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Research Article

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Phytochemical effect of the indigenous plant *Tecoma stans* on the Yellow fever mosquito, *Aedes aegypti* (L).

Sathiyamoorthy P, Dharani P, Deepalakkshmi B, Gowsalya K and Dr. K Elumalai*

Department of Zoology, Government Arts College for Men (Autonomous), Nandanam, Chennai- 600035, Tamil Nadu, India

*Corresponding Author E- mail: *sathiyamoorthy0518@gmail.com*

Abstract

Considering plants are a rich source of bioactive phytochemicals that are risk-free and biodegradable into non-toxic byproducts, which might be tested for insecticidal effects and mosquito repellant, interest in plant-based repellents has recently increased. Numerous studies have documented the effectiveness of plant extracts or essential oils as insect repellents against mosquito vectors all over the world. Millions of people die each year from deadly diseases that mosquitoes spread, and as they become resistant to modern insecticides, their vectorial capability is recovering. Alternative sources of mosquito repellents may include plants. In the current study, *Tecoma stans* effectiveness against the common disease vector *Aedes aegypti* was evaluated. Additionally, tests were done on the plant extract's potential phytochemical components, antioxidant activity, and antibacterial activity. The findings show that *Tecoma stans* can be a powerful source of antibacterial, antioxidant, and mosquito repellent.

Keywords: *Tecoma stans, Aedes aegypti,* antimicrobial and mosquitocidal activities.

Introduction

Plants have long been used as traditional remedies, and they are now being used to treat some of the biggest killers of people, such as heart disease and cancer. In order to maintain human health, insecticidal, antivenomous, and related substances have so continued to be important sources of therapeutic compounds since ancient times.

Tecoma stans is a type of perennial flowering shrub that is indigenous to the Americas and belongs to the Bignoniaceae (Trumpet vine) family. Yellow trumpet bush, yellow elder, yellow bells, and ginger-thomas are examples of common names (GRIN, ARS, USDA, 2017). *T. stans* shown anticancer, antibacterial, anti-inflammatory, anti-free radical,

hypoglycemic, and antidiabetic activities (Pelton, 1964).

This plant is known to contain monoterpene alkaloids, and two of them are thought to be responsible for the plant's hypoglycaemic effects (Luke *et al.*, 2003). Due to the presence of Tecomine, it is well known that *T. stans* exhibits significant alkaloidic variability depending on where it grows (Dickinson and Jones, 1969).

Furthermore, it has been demonstrated that giving *T.stans* leaves intravenously to healthy dogs causes an early hyperglycemic response, which is followed by a delayed decline in blood glucose levels and a concurrent increase in triglycerides; no significant changes in insulin levels were seen (Meckes, 1985).

Aedes aegypti is a vector for many infections to be spread. As of 2022, the Walter Reed Biosystematics Units state that it is connected to 54 viruses and two types of Plasmodia, including *Plasmodium gallinaceum*, *Plasmodium lophurae*, yellow fever virus, Dengue virus, Murray Valley Encephalitis virus, and many others.

Materials and Methods

Collection of plant samples

Tecoma stans freshly leaves were collected in the Chengi district, both young and old, and brought into the lab. The leaves were scrubbed and allowed to air dry in the shade for ten to fifteen days in the lab. Using an electrical blender and a sieve, the leaves were ground into a consistent, fine powder. After that, it is maintained in a glass container with a secure lid.

Preparation of extraction

The leaves powder was extracted using the maceration process using ethanol as the solvent. The extracts were filtered via No. 1 Whatman filter paper. The crude extract was then dried in a rotating evaporator at 50 degrees Celsius, then collected and stored under sterile conditions.

Antimicrobial activity

The Well Diffusion method, According to Devillers *et al.*, (1989), was used to evaluate the antibacterial and antifungal properties of *T. stans.* Gram positive strains of *Staphylococcus aureus*, *Enterococcus faecalis*, and gram-negative Strains of *Escherichia coli, Klebsiella pneumoniae*, were used to test the effectiveness of the *T. stans* antibacterial nature, while the pathogenic yeast *Candida albicans* was used to estimate the plant antifungal potency.

