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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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KEYWORDS

Major chemical compound, larvicidal effect, mosquito species, *Mukia maderaspatana*

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, flavaonoids, 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.

1. 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 [1-3]. 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 [4,5]. 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

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because there is currently no vaccine or drug available to treat diseases spread by this vector [6,7]. These compounds are intensively explored in search of new bioactive metabolites [8], 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 [9,10].

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 [11]. 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).

2. MATERIALS AND METHODS

2.1. 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), *Phylianthus 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 ºC until a subsequent bioassay [12].

Table 1. Medicinal plants of LME

S.No.	Medicinal plants	EQ (g)	YP (%)
1	*Trigonell foenum*	1.114	11.14
2	*Phyllanthus niruri*	0.6396	6.4
3	*Senna auriculata*	2.0844	20.84
4	*Mukia maderaspatana*	0.4745	4.74
5	*Justicia adhatoda*	0.7126	7.13
6	*Andrographis paniculata*	0.7577	7.58
7	*Hybanthus enneaspermus*	0.4872	4.87
8	*Cardiospermum corundum*	0.8817	8.82
9	*Azadirachta indica*	0.9781	9.78

EQ- Extract Quantity; YP- Yield Percentage

Fig. 1 Medicinal plant extracts powder (*Trigonell foenum, Phyllanthus niruri, Senna auriculata, Mukia maderaspatana, Justicia adhatoda, Andrographis paniculata, Hybanthus enneaspermus, Cardiospermum corundum* and *Azadirachta indica*).

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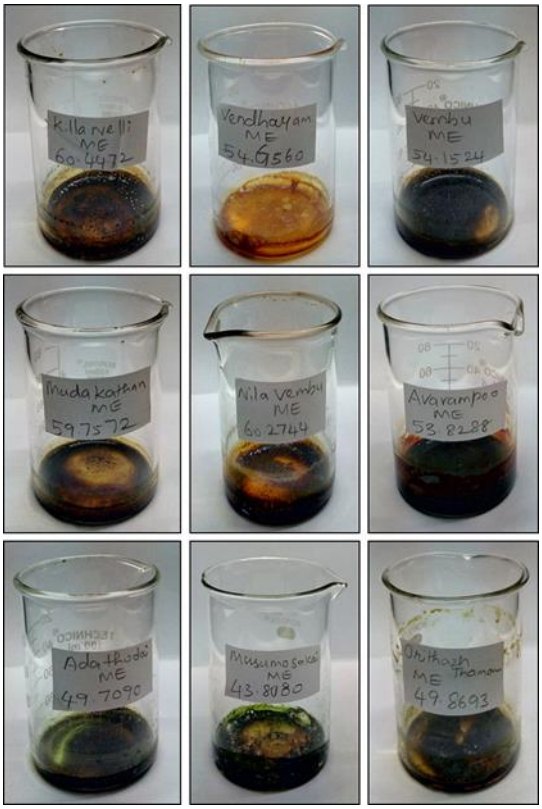

Fig. 2 Preliminary screening of medicinal plant MLEs

2.2. 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±3ºC). 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. [13] to determine the LC_{50} and LC_{90} values at 24 h and 48 h exposure under entomology laboratory climatic conditions by using protocol of World Health Organization [14]. 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 [15]. The extracts with greater activity were taken into consideration for more research:

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

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., [16]. The following are examples of phytochemicals: phenolic compounds, terpenoids, flavonoids, tannins, alkaloids, cardiac glycosides, saponins, coumorins 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 [17].

Gas Chromatography–Mass Spectrometry analysis

Utilizing a mass detector Turbo mass gold-Perkin Elmer exact identifier and an Elite-5MS (5% Diphenyl/ 95% Dimethy poly siloxane), 30×0.25mm×0.25µm df) thin section, gas chromatography-mass spectroscopy (GC-MS) was performed. The delta and interface temperatures were 200 and 280 ºC, and the stove was programmed to rise from 50 to 280 ºC at a rate of 5 ºC 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 (2µl). at 70eV, electron mass spectrometry (ESMS) was applied. The

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fourfold temperature and particle source were maintained at 250 and 200 ºC, respectively [18].

