

PHYTOCHEMICAL SCREENING & ANTIFUNGAL ACTIVITY OF *TECOMA STANS*

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ABSTRACT

Tecoma stans is a widely cultivated, cool season with climbing growth habit. In the present investigation an attempt was made to screen the anti-fungal activity of *Tecoma stans* leaf. In view of the percentage inhibition also the plant was studied, in which the plant extract was found effective. The antifungal activity was carried out using different extracts of n-hexane, ethyl acetate and ethanolic against fungi such as *Aspergillus niger* and *Penicillium* by the cup-plate assay method and zone of inhibition. The different extracts showed moderate activity against zone of inhibition of ethanolic extract was *Aspergillus niger* and *Penicillium*. The overall result of this study indicates that the n-hexane, ethyl acetate and ethanolic extract of *Tecoma stans* have anti-fungal property.

Keywords: *Infection, Tecoma stans, anti-fungal, n-hexane, ethyl acetate and ethanolic.*

1. INTRODUCTION

Medicinal plant is defined as any substance with one or more of its organ containing properties that can be used for therapeutic purposes or which can be used as precursors for the synthesis of various drugs. Medicinal plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols etc. Traditional medicine refers to health practices, knowledge and beliefs incorporating plants, animals and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well being. Over the years, medicinal plants have been found useful in the treatment and management of various health problems. Traditional medicine is undoubtedly a reliable alternative approach to health care delivery in the metropolis because it is cheap, easily accessible and efficacious. Herbal drugs are invariably single plant extracts or fractions thereof or mixtures of fractions/extracts from different plants. Traditional plant medicines might offer a natural key to treat various human ailments. In recent years, there has been an increasing interest by researchers in the use of naturally occurring biologically active compounds of medicinal value. Plants have been used in virtually all cultures as a source of medicine. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have

made large contributions to human health and well being. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). Genetic biodiversity of traditional medicinal herbs and plants is continuously under the threat of extinction as a result of growth exploitation, environment-unfriendly harvesting techniques, loss of growth habitats and unmonitored trade of medicinal plants [1-2]. The plant is fast growing evergreen plant with 20-30 ft in height, having moderate growth and yellow flowers. Leaves are green, compound, imparipinnate, and lanceolate with serrate margin. Fruits are elongated and clustered. Ginger thomas leaves, bark and roots contains biologically active chemicals, and extracts from those tissues are in use as traditional folk medicines. Plant is in use through Mexico, India and Central America for diabetes, roots for diuretic and urinary disorder control. *Tecoma stans* was also investigated for antifungal effect in roots. Standardization of a plant is first requirement for its use in herbal medicines [3]. Synonyms: Ginger-Thomas, Yellow Trumpet/ Yellow Bells/ Yellow-Elder.

Vernacular Name: Hindi – Piliya/ Pila kaner English – Yellow bells Kannada – Koranekelar Tamil –sonnapatti Telugu – Pachagotla Bengali –chandaprabha Marathi – Ghanti ful [4]

Description of Tecoma Stans: *Tecoma stans* is a promising species in the trumpet vine family, Bignoniaceae that is native to the Americas with many synonyms and common names. Synonyms include *Bignonia stans* L., *Gelseminum stans* (L.) Kuntze, *Stenolobium stans* (L.) Seem and Common names, Yellow Trumpet bush, Yellow Bells, Yellow Elder, Ginger-Thomas, and *Esperanza*. *Tecoma stans* is the official flower of the United States Virgin Islands and the national flower of The Bahamas. It is a flowering perennial shrub or small tree, 5-7.6 m in height. Bark is pale brown to grey and roughens with age. Leaves are opposite, compound and imparipinnate with 2 to 5 pairs of leaflets and a larger single terminal leaflet. Leaflets are lanceolate, up to 10 cm long, with serrated margins, mid-green above and soft to the touch. Flowers occur in clusters at the ends of the branches and are trumpet shaped with 5 rounded lobes, 6 cm long, pale to bright yellow, with faint orange stripes at the throat. Fruits are narrow, slightly flattened to pointed capsules, up to 20 cm long, containing many winged seeds; green when young, pale brown on ripening and remain on the tree in untidy clusters for many months [5].

- The main objective of the study is to obtain the ethanolic, n-hexane and ethyl acetate extracts of *Tecoma stans* by soxhlation method and to evaluate anti-fungal activity of ethanolic, n-hexane and ethyl acetate extracts of *Tecoma stans*.
- To explore the phytochemical screening (the presence of various chemical constituents in plant extract)

2. Materials and Methods

Collection of plant:

Tecoma stans leaves are obtained from the local premises.

Preparation of plant extract:[6]

The collected leaves were sun dried, pulverized by a mechanical grinder, sieved through 40mesh. About 50gms of powdered material is extracted using methanol and water as solvents using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extracts is then concentrated by distillation process and dried under reduced pressure. The solvent free powdered mass thus obtained is used for the experiment.

Pharmacognostic Studies: Physico-chemical parameters:[7-9]

The leaves of the plant were subjected for investigation of physico-chemical parameters like organoleptic evaluation, microscopical studies, determination of ash value, extractive values, loss on drying, crude fibre content and p^H .

