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Phytochemical, Physico-Chemical and In-Vitro Anthelmintic Activity of *Tecoma stans*

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ABSTRACT:

The plant *Tecoma stans* belongs to family "Bignoniaceae". The plant is used as anthelmintic, diuretic, to treat syphilis or for intestinal worms, powerful diuretic, vermifuge, tonic, diabetes and stomach pains,snake and rats bites and cancer etc. **Aim**;The aim of the study is to investigate Phytochemical screening of the methanolic and Aqueous extract of *Tecoma stans*and the presence of different secondary metabolites responsible for the therapeutic values of the drug and also to find out the anthelmintic activity study by *in-vitro* test species *Pheretima posthuma* responded towards the plant extracts by showing the sign of paralysis and death finally. **Results**; The % of yield of methanolic extract and Aqueous extract were found to 8.76 % w/w and 3.57 %w/w. The Powder analysis and fluorescence and TLC was observed in ultra short, ultra long and visible. The results were shown in (table-11,12 and 13). **Conclusion:** The %yields of both extract were found to 8.76 % w/w and 3.57 %w/w. The preliminary Phytochemical screening on the leaf extract was carried out by subjecting the different extracts to qualitative test for the identification of various plant constituents. It showed the presence of alkaloids, Carbohydrates and glycoside, saponins, terpenoids, (Table-10) but do not shows the presence of Anthocyanin Gums and mucilage Fixed oil. The data revealed that the aqueous extract has a better wormicidal effect than Petroleum Ether extract.

Keyword:*Tecoma stans*, medicinal use, phytochemical screening, Albendazole, Anthelmintic Activity, Pheretima posthuma.

INTRODUCTION:

All increasing technological horizons (excluding genes) aimed at tapping myriads of techniques of altering plants around us are circumscribed by herbal technology. To harvest the abundant products that the plants produce, such as natural dyes, biofertilizers, biopesticides, and biofuel, a variety of systems have been created.[1]The first step in codifying concepts and creating scientific procedures of this new concept of profitably controlling the plants around us was Herbal Technology. Herb Technology has been at the forefront of herbal medicine development for nearly two decades.

Allopathic medicine is the back bone of present medicine system has been developed through rigorous scientific procedure and has found to be quite effective but still it tend to have adverse drug reaction .how ever on the other hand, the natural products, being the most important source of therapeutics, is still at infancy and need more research to make it more effective and safer as compared to the existing form. **[2]**

Medicinal plants are of great value in the field of treatment and cure of disease. Over the years, scientific research has expanded our knowledge of the chemical effect and composition of the active constituents, which determine the medicinal properties of plant. Crude drugs of natural origin, i.e. obtains from the plants, animals and mineral sources and their active chemical constituents is the core subject Matter of pharmacognosy. These are not only used for treatment of various diseases but also in cosmetic, textile and food industries. During the fast half of the nineteenth century apothecaries of herbal tea mixtures, all kinds of tinctures, extracts and juices which in turn were employed preparing medicinal drops, syrups, infusions, ointments and liniments[3].

In May 2003 a resolution on Traditional Medicine was developed at the 56th World Health Assembly. The resolution requested W.H.O to support and also evidence based information to assist member states in formulating policy and regulations for the safety, efficacy and quality of Traditional Medicines. Indian System of Medicine is developing no doubt. The growth is slow when compared to the gigantic population. It is the time for the Indian Medicines to be given their rightful place in the society [3].

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TAXONOMY AND ETHNO MEDICINE OF TECOMA STANS [4-7]

Synonyms-

Tecoma tronadora (loes) Johnst

Tabebuia stans Juss

Bignonia Stans Linn

Stenolobium stans

Stenolobiumincisum Rose and stand

Gelseminum stans (L) Kuntze

Family:

The species belongs to family " Bignoniaceae ".

Vernacular Names

TEL	Pachagotla
Tam:	Sonapatti,Sornapatti, Nagasambagam.
Kan	Koreneklar
Canarese :	Koranekelar
French :	Bois de pissenlit
Hind	Sonapatti
Satar	Puttane
Arabic	Tacoma
English	Ginger Thomas

DISTRIBUTION

Tecoma stans comes from desert shrub lands and dry forests in the redion from Texas and Arizona southward to Argentina. It has become established in many parts of the Pacific and is naturalizing in South Florida. In South Florida, it invades dry disturbed sites, pine Rockland, and Rockland hammocks. In the Pacific, it prefers wet or mesic sites.

GEOGRAPHICAL DISTRIBUTION

These are planted in gardens in the plains throughout India and in the hills, up to an altitude of 1,500 m. it is naturalized in most parts of India, and is also found as an escape in the waste, dry places near gardens and houses.

