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Separation And Analytical Evaluation Of The Antidiabetic Activity Of Alkaloids Of *Tecoma Stans*

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Abstract: In this current research, a detailed protocol has been programmed. The leaves of Tecoma stans were collected from nearby areas of district Amroha in December. A herbarium was prepared and authenticated from NISCAIR, Delhi. Dried leaves were extracted using solvent ethanol by the soxhlet apparatus. The extract was dried using a rotary evaporator. The obtained extract was subjected to preliminary phytochemical tests. Qualitative phytochemical tests confirmed the presence of alkaloids, glycosides, saponins, flavonoids, steroids, and phenols. Alkaloids were isolated from the obtained extract; confirmation was done through preliminary phytochemical and TLC analysis. The isolated alkaloids of leaves of Tecoma stans was subjected to in-vitro antidiabetic activity. The isolated alkaloids produced 94.16±0.74% inhibition of α-glucosidase activity at maximum concentrations 25μg/ml, and its IC50 was found to be 44.62±0.65μg/ml. The standard drug acarbose exhibited 98.19±0.32% inhibition of α-glucosidase activity at maximum concentrations of 2.5μg/ml, and its IC50 was found to be 48.97±0.73μg/ml. The isolated alkaloids inhibit 92.06±0.34% α- amylase activity at maximum concentrations of 200μg/ml, and its IC50 was found to be 45.25±0.41μg/ml. The standard drug acarbose exhibited 98.39±0.71% inhibition of α-amylase activity at maximum concentrations 120μg/ml, and its IC50 was found to be 42.03±0.89μg/ml. the study revealed that alkaloids of Tecoma stans leaves showed significant antidiabetic potential (In-vitro) compared with standard compound Acarbose.

***Index Terms* - Tecoma stans, alkaloids, α-glucosidase, α-amylase, acarbose.**

1. INTRODUCTION

1.1. Diabetes Mellitus

Diabetes mellitus is more commonly known simply as diabetes. Diabetes mellitus (DM) is commonest endocrine disorder that affects more than 100 million people worldwide (6% population). It is caused by deficiency or ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood. It is found to damage many of body systems particularly blood vessels, eyes, kidney, heart and nerves. But diabetes insipidus is a rare condition that has nothing to do with the pancreas or blood sugar. Diabetes insipidus occurs when the body can't regulate how it handles fluids. The condition is caused by a hormonal abnormality and isn't related to diabetes. In addition to extreme thirst and heavy urination, other symptoms may include getting up at night to urinate, or bed-wetting.

1.1.1 Classification of Diabetes Mellitus

Diabetes mellitus has been commonly classified into two types- insulin dependent diabetes mellitus (IDDM, Type I) and non-insulin dependent diabetes mellitus (NIDDM, Type II), other type of diabetes are-

•Pre-diabetes: a condition in which blood sugar is high, but not high enough to be type 2 diabetes.

•Gestational diabetes: a condition in which your blood sugar levels become high during pregnancy. Neonatal diabetes: a rare form of diabetes that is usually diagnosed in children less than 6 months of age.

This early occurring type of diabetes is caused by one of a number of genetic mutations and is therefore described as a monogenic form of diabetes (Antar S. A.et al., 2023).

1.1.2 Epidemiology

It is estimated that 366 million people had DM in 2011; by 2030 this would have risen to 552 million. The number of people with type 2 DM is increasing in every country with 80% of people with DM living in low- and middle-income countries. DM caused 4.6 million deaths in 2011. It is estimated that 439 million people would have type 2 DM by the year 2030.The incidence of type 2 DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors.

It is predicted that the prevalence of DM in adults of which type 2 DM is becoming prominent will increase in the next two decades and much of the increase will occur in developing countries where the majority of patients are aged between 45 and 64 years (Ramchandran A. 2010). In India according to recent estimates, approximately 285 million people worldwide (6.6%) in the 20–79-year age group will have diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to have diabetes. India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the “diabetes capital of the world”. According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken.

