

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *TECOMA STANS* CRUDE EXTRACTS

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ABSTRACT

Medicinal plants are the wealthy source of antibacterial agents and curatives. *Tecoma stans* are commonly practiced medicinal plants in the villages of Salem District, Tamilnadu (India). Plants grown in this region are not systematically tested for their biological activities in general and antimicrobial activity in particular. Hence, In vitro antibacterial activity of leaf, seeds, Flowers extracts of plant was tested by agar plate method against three bacterial organisms E.Coli, Enterobacter, bacillus creuseus. Gram-negative bacterial strains were more susceptible to the crude extracts as compare to gram-positive. However, this study revealed maximum growth inhibition and effectiveness was remarkably observed in the extracts of *Tecoma stans*. These results indicate that leaves, seeds, flowers have a potential broad spectrum antibacterial activity.

KEYWORDS: Tecoma stans, Antibacterial activity, Agar plate method.

INTRODUCTION

Tecoma stans Plants are Cultured everywhere not in specific place. It useful to human health and well being. The plant is fast growing plant with 30 feet in height contents yellow flowers and leaves with green. The t.stan is useful to treat diabetis in mostly countries like Mexico,

India and America and the roots is used to treat diuretic and Anti-fungal. The first prefer for this plant is herbal medicines.^[1,2]

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 sessile, 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".

Medicinal use

A leaf infusion can be taken orally for treating diabetes and stomach pains

A strong leaf and root decoction is taken orally as a diuretic, to treat syphilis or for intestinal worms.

The flowers are diuretic.

Trees can be planted as a live hedge.

The light brown wood is hard and very durable.

It is used in cabinet making, turnery, to make tools, and in the construction of buildings.^[3-5]

PHYTOCHEMICAL EVALUATION OF POWDERED EXTRACTS 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.

Carbohydrates

Molisch's test: About 2ml of powdered 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.

Alkaloids

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

Tannins

Small quantity of powdered 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.^[6]

Glycosides

The powdered 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.

Saponins

About 0.2 g of the powdered 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.

Flavonoids

Powdered 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.

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.

Proteins

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

Amino acids

The powdered flower was treated with Million's reagent. Red colour showed the presence of amino acids.^[7]

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.

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.^[8]

MATERIALS AND METHODS**Experimental organisms****Escherichia coli**

Escherichia coli (abbreviated as **E. coli**) are bacteria found in the environment, foods, and intestines of people and animals.

Enterobacter is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria of the family Enterobacteriaceae. Several strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised (usually hospitalized) hosts and in those who are on mechanical ventilation.

Bacillus cereus is a Gram-positive, rod-shaped, aerobic, facultatively anaerobic, motile, beta hemolytic bacterium commonly found in soil and food. Some strains are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals.

Chemicals used

Ethanol,

Distilled water,

Dimethyl sulphoxide(DMSO).

Apparatus

Table 1: apparatus.

| Serial No. | List of Equipments | Company |
|------------|--------------------|--------------------|
| 1 | Incubator | Bio-Technics India |

Extraction procedure

Collection of plant material

Tecoma stans leaves, seeds and flowers were collected from local areas of karimnagar, Telangana, India.

Preparation of flower extract

Tecoma stans flowers (6.8 g) were collected from the karimnagar district Telangana in the month of jan. Flowers were dried under shade for 7 days. Dried flowers were subjected to grinding in Grinder to 20 mesh size, and a homogenous yellow powder was obtained and stored in an air-conditioned room before preparation of the extracts for analysis.then the powerd were extracted by using Soxhlet apparatus.

Preparation of leaves extract

Tecoma stans leaves (5 g) were collected from the karimnagar district Telangana in the month of jan. Leaves were dried under shade for 7 days. Dried leaves were subjected to grinding in Grinder to 20 mesh size, and a homogenous yellow powder was obtained and stored in an air-conditioned room before preparation of the extracts for analysis.then the powder were extracted by using Soxhlet apparatus.

Preparation of seeds extract

Tecoma stans seeds (3.8 g) were collected from the karimnagar district Telangana in the month of jan. Flowers were dried under shade for 7 days. Dried seeds were subjected to grinding in Grinder to 20 mesh size, and a homogenous yellow powder was obtained and stored in an air-conditioned room before preparation of the extracts for analysis.then the powerd were extracted by using Soxhlet apparatus.

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 15min at 15lb pressure.

Agar Plate Method

Two different leaf and flower extracts of *Tecoma stans* were tested for anti-microbial activity using AGAR PLATE 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 cereus*, and *Enterobacter* were spreaded with the spreader into the petri plates and do wells with borear. Then the different solvent extracts like ETHANOLIC LEAF, AQUEOUS SEEDS and AQUEOUS FLOWER. (All are done in laminar air flow chamber). Then the dried plates were placed in incubator at room temperature & also prepared control and standard (chloramphenicol). Then the zone of inhibition was measured after 48hrs.^[9,10]

RESULTS

Anti bacterial activity

The zone of inhibition (mm) of different leaves, seeds and flower extracts for anti-bacterial activity was determined by using Agar Well Diffusion Method.

