



Oviposition deterrent efficacies of *Terminalia catappa* Linn. (Combretaceae) methanol and acetone leaf extracts against *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Mosquitoes cause major health hazards to human beings as they are the carrier of various diseases. The most important thing to reduce the incidence of vector borne diseases is controlling the mosquito population, Vector control is facing a that due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either adopting counter measures or development if newer insecticides. Plants acts as alternative agents for control of vectors because they are rich in bioactive chemicals. Therefore in the current study we investigate the ovipositional deterrence activity of *Terminalia catappa* methanol and acetone leaf extracts on *Aedes aegypti*. The effect of methanol and acetone leaf extracts of *T. catappa* on oviposition of *Ae. aegypti* was determined under two sets of conditions (choice and no choice oviposition assay) as suggested by Chen *et al.*, (1996) [9]. Among the total number of eggs laid, the control medium possessed high percentage of eggs viz. 39.90% (methanol) and 33.89% (acetone) when placed along with ovitraps with 0.1, 0.50, 1.0, 1.50 and 2.0% in which appeared 28.15, 20.19, 8.045, 3.666, 0.040% (methanol) and 29.69, 23.53, 9.715, 3.131 and 0.032% eggs (acetone) respectively (choice oviposition test). Percent oviposition in 0.1, 0.50, 1.0, 1.50 and 2.0% methanol and acetone leaf extracts of *T. catappa* was 31.50, 18.20, 10.72, 3.187 and 0.094 % (methanol) and 33.64, 22.62, 13.82, 6.367 and 0.123% (acetone) which were less compared to their control 68.49, 81.79, 89.27, 96.81 and 99.90 % (methanol) and 66.35, 77.37, 86.17, 93.63 and 99.87 % (acetone) respectively (no choice oviposition test). Result of the present study revealed that the phytochemicals which is present in methanol and acetone leaf extracts of *T. catappa* may be utilized as a oviposition deterrent.

Keywords: *Terminalia catappa*, methanol and acetone leaf extracts, GC-MS, *Aedes aegypti*, oviposition deterrent assay

Introduction

Mosquitoes are the most imperious group of insects, adversely influencing the health status of human beings. More than 3500 species of mosquito are distributed worldwide of which thousands feeds on the blood of various animals and about 20% are the potential vector of diseases in human and other animals (Harbach, 2021) [16]. Mosquitoes are transmitting many dreadful vector borne diseases (VBDs) to public as a results causing millions of mortality has been occurring every year. *Aedes aegypti* exhibits ecological plasticity. *Ae. aegypti* breeds in different household water containers (Albaba *et al.*, 2015) [4]. *Ae. aegypti* is vector for transmitting the dengue, dengue haemorrhagic fever, chikungunya, yellow fever and zika fever (WHO, 2009) [33]. According to the NVBDCP number of dengue, chikungunya cases from 2000 to 2022 in India was 10,43,951 and 1633 deaths and in Tamil Nadu was 57,583 and 100 deaths; in India was 13,084,190 and no deaths and in Tamil Nadu was 64,877 and no deaths respectively.

Enormous effort have been put to control these disease. Synthetic chemical pesticides (SCPs) are the most common effective methods of control strategy against HVMs (Human Vector Mosquitoes). Recently, unadvisable application of SCPs are increased and cause negative health issues on aquatic, terrestrial organisms and also likelihood of mosquito resistance to the pesticide (Abinaya *et al.*, 2018) [1]. So an alternative strategy needs to overcome these problem. Natural products of plant origin are safe to use than synthetic insecticide to control mosquitoes. Phytochemicals such as alkaloids, flavonoids, tannins and phenols etc., have been reported to show mosquitocidal effects. Methanolic extract of *Senna alata* (Karthiyayni *et al.*, 2018) [20]; methanolic extract of *Malvastrum coromandelianum*, *Mimusops elengi* (Irudayaraj and Mary Fabiola, 2022) [18]; methanolic extract of *Lantana camara* (Ajaegbu *et al.*, 2022) [3]; aqueous extract of *Lantana camara*, *Bougainvillea spectabilis*, *Tephrosia villosa* (Hubert *et al.*, 2022) [17]; hexane extract of *Ocimum basilicum* (Chan *et al.*, 2022) [8].

No reports is available on the ovipositional deterrent effects of the *Terminalia catappa* methanol and acetone leaf extracts against *Ae. aegypti*. So, the above facts prompted us to undertaken investigation.

