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# Fractional extraction, isolation and identification of biologically active compounds from *Melaleuca leucadendron* L. to control the vectors of dengue and filariasis

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**Abstract**
**Objectives:** To find out the larvicidal activities of *Melaleuca leucadendron* (L.) against *Culex quinquefasciatus* Say and *Aedes albopictus* (Skuse), and to isolate the active compounds present in the most active fraction of the plant extracts.
**Methods:** Cold extracts of *M. leucadendron* leaves were taken using methanol as solvent and fractionation was done using the solvents n-hexane, ethyl acetate and acetone by column chromatographic method. Bioassays were conducted using all these extracts and $LC_{50}$ were calculated. The structural elucidation of the compounds of most active fraction isolated from chromatographic studies after bioassay was done by LCMS and NMR spectroscopy.
**Results:** *M. leucadendron* treated larvae shown restless activity in *Ae. albopictus* more than in *Cx. quinquefasciatus*. The activity of column fractions of *M. leucadendron* was in the order Chloroform > Hexane (H) >: Ethyl acetate (EA). The compounds present in the most active fraction was citral and α-gurjunene.
**Conclusion:** From the results it has been proved that the plant is having potential larvicidal activity.

**Keywords:** *Melaleuca leucadendron, Aedes albopictus*, *Culex quinquefasciatus*, larvicidal activity

## Introduction
In view of public health importance, mosquitoes identify themselves as vectors of capital diseases like malaria, filariasis, dengue, Japanese encephalitis etc. Over centuries, scientists have been experimenting with various methods, which include use of synthetic insecticides, to encounter threats from mosquito borne diseases. The commodious, disproportionate and repeated use of organic insecticides such as carbamates, organophosphates and organochlorines has led to severance of natural biological control systems. This has eventually led to revitalization and development of resistance in target species and destruction of non-target flora and fauna inhabiting the same aquatic habitat. The remnants of the pesticides in the field are known to exhibit bio-magnification by entering into the ecosystem and circulating through the food web, ultimately triggering environmental imbalance.
Recent studies stimulated the investigation of insecticidal properties of plant-derived extracts; and concluded that they are environmentally safe, degradable and target specific [1]. Phytochemicals with mosquitocidal potentials are now recognized as potent alternative to replace synthetic insecticides in mosquito control programs due to their excellent ovicidal, larvicidal, adulticidal and repellent properties [2].
Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varities and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. The plant world comprises a rich untapped pool of phytochemicals that may be widely used in place of synthetic insecticides in mosquito control programme. Kishore *et al.* [3] reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potentiality of several plant derived secondary materials, such as, alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpans and lignans.
In certain instances, the same phytochemical toxin from a single plant species exhibits various degrees of toxicity to different mosquito species. Minijas and Sarda showed that crude extracts

containing saponin from fruit pods of *Swartzia madagascariensis* produced higher mortality rate in larvae of *An. gambiae* than in larvae of *Ae. aegypti* and no mortality was induced in larvae of *Cx. Quinquefasciatus* [4].

The present study aims to explore the larvicidal activity of *Melaleuca leucadendron* extracts against *Cx. quinquefasciatus* and *Ae. albopictus* and also to study the potential compounds from the plants for an alternative to the synthetic insecticides to control mosquito vectors.

## Materials and Methods

Test organism: The mosquito species *Culex quinquefasciatus* Say and *Aedes albopictus* Skuse were used for the study.

Plant selected for the study: *Melaleuca leucadendron* L. Commonly called as Cajeput, white tea tree, swamp tea tree etc., included in the family Myrtaceae. *M. leucadendron* grows to a tree of nearly 20 m height and has a long flexible trunk with irregular ascending branches covered with a pale thick, lamellate bark, it is soft and spongy and from time to time throw off its outer layer in flakes; leaves entire, linear, lanceolate ash colour, alternate on short foot stalks; flowers sessile white on long spike. The leaves have a very aromatic odour and the oil is distilled from the fresh leaves and twigs, and is volatile and stimulating with an aroma like camphor, rosemary or cardamom seeds. Taste bitter, aromatic and camphoraceous.

