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TECOMA STANS: A PHYTOCHEMICAL ANALYSIS & ANTHELMINTIC STUDY OF IT'S DIFFERENT EXTRACT

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ABSTRACT

The plant *Tecoma stans* belongs to family "Bignoniaceae". The leaf of *Tecoma stans* is used in the treatment of diabetes, stomach pain, cancer, diuretic, syphilis, intestinal worms, snake rats bites and anticancer. **Aim**; The aim of the study is to investigate Phytochemical screening of the Petroleum ether and methanol extract of *Tecoma stans* powdered 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 petroleum extract and methanolic extract were found to 4.66% w/w and 8.76%w/w. In fluorescence analysis the powder drug was treated with different reagent which showed light green, pale green, green, in day light and green, deep green, yellowish green, brown in U.V light. Similarly methanolic extract showed light green, pale green, brown, yellow, green, dark green in day light and green, crimson, deep brown, deep yellow and yellow are appeared. TLC was carried out for the extract. The methanolic leaf extract showed different R_f spots in two solvent systems. **Conclusion:** The % yields of both extract were found to 4.66% w/w and 8.76%w/w. The preliminary phytochemical screening showed the presence of alkaloids, glycosides, steroids, tannins, terpenoids, flavonoides, quinines, saponins. In fluorescence analysis the powder drug and methanolic extract was treated with different reagent which showed different colours in U.V light. The data revealed that the and methanolic extract has a better wormicidal effect than Petroleum Ether extract.

KEYWORDS: Tecoma stans, medicinal use, phytochemical screening, Phytochemical, Albendazole, Anthelmintic Activity, Pheretima posthuma.

INTRODUCTION

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.[1] Despite advances in Western System of Medicine and Medical Technology world over, it is increasingly being realized that if we have to support the health care requirements of our ever-increasing population, we will have to resort to cheaper, yet effective alternatives and there cannot be a better alternative than herbal drugs, which have had a long history of safe usage different parts of the world, including India. 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. In the current phase of the health care delivery system, people are turning towards herbal treatment. Indian systems of Medicine mainly focus on herbs for prevention of disease, to increase the longevity and for cure of much chronic disease. Indian has very rich resources of these herbs duo to broad bio diversity.[2] Tradition of medicinal plants use in India is about 4000 years old. Even today millions of people across the country depend on this tradition, which includes two streams, the modified system and folk system. Ethnic communities all over the country practice folk medicine. The modified systems include Ayurveda, Siddha, Unani and Tibetan. As per estimates India has about 15000 species of plants with medicinal properties. Presently about 800 of these are in use as follows[3] from where auyrveda has 1769 species, siddha has 1121 species, Unani has 751 species,Tibetan has 279 species and folk has 4671 species**.** Traditional Medicine is defined by W.H.O as health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manuals techniques and exercises applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well being. The term traditional medicine is applied due to the fact that they had their origin in the remote past and most of these are still practiced almost in the same manner as have been in the past, maintaining the tradition. The basic principle involved in traditional medicine is that these are holistic in nature and rather than treating in isolation they are believed to eradicate the root

cause of the disease. 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.[4]

TAXONOMY AND ETHNO MEDICINE OF *TECOMA STANS*[5-8]

The synonym of this plant *Tecoma stans* are Tecoma tronadora (loes) Johnst, Tabebuia stans Juss, Bignonia Stans Linn, Stenolobium stans, Stenolobium incisum Rose and stand, Gelseminum stans (L) Kuntze.This plant belongs to the family "Bignoniaceae". It has many vernacular names as in Telugu-Pachagotla, Tamil-Sonapatti, Sornapatti, Nagasambagam, Kannada- Koreneklar, French- Bois de pissenlit, Hindi- Sonapatti, English- Ginger Thomas, Arabic- Tacoma, Spanish- Roble Amarillo, Sauco Amarillo.

