

Phytochemical Investigation & Diuretic Activity of Tecoma Stans Leaf Extract.

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KEYWORDS

Tecoma stans, diuretic activity, phytochemical investigation, Hydroethanolic extract, Wistar rats, molecular docking.

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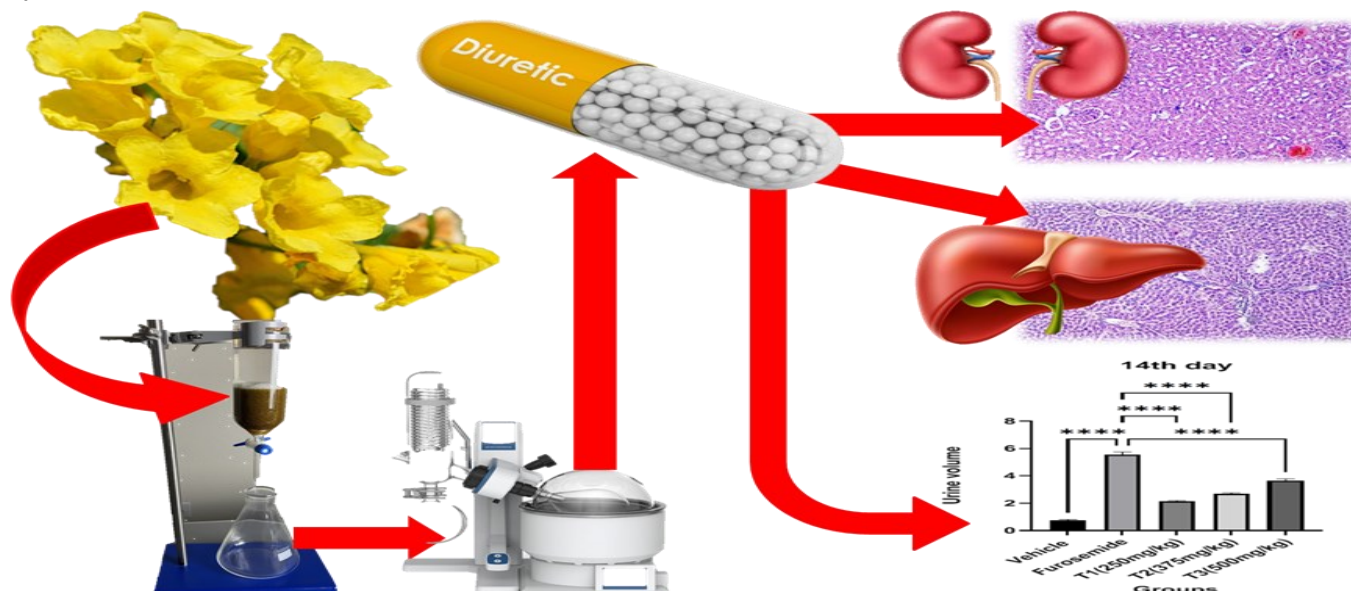
ABSTRACT

Tecoma stans leaves were collected, dried, and processed to obtain a Hydroethanolic extract using percolation. The extract was then administered to adult male Wistar rats in varying doses (250 mg/kg, 375 mg/kg, and 500 mg/kg) to evaluate its diuretic activity over 14 days. Furosemide, a standard diuretic drug, served as the positive control. Urine volume, pH, and diuretic index were measured on days 1st, 7th, and 14th. Histopathological examinations of kidney and liver tissues were conducted post-experiment. Additionally, molecular docking studies were performed to understand the binding interactions of the extract's bioactive compounds with target proteins.

The study demonstrated that the Hydroethanolic extract of Tecoma stans significantly increased urine output in a dose-dependent manner. The highest dose (500 mg/kg) produced a diuretic index of 4.80 and a Lipschitz value of 0.65 on the 14th day, indicating potent diuretic activity comparable to the standard drug furosemide. Histopathological analysis revealed no adverse effects on the kidneys and liver. Molecular docking studies suggested strong binding affinities of the extract's bioactive compounds with diuretic target proteins.