Antioxidant Assay

Using DPPH assay, the radical scavenging capacity of plant extract was assessed. After fully mixing the antioxidant into the cuvette containing 2.960 l of 0.1 ml of plant extract, the reduction in DPPH solution absorbance at 519 nm was determined. After 20 minutes, the setup was checked for absorption using a UV spectrophotometer while it was still dark and at room temperature. Control substances included ascorbic acid. The following equation was used to determine the plant extract's capacity to scavenge DPPH radicals.

$$\text{Percentage of Inhibition} = \frac{\text{Absorbance of control - absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Mosquitocidal Activity

Larvicidal activity against *A. aegypti*

The larvicidal activity of *A. officinarum* was investigated at doses of 100, 200, 300, and 400 l/L using standard WHO Protocols (WHO 2005) for the aforementioned bioassays. There were no meals provided to the treated larvae, therefore mortality and survival were recorded at 24, 48, and 72 hours. The symptoms of the treated larvae were evaluated and reported immediately and at intervals.

Corrected mortality =

$$\frac{\text{Observed mortality in treatment - Observed mortality in Control}}{\text{100-Control mortality}} \text{ X100}$$

Pupicidal activity against *A. aegypti*

To prevent adult mosquitoes from emerging during pupicidal activity, the entrance of each bowl containing pupae was covered with a muslin cloth. Pupae mortality was noted after 24 hours. As a control, pupae were exposed to dechlorinated water without biopesticide. Using Abbott's formula (Abbott's, 1925), the control mortalities were adjusted.

Percentage mortality=

$$\frac{\text{Number of dead larvae/ pupae}}{\text{Number of larvae /pupae introduced}} \text{ X100}$$

Repellent activity against *A. aegypti*

In a repellent test chamber (30 x 30 x 62.5 cm), the repellent action was carried out in a lab setting. On the hands of human test subjects, five repellent concentrations—100, 200, 300, and 400 ppm—that were dissolved in ethanol were tested. Through the top opening, female mosquitoes were let into the repellent chamber. Each patient had one hand with 0.3 ml of the test solution spread on the dorsal side. The hand was placed inside the repellent chamber for 10 minutes through a hole up to the wrist and closed with cotton to prevent mosquito escape so that the female mosquitoes could bite on the hand more easily after 30 minutes of application. Every 15 minutes, the test was repeated.

Results

Phytochemical Analysis of *T. stans*

In the phytochemical investigation of *T. stans*, the presence of tannins, alkaloids, glycosides, saponins, terpenoids, flavonoids, phytosterols, and phenolics was determined.

DPPH Assay - Antioxidant Activity of *Tecoma stans*

Table 1: Antioxidant activity of *T. stans* evaluated by DPPH Assay

Concentrations (µg/ml)	Absorbance		Average	Inhibition (%)
	I	II		
Control	1.123	1.126	1.1245	0
20	0.924	0.927	0.9255	17.69675
40	0.731	0.729	0.73	35.08226
60	0.462	0.460	0.461	59.0040
80	0.281	0.276	0.2785	75.23344
100	0.079	0.076	0.0775	93.10805

The inhibitory concentration of *T. stans* at 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml was found to be 17.7%, 35.08%, 59%, 75.23% and 93.11% respectively. The results reveal that the *T. stans* extract is found to have a potent antioxidant and radical scavenging activity against free radicals.