Terminalia catappa Linn. (Indian almond), commonly knows as Laal Badam (RWD almond), belonging to Combretaceae Family and native to Asia, Australia and Madagascar. The tree grows to 35 meters tall with an upright and horizontal branches. The leaves are large, broad, ovoid, glossy dark green and leathery. They are

dry-season deciduous, before falling they turn pinkish – red or yellow – brown (Orwa *et al.*, 2009) [26]. The various extracts of leaves and bark of *T.catappa* have been reported to be antimicrobial (Aimola *et al.*, 2014) [2]; hepato-protective (Lin *et al.*, 1997) [23]; hypoglycemic (Berry *et al.*, 2007) [6]; analgesic and modulatory activity (Aimola *et al.*, 2014) [2]; radical scavenging activity (Ko *et al.*, 2002) [21].

The aim of the present study:

- Qualitative phytochemical analysis of *T.catappa* methanol and acetone leaf extracts.
- Gas Chromatography and Mass Spectrometry analysis of methanol and acetone leaf extracts of *T. catappa*.
- Ovipositional deterrent activity of *Ae.aegypti* to methanol and acetone leaf extracts of *T. catappa*.

Materials and Methods

The eggs of *Ae. aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore (DT), Tamil Nadu, India. They were hatched, reared and have been still maintained for many generations in the laboratory. The larvae were reared in plastic cups and provided with commercial fish food *ad libitum* (Lymio *et al.*, 1992) [25]. The pupae were collected from culture trays and were transferred to glass beakers. The pupae containing glass beaker were kept inside mosquito cage for adult emergence. The adult female *Ae. aegypti* were fed by human arm Judson (1967) [19] and Briegel (1990) [7]. Both females and males were provided with 10% glucose solution on cotton wicks Villani *et al.*, (1983) [32]. A plastic cups (200 ml) (egg traps) lined with filter paper containing water was kept in the cage.

Collection and preparation of plant extracts

T.catappa leaves were collected from Anaikatti, Coimbatore, Tamil Nadu, Southern India. The identification of the plant was authenticated at BSI, Coimbatore. The leaves washed with distilled water and then they kept for drying under shade at room temperature ($27 \pm 2^\circ\text{C}$) for about 2 weeks till they dried completely. The dried leaves were finely powdered using electric grinder. Powdered plant materials (100g) was soaked in methanol, acetone (1000 ml) in airtight wide mouth bottle and kept separately for 4 days with periodic shaking. After that, the extract was filtered using Whatman No.1 filter paper and kept in Petri dishes for drying at room temperature (Kongkathip, 1994) [22]. Dried extract were then used for the preparation of stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae.

Qualitative phytochemical analysis of methanol and acetone leaf extracts of *T. catappa*

Qualitative phytochemical analyses of the plant extracts was carried out using the standard protocol of Harbone (1984) [15] and Trease and Evans (1989) [30].

Gas Chromatography- Mass Spectrometry (GC-MS) analysis of methanol and acetone leaf extracts of *T. catappa*

The GC-MS analysis was conducted at SITRA, Coimbatore, Tamil Nadu.

Oviposition assay

Choice oviposition test

15 pairs of adult *Ae. aegypti* were kept in a cage (30 cm x 30 cm x 30 cm) and maintained under room condition of ($27.2 \pm 2.0^\circ\text{C}$). The adult (males) were provided with 10% glucose solution. Females were blood fed by human arm every alternate day. Five egg traps (200 ml plastic cups) containing any one of the concentrations of the test compound (0.1, 0.50, 1.0, 1.50 and 2.0%) and control were placed inside the cage. After 24 hours, the egg traps were taken out and the eggs present in each were separately counted. A support for oviposition was provided by placing a piece of filter paper (Whatman No.1) on the inner surface of each egg traps so that the lower half of it was submerged in the test solution and control in order for the whole paper to get moistened while the upper half of it was above the solution where the mosquitoes would lay their eggs on. The test was replicated 3 times. The untreated and treated egg traps (plastic cups) were placed at alternate diagonally opposite locations for each replicate so as to nullify any effect of their locations on oviposition (Chen *et al.*, (1996) [9] and Dhanakkodi *et al.*, (1999) [13].

No-choice oviposition test

15 pairs of adult *Ae. aegypti* were kept in a cage (30 cm x 30 cm x 30 cm) and maintained under room condition of ($27.2 \pm 2.0^\circ\text{C}$). The adult (males) were provided with 10% glucose solution. Females were blood fed by human arm every alternate day. Egg trap (200 ml plastic cups) containing any one of the test concentrations of any of the test compound (0.1, 0.50, 1.0, 1.50 and 2.0%) was placed at a time in the cage along with a control trap. After 24 hours, the egg traps were removed and the number of eggs were counted. A support for oviposition was provided by placing a piece of filter paper (Whatman No.1) on the inner surface of each egg traps so that the lower half of it was submerged in the test solution and control in order for the whole paper to get moistened while the upper half of it was above the solution where the mosquitoes would lay their eggs on. The test was replicated 3 times. The untreated and treated egg traps (plastic cups) were placed at alternate diagonally opposite locations for each replicate so as to nullify any effect of their locations on oviposition (Chen *et al.*, (1996) [8] and Dhanakkodi *et al.*, (1999) [13].

