

Eco-friendly control of disease-transmit mosquito vectors using the mosquito fish *Gambusia affinis* and low dosages of *Mukia maderaspatana* extracts

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Abstract

In many countries, mosquitoes pose a serious threat to public health, and managing mosquito populations is one of the most difficult aspects of mosquito control programmes. There is a need for decision biological active molecules to control mosquito in order to prevent dengue virus transmission. In the present investigation, ethanol and methanol using mosquitocidal compounds isolated against disease-transmitting mosquitoes. The highest preliminary larvicidal effective was found in the methanol leaf extract of *Mukia maderaspatana* (MLE-*M. maderaspatana*), followed by *Trigonella foenum*, *Phyllanthus niruri*, *Senna auriculata*, *Justicia adhatoda*, *Andrographis paniculata*, *Hybanthus enneaspermus*, *Cardiospermum corundum* and *Azadirachta indica*. The MLE and ELE-*M. maderaspatana* had the highest mortality rates at 48 hours, with LC₅₀ values of 4.46 ppm and 60.55 ppm against *An. stephensi*, respectively. The phytochemical studies by quantitative and qualitative methods were MLE and ELE-*M. maderaspatana*, showed presence of saponins, glycosides, alkaloids, flavanoids, terpenoids, phenolic compounds, cardiac glycosides, coumorins, and steroid. The functional groups where FT-IR analysis was found included secondary profiling, which is obviously a phytochemical and may function as a geranylgeraniol molecule. It is evident from the Nuclear magnetic resonance (NMR) spectrum that MLE-*M. maderaspatana* was the source of the geranylgeraniol compound. MLE-*M. maderaspatana* exhibited a more potent antioxidant activity in DPPH, ABTS⁺, H₂O₂ tests than ascorbic acid. The primary component of the 26 MCCs found in the MLE-*M. maderaspatana* was geranylgeraniol. The results so show that MLE-*M. maderaspatana*, a component from a medicinal plant, may be a more effective mosquito control agent than readily available insecticides.

Introduction

Global warming and pollution are two of the most serious issues facing the globe today. The main reason why diseases carried by mosquitoes are spreading is because of these issues (Hussain et al. 2023). These problems are the main cause of spreading of mosquito borne disease e.g., dengue, chikungunya, malaria, filariasis, yellow fever, dirofilariasis tularemia, and many other diseases (Brugman et al. 2018; Habeeb and Al-Solami 2021). Malaria, transmitted by mosquito, is the most well-known and lethal of the vector-borne diseases, but there are others. Dengue and yellow fever, for example, are known for erupting in massive outbreaks that can paralyse health systems and create significant economic and social upheaval. Lymphatic filariasis can be eliminated by stopping the spread of infection through prevention chemotherapy with safe medicine combination repeated annually. Controlling the vector population is the primary method of control available because there is currently no vaccine or drug available to treat diseases spread by this vector (Pliego-Pliego et al. 2020; Inana et al. 2021). These compounds are intensively explored in search of new bioactive metabolites (Baranitharan et al. 2021), including against the mosquitoes. So, hereby new inhibitor development is required with practical value and isolation of new compound with both potent and highly selected isolation is required. Despite decades of intensive research, the effective and sustainable management of mosquito vector populations is still a hard challenge to deal with (Pavela et al., 2019; Dhanasekaran et al. 2022a).

A climber that grows in the tropics of both India and Sri Lanka, *Mukia maderaspatana* is a medicinal and therapeutic plant that contains a variety of phytochemicals, including flavonoids, saponins, terpenoids, alkaloids, carbohydrates, phenolic compounds, tannins, steroids, and cardiac glycerides. The leaves, root, and fruits are used in Siddha and Ayurvedic medicine for their anti-inflammatory, carminative, stomachic, anti-hyperlipidemic, diuretic, expectorant, antiulcer, antipyretic, anti-hyperglycemic, antirheumatic, antibacterial, hepatoprotective, and antioxidant qualities (Sujeethasai 2020). The aim of this present study is to research the valuable phyto-chemical compound and understand the mechanisms of bioactive compounds that bind the active site of the mosquito juvenile hormone protein (MJHP).

Materials and methods

Plant material and extraction

A total of 18 medicinally important leaves such as *Trigonell foenum* (vendhayam), *Phyllanthus niruri* (Keelanelli), *Senna auriculata* (Avarampoo), *Mukia maderaspatana* (Musumusukkai), *Justicia adhatoda* (Adathoda), *Andrographis paniculata* (Nilavembu), *Hybanthus enneaspermus* (Orithal thamarai), *Cardiospermum corundum* (Mudakathan), *Azadirachta indica* (Veppilai), *Ocimum sanctum* (Tulsi), *Aloe vera* (Katrai), *Solanum nigrum* (Manathakkali), *Syzygium jambolanum* (Naval), *Eclipta allopa* (Karisalankanni), *Phyllanthus emblica* (Nelli) and *Andrographis paniculata* (Siriya nankai) was carried out during the growing season of 2023 from varies location of Yercaud (11°77'48" N and 78°20'97" E), Salem District, Tamil Nadu, India (Table 1 and Fig. 1). All undesired dirt was removed by repeatedly washing newly formed leaves. After washing, the shade dried and was put in a 50°C hot air oven for half an hour before being ground in an electric blender. Methanol was utilized as a solvent for the next 72 hours of the extraction process after a Soxhlet device containing 500 g of ground plant material was loaded (Fig. 2). A rotary evaporator (Heidolph, Germany) was used to evaporate the solvent under vacuum, and the dry extract was kept at 4 4 °C until a subsequent bioassay (Baranitharan et al. 2016a).

Table 1
Medicinal plants of LME

S.No.	Medicinal plants	EQ (g)	YP (%)
1	<i>Trigonell foenum</i>	1.1140	11.14
2	<i>Phyllanthus niruri</i>	0.6396	6.40
3	<i>Senna auriculata</i>	2.0844	20.84
4	<i>Mukia maderaspatana</i>	0.4745	4.74
5	<i>Justicia adhatoda</i>	0.7126	7.13
6	<i>Andrographis paniculata</i>	0.7577	7.58
7	<i>Hybanthus enneaspermus</i>	0.4872	4.87
8	<i>Cardiospermum corundum</i>	0.8817	8.82
9	<i>Azadirachta indica</i>	0.9781	9.78
EQ- Extract Quantity; YP- Yield Percentage			

Preliminary larvicidal effect

Using the method at 24 and 48 hours, 18 collected medicinal herbs were subjected to a preliminary larvicidal effect. Following WHO procedure (WHO 2005), the larvicidal effect was tested at room temperature (25 ± 3°C). The experiment was conducted o 25 larvae in their instar after combining the MLE-*M. maderaspatana* (249 ml) with triple-distilled water.

Larvicidal effect

Larval test was conducted as previously described by Dhanasekaran et al. (2022) to determine the LC₅₀ and LC₉₀ values at 24 h and 48 h exposure under entomology laboratory climatic conditions by using protocol of World Health Organization (WHO, 2005). A standard stock solution of MLE and ELE-*M. maderaspatana* was prepared in distilled water at 1000 ppm. Subsequently, 5 duplicates of the produced plant extract (1.0 ml) were mixed along with 249 ml of water. The duplicates were exposed for 24 and 48 hours, whereas the control group did not receive any extract. Next, using Equation to calculate the mortality rate from the mean of the triplicates, the number of live larvae was reported (Finney 1971). The extracts with greater activity were taken into consideration for more research:

$$\% \text{ of mortality} = \frac{\text{Number of larvae died}}{\text{Total number of larvae exposed}} \times 100$$

Phytochemical screening of medicinal plants

In order to identify the secondary metabolites contained in the MLE and ELE-*M. maderaspatana*, a phytochemical screening qualitative analysis was carried out using the techniques outlined by Sathish Kumar et al., (2013). The following are examples of phytochemicals: phenolic compounds, terpenoids, flavonoids, tannins, alkaloids, cardiac glycosides, saponins, coumarins and steroid.

