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Phytochemical Composition, Anti-inflamatory and Analgesic Activities of *Tecoma stans* Linn. (Bignoniaceae)

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Abstract

Tecoma Stans Linn. is known to have various medicinal and therapeutic properties. Its anti-inflammatory and analgesic properties were assessed in this study. The extracts and Indomethacin were found to inhibit carrageenan-induced paw oedema on albino rats with a strong activity in Indomethacin 5 mg/kg having 53.88% inhibition while *Tecoma stans* Linn 200 mg/kg, 400 mg/kg have 18.09% and 49.38% inhibition respectively. It elicited mild to moderate analsesic activity in acetic acid induced writhing in mice as compared with the control group. Phytochemical studies on the plants revealed the presence of bioactive components comprising flavonoids (5.70 mg. 100 g^{-1}), alkaloids (5.40 mg. 100 g^{-1}), tannins (0.40 mg. 100 g^{-1}), saponins (0.38 mg. 100 g^{-1}) and phenols (0.10 mg. 100 g^{-1}). These bioactive compounds may be responsible for the medicinal properties of *Tecoma stans* Linn. The plant thus deserves further developmental studies.

Keywords: *Tecoma stans* Linn, Bioactive compounds, Anti inflammatory, Analgesic, ethanolic extracts.

Introduction

Tecoma stans (L.) from Bignoniaceae family is a semi-evergreen ornamental tropical shrub or small tree originally from Latin American which has been cultivated in Iran (particularly in west and southwest parts) recently. It is also found in different parts of India. 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. Flower infusion can be taken orally for diabetes and stomach pains. A strong flower and root decoction is taken orally as a diuretic, to treat syphilis or for intestinal worms. The root is considered an effective remedy for snake and rats bites and for scorpion sting. The roots are used as a powerful diuretic, vermifuge and tonic. Flower and leaves have some medicinal value for the treatment of various cancer (Kiritikar,1999). Its leaves are used traditionally in Mexico to control diabetes (Roman-Ramos *et al*.,1991; Costantino *et al*.,2003). The plant contains tecomonine, tannins, flavonoids, alkaloids, quinones and traces of saponins (Lozoya *et al*.,1985).

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The leaves contain flavonoid, alkaloids such as tecomine and tecostidine. According to recent studies a new phenylethanoid, and a novel monoterpene alkaloid, along with eleven known compounds were isolated from the fruits and luteolin 7-O-beta-D-glucuronopyranoside, diosmetin 7-O-beta-D-glucuronopyranoside, diosmetin 7-O-beta-D-glucopyranoside, diosmetin 7-O-beta-D-glucuronopyranoside methyl ester and acetoside were isolated from the flowers (Marzouk *et al*., 2006). The tecostanine isolated from the leaves is suggested for antihyperglycemic effect . *Tecoma stans* Linn. growing in Egypt has two alkaloids called tecomine-1 and tecostanine-2 with hypoglycemic effect in fasting rabbits which are inactive in the absence of pancreas (Hammouda *et al*.,1964; Hammouda *et al*., 1996). *Tecoma stans* Linn. is not a toxic because this plant is used in Latin America as a remedy for diabetes and moreover for feeding cattle and goats in Mexico (Susano *et al*.,1981). The aim of the present study, therefore, was to investigate the composition of phytoconstituent and their anti-inflammatory and analgesic properties of alcoholic leaf extract on albino rats and swiss mice.

Experimental

Tecoma Stans leaf were collected from the local area of Salipur, Cuttack, Orissa, during January-February and authenticated by Dr. P. Jayaraman,

Director, Plant Anatomy Research Center (PARC), Tambaram, Chennai, India, and a voucher specimen holding No. PARC/2007/83 was deposited in the same center.

Preparation of plant extracts

The leaves of *Tecoma stans* Linn. were air-dried for 10 days and then ground into a uniform powder. The powdered materials (1000 g for each sample) were stored in airtight bottles for chemical analysis.

Quantitative determination of chemical constituents

Preparation of fat free sample

The sample was de-fatted with diethyl ether using a soxhlet apparatus for 2h.