Antimicrobial activity of *Tecoma stans:*

Table 2: Antimicrobial activity of *T. stans*

S. No.	Pathogens	Zone of inhibition (mm in diameter)	
		Positive control (PC)	Ethanol extract of *Tecoma stans* (100 µg/ml)
1	*Staphylococcus aureus*	29	23
2	*Enterococcus faecalis*	30	19
3	*Escherichia coli*	32	24
4	*Klebsiella pneumoniae*	28	21
5	*Candida albicans*	31	22

Positive controls -

- Bacteria – Gentamycin
- Fungus – Clotrimazole

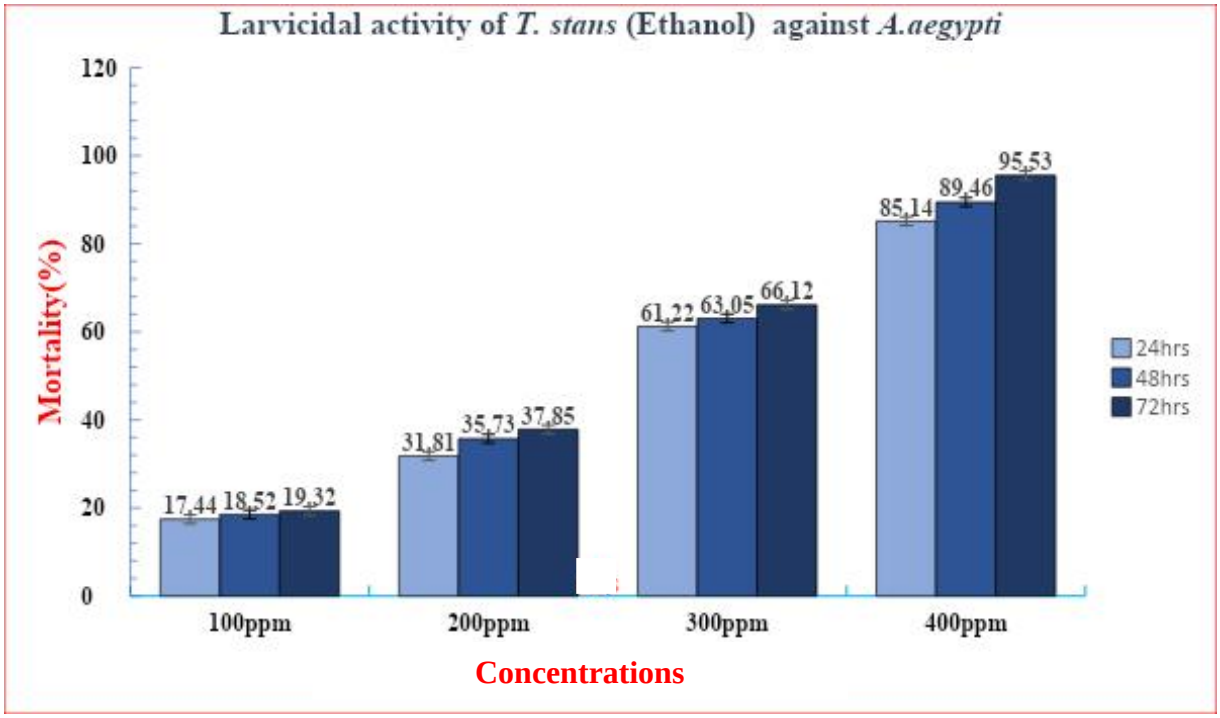
Table: 3 Larvicidal activities of Ethanol solvent extracts of *T. stans* against third instar larva of *A. aegypti*

Concentrations	*Tecoma stans* ethanol extract Exposure periods in (hrs)		
	24	48	72
100ppm	$17.44\pm2.56_a$	$18.52\pm1.74_a$	$19.32\pm2.16_a$
200ppm	$35.81\pm2.08_b$	$35.73\pm1.49_b$	$37.85\pm1.89_b$
300ppm	$61.22\pm1.40_c$	$63.05\pm0.87_c$	$66.12\pm1.27_c$
400ppm	$85.14\pm0.73_d$	$89.46\pm0.54_d$	$95.53\pm0.31_d$
Neem Azal	100.0±0.00	100.0±0.00	100.0±0.00

Values represent mean ± SD of five replications. Different alphabet in the column shows statistically significant at $p<0.05\%$ (Tukey test)

The larvicidal activity of *T. stans* was tested against the third instar larvae of *Ae. aegypti* and the data obtained in the experiment are shown in table 3 and figure 1. The maximum larvicidal activity was observed in the 400ppm concentration of the DCM extract of *T. stans* at 72hrs (95.53±0.31%). The trend was followed by the 300ppm concentration (66.12±1.27%).