2. PLANT PROFILE

Tecoma stans (Bignoniaceae) is a shrub or a small tree which can reach a height of 8 m, rarely 10 m, and with stem diameters of up to 25 cm. Plants in dense stands are usually smaller with heights of 5-6 m. It remains evergreen in moist and warmer regions but changes to deciduous in more temperate regions with a pronounced dry season (Pelton, 1964). The pinnate leaves are bright green above, paler below and can be smooth or hairy, often around the veins, depending on the region (subspecies). The leaf size is also dependent on the variety and can be large, 100-200 mm long, pinnate with 3-17 leaflets 2.4-15 cm long, 0.8-6 cm wide, progressively larger distally (Gentry, 1992). Most leaves have 5-7 leaflets including the terminal one. Leaf margins can be sharply toothed as in *T. stans* var. angustata or less toothed as in the other varieties. Inflorescences are terminal or subterminal with up to 20 bright yellow showy trumpet-shaped Flowers, about 50 mm long. In some varieties the corolla is slightly orange-yellow with pinkish lines in the throat. The fruit is a linear shiny capsule, 12-22 cm long and about 1 cm thick, pointed at the end. The two-valve dehiscent capsule splits open to release up to 77 (mean 42) papery- winged seeds which are primarily wind, and to a lesser extent water, dispersed.

Figure: 1 *Tecoma stans* L. Plant

2.1 Scientific Classification (India biodiversity and CABI.org)

Domain:	Eukaryota
Kingdom:	Plantae
Phylum:	Spermatophyta
Subphylum:	Angiospermae
Class:	Dicotyledonae
Order:	Scrophulariales
Family:	Bignoniaceae
Genus:	*Tecoma*
Species:	*Tecoma stans*

2.2 Preferred Scientific Name

Tecoma stans (L.) Juss. ex Humb., Bonpl. & Kunth

2.3 Phytochemical Investigation

Crude extracts of whole plant contain 5- deoxy stansioside and iridoid glycoside, tecomine, chrysoeriol, apigenin, and other polyphenols, leaves contain indolic compounds, fruits and flowers contain monoterpenic alkaloids, 5-hydroxy skythanthine hydrochloride. Chemical structure of phytochemicals given blow (Sunita Verma 2016).

3 RESEARCH METHODOLOGY

3.1 Plant Material and Extraction

Tecoma stans leaves were collected during December 2025 from Amroha Uttar Pradesh, India, and authenticated by Dr. Sunita Garg, Emeritus Scientist, CSIR-NISCAIR, and Mr. RS Jayasomu, Senior Principal Scientist, Head RHMD, Reference number NISCAIR/RHMD/Consult/2020/3672-73. A voucher specimen of the plant was preserved at Raw material herbarium and museum, Delhi (RHMD) for future reference. The dried leaves were subjected to size reduction with the help of stainless-steel grinder and collect the fine powder of the leaves. Extraction was done with a hot soxhlet extraction process using ethanol as solvent then the extract was concentrated to dryness with the help of a water bath and finally air-dried. The obtained dried extracts of *Tecoma stans* leaves were weighed and extractive value was calculated. It was kept in an airtight container and stored in a desiccator and used for investigation of their potential.

3.2 Preliminary Phytochemical Studies

Leaves extracts of the plant of *Tecoma stans* were subjected to chemical tests for the identification of their active constituents such as alkaloids, flavonoids, glycosides, saponins, fat and oil etc.

3.3 Thin Layer Chromatography (TLC)

Thin layer chromatography is a very old method of analysis. TLC is used for the separation and identification of compound from a particular mixture of compounds. It is very popular in qualitative analysis. The principle of TLC is based on the ''affinity'' of the solvent with stationary and mobile phases. The compounds which have the great affinity to the stationary phase run slow while the compound which have the great affinity to the mobile phase run faster with mobile phase.

3.3.1 Sample application

An origin line was marked before the sample was applied on the plates, which was usually 2.0-2.5 cm from the bottom of the plates. About 1, 2 and 5µl sample of EE was applied on the origin line with the help of a fine capillary.

3.3.2 Development solvents

A development solvent should not react chemically with the substance in the mixture under examination. Carcinogenic solvents (benzene etc.) should always be avoided. Commonly used solvents are petroleum ether, ethyl acetate, n-hexane, acetone and chloroform are used.

3.3.3 Location of spots

The positions of various solutes separated by TLC were located by keeping the plates in iodine camber for some time. After visualizing the spots, the distance travelled by the unknown substance to be identified as well as by the solvent front was measured and put in the following formula to calculate the R*f* values.