PLANT PROFILE:**[8]**

Description :

Tecoma stans is a shrub or small tree. It is a Central and South American tree that grows to 25 ft (7.6m).

Leaves: Leaves opposite, odd-pinnate, up to 20 cm in length; leaflets 5 or 7, lanceolate to oblong-lanceolate, 6-13 cm long, long and slenderly acuminate, base acute or acuminate, margins sharply serrate.

Flowers: Flowers yellow, fragrant, in terminal panicle, found throughout the year,. flowers racemosely arranged on the few branches . Calyx green , 5 mm. long 5-toothed,Corolla yellow, 4-4.5cm long, tube inflated upward., Capsules linear, about 15 cm long , 8 mm. wide, acuminate, compressed.

Bark : The bark on the main trunk is light brown and becomes corky with age.

Fruit: 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 the tree in untidy clusters for many months.

Seeds : Seeds numerous, each with 2 thin wings.

Figure.1– *Tecoma stans*

CHEMICAL CONSTITUENTS:[9-14]

The plant contain triterpenes, hydrocarbons, resins and a volatile oil. The leaves contain flavonoid, the alkaloids, tecomine($C_{11}H_{21}NO$) and tecostidine. According to recent study,six alkaloids, viz. tecomanine, 4-noractinidine, an N-nor-methyl skytanthine, boschniakine, and two other-unnamed alkaloids have been isolated from the plant. The flower contain β-carotene and zeaxantin.Seeds yield 23 percent of fatty oil having the following composition: palmitic,6; stearic, 3;octadecenoic, 7;octadecadienoic, 24;octadecatrienoic, 41;and octadecatertaenoic.

TRADITIONAL USES[15-16]

Its primary applications have been in treating diabetes and digestive problem. Extracts from *Tecoma stans* leaves have been found to inhibit the growth of the yeast infection. Leaf infusion can be taken orally for diabetes and stomach pains. A strong leaf and root decoction is taken orally as a diuretic, to treat syphilis or for intestinal worms.

The root is considered in the Satara District an effective remedy for snake and rats bites and for scorpion sting. The root is ground with lemon juice or if this cannot be had, with water, and applied to the affected part, while a table spoonful is given internally. The roots are used as a powerful diuretic, vermifuge and tonic.

Flower and leaves have some medicinal value for the treatment of various cancer.

MATERIALS AND METHODS

The following drugs and chemicals were used for the different experimental study. The Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. Methanol and Petroleum ether was purchased from Qualigens chemicals. Mumbai. And all others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer.

Plants collection, Identification and processing:

The stems of *Tecoma stans*were collected from adjoining area of Barpali (Dist-Bargarh, Orissa) in the month of December 2024. The plant was identified by and authenticated By Prof. (Dr.) Santosh Kumar Dash, Retired Professor and H.O.D., P.G Dept. of Biosciences, C.P.S, Mohuda, Berhampur, Ganjam, Odisha. on dated 10-12-2024. The plant was washed properly with water to remove the mud or dust, and then it was dried in sun light for 1 hour and kept in shade dried and powdered by the help of mechanical process. The coarse powder have stored in air tight container for further studies.

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QUANTITATIVE MICROSCOPY [17-24]

Lower Epidermis

Table-1 Determination of stomatal number

Field.No	No. of stomata per sq.mm
I	4
II	2
III	3
IV	3

Average stomatal number=3

Table-2 Determination of stomatal index

Field. No	No. of stomata per sq mm(S)	No. of Epidermal cells per sq. mm.	Stomatal index I= S/E+S × 100
I	4	26	13.33
II	2	21	8.69
III	3	20	13.04
IV	3	18	14.28
V	3	15	16.66

Average stomatal index=13.20

Table-3 Determination of palisade ratio

Field No.	No.of Epidermal cells per sq mm(E).	No .of Palisade cells(P).	Palisade ratio
I	4	17	4.25
II	4	15	3.75
III	4	13	3.25
IV	4	13	3.25
V	4	18	4.5

Average palisade ratio=3.8

Table-4 Determination of Vein-islet number

Field No.	Vein-islet number per sq. mm.(A).	No.of vein-islet in 4mm.Area(B).B=A×4
I	4	16
II	3	12
III	2	8
IV	4	16
V	4	16

Average vein-islet number=13.6

Table-5 Determination of vein termination number

Field No.	Vein termination per sq.mm.(A).	No. of vein termination in 4mm.Area(A×4
I	3	12
II	2	8
III	3	12
V	4	16
IV	3	12

Average vein termination=12

Table-6 Determination of ash value

Parameter	Leaf
Total ash	4.2% w/w
Water soluble ash	4% w/v
Acid insoluble ash	1% w/v

Table-7 Determination of extractive value

Parameter	Leaf
Water soluble	16.8% w/v
Alcohol	8.8% w/v

Table-8 Determination of moisture content

Parameters	Leaf
Loss on drying	14.74% w/w

PREPARATION OF EXTRACTS [25-28]

The leaf of Tecoma stans was shade dried and coarsely powder. The leaves of the plant were extracted by decoction with the petroleum ether (60-80o C) for 18 hours and Methanol by heating in refluxed condenser for 18 hours.