Figure: 1 Larvicidal activities of ethanol extracts of *T. stans* against third instar larva of *A. aegypti*



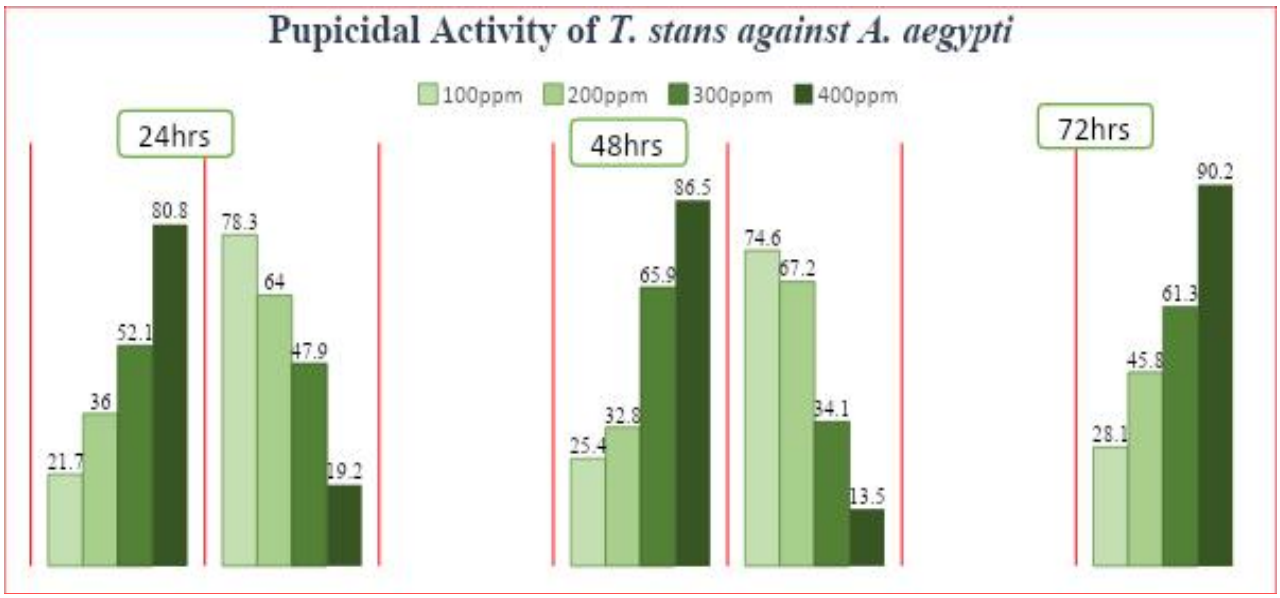
Pupicidal activity

Table 4. Pupicidal activity of Ethanol extract of *T. stans* tested against pupae of *A. aegypti*.

Concentrations (ppm)	Exposure period (in Hrs)					
	24 % Activity		48% Activity		72% Activity	
	Pupal mortality	Adult emergence	Pupal mortality	Adult emergence	Pupal mortality	Adult emergence
100	21.7±2.32	78.3±1.88	25.4±1.26	74.6±1.82	28.1±2.08	71.9±2.74
200	36.0±1.83	64.0±1.55	32.8±1.58	67.2±1.93	45.8±1.91	54.2±1.77
300	52.1±1.07	47.9±1.10	65.9±1.40	34.1±0.58	61.3±1.24	38.7±0.78
400	80.8±0.29	19.2±0.64	86.5±0.15	13.5±0.24	90.2±1.08	9.8±0.21
Neem Azal	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