FTIR spectra analysis MLE-M. maderaspatana

The distinctive functional groups in the LE were found using an FTIR spectrum (Shimadzu, IR prestige-21). A minimum of 50 mg of Les was dispensed after the potassium bromide (KBr) was dried. To create a thin disc of Br, the mixture was well combined in a mortar under 6 bar of press pressure for two minutes. The disc was then put inside a diffuse reflectance accessory sample cup. To improve the signal to noise ratio, the sample was then scanned 16 times for transmittance between 4000 and 400 cm^{-1} (the mid-IR region). Each peak in the infrared spectrum was printed along with a label indicating its matching wavelength (Baranitharan et al. 2019).

Gas Chromatography–Mass Spectrometry analysis

Utilizing a mass detector Turbo mass gold-Perkin Elmer exact identifier and an Elite-5MS (5% Diphenyl/ 95% Dimethyl poly siloxane), 30×0.25mm×0.25 μm df) thin section, gas chromatography-mass spectroscopy (GC-MS) was performed. The delta and interface temperatures were 200 and 280 °C, and the stove was programmed to rise from 50 to 280 °C at a rate of 5 °C min^{-1} . The temperature was held at this level for 36 minutes. The heat stream rate of the transporter gas employed was 1.0 ml min^{-1} (consistent stream). A 10:1 split was used while injecting the sample (2 μl). at 70eV, electron mass spectrometry (ESMS) was applied. The fourfold temperature and particle source were maintained at 250 and 200 °C, respectively (Baranitharan et al. 2017).

Nuclear Magnetic Resonance (NMR) spectral analysis

Using the India Institute of Technology's database, located at Chennai-600036, Tamil Nadu, the structure of the chemical was identified using NMR^C and NMR^H spectra data. Then, they were obtained using a spectrometer running at 300.1299740 MHz. a Varian Unity Plus (International Equipment Trading Ltd., Mundelein, Illinois-60,060, USA). The BRUKER Avance-300 spectrometer (7.05 T) with DRX 300 MHz was used to record the NMR^C and NMR^H for *M. maderaspatana*-MLE. The solvent used was CDCl_3 . Version 3.0 of the TOPSPIN NMR system software was being used by the spectrometer. Parts per million (PPM) of chemical shift (δ) were reported in relation to TMS as the internal standard (s-singlet; d-doublet; t-triplet; m-multiplet)

Predation efficiency

The effectiveness of *G. affinis* predation on III instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* was evaluated in an environment treated with ELE-*M. maderaspatana* and MLE. Fish of the species *G. affinis* was obtained from the Fisheries Department of Nagapattinam, Tamil Nadu, India, and kept in cement tanks (125 cm in diameter and 65 cm in depth) with field-collected water at $27 \pm 3^\circ\text{C}$ and R.H. 90%. 200 larvae were exposed to one *G. affinis* in a 2.5 L glass beaker arena containing 75 ppm of *M. maderaspatana* and dechlorinated water for each mosquito species that was examined at the III instar stage (Jebanesan et al. 2020). For the III instar, five replications were carried out, along with one control (dechlorinated water containing mosquito larvae without tadpoles). For five days, all beakers were examined every 24 hours, and the quantity of missing prey was noted. After every daily examination, missing mosquito larvae were replaced with fresh ones. The predatory efficiency was calculated using the formula below:

$$\text{Predation efficiency} = (\text{number of missing mosquitoes} / \text{total number of mosquitoes}) \times 100$$

Invitro free radical scavenging and antioxidant assays

DPPH free radical scavenging activity

According to Mamta et al. (2015), the scavenging activity of DPPH (1,1-Diphenyl-2-Picryl-hydrazyl) free radicals was used to determine the antioxidant activity of *M. maderaspatana* when it was extracted in various organic solvent extracts, with ascorbic acid as reference material. DPPH solution containing 0.004% MeOH was mixed with extracts (50, 150, 250, and 500 µg/ml) for this antioxidant. The scavenging activity % inhibition was computed using the formula below:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

ABTS⁺ radical cation decolourisation assay

Using ABTS (2,2 azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) as free radicals, the free radical scavenging activity of *M. maderaspatana* extracts was also calculated (Re et al. 1999). Gallic acid, a common antioxidant molecule, was used to compare the ABTS radical scavenging ability of *M. maderaspatana* extract. The plant extracts 50, 150, 250, and 500 µg/ml free radical scavenging potentials were calculated using the following formula:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

Scavenging of hydrogen peroxide (H₂O₂)

The method of Ruch et al. (1989) was used to determine *M. maderaspatana* capacity to scavenge H₂O₂. Using spectrophotometry and absorption at 230 nm in a spectrophotometer, the concentration of H₂O₂ in a solution with a pH of 7.4 was found. Extracts (50, 150, 250, and 500 µg/ml) were combined with distilled water to create an H₂O₂ solution. The H₂O₂ absorbance at 230 nm was measured after 10 minutes and compared to a blank solution that had phosphate buffer but no H₂O₂. *M. maderaspatana* percentage of H₂O₂ scavenging was calculated using the following formula; each test was conducted three times.

$$\text{Scavenged H}_2\text{O}_2 \text{ Percentage} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The means and standard deviations of each set of data were reported. Probit analysis was used to determine the LC₅₀ values based on average larval mortality data. By using a probit regression model, their statistics were estimated at the 95% upper confidence limit (UCL) and lower confidence limit (LCL) values. The statistical software tool, SPSS version 16.0, was used to calculate all of the analyses. Statistical significance was defined as $p < 0.05$ for results.

Results and discussion

Preliminary larvicidal activity

Table 2 display the findings of the preliminary larvicidal activity of the extract of several medicinal herbs. Among the 18 plant extracts, *Trigonell foenum*, *Phyllanthus niruri*, *Senna auriculata*, *Mukia maderaspatana*, *Justicia adhatoda*, *Andrographis paniculata*, *Hybanthus enneaspermus*, *Cardiospermum corundum*, *Azadirachta indica*, *Ocimum sanctum*, *Aloe vera*, *Solanum nigrum*, *Syzygium jambolanum*, *Eclipta allopa*, *Phyllanthus emblica* and *Andrographis paniculata* shows positive effect after 24h and 48h exposure. The MLE-M. *maderaspatana* had the highest death rate, followed by MLE-T. *foenum*. Based on the mortality of preliminary activity *Mukia maderaspatana*, *Trigonell foenum*, *Phyllanthus niruri*, *Senna auriculata*, *Justicia adhatoda*, *Andrographis paniculata*, *Hybanthus enneaspermus*, *Cardiospermum corundum* and *Azadirachta indica* were for larvicidal activity.

Table 2
Preliminary larvicidal bioassay of MLE

Sl.No	Sample Name	24 hours	48 hours
1	<i>Phyllanthus niruri</i>	+	+
2	<i>Trigonella foenum graecum</i>	+	+
3	<i>Senna auriculata</i>	+	+
4	<i>Mukia maderaspatana</i>	+	+
5	<i>Justicia adhatoda</i>	+	+
6	<i>Andrographis paniculata</i>	+	+
7	<i>Hybanthus Enneaspermus</i>	+	+
8	<i>Cardiospermum corundum</i>	+	+
9	<i>Azadirachta indica</i>	+	+
10	<i>Ocimum sanctum</i>	-	-
11	<i>Aloe vera</i>	-	-
12	<i>Solanum nigrum</i>	-	-
13	<i>Syzygium jambolanum</i>	-	-
14	<i>Eclipta allosa</i>	-	-
15	<i>Phyllanthus emblica</i>	-	-
16	<i>Andrographis paniculata</i>	-	-
17	<i>Hibiscus rosa sinensis</i>	-	-
18	<i>Citrus limon</i>	-	-