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 NMRC and NMRH spectra data. Then, they were obtained using a spectrometer running at 300.1299740 MHz. a Varian Unity Plus (International Equipment Trading Ltd., Mundelein, lllinois-60,060, USA). The BRUKER Avance-300 spectrometer (7.05 T) with DRX 300 MHz was used to record the NMRC and NMRH for M. maderaspatana-MLE. The solvent used was CDCl3. 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±3 °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 [19]. 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:

Predation efficiency = (number of missing mosquitoes/total number of moquitoes) × 100

DPPH free radical scavenging activity

According to Mamta et al. [20], 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:

DPPH scavenging activity

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

ABTS+ radical cationdecolourisation assay

Using ABTS (2,2 azino-bis(3- ethylbenzothiazoline-6-sulfonic acid) as free redicals, the free redical scavenging activity of M. maderaspatana extracts was also calculated [21]. 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 (H_2O_2)

The method of Ruch et al. [22] 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.

$$\text{Scavenged H2O2 Percentage} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The means and standard deviations of each set of data were reported. Probit analysis was used to determine the LC_{50} 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

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software tool, SPSS version 16.0, was used to calculate all of the analyses. Statistical significance was defined as $p<0.05$ for results.

3. RESULTS AND DISCUSSION

3.1. 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, Phylianthus 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	*Phyllanthus niruri*	+	+
2	*Trigonella foenum graecum*	+	+
3	*Senna auriculata*	+	+
4	*Mukia maderaspatana*	+	+
5	*Justicia adhatoda*	+	+
6	*Andrographis paniculata*	+	+
7	*Hybanthus Enneaspermus*	+	+
8	*Cardiospermum corundum*	+	+
9	*Azadirachta indica*	+	+
10	*Ocimum sanctum*	-	-
11	*Aloe vera*	-	-
12	*Solanum nigrum*	-	-
13	*Syzygium jambolanum*	-	-
14	*Eclipta alloa*	-	-
15	*Phylianthus emblica*	-	-
16	*Andrographis paniculata*	-	-
17	*Hibiscus rosa sinensis*	-	-
18	*Citrus limon*	-	-

The results, larvicidal activity of MLE and ELE-*M. maderaspatana* tested against *Cx. quinquefasciatus, An. stephensis* 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. stephens*i 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 ppm/9.25 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}/LC_{90} 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).

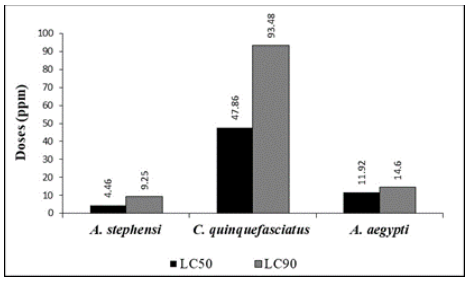

Fig. 3 LC_{50} and LC_{90} values of MLE-*M. maderaspatana* against disease-transmission mosquitoes.

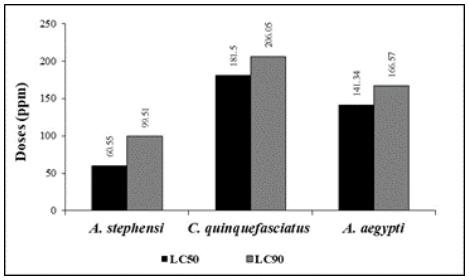

Fig. 4 LC_{50} and LC_{90} values of ELE-*M. maderaspatana* against disease-transmission mosquitoes

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

Extracts	Target species	Duration	NEX	Mean ± SD	Mortality (%)
MLE	*C. quinquefsciatus*	24 hours	25	23.5 ± 0.2	94%
	A. stephensi		25	24.3 ± 0.4	97%
	A. aegypti		25	23.7 ± 0.2	95%
	C. quinquefasciatus	48 hours	25	23.8 ± 0.3	95%
	A. stephensi		25	25.0 ± 0.0	100%
	A. aegypti		25	24.2 ± 0.4	97%
ELE	*C. quinquefsciatus*	24 hours	25	14.8±4.2	59%
	A. stephensi		25	23.2±2.3	93%
	A. aegypti		25	25.0±0.0	100%
	C. quinquefasciatus	48 hours	25	20.0±0.6	80%
	A. stephensi		25	24.2±1.6	97%
	A. aegypti		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 4. LC_{50}/LC_{90} 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'' ($\delta\phi = 6$)
					LCL	UCL		LCL	UCL	
MLE	*A. stephensi*	1.64	2.24 ± 0.08	4.38	3.69	5.28	9.37	7.32	12.46	12.3 (6)
	C. quinquefasciatus	1.87	2.16 ± 0.04	45.76	43.38	52.67	92.51	73.37	119.26	17.2 (6)
	A. aegypti	5.25	9.54 ± 0.04	10.89	9.27	12.73	13.74	12.93	15.85	1.4 (6)
ELE	*A. stephensi*	3.43	9.51 ±0.03	60.55	64.06	73.35	99.51	87.22	113.53	2.7 (6)
	C. quinquefasciatus	10.1	47.57 ±0.01	181.5	177.24	185.94	206.05	196.8	215.67	2.4 (6)
	A. aegypti	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.