Pupicidal activity of ethanol extract of *T. stans* was tested against the freshly moulted pupae of *A. aegypti* (24hrs, 48hrs & 72hrs). It was observed that the pupal mortality of 21.7, 25.4 &28.1% caused by 100ppm extract and the adult emergence of 78.3, 74.6 &71.9 % was noticed against it. In the same way, 200ppm showed 36.0, 32.8 &45.8 % of pupal mortality with the adult emergence of 64.0, 67.2 & 54.2 %. Besides, 52.1, 65.9 & 61.3 % of pupal mortalities were noticed 300ppm with adult emergence of 47.9,34.1& 38.7% and 400ppm concentrations of the same extract with the pupal mortalities were 80.8,86,5 &90.2 and 19.2, 13.5 &9.8% adult emergence respectively (Table .4; figure 2).

Figure 2. Pupicidal activity of ethanol extract of *T. stans* against pupae of *A. aegypti*.



Repellent activity

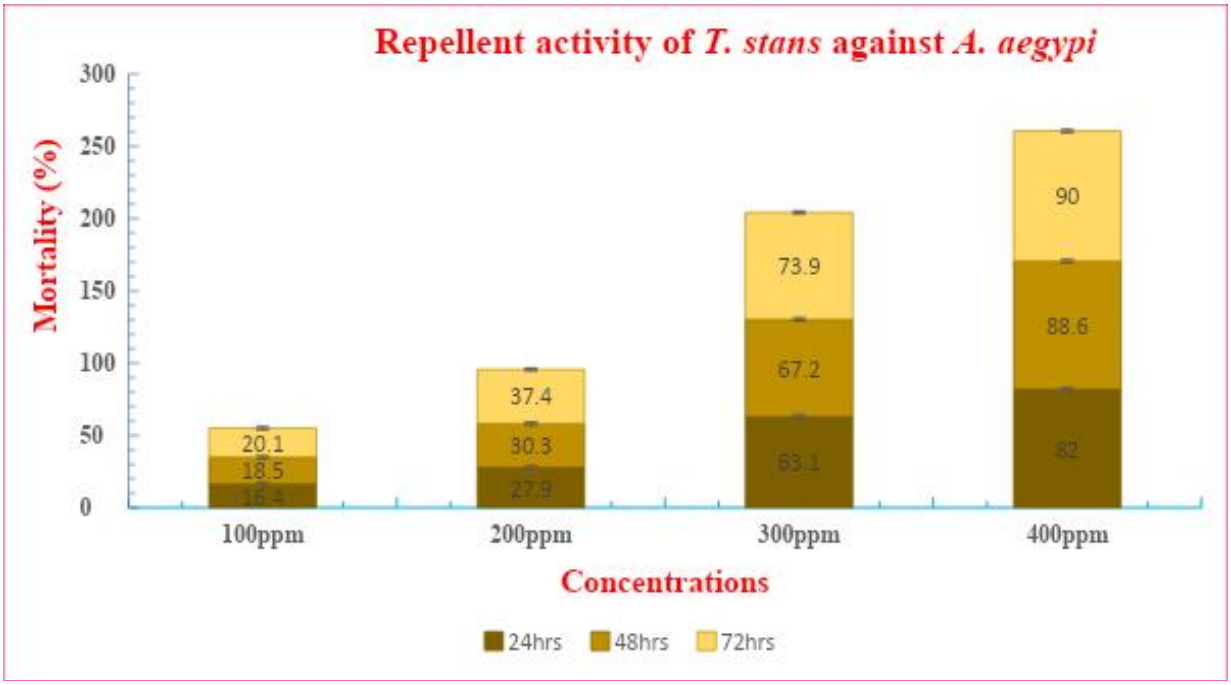
Table 5. Repellent activity of ethanol extract of *T. stans* tested against adult *A. aegypti.*

