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Research article

Phytochemical, pharmacognostic and hepatoprotective activity of the leaves of *Tecoma stans*

Singh Anju[1,2]*, Kumar Vikas[1], Rajendiran A[2], Gupta Pooja[2]

1. Sam Higgin bottom University of Agricultural Sciences and Technology, Nain, Prayagraj, Uttar Pradesh, India
2. University Institute of Pharmacy, C.S.J.M.U. Kanpur, Uttar Pradesh, India

ABSTRACT

Tecoma stans a woody shrubs belonging to the Bignoniaceae family and is commonly known as Pelli Kaner. The present paper shows various pharmacognostic standards such as taxonomy of leaf which shows leaf is found to be simple with the alternate arrangement, serrulate margin with obovate shape, obtuse apex and oblique, rounded base, pinnate venation. Secondly, microscopic characters are determined by cutting its leaf transverse section as well as powder microscopy, these studies provide various information about the arrangement of cell-like xylem, phloem, the upper and lower epidermis. Vein islet and vein termination number etc. Physical and chemical parameters such as total ash, acid insoluble ash, water-soluble ash, moisture content, water-soluble, and extractive value of leaf are calculated. Various chemical reagents are used to identify the chemical nature of leaf extract. Ethanolic extract of leaf is found to be active and 200 and 400 mg when in vivo evaluated for hepatoprotective activity. Ethanolic extract of the leaf also contains total phenolic content of 1.36 ± 0.02 mg/gm of dried extract equivalent to Gallic acid, the total flavonoids content of the extract was evaluated to be 4.41± 0.02 mg/gram and potential antioxidant activity which is comparable to the standards.

Keywords: *Tecoma stans* (TS), Phenolic content, Flavonoids content, Antioxidant activity, Hepatoprotective activity

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Correspondence: Singh Anju* ✉ anjutsingh@gmail.com

Sam Higgin bottom University of Agricultural Sciences and Technology, Nain, Prayagraj, Uttar Pradesh, India

INTRODUCTION

The *Tecoma stans* L. (Family- Bignoniaceae), locally known as "peeli kaner" is found in India, South America, also known as Yellow trumpet bush is an attractive plant that is cultivated as an ornamental. It has sharply toothed, large green leaves with light golden yellow coloured bell-shaped flowers. [1, 25]

It can withstand drought and thrives in hotter temperatures. Tecomine (the alkaloids derived from the plant picked in Egypt) was proven to be one of the chemicals responsible for the hypoglycaemic action, which is noteworthy given the interest in medicines that can treat type II diabetes.[2] Flowers, as well as bark, were used in the treatment of various cancers while flowers are traditionally used to treat eye disorders. At the same time, it also acts as anti-diabetic [3], antispasmodic [4], antioxidant [4], anti-proliferative [5], wound healing [5], cytotoxic [6], antimicrobial [5], anti-fungal [7]. Other chemical constitutes are phytosterols, flavonoids, quinones, amino acids, monoterpenes, triterpene, glycosides, alkaloids, phenols, saponins, and tannins. [8]

Tecoma stans is a plant that has been used in traditional medicine for the treatment of diarrhoea, dysentery, conjunctivitis, edema, inflammation, swellings, and muscular soreness by people from all over the world. This is commonly used in many nations to treat dysentery, diarrhea, muscular pain, and abdominal colic's and its extract possesses has antibacterial, antiviral, and antifungal functions. [16]

Figure 1: *Tecoma stans* Linn.

MATERIALS AND METHODS

Plant material: Collection of leaves was carried out from the locality of Kanpur, in the month of October 2015. The plants were identified & authenticated in the herbarium lab of the National Botanical Research Institute (N.B.R.I), Lucknow (U.P.) The accession number for the specimen is LWG-69.