R*f* = Distance travelled by the spots from solvent front/Distance travelled by solvent from origin front.

(Bele, et al., 2011)

4. ISOLATION OF ALKALOIDS

The isolation of alkaloids was performed with slight modification in the method of Mushtaq A., 2017. Dried ethanolic extract (5g) was taken in a separate funnel and extracted with dilute aqueous tartaric acid solution and ethyl acetate. The mixture was sown for 15 minutes and kept aside. The two layers were separated. The aqueous layer was collected and neutralized with NH3 and then extracted with ethyl acetate. The organic layer was collected and the solvent evaporated to obtain alkaloids. The isolated alkaloids sample was confirmation with the help of preliminary phytochemical and TLC analysis.

5. ASSESSMENT OF IN VITRO ANTIDIABETIC ACTIVITY

The antidiabetic effect of plants and their active principles can be assessed in vitro using a variety of biological test systems. They play a major role in the evaluation of antidiabetic properties as an initial screening tool before in vivo studies. Cases of diabetes mellitus around the world are increasing day by day. It has been predicted that India, China, and the United States will be having the largest number of diabetic patients by 2030. Therefore, the anti-diabetic study is attracted a huge number of scientists and various in-vitro and in-vivo models have been developed for the anti-diabetic study.

In drug development, in-vitro techniques are an important method of confirming a particular biological activity of a compound before animal experiments *In vitro* cell lines are an alternative to animal models to study the particular activity of drugs. To produce more reproducible results, the *in-vitro* cell lines from clonal cells, cell lines are used (Antony J. et al. 2019).

5.1 Inhibition of α-Amylase Activity

Starch solution (0.5% w/v) was prepared by stirring potato starch (0.125 g) in 20 mM sodium phosphate buffer with 6.7 mM sodium chloride (pH 6.9; 25 ml) in a boiling water bath for 15 min. The α-amylase solution was prepared by mixing 1 U/ml of α- amylase in the same buffer. The colorimetric reagent was prepared by mixing an equal volume of sodium potassium tartrate tetrahydrate solution and 96 mM 3, 5-dinitro salicylic acid (DNS) solution. Starch solution (1000 μL) was mixed with increasing concentration of Acetone and Isolated alkaloids extract (25, 50, 100, 200, 400, and 800 μg/ml), acarbose (10– 320 μg/ml), and to this 1000 μL of the α-amylase solution was added and incubated at 25°C for 3 min to react with the starch solution.

A 1000 μL of 96 mM DNS reagent was added to the above solution, and the contents were heated for 15 min on a boiling water bath. The final volume was made up of distilled water, and the absorbance was measured at 540 nm using UV-1800, Shimadzu Corporation, Kyoto, Japan. The percentage inhibition and 50% inhibitory concentration (IC_{50}) values were calculated.

5.2 Inhibition of α-Glucosidase Activity

The α-glucosidase enzyme inhibition activity was determined by incubating 100 μL of α-glucosidase enzyme (1 U/ml) solution with 100 μL of phosphate buffer (pH 7.0) which contains 100 μL of Acetone and Isolated alkaloids extract (25–800 μg/ml), acarbose (0.1–3.2 μg/ml) at 37°C for 60 min in maltose solution. To stop the α-glucosidase action on maltose, the above reaction mixture was kept in boiling water for 2 min and cooled. To this, 2 ml of glucose reagent was added and its absorbance was measured at 540 nm to estimate the amount of liberated glucose by the action of the α-glucosidase enzyme. The percentage inhibition and 50% inhibitory concentration (IC_{50}) values were calculated. (Kuppusamy A. *et al.* 2011)

6. STATISTICAL ANALYSIS

All analyses were performed using GraphPad Prism version 5, 2007 (GraphPad Software, San Diego, USA). The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The intergroup significance was analyzed using Dunnet's t-test. A p-value < 0.05 was considered to be significant. All the values were expressed as mean ± SEM.

7. RESULTS AND DISCUSSION

7.1 Extraction Yield (%) and Physical Characteristics of Extracts Of Leaves Of *Tecoma stans*

Results of % extraction yield and physical characteristics (Appearance and Consistency) of extracts are presented in Table 1.