Oviposition Active Index (OAI) was calculated as detailed by Vasuki (1991) [31] using the formula,

$$OAI = \frac{Nt - NS}{Nt + NS}$$

Where

Nt- is the total number of eggs in test solutions

NS- is the total number of eggs in control

This would indicate whether the effect of the test compound on oviposition is positive or negative. OAI was in the range of -1 and +1.

Further, the percentage of oviposition deterrence (oviposition deterrent index of Lundgren, 1975) was determined according to the formula given by Dimetry *et al.*, (1995) ^[14].

$$\text{Oviposition Deterrent Index (ODI)} = \frac{B-A}{A+B} \times 100$$

Where

A – is the number of eggs laid on treated

B – is the number of eggs laid on control.

The data were statistically examined using Student's *t*- test.

Results

Phytochemical analysis of methanol and acetone leaf extracts of *T.catappa*

Carbohydrates, tanins, quinones, cardiacglycosides, terpenoids, triterpenoids, coumarins, steroids (methanol) and carbohydrates, tanins, alkaloids, quinones, terpenoids, triterpenoids, phenols, steroids (acetone) (Table-1).

Gas Chromatography- Mass spectrometry (GC-MS) analysis methanol and acetone leaf extracts of *T.catappa*

3-Methoxy-4-methylaniline, Aziridine,1-propyl, Succinic acid, 2,4,6-trichlorophenyl 2-naphthylmethyl ester, 2-Methylthioacetic acid, Phosphorous P4, Myristic acid,4-methoxyphenyl ester (methanol) and 3-Octanamine, Isoxazole, Acetone, 1-Butanol 1,2- amino-, 1,3-Dioxol-2-one, (acetone) (Table 2 & 3).

Effect of methanol and acetone leaf extracts of *T. catappa* on the oviposition of *Ae. aegypti*

Choice oviposition test

The total number of eggs laid in ovitraps containing any concentration of the methanol and acetone leaf extracts of *T. catappa* was always less than that in the control. Among the total number of eggs laid, the control medium possessed high percentage of eggs *viz.* 39.90% (methanol) and 33.89% (acetone) when placed along with ovitraps with 0.1, 0.50, 1.0, 1.50 and 2.0% in which appeared 28.15, 20.19, 8.045, 3.666 and 0.040% (methanol); 29.69, 23.53, 9.715, 3.131 and 0.032% eggs (acetone) respectively (Table - 4).

Rate of oviposition in ovitraps with any concentration of test compound was significantly ($P < 0.001$) less than in control.

No - choice oviposition test

Percent oviposition in 0.1, 0.50, 1.0, 1.50 and 2.0% methanol and acetone leaf extracts of *T. catappa* was 31.50, 18.20, 10.72, 3.187 and 0.094 % (methanol); 33.64, 22.62, 13.82, 6.367 and 0.123% (acetone) which were significantly ($P < 0.001$) less compared to their control counterparts 68.49, 81.79, 89.27, 96.81 and 99.90 % (methanol); 66.35, 77.37, 86.17, 93.63 and 99.87 % (acetone) respectively (Table - 5).

The data of choice and no - choice oviposition test clearly exhibited interference of methanol and acetone leaf extracts of *T. catappa* on the oviposition preference of mosquitoes. Every OAI value recorded for each concentration of methanol and acetone leaf extracts exhibit negative values which indicates strong deterrent of mosquitoes towards test extracts.