Preparation of the extracts of leaves of the plant *Tecoma stans*powdered of entire plant by using following solvents:

(a) Methanol Extract

(b) Aqueous Extract

(a) Metnnol Extract

The shade dried course powder of the entire plant (200 gm) was packed well in soxhlet apparatus and was subjected for continuous hot extraction with Methanol extract with(40-45^{O} C) for 18 hours in a refluxed condenser. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. Dried and kept in a desiccator till experimentation. Obtained extract (Brown) was weighed and percentage yield was calculated in terms of air-dried powdered crude material.

(b) Aqueous extract

The shade dried course powder of the entire plant(200 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with distilled water until the completion of extraction at a temperature of (40-45^{O}C) for 18 hours in a refluxed condenser. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the distilled water completely. It was finally dried and kept in a desiccator till experimentation. Obtained extract (Dirty Green) was weighed and percentage yield was calculated in terms of air-dried powdered crude material.

The yield and % yield of both Methanol and aqueous extracts of powdered of entire plant *Tecoma stans* were reported below.

Table-9 Extraction values of *Methanol*and aqueous extracts of*Tecoma stans.*

Sl. No.	Extracts	% Yield (w/w)	Colour of extract
1.	Methanol	8.76%	Brown
2.	Aqueous	3.57%	Dirty Green

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PRELIMINARY PHYTOCHEMICAL SCREENING:

The plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as Carbohydrates, Protein and Lipids that are utilized as food by human being but also for a multitude of compounds like Glycosides, Alkaloids, Volatile oils, Tannins etc., that exerts a physiological effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents. The different qualitative chemical tests can be performed for establishing profile of given extract for its chemical composition. The following tests may be performed on extracts to detect various phytoconstituents present in them.

Table-10 Qualitative Phytochemical analysis of Methanolic and Aqueous extracts of *Tecoma stans*(Leaves part)

Phytochemical test	**Methanolic Extract**	**Aqueous Extract**
Alkaloid test		
Mayer's test	Present	Present
Wagner's test	Present	Absent
Hager's test	Present	Present
Dragendorff's test	Present	Absent
Carbohydrates and glycoside		
Molish's test	Present	Present
Fehling's test	Present	Absent
Keller-killiant test	Present	Absent
Benidict's test	Present	Present
Borntrager's test	Present	Present
Saponins		
Foam test	Present	Present
Proteins & amino acid		
Millon's test	Present	Absent
Biuret's test	Present	Absent
Ninhydrin test	Present	Absent
Phenolic compounds & flavonoids		
Ferric chloride test	Absent	Absent
Lead acetate test	Present	Absent
Alkaline test	Present	Present
Phytosterol		
Libermann-Burchard's test	Absent	Absent
Salkowski reaction	Present	Absent
Amino acid		
Ninhydrin test	Absent	Absent
Triterpenoid		
Noller's test	Absent	Present
Anthocyanin		
With sodium hydroxide	Absent	Absent
With H_2SO_4	Absent	Absent
Gums and mucilage		
Sweling test	Absent	Absent
Fixed oil		
Spot test	Absent	Absent
Saponification test	Absent	Absent

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Table-11 Powder analysis with chemical reagent

Treatment	Day light	U.V light
Drug powder + 1 N HCL	Light green	Yellowish Green
Drug powder + 50% HCL	Pale green	Light Green
Drug powder + 50% H_2 SO_4	Deep Green	green
Drug powder +50% HNO_3	Yellowish brown	Green
Drug powder + 1N NaOH	Yellow	Yellowish green
Drug powder + al. NaOH	Dark green	Brown
Drug powder + water	Light Green	Light Yellow
Drug powder + methanol	Dark green	Deep Green

