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REGULAR ARTICLE

ESTABLISHMENT OF TWO VARIETIES IN *TECOMA STANS* OF INDIAN ORIGIN PHARMACOGNOSTICALLY AND PHARMACOLOGICALLY

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SUMMARY

The plant *Tecoma stans* (L.) Kunth, belongs to family Bignoniaceae and commonly known as "Pachagotla" is a dicotyledonous herb popularly grown for its flowers as an ornamental / garden plant in normal gardens and temples. It is also known as *Bignonia stans* L. Almost all parts of the plant is reported for its medicinal use. This plant is considered to be very effective in the treatment of diabetes. The leaves contain the alkaloids tecomine and tecostamine which is potent hypoglycaemic agent when given intravenously. Anthranilic acid is responsible for the antidiabetic activity, roots are powerful diuretic and vermifuge and tonic. In order to ensure the use of only genuine and uniform material in preparation of herbal formulation, work on standardization was carried out. Morphological and anatomical aspects as well as differential microchemical response have been worked out to identify the diagnostic features of the leaf. Physical constant values involving moisture content, ash and extractives as well as qualitative and quantitative estimation of various phytochemicals have been studied. The presence of alkaloids, carbohydrates, glycosides and proteins were the chemical constituents recorded. Pharmacologically the alpha – glucosidase inhibitory activity of alcoholic and aqueous extracts were carried out by using the goat intestine method.

Key words: *Tecoma stans*, Bignoniaceae, Variant, Diabetes, Alpha – glucosidase

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1. Introduction

Tecoma stans (L.) Kunth or Yellow Trumpet bush belonging to the family Bignoniaceae is generally described as a perennial tree or shrub. This dicot (dicotyledon/magndiophyta) is native to the United States and Africa has its most active growth period in the spring and summer. The Yellow Trumpet bush has green foliage and inconspicuous yellow flowers, with an abundance of conspicuous brown fruits or seeds. The greatest bloom is usually observed in the indeterminate, with fruit and seed production starting in the summer and continuing until fall. Leaves are not retained year to year. The plant is noted as dangerous invasive plant and spread through cross pollination by insects, ants, humming birds and honey bees. Leaves contain the alkaloids tecomine and tecostamine are potent hypoglycaemic agent when given intravenously. Anthranilic acid is responsible for the antidiabetic activity; roots are powerful diuretic and vermifuge [1, 2, 3]. A literature survey and screening of scientific data revealed that a large number of indigenous drugs have already been investigated as regard their botany and chemistry is concerned, however, a systematic standardization including pharmacognostical and physico-chemical study is still lacking. The present investigation is on the establishment of the two new varieties of *Tecoma stans* (L.) Kunth by taking the pharmacognsotical, phytochemical and pharmacological parameters as the main investigating elements (Figure 1).

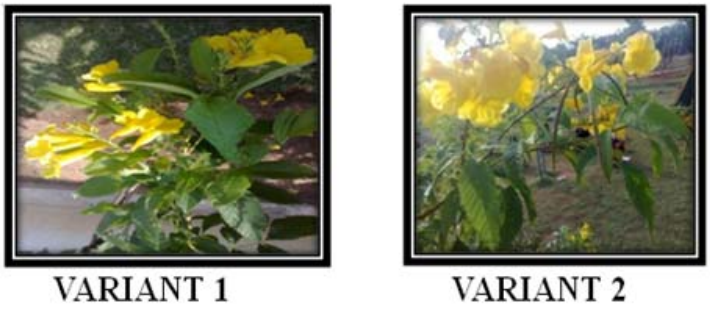

Figure 1: Pictures of Variant 1 and Variant 2 of *Tecoma stans* (L.) Kunth

2. Materials and Methods

Two plant variants of *Tecoma stans* (L.) Kunth were collected, which were differing in their morphology, and having major morphological variations. The fresh leaves of both the varients were collected in the month of December (2009) to February (2010) from Warangal and Nalgonda districts of Andhra Pradesh, India. The vegetal material was used to prepare a herbarium and they were authenticated by the taxonomist of the Department of Botany,Dr. Vatsavaya S. Raju, M.Sc., Ph. D., D. A. S., FBS, FIAT, Kakatiya University, Warangal. The plants were certified as *Tecoma stans* (L.) Kunth, Family-Bignoniaceae (Figure 2). Voucher no: NCOP-NLG/Ph'cog/2009-10/012 and NCOP-NLG/Ph'cog/2009-10/013.The voucher variants were deposited at the Department of Pharmacognosy, Nalanda College of Pharmacy, Nalgonda. The line drawings of both the variants were made (Figure 3). Collected fresh leaves were washed and used for the study of macroscopic and microscopic characteristics. The dried leaves of the plant was powdered and passed through 40 mesh size and stored in an air tight container for further studies [4].