Tecoma stans leaf extract exhibits significant diuretic activity, suggesting its potential as a natural alternative for managing body fluids. Further research is warranted to isolate specific bioactive compounds and elucidate their mechanisms of action.

Graphical Abstract



INTRODUCTION

Tecoma stans, commonly known as Yellow Trumpetbush or Yellow Bells, is a perennial shrub belonging to the family Bignoniaceae. Native to the Americas, particularly the southwestern United States, Mexico, and Central America, *Tecoma stans* is renowned for its ornamental beauty and medicinal properties. Among its various plant parts, leaves are of particular interest due to their rich reservoir of phytoconstituents, which are bioactive compounds responsible for its medicinal attributes.^[1-3]

The leaves of *Tecoma stans* are characterized by their lanceolate shape, with serrated edges and a glossy green hue. They grow in clusters along the stems, providing an attractive backdrop to the plant's showy flowers. While the flowers steal the spotlight with their visual appeal and fragrance, the leaves play a crucial role in the plant's physiology and ecological functions.^[4]

Among the phytoconstituents found in *Tecoma stans* leaves are alkaloids, flavonoids, phenolic compounds, terpenoids, and tannins. These bioactive compounds exhibit various pharmacological activities, including antioxidant, anti-

inflammatory, antimicrobial, and anticancer properties. As such, extracts derived from *Tecoma stans* leaves have been used in traditional medicine for centuries to treat a range of ailments, from fevers and digestive disorders to skin conditions and respiratory infections.^[5-7]

In recent years, scientific research has focused on exploring the therapeutic potential of *Tecoma stans* leaves and their phytoconstituents, leading to a better understanding of their pharmacological mechanisms and potential applications in modern medicine. By unravelling the chemical composition and biological activities of these leaves, researchers aim to harness their medicinal properties for the development of novel drugs and natural remedies.^[8]

2. MATERIALS & METHODS

2.1 Collection of Plant Material

Tecoma stans

The leaves of *Tecoma stans* were collected from lawns behind the college building, SGRS College of Pharmacy, Saswad Pune. The collected leaves were dried and grinded (coarse powder) for extraction of the crude drug.

Sr. No.	Common Name of Plant	Biological Source
1.	Ghanti Ful	<i>Tecoma stans</i>

2.2 Authentication:

The plant was identified by Dr. H. S. Patil, Head of the Department of Botany, Vidya Pratishthan's Arts, Science & Commerce College, Vidyanagari, Baramati, Dist. Pune.

2.3 Method: The leaves were dried in the shade for 10-12 days. After complete drying, the leaves were pulverized to a coarse powder of 40 mesh size in a mechanical grinder.

3. Extraction of *Tecoma stans*

✓ Solvent: Hydroethanolic solution (50:50 v/v ethanol: water).

✓ Method for extraction: Percolation

A 500 g amount of plant material was extracted twice by percolation using 5 L of 50% of Hydroethanolic solution (50:50

v/v ethanol: water). The resulting extracts were pulled together and concentrated using a Rotary evaporator at 60°C under pressure. The extract was freeze-dried to obtain the powdered form of the *T. stans* extracts (TSE).^[9]

4. Animals

Adult male Wistar rats, each in the weight range of 180-200g, were obtained for this study. Animals were randomly allocated to five treatment groups of 6 animals each and kept in cages and housed under standard conditions of temperature, humidity and dark light cycle [12h-12h]. They were provided with regular rat chow and distilled water ad libitum.

5. Experimental protocol

Table No.1: Experimental design

Groups	Name of Group	Treatment	No. of Animals
I	Normal Control	Vehicle	6
II	Positive Control	Furosemide 10 mg/kg [10]	6
III	Test I	Low dose (250mg/kg) [11] of Hydroethanolic extract of <i>Tecoma stans</i> leaves	6
IV	Test II	Medium dose (375mg/kg)[11]Hydroethanolic extract of <i>Tecoma stans</i> leaves	6
V	Test III	High dose (500mg/kg)[11]Hydroethanolic extract of <i>Tecoma stans</i> leaves	6

6. In Vivo Model:

Model: LIPCHITZ TEST

Duration of Study: 15 days

Standard: Furosemide

6.1 Purpose and Rationale:

A method for testing diuretic activity in rats has been described by Lipschitz et al. (1943). The test is based on water and sodium excretion in test animals and compared to rats treated with a high dose of urea. The "Lipschitz-value" is the quotient between excretion by test animals and excretion by the urea control.