Table 1 Extraction yield (%) of ethanolic extract

Extracts	Extraction Yield (%)	Appearance	Consistency
Ethanolic extract	29.5	Greenish-brown	Greasy

7.2 Preliminary Phytochemical Screening of Ethanolic Extract

Preliminary phytochemical screening of ethanolic extract revealed the presence of alkaloids, glycosides, saponins, phenols, Flavonoids, and Steroids (Table 2). (Kokate 2008)

Table 2 Qualitative phytochemical screening of *Tecoma stans* leaves extract.

S. No.	Phytochemicals	Ethanolic Extract
1	Carbohydrates	-
2	Proteins	-
3	Alkaloids	+
4	Glycosides	+
5	Saponins	+
6	Amino acids	-
7	Steroids	+
8	Flavonoids	+
9	Tannins	-
10	Phenols	+
11	Fixed oils and Fats	-

Present (+), Absent (-)

Presence of alkaloid was confirmed by Hager's & Dragendorff's test. Presence of phenol was confirmed by litmus, ferrous chloride and phthalein dye test. The presence of saponin was confirmed by foam test

For the confirmation of flavonoids sulfuric acid test and lead acetate test was performed. Glycosides were confirmed by legal test while presence of steroids were confirmed via Libermann Burchard test

8. ISOLATION AND CONFIRMATION OF ALKALOIDS

The isolation of alkaloids was performed with slight modification in the method of Mushtaq A., 2017. The confirmation of alkaloids was done through preliminary phytochemical analysis of obtained product material (alkaloids sample). The alkaloid sample's preliminary phytochemical analysis revealed that only alkaloids were present while Carbohydrates, Fat and oils, Phenol, Steroids, Flavonoids, Tannins, and glycosides are absent in the sample. (Table 3)

Table 3 Alkaloids sample's preliminary phytochemical analysis

S. No.	Phytochemicals	Findings in isolated alkaloids sample
1	Alkaloid	+
2	Cardiac glycoside	-
3	Anthraquinone glycoside	-
4	Saponin glycoside	-
5	Coumarin glycoside	-
6	Cyanogenetic glycoside	-
7	Tannins	-
8	Flavonoids	-
9	Steroids	-
10	Phenols	-
11	Fat and oils	-
12	Carbohydrates	-

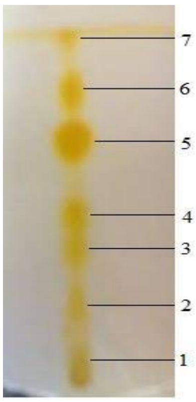
8.1 TLC Findings of Isolated Alkaloids Sample

The thin layer of chromatography analysis of extract was done using n-hexane and acetone (3:1.5). The measurement of the R*f* value using TLC indicates the distance traveled by individual spots. The figure depicts the spots of components observed on TLC plates.

Table 4 Findings of the TLC study of isolated leaves alkaloids

S. No.	R*f* Values	Color
1.	0.05	Yellow
2.	0.09	Yellow
3.	0.20	Yellow
4.	0.43	Brown
5.	0.58	Brown
6.	0.78	Yellow
7.	0.95	Yellow

Figure 1 TLC image of isolated leaves alkaloids



9. IN VITRO ANTIDIABETIC ACTIVITY

9.1 Inhibition of *α*-Amylase Activity

Isolated alkaloids produced 17.52±0.12% inhibition of α-amylase activity at 25μg/ml and 92.06±0.34% at 200μg/ml concentrations, respectively, and its IC_{50} was found to be 45.25±0.41μg/ml.

The standard drug acarbose exhibited 20.12±0.3% inhibition of α-amylase activity at 20μg/ml and 98.39±0.71% at 120μg/ml concentrations, respectively, and its IC_{50} for acarbose was found to be 42.03±0.89μg/ml (Table 5.5).

Table 5 *α*-amylase inhibitory activity of *Tecoma stans* leaves alkaloids.