The results, larvicidal activity of MLE and ELE-*M. maderaspatana* tested against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* for the duration of 24 h and 48 h were shown in Table 3. The highest mortality 100% and 97% was observed MLE and ELE-*M. maderaspatana* against *An. stephensi* at 24h and 48h. Followed by, the mortality 97% and 95% (*Ae. aegypti*), 95% and 94% (*Cx. quinquefasciatus*). Table 4 shows the results of testing MLE and ELE-*M. maderaspatana* larvicidal effectiveness against significant larvae of mosquito vector. MLE-*M. maderaspatana* was highly effective ($LC_{50}/LC_{90} = 4.46 \text{ ppm}/9.25 \text{ ppm}$) against the larvae of *An. stephensi*. Followed by, LC_{50}/LC_{90} values were *Ae. aegypti* (11.92 ppm/14.60 ppm) and *Cx. quinquefasciatus* (47.86 ppm/93.48 ppm) (Fig. 3). The highest mortality ($LC_{50} \text{ ppm}/LC_{90} \text{ ppm}$ values) was 60.55 ppm/99.51 ppm from the ELE-*M. maderaspatana* against *An. stephensi*. Followed by, LC_{50}/LC_{90} values were *Ae. aegypti* (141.34 ppm/166.57 ppm) and *Cx. quinquefasciatus* (181.5 ppm/206.05 ppm) (Fig. 4). A previous work examined the larvicidal efficacy of twelve compounds extracted from the leaf essential oil of two species of *Eucalyptus* against *Ae. aegypti* and *Ae. albopictus* (Cheng et al., 2009). According to Kovendan et al., (2012), an ethanol extract of entire sections of *Leucas aspera* tested against *An. stephensi* larvae revealed an LC_{50} of 9.695, respectively. When tested against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*, *Sesamum indicum* methanol extract had the highest larvicidal effect (LC_{50}) values of 349.88, 338.27, and 254.85 mg/L (Baranitharan et al. 2015). The larvicidal activity of extracts from *Plumbago zeylanica* and *Cestrum nocturnum* was investigated against *Ae. aegypti* (Patil et al. 2011). When evaluated against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*, the LC_{50} value of the ethyl acetate extract of *Commiphora caudata* was 97.19, 96.04, and 94.76 mg/L (Baranitharan and Dhanasekaran 2014). To test *Pedaliium murex* methanol extract against *Cx. quinquefasciatus* and *Ae. aegypti*, the maximum larvicidal effect (111.66 and 127.08 mg/L) was observed (Gokulakrishnan et al. 2016). *Ficus racemosa* methanol extract has a lethal impact (LC_{50}) of 64.76 ppm against *Ae. aegypti*, according to Baranitharan et al. (2016). Linalool

from the essential oil of *Lavender augustifolia* had the largest larval impact, its LC₅₀ values against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* were 36.26, 36.81, and 37.49 ppm (Baranitharan et al. 2021).

Table 3
Mortality percentage of MLE and ELE-*Mukia maderaspatana* against mosquito larvae

Extracts	Target species	Duration	NEX	Mean ± SD	Mortality (%)
MLE	<i>C. quinquefasciatus</i>	24 hours	25	23.5 ± 0.2	94%
	<i>A. stephensi</i>		25	24.3 ± 0.4	97%
	<i>A. aegypti</i>		25	23.7 ± 0.2	95%
	<i>C. quinquefasciatus</i>	48 hours	25	23.8 ± 0.3	95%
	<i>A. stephensi</i>		25	25.0 ± 0.0	100%
	<i>A. aegypti</i>		25	24.2 ± 0.4	97%
ELE	<i>C. quinquefasciatus</i>	24 hours	25	14.8 ± 4.2	59%
	<i>A. stephensi</i>		25	23.2 ± 2.3	93%
	<i>A. aegypti</i>		25	25.0 ± 0.0	100%
	<i>C. quinquefasciatus</i>	48 hours	25	20.0 ± 0.6	80%
	<i>A. stephensi</i>		25	24.2 ± 1.6	97%
	<i>A. aegypti</i>		25	25.0 ± 0.0	100%
Signification at p > 0.05 level, SD- Standard Deviation, NEX- Number of larvae exposed, Mortality (%) - percentage of mortality.					

Table 3
Hydrogen peroxide Activity of Solanumincanum crude extracts

Solvents	Concentrations (µg/ml)				IC ₅₀ value (mg/ml)
	50	150	250	500	
Hexane	17.11 ± 0.09	23.35 ± 0.06	27.50 ± 0.02	37.35 ± 0.03	584.511
Chloroform	19.89 ± 0.06	25.76 ± 0.02	29.45 ± 0.02	41.15 ± 0.02	597.87
Ethyl acetate	32.70 ± 0.05	37.75 ± 0.05	43.45 ± 0.03	55.96 ± 0.02	367.647
Methanol	38.46 ± 0.03	44.60 ± 0.07	54.46 ± 0.01	65.64 ± 0.01	133.641
EDTA	41.42 ± 0.75	67.30 ± 0.03	75.45 ± 0.50	84.50 ± 0.50	82.715

Table 4
LC₅₀/LC₉₀ values of MLE and ELE-*Mukia maderaspatana* against mosquito larvae

Extract	Target species	Intercept	Slope	LC ₅₀ (ppm)	95% confidence limit (ppm)		LC ₉₀ (ppm)	95% confidence limit (ppm)		χ^2 (df = 6)
					LCL	UCL		LCL	UCL	
MLE	<i>A. stephensi</i>	1.75	2.38 ± 0.06	4.46	3.93	5.05	9.25	7.09	12.06	11.1 (6)
	<i>C. quinquefasciatus</i>	1.91	2.39 ± 0.06	47.86	42.53	53.85	93.48	72.54	120.48	16.1 (6)
	<i>A. aegypti</i>	6.35	10.75 ± 0.02	11.92	11.49	12.37	14.60	13.62	15.60	1.3 (6)
ELE	<i>A. stephensi</i>	3.43	9.51 ± 0.03	60.55	64.06	73.35	99.51	87.22	113.53	2.7 (6)
	<i>C. quinquefasciatus</i>	10.10	47.57 ± 0.01	181.5	177.24	185.94	206.05	196.8	215.67	2.4 (6)
	<i>A. aegypti</i>	7.79	33.58 ± 0.02	141.34	137.02	145.8	166.57	157.02	176.69	2.5 (6)
LC ₅₀ = Lethal Concentration brings out 50% mortality and LC ₉₀ = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Slope; Chi-square.										

Phytochemical screening of MLE and ELE- *M. maderaspatana*

Phytochemical screening of MLE and ELE-*M. maderaspatana* revealed a wide number of bioactive compounds present (Table 5). Bioactive substances found in LE include coumorins, glycosides, cardiac glycosides, terpenoids, alkaloids, saponins, tannins, and flavonoids. Thin layer chromatography in a methanol solvent solution was used to investigate phytochemical screening. Alkaloids, terpenoids, phenolic compounds and glycosides are among the phytochemical group members that contain it in large quantities. The phytochemical group's presence (flavonoids, saponins, tannins and steroid) and the phytochemical group's trace amounts (cardiac glycosides and coumorins) come next. The phytochemical group flavonoid was abundantly, alkaloids and saponins is presence in ethanol extract, while tannins, glycosides, and steroids were present in trace amounts. The results of this work are comparable to those of an MPC screening of *Coleus aromaticus* leaf fractions, which identified a variety of bioactive chemicals including tannins, terpenoids, tri-terpenoids, steroids, saponins, phenol, proteins, alkaloids and glycosides (Baranitharan et al. 2017). According to Yadav and Agarwala (2011), phytochemicals like carbohydrates, tannins, saponins, proteins, phenols, and flavonoids were identified from various solvent extracts of *Bryophyllum pinnaum*, *Ricinus communis*, *Ipomea aquatic*, *Tinospora cordifolia*, *Terminalia bellerica*, *Xanthium strumarium* and *Oldenlandia corymbosa*. According to Anindita and Bikramjit (2017), primary phytochemical screening of *Rouvolfia serpentina* and *Moringa olifera* revealed the presence of metabolites such as glycosides, alkaloids, phenolic compounds, tannins, steroids, flavonoids, saponins. Aqueous and methanol extracts of some therapeutic plants revealed the presence of steroids, alkaloids, tannins, glycosides, phenols, flavonoids, cards, and terpinoids (Padmapriya et al. 2020). *Bischofia javanica* and *Curcuma domestica* showed presence of compounds such as terpenoids, saponins, alkaloids and flavonoids (Aththorick and Berutu 2018).