Extraction and Fractionation: Fresh leaves of *M. leucadendron* were collected from Calicut University campus. The leaves were thoroughly washed with water and shade dried in the room temperature. The dried materials of selected plants were powdered using a mixer grinder and sifted through a fine mesh of sieve. The powders were packed in airtight ziplog bags (half a kg capacity) and stored at -20 ºC.

Extracts were taken by cold solvent extraction method using acetone as solvent. Fractionation of miscella was done by column chromatography using glass column of 50cm length and Silica gel powder (60/120 mesh) and 5g of miscella. Then the miscella was eluted with different solvents (HPLC grades) and solvent systems such as n-heaxane, ethyl acetate and chloroform. Elutes collected were dried at room temperature and the extracts were further diluted and used in bioassays.

Bioassay: Different extracts of selected plant materials were tested against 3rd instar larvae of *Cx. quinquefasciatus* and *Ae. albopictus*. Observations were taken after 1hour, and the mortality of the larvae at the end of 24 hour was recorded and percentage mortality is estimated. $LC_{50}$ were calculated using a Probit programme developed by Finney, 1971 [5].

## NMR spectra and LCMS analysis

To identify the compounds present in the most active fraction, the chloroform fraction again fractionated using chloroform and n- hexane (1:1) and carried out the structural elucidation of the compounds isolated from chromatographic studies. Preliminary structural analysis using LC-MS, NMR spectroscopic techniques was carried out in collaboration with Department of Chemistry, Annamalai University. The NMR spectra ($^1H$ & $^{13}C$ NMR) were recorded at 400/100MHz using DMSO-$d^6$ as a solvent system and tetramethylsilane (TMS) used as an internal standard [6]. The mass spectrometry used to calculate the mass to charge measurement after assigning the most abundant ion 100%.

## Results

The table 1 and 2 represents the values in ppm for 24 hr $LC_{50}$ and $LC_{90}$ of the different column fractions of *M. leucadendron* tested against III instar larvae of *Cx. quinquefasciatus* and *Ae. albopictus*. The 24 hr $LC_{50}$ of Hexane, EA and Chloroform fractions of *M. leucadendron* against the III instar larvae of *Cx. quinquefasciatus* were 23.67, 42.4 and 14.12 ppm respectively (table 41). 24 hr $LC_{50}$ ($LC_{90}$) values (ppm) obtained for the Hexane, EA and Chloroform fractions of *M. leucadendron* tested against the III instar larvae of *Ae. albopictus* were 20.42 (50.76), 40.38 (82.66) and 11.66 (38.76) ppm respectively.

**Table 1:** 24 hr $LC_{50}$ and $LC_{90}$ (ppm) and associated statistics of different column fractions of *M. leucadendron* against III instar of *Cx. Quinquefasciatus*

| Column Fractions: | $LC_{50}$ (LFL-UFL) | $LC_{90}$ (LFL-UFL) | $\chi^2$ |
|---|---|---|---|
| Hexane | 23.67 (15.56-36.48) | 55.76 (46.62-68.24) | 5.2 |
| Ethyl acetate | 42.4 (36.54- 54.28) | 98.66 (80.54-112-44) | 6.4 |
| Chloroform | 14.12 (6.56-22.98) | 39.76 (30.67-48.6) | 4.2 |

**Table 2:** 24 hr $LC_{50}$ and $LC_{90}$ (ppm) and associated statistics of different column fractions of *M. leucadendron* against III instar of *Ae. Albopictus*

| Column Fractions: | $LC_{50}$ (LFL-UFL) | $LC_{90}$ (LFL-UFL) | $\chi^2$ |
|---|---|---|---|
| Hexane | 20.42 (12.68-32.48) | 50.76 (44.65-62.22) | 4.4 |
| Ethyl acetate | 40.38 (32.22- 52.24) | 82.66 (70.54-92-42) | 5.2 |
| Chloroform | 11.66 (5.44-18.44) | 38.76 (28.88-42.55) | 3.8 |

## LCMS and NMR data of *Melaleuca leucadendron*

The most active chloroform fraction again fractionated using Chloroform and Hexane in different concentration (1:1, 1:2, 1:3, 1:4, 4:1, 3:1, 2:1) and subjected to bioassay. The most active fraction was found to be chloroform: hexane 1:1 fraction with $LC_{50}$ (LFL-UFL) values (in ppm) 8.56 (3.87-13.25) and 9.87 (4.58- 16.42) for *Ae.albopictus* and *Cx. quinquefasciatus* respectively. After NMR and LCMS, two compounds were elucidated and its structure and details were as follows.

