dried under shade drying for 4-5 days. Then the dried leaves and flowers were grinded sieved to get nearly fine amorphous powder.

3) EXTRACTION

Extraction is the process of obtaining the constituents by separating them from crude drug by the use of solvents. Powdered material was extracted with suitable solvent or mixture of solvents for extracting the various phytoconstituents present in the crude drug.

SOXHLEATION: 120gm of each powdered leaf and flower were used for carrying out soxhlation extraction with 360ml of METHANOL and 360ml of WATER for 6hrs at room temperature. All the extracts were collected and evaporated at room temperature. The dried residues were weighed and % yield of each extract was calculated.



Fig-13 Soxhlation apparatus

4) PHYTOCHEMICAL EVALUATION OF POWDERED FLOWER EXTRACT OF *Tecoma stans*

The powdered flower sample of *Tecoma stans* was taken and phytochemical screening was done to check the phytoconstituents present using standard reagents.

a) Carbohydrates

- *Molisch's test:*

About 2ml of powdered flower extract was mixed with 0.2 ml of alcoholic solution of α -naphthol 10% in addition to 2 ml of sulphuric acid, a bluish violet zone is formed this indicates the presence of carbohydrates and /or glycosides.

b) Alkaloids

About 0.2 g of the powdered flower extracts was warmed with 2% H₂SO₄ for two minutes. It was filtered and few drops of Dragendorff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

c) Tannins

Small quantity of powdered flower extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

d) Glycosides

The powdered flower extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

e) Saponins

About 0.2 g of the powdered flower extract was shaken with 5ml of distilled water and then heated to boil. Frothing (Appearance of creamy mass of small bubbles) shows the presence of saponins.

f) Flavonoids

Powdered flower extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

g) Steroids (LB test)

2 ml of acetic anhydride was added to 0.5 g of the powdered flower of each with 2 ml of H₂SO₄. The colour changed from violet to blue or green in samples indicating the presence of steroids.

h) Proteins

To the powdered flower extract, 5% NaOH and 1% copper sulphate solution were added. Violet color produced shows the presence of proteins.

i) Amino acids

The powdered flower was treated with Million's reagent. Red colour showed the presence of amino acids.

j) Phenolic compounds

Small quantities of powdered flower samples were taken separately in water and test for the presence of phenolic compounds was carried out by using reagents like 5% ferric chloride solution, 1% gelatin solution containing 10% NaCl and 10% lead acetate.

k) Gums and Mucilage

A small quantity of powdered flower extracts were added separately to 25ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gums and mucilage.

5) ANTI-MICROBIAL ASSAY

Anti-microbial activity is a process of killing or inhibiting the growth of microbes. Anti-microbial agent either kills (bactericidal) the microbes or inhibits the growth (bacteriostatic) of microbes. The standard bacterial test organisms were sub cultured on freshly prepared nutrient agar and the extracted samples were inoculated into the culture using paper disc diffusion method.

PREPARATION OF NUTRIENT AGAR MEDIUM

Nutrient agar was prepared by dissolving it in required quantity of distilled water by heating it on hot plate. Then the agar medium was sterilized in an autoclave at 121°C for 15 min at 15 lb pressure.

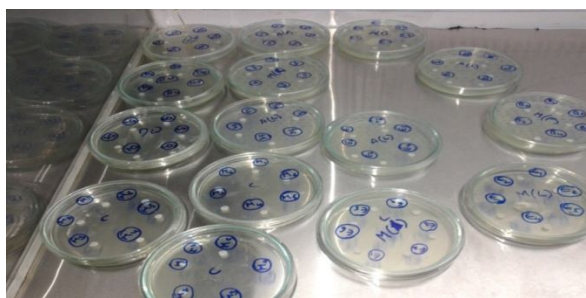


Fig-14: Nutrient agar plates

PAPER DISC DIFFUSION METHOD

Two different leaf and flower extracts of *Tecoma stans* were tested for anti-microbial activity using PAPER DISC DIFFUSION METHOD. Nutrient agar medium was prepared, sterilized and used as growth medium for bacterial culture. 15ml of sterilized medium was poured into each petri plate, covered semi half and allowed to solidify. Then the test micro organisms like *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* were inoculated into the petri plates using sterile cotton swabs. The sterilized paper discs were soaked in different solvent extracts like METHANOLIC LEAF, METHANOLIC FLOWER, AQUEOUS LEAF and AQUEOUS FLOWER (1µg/ml) and were dried at 50°C. Then the dried discs were placed on medium plated seeded with microorganisms & also prepared control and standard (Amoxicillin 1µg/ml). Then plates were incubated at 37°C. Then the zone of inhibition was measured after 24hrs. Three Petri plates with *controlled* and *standard samples* (1µg/ml) were taken and then discs were placed into the medium plates and incubated at 37°C. Then zone of inhibition was measured after 24hrs

RESULTS AND DISCUSSION

The leaves and flowers of *tecoma stans* were taken, shade dried, grinded and sieved and the leaf and flower powders were weighed to obtain % yield.

CRUDE DRUG EXTRACT YIELD

The leaf and flower powders which were taken for the extraction process by Soxhlation method has shown the yield of the following extracts:

Table-2

Name of the Extract	Part of the Plant	Nature of the drug	% yield
Methanolic leaf	Leaf	Resinous mass	49.10%
Methanolic flower	Flower	Resinous mass	80.75%
Aqueous leaf	Leaf	Dried powder	70.5%
Aqueous flower	Flower	Dried powder	75.2%

Extraction process was done to the leaf and flower extracts of *Tecoma stans* by Soxhlation method and it was observed that Methanolic flower extract has high % yield compared to other extracts.

