

SCIENTIFIC VALIDATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *TECOMA STANS* (L) JUSS. LEAF

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Article Received: 16 January 2025 | Article Revised: 05 February 2025 | Article Accepted: 27 February 2025

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DOI: <https://doi.org/10.5281/zenodo.14966505>

How to cite this Article: Bhupendra Rodle and Dr. Sachin K. Jain (2025). SCIENTIFIC VALIDATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *TECOMA STANS* (L) JUSS. LEAF. World Journal of Pharmaceutical Science and Research, 4(1), 921-928. <https://doi.org/10.5281/zenodo.14966505>



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ABSTRACT

The objective of this study was To Scientific Validation of Antidiabetic Activity of Ethanolic Extract of Tecoma Stans (L) Juss. Leaf. The plant leaves were collected locally from the herbal store and botanical garden of the garden of the botany central council for Research Ayurveda and Siddha Govt. of India .The plant was identified and authenticated by comparison with herbarium specimens. The leaves of Tecoma Stans (L.) juss ex kunth were authenticated by comparison with herbarium specimens and authentication No. BSI/SRC5/23/2016/Tec/1993. The different extracts (alcoholic and aqueous) of Tecoma Stans were subjected to physicochemical analysis. Tests for carbohydrates, phenols, tannins, alkaloids, flavonoids, fats, glycosides, steroids, amino acids, proteins carbohydrates, proteins, amino acids, flavonoids, saponins, phenol and tannins which may probably responsible for their expected pharmacological action. In the toxicity studies ethanolic extract did not show any signs or symptoms of toxicity in ratsat doses up to 2000 mg/kg p.o., indicating that it has no toxicity at the maximal doses tested in this work. The extract with maximum number of phytoconstituents and extractive value identified (ethanolic) is used in the further evaluations. Toxicity study shows the safety nature of the extract and also acute and sub-acute toxicity studies do not produce any toxic symptoms upto 500 mg/kg. The extract was pre-clinically evaluated against STZ induced diabetic rats models for its antidiabetic activity. The extract showed insulin mimetic activity and control of blood sugar level which are comparable to the reference drug glibenclamide at a dose of 10mg/kg. as the invivoresults indication has been concluded 50% ethanolic extract of Tecoma Stans (L), which may be containing structurally insulin resembled compounds. In conclusion the extract is safe and can be used to treat diabetic conditions without any harmful effects.

KEYWORDS: Herbarium specimens, Tecoma Stans (L) Juss. Leaf, Zeta potential, antidiabetic, Validation.

1. INTRODUCTION

Diabetes mellitus is one of the most common and challenging disease conditions of the 21st century. It is a chronic complex progressive and multisystemic disorder with life threatening micro and macrovascular complications. WHO defined Diabetes mellitus as a metabolic disorder of multiple etiologies characterised by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. It is a major cause of morbidity and mortality. Prevalence of DM is about more than 150 million diabetics across the world and more than one fifth of them are Indians. International Diabetes Federation, India has been declared India as "Diabetic Capital of the World" at the recent Conference in Paris.

The common herbs which can be used against diabetes include turmeric, neem, coccinia indica, amalaki, triphala, bitter gourd, rose apple, leaves of bilva, cinnamon, gymnema, fenugreek, bay leaf and aloe vera. The Ayurvedic preparations 'Vasant Kusumakar Ras' and 'Chandraprabha Vati' are used to treat diabetes mellitus. Proprietary Ayurvedic medications are also used to treat diabetes.

Although in rural India the prevalence of diabetes is much lower than in the urban population, even here the prevalence of diabetes is rapidly rising. Diabetes is fast becoming the epidemic of the 21st century. Type diabetes, which is more prevalent (more than 90% of all DM cases) and the main driver of the diabetes epidemic, now affects 5.9% of the world's adult population with almost 80% of the total in developing countries. The World Health Organization (WHO) reported that 32 million Indians had diabetes in the year 2000.