Discussion

The result of the present study is in agreement with the earlier findings on the ovipositional deterrent effect of different plants origin. Acetone leaf extract of *Prosopis juliflora* found to be strong oviposition-deterrent of *Ae. albopictus* at 100 ppm (Yadav *et al.*, 2014) ^[34]; the concentrations of the acetone extract of *Laggera aurita* ranging between 0.03% and 0.5% showed less egg laying by female mosquitoes *An. stephensi* in treated bioassay than untreated control indicating oviposition deterrent activity (Singh and Mittal, 2015) ^[29]; the ovipositional determinant nature of the test solutions was studied at 0.1% by choice based oviposition of gravid vector mosquitoes. In hexane, ethyl acetate and methanol *Cascia occidentalis* leaf extracts the effective deterrence was 97.0%, 92.5% and 93.7% in *An.stephensi*; 87.8%, 90.4% and 73.0% in *Cx. quinquefasciatus*; 94.0%, 96.2% and 99.0% in *Ae. aegypti* respectively (Raja *et al.*, 2016) ^[27]; the methanol leaf extract of *Pelargonium graveolens*, *Commiphora berryi* and *Ceiba pentandra* showed 100%, 87.4% and 58.8% oviposition deterrent activity at 350 ppm concentration against *Ae. aegypti* (Baranitharan *et al.*, 2016) ^[5]; the methanol leaf extract of *Golchidion neilgherrense* and *Cinnamomum wightii* showed ovipositional deterrence and effective repellence against *Cx quinquefasciatus* at different concentrations (Deepalakshmi and Jeyabalan, 2017) ^[12]; ethanol extract of *Pometia pinnata* leaves have anti- oviposition effect on female mosquito *Ae.aegypti* at 400 ppm (Luthfi *et al.*, 2020) ^[24].

If oviposition is prevented the mosquito life cycle is disrupted and population growth is reduced. The present oviposition study shows that the methanol and acetone leaf extracts of *T. catappa* act as oviposition deterrent, this indicates that *Ae. aegypti* mosquitoes were acutely sensitive to phytochemical stimuli and respond to the odour of the leaf extracts. The strong odour of the leaf extracts produce maximum effective repellence against oviposition. As female mosquitoes approach an oviposition site, they use a site-specific olfactory cue as a short-range signal for determining its quality. Volatile chemical emanated from an oviposition site is sensed and evaluated by the olfactory receptors located on the antennae, palps, labrum and tarsi (Choo *et al.*, 2015^[10]; Day, 2016^[11]). According to Rosell *et al.*, (2008)^[28] direct toxicity, oviposition, repellency and attraction all appear to be caused through interactions of phytochemicals with the insect nervous system either through acetylcholinesterase inhibition or octopamine receptor antagonism. Through the route of action in our study was not determined, it is possible that the methanol and acetone leaf extracts have similar impact on *Ae. aegypti*.

Table 1: Qualitative phytochemical analysis of *T. catappa* methanol and acetone leaf extracts

Phytochemical constituents	Methanol	Acetone
Carbohydrates	+	+
Tannins	+	+
Flavonoids	-	-
Alkaloids	-	+
Quinones	+	+
Glycosides	-	-
Cardiac Glycosides	+	-
Terpenoides	+	+
Triterpenoides	+	+
Phenols	-	+
Coumarins	+	-
Steroides	+	+
Phytosteroides	-	-
Phlobatannins	-	-
Anthraquinones	-	-
Saponins	-	-

(+): Present

(-): Absent

Table 2: Important compounds identified in the GC-MS analysis of methanol leaf extract of *T. catappa*

S. No	Retention Time	Compound Name	Chemical Formula	Area (%)
1	4.3903	3-Methoxy-4-methylaniline	C ₈ H ₁₁ NO	289740.1
2	13.5078	Aziridine,1-propyl	C ₅ H ₁₁ N	4439952.2
3	18.0024	Succinic acid, 2,4,6-trichlorophenyl 2-naphthylmethyl ester	C ₂₁ H ₁₅ Cl ₃ O ₄	617257.6
4	19.4860	2-Methylthioacetic acid	C ₃ H ₆ O ₂ S	34884.3
5	19.9561	Phosphorous P4	P ₄	187070.3
6	20.1939	Myristic acid,4-methoxyphenyl ester	C ₂₁ H ₃₄ O ₃	202302.6
7	20.3731	2-Fluoro-5-methylaniline	C ₇ H ₈ FN	117265.7
8	20.7945	N-tert-Butyl methylamine	C ₅ H ₁₃ N	293663.2
9	21.1043	Emodin,3TMS derivative	C ₂₄ H ₃₄ O ₅ Si ₃	3762.6
10	21.1556	5-[Isopropyl amino]-2-nitrobenzonitrile	C ₁₀ H ₁₁ N ₃ O ₂	819229.4

Table 3: Important compounds identified in the GC-MS analysis of acetone leaf extract of *T. catappa*