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* [23]. According to Kovendan et al., [24], an ethanol extract of entire sections of *Leucas aspera* tested against *An. stephensi* larvae revealed an LC50 of 9.695, respectively. When tested against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*, *Sesamum indicum* methanol extract had the highest larvicidal effect (LC50) values of 349.88, 338.27, and 254.85 mg/L [25]. The larvicidal activity of extracts from Plumbago zeylanica and Cestrum noctumum was investigated against Ae.

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aegypti [26]. 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 [27]. To test Pedalium murex methanol extract against *Cx. quinquefasciatus* and *Ae. aegypti*, the maximum larvicidal effect (111.66 and 127.08 mg/L) was observed [28]. *Ficus racemosa* methanol extract has a lethal impact (LC50) of 64.76 ppm against *Ae. aegypti*, according to Baranitharan et al. [29]. Linalool from the essential oil of *Lavender augustifolia* had the largest larval impact, its LC_{50} values against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* were 36.26, 36.81, and 37.49 ppm [30].

3.2. 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 pytochemical 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 pinnaaum, Ricinus communis, Ipomea aquatic, Tinospora cordifolia, Terminalia bellerica, Xanthium strumarium* and *Oldenlandia corymbosa*. According to Anindita and Bikramjit [31], primary phytochemical screening of Rouvolfia serpentine and *Moringa olifera* revealed the presence of metabolites such as glycosides, alkaloids, phenolic compounds, tannins, steroids, flabinoids, saponins. Aqueous and methanol extracts of some therapeutic plants revealed the presence of steroids, alkaloids, tannins, glycosides, phenols, flavonoids, cards, and terpinoids [32]. *Bischofia javanica* and *Curcuma domestica* showed presence of compounds such as terpenoids, saponins, alkaloids and flavonoids [33].

3.3. 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) 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 amides, alkanes, 1* amines, aliphatic amines and alkyl halides [34]. FTIR spectroscopy analysis was found OH stretching and C=O vibration functional groups from *Punica granatum*-MLE [19]. *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]-á-methyl-, methyl ester [35]. Further, Citrus limetta-MLE contained phytocompound of Corynan-17-01, 18, 19-didehydro-10-methoxy-, acelate (ester) [36].

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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	++	++	+++	+++	++
		Alkaline reagent	++	+	-	+++	+++
2	Flavonoids test	Lead acetate test	++	+++	-	++	-
		Ammonia test	++	+	-	+++	+++
		Benedicts reagent	-	-	-	+++	-
3	CHO test	Fehling's reagent	+++	+++	++	+++	-
		Conc. H_2SO_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	++	-	+++	+++	-
		Libermann 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	+	+	-	-	-

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+++: 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.