2. MATERIALS AND METHOD

2.1 MATERIALS

2.1.1 Plant

The plant leaves were collected locally from the herbal store and botanical garden of the garden of the botany central council for Research Ayurveda And Siddha Govt. of India. The plant was identified and authenticated by comparison with herbarium specimens. The leaves of *Tecoma Stans* (L.) juss ex kunth were authenticated by comparison with herbarium specimens and authentication No. BSI/SRC5/23/2016/Tec/1993.

2.1.2 Animals

Wistar rats (150 – 250 g) used for the study were obtained from the animal house of the Department of Pharmacology, Oriental College of Pharmacy, Indore, Madhya Pradesh. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions. The animals were housed three per cage in a polypropylene cage and maintained in standard laboratory conditions with free access to food and water ad libitum. All animal experiments were conducted in compliance with (Organization for Economic Cooperation and Development) OECD Guideline and approved by the Institutional Animal Ethics Committee, Oriental College of Pharmacy.

2.1.3 Chemicals, Drugs and Instruments

Streptozotocin, citric acid, sodium citrate were collected from a private chemical store Coimbatore (Ponmani and co). Other important chemical used in phytochemical analysis like alcohol, hydrochloric acid, ∞ - naphthol, Sulphuric acid, Fehling A&B, Benedict reagent, sodium hydroxide nitric acid, ammonia, lead acetate, ninhydrin, sudan red III reagent, glycerin, picric acid, chloroform, acetic anhydride, ferric chloride, zinc, dragendorff's reagent, Wagner's reagent,

Mayer's reagent, sodium. Chloride and bromine water were collected from the store of Oriental College of Pharmacy. All the chemicals used in the study are of analytical grade.

2.2 EXPERIMENTAL METHOD

2.2.1 Extraction Procedure by Continuous Hot percolation Method

The leaves of the plant, dried under shade, are carefully removed and grinded using a blender. The coarse powder obtained was used for the extraction by successive solvent extraction by Soxhlet apparatus using various solvents.

Table No. 1: Soxhlet Extraction of Tecoma Stans (L.) Juss. EX KUNTH.

Plant	Part used	Method of Extraction	Solvents	Average value of extractive(% W/V)
Tecoma Stans (L.) juss.ex kunth	Dried Leafs	by Soxhlet apparatus	Ethanol (50%)	33.2%

2.2.2 Phytochemical Analysis

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. Phytoconstituents are the contributors of pharmacological activities of a plant. The individual extracts are subjected to qualitative tests for identification of various plant constituents.

2.2.3 Experimental Design

In this study, 4 groups of 6 rats each were given with 5, 50 and 300 and 2000 mg/kg of the extract (p.o.). After drug administration the food is withheld for 3 hours. The animals are observed continuously for the first 2 hours, then occasionally up to 6 hours and then daily up to 14 days, post treatment to observe for any symptoms of toxicity and mortality. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), autonomic effects (salivation, lacrimation, gauntness and piloerection) and central nervous system (gait, tremors and convulsion) were carried out and changes were noted (OECD, 2001).

2.2.4 Induction of Diabetes in Experimental Animal

Experimental diabetes was induced by single intraperitoneal injection of 25 mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer (pH 4.5) after 15 min of intraperitoneal injection of nicotinamide (110 mg/kg) prepared in normal saline. Rats with marked glycosuria (fasting blood glucose level greater than 200 mg/dL) after one week of administration of STZ were used for the study. Diabetes was confirmed after 48 hr of streptozotocin injection, the blood samples were collected through tail vein and plasma glucose levels were estimated by glucose oxidase method (accurate active glucometer). The rats having fasting plasma glucose levels more than 200 mg/dL were selected and used for the present study.

2.2.5 Experimental protocol

All hyperglycaemic rats were randomly divided into four groups of six rats in each group, 24 rats (18 diabetic rats and 6 normal rats).

Group I – Normal control (Distilled Water) .

Group II – Diabetic control (Distilled Water)

Group III – Streptozotocin + Glibenclamide (10 mg/kg p.o)

Group IV – Streptozotocin + Ethanolic extract (300 mg/kg p.o.)