Macroscopy

Its taxonomic characters and organoleptic properties of leaves were noted such as leaf size and its colour, taste, odour, surfaces, venation, margin, base, apex, lamina, and texture. [10]

Microscopy

The transverse sections of the fresh leaves were cleaned in KOH (as Choral hydrate is banned), mounted with glycerine, and examined under a compound microscope at projection 10 xs & 40 xs for microscopic evaluation. The epidermal cells including stomata (lower and upper), covering trichomes, phloem, xylem, stomata (type and distribution), and collenchyma were all found to be present or absent. [11]

A tiny number of powdered leaves, as well as transverse sections of fresh leaves through the lamina and midrib, were cleaned, mounted, and inspected. Research that is quantitative on epidermal strips, quantitative leaf microscopy was used to estimate vein islet number, vein termination number, stomata index, and stomata number. [12]

Quantitative investigation

On epidermal strips, quantitative leaf microscopy was used to estimate vein islet number, vein termination number, stomata index, and stomata number. [13]

Physicochemical parameters

Parameters of physicochemical properties were studied according to the method provided by Sailor et al. was slightly modified to determine total ash, acid insoluble ash, and moisture content. [14]

Preliminary phytochemical screening

Screening for phytochemical in the preliminary stage Hexane, ethyl acetate, chloroform, and ethanol was used to extract the powder drug weighing 20 g. The extracts obtained after successive solvent extraction were subjected to various qualitative chemical tests for the presence of a mixture of phyto constituents such as alkaloids[15], carbohydrates, glycosides, steroids, tannins, proteins, flavonoids, and saponins as well as detection methods.[16, 17]

Total Phenol Contents

The Folin-Ciocalteu procedure was used to assay the total phenol content of the extracts. In a test tube, 1 ml of the extract (1 mg/ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent and 1.5 ml of sodium carbonate. After shaking, it was kept for 30 min. for reaction. The absorbance reading was measured at the wavelength of 765 nm. Gallic acid monohydrate (10-50 µg/ml), the standard curve was used. [18]

Total Flavonoid Contents

The flavonoid content determination is based upon the formation of a flavonoid-aluminum complex. One millilitre of the extract at a concentration of 1mg/ml was prepared by adding water (distilled). Stated in zero time, 0.3 ml of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% $AlCl_3$ was added, and then at 6 min, 2 ml of 1 M NaOH was also added to the mixture, followed by volume makeup to 10 ml with distilled water. Immediately, the absorbance reading was measured at the wavelength of 510 nm. Quercetin was considered as a standard for the estimation of flavonoid content. [19]

Antioxidant activity by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) [20]

The ability of the matching extract and some pure chemicals to donate hydrogen atoms or electrons was determined by bleaching a purple-coloured methanol solution of DPPH. The capacity of the extracts to scavenge the DPPH free radical was determined using the method described in the paper. In a nutshell, a 0.1 mM DPPH solution in 100% MeOH was made. 4 ml of the sample solution in 40% MeOH at various concentrations (1-160 g/ml) were added to 1 ml of this solution. The mixture was vigorously agitated and incubated at room temperature for 30 minutes until stable absorbance readings were obtained. Continuous monitoring of the decline in absorbance at 517 nm was used to track the lowering of the DPPH radical. For the control, 40% MeOH was used instead of samples. Higher free radical scavenging activity was shown by lower absorbance in the reaction mixture. The following equation was used to compute the DPPH radical scavenging activity:

$$\text{Scavenging effect}(\%) = \left(1 - A_{\text{samples }517} / A_{\text{control}517}\right) \times 100$$

Where, A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Quercetin was considered as a standard here. The IC_{50} value represents the sample concentration required to scavenge 50% of the DPPH free radical. [22]

IN-VIVO HEPATOPROTECTIVE STUDIES

Experimental animals

Animals used in research Wistar rats (160–200 g) of either sex, male or female were obtained and kept for the duration of one week under controlled conditions of temperature (27 °C) and ratio 44–56 percent, light and dark cycles of twelve hours. The animals were fed a conventional mouse pellet diet (Amrut, India) for 18–24 hours before the experiment, though the water was available at all times.[21] The Institutional Animal Ethics Committee accepted the protocol for this study based on the guidelines of the CPCSEA, New Delhi, with the number IEAC/ UIP/ March-2019/107.