Phyto chemical screening:

To perform Phyto chemical analysis, the powdered drug is taken and macerated for 48hrs using methanol and water as solvents. After 48hrs the filtrates were obtained which are further concentrated and dissolved in acetone to perform the screening.

Test for Carbohydrates:

- **Molisch's test:** To 2-3ml aqueous extract, add few drops of alpha-naphthol solution in alcohol shake and add conc. H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids.

Test for reducing sugars:

- **Fehling's test:** Mix 1 ml Fehling's A and 1 ml Fehling's B solutions, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10min. First yellow, then brick red ppt. is observed.

Test for Monosaccharides:

- **Barfoed's test:** Mix equal volume of barfoed's reagent and test solution. Heat for 1-2min in boiling water bath and cool. Red ppt. is observed.

Test for proteins:

- **Biuret test:** To 3 ml test solution add 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour appears.
- **Millon's test:** Mix 3 ml test solution with 5 ml Millon's reagent. White ppt is formed. Warm the ppt which turns brick red or the ppt dissolves giving red coloured solution.

Test for amino acids:

- **Ninhydrin test:** Heat 3 ml test solution and 3 drops of 5% ninhydrin solution in boiling water bath for 10 min. Purple or bluish colour appears.

Test for steroids:

- **Salkowskireaction:** To 2 ml of extract, add 2 ml of chloroform and 2 ml conc. H_2SO_4 . Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
- **Liebermann-Burchard reaction:** Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of the test tube. First red, then blue and finally green colour appears.

Test for glycosides:

- **Legal's test:** To aqueous or alcoholic extract, add 1 ml pyridine and 1 ml sodium nitroprusside. Pink to red colour appears.

Test for saponins:

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

Test for flavonoids:

- **Shinoda test:** To dry powder or extract, add 5 ml 95% ethanol, few drops of conc. HCL and 0.5 mg Magnesium turnings. Orange, pink, red to purple colour appears.
- **Sulphuric acid test:** On addition of sulphuric acid (66% or 80%) flavones and flavonols dissolve into it and give a deep yellow solution.

Test for alkaloids:

- **Dragendorff's test:** To 2-3 ml filtrate, add few drops Dragendorff's reagent. Orange brown ppt. is formed.
- **Mayer's test:** To 2-3 ml of filtrate add few drops of Mayer's reagent gives ppt.
- **Hager's test:** To 2-3 ml of filtrate add Hager's reagent gives yellow ppt.
- **Wagner's test:** To 2-3 ml of filtrate add Wagner's reagent gives reddish brown ppt.

Test for tannins:

- **5% FeCl₃:** To 2-3 ml of aqueous or alcoholic extract, add few drops of 5% FeCl₃ reagent gives deep blue-black extract.
- **Gelatin solution test:** To 2-3 ml of aqueous or alcoholic extract, add solution of gelatin gives white ppt.

Test for volatile oils:

- Volatile oils have characteristic odour..
- Filter paper is not permanently stained with volatile oil.
- Solubility test: Volatile oils are soluble in 90% alcohol.

Antifungal activity: Well Diffusion Method:[10-12]

Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of anti-bacterial or anti-fungal activities of the plant samples (Perez et al., 1990). A sterile swab was used to evenly distribute bacterial or fungal culture over the appropriate medium as stated previously. The plates were allowed to dry for 15 minutes before use in the test. Wells were then created and a pipette was used to place 50 µl of the crude extract of *C. 3 viminalis* into each well. The same extract was used on each plate; with a total of two plates used for each extract including two wells for the positive and negative controls. The negative and positive controls were the same as used in the tube dilution assay. The plates were incubated at 37 °C for 24 hours after which they were examined for inhibition zones. A caliper was used to measure the inhibition zones. Twelve replicates were done for each concentration of the different extracts, and each experiment was repeated six times to ensure reliability.

Czapek Dox Agar medium was prepared and autoclaved at 121 degree Celsius, 10lb pressure for 20 mins. Allow the media to cool, before the media gets solidify fungal spore suspension was

added, and allowed to mix thoroughly with the media then the media was poured into the petriplates. The plates were kept aside for solidification. All the plates were labeled accordingly. The antifungal activity was tested by agar well diffusion technique. Wells are punched into the agar using gel borer. Aqueous extracts by soxhlation (10mg/ml in DMSO) were loaded into the wells as per the labeling and incubated at 25 degree Celsius for 4-5 days. The plates were observed for zone of inhibition every day up to 5 days and measured the zone of inhibition.