The test drug was administered orally using an oral feeding needle once daily for 28 days. The body weight, food and water intake behaviour of the animals were measured at the onset of the study and at the regular intervals of every week up to 28 days.

Group I animals (normal rats) were administered orally with distilled water whereas group II animals (diabetic) received distilled water, group III animals (diabetic) received glibenclamide (10 mg/kg p.o) and group IV animals (diabetic) received extract 300 mg/kg body weight for 28 consecutive days.

The blood samples collected from the tail vein of rats on 0, 7, 14, 21 and 28 days after administration of formulation. The blood glucose levels were determined by the glucose oxidase method using glucometer.

2.2.6 Statistical Analysis

All values are expressed as mean \pm SEM. Statistical analysis was performed by One-way Anova, analysis of variance (ANOVA) followed by Dunnet's t-test. A 'p' value less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Evaluation

Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases. Phytochemical analysis of ethanol extract shows alkaloids, carbohydrates, saponins, proteins, amino acids, flavonoids and tannins. The combination of above mentioned phytochemicals may be the reason behind the and diabetic properties of the plant.

Table No. 02: Preliminary Phytochemical Evaluation of Tecomastans (L.) Juss. EX KUNTH leaf Extracts.

S. No.	Phytoconstituents	Ethanol
1	Alkaloids	+
2	Carbohydrates & Glycosides	+
3	Phytosterols	-
4	Fixed oils	-
5	Saponins	+
6	Tannins and Phenols	+
7	Proteins and Amino acids	+
8	Gums and Mucilages	-
9	Flavonoids	+
10	Tannins	+

3.2 Acute Toxicity Study

There was no mortality or signs of toxicity up to the limit dose of 2000 mg/kg in treated rats. All 24 rats were normal throughout the study and survived until the end of the 14-day experiment period.

Table No. 03: Changes in wellness parameters observed for ethanolic extract treated wistar rats.

S. No	Response	Group 1 (5mg/kg)		Group 2 (50 mg/kg)		Group 3 (300 mg/kg)		Group 4 (2000 mg/kg)	
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Anxiety	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4	Roaming	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Tremor	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

7	Depression	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Scratching	Present	Present	Present	Present	Present	Present	Present	Present
10	Defecation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
12	Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
17	Pilo erection	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
18	Nail status	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
19	Gauntness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
20	Gait	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
21	Diarrhoea	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
22	Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
23	Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
24	Lethargy	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
25	Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

3.3 Effect of ethanolic extract on Glucose-Loaded Rat (OGTT Model)

Vehicle treated group and GL (10 mg/kg body wt) treated group showed significant rise in serum glucose level (SGL) after one hour of glucose administration, whereas groups II and III showed significant increase in SGL respectively. From the study, it is found that both 200 mg/kg and 400 mg/kg of ethanolic extract possess significant hypoglycemic activity in normal rats.

Table No. 04: Effect of ethanolic extract on serum glucose levels in OGTT model in normal rats.

S. No	Drug/Control	Body weight	Blood glucose level (mg/dL)				
			0 hour	1 hour	2 hour	3 hour	4 hour
1	Group-1 control (distil led water)	180.0±2.0	92.0±2.5	132.0±3.5	117.0±0	119.0±1.0	100.5±1.5
2	Group-2 extract (200 mg/kg)	164.1±2.7	102.0±1.0**	123±0**	107.0±2.0**	101.0±3.0*	98.0±2.0*
3	Group-3 exotract (400 mg/kg)	152.6±3.4	99.0±1.5**	120.0±1.5**	100.0±2.5**	96.0±3.0*	88.5±1.5*
4	Group-4 GL (10 mg/kg body wt)	151.3±2.3	111.0±4.5**	121.0±3.1**	117.0±3.6**	114.0±2.6*	112.5±1.2*

Values are represented as mean ± SEM (n=6 rats).

Values are statistically significant at *P < 0.05,** P < 0.01. GL = Glibenclamide.