Liver toxicity induced by Carbon tetrachloride (CCl4)

Animals were divided into five groups (n = 6). Group I served as vehicle control, receiving liquid paraffin. For 14 days, Groups II-V were given intraperitonially CCl4 in liquid paraffin (1:2) at a dose of 1 ml/kg body weight once every 72 hours [23]. Ethanolic extract of *Tecoma stans* of the leaf was given to Groups IV and V at doses of 200 and 400 mg/kg body weight, respectively, while silymarin was given to Group V at a dose of 25 mg/kg body weight through oral administration [23, 24].

Group I: CCl4 control received liquid paraffin;

Group II: CCl4 as natural recovery group (1 ml/kg, i.p.);

Group III: CCl4 + treated with a daily dose of Silymarin (25 mg/ kg

body weight);

Group IV: CCl4 + treated with a daily dose of Ethanolic extract of Tecoma stans of the leaf (200 mg/kg, p.o.);

Group V: CCl4 + treated with a daily dose of Ethanolic extract of Tecoma stans of the leaf (400mg/kg, p.o.).[21]

Data Analysis

The results were analyzed statistically using a two-way analysis of variance (ANOVA) followed by Bonferroni post-tests to calculate the level of significance. The values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *p<0.05, **p<0.01, ***p<0.001 when compared with the control group. [21]

Evaluation of liver damage

At the end of the experiment blood was collected from retro-orbital plexus from the overnight fasted animals, after being anesthetized with 100 mg/kg ketamine, i.p. The blood samples were collected with 20 µl EDTA (5%) in each Eppendorf and centrifuged at 5000 rpm for 20 min (Sigma 3K30, UK). The supernatant (serum) was separated with the help of a micropipette and placed in the new Eppendorf with well labeled and stored at -80°C for further analysis. The homogenate was centrifuged at 4°C for 5 min at 3 000 r/min and the supernatant was used for estimation of viscus oxidative stress markers. (Biochemical and antioxidant estimation). The opposite liver tissue specimens were used for histopathological examination. [2]

RESULTS AND DISCUSSION

Macroscopic character

The macroscopic characters were useful in the rapid identification of plant material and also serve as a significant standardization parameter. Macroscopically, the leaf of *Tecoma stans* is alternate, entire, having a symmetrical base. Leaf having an obtuse apex, pinnate type of venation, serrulate margin, upper epidermis green in Colour, lower epidermis lighter in Colour with an aromatic odour, and pungent taste are reported in Table 1.

Table 1: Morphology of leaf of Tecoma stans

Morphological parameter	Observation
Arrangement	Alternate, entire
Size	5-10 cm
Base	Symmetrical
Venation	Pinnate
Margin	Serrulate
Apex	Obtuse
Shape of lamina	Entire
Odour	Aromatic
Colour-outer surface -Inner surface	Green Green
Taste	Pungent

Microscopic character

The microscopic character of leaves of *Tecoma stans* serves as the diagnostic character and is helpful in the differentiation of species as well as identification of particular herb (Fig 6). The transverse section passing through the midrib shows single-layered upper epidermis, lower epidermis, followed by single-layered palisade cells in the lamina portion. Midrib shows vascular bundles, the lower portion of the midrib is occupied by colleen chymatous cells, covering trichomes, xylem, and phloem under the light microscope.

Figure 2: Trasverse section of leaf

Figure 3: Stomata at 10X

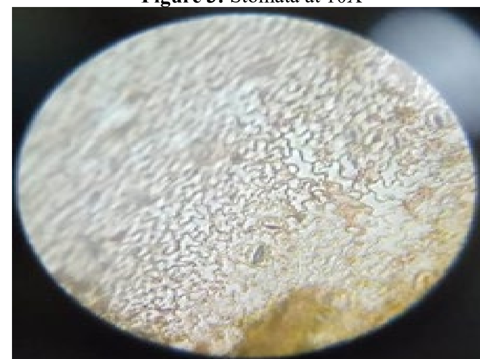
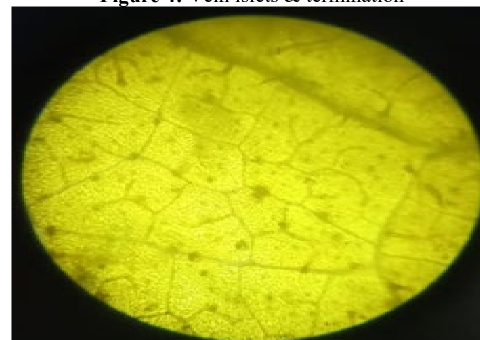


