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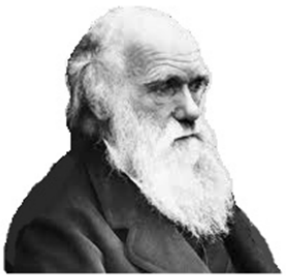

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Entomotoxicity properties of eco-friendly methanol extract fractions from *Phyla nodiflora* (L.) Greene leaf exhibits mosquito larvicidal, pupicidal and antimicrobial activity

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ABSTRACT

Mosquitocidal activity of *Phyla nodiflora* methanol leaf extract fractions (*Pn*-MLEFr) was controlled and using main components on malarial vector, *Anopheles stephensi* (*An. stephensi*). *Pn*-MLE were characterized utilizing tools containing TLC, CC, FTIR and GCMS, and it against 3rd instars larvae of *An. stephensi*, followed by pupicidal activity was determined to concentration of 1, 3, 5 ppm. In phytochemical, characterized by GCMS analysis was carried out to be eligible for the constituents of the MLE. *Pn*-MLE Fr-6 showed the highest LC_{50} and LC_{90} values of 25.80 and 68.45 ppm, respectively. Fr-6 was found to be most effective for this activity provided pupa stage at death and stage of non-mortality values were 22.85 (NDP), 76.1 (TDP), 1.2 (DP), 0.7 (DA), 78% (TM%) and 6.6 (NAE), 22 (AE%) at 1 ppm against *An. stephensi*. Moreover, 3 and 5 ppm were provided TM% and AE% values of 94.43% and 5.57%; 100% and no adults emerged. In GC-MS analyzes, a total of 21 compounds were identified in the *Pn*-MLE, the main component was Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-. Further, the AgNPs synthesized using *Fm*-ALE showed enhanced anti-bacterial activity. The reports revealed the Ergosta-5,22-dien-3-ol, acetate, (3á,22E)- was the most one of the important a compound provides malarial vector control from *Pn*-MLEFr.

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***Keywords*:** *Phyla nodiflora*, *Anopheles stephensi*, mosquito larvae, GC-MS

1. INTRODUCTION

In many countries in sub-Sahara Africa, malaria remains a leading cause of mortality and morbidity among the populace, with detrimental effects on health care programs in these countries, and World Health Organization (WHO) reported in 2018 that malaria is still endemic in 80 countries and territories (WHO, 2019; Baranitharan *et al.*, 2020; Subash *et al.*, 2019). Various interventions have been developed for both vector and parasites to curb malaria infection in humans. *Anopheles stephensi* has recently emerged as an efficient and breeds predominantly in urban settings, prefers water storage containers (Surendran *et al.*, 2019), and is found throughout the horn of Africa (WHO, 2020). The introduced *An. stephensi* exhibits resistance to several classes of insecticides, posing challenges in controlling the spread to new areas. The recent negative impacts of chemical insecticides should be removed urgently and search efforts towards the development of new environment friendly, naturally available vector control methods by using realistic mediators. *Phyla nodiflora* (Verbenaceae) is gathered from the wild for local domestic medicinal use, and found such as astringent, anodyne, antibacterial, deobstruent, emmenagogue, refrigerant, febrifuge, parasiticide, diuretic, emollient, and be useful in the treatment of ischuria, pain in the knees, constipation, blenorrhoea, lithiasis and blenorrhoea (Duke and Ayensu, 1985). The plant juice is cooling and used to relieve minor gastric troubles, fever, coughs and colds. The aroma of the inhaled plant is breathed in to treat coughs and colds (PRSA, http://proseanet.org/). In this investigate, *Pn*-MLE using FT-IR, GC-MS and further investigated larvicidal, pupicidal and antimicrobial activity Raj, et al., 2017

2. MATERIALS AND METHODS

2. 1. Plant material and extraction

Figure 1. *Pn*- leaf powder

P. nodiflora leave was carried out during the growing season (December to February) of 2019 from different places of Yerkadu, Salem District of the Tamilnadu. The samples were authenticated at the Department of Botany of Annamalai University. The leaves were washed many times with water to remove all the unwanted impurities. Then, the leaves were shade-dried under room temperature and kept in a hot air oven for 50 ºC for half and h. After that, the material was ground by using electric blender (Figure 1). 500 g of powdered plant material was packed inside a Soxhlet apparatus, and successive extraction was carried out usipng as solvent methanol for 72 h. The solvent was evaporated under vacuum in a rotary evaporator (Heidolph, Germany), and the dried extract was stored at 4 ºC until further bioassay.

2. 2. Test mosquito larvae

An. stephensi larvae were separately reared in laboratory Department of Zoology, Poompuhar College (Autonomous), Mayiladuthurai District. The pupae were collected from the culture trays and that are transferred into the plastic containers (12×12 cm) containing 500 mL of water with the help of a dipper. It was kept up at 27 ±20 ºC, 75-85 relative mugginess. The hatchlings were bolstered with dog biscuits and yeast at 3:1 proportion.