Concentrations	Exposure period (hrs)		
	24	48	72
100ppm	$16.4\pm2.01_a$	$18.5\pm1.89_a$	$20.1\pm2.79_a$
200ppm	$27.9\pm1.76_b$	$30.3\pm1.73_b$	$37.4\pm1.29_b$
300ppm	$63.1\pm1.33_c$	$67.2\pm1.11_c$	$73.9\pm1.06_c$
400ppm	$82.0\pm0.87_d$	$88.6\pm0.73_c$	$90.0\pm0.23_d$
Control	98.0±0.00	98.0±0.00	98.0±0.00

Values represent mean ± SD of five replications. Different alphabet in the column shows statistically significant at $p<0.05\%$ (Tukey test)

The repellent activity of ethanol extract of *T. stans* that was tested against adult of *A. aegypti* (Table 5). The repellent activity of the extract showed statistically significant activity at higher concentrations of 400 ppm 82.0, 88.6 & 90.0% for 24, 48 & 72hrs. In the same way, experimental groups exposed to 300, 200 & 100ppm of treatment showed maximum repellent activity than the other two exposure periods.

Figure 3. Repellent activity of ethanol extract of *T. stans* tested against adult of *A. aegypti*



Discussion

Individually or in combination, phytoconstituents such phytosterol, triterpene, glycosides, phenols, flavonoids, saponins, and tannins may have a synergistic effect on the healing of wounds (Das *et al.*, 2010). The extracts' powerful bioactivities, which are known to contain strong antioxidants (Zhang and Lin, 2008), may be explained by the tannins and saponins present in them (Gulcin *et al.*, 2004).

T. stans callus tissues were examined for the manufacture of these monoterpene alkaloids, as well as for the presence of lapachol and other primary and secondary plant metabolites such sugars, triterpenoids, p-sitosterol, and phenolics (Dohnal, 1976 and Dohnal, 1977). 2-(3,4-dihydroxyphenyl) ethyl-2-O- [6-deoxyalpha-L-mannopyranosyl- 4- (3, 4-dihydroxyphenyl)-2-propenoate] is a novel phenylethanoid. *T. stans* was found to include beta-D-glucopyranoside, a far-out monoterpene alkaloid called 5-hydroxy-skytanthine hydrochloride, as well as eleven other substances (Marzouk *et al.*, 2006).

The Tecoma genus contains a variety of bioactive chemicals that have been shown to have a range of pharmacological effects, including antioxidant, antibacterial, antifungal, antitumor, antioxidant, antimicrobial, hypoglycaemic, free radical scavenging, anti-inflammatory and antidiabetic properties (Verma, 2016). *T. stans* extracts in chloroform, butanol, and ethyl acetate exhibited strong inhibitory effects against *E. coli*, *P. aeruginosa*, and *S. aureus*. While the ethyl acetate extract of *T. stans* exhibits outstanding effectiveness against *Aspergillus niger*, its n-hexane extract totally inhibited *Fusarium solani* (Javid *et al.*, 2015).

According to the findings of a study by Oyewole *et al.*, leaf extract has been proven to have insecticidal, antifeedant, and repellent properties and concluded that the presence of various chemical components in the plant may be the cause of the insecticidal activity.

The larvicidal and delayed effects of *Tecoma stans* (Bignoniaceae) leaf extract against the medically significant mosquito species *Culex pipiens* L. were examined (Diptera: Culicidae) and summarised that the ethanolic extract of *T. stans* might be a possibility for application as a long-lasting botanical insecticide for mosquito control (Hafsi *et al.*, 2022).

Conclusion

In conclusion, our research shown that *Tecoma stans* leaf extracts in ethanol can be converted into an environmentally beneficial mosquitocidal agent. Additionally, our findings provide a basic framework for additional research into the effectiveness of natural product extracts' antibacterial, antioxidant, and mosquitocidal capabilities.

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