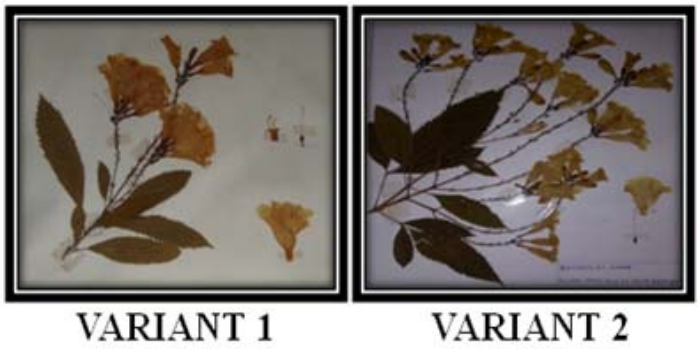

Figure 2: Herbarium of two variants of *Tecoma stans* (L.) Kunth

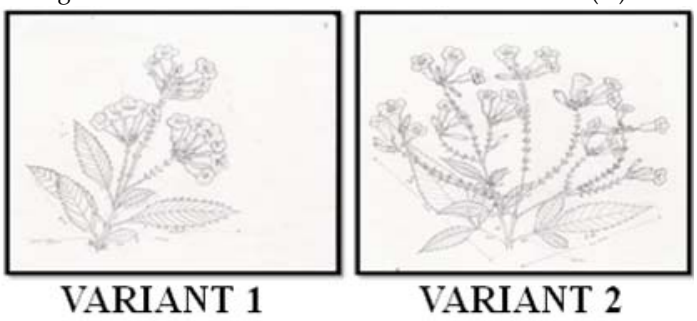

Figure 3: Line drawing of two variants of *Tecoma stans* (L.) Kunth

Microscopic studies

For microscopic studies, the epidermal peelings and transverse sections of leaf were performed by free hand sectioning. The sections were then stained in safranin(1%), alcohol(50%), haematoxyline, alcohol 30%, 50%, 75%, 90% and 100% and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerine [5].

Quantitative microscopy

The leaf epidermal studies were carried out on fresh variants. Peels were removed by free hand. They were stained in phluroglucinol and concentrated hydrochloric acid and mounted in glycerine. Stomatal index and stomatal numbers were calculated.

The powder microscopic studies were carried out on the dried powders of both the variants separately. The powder was stained in phluroglucinol and concentrated hydrochloric acid and mounted in glycerine, this method was for identification of powder characteristics and their measurements. The powder was also examined in glycerine for the examination of calcium oxalate crystals and their measurements [5, 6].

Physico chemical characters

Physico-chemical parameters of the powdered drug such as loss on drying (moisture content), ash values, extractive values were performed as per standard references. The percentages of dry extracts were calculated in terms of air dried leaf powder.

Extraction

The leaf powders of both the variants weighing about 10g were taken and extracted by maceration using the solvents water and alcohol at room temperature. Before the commencement of the extraction specific gravity of both the solvents were noted. The specific gravity was noted intermittently and the extraction was concluded when the value was found same as the initial value. The extracts were collected and concentrated under vacuum. It was then subjected to phytochemical screening.

Phytochemical evaluation

Phytochemical studies such as qualitative and quantitative parameters were carried out from the shade-dried powdered material by extracting with water and alcohol by cold maceration process. For qualitative and quantitative phytochemicals the recommended procedures were followed [5, 6, 7, 8, 9].

Thin layer chromatographic studies
Alkaloids

Precoated TLC plates were used. The solvent system selected was Toulene : ethyl acetate : diethyl amine in the ratio70:20:10 and the detection or visualization is carried out in UV chamber, where the fluorescent orange colour spots were detected[10].

Anthranilic acid

Precoated silica gel F plates were used to perform the experiment.The solvent system selected was n-butanol: acetic acid: water in the ratio 4:1:1.The obtained spots were detected under UV chamber in short and long wavelength.