3.RESULTS

Extractive values of *tecoma stans* :

Solvents	In %w/w
	Leaf
Methanol	14
Aqueous	24

Phyto chemical screening:

Preliminary Phytochemical screening of different extracts:

Name of the phytoconstituent	n-hexane	Ethyl acetate	Ethanol
	Leaf	Leaf	Leaf
Alkaloids	+	+	+
Carbohydrates	+	+	-
Amino acids	+	+	-
Tannins	+	+	-
Steroids	-	-	-
Saponins	+	+	-
Flavanoids	+	-	+
glycosides	+	+	-
Mucilages	-	+	-
Proteins	+	+	-

Anti-fungal activity:

		Aspergillus	Pencilium
1	n-Hexane	0	0
2	Ethyl acetate	1.44	1.32
3	Ethanol	0	0
4	Ketoconazole	1.8	2.3



Zone of inhibition of ketoconazole against pencillium and aspergillus specie



Zone of inhibition of ethyl acetate extract against pencillium and aspergillus species

4.DISCUSSION:

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are glycosides, flavanoids, alkaloids, tannins and phenolic compounds. The preliminary phytochemical screening of fractions of stem and leaves of *tecoma stans*. ethanolic , ethyl acetate,n-hexane extract revealed the presence of alkaloids, carbohydrates, amino acids,

glycosides, saponins, tannins and flavanoids. Anti fungal activity of extracts may be due to its high contents of tannins and flavanoids.

Currently, medicinal plants continue to play an important role in the management of diabetes mellitus, especially in developing countries, where many people do not have certain drawbacks and therefore there is a need to find safer and more effective anti diabetic drugs. This study was under taken to evaluate the anti hyperglycemic activity of *Trianthea decandra* Linn. plant and no mortality were observed indicating that there were no acute toxic effects of *tecoma stans*.

The anti fungal activity of *Basella alba* leaf extracts was performed against the fungi *Aspergillus niger* and *penicillium specius* by pour plate method. The n-hexane and ethonalic extract had shows no activity against both the fungi. ethyl acetate activity was observed against both *Aspergillus niger* and *penicillium specius*. the standard drug used for anti fungal activity was ketoconazole (50µg/ml). Activity of ethyl acetate extract of leaf (1.4 cm) had exhibited slightly lower activity compared to the standard (2.1 cm) against both the fungi.

5. CONCLUSION:

The results obtained from the present study show that the leaf of *tecoma stans* has beneficial effects in decreasing fungal infections. *tecoma stans* appears to be an attractive material for further studies, leading to possible drug development for fungal diseases. Development of phytomedicine is relatively less expensive and less time consuming. So it is more suited to our economic conditions than allopathic drug development which is more expensive and spread over several years. In conclusion, the results from the present study give a path for the further study of use of *tecoma stans* in folklore medicine for the treatment of microbial infections. From the observation it can be concluded that ethyl acetate extract of *tecoma stans* leaf had good Anti fungal activity and can be further employed for the molecules responsible for its Anti fungal activity.

6. REFERENCES

1. www.pharmaceutical.org, Aug 2013 (2), 13-2
2. Research Journal of Pharmaceutical, Biological and Chemical Sciences March-April 2014 RJPBCS 5(2) Page.No. 605
3. Divya Sri et al.: Journal of Medical and Pharmaceutical Innovation; 1(2)2014; 1-417-50-1-PB-14. Khare C.P. Indian medicinal plant. Springer. 2007: 649-650.
4. S Raju et al./JPBMS, 2011, 8(07)
5. www.wjpr.net Vol 3 Issue 2, 2014
6. Govindappa M, Sadananda TS, Channabasava R and Vinay B. Raghavendra, in vitro anti-inflammatory, lipoxigenase, xanthine Oxidase and acetylcholinesterase inhibitory activity of *Te comastans* (L.) Juss. Exkunth, International Journal of Pharma and Bio Sciences, 2011, 2(2): 275-85.
7. Jinqiu Zhu, Rene Vinas, Ernest E. Smith, In vitro evaluation of human liver cancer cells

- and the potential cytotoxicity of *Tecoma stans* (Bignoniaceae) and *Brickellia cavaniilesi* (Asteraceae) both single and in combination, *Toxicological & Environmental Chemistry*, 2008, 90(4):801–08.
8. Gaitan I, Paz AM, Zacchino SA, Tamayo G, Gimenez A, Pinzon R, Caceres A, Gupta M. Subcutaneous antifungal screening of Latin American plant extracts against *Sporothrix schenckii* and *Fonsecaea pedrosoi*. *Pharm Biol*. 2011 May 19. Epub ahead of print.
 9. Indra Gandhi M and Ramesh S, Antifungal and haemolytic activities of organic extracts of *Tecoma stans* (Bignoniaceae), *Journal of Ecobiotechnology*, 2010, 2(2). Epub ahead of print.
 10. Robles-Zepeda RE, Velazquez-Contreras CA, Garibay-Escobar A, Galvez-Ruiz JC, Ruiz-Bustos E, Antimicrobial Activity of Northwestern Mexican Plants Against *Helicobacter pylori*, *Journal of Medicinal Food*, 2011 June 11. Epub ahead of print <http://biofrontriers.org/?p=177>
 11. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. Agroforestry Database: a tree reference and selection guide version 4.0 (<http://www.worldagroforestry.org/af/treedb/>)
 12. Das C, Mohanty A, Sahoo DC, Dash S, Wound healing potential of methanolic extract of *Tecoma stans* Linn, Leaf, *Drug Invention Today*, 2010, 2(8):373-75.