GEOGRAPHICAL DISTRIBUTION

These plants are distributed all over the world as in Argentina, Bolivia, Brazil, Chile, Colombia, French Guina, Guatemala, Guyana, Haiti, Honduras, Mexico, Nicaragua, Panamma, Paraguay, Puerto Rico, Surinam, Uruguay, Venezuela. Ghana, India, Kenya, Liberia, Mali, Mauritania, Niger, Nigeria, Pakistan, Rwanda, Snegal, Sierra Leone, Sudan, Tanzania, Togo, Uganda, United State Of America.[9-10] 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

Tecoma stans is a shrub or small tree.[11] It is a Central and South American tree that grows to 25 ft (7.6). The Leaves are 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. The Flowers are 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. The bark on the main trunk is light brown and becomes corky with age. The 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 an the tree in untidy clusters for many months and the Seeds are numerous, each with 2 thin wings. These plants grow on clay loams soil, but it particularly tolerant of alkaline condition. This type of plant species needs full of sun. The seed

germinates readily in sandy soil in the spring. This plant grows at an altitude of 0-2000m. Mean annual rainfall: 600mm. These plants are pollinated by humming birds.

CHEMICAL CONSTITUENTS

The plant contains triterpenes, hydrocarbons, resins and a volatile oil.[12-17] 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.

MEDICINAL USES

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.[18-19] The root is considered as 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 plant specimens were collected from adjoining area of Barpali (Dist-Bargarh, Odissa) in the month of January-2018. The plant was identified by Botanist Prof. (Dr.) Santosh Kumar Dash, Retired Professor and H.O.D., P.G Dept. of Biosciences, C.P.S, Mohuda, Berhampur,

Ganjam, Odisha. The plant was washed properly with water to remove the mud or dust, and then it was dried in sun light for 1 h 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.

Figure-1 plant parts of *Tecoma stans.*

Preparation of extracts[20]

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

Petroleum ether extract

The powder of the leaf was extracted with petroleum ether (60-80^{0} C) by heating in refluxed condenser for 18 hours. The extract was evaporated to dryness under vacuum. The dried extract was stored in vacuum desiccators.

Methanol extract

The marc left after petroleum extract was dried and extracted with methanol by heating in refluxed condenser for 18 hours. The extract was evaporated to dryness under vacuum. The dried extract was stored in vacuum desiccators.

Table-1 Percentage yield of leaf extract of *Tecoma stans.*

Extract	Percentage Yield	Colour
Petroleum ether	4.66%	Brown
methanol	8.76%	Dark brown

QUALITATIVE CHEMICAL EXAMINATION

The preliminary phytochemical screening[20,21] on the leaf extract was carried out as per the method. These extracts were subjected to qualitative test for the identification of various plant constituents.

Table-2 Preliminary phytochemical screening.

SL.No	Constituent	Test	Petroleum extract	Methanol extract
1	Alkaloids	a) Mayer's reagent b) Dragendroff's reagent c) Hager's reagent d) Wagner's reagent	Present Present Present Present	Present Present Present Present
2	Carbohydrates and glycoside	a) Molish's reagent b) Borntrager's test c) Keller-killiant test d) Benedict's tesr	Present Present Present Present	Present Present Present Present
3	Steroid	a) Liebermann-burchard b) Salkowski reaction	Absent Absent	Present Present
4	Saponin	a) Foam test	Present	Present
5	Protien	a) Million's test b) Biuret test	Absent	Absent
6	Amino acid	a) Ninhydrin test	Absent	Absent
7	Flavonoid	a) Shinoda test	Absent	Present
8	Tannins	b) lead acetate sol	Absent	Present
9	Triterpenoid	a) Noller's test	Absent	Present
10	Quinine	a) With sodium hydroxide	Absent	Present
11	Coumarin	a) 10% sodium hydroxide	Absent	Absent
12	Anthocyanin	a) With sodium hydroxide b) With H_2SO_4	Absent	Absent
13	Gums and mucilage	a) Sweling test	Absent	Absent
14	Fixed oil	a) Spot test b) Saponification test	Absent	Absent

FLUORESCENCE ANALYSIS[22-24]

Fluorescence analysis of the drug was observed in day light and Ultra-Violet light (254nm) using powder and various solvent extracts of the drug. The drug powder were treated with different solvent in different test tubes. The solvents used are methanol, water, alcoholic sodium hydroxide, 1N sodium hydroxide, 50% nitric acid, 50% sulphuric acid, 50% hydrochloric acid, 1N hydrochloric acid. Then they are subjected to fluorescence analysis in day light and ultra violet light. The results are given in the Table No-3. The extract is treated with similar reagent and subjected to fluorescence in day light and ultraviolet light. The results are given in the Table No-4.

Table-3 Fluorescence Analysis of Drug Powder.