Alkaloids determination

5g of the sample was weighed into a 250ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was washed with dilute ammonium hydroxide solution and then filtered. (Harborne, 1973). The residue, which was taken as the crude alkaloid was weighed.

Total phenol determination

For the extraction of the phenolic constituents, the fat free sample was boiled with 500 ml of ether for 15 min. 5ml of the extract was pipette into a 50ml flask, and then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for color development. The absorbance of the solution was read using a spectrophotometer at 505nm wave length (Obadoni *et al*., 2001).

Flavonoids determination

10g of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No.42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over water bath and weighed (Boham *et al*., 1994).

Saponins determination

The samples were ground 20g of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4h with continuous stirring at about 55^{o}C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^{o}C. The concentrate was transferred into a 250ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 ml 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage (Obadoni *et al*., 2001)

Tannin determination

500 mg of the sample was weighed into 100ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml of the volumetric flask and made up to mark. Then 5ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1 m $FeCl_3$ in 0.1 N HCl and 0.008 m potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 605nm wavelength within 10 min. A blank sample was prepared and the color developed and need at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (Van-Burden *et al*.,1981).

Extraction

Each of the powdered plant materials (100 g) was packed into a soxhlet apparatus (2L) and extracted exhaustively with 500 ml of ethanol for 6 h. The ethanol was evaporated using a water bath and then left at laboratory temperature for two days for evaporation of the remaining ethanol. The extract was stored in the refrigerator for proper preservation until when needed. The yields were 8.20 g for *T. stans.* The concentration was made in 0.9% saline for the experimental studies.

Animal studies

Male Sprague Dawley albino rats (150) Wister strain and Mature male Swiss Mice (25) of were used for the anti-inflammatory and analgesic studies. They were obtained from Central Animal House of Institute of Pharmacy and Technology Salipur, Cuttack, Orissa India. The animals were grouped in polycyclic cages (38 cm X23 cm X 10 cm) with five animals per cage and maintained under standard laboratory conditions (temperature 25 ± 6^{o}C). They were allowed free access to standard dry pellet diet and water *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment.

Anti-inflammatory activity of carrageenan induced rat paw oedema

Carrageenan pedal inflammatory in rats was induced according to the method described by Onasanwo *et al*. The rats were divided into four groups each having six animals. The animals in the test group were treated orally with 200 and 400 mg/kg of plant extracts, one hour before carrageenan injection. At the same time, the control group received 0.9% saline and the reference group received 5 mg/kg Indomethacin. An injection of 0.1 ml of 1% Carrageenan was given into the right hind foot of each rat under the sub plantar aponeurosis. The measurement of the increase in paw size was done immediately before and after 3 h following carrageenan injection. The inhibitory activity after 3h was taken as a measure of paw oedema (Onasanwo *et al*., 2005)

The inhibitory activity was calculated according to the formula

$$\% \text{ of inhibition} = \frac{[C\ t - C\ o]\ \text{control} - [C\ t - C\ o]\ \text{test}}{[C\ t - C\ o]\ \text{control}} \times 100$$

Where: [C t – C o] = Change in paw size

Analgesic activity by acetic acid induced writhing in mice

A group of mice were administered 0.1 ml/10g of 0.3% (v/v) acetic acid ip. The mice exhibiting the writhing episode (stretching of hind limbs and bending of trunk) were selected for the study. The mice were randomly divided into four groups each containg six animals. The mice were administered *Tecoma stans* Linn. leaf extract 200mg/kg and 400mg/kg and Indomethacin (5mg/kg) orally 1hr. prior the acetic acid injection. The number of writhing episodes was counted for 30minutes following acetic acid administration. (Witkin *et al*.,1961).

Statistical analysis

All measurements were replicated three times for phytochemical and 6 times for pharmacological analysis and standard deviation determined. The student's-test at $p < 0.05$ was applied to assess the difference between the means.