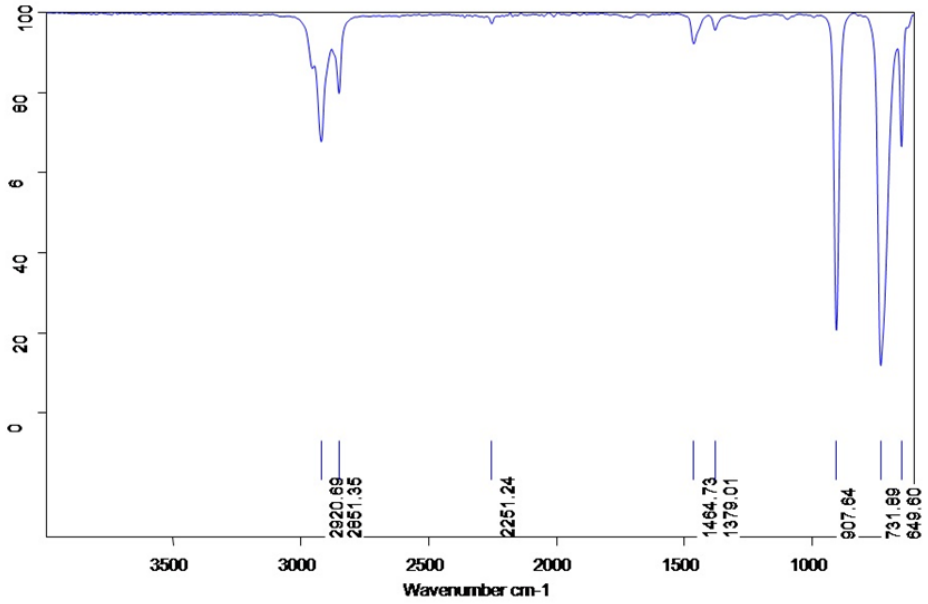

Fig. 5 Spectra analysis of MLE-*M. maderaspatana*

3.4. 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 ($C_{20}H_{34}O$ and 16.04%) (Fig. 7), Isodecyl diphenyl phosphate ($C_{22}H_{31}O_4P$ 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 ($C_5H_{10}O_4$ and 8.76%), Diphenylamine (C12H11N and 5.11%), Citronellol epoxide ($C_{10}H_{20}O_2$ and 5.00%), Isopropyl Myristate ($C_{17}H_{34}O_2$ and 4.84%), 9-Cedranone ($C_{15}H_{24}O$ and 4.75%), Oleyl alcohol ($C_{18}H_{36}O$ and 3.59%), n-Butyl myristate (C18H36O2 and 3.66%), benzoyl peroxide ($C_{14}H_{10}O_4$ and 3.42%), 2,5-Pyrrolidinedione, 1-butyl- ($C_8H_{13}NO$ and 3.27%), 4-tert-Butyl-2,6-diisopropylphenol ($C_{16}H_{26}O$ and 3.27%), Limonen-6-ol, pivalate ($C_{15}H_{24}O_2$ and 2.73%) and 5,5-Dibutylnonane ($C_{17}H_{36}$ 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 [37]. 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 [35]. Medicinal plant, *Erythrina variagata* LME was found 12-octadecenoic acid, methyl ester and it had predominant toxicity against HVMs [17]. *Petalonema alatum* LME was found different major phyto-compounds (MPCs) like 5-thio-D-glucose, 5-allylsulfanyl-l-(4-methoxy-phenyl)-1H-tetrazole, E)-10-heptadecen-8-ynoic acid methyl ester, and Z-11-hexadecenoic acid [38]. Twenty compounds, or 100% of the compounds, had their chemical constituents identified in the methanol extract following GC-MS analysis of the LME-*Loranthes pentandrus*. These investigations were conducted to determine the principal phytocompounds [39]. *Citrus limetta*-MPCs was showed six compounds, the main Corynan-17-01, 18, 19-didehydro-10-methoxy-, acelate (ester) [36]. In the *Ageratina adenophora*-MLE secondary metabolic study, twenty-one chemicals were found, with 5-isopropyl-2-methylphenol being the predominant one [10].

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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
$C_5H_{10}O_4$	1-Monoacetin	5.492	679233	8.76	134.13	69079	4.04	*RI-MS*
$C_9H_{14}O$	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*
$C_9H_{10}O_3$	Ethyl 3-hydroxybenzoate	8.925	144043	1.86	166.17	35032	2.05	*RI-MS*
$C_4H_4O_4$	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*
$C_8H_{13}NO$	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*
$C_3H_4O_4$	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*
$C_8H_8O_2$	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*

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MF=	$C_{15}H_{24}O_2$	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*
	$C_{18}H_{38}N_2O$	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*
	$C_6H_{10}O_2$	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*
	$C_{22}H_{31}O_4P$	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*
	$C_{17}H_{23}N_5O$	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*

