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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS OF *TECOMASTANS* (L.) JUSS EX KUNTH LEAVES FROM PUTTAPARTHI, ANDHRA PRADESH, INDIA

***Mukul Anand and Basavaraju, R.**

Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Puttaparthi-515134, District Anantapur, Andhra Pradesh, India

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ABSTRACT

The aim of the study was to determine the levels of certain phytochemicals in the leaves of *Tecomastans* during two physiological stages i.e., flowering and pre-flowering. The study revealed that the physiological developmental stages significantly affected the levels of studied phytochemicals. The flowering stage revealed higher levels of polyphenols, flavonoids and tannins in comparison to pre-flowering stage. However, alkaloids were observed to decline during flowering phase of the plant. Increased levels of bioactive components during generative period of the plant could be extracted using different solvent systems with maximum solubility and exploited bypharmaceutical industries in certain formulations for medicinal purposes.

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INTRODUCTION

Our mother nature perform a unique and the most important duty of delivering the human and animal systems with fundamental (glucose, amino acids and fatty acids) and magical (phytochemicals) molecules which not only assist in our development but also prevent and cure health disorders and diseases. These nutrients and important components find their way to reach our systems through diet which other than their fundamental roles helps us to receive these genius molecules and perform their health beneficial effects such as regulating blood sugar and lipid levels, immune modulation, brain health promotor, act as diuretics, helps respiratory and gastro disorders, fight against bacterial, fungal and viral infections, liver and renal protection, pain reliever, weight reduction, reduce over production of stress generated radicals and thereby maintain balance between oxidants and anti-oxidants in the system. Indian system of medicine is known to use more than 6,000 species of medicinal plants which is almost double the value of officially documented medicinal plant species, one such less explored species is *Tecoma stans*. It is a perennial shrub which belongs to the family Bignoniaceae, has green foliage, inconspicuous yellow colour bell shaped flowers with an abundance of conspicuous brown seeds (Parrotta, 2001; Khare, 2007; Kandakatla *et al.,* 2010). It is an indigenous medicinal plant which is gradually gaining popularity throughout the world. All the parts of the plant have been in use traditionally as preventive and curative agents against various disorders and diseases. They have marked their use as efficient hypoglycemic agents, powerful diuretics and effective against infections (Aguilar *et al.,* 1993; Alonso-Castro *et al.,* 2010; Raju *et al.,* 2011; Salem *et al.,* 2013). Very few studies have been undertaken to determine the phytochemicals present in the plant at national and international level. Certain health beneficial compounds reported from the roots, leaves, flowers and bark include alkaloids (Tecomine, Tecostamine, γ- skythanthine, boshniakine, 5-dehydro-skythanthine, 4-noractenidine, N-normethyl-skythanthine), polyphenols, phenolic acids (Chlorogenic acid, caffeic acid, rutin, vanillic acid, o-couumaric acid, spinapcin acid), p-sitosterol, anthranilic acid, lutein, flavonoids, anthraquinones, tannins, sterols, terpenes, saponins, β- carotene, zeaxanthine, ascorbic acid, essential amino acids etc. (Costantino *et al.,* 2003; Dash *et al.,* 2011; Govindappa *et al.,* 2011; Torane *et al.,* 2011; Singh *et al.,* 2011). Toxicity studies carried out on the plant have revealed it to be non-toxic. In addition, this shrub is also utilized as forage for cattle in Mexico with no evidence of foliage toxicity (Jimenez-Ferrer *et al.,* 2007). To our

Corresponding author: Mukul Anand,
Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Puttaparthi-515134, District Anantapur, Andhra Pradesh, India.

knowledge there have been no studies carried out at Puttaparthi, Andhra Pradesh (India) on this miraculous evergreen plant till date. Hence, the aim of the study was to explore this plant with huge medicinal potential and generate data on few phytochemicals present in the leaves of the selected plant.