Results and Discussion

The phytochemical content of *Tecoma stans* leaves is shown in Table-1. The flavonoids content was 5.70mg 100 g^{-1} in *T. stans* leaf. Flavonoids act as a protective agent against inflammatory disorders. They reduce oedma formation and inhibit the synthesis of prostaglandin and thromboxane (Okwu *et al*., 2005).

Table -1. Phytochemical composition of *Tecoma stans* Linn. leaves on dry weight basis(mg 100^{-1})

Phytochemical	*Tecoma stans* Linn. leaves
Alkaloids	5.40 ± 0.03
Flavonoids	5.70 ± 0.03
Tannins	0.40 ± 0.03
Saponins	0.38 ± 0.01
Phenols	0.10 ± 0.30

Table 2. Effects of crude extracts of *Tecoma stans* Linn. leaf and Indomethacin on carrageenan induced paw oedema rats

Group	Dose mg/kg	Change in paw size, cm	Inhibition in paw thicking %
Normal saline	10 ml/kg	0.74 ± 0.06	0
EETS	200 mg/kg	0.58 ± 0.04	18.09
EETS	400 mg/kg	0.36 ± 0.01*	49.38
Indomethacin	5 mg/kg	0.38 ± 0.02*	53.88

Values are expressed as means ± S.E.M(n=6).
**P*<0.01 compared with Normal saline group; student's *t*- test
EETS: ethanolic extract of *Tecoma stans* Linn.

Table-3: Effects of crude extracts of *Tecoma stans* Linn. leaf and Indomethacin aceticacid induced writhing in mice

Time	Control (1ml/mg normal saline)	EETS (200mg/kg)	EETS (400mg/kg)	Indomethacin (5mg/kg)
1	14.93± 0.83	13.67± 0.61	12.86± 0.31*	7.7± 0.48**
2	19± 0.73	17±0.85	13.85± 0.42**	9± 0.51**
3	5.69± 0.67	6±0.58	4.68± 0.42**	2.18±0.31**
Total	38.7± 2.13	34.35± 1.76	31.33± 1.62**	18.33± 0.82**

Values are expressed as means ± S.E.M(n=6).
**P*<0.01 compared with Normal saline group; student's *t*- test
EETS: ethanolic extract of *Tecoma stans* Linn.

As a result of the availability of flavonoids in *Tecoma stans* Linn. the extracts prevent platelet stickiness and hence platelet aggregation. High quantity of alkaloids was found in *Tecoma stans* Linn. *which* contained 5.40 mg 100 g^{-1} of alkaloids. Some alkaloids present in the plant function as spasmolytic, anti-cholinergic and anesthetic agents. Tannins content was found to be in *Tecoma stans* Linn 0.40 mg 100 g^{-1} of tannins. The value of saponins in *T. stans* was 0.38mg $100g^{-1}$ of saponins. The identification of saponins and tannins in the leaves of *Tecoma stans* Linn. may be the reason for the haemostatic activity of the plant. Leaves of *Tecoma stans* contained 0.10mg $100g^{-1}$ of phenol. The presence

of phenols in the plant may be responsible in having the ability to block specific enzymes that causes inflammation (Duke,1992). The anti-inflammatory property of *T. stans* leaf extracts on carrageenan - induced paw oedema in rats is shown in Table- 2. The extracts and Indomethacin were found to inhibit paw oedema in rats with a strong activity in Indomethacin having (53.88%) inhibition. The leaf extracts of *Tecoma stans* Linn. 400mg/kg, 200mg/kg showed 49.38% and 18.09% inhibition respectively.

The ethanolic extract 400mg/kg of *Tecoma stans* Linn. produced a marked anti-inflammatory and analgesic activity. It reduced the size of pedal swelling induced by carrageenan and elicited mild to moderate analsesic activity in acetic acid induced writhing in mice as compared with the control group shown in Table 3. The anti-inflammatory and analgesic effects of plants may be due to the presence of bioactive compounds such as flavonoids, saponins and phenolic compounds.

Conclusion

The present study indicates that *Tecoma stans* Linn. leaf have a potent anti inflammatory and analgesic activity and therefore it can be used for development of herbal drug for anti inflammatory and analgesic condition.

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