PHYTOCHEMICAL EVALUATION OF POWDERED FLOWER

The powdered folwer extract obtained was subjected to phytochemical screening using standard procedure by dissolving the sample in sufficient amount of respective solvents and tested for various constituents.

Table-3

S.no.	Name of chemical test	Methanolic flower extract
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Tannins	—
4.	Flavonoids	+
5.	Glycosides	—
6.	Proteins	—
7.	Mucilage	+
8.	Gums	—
9.	Saponins	+
10.	Phenolic compounds	+
11.	Amino acids	—

By performing the phytochemical screening of powdered flower extract of *Tecoma stans*, it was observed that the flower extract mainly contains Alkaloids, Carbohydrates, Tannins, Saponins and phenolic compounds.

ANTI-MICROBIAL ACTIVITY: The zone of inhibition (mm) of different leaf and flower extracts for anti-microbial activity was determined by using PAPER DISC DIFFUSION METHOD.



Fig -15: Laminar air flow unit

ZONE OF INHIBITION (mm) OF DIFFERENT LEAF EXTRACTS**Table-4**

TEST ORGANISM	EXTRACTS			
	Methanolic leaf (1µg/ml)	Aqueous leaf (1µg/ml)	Controlled (1µg/ml)	Standard(Amoxicillin) (1µg/ml)
Escherichia coli	12.03	15.71	-	25.3
Bacillus subtilis	10.13	11.25	-	22.85
Staphylococcus aureus	8.33	14.5	-	24.5

By performing the zone of inhibition (mm) of different leaf extracts, the zone of inhibition (mm) of aqueous leaf extracts was found to inhibit the microbes to a greater extent.

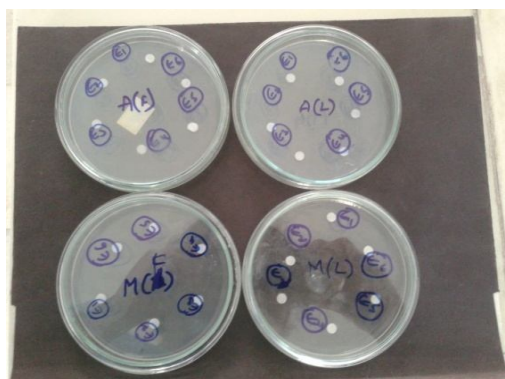
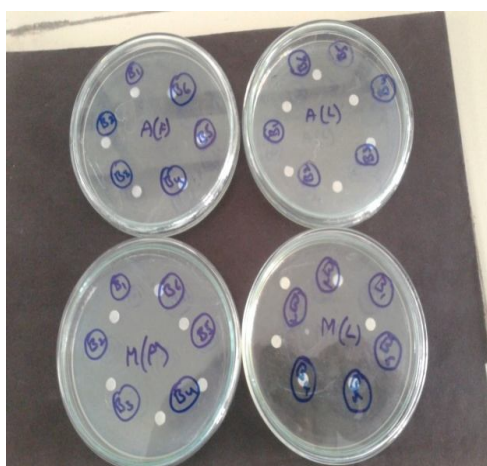
ZONE OF INHIBITION (mm) OF DIFFERENT FLOWER EXTRACTS

TEST ORGANISM	EXTRACTS			
	Methanolic flower (1µg/ml)	Aqueous flower(1µg/ml)	Controlled (1µg/ml)	Standard(Amoxicillin) (1µg/ml)
Escherichia coli	16.25	17.7	-	25.3
Bacillus subtilis	13.05	17.73	-	22.85
Staphylococcus aureus	11.13	18.33	-	23.2

Table-5

By performing the zone of inhibition (mm) of different flower extracts, the zone of inhibition (mm) of aqueous flower extracts was found to inhibit microbes to a greater extent.

ZONE OF INHIBITION**Fig-16: Incubation of petri plates in BOD incubator**

**Fig-17: Escherichia coli****Fig-18: Staphylococcus aureus****Fig-19: Bacillus subtilis****Fig-20: Amoxicillin**

DISCUSSION

In ethnomedicinal practices, the Tecoma stans (Yellow bells) was used in the treatment of cancer, diabetes, arthritis, syphilis and stomach pains. The flower extract of tecoma stans possess good hepatoprotective activity. In the present study, powdered flower extract was subjected to phytochemical evaluation using different chemical reagents and they showed the presence of alkaloids, flavonoids, carbohydrates, mucilage, saponins, and phenolic compounds which are highly active against gram positive and gram negative bacteria. The powdered was subjected to extraction with various solvents like METHANOL and WATER by successive soxhlation method based on polarity and concentrated extracts (1µg/ml) were used for anti-microbial assay.

All the extracts from Tecoma stans powder showed mild to strong activity against most tested micro organisms. The results were compared with those of Amoxicillin as standard antibiotic. Compared to all the four extracts, Methanolic leaf extract and controlled sample showed no activity against gram positive and gram negative bacteria. Aqueous flower extract displayed

excellent activity against gram positive bacteria *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Methanolic flower extract showed considerable activity against *Escherichia coli* and *Bacillus subtilis* compared with leaf extracts (methanol & Aqueous).

CONCLUSION

Tecoma stans is used to treat various types of cancer, diabetes, arthritis etc. The main aim of this work is to find out the anti-microbial activity in *tecoma stans* leaf and flower extracts. The phytochemical screening of powdered flower extract contains alkaloids, flavonoids, carbohydrates, tannins and phenolic compounds. Due to the presence of more amount of flavonoids and saponins, there may be chances of invading several other human pathogenic microbes. The microbial study of various *tecoma stans* extracts (METHANOL and WATER) of flower were found to be effective against various gram positive and gram negative bacteria compared to leaf extracts. So in future the components of the *tecoma stans* flower extract may also reveal still more activities in inhibiting several pathogenic microbes.

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