Figure 4: Vein islets & termination



Quantitative investigation

The stomatal index, stomatal number, vein islet number, vein termination numbers are comparatively constant for plants and can be used to make different closely related species. The results are depicted in Table 2.

Table 2: Quantitative leaf microscopy of Tecoma stans

Parameter	Range
Vein islet no.	3-5/mm2
Vein termination no.	6-9/mm2
Stomata no. upper surface	40-45/mm2
Stomata no. lower surface	50-55/mm2
Stomata index upper surface	1.35- 1.98
Stomata index lower surface	1.1-1.5

Physicochemical parameters

Various physicochemical parameter of the powdered drug has been investigated and reported in Table 3. The moisture content of drugs might be a minimum level to dispirit the reduction of bacteria, fungi, or yeast through storage. Ash values are used to find out the quality and purity of the unsophisticated drug. It indicates the

existence of a mixture of impurities like carbonate, oxalate, and silicate. The acid-insoluble ash consists mainly of silica and indicates contamination with earthy material. The water-soluble ash is used to estimate the number of inorganic elements present in drugs. The extractive values are valuable to estimate the chemical constituents present in the crude drug and assist in the evaluation of definite constituents soluble in a particular solvent Table 4. Extractive values results indicate that Ethanolic extract has maximum extractive value.

Table 3: Physicochemical parameter of powder of *Tecoma stans*

Physicochemical parameter		Means(%w/w)
Ash values	Total ash	0.59
	Acid insoluble ash	0.65
Moisture content	Loss on drying at 110ºC	0.79
Bulk Density		0.11
Tapped Density		0.15
Carr's Index		39

Table 4: Colour, consistency, odour, taste, and extractive values of successive solvent extraction and direct extraction of *Tecoma stans* leaves

Name of extracts	Consistency	Odour	Taste	Colour	Extractive value
Hexane	Semi-solid	Characteristic	Bitter	Dark-green	3.6%
Chloroform	Semi-solid	Characteristic	Bitter	Dark-green	3.1%
Ethyl acetate	Semi-solid	Characteristic	Bitter	Green	2.9%
Ethanol	Semi-solid	Pungent	Sweet	Green	6.4%

Preliminary phytochemical screening

The outcomes of extractive values of the powdered drug in different solvents obtained by successive extraction are reported in Table 4. All extracts subjected to the qualitative chemical test and results are exposed in Table 5. The result shows that maximum constituents found an ethanolic extract of *Tecoma stans* including phenols, terpenoids, and flavonoids. Such preliminary phytochemical screening was helpful in the prediction of the nature of drugs and also for the detection of different constituents present in different polarity solvents. So it could be helpful to extract out particular constituents by solvent.

Table 5: Phytochemical analysis of *Tecoma stans* leaves extract

Phytoconstituents	Hexane	Chloroform	Ethyl acetate	Ethanol
Alkaloids	+	+	+	+
Carbohydrate	+	+	+	+
Tannins	+	+	-	+
Steroids	-	-	-	+
Saponins	-	+	-	+
Glycosides	+	+	+	+
Protein	-	+	-	+
Flavanoids	-	-	+	+

(+): Present, (-): Absent.

Result of DPPH activity, total flavonoid, and total phenolic activity.