Table 5
Phytochemical screening of *Mukia maderaspatana* leaf extract

S.No.	Phytoconstituents	Reagent used	CLE	BCL	PELC	ELE (50%)	WLE
1	Alkaloids	Wagner's test	+	+++	+++	++	++
		Mayer's test	++	-	+++	+++	-
		Picric acid test	++	++	+++	+++	++
2	Flavonoids test	Alkaline reagent	++	+	-	+++	+++
		Lead acetate test	++	+++	-	++	-
		Ammonia test	++	+	-	+++	+++
3	CHO test	Benedicts reagent	-	-	-	+++	-
		Fehling's reagent	+++	+++	++	+++	-
		Conc. H ₂ SO ₄ test	+++	++	-	+++	-
4	Proteins & amino acids	Xanthoproteic test	++	+	-	+++	+++
		Biuret test	++	-	-	+++	-
5	Glycosides test	Modified Bomtrager's test	+	+	+	+++	++
		Keller killiani test	+++	+++	+++	-	-
6	Steroids & terpenoids test	Salkowski's test	++	-	+++	+++	-
		Liebermann Burchard test	+++	-	+	-	-
7	Inorganic compound test	Sulphate test	+++	+++	+++	-	-
		Carbonate test	-	-	-	-	+++
8	Saponins	Froth test	++	+++	+++	-	-
9	Anthrax Quinones	Bomtrager's test	-	-	-	-	-
10	Tannins & Phenol	FeCl ₃ test	-	-	-	-	-
		Galatin test	+++	+++	+++	+	+
11	Resins	Acetone test	+++	+	+++	+	+
12	Gum & Mucilage	Ppt by Alcohol	-	+++	-	-	-
13	Fixed oils & fats	Spot test	++	-	++	-	+
14	Lipids	Dichromate	-	+++	+++	+++	-
15	Starch	Lugol's iodine	+	+	-	-	-
+++: Abundance of the phytochemical group; ++ : presence of the phytochemical group; + : trace of the phytochemical group; - : absence of the phytochemical group. CLE: Chloroform Leaf Extract; BLE: Benzene Leaf Extract; PELE: Petroleum ether Leaf Extract; ELE (50%): 50% Ethanol Leaf Extract; WLE: Water Leaf Extract							

FTIR spectra analysis of MLE- *M. maderaspatana*

FTIR spectra analysis was identified the functional groups of the MLE-*M. maderaspatana*; FT-IR spectra clearly exhibited absorption in the different range 2920.69 cm⁻¹ to 649.60 cm⁻¹ (Fig. 5). The peak values corresponded to functional groups like alkanes (C-H stretching 2920.69 cm⁻¹ to 2851.35 cm⁻¹) medium bonding, alkynes (-C≡C- stretching 2251.24 cm⁻¹) weak bonding, aromatics (C-C stretching (in ring) 1464.73 cm⁻¹) medium bonding, carbonyl (general) (C=O stretching 1379.01 cm⁻¹)

¹) very weak bonding, 1*, 2* amines (N-H wag 907.64 cm⁻¹) strong and broad bonding, and alkyl halides (-C ≡ C-H: C-H bend 649.60 cm⁻¹) medium bonding. The functional groups such as alkanes, alkynes, aromatics, carbonyl (general), 1*, 2* amines and alkyl halides confirmed their presence in MLE-*M. maderaspatana*. By using FTIR analysis, similar findings were made for *Phyla nodiflora*-MLE, which contained functional groups such as amides, alkanes, 1* amines, aliphatic amines and alkyl halides (Irrusappan et al. 2022). FTIR spectroscopy analysis was found OH stretching and C = O vibration functional groups from *Punica granatum*-MLE (Jebanesan et al. 2020). *Jussiaea repens*-ELE, the major component, included the functional groups that were discovered by FTIR analysis, such as 4-piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]- α -methyl-, methyl ester (Krishnappa et al. 2020). Further, *Citrus limetta*-MLE contained phytochemical of Corynan-17-01, 18, 19-didehydro-10-methoxy-, acetate (ester) (Baranitharan et al. 2020).

Major chemical compound analysis

The twenty-one compounds of MLE-*M. maderaspatana* were found in their mass spectra, representing 100% of the sample. Table 6 and Fig. 6 display the chemical formula and concentration as percentages (%). The MCC in MLE-*M. maderaspatana* are Geranylgeraniol (C₂₀H₃₄O and 16.04%) (Fig. 7), Isodecyl diphenyl phosphate (C₂₂H₃₁O₄P and 10.98%), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (C₁₇H₂₄O and 10.03%), 1-Monoacetin (C₅H₁₀O₄ and 8.76%), Diphenylamine (C₁₂H₁₁N and 5.11%), Citronellol epoxide (C₁₀H₂₀O₂ and 5.00%), Isopropyl Myristate (C₁₇H₃₄O₂ and 4.84%), 9-Cedranone (C₁₅H₂₄O and 4.75%), Oleyl alcohol (C₁₈H₃₆O and 3.59%), n-Butyl myristate (C₁₈H₃₆O₂ and 3.66%), benzoyl peroxide (C₁₄H₁₀O₄ and 3.42%), 2,5-Pyrrolidinedione, 1-butyl- (C₈H₁₃NO and 3.27%), 4-tert-Butyl-2,6-diisopropylphenol (C₁₆H₂₆O and 3.27%), Limonen-6-ol, pivalate (C₁₅H₂₄O₂ and 2.73%) and 5,5-Dibutylnonane (C₁₇H₃₆ and 2.03%). The present study's results are comparable to the LE's GC-MS analysis, which revealed the existence of twenty compounds primarily composed of palmitic acid, neophytadiene, and limonene dioxide (Mamudha and Sunilson, 2021). Continuously, the mass spectral analysis also confirmed these compounds and molecules consist of carbon 8 atoms, hydrogen 15 atoms and oxygen 4 atoms were indicated in different colors (Krishnappa et al. 2020). Medicinal plant, *Erythrina variagata* LME was found 12-octadecenoic acid, methyl ester and it had predominant toxicity against HVMs (Baranitharan et al. 2019). *Petalonema alatum* LME was found different major phytochemicals (MPCs) like 5-thio-D-glucose, 5-allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole, E)-10-heptadecen-8-ynoic acid methyl ester, and Z-11-hexadecenoic acid (Saravanakumar et al. 2016). Twenty compounds, or 100% of the compounds, had their chemical constituents identified in the methanol extract following GC-MS analysis of the LME-*Loranthus pentandrus*. These investigations were conducted to determine the principal phytochemicals (Krishnappa et al. 2019). *Citrus limetta*-MPCs was showed six compounds, the main Corynan-17-01, 18, 19-didehydro-10-methoxy-, acetate (ester) (Baranitharan et al. 2020). In the *Ageratina adenophora*-MLE secondary metabolic study, twenty-one chemicals were found, with 5-isopropyl-2-methylphenol being the predominant one (Dhanasekaran et al. 2022b).