6.2 Procedure:

Select Albino rats weighing between 180-250 g of either sex (male or female), and divide them into 5 groups, 6 six animals in each group. Weigh all the animals before drug administration.

Group I was kept as normal control, Group II was kept as positive control, and received Furosemide 10 mg/kg will be used orally as a standard drug. The group III, IV, and V receive test drugs in low (250mg/kg), medium (375mg/kg) and high doses (500mg/kg) respectively will be administered orally daily for 14 days. Immediately after the test drug treatment, all animals were placed in the metabolic cages. After 4- hours total urine volume was collected on the 1st, 7th, and 14th day. Histopathological study of organs like kidneys and liver will be performed on the 15th day.

7. Preparation of Solution and test samples

7.1 Preparation of Furosemide solution:

Furosemide was given at a dose of 10 mg/kg of body weight of experimental animals. An accurately weighed quantity of

Furosemide dissolved in 1 ml of distilled water was given to animals using a clean and dry oral feeding needle i.e., a cannula for 14 days orally.

7.2 Preparation of Test samples:

250 mg/kg, 375 mg/kg, and 500 mg/kg doses of extract of *Tecoma stans* in a 1:1 ratio were dissolved in distilled water according to their body weight. The solution of test samples was administered to the animals using a clean and dry oral feeding needle i.e., a cannula for 14 days by orally.

8. Molecular Docking Studies

Molecular docking is a compelling framework for comprehending drug bio-molecular interactions, which is useful for both mechanistic research and rational drug design and discovery. It aligns a ligand with the preferred binding site of a target-specific DNA or protein (receptor) region, primarily through non-covalent means, to form a stable complex with increased specificity and potential efficacy. The data gathered using the docking method can be utilized to infer complex stability, free energy, and binding energy. The three-dimensional structure of

any complex is predicted based on the binding characteristics of the ligand and target.

A scoring algorithm in the program is used to rank and sort various potential adduct structures that are produced by molecular docking. Reaching an optimal conformation and a reduced binding free energy in a ligand-receptor combination is the primary goal of molecular docking. A data bank for target searches in the appropriate Protein Drug Bank (PDB) format and a procedure for ligand preparation as a PDB file is necessary for the practical implementation of molecular docking. To do this, the ligand can be created in PDB format using a variety of software programs (Discovery Studio, for example). These instruments give ligands the structure they need to bind with specific target proteins or DNA. [12]

8.1 Protein-ligand interactions

Docking simulations predict the binding orientation of drug candidates to their protein targets. Docking simulations were performed in Dell precision T-1500 workstation Intel (R) Core (TM) i7 CPU 860 @GHz; 12.0 GB RAM, 1 TB Hard disk. Protein-ligand interactions were visualized using Vlife MDS

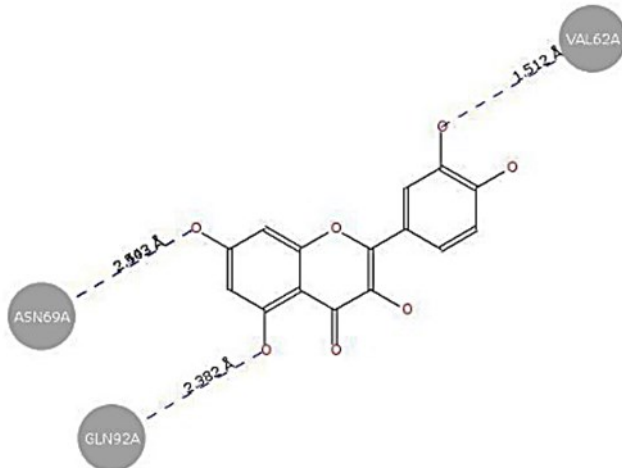


Figure no.1: Hydrogen bonding interactions with PDB ID: 1AZM with VAL 62A, GLA92A and ASN 69A