## Compound 1

It is interesting to note that the structure isolated resembles the structure of (E)-3, 7-dimethylocta-2, 6-dienal. IUPAC Name: (E)-3, 7-dimethylocta-2, 6-dienal, Chemical Formula: $C_{10}H_{16}O$.

$^1H$ NMR (400 MHz, $CDCl_3$), δ (ppm): 1.837 (s, 3H, H(12), H(13) and H(14), 1.935 (s, 3H, H(25), H(26), H(27), 2.927 (t, 2H, H(16), H(17), J=1.2Hz), 3.644 (t, 2H, H(18), H(19), J=1.0), 2.250 (s, 3H, H(22), H(23), H(24), 4.923 (t, 1H, H(15) J=2.1Hz), 5.037 (d, 1H, H(20), J=1.0 Hz), 7.314 (s, 1H, H(20) (PlateNo. 1A).

$^{13}C$ NMR (100 MHz, $CDCl_3$), δ (ppm): 22.81 (C1), 29.55 (C11), 28.44 (C10), 44.13 (C4), 52.82 (C5), 112.10 (C3), 139.83 (C7), 174.46 (C8) (PlateNo. 1B).

Mass spectra ESI [M-H] $^-$151.27 (Actual Mol. Wt. 152.23) (PlateNo.1C).