S. No	Retention time	Compound name	Chemical formula	Area (%)
1	4.1243	3-Octanamine	C ₈ H ₁₉ N	231854560.1
2	4.1298	Isoxazole	C ₃ H ₃ NO	396254.2
3	4.2004	Acetone	C ₃ H ₆ O	1114599.1
4	4.2417	1-Butanol,2-amino-	C ₄ H ₁₁ NO	763060.1
5	5.6422	1,3-Dioxol-2-one	C ₃ H ₂ O ₃	79537.0
6	7.9081	1,5-Diazabicyclo[3.1.0]hexane	C ₄ H ₈ N ₂	264323.8
7	8.0380	5-Ethoxy-3,4-dihydro-2H-pyrrole-2-carboxylic acid, ethyl ester	C ₉ H ₁₅ NO ₃	931624.8
8	9.9448	5-Amino-1-ethylpyrazole	C ₅ H ₉ N ₃	317856.5
9	9.9544	Isoxazole	C ₃ H ₃ NO	1223696.1
10	10.0741	Cyclopropylamine, N-isobutylidene-	C ₇ H ₁₃ N	2374063.0

Table 4: Changes in the oviposition deterrence of different concentrations of the methanol and acetone leaf extracts of *T.catappa* against *Ae.aegypti* under choice oviposition test

Parameters	Control	Concentrations (%)				
		0.1	0.50	1.0	1.50	2.0
Total number of eggs laid [#]	992(m)	700(m)	502(m)	200(m)	91(m)	1(m)
	1050(a)	920(a)	729(a)	301(a)	97(a)	1(a)
Percent oviposition	39.90(m)	28.15*(m)	20.19*(m)	8.045*(m)	3.666*(m)	0.040*(m)
	33.89(a)	29.69*(a)	23.53*(a)	9.715*(a)	3.131*(a)	0.032*(a)
Percent reduction in oviposition over control		70.56(m)	50.60(m)	20.16(m)	9.173(m)	0.100(m)
		87.61(a)	69.42(a)	28.66(a)	9.238(a)	0.095(a)
Reduction in number of eggs compared to control		292(m)	490(m)	792(m)	901(m)	991(m)
		130(a)	321(a)	749(a)	953(a)	1049(a)
Oviposition Active Index (OAI)		-0.172(m)	-0.328(m)	-0.664(m)	-0.831(m)	-0.997(m)
		-0.065(a)	-0.180(a)	-0.554(a)	-0.830(a)	-0.998(a)
Oviposition Deterrent Index (ODI)		17.25(m)	32.84(m)	66.44(m)	83.19(m)	99.79(m)
		6.598(a)	18.04(a)	55.44(a)	83.08(a)	99.80(a)

[#], Total number of eggs laid in 3 replicates.

^{*}, Significantly different from control (P<0.001).

(m): methanol leaf extract.

(a): acetone leaf extract.

Table 5: Changes in the indices of oviposition deterrence of different concentration of the methanol and acetone leaf extracts of *T.catappa* against *Ae.aegypti* under no-choice oviposition test

Parameters	Concentrations (%)									
	C	0.1	C	0.50	C	1.0	C	1.50	C	2.0
Total number of eggs laid [*]	450(m)	184(m)	593(m)	132(m)	841(m)	101(m)	972(m)	32(m)	1052(m)	1(m)
	1004(a)	509(a)	1122(a)	329(a)	1321(a)	212(a)	1500(a)	102(a)	1622(a)	2(a)
Percent oviposition	68.49(m)	31.50#(m)	81.79(m)	18.20#(m)	89.27(m)	10.72#(m)	96.81(m)	3.187#(m)	99.90(m)	0.094#(m)
	66.35(a)	33.64#(a)	77.37(a)	22.62#(a)	86.17(a)	13.82#(a)	93.63(a)	6.367#(a)	99.87(a)	0.123#(a)
Percent reduction in oviposition over control		46.00(m)		22.25(m)		12.00(m)		3.292(m)		0.095(m)
		50.69(a)		29.24(a)		16.04(a)		6.800(a)		0.123(a)
Reduction in number of eggs compared to control		216(m)		461(m)		740(m)		940(m)		1051(m)
		495(a)		796(a)		1109(a)		1398(a)		1620(a)
Oviposition Active Index(OAI)		-0.369(m)		-0.635(m)		-0.785(m)		-0.936(m)		-0.998(m)
		-0.327(a)		-0.547(a)		-0.723(a)		-0.872(a)		-0.997(a)
Oviposition Deterrent Index(ODI)		36.98(m)		63.58(m)		78.55(m)		93.62(m)		99.81(m)
		32.71(a)		54.74(a)		72.34(a)		87.26(a)		99.75(a)

^{*}, Total number of eggs in laid in 3 replicates.

[#], significantly different from control (P<0.001).

(m): methanol leaf extract.

(a): acetone leaf extract.

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