Table 6
Components identified in the MLE-*Mukia maderaspatana* using GC-MS

MF	Name of Compound	RT (min)*	PA	PA (%)	MW (g/mol)	Height	Height (%)	MI
C ₅ H ₁₀ O ₄	1-Monoacetin	5.492	679233	8.76	134.13	69079	4.04	RI-MS
C ₉ H ₁₄ O	trans,cis-2,6-Nonadienal acetate	7.800	90339	1.16	138.21	27715	1.62	RI-MS
C ₁₅ H ₂₄ O	9-Cedranone	8.094	368347	4.75	220.35	77678	4.55	RI-MS
C ₉ H ₁₀ O ₃	Ethyl 3-hydroxybenzoate	8.925	144043	1.86	166.17	35032	2.05	RI-MS
C ₄ H ₄ O ₄	Fumaric acid	8.992	104537	1.35	116.07	44792	2.62	RI-MS
C ₁₂ H ₂₂ O ₂	Vinyl decanoate	9.414	60151	0.78	198.30	13499	0.79	RI-MS
C ₁₂ H ₁₁ N	Diphenylamine	10.685	396688	5.11	169.22	77731	4.55	RI-MS
C ₈ H ₁₃ NO	2,5-Pyrrolidinedione, 1-butyl-	10.859	253549	3.27	99.09	31147	1.82	RI-MS
C ₁₄ H ₁₀ O ₄	benzoyl peroxide	11.207	265578	3.42	242.23	60392	3.53	RI-MS
C ₁₇ H ₃₆	5,5-Dibutylnonane	13.586	157468	2.03	240.5	46894	2.74	RI-MS
C ₁₆ H ₂₆ O	4-tert-Butyl-2,6-diisopropylphenol	13.586	253746	3.27	234.38	54830	3.21	RI-MS
C ₃ H ₄ O ₄	Malonic acid,	14.436	134554	1.73	104.06	33332	1.95	RI-MS
C ₁₇ H ₃₄ O ₂	Isopropyl Myristate	14.841	375430	4.84	270.45	118132	6.91	RI-MS
C ₈ H ₈ O ₂	Phenylacetic acid	15.417	27440	0.35	136.15	10647	0.62	RI-MS
C ₁₇ H ₂₄ O	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	17.273	777931	10.03	276.37	197927	11.58	RI-MS
C ₁₅ H ₂₄ O ₂	Limonen-6-ol, pivalate	19.879	211486	2.73	236.35	56425	3.30	RI-MS
C ₁₉ H ₃₈ O ₂	Isopropyl Palmitate	19.996	138718	1.79	298.5	37811	2.21	RI-MS
C ₁₈ H ₃₈ N ₂ O	Stearic acid hydrazide \$\$ Stearic hydrazide \$\$	22.736	115686	1.49	298.5	28717	1.68	RI-MS
C ₁₈ H ₂₆ O	2-Ethylhexyl trans-4-methoxycinnamate	23.810	135157	1.74	290.39	29767	1.74	RI-MS
C ₁₈ H ₃₆ O ₂	n-Butyl myristate	24.351	284249	3.66	284.5	64298	3.76	RI-MS

MF = Molecular formula, *RT = Retention time (min), PA = Peak area, MW = molecular weight, MI = Mode of identification

MF	Name of Compound	RT (min)*	PA	PA (%)	MW (g/mol)	Height	Height (%)	MI
C ₆ H ₁₀ O ₂	Caprolactone oxime	27.258	16884	0.22	114.14	8693	0.51	RI-MS
C ₁₀ H ₂₀ O ₂	Citronellol epoxide	28.999	387834	5.00	172.26	71828	4.20	RI-MS
C ₂₂ H ₃₁ O ₄ P	Isodecyl diphenyl phosphate	30.782	851567	10.98	390.5	201014	11.77	RI-MS
C ₁₈ H ₃₆ O	Oleyl alcohol	32.552	278499	3.59	268.47	52912	3.10	RI-MS
C ₁₇ H ₂₃ N ₅ O	3,5-Ethanol Quinolin-10-ol	41.908	2738	0.04	313.4	4227	0.25	RI-MS
C ₂₀ H ₃₄ O	Geranylgeraniol	43.620	1244158	16.04	290.48	254014	14.87	RI-MS
			7756010	100.00		1708533	100.00	RI-MS
MF = Molecular formula, *RT = Retention time (min), PA = Peak area, MW = molecular weight, MI = Mode of identification								

Nuclear Magnetic Resonance (NMR) analysis

The MLE-*M. maderaspatana* underwent NMR spectral analysis, and the spectral peaks that emerged are displayed (Figs. 8 & 9). The observed peaks were examined, their potential structures were postulated and verified using the available information, and they were contrasted with the NIST chemical library. The band that was observed at 907.64 cm⁻¹ is a result of the N-H wag stretching vibration, which is a strong and wide bonding. The spectrum unequivocally shows that MLE-*M. maderaspatana* was used to extract the geranylgeraniol molecule. Subsequently, components were detected in the isodecyl diphenyl phosphate compound, exhibiting almost 80% similarity. In a similar vein, *Blumea mollis*-LAE was subjected to ¹H and ¹³C NMR spectral analysis, the results of which clearly revealed the molecule Atalantin (Elumalai et al. 2020). The signals found in the phenol, 2-methyl-5-(1-methylethyl) molecule from *Punica granatum* 1H NMR (400 MHz, CDCl₃) spectrum (Jabanesan et al. 2020).

Predation efficiency of *G. affinis*

Following a 24-hour period of treatment with modest dosages of MLE-*M. maderaspatana*, the predation efficiency of *G. affinis* was observed against III instar larvae of *An. stephensi* (93.12%), *Cx. quinquefasciatus* (82.82%), *Ae. aegypti* (87.90%). Followed by, ELE-*M. maderaspatana* tested against *An. stephensi* (90.34%), *Cx. quinquefasciatus* (78.66%) and *Ae. aegypti* (85.66%) (Table 7). For the eight days following treatment (i.e., post treatment observation period), no discernible toxicity effects were seen in one *G. affinis* exposed to the contaminated aquatic environment caused by MLE and ELE-*M. maderaspatana*. Subramaniam et al. (2015) found that in environments contaminated with *Mimusops elengi* extract, *G. affinis* had a predation efficiency of 86.2% against III instar larvae of *An. stephensi* and 81.7% against *Ae. albopictus*. Chobu et al. (2015) found that *G. affinis* outcompetes *Carassius auratus*, a goldfish from the Cyprinidae family, as a predator of *An. gambiae* III instar larvae. A well-known biocontrol agent that is particularly effective in preventing mosquito larvae from developing is the mosquito fish (Griffin and Knight 2012).

Table 7
Predation efficiency of the *G. affinis* fish against III instar larvae of mosquito larvae

Extracts	Target species	Predated larvae (n)					Predation (%)	Predation efficacy per day
		Day 1	Day 2	Day 3	Day 4	Day 5		
MLE	<i>A. stephensi</i>	183.4 ± 3.71 ^{cd}	188.6 ± 4.21 ^d	185.6 ± 4.03 ^d	186.4 ± 3.57 ^d	187.2 ± 3.63 ^d	93.12	186.24
	<i>C. quinquefasciatus</i>	160.4 ± 2.70 ^{ab}	166.2 ± 3.27 ^b	167.4 ± 4.15 ^b	164.6 ± 4.44 ^b	169.6 ± 4.61 ^{bc}	82.82	165.64
	<i>A. aegypti</i>	171.8 ± 3.78 ^{bc}	174.6 ± 4.27 ^c	178.4 ± 3.71 ^{cd}	175.4 ± 2.50 ^c	178.8 ± 4.32 ^{cd}	87.90	175.80
ELE	<i>A. stephensi</i>	179.6 ± 4.44 ^{cd}	176.8 ± 3.83 ^c	180.4 ± 3.64 ^{cd}	182.2 ± 4.49 ^{cd}	184.4 ± 4.15 ^d	90.34	180.68
	<i>C. quinquefasciatus</i>	152.4 ± 4.27 ^a	157.8 ± 2.94 ^{ab}	155.4 ± 3.84 ^a	159.8 ± 4.08 ^{ab}	161.2 ± 3.49 ^{ab}	78.66	157.32
	<i>A. aegypti</i>	165.4 ± 3.04 ^b	169.4 ± 3.36 ^{bc}	171.4 ± 3.71 ^{bc}	173.6 ± 4.66 ^{bc}	176.8 ± 4.86 ^c	85.66	171.32

Predation rate are mean ± SD of five replications (i.e. 1 *G. affinis* adult vs 200 mosquito larvae pre replication). No mortality in control (i.e. clean water without *G. affinis*). Within each column, means followed by the same letter are not significantly different ($P < 0.05$)

Antioxidant assay

The hexane extract exhibited the highest amount of DPPH radical scavenging activity, with an EC₅₀ value of 411.485 mg/ml, higher than that of the crude extracts from *M. maderaspatana* (154.028 mg/ml), ethyl acetate (104.21 mg/ml), and methanol (64.721 mg/ml). Conversely, the ascorbic acid extract (20.221 mg/ml) showed the lowest value of activity. Hexane, chloroform, ethyl acetate, methanol, and ascorbic acid were the solvents whose scavenging action was reduced by the ascorbic acid, according to the results (Table 8 and Fig. 10). The extracts with the highest IC₅₀ values in the ABTS⁺ radical scavenging assay were hexane (261.872mg/ml), followed by chloroform (198.495mg/ml), ethyl acetate (138.833mg/ml), methanol (63.541mg/ml), and ascorbic acid (46.935ml/ml), in that order. The findings demonstrate how different solvents scavenging activities reduced ascorbic acid in the following order: hexane, chloroform, ethyl acetate, methanol, and ascorbic acid (Table 9 and Fig. 11).