8. Results & Discussion:

8.1: Urine volume:

Table no.2: Effect of Hydroethanolic extract of *Tecoma stans* on urinary volume on 1st day

Group	Extract & dose [mg/kg]	Volume of urine (ml/6h)	pH	Diuretic index	Lipschitz value
1.	Vehicle	0.47 ± 0.05	6.9	-----	-----
2.	Furosemide 10mg/kg	5 ± 0.54	7.0	10.50	-----
3.	TSE 250mg/kg	1.04 ± 0.05	7.1	2.18	0.20
4.	TSE 375mg/kg	1.23 ± 0.02	6.8	2.58	0.24
5.	TSE 500 mg/kg	1.40 ± 0.07	7.1	2.94	0.28

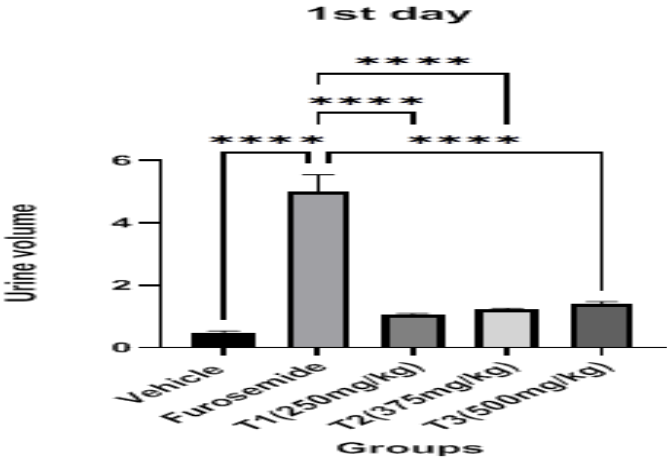
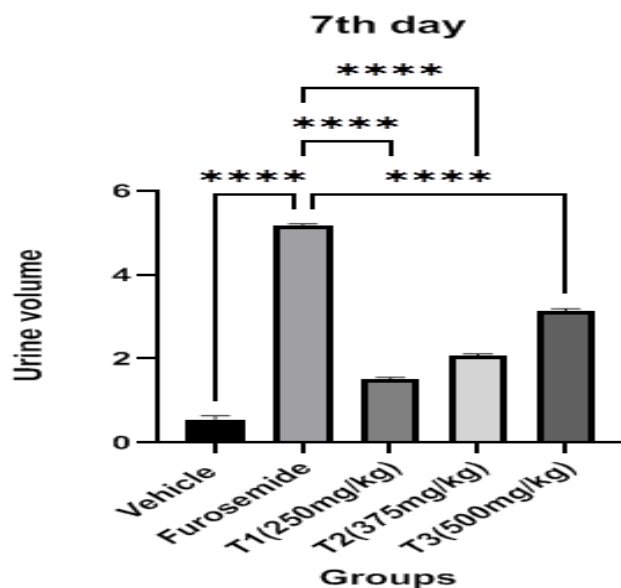


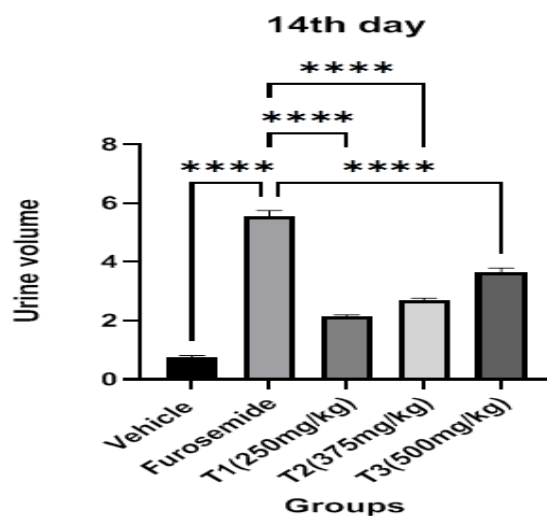
Figure no.2: Urine volume of animals on 1st day