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

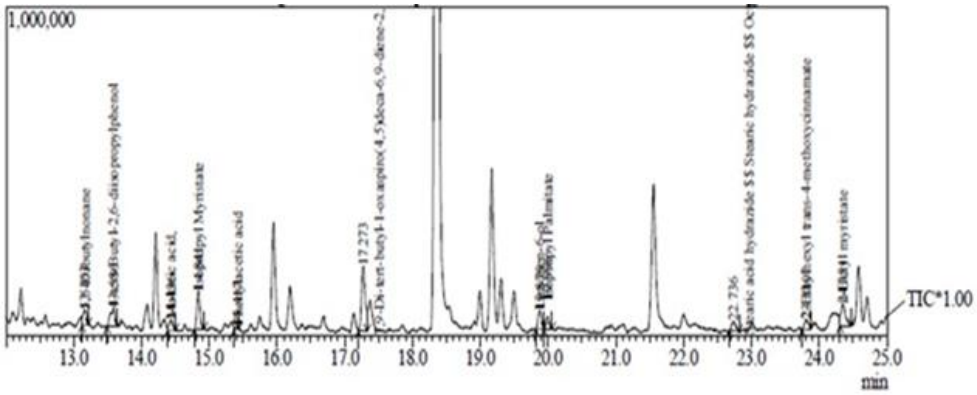

Fig. 6 Chemical compounds analysis of MLE-*M. maderaspatana*

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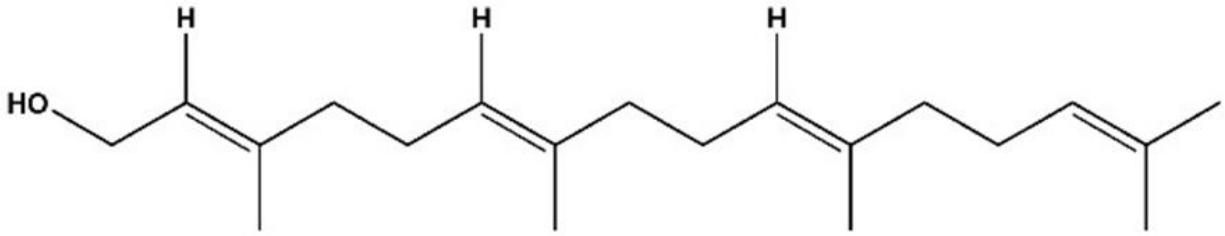
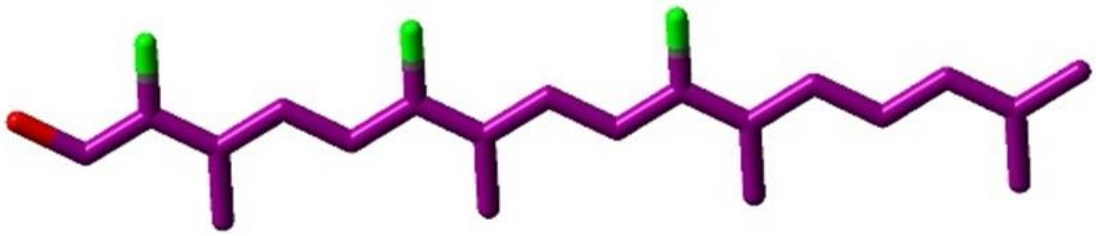


2D structure of Geranylgeraniol



3D structure of Geranylgeraniol

Fig. 7 2D & 3D structure of Geranylgeraniol compound from MLE-*M. maderaspatana*

3.5. Nuclear Magnetic Resonance (NMR) analysis

The MLE-*M. maderaspatana* underwent NMR spectral analysis, and the spectral peaks that emerged are displayed (Fig. 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. The signals found in the phenol, 2-methyl-5-(1-methylethyl) molecule from *Punica granatum* 1H NMR (400 MHz, CDCl3) spectrum [19].

3.6. 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. [40] 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. [41] found that *G. affinis* outcompetes *Carassius auratus*, a goldfish from to 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 [42].