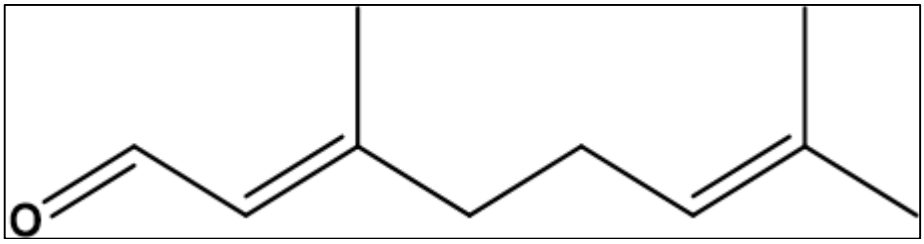

**Fig 1:** The structure of the compound isolated from *M. leucadendron*.

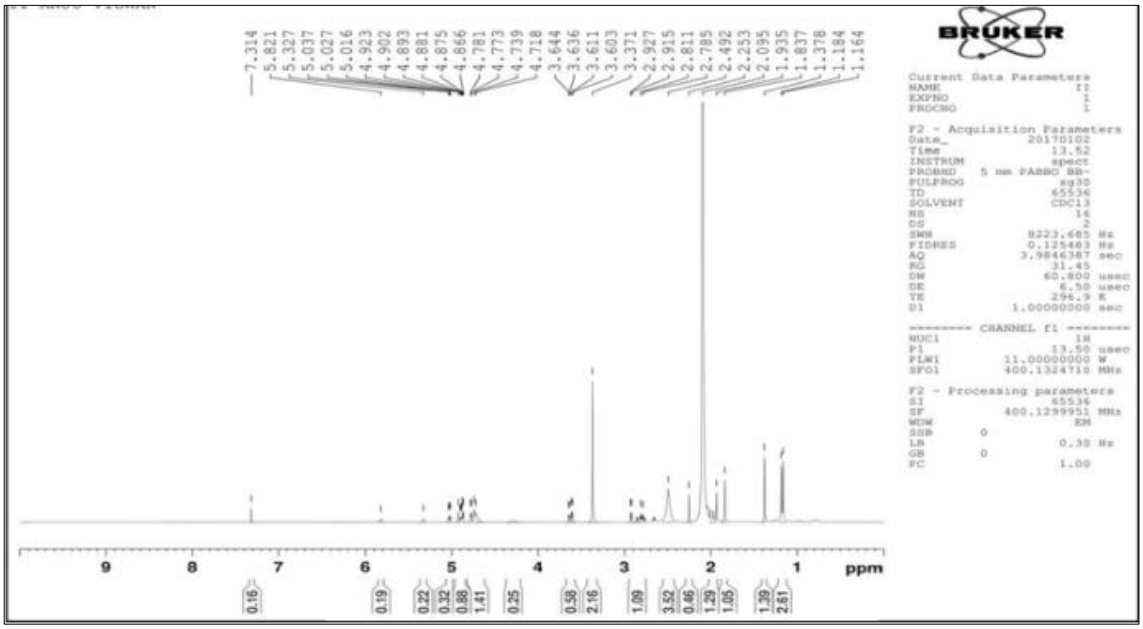


**Plate 1a:** $^{1}$H NMR spectrum of Compound 1

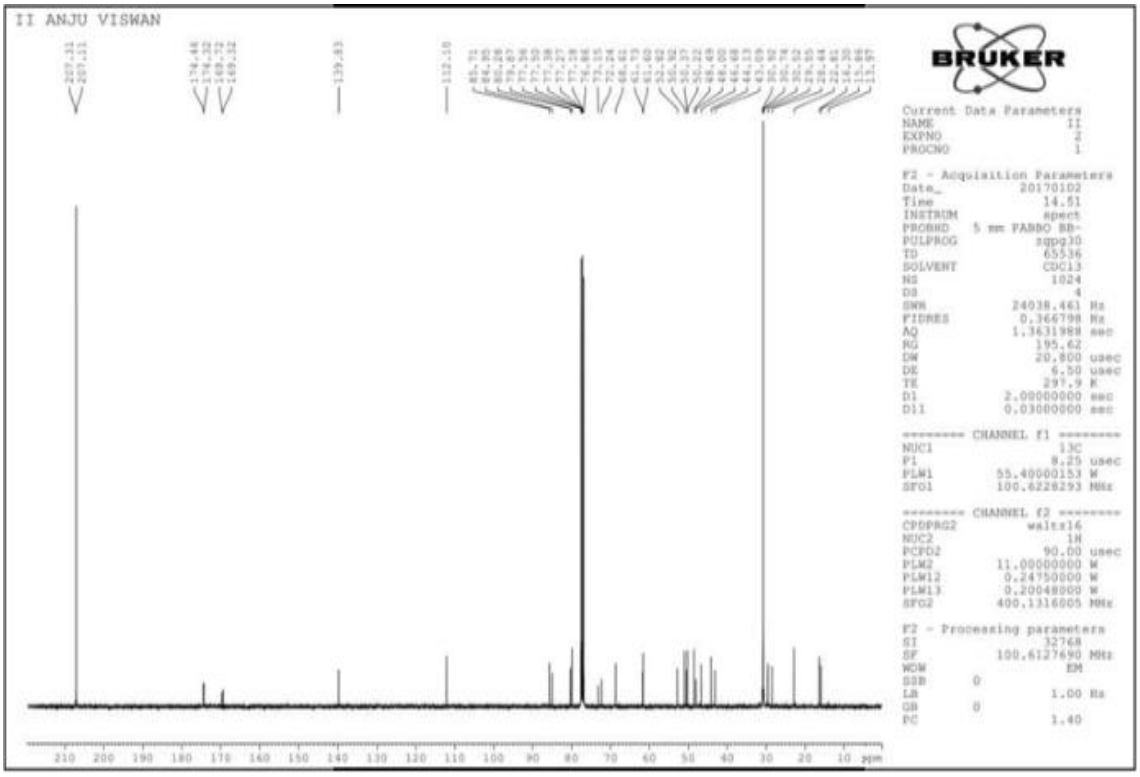


**Plate 1b:** $^{13}$C NMR spectrum of Compound 1

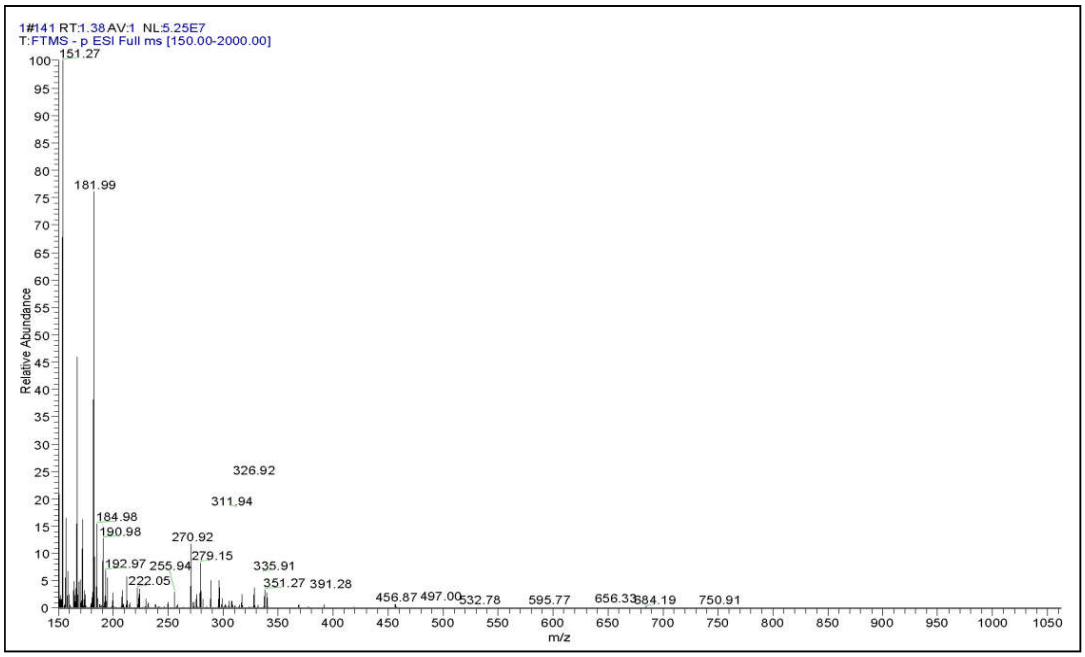


**Plate 1c:** LCMS spectrum of Compound 1

**Compound 2**

It is interesting to note that the structure isolated resembles the structure of 1, 1, 4, 7-tetramethyl-1a, 2, 3, 4, 4a, 5, 6, 7b-octahydro-1H-cyclopropa[e]azulene. IUPAC Name: 1,1,4,7-tetramethyl-1a, 2, 3, 4, 4a, 5, 6, 7b-octahydro-1H-cyclopropa[e]azulene, Chemical Formula: $C_{15}H_{24}$.