Pharmacological activity

The aqueous and alcoholic extracts of both the variants were taken along with acarbose as standard in test tubes. To all the test tubes, 500µl of enzyme homogenate was added and incubated for 37^0C for 15min. Then 500 µl of 2% maltose solution was added to the test tubes and again incubated for 15min at 37^0C. It was then centrifuged for 5min. After centrifugation, 0.8ml of resulting supernatant liquid was collected from each test tube and was estimated by *Folin wu* method. In this method the supernatant liquid was transferred to different volumetric flasks. To this 0.8 ml of alkaline copper sulphite solution was added and heated for 8 min and then cooled. After cooling 0.8ml of phosphomolybdic acid was added into all the volumetric flasks. Finally the volume was made to 10ml with distilled water. Absorbances of all these solutions were

observed by UV spectrophotometer at a wavelength of 660nm [12, 13, 14]. For aqueous extract water used as control and for alcoholic extract ethanol was the control. The percentage inhibition of Alpha glucosidase was calculated by using formula,

$$\text{Inhibitory ratio} = \frac{(C - T) \times 100}{T}$$

Where, C is the absorbance of control,

T is the absorbance with addition of plant extract/acarbose.

3. Results and Discussion

Microscopical studies

Similarities of transverse section of leaf of two variants

A thin TS of the leaf of two variants (variant 1 and variant 2) showed dorsiventral nature.. Following tissues are present in midrib and lamina (Figure: 4,5a,5b).

Figure:4 Transverse section of simple leaf of variant 1

Figure:5a. Transverse section of simple leaf of variant 2

Figure:5b. Transverse section of compound leaf 3 of variant 2

Midrib

Epidermal layers of lamina were continuous in the midrib region also strips of collenchyma appeared below the upper and above the lower epidermis. This was followed by cortical parenchyma. An arc shaped vascular bundle was present more towards the dorsal surface (lower epidermis) of the midrib. Distinct phloem tissue could be seen on the dorsal surface and well developed xylem tissue towards the ventral surface of the midrib.

Lamina

The epidermal cells of the lamina were square shaped with outer wall and thin

cuticle. The palisade cells were single layered, compact and cells radially elongated. The spongy parenchyma cells were 3-4 layered, loosely arranged with intercellular spaces. Stomata were present on either side. The upper epidermis and lower epidermis were single layered rectangular cells with cuticulized outer walls consisting of unicellular covering trichomes and glandular trichomes. The covering trichomes were uniseriate, multicellular, bent, and warty with sharp tips and glandular trichomes were found to be sessile with 6-8 celled head.

Similarities of transverse section of petiole of the two variants

Transverse section of the petiole appears more or less cylindrical. The petiole is winged, and the outer surface is covered by the sessile glandular trichomes. The epidermis is made up of small-thick-walled cells with thin cuticle on the outer walls the ground tissue is differentiated into outer 5 layers of collenchyma and inner parenchyma. Vascular strands of the petiole occur as a large median arc. An arc shaped central vascular strand where xylem is towards upper side and phloem is towards lower side, the medullary rays are also seen in between each strand of xylem and phloem. Small patches of the xylem and phloem are observed at the upper surface of the petiole. Polygonally packed parenchymatic cells without any inter cellular spaces are observed. Calcium oxalate crystals are seen in which are solitary in the cells of parenchymatous vascular tissue (Figure 6,7).