Table 8
DPPH activity *M. maderaspatana* crude extracts

Solvents	Concentrations (µg/ml)				IC ₅₀ value (mg/ml)
	50	150	250	500	
Hexane	29.54 ± 0.13	39.20 ± 0.14	52.18 ± 0.98	71.20 ± 0.50	411.485
Chloroform	38.64 ± 0.19	48.70 ± 0.50	61.31 ± 0.28	78.68 ± 0.76	154.028
Ethyl acetate	45.35 ± 0.50	52.60 ± 0.33	65.38 ± 0.78	84.12 ± 0.28	104.21
Methanol	49.20 ± 0.20	58.78 ± 0.78	73.88 ± 0.76	94.30 ± 0.50	64.721
Ascorbic acid	54.17 ± 0.12	67.16 ± 0.32	78.70 ± 0.05	98.28 ± 0.78	20.221

Table 9

ABTS⁺ activity of *M. maderaspatana* crude extracts

Solvents	Concentrations (µg/ml)				IC ₅₀ value (mg/ml)
	50	150	250	500	
Hexane	26.06 ± 0.44	37.74 ± 0.48	48.20 ± 0.50	59.21 ± 0.70	261.872
Chloroform	38.50 ± 0.50	43.16 ± 0.27	57.27 ± 0.35	72.61 ± 0.21	198.495
Ethyl acetate	41.01 ± 0.28	48.76 ± 0.68	64.00 ± 0.50	79.65 ± 0.55	138.833
Methanol	46.18 ± 0.01	65.57 ± 0.02	75.02 ± 0.32	89.32 ± 0.93	63.541
Ascorbic acid	49.18 ± 0.12	66.57 ± 0.35	75.02 ± 0.32	95.12 ± 0.29	46.935

Using the same quantities, EDTA (82.715mg/ml) was shown to have a lower EC₅₀ value in the H₂O₂ scavenging assay than chloroform extracts (597.87mg/ml), hexane (584.511mg/ml), ethyl acetate (367.647mg/ml) methanol (133.641mg/ml). The findings show that, in the order of chloroform > hexane > ethyl acetate > methanol > EDTA, the values on the H₂O₂ radical scavenging activity of the various solvents of *M. maderaspatana* decrease relative to that of EDTA (Table 10 and Fig. 12). According to Baruah et al. (2023), the LC₅₀ values for different antioxidant activities varied from 27.94 to 114.15 µg/ml for DPPH, 15.05 to 707.74 µg/ml for ABTS, and 40.23 to 338.91 µg/ml for TBARS. In the DPPH, FRAP, and ABTS tests, *Ocimum basilicum* shown a stronger antioxidant activity than *Ocimum americanum* (Mahendran and Vimolmangkang 2023). They found that conventional antioxidants had greater activity than essential oils (Horvathova et al. 2014; Hazrati et al. 2020). Additionally, an examination of antioxidants revealed that the leaf oil and inflorescence oil had effective concentrations (EC₅₀) of 22.76 µg/ml and 26.18 µg/ml, respectively, which translates to 17.57 µg/ml of ascorbic acid (John et al. 2022).

Conclusion

The findings of the research showed that MLE-*M. maderaspatana*, the main bioactive compound Geranylgeraniol, was well done investigated larvicidal effect against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. As such, for insecticide and antioxidant effects to be successful, we have to improve our understanding of the insecticide susceptibility and behavior to ensure we develop the most future-proof and holistic vector control strategies.

Declarations

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Compliance with ethical standards

We adhered to all relevant national and international regulations. The institution or practice where the studies were conducted has ethical criteria that were followed in all procedures carried out during the study.

Conflicts of interest

The authors declare that there was no conflict of interest.

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Figures



Figure 1

Medicinal plant extracts powder (*Trigonell foenum*, *Phyllanthus niruri*, *Senna auriculata*, *Mukia maderaspatana*, *Justicia adhatoda*, *Andrographis paniculata*, *Hybanthus enneaspermus*, *Cardiospermum corundum* and *Azadirachta indica*).