$^{1}$H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.013 (d, 3H, H(16), H(17) and H(18) J=1.1Hz), 1.100 (s, 6H, H(26), H(27), H(28), H(29), H(30) and H(31), 1.285 (m. 1H, (H24) J=0.4Hz, 1.355 (d, 1H, H(25) J=5.6Hz, 1.477 (m, 2H, H(20), H(21) J=1.8Hz, 1.561 (m, 1H, H(19) J=1Hz, 0.983 (s, 3H, H(37), H(38) and H(39), 1.784 (m, 2H, H(34), H(35) J=1.1Hz), 1.912 (m, 1H, H(36) J=1.8), 4.030 (t, 2H, H(32), H(33) J=2.1Hz) (PlateNo. 2A).

$^{13}$C NMR (100 MHz, $CDCl_3$), δ (ppm): 25.73(C15), 27.89 (C1), 28.90 (C8 and C9), 29.24 (C3), 31.84 (C7), 33.70 (C6), 34.78 (C5), 35.99 (C13), 36.34 (C2), 41.59 (C12), 46.84 (C14), 76.43 (C10) and 77.06 (C11) (Plate No. 2B).

Mass spectra ESI $[M-H]^{-}$203.37 (Actual Mol. Wt. 204.35) (Plate No. 2C).