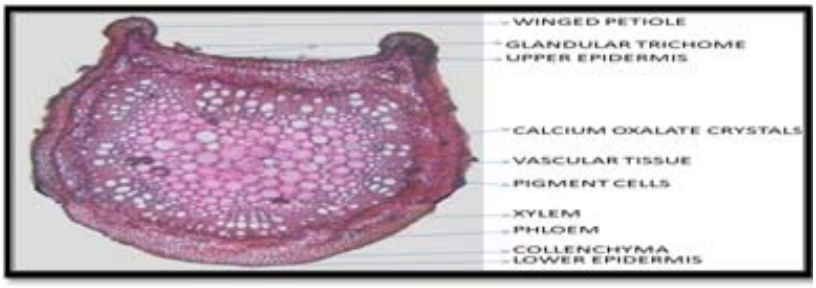

Figure 6: T.S of petiole of variant 1

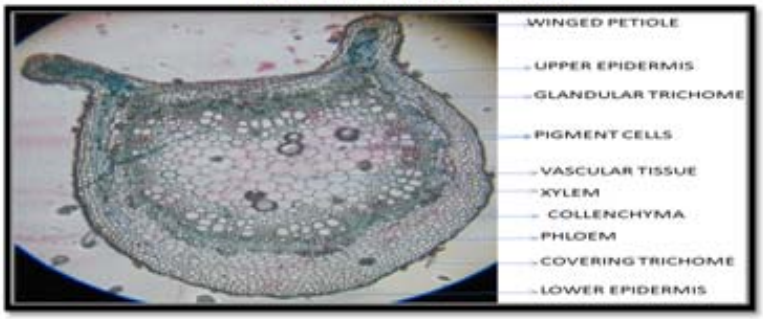

Figure 7: T.S of petiole of variant 2

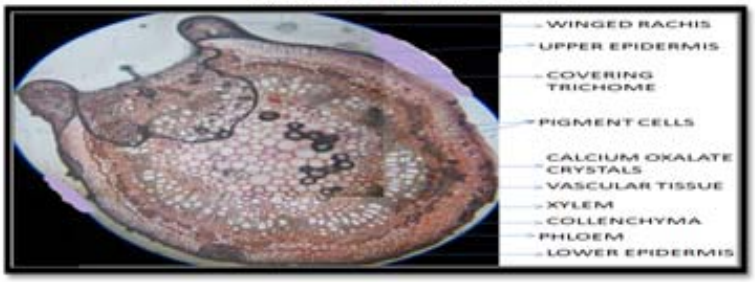

Figure 8: T.S of rachis of compound leaf 3of variant 2

Microscopy of transverse section of rachis

Since Variant 1 is simple leaf rachis was found to be absent, hence the T.S of the rachis of variant 2 was taken.In Transverse section, rachis appears more or less cylindrical or less cylindrical. The rachis is winged the gap between winged rachis is broader, and the outer surface is covered by the covering trichomes. The epidermis is made up of small-thick-walled cells with thin cuticle on the outer walls the ground tissue is differentiated into outer 5 layers of collenchyma and inner parenchyma. Vascular strands of the rachis occurs as a large median arc. An arc shaped central vascular strand where xylem is towards upper side and phloem is towards lower side, the medullary rays are also seen in between each strand of xylem and phloem. Small patches of the xylem and phloem are observed at the upper surface of the rachis. Polygonally packed parenchymatic cells without any inter cellular spaces are observed. Calcium oxalate crystals are seen in which are solitary in the cells of parenchymatous vascular tissue. Some parenchymatous vascular tissue contains pigment cells (Figure 8).

Table 1: Differences of transverse section of leaf, petiole and rachis of two variants

Variant. No	Leaf/ Leaflet	Transverse section of leaf/petiole/ rachis	Epidermis		Collenchyma	Distance between vascular bundle and lower epidermis	Calcium oxalate crystals	The groove
			Upper epidermis	Lower epidermis				
1.	Simple leaf	T.S of leaf	A	N	2-3 layered	AA	Absent	-
		T.S of petiole	B	B	2-3 layered	BB	Present	Wider
2.	A. Simple leaf	T.S of leaf	C	N	2-3 layered	CC	Absent	-
		T.S of petiole	B	A	3-4 layered	CC	Absent	Wider
	B. Compound leaflet 3	T.S of leaf	D	F	2-3 layered	CC	Absent	-
		T.S of rachis	D	B	2-3 layered	CC	present	Wider

A. Unicellular covering trichomes and sessile glandular trichomes are present, B. Sessile glandular trichomes are present, C. Trichomes are absent, D. Unicellular covering trichomes are present, E. Abundant number of Multicellular covering trichomes along with sessile glandular trichomes are present, F. Unicellular and multicellular covering trichomes along with sessile glandular trichomes are present

AA. Vascular bundle is just radiating towards lower epidermis, BB. Vascular bundle is more radiating towards lower epidermis, CC. Vascular bundle is just radiating towards lower epidermis

Quantitative microscopy

The stomatal index, stomatal number are given in Table 2 and measurements of powder microscopy values are given in Table 3. The variant no: 1 was showing actinocytic type of stomata and variant no: 2 were showing anomocytic stomata (Figure 9). The stomatal number and stomatal index were varying from each other.