ZONE OF INHIBITION OF DIFFERENT LEAF EXTRACT

Table 2: Zone of inhibition of leaf extract values.

| Test organisms | EXTRACT |
|-------------------------|------------------------------------|
| | Ethanolic leaf extract in diameter |
| <i>Escherichia coli</i> | 1.8cm |
| <i>Enterobacter</i> | 1.55cm |
| <i>Bacillus cereus</i> | 2.27cm |

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

ZONE OF INHIBITION OF DIFFERENT FLOWER EXTRACT**Table 3: Zone of inhibition of flower extract values.**

| Test organisms | EXTRACT |
|------------------|------------------------------------|
| | Aqueous flower extract in diameter |
| Escherichia coli | 1.52cm |
| Enterobacter | 2.3cm |
| Bacillus cereus | 1.2cm |

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

ZONE OF INHIBITION OF DIFFERENT SEEDS EXTRACT**Table 4: Zone of inhibition of different seeds extract values**

| Test organisms | EXTRACT |
|------------------------|-----------------------------------|
| | Aqueous seeds extract in diameter |
| Escherichia coli | 1.67cm |
| Enterobacter acrogenes | 1.87cm |
| Bacillus cereus | 1.2cm |

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

Minimum inhibitory concentration(MIC)**ZONE OF INHIBITION OF DILUTION 10mg,25mg,50mg LEAVES EXTRACT****Table 5: Zone of inhibition of dilution 10mg,25mg,50mg leaves extract values.**

| Test organisms | EXTRACT | | |
|-----------------------|-------------------------------------|--------|--------|
| | Ethanollic leaf extract in diameter | | |
| | 10mg | 25mg | 50mg |
| Escherichia coli | 0.3cm | 0.83cm | 1.8cm |
| Enterobacter acrogene | 0.2cm | 0.4cm | 1.55cm |
| Bacillus cereus | 0.23cm | 0.43cm | 2.27cm |

ZONE OF INHIBITION OF DILUTION10mg, 25mg, 50mg FLOWERS EXTRACT**Table 6: Zone of inhibition of dilution10mg, 25mg, 50mg flower extract values.**

| Test organisms | EXTRACT | | |
|-----------------------|------------------------------------|--------|--------|
| | Aqueous flower extract in diameter | | |
| | 10mg | 25mg | 50mg |
| Escherichia coli | 0.26cm | 0.76cm | 1.52cm |
| Enterobacteracrogenes | 0.2cm | 0.5cm | 2.3cm |
| Bacillus cereus | 0.33cm | 0.6cm | 1.2cm |

ZONE OF INHIBITION OF DILUTION 10mg, 25mg, 50mg SEEDS EXTRACT**Table 7: Zone of inhibition of dilution 10mg, 25mg, 50mg seed extract values.**

| Test organisms | EXTRACT | | |
|-----------------------|-----------------------------------|--------|--------|
| | Aqueous seeds extract in diameter | | |
| | 10mg | 25mg | 50mg |
| Escherichia coli | 0.3cm | 0.73cm | 1.67cm |
| Enterobacteracrogenes | 0.3cm | 0.63cm | 1.87cm |
| Bacillus cereus | 0.23cm | 0.6cm | 1.62cm |

DISCUSSION

In ethnomedicinal practices, the *Tecoma stans* (Yellow bells) was used in the treatment of cancer, diabetes, arthritis, syphilis and stomach pains. The flower, seeds and leaves extract of *tecoma stans* possess good antibacterial activity. In the present study, powdered flower, seeds and leaves 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 ETHANOL and WATER by successive soxhlation method based on polarity and concentrated extracts were used for anti-bacterial 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 CHLORAMPHENICOL as standard antibiotic. Ethanolic leaf extract displayed excellent activity against Entero bacter, *Escherichia coli* and *Bacillus cereus*. Flower, seedsn extract showed considerable activity against Entero bacter and *Bacillus cereus* compared with leaf extracts(ethanolic).

Invitro antibacterial activity was examined for ethanolic and aqueous extract of *tecoma stans* leaves, seeds and flowers. Antibacterial activity was evaluated by Agar well diffusion method against *Escherichia coli*, Entero bacter and *Bacillus cereus* and minimum inhibitory concentration[MIC] was carried out for the EXTRACTS leaves, seeds and leaves. The micro organisms (10ml) were seeded on the medium by spread plate method.

Maximum antibacterial activity was exhibited by leaf *Bacillus cereus*{2.27cm} followed by flower extract against *Enterobacter acrogenes* {2.23cm} other extracts also had shown good antibacterial activity. Therefore minimum inhibitory concentration for all three extracts

against *Escherichia coli*, *Enterobacter aerogenes* was performed. 50mg/ml, 25mg/ml, 10mg/ml and 50mg/ml concentrations were prepared and used for MIC.

MIC of the ethanolic extract of leaf was observed as 10mg/ml against *Escherichia coli*, *Enterobacter aerogenes* and *Bacillus cereus*. The aqueous flower extract also exhibited MIC of 10mg/ml. The seed extract also had shown MIC of 10mg/ml. Due to the presence of more flavanoids and phenolic compounds the antibacterial activity was shown.

Comparative studies against standard drug for Anti bacterial activity

The comparative study of antibacterial was done with chloramphenicol standard drug against different microorganisms like *Bacillus cereus*, *Escherichia coli* and *enterobacter aerogenes*. The diameter of zone of inhibition was found to be *Bacillus cereus* {2.27cm}, *Escherichia coli* {1.8cm} and *enterobacter aerogenes* {2.3cm}.

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-bacterial activity in *tecoma stans* leaf, Seeds and flower extracts. The phytochemical screening of powdered flower, Seeds and leaves 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 bacterial study of various *tecoma stans* extracts (ETHANOL and WATER) of flower, seeds and leaves were found to be effective against various gram positive and gram negative bacteria compared to leaf, seed 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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