Treatment	Day light	U.V light
Drug powder + 1 N HCL	Light green	Green
Drug powder + 50% HCL	Pale green	Green
Drug powder + 50% H_2 SO_4	Green	Deep 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	Green	Green
Drug powder + methanol	Dark green	Green

Table-4 Fluorescence Analysis of methanolic extract.

Treatment	Day light	U.V light
Methanol extract + 1N HCL	Light green	Green
Methanol extract + 50% HCL	Light green	Green
MethanoL extract + 50% H_2SO_4	Pale orange	Crimson
Methanol extract + 50% HNO_3	Brown	Deep brown
Methanol extract +1N NaOH	Yellow	Deep yellow
Methanol extract + al. NaOH	Light yellow	Yellow
Methanol extract + water	Green	Green
Methanol extract + methanol	Dark green	green

THIN LAYER CHROMATOGRAPHY[25]

It is one of the methods of separating and isolating plant constituents, the chromatographic procedure originated by Tswett is one of the most useful techniques for general application. All finely divided solids have the power to adsorb other substances on their surfaces to a greater or lesser extent. Similarly all substances are capable of being adsorbed, some much more readily than other. This phenomenon of selective adsorption is the fundamental principle of chromatography. The TLC was carried over plates (20cm ×5cm) coated with silica gel G containing 15% $CaSO_4$ ½ H_2O $_2$ as binder. The TLC was done for Methanolic extract by using iodine vapour as detecting agent and finally the different R_f values were calculated.

Table-5 TLC of methanolic leaf extract of *Tecoma stans*.(Linn.).

Test extract	Solvent system	Detecting agent	Number of spots	Colour of the spot	R_f value
	Solvent I				
Methanol	a) Tolune: Ethyl acetate: Formic acid: 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 BIOLOGICAL (ANTHELMINTIC) ACTIVITY[26]

The anthelmintic study was done by using one in-vitro species adult earthworms Pheretima posthuma. 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 Pheritima posthuma 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 6: Anthelmintic Effect of *Tecoma stans* extracts.

Group	Concentration of Extract (%)	Time in minutes (Mean ± SEM)	
		Paralysis time(Min)	Death time(Min)
Albendazole (std)	10 mg/ml	15min,16 sec ±17	19min,15 sec ±48
	30 mg/ml	13min,26 sec ±12	16 min,26 sec ±12
	50 mg/ml	10 min,48 sec ±14	14min,48 sec ±14
Petroleum Ether extract	15 mg/ml 6	25min,16 sec ±17	31min,15 sec ±48
	30 mg/ml 6	20min,26 sec ±12	26 min,26 sec ±12
	50 mg/ml 3	18 min,48 sec ±14	21 min,48 sec ±14
Methanolic extract	15 mg/ml 6	13min,19 sec ±17	19min,15 sec ±48
	30 mg/ml 4	12min,26 sec ±12	16 min,26 sec ±12
	50 mg/ml 3	10 min,48 sec ±14	13 min,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 DISCUSSION

The percentage yield of petroleum extract and methanolic extract were found to 4.66% w/w and 8.76%w/w (Table-1). 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, glycosides, steroids, tannins, amino acids, terpenoids, flavonoides, quinines, saponins. (Table-2). The fluorescence analysis of the drug was observed in day light and ultra violet light using powder and methanolic extract of the drug. The results are shown in the (Table-3, 4). In fluorescence analysis the powder drug was treated with different reagent which showed light green, pale green, green, yellowish brown, yellow dark green in day light and green, deep green, yellowish green, brown in U.V light. Similarly methanolic extract showed light green, pale green, brown, yellow, green, dark green in day light and green, crimson, deep brown, deep yellow and yellow are appeared. Thin layer chromatography was carried out for the extract. The methanolic leaf extract of leaf showed five spots in two solvent systems. The R_f value of spots in solvent-I (Plate A) were found to be 0.18, 0.29, 0.55, 0.79, 0.87 and for solvent-II (Plate B) 0.18, 0.30, 0.57, 0.83, 0.88. (Table-5).

The results (as shown in Table-6) 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 and methanolic extract has a better wormicidal effect than Petroleum Ether extract. The results were compared with the standard drug, Albendazole. Further study is required to find out the novel phytoconstituents responsible for anthelmintic action against various helminthes.

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