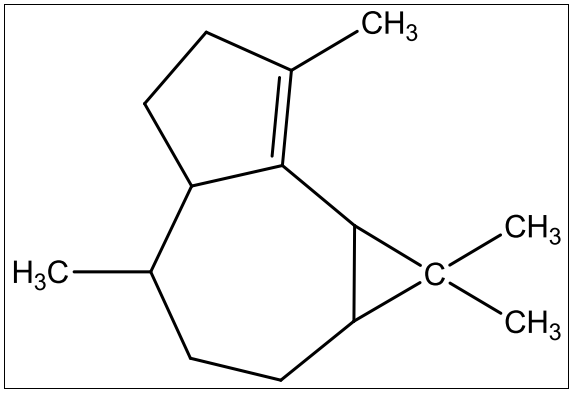


**Fig 2:** The structure of the compound isolated from *M. leucadendron*

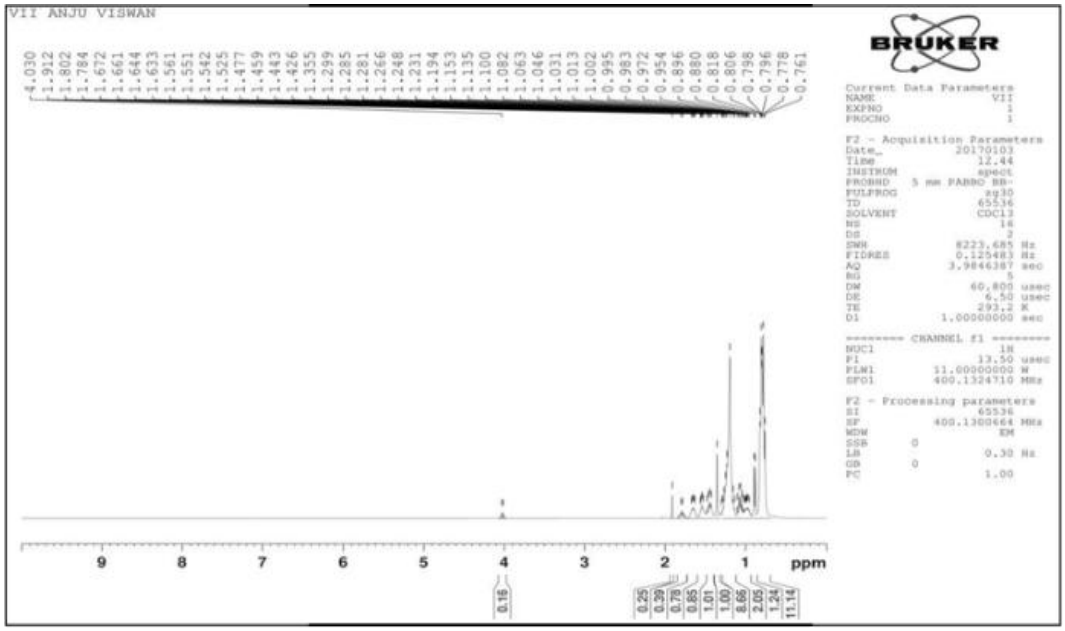


**Plate 2a:** $^{1}$H NMR spectrum of Compound 2

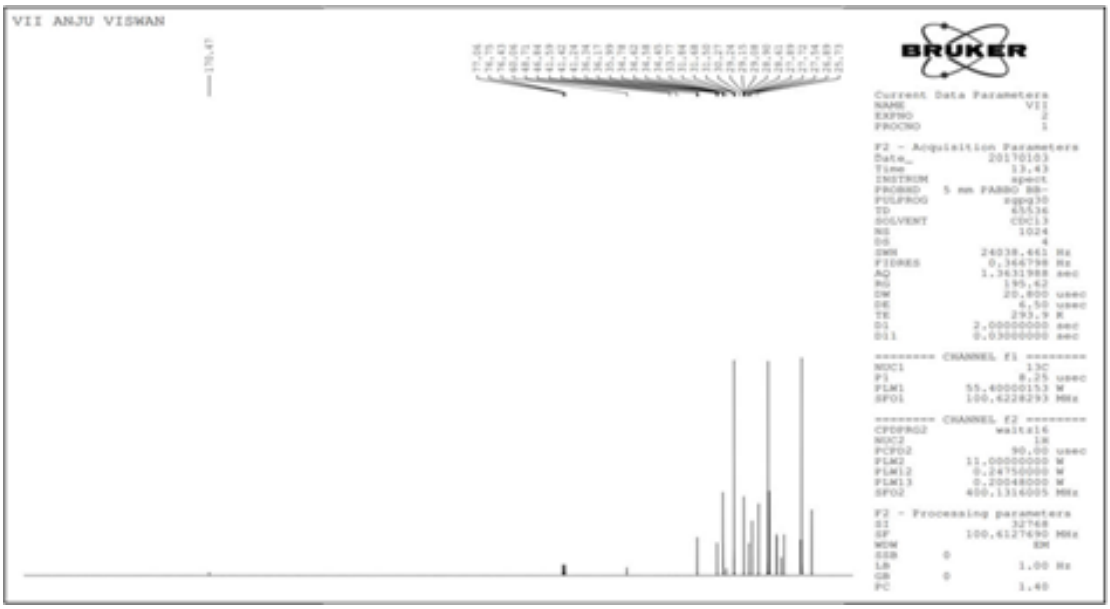


**Plate 2b:** $^{13}C$ NMR spectrum of Compound 2

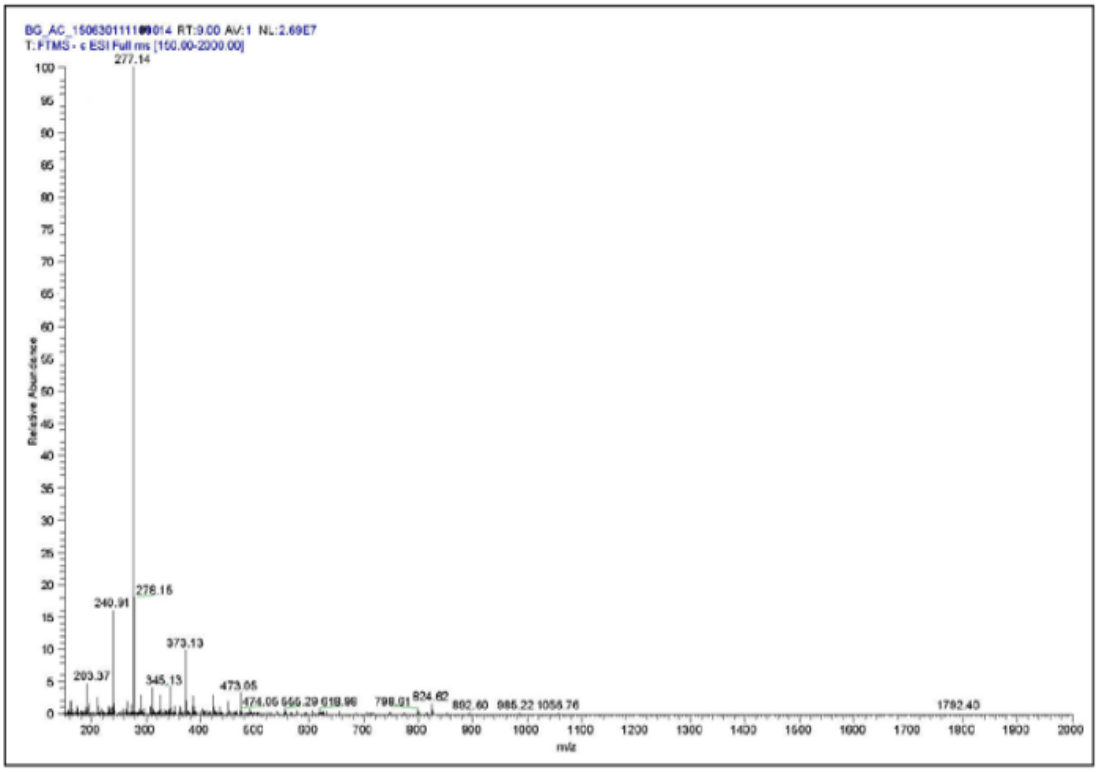


**Plate 2c:** LCMS spectrum of Compound 2

## Discussion

Essential oil of *M. leucadendron* showed 100% repellency against *Cx. quinquefasciatus* and 80.9% repellency against *Ae. aegypti* at its 8th hour of exposure. Adulticidal activities of the essential oils observed after 24hour exposure showed 100% adult mortality, indicating their potential as biocontrol agents for mosquitoes [7]. Amer and Mehlhorn, reported forty one essential oils against different species of mosquitoes and found out five most effective essential oils *viz*; *Litsea cubeba*, *M. leucadendron*, *M. quinquenervia*, *Viola odorata* and *Nepeta cataria* which induced 100% repellency over a protection period of 480 min against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*[8]. The essential oil from *M. leucadendron leaves* are green in color with a camphor odor and is medicinally farming such as antiseptic, stomachic stimulant, analgesic, anti-rheumatic, expectorant, and for treatment of intestinal worms [9, 10].