Table 2: Quantitative microscopy of epidermis

Variant no:	Type of stomata	Stomatal number	Stomatal index
1.	Actinocytic	2	16.66
2.	Anomocytic	3	25

Powder Microscopy

Powder microscopy of leaf (Figure 10,11)

Stomata: Actinocytic type of stomata was seen.

Trichomes: Two kinds of trichomes were seen. The covering trichome was found to be blunt and fine warty, uniseriate, unicellular to multi-cellular, 3-4 celled with pointed tips slightly bent at apex. Sessile glandular trichomes consists of multicellular head.

Calcium oxalate crystals were of prism type.

Xylem vessels were lignified.

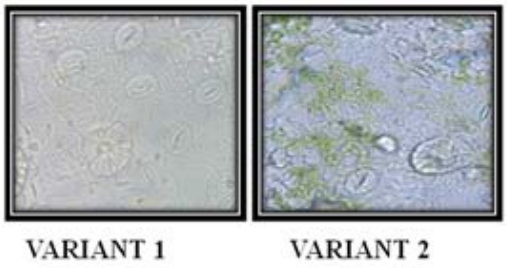

Figure 9: Stomata of Variant 1 and Variant 2

Figure 10: Powdered microscopical features of Variant 1

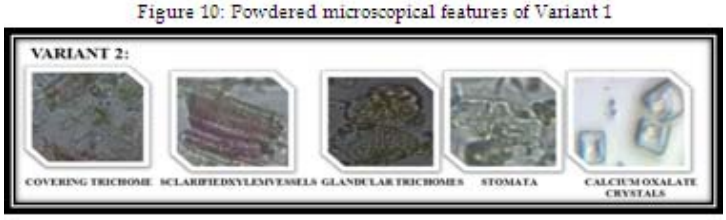

Figure 11: Powdered microscopical features of Variant 2

Table 3: Measurement of cell characters

Variant. no	Length of covering trichome	Diameter of the glandular trichome	Diameter of calcium oxalate crystals
1.	15.6µ-56.94µ-124.8µ	7.8 µ-13.26 µ-15.6 µ	3.9 µ-7.41 µ-15.6 µ
2.	31.2 µ-83.07µ-156µ	7.8 µ-11.7µ-15.6µ	3.9 µ-8.19 µ-11.7µ

The length of covering trichomes , diameter of glandular trichomes and calcium oxalate crystals were varying from one variant to the other variant which are shown in Table 3.

Physico chemical standardization:

The physicochemical characters such as moisture content, total ash, acid insoluble ash, acid soluble ash, and extractive values in alcohol and water of the dried leaf powder were calculated in terms of air dried sample. Ash values indicate the purity of drug, extractive values represent the presence of polar or non polar compound and loss on drying values indicate whether the drug is safe regarding the microbial contamination. All the parameters were found to be different from variant another.

Table 4: Loss on drying

Variant no.	Loss on drying/ moisture content (%)
1.	6.92
2.	5.90

Table 5: Ash values

Variant no.	Total ash	Acid insoluble ash	Water insoluble ash
1.	13	0.06	0.12
2.	10	0	0.10

Table 6: Extractive values

Variant no.	Weight of total alcoholic extracts(grams)	Weight of total aqueous extracts(grams)
1.	5.33	5.19
2.	5.00	5.35

Phytochemical screening

The results of qualitative phytochemical tests are tabulated in Table 7. The different chemical compounds such as alkaloids, carbohydrates, glycosides, proteins were detected in aqueous and alcoholic extracts of the variants of *Tecoma stans* which could make the plant useful in treating different ailments and having potential for providing useful drug for human use.

Table 7: Preliminary phytochemical screening of alcoholic extracts

S. no	Chemical tests	Alcoholic extracts	
		Variant 1	Variant 2
1.	Alkaloids	+	+
	Dragendroffs test	+	+

	Mayers test	+	+
	Hagers test	-	+
	Wagners test	+	-
2.	Amino acids	-	-
3.	Carbohydrates	+	+
4.	Glycosides	+	+
5.	Proteins	-	-
6.	Saponins	-	-
7.	Steroids	-	-
8.	Tannins and phenolic compounds	-	-