Concentration (*μ*g/ml)	**% Inhibition Isolated Alkaloids**	**Concentration (*μ*g/ml)**	**% Inhibition Acarbose**
25	17.52±0.12	20	20.12±0.3
50	33.91±0.45	40	39.09±0.78
75	45.16±0.19	60	50.39±0.9
100	54.78±0.68	80	71.01±0.54
150	73.45±0.1	100	86.34±0.62
200	92.06±0.34	120	96.39±0.71
IC_{50} (μg ml^{-1})	45.25±0.41	**IC_{50} (μg ml^{-1})**	42.03±0.89

9.2 Inhibition of *α*-Glucosidase Activity

Isolated alkaloids produced 16.93±0.44% inhibition of α-glucosidase activity at 1μg/ml and 94.16±0.74% at 25 μg/ml concentrations, respectively, and its IC_{50} was found to be 44.62±0.65μg/ml.

The standard drug acarbose exhibited 25.18±0.15% inhibition of α-glucosidase activity at 0.1μg/ml and 98.19±0.32% at 2.5μg/ml concentrations, respectively, and its IC_{50} for acarbose was found to be 48.97±0.73μg/ml (Table 5.6).

Table 6 *α*-glucosidase inhibitory activity of *Tecoma stans* leaves alkaloids and acarbose.

Concentration (*μ*g/ml)	**% Inhibition Isolated Alkaloids**	**Concentration (*μ*g/ml)**	**% Inhibition Acarbose**
1	16.93±0.44	0.1	25.18±0.15
5	35.19±0.1	0.5	49.05±0.17
10	53.69±0.9	1	60.19±0.23
15	69.53±0.84	1.5	76.01±0.61
20	85.03±0.34	2	96.02±0.10
25	96.16±0.74	2.5	98.19±0.32
IC_{50} (μg ml^{-1})	44.62±0.65	**IC_{50} (μg ml^{-1})**	48.97±0.73

10. SUMMERY & CONCLUSION

Nowadays, plants are considered a very important natural source for discovering new pharmaceutical products having therapeutic benefits with no side effects. Numbers of Herbal drugs are known to treat various ailments. In the present investigation, the objective of the study was to extract the *Tecoma stans* leaves and evaluate their phytochemical and anti-diabetes potential. A detailed protocol has been programmed. The leaves of *Tecoma stans* were collected from nearby areas of district Amroha in December. A herbarium was prepared and the collected root sample along with plant specimen was authenticated by Dr. Sunita Garg, Emeritus Scientist, CSIR-NISCAIR, and Mr. RS Jayasomu, Senior Principal Scientist, Head RHMD, Reference number NISCAIR/RHMD/Consult/2020/3672-73. A voucher specimen of the plant was preserved at Raw material herbarium and museum, Delhi (RHMD) for future reference. (Annexure: Appendix A). Dried leaves were powdered and extracted using solvent ethanol by soxhlet apparatus. The extract was dried using a rotary evaporator. % yield of extract was found to be 29.5%. The obtained extract was subjected to preliminary phytochemical tests. Qualitative phytochemical tests confirmed the presence of alkaloids, glycosides, saponins, flavonoids, steroids, and phenols. Alkaloids were isolated from the obtained extract; confirmation was done through preliminary phytochemical and TLC analysis. TLC was performed to get the maximum number of bends/ spots that revealed the existence of 7 different alkaloids in the isolated alkaloids sample. The isolated alkaloids of leaves of *Tecoma stans* was subjected to *in-vitro* antidiabetic activity. Isolated alkaloids showed good antidiabetic potential against the α- amylase and *α*-glucosidase. The isolated alkaloids produced 94.16±0.74% inhibition of α-glucosidase activity at maximum concentrations 25μg/ml, and its IC_{50} was found to be 44.62±0.65μg/ml. The standard drug acarbose exhibited 98.19±0.32% inhibition of α-glucosidase activity at maximum concentrations of 2.5μg/ml, and its IC_{50} was found to be 48.97±0.73μg/ml The isolated alkaloids inhibit 92.06±0.34% α-amylase activity at maximum concentrations of 200μg/ml, and its IC_{50} was found to be 45.25±0.41μg/ml. The standard drug acarbose exhibited

98.39±0.71% inhibition of α-amylase activity at maximum concentrations 120μg/ml, and its IC_{50} was found to be 42.03±0.89μg/ml. Finally, it was concluded that isolated alkaloids of *Tecoma stans* leaves showed significant antidiabetic potential (*In-vitro*) compared with standard compound acarbose.

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