Table no.3: Effect of Hydroethanolic extract of *Tecoma stans* on urinary volume on the 7th day

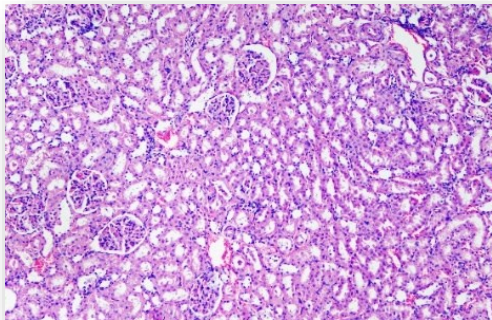
Group	Extract & dose [mg/kg]	Volume of urine (ml/6h)	pH	Diuretic index	Lipschitz value
1.	Vehicle	0.542 ± 0.09	7.1	-----	-----
2.	Furosemide 10mg/kg	5.16 ± 0.05	7.0	9.52	-----
3.	TSE 250mg/kg	1.5 ± 0.05	7.1	2.76	0.29
4.	TSE 375mg/kg	2.06 ± 0.05	6.7	3.80	0.39
5.	TSE 500 mg/kg	3.14 ± 0.05	7.2	5.79	0.60

Figure no.3: Urine volume of animals on 7th dayTable no.4: Effect of Hydroethanolic extract of *Tecoma stans* on urinary volume on the 14th day

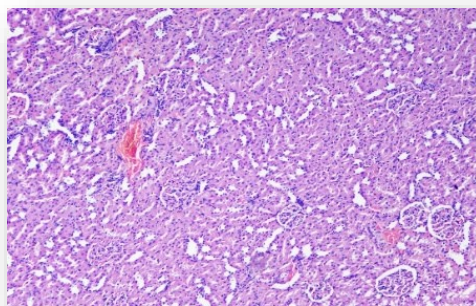
Group	Extract & dose [mg/kg]	Volume of urine (ml/6h)	pH	Diuretic index	Lipschitz value
1.	Vehicle	0.76 ± 0.05	6.9	-----	-----
2.	Furosemide 10mg/kg	5.56 ± 0.19	7.2	7.31	-----
3.	TSE 250mg/kg	2.14 ± 0.05	7.1	2.81	0.38
4.	TSE 375mg/kg	2.69 ± 0.08	7.0	3.53	0.48
5.	TSE 500 mg/kg	3.65 ± 0.13	6.9	4.80	0.65

Figure no.4: Urine volume of animals on 14th day

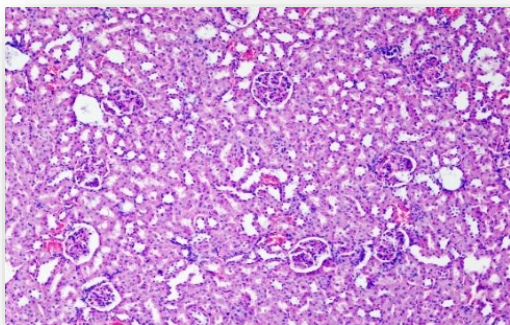
8.2: Histopathological study:
1. Kidney



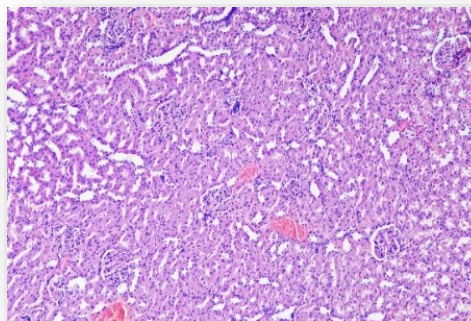
Group I: Vehicle



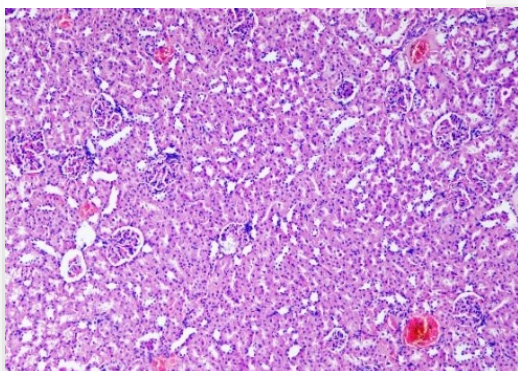
Group II: Furosemide 10mg/kg



Group III: T1 (250mg/kg)