Table-12 Fluorescence analysis of Methanolicleaf Extract

Reagent	Colour observed (naked eye)	Colour observed (U.V short wave length)	Colour observed (U.V long wave length)
Powder as such	Light Brown	Light Brown	Dark brown
Powder + 1N NaOH in methanol	Yellowish brown	Light green	Dark brown
Powder + NaOH in water	Light brown	Light brown	Dark brown
Powder + 50% HCl	Deep Brown	Light green	Light Black
Powder +50% H_2SO_4	Light brown	Light green	Black
Powder +50% HNO_3	Light brown	Green	Brown
Powder + petroleum ether	Light brown	Light brown	Brown
Powder + chloroform	Brown	Green	Dark brown
Powder + picric acid	Yellow	Light Green	Black
Powder + 5% $FeCl_3$	Yellow green	Green	Black
Powder + 5% iodine solution	Green	Dark green	Black
Powder + methanol	Light brown	Brown	Dark brown
Powder + (HNO_3 + NH_3)	Brown	Green	Dark brown

Table-13 Thin layer chromatography of Methanolic leaf extract of *Tecoma stans*.(Linn.)

Test extract	Solvent system	Detecting agent	Number of spots	Color of the spot	R_f value
	Solvent I				
Methanol	a)Tolune:Ethylacetate:Formicacid:Water (7:2:0.5:0.5)	Iodine vapour	5	Green Light green Yellow Light yellow	0.18, 0.29, 0.55, 0.79, 0.87.
	Solvent II				
Methanol	b)Dichloromethane:Methanol (10:1)	Iodine vapour	5	Light yellow Light green .	0.18, 0.30, 0.57, 0.83, 0.88.

DETERMINATION OF ANTHELMINTIC ACTIVITY [29]:

The anthelmintic study was done by using one in-vitro species adult earthworms Pheretimaposthuma. Earthworms were collected near the swampy water in our locality. The average size of the round worm was 5-7 cm; average size of the earthworm was 8-9 cm. These earthworms were identified and services of veterinary practioner were utilized to confirm the identity of worms. The

suspensions of various extracts were prepared in 2% gum acacia solution to obtain 1, 2.5 and 5% concentrations. Solutions of similar concentrations of the standard drug albendazole were also prepared in distilled water.

Two ml of each concentration of various extracts of *Tecoma stans* and standard drug albendazole were diluted to 10 ml separately with normal saline and poured in petridishes. 2ml of 2% gum acacia solution was diluted to 10ml with normal saline to serve as control. Six earthworms of nearly equal size were placed in each Petridis at room temperature. Time was recorded at the time of releasing the earthworms to each concentration. The time taken (minutes) for the complete paralysis and death were recorded. The mean paralysis time for each sample was recorded. The anthelmintic activity was evaluated on adult Indian earthworm Pheritimaposthuma due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body colour.

Table 14: Anthelmintic effect of *Tecoma stans* extracts**.**

Group	**Concentration of Extract (%)**	**Time in minutes (Mean ± SEM)**	
		Paralysis time(Min)	**Death time(Min)**
Albendazole (standard)	10 mg/ml	14min,18 sec ±17	18min,18 sec ±42
	30 mg/ml	10min,21 sec ±12	14 min,12 sec ±10
	50 mg/ml	8 min,12 sec ±14	12min,40 sec ±11
Methanolic Extract	15 mg/ml	27min,16 sec ±17	32min,15 sec ±48
	30 mg/ml	23min,26 sec ±12	30 min,26 sec ±12
	50 mg/ml	21 min,48 sec ±14	24 min,48 sec ±14
Aqueous extract	15 mg/ml	13min,19 sec ±17	19min,15 sec ±48
	30 mg/ml	12min,26 sec ±12	15 min,26 sec ±12
	50 mg/ml	9 min,48 sec ±14	11min,14 sec ±10
Control	-	-	-

Results are expressed as mean ± SEM from six observations, *Control worms were alive upto 24 hrs. of observation,* N/A= No Activity shown within 24 hours.

Results and Discussions:

The percentage yield of Methanol and aqueous extract were found to 8.76 %w/w and 3.57 %w/w (Table-19). The preliminary phytochemical screening on the leaf extract was carried out by subjecting the different extracts to qualitative test for the identification of various plant constituents. It showed the presence of alkaloids, Carbohydrates and glycoside, saponins, terpenoids, (Table-10) but do not shows the presence of Anthocyanin Gums and mucilage Fixed oil. The Powder analysis and fluorescence and TLC was observed in ultra short, ultra long and visible. The results were shown in (Table-11,12 and 13).

The results (Table-14) depict the time taken for paralysis and death of earthworms after the treatment with the test extracts at the selected concentrations. The data revealed that the aqueous extract has a better wormicidal effect than Petroleum ether extract with compared with the standard drug Albendazole. Further study is required to find out the novel phytoconstituents responsible for anthel Bibliographymintic action against various helminthes.

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Conflict of Interest Statement: All authors have nothing else to disclose.