MATERIALS AND METHODS

Collection and identification of leaf sample

Fresh leaf samples from top, middle and bottom portion of the plant were collected from two different areas of Puttaparthi, Andhra Pradesh, India during the month of May, 2015. Confirmation of the collected leaf samples was done by Prof. R. Basavaraju, Sri Sathya Sai Institute of Higher Learning; Prof. T. N. Lakhanpal, University of Shimla, Himachal Pradesh and Prof. P. S. Srivastava, Jamia Hamdard University, New Delhi. The samples were certified as *Tecoma stans* (L.) Juss Ex. Kunth, Family- Bignoniaceae, voucher no: SSSIHL/DBS/2015-2016/001-002. The voucher specimens were deposited at the department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Puttaparthi.

Processing of the leaf sample

Collected fresh leaf samples were washed to remove dust and foreign particles, shade dried, powdered and sieved through 2 mm sieve. Thereafter, the powdered leaf samples were stored in air tight containers at room temperature until analyzed for certain phytochemicals.

Phytochemical analysis

The qualitative screening was carried out using five different solvent systems viz., water, methanol, ethyl acetate, chloroform and hexane. The sample solvent mixture (1:10) was extracted using mechanical shaker and filtered through Whatman no. 1. The filtrates were assessed for the presence of various phytochemicals using qualitative screening protocols by Sofowara (1993) and Trease and Evans (2002). The quantitative assessment of bioactive components was carried out for total polyphenols (Singleton and Rossi, 1965), flavonoids (Marinova *et al.,* 2005), tannins (Price *et al.,* 1978) and alkaloids (Harborne, 1973).

Statistical analysis

The data obtained was expressed as Mean ± SD.

RESULTS AND DISCUSSION

The current study was undertaken in leaf samples collected from two different spots of Puttaparthi. In India, Tecoma starts flowering as early as April and continues into autumn season which falls between August and October. The leaf samples were collected during the month of May, 2015. It was observed during sample collection that for selected plant at spot A flowering stage had not yet arrived while plant at spot B had few flowers. This propelled us to discuss the results obtained comparatively between the spots as both were in two different physiological stages i.e., budding (pre-flowering) and flowering. Moreover, a positive correlation exists between the amount of secondary metabolites produced in the plants and their utilization and accumulation during the different physiological stages such as germination, budding, flowering and fruiting. The dried leaf samples were screened qualitatively for the presence and absence of certain phytochemicals such as polyphenols, flavonoids, tannins, alkaloids, terpenoids and saponins (Table 1). For this purpose five different solvent extracts were prepared using water, methanol, ethyl acetate, hexane and chloroform. Solubility of the screened phytonutrients in different solvents used showed the absence or presence as high, medium and low. Plant in the pre-flowering stage showed medium concentration of polyphenols, flavonoids, tannins in comparison to high concentration exhibited at flowering phase. Of the different solvents used aqueous and methanolic extracts showed higher profile of phenolic compounds. However, alkaloids were found to be high in pre-flowering with maximum solubility in methanol and chloroform extracts. Saponins and terpenoids did not show any variation on the basis of the two physiological stages and were found to have maximum solubility in methanol in comparison to ethyl acetate, hexane and chloroform. Phenolic compounds are known for playing key roles in the growth, regulation and structuring of plants. Polyphenols, flavonoids and tannins were found to be higher in the leaves at the flowering stage than during the vegetative stage. The amount of polyphenols found in the studied leaf samples were 2236.8 and 3984.2 mg/100 g on dry weight basis before and during flowering respectively.