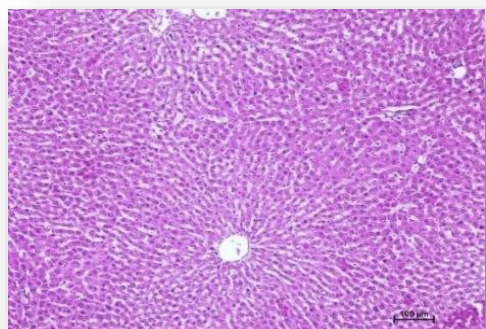
Group IV: T2 (375mg/kg)



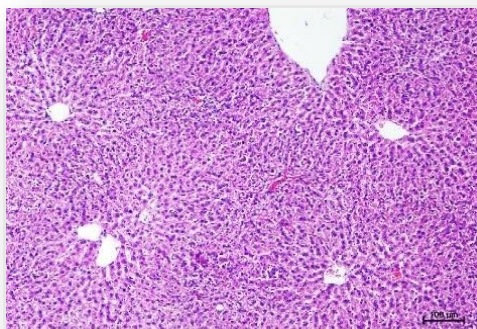
Group V: T3 (500mg/kg)

Figure no.5: Histopathology of kidneys of rats

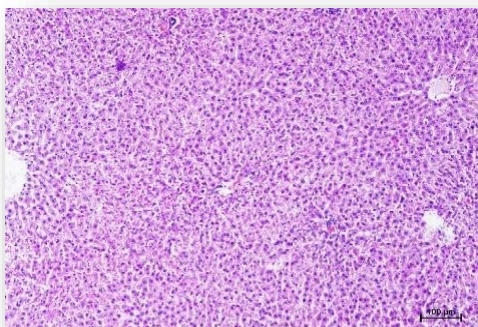
2. Liver:



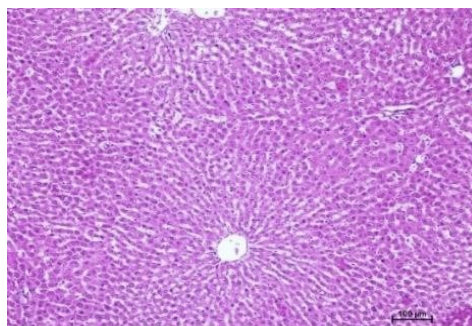
Group I: Vehicle



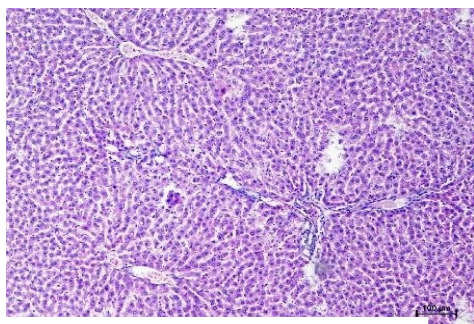
Group II: Furosemide 10mg/kg



Group III: T1 (250mg/kg)



Group IV: T2 (375mg/kg)



Group V: T3 (500mg/kg)

Figure no.6: Histopathology of the liver of rats

Tecoma stans leaf extract exhibits significant diuretic activity, suggesting its potential as a natural alternative for managing body fluids. Further research is warranted to isolate specific bioactive compounds and elucidate their mechanisms of action.

CONCLUSION

The results of the diuretic investigation on the herbal plant showed that *Tecoma stans* showed significant diuretic activity and hence, it may be utilized to successfully control body fluids.

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