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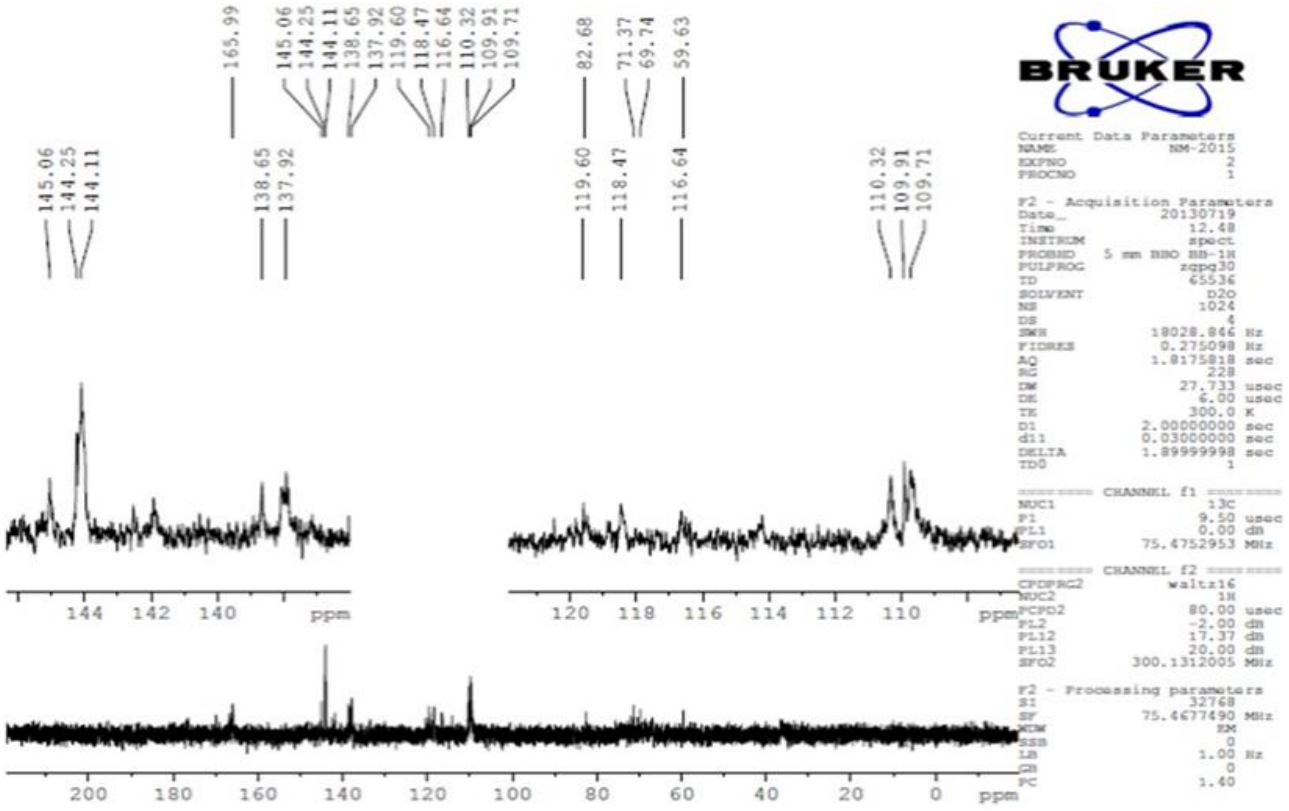