Table 1. Phytochemicals identified in leaves of *Tecoma stans*

Phytochemical	Pre- flowering stage					Flowering stage				
	AE	ME	EE	CE	HE	AE	ME	EE	CE	HE
Polyphenols	++	+	+	-	-	+++	++	++	-	-
Flavonoids	++	+	+	-	-	+++	++	++	-	-
Tannins	++	++	+	-	-	+++	+++	++	-	-
Alkaloids	-	+++	+	+++	+	-	++	+	++	+
Terpenoids	-	++	+	+	+	-	++	+	+	+
Saponins	++	++	+	+	-	++	++	+	+	-

AE-Aqueous extract, ME-Methnaolic extract, EE-Ethyl acetate extract, CE- Chloroform extract and HE-Hexane extract
+++ = High concentration, ++ = Medium concentration, + = Low concentration, - = absent

Table 2. Phytochemical analysis of selected leaf samples

Phytochemical (mg/100g)	Pre- flowering stage	Flowering stage
Polyphenols	2236.8 ± 111.6	3984.2 ± 88.8
Flavonoids	118.4 ± 5.58	199.2 ± 4.42
Tannins	11.5 ± 0.22	14.8 ± 0.899
Alkaloids	2450 ± 12.47	1783 ± 24.94

Values are ± SD of three replicates on dry weight basis

Similar tendency was recorded in the leaf samples for flavonoids and tannins (mg/100g) exhibiting 118.4 and 11.5 before flowering and 199.2 and 14.8 during flowering respectively. Studies carried out on other leaf samples viz., *Limmonium delicatulum* and *Bryoniadioica*, also support the observed values of phytochemicals viz., polyphenols, flavonoids and tannins before and during flowering stage (Gholivand and Piryaei, 2012; Medini *et al.,* 2014). The observed values of polyphenols and flavonoids in the studied leaf samples are comparable to the reported values of 1200 and 120 mg/100g respectively (Alonso-Castro *et al.,* 2010). This could be due to the use of water for the extraction of the phytochemicals. This is further supported by a study where different solvents were used for the extraction purpose reporting lower polyphenol and flavonoid values of 144.7 and 12.8, 37.33 and 50.1, 461.4 and 38 mg/100g on dry weight basis in acetone, methanolic and ethanolic leaf extracts of *Tecoma stans* respectively (Torane *et al.,* 2011). Other than the generative stage of the plant, type of solvent used for extraction and solubility of phenolic compounds in the solvent there are also other important factors which could stimulate and allow biosynthesis and accumulation of these substances in the leaves. These include age of the plant and leaf, resistance towards stress conditions (hot temperature, drought, high solar exposure, and soil salinity) and fruiting stage of the plant (Medini *et al.,* 2014). A preceding study has shown that leaves tend to accumulate more polyphenols and flavonoids during the seed bearing stage and decline during seedless phase (Chauhan *et al.,* 2004). Alkaloids isolated from the studied sample are monoterpens and are known to be potent hypoglycemic agents (Costantino *et al.,* 2003). In the present study alkaloids were estimated by gravimetric method and were found to be 2450 and 1783 mg/100g on dry weight basis for pre-flowering and flowering phase respectively. It has been reported that alkaloid levels vary between vegetative and generative stages of a plant. Throughout the vegetative period alkaloid levels undergo changes and before the generative (flowering) period starts i.e., during the budding stage alkaloid levels in leaves are at their peak and the levels continue to increase significantly during the seed bearing stage and decline subsequently as the seed ripens. Towards the end alkaloids tend to accumulate in roots and stems (Brummund, 1988; Gataulina, 2002; Maknickiene and Asakaviciute, 2008). The observed alkaloid values are in line with the reported content of 2050 mg/100 g in *Tecoma stans* leaves (Alonso-Castro *et al.,* 2010). However, lower content of 51.80 and 640 mg/100 g have been reported in the studied leaf sample by Rama Krishna *et al.* (2009), Hussain *et al.* (2011) respectively. The other major factors which play important role in accumulation and utilization of alkaloids is location, environmental stress conditions, soil type and salinity.

Conclusion

Tecoma stans is an ornamental evergreen shrub which is a store house of number of phytonutrients which are known for their biological properties. Leaf samples exhibited higher concentration of studied phytochemicals at the flowering stage than at the vegetative stage. The results indicate that the extraction of bioactive molecules from the natural unexplored sources at a specific growth stage with appropriate solvents can provide fractions with biological activities that could be used in the formulation of novel drugs.

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