3.4 Effect of ethanolic extract on serum glucose level of diabetic rats

Diabetic control rats showed consistent and gradual rise in SGL during the study. GL (10 mg/kg body wt) and ethanolic extract 400 mg/kg treated rats showed a significant reduction 7th, 14th, 21st, and 28th day of the study and the results were found to be statistically significant (P<001) as compared to diabetic control which is shown in Table 08. The effect was found to be time dependent up to 28th day of the study. Decrease in SGL was more significant (P<0.001) on 28th day when compared with standard drug.

Table No. 05: Effect of 27 days treatment of ethanolic extract on serum glucose levels of STZ-induced diabetic rats.

S. No.	Treatment	Initial	7 th day	4 th day	21 st day	28 th day
1	Normal control	89.3±3.8	91.0±1.5	95.0±1.0	92.8±2.1	89.0±1.7
2	Diabetic control	221.5±3.2	267.3±3.5	310.3±2.2	383.0±2.8	405.3±3.2
3	Diabetic+Glibenclamide (10 mg/kg)	281.0±1.9***	261.0±3.6**	153±3.8***	140.1±3.1***	129.5±2.7***
4	Diabetic+extract (400 mg/kg)	240.1±2.2***	210.6±3.3***	160.3±3.7***	121.3±1.4***	96.8±1.7***

Values are represented as Mean ± SEM (n=6 rats).

Values are statistically significant at ** P < 0.01, *** P < 0.001.

Diabetic + ethanolic extract compared with diabetic + glibenclamide and normal control rats.

3.5 Effect of ethanolic extract treatment on body weight

There was also a significant reduction in body weight in diabetic animals, however, the animals treated with 400 mg of ethanolic extract and GL showed significant (P<0.001) check on the loss of body weight on days 21 and 28 in comparison to the day of onset of the study.

Table No. 06: Effect of ethanolic extract treatment on body weight in STZ-induced diabetic rats on 21st day and 28th day.

S. No.	Drug/Control	Body weight(g)		
		Baseline	21st day	28th day
1	Normal control	180.0±2.0	180.9±3.2	182.2±3.1
2	Diabetic control	164.1±7.1	155.0±7.0	123±10.2**
3	Diabetic+ Glibenclamide (10mg/kg)	152.6±8.4	153.8±9.5**	155.1±6.7***
4	Diabetic + extract (400mg/kg)	151.3 ±7.3	152.0±5.1**	156.0±7.3***

Values are represented as Mean ± SEM (n=6 rats).

Values are statistically significant at ** P < 0.01, *** P < 0.001.

Diabetic + ethanolic extract compared with diabetic + glibenclamide and normal control rats.

4. SUMMARY AND CONCLUSION

The current anti-diabetic drug research is facing complex challenges. As times go on it demands an integrated approach towards the health care system. There has been a growing interest in natural medicinal plant related research.

Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases. Phytochemical analysis of ethanol extract shows alkaloids, carbohydrates, saponins, proteins, amino acids, flavonoids and tannins. The combination of above mentioned phytochemicals may be structural similarity of compound of the plant.

The acute toxicity study indicated that ethanolic extract at a dose 2000 mg/kg caused neither visible signs of toxicity nor mortality. The LD50 and ED50 of the drug were estimated as 2000 mg/kg and 200 mg/kg respectively. If LD50 is 2000 mg/kg, it could be generally regarded as safe (GRAS). This finding is in agreement with Clarke and Clarke⁴⁵, who reported that any compound or drug with oral LD50 estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe. However, it is suggested that variables such as animal species, strain, age, gender, diet, bedding, ambient temperature, caging conditions, and time of the day can all affect the LD50 values obtained and as such are considerable uncertainties in extrapolating the LD50 obtained for species to other species. This finding is suggestive that LD50 may not be considered as a biological constant.

The extract was pre-clinically evaluated against STZ induced diabetic rats models for its antidiabetic activity. The extract showed insulin mimetic activity and control of blood sugar level which are comparable to the reference drug glibenclamide at a dose of 10mg/kg. as the *In vivo* results indication has been concluded 50% ethanolic extract of *Tecoma Stans* (L), which may be containing structurally insulin resembled compounds. In conclusion the extract is safe and can be used to treat diabetic conditions without any harmful effects.

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