Fig. 8 NMR^{C} spectrum of the methanol extract isolated from *M. maderaspatana*

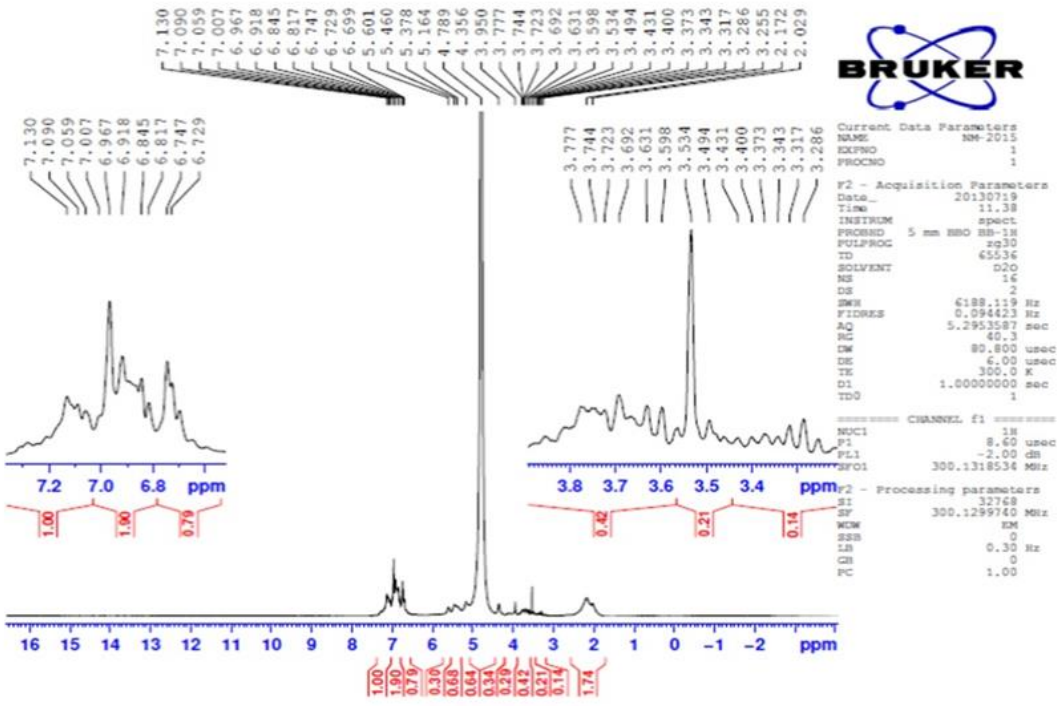

Fig. 9 NMR^{H} spectrum of the methanol extract isolated from M. maderaspatana

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Table 7 Predation efficiency of the G. affinia 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	*A. stephensi*	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
	C. quinquefsciatus	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
	A. aegypti	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	87.9	175.8
ELE	*A. stephensi*	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
	C. quinquefsciatus	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
	A. aegypti	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)

3.7. Antioxidant assay

The hexane extract exhibited the highest amount of DPPH radical scavenging activity, with an EC_{50} 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 IC_{50} 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).

Using the same quantities, EDTA (82.715mg/ml) was shown to have a lower EC_{50} 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. [43], the LC_{50} 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 basilicu*m shown a stronger antioxidant activity than *Ocimum americanum* [44]. They found that conventional antioxidants had greater activity than essential oils [45,46]. Additionally, an examination of antioxidants revealed that the leaf oil and inflorescence oil had effective concentrations (EC_{50}) of 22.76 µg/ml and 26.18

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µg/ml, respectively, which translates to 17.57 µg/ml of ascorbic acid [47].

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

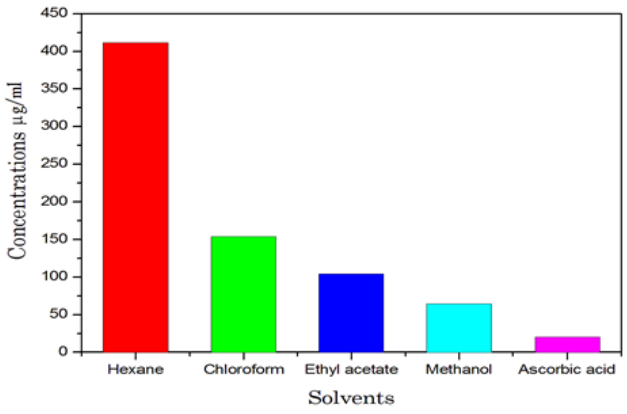

Fig. 10 DPPH radical scavenging activities of *Solanum incanum* crude extract

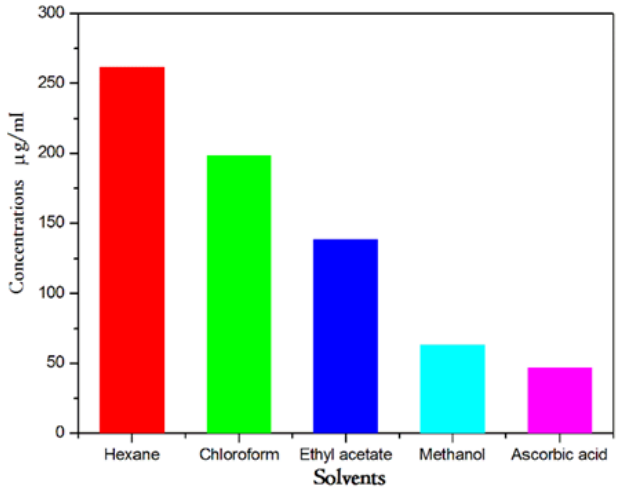

Fig. 11 ABTS Activity of *Solanum incanum* crude extracts

4. 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.

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