Table 8: Preliminary phytochemical screening of aqueous extracts

S. no	chemical tests	Aqueous extracts	
		Variant 1	Variant 2
1.	Alkaloids	+	+
2.	Amino acids	-	-
3.	Carbohydrates	+	+
4.	Glycosides	+	+
5.	Proteins	+	+
6.	Saponins	-	-
7.	Steroids	-	-
8.	Tannins and phenolic compounds	-	-

Thin layer chromatographic analysis

TLC for alkaloids

Table 9: TLC for alkaloids

Variant no:	R_f value of spot –A	R_f value of spot –b
1.	0.72	0.45
2.	0.61	0.34

TLC for anthranilic acid

Table 10: TLC for anthranilic acid

Variant no.	R_f VALUE	
	Alcoholic extract	Aqueous extract
1.	0.80	0.86
2.	0.85	0.87

The Standard R_f value of Anthranilic acid was 0.88[11]

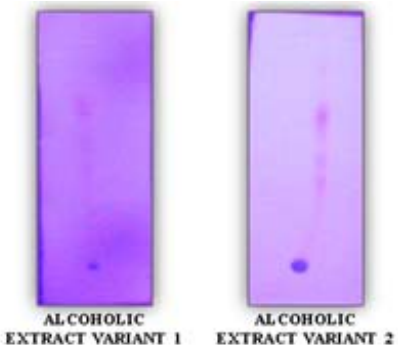

Figure 12: TLC for alkaloids

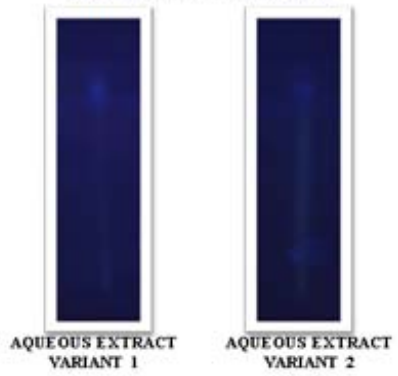

Figure 13: TLC for anthranilic acid

The TLC for anthranilic acid was carried out for both variants using alcoholic and aqueous extracts. By comparing the Rf values of aqueous and alcoholic extracts of both variants with that of the standard Rf values of anthranilic acid, it was found that anthranilic acid was present in both the variants (Figure 12,13).

Pharmacological activity

Invitro Hypoglycaemic Activity: Alpha - Glucosidase Inhibitory Activity

Table 11: Percentage Inhibition of aqueous and alcoholic extracts

	Alcoholic extracts	Aqueous extracts
Standard (acarbose)	30.58	45.77
Variant 1	13.24	18.4
Variant 2	17.62	20.39

The Table 11 and Figure 14 shows the % inhibition of aqueous and alcoholic extracts of both the variants compared with that of the standard Acarbose. The % inhibition was varying from one variant to the other compared to that of the standard Acarbose.

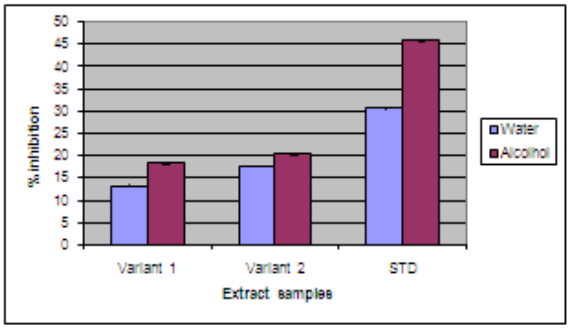

Figure 14: Graphical representation of % inhibition of two variants

4. Conclusion

Tecoma stans is exotic species introduced into tropics including India for its horticultural value. Presently, it is found naturalized at times as on Simhachalam and Tirumala hills though largely it is found planted as hedge, in temple premises, house compounds, etc. for its blossoms. The plant is recognized by its bell-shaped golden yellow flowers.

From all the above works it was conclude that the two variants are having variations in many aspects compared to the plant one which was taken as a standard that is *Tecoma stans* (L.) Kunth. So, from this work it was conclude that the two variants are considered as new cultivars of *Tecoma stans*. The suggested names are as follows:

Tecoma stans (L.) Kunth cv. Nalgonda 1

Tecoma stans (L.) Kunth cv. Warangal 1

The above two variants of *Tecoma stans* (L.) Kunth, in view of their constituent variations and distinctions in the external morphology, they were described formally and named them as Nalgonda 1 and Warangal 1.

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