Three fractions of *M. leucadendron* leaf extracts *viz*, hexane, ethyl acetate and chloroform were taken using the column chromatography and analysed their larvicidal activity against 3rd instar larvae of both *Cx. quinquefasciatus* and *Ae. albopictus*. The mortality rates were recorded and the $LC_{50}$ values were calculated. The chloroform fraction of *M. leucadendron* has more activity than hexane and ethyl acetate fractions. A slight difference is seen in the activity against *Cx. quinquefasciatus* and *Ae. albopictus*. With all three fractions, *Ae. albopictus* shows less $LC_{50}$ values as compared with *Cx. quinquefasciatus*, as in the case of *A. cocculus* extracts.

The fractions isolated from the chloroform fraction of *M. leucadendron* were II and VII. The fractions II produced the

molecular ion signal (m/z) of 151.27 and the identified compound may be (E)-3, 7-dimethylocta-2, 6-dienal and its molecular formula is $C_{10}H_{16}O$ (Citral). The fractions VII produced the molecular ion signal (m/z) of 203.37 and the identified compound may be 1,1,4,7-tetramethyl-1a,2,3,4,4a,5,6,7b-octahydro-1H-cyclopropa[e]azulene and its molecular formula is $C_{15}H_{24}$ (α-Gurjunene).

In conclusion, the $^{1}H$ and $^{13}C$ NMR as well as LCMS spectrum confirms two chemical constituents (citral & α-Gurjunene) obtained from the most active fraction of the plant extracts of *M. leucadendron.* The identified compounds may lead the way to define its potential biological activity. These molecules can be further theoretically tested for its biological activity using *in silico* methods. In future, based on its theoretical activity the compounds can be further redesigned for its potent biological activity.

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**References**

1. Tehri K, Singh N. The role of botanicals as green pesticides in integrated mosquito management-A review. Int. J Mosq. Res. 2015; 2:18-23.
2. Shaalan EAS, Canyon D, Younesc MWF, Abdel Wahaba H, Mansoura AH. A review of Botanical phytochemicals with mosquitocidal potential. Environ. Int. 2005; 3:1149-66.
3. Kishore N, Mishra BB, Tiwari VK, Tripathi V. A review on natural products with mosquitosidal potentials. In: Tiwari VK, editor. Opportunity, challenge and scope of natural products in medicinal chemistry. Kerala: Research Signpost, 2011, 335-65.
4. Minjas JN, Sarda RK. Laboratory observations on the toxicity of Swartzia madagascariensis (Leguminosae) extract to mosquito larvae. Trans R Soc Trop Med Hyg. 1986; 80(3):460-61.
5. Finney DJ. Probit Analysis. Cambridge University Press, 1971.
6. Hari KM, Thriveni P. Synthesis of coumarins via Pechmann reaction using Cr(NO3)3.9H2O as a Catalyst under microwave irradiation. Der Pharma Chemica. 2016; 8(10):94-8.
7. Pushpalatha E. Anju Viswan K. Adulticidal and repellent activities of *Melaleuca leucadendron* (L.) and *Callistemon citrinus* (Curtis) against filarial and dengue vectors. Entomon. 2013; 38(3):149-54.
8. Amer A, Mehlhorn H. Repellency effect of forty-one essential oils against Aedes, Anopheles, and Culex mosquitoes. Parasitol. Res. 2006; 99(4):478-90.
9. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc. 1991; 7:210-37.
10. Kudom AA, Mensah BA. Botchey MA. Aqueous Neem Extract Versus Neem Powder on *Culex quinquefasciatus*: Implications for Control in Anthropogenic Habitats. J Insect Sci. 2011; 11:1-9.