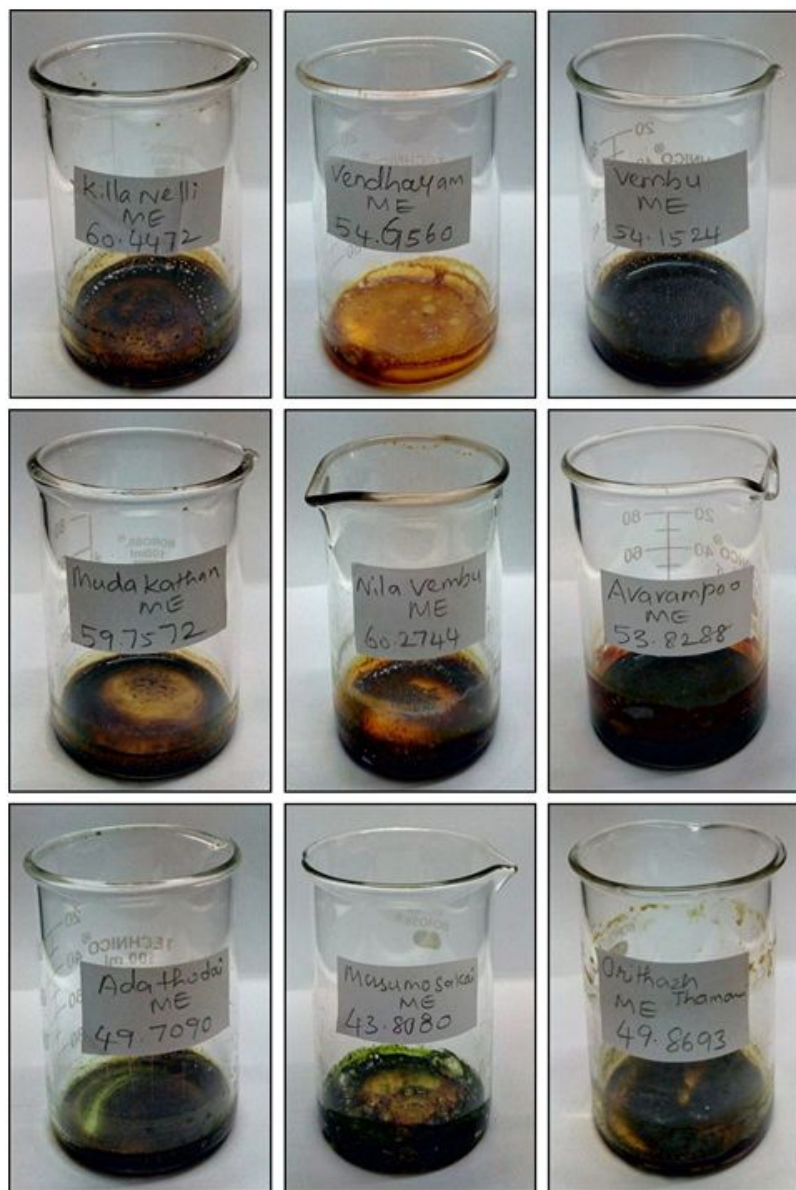


Figure 2

Preliminary screening of medicinal plant MLEs

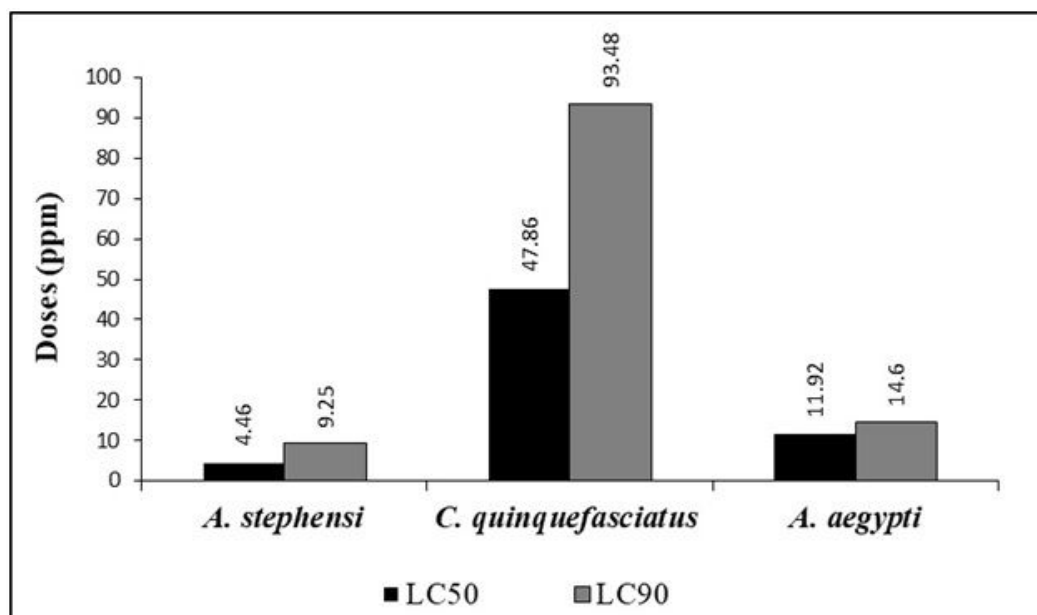


Figure 3

LC₅₀ and LC₉₀ values of MLE-*M. maderaspatana* against disease-transmission mosquitoes

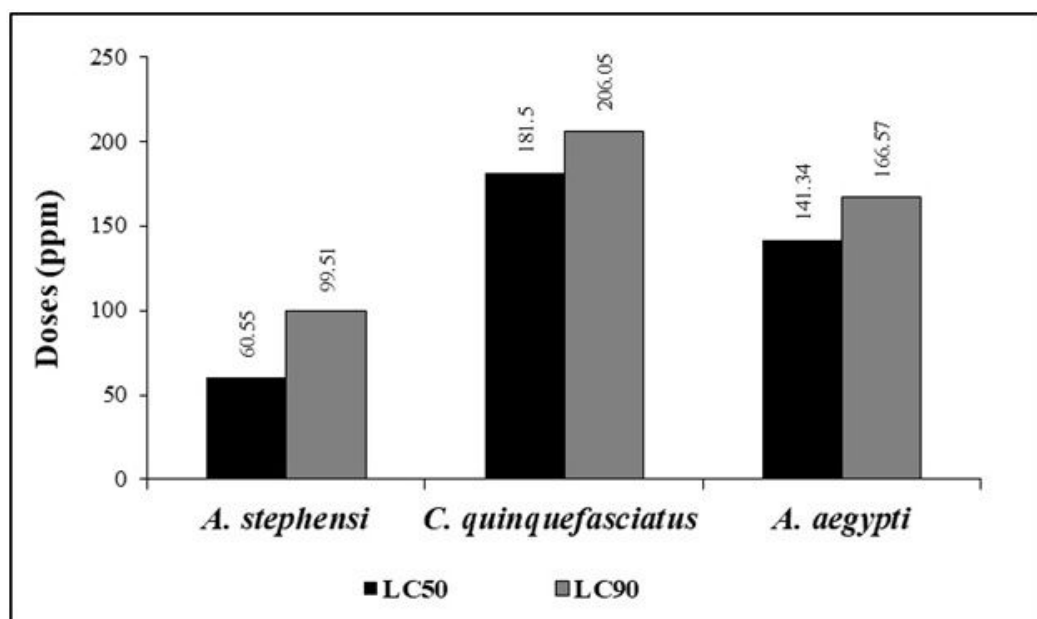
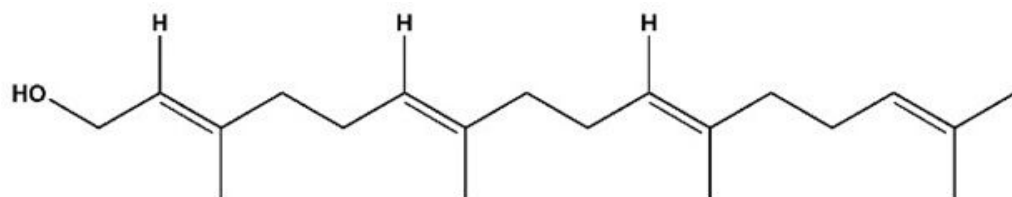


Figure 4

LC₅₀ and LC₉₀ values of ELE-*M. maderaspatana* against disease-transmission mosquitoes