Table 6: DPPH activity, Total Flavonoids & Phenol Contents of *Tecoma stans* leaves extract

Extract	Concentration (µg/ml)		
	DPPH activity	Total Flavonoids Contents	Total Phenol Contents
Hexane	7.65	21.68	4.57
Chloroform	2.2	44.84	9.46
Ethyl acetate	0.47	19.73	4.16
Ethanol	17.6	45.31	9.56

Table 6 is the complied result of all assays. It shows that ethanolic extract shows a significant potential anti-oxidant activity. The ethanolic extract also contains the highest concentration of phenol and flavonoids.

Result of CCl4 induced hepatotoxicity

Table 7: shows all the results of liver weight and compared with standard and control

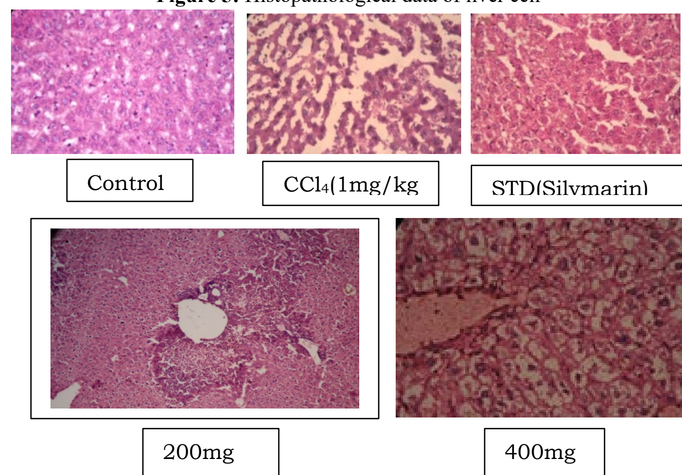
Groups`	Dose (mg/kg)	Liver weight g /100g of body weight
Vehicle Control	NC	3.80 ± 0.044
CCl_4	1ml/kg	6.04 ± 0.07
Silymarin + CCl_4	25mg/kg	4.20 ± 0.062
TS_1+ CCl_4	200mg/kg	4.98 ± 0.07*
TS_2 + CCl_4	400mg/kg	4.25 ± 0.05**

All values are statistically expressed as Mean ± SEM (n=6) one-way ANOVA. *represents significant at $p<0.05$ and **represents highly significant at $p<0.01$ when compared with control.

Table 7: Effect of Ethanolic leaves extract *Tecoma stans* on liver weight in CCl4 induced hepatotoxicity.

Evaluation of liver damage

Figure 5: Histopathological data of liver cell



Control

The liver segment of normal control rats showing normal architecture

Toxic group (CCl_4)

The liver segment of CCl_4 treated rats showing huge fatty changes, necrosis, ballooning degeneration, and severe infiltration of the lymphocytes and therefore the loss of cellular boundaries

Standard group silymarin

Liver section of rats treated with CCl_4 and 25mg/kg of silymarin showing signs of inflammatory cascade around central vein indicating a light degree of the fatty amendment, and necrosis and focal necrosis (dilatation).

Tecoma stans (TS) 200mg/kg

The liver segment of rats treated with CCl_4 and 200 mg/kg of TS showing fewer inflammatory cells around the central vein, absence of necrosis.

Tecoma stans (TS) 400mg/kg

The liver segment of rats treated with CCl_4 and 400 mg/kg of TS showing: minimal inflammatory cellular infiltration, large septa

of connective tissue flowing together and penetrating the parenchyma. There is the regeneration of hepatocytes evident.

CONCLUSION AND FUTURE WORK

Based on current research, a variety of standardization of *Tecoma stans* such as macroscopy, microscopy, physical, chemical, and phytochemical investigations was completed. The presence of alkaloids, steroids, flavonoids, tannins, and saponins was observed. Following assay such as total phenol content, total flavonoids content, and DPPD has been performed and ethanolic extract of leaves have 9.56 µg/ml 45.31 µg/ml & 17.6µg/ml respectively. Furthur, ethanolic extract of leaves shows good hepatoprotective activity at 200 mg /100g & 400 mg /100g as it significantly reduces liver weight and has good histopathological data when compared with standard and control. A good hepatoprotective effect may be present because of polyphenolic constituents in the ethanolic extract of leaves.

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