2D structure of Geranylgeraniol



3D structure of Geranylgeraniol

Figure 7

2D & 3D structure of Geranylgeraniol compound from MLE-*M. maderaspatana*

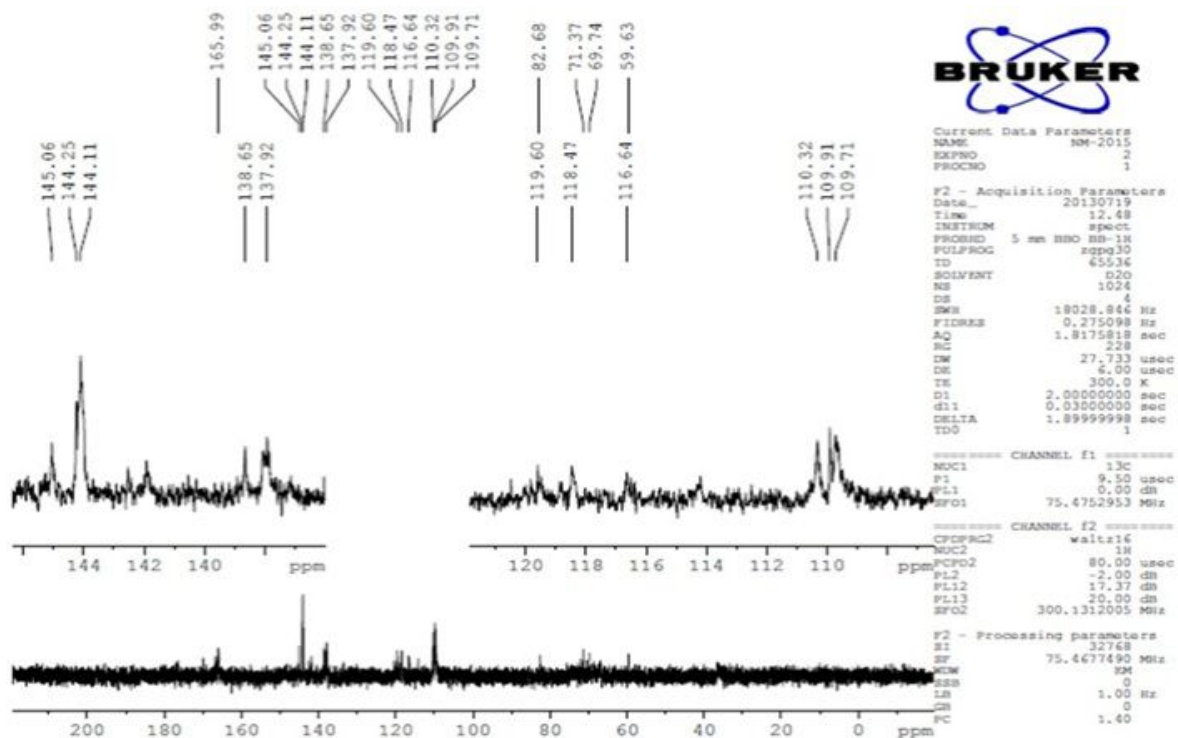


Figure 8

NMR^C spectrum of the methanol extract isolated from *M. maderaspatana*

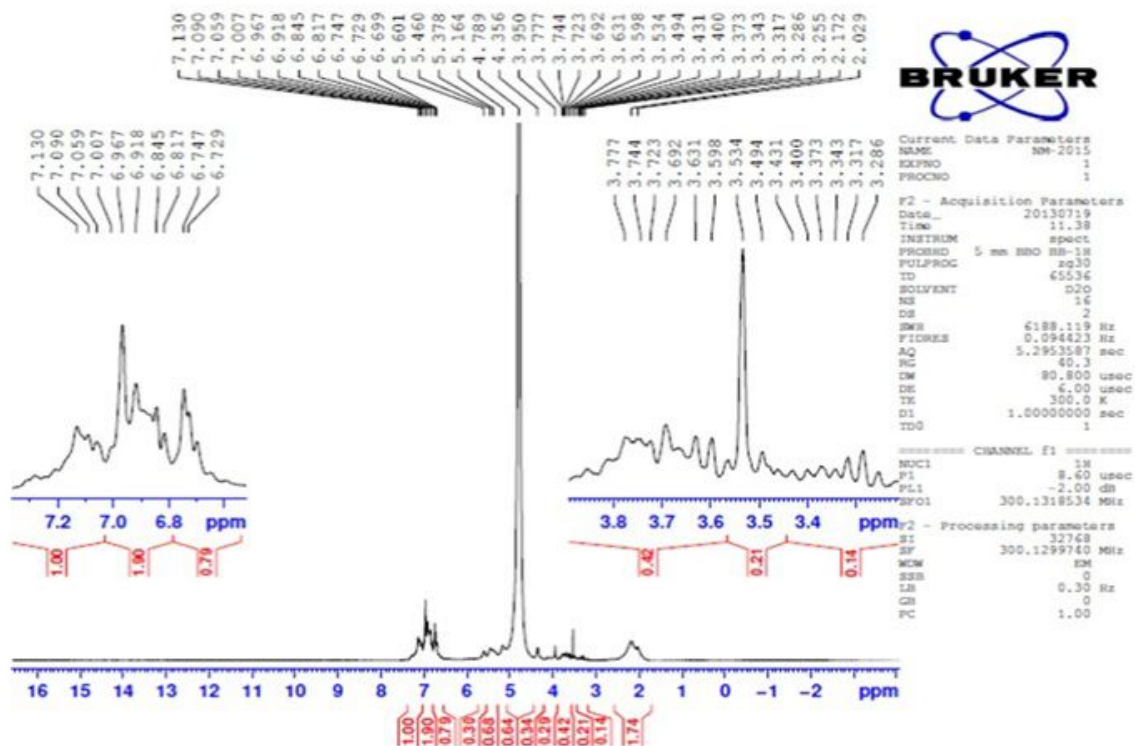


Figure 9

NMR^H spectrum of the methanol extract isolated from *M. maderaspatana*

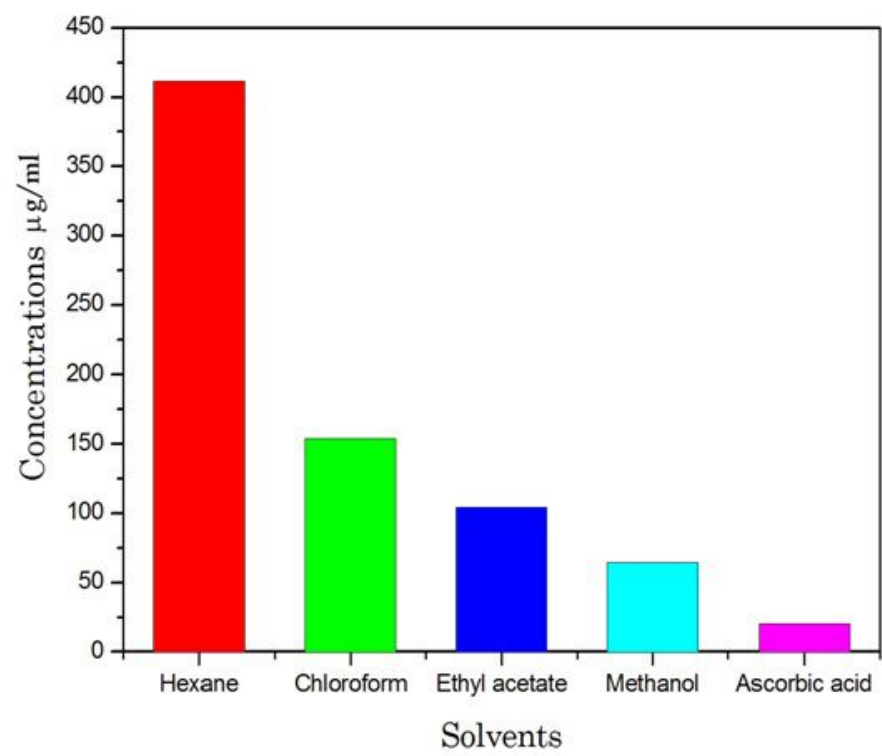


Figure 10

DPPH radical scavenging activities of *M. maderaspatana* crude extract

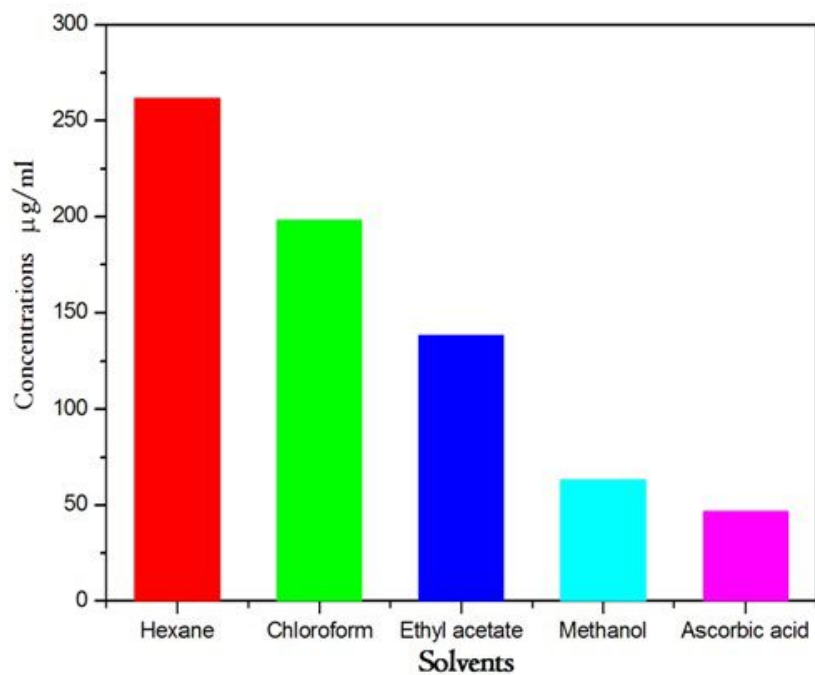


Figure 11

ABTS activity of *M. maderaspatana* crude extract

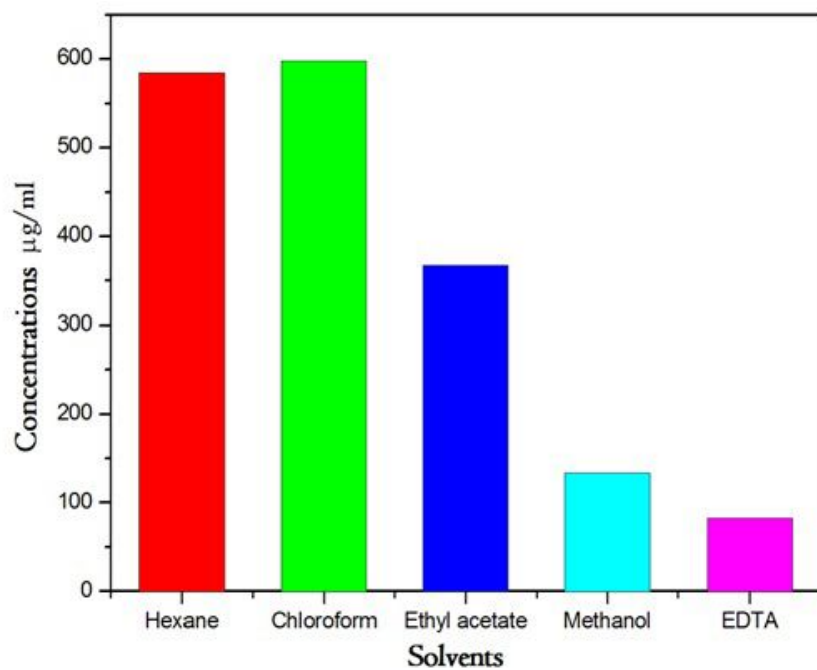


Figure 12

Hydrogen peroxide scavenging activity of *M. maderaspatana* crude extract