2. 3. Larvae bioassay

One gram of each *P. nodiflora* extract was solved in 1 ml dimethyl sulfoxide (DMSO) and dilueted in 249 ml of dechlorinated (DCL) water. Control (without extract) was 1 ml of DMSO in 249 ml of DCL water. Per each tested, 25 singles per replicate were stored in 249 ml of DCL water and 1 ml of DMSO + the required dose. The larvae bioassay was assessed as per procedure WHO (2005), all dose ranging from 50 to 250 mg/lit were prepared, and were tested on early third instars of the targeted mosquito, *An. stephensi*. Larval mortality was recorded after 24 h, and each dose, 5 replicates were carried out. Percent mortality was rectified for control mortality utilizing the formula by Abbott's (1925).

$$Mortality\ (\%) = \frac{Number\ of\ larvae\ died}{Total\ number\ of\ larvae\ Exposed} \times 100$$

2. 4. Pupae bioassay

Groups of 25 pupae were introduced into 500 ml of the extract, containing specific concentrations of the extract in a plastic cups in 5 replications. In control, the same number of pupae was maintained in 500 ml DCL water containing acceptable volume of DMSO. All plastic cups were maintained at room temperature (28 ±2 °C) with naturally prevailing photoperiod (12:12h/L:D) within the laboratory. Any pupa was thought-about to be dead if did not move once poked repeatedly with a mushy brush. Mortality of each pupa was recorded when 24 h of exposure to the extract.

$$\text{Percentage mortality}\ (\%) = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} \times 100$$

2. 5. FTIR analysis

Infrared analysis was used to probe bond vibrations and bending in molecules and to

reveal the types of functional groups present in *P. nodiflora*-MLE. Functional group region is in the range from 3650.991 to 662.76 cm^{-1} and finger print region is from 1640.75 to 704.32 cm^{-1} (Vivek *et al.*, 2011).

2. 6. GC-MS analysis

Chromatography analysis was performed using a mass detector Turbo mass gold-Perkin Elmer particular identifier and an Elite-5MS (5% Diphenyl / 95% Dimethy poly siloxane), 30×0.25 mm ×0.25 µm df) slender segment. The stove temperature was customized from 50 to 280 ºC at the rate of 5 ºC min^{-1} and stopped at this temperature for 36 min. The delta and interface temperatures were 200 and 280 ºC, respectively. The transporter gas was heat a stream rate of 1.0 ml min^{-1} (consistent stream). The sample (2µl) was injected at a split of 10:1. Electron sway mass spectrometry was conveyed at 70eV. Particle source and fourfold temperature were kept up at 250 and 200ºC separately (Kumaravel *et al.*, 2010).

2. 7. Anti-bacterial activity of AgNPs

The antibacterial role of the synthesized AgNPs on four bacterial pathogens (gram-negative and gram-positive) was decisive in the disc diffusion pattern utilizing sterilized MHA (Mueller Hinton Agar) media (Merck millipore's, Analytical Research Centre, Puducherry) (37). The test organisms were inoculated in Luria bertani (Bacteria) (pH 7.4) for 18-24 h at 37 °C, further agar plates utilizing cotton cloths, and concentrations ranging from 50 µg to 500 µg. MHA outward bored utilizing sterilized gel borer to made wells, after that triplicate plates were protected for bacterial. The explored four most common infection-causing bacteria, such as *B. substils, K. pneumoniae, E. coli, S. aureus*.

2. 8. Data analysis

The average larvae bioassay (%) data were subjected to different statistical baggage, LC_{50}, LC_{90}, LCL/UCL, regression (R^2), chi-square (χ^2) etc (Finney, 1971). Pupae data were analyzed using two-way ANOVA (factors: the tested fraction and the tested dose) followed by Tukey's HSD test. The significance level was set at $P<0.05$.

3. RESULTS

3. 1. Larval bioactivity

Table 1 is showing that, *Pn*-MLE six fractions were checked for their larvae kill activity against the selected mosquito species, which have been tested for their larvicidal activity of *Ae. aegypti*. Fr-1 showed the highest LC_{50}/LC_{90} values of 98.21/135.77 ppm, respectively, followed by the LC_{50}/LC_{90} values of 86.49/128.54 ppm, for Fr-2; the LC_{50}/LC_{90} values of 39.72/115.25 ppm, for Fr-3; the LC_{50}/LC_{90} values of 31.46/104.88 ppm, for Fr-4; the LC_{50}/LC_{90} values of 27.19/85.26 ppm, for Fr-5 than LC_{50}/LC_{90} values of 25.80/68.45 ppm, for Fr-6, respectively.

3. 2. Pupicidal bioactivity

Table 2 is showing that, statistically significant pupicidal activity was tested with *Pn*-MLE six Fr. The Fr-6 was found to be most effective for this activity provided pupa stage at

death (NDP, TDP, DP, DA and TM%), and stage of non-mortality (NAE and AE%) values were 22.85 (NDP), 76.1 (TDP), 1.2 (DP), 0.7 (DA), 78% (TM%) and 6.6 (NAE), 22 (AE%) at 1 ppm. Moreover, 3 and 5 ppm were provided TM% and AE% values of 94.43% and 5.57%; 100% and no adults emerged. Fr-5 to 1 was showed TM% and AE% values of 96.87% and 3.13%; 89.21% and 10.79%; 81.28% and 20.72%; 70.46% and 29.54%; 59.9% and 40.1% at 5 ppm, respectively.

Table 1. Larvicidal activity of *Pn*-MLE tested against freshly molted third instar larvae o f *An. stephensi.*

Fractions	LC_{50} (ppm) LCL/UCL	LC_{90} (ppm) (LCL/UCL)	R^2	χ^2 (df = 3)
Fr-1	98.21 (91.64/108.29)	135.77 (102.52/158.24)	y=4.22893x+0.21148	10.4571[a]
Fr-2	86.49 (75.12/105.86)	128.54 (115.68/144.38)	y=4.05017x+0.03826	11.2914[a]
Fr-3	39.72 (31.55/49.18)	115.25 (94.27/158.65)	y=3.60428x+1.27354	14.5092[a]
Fr-4	31.46 (26.65/44.59)	104.88 (81.56/149.52)	y=2.32084x+0.21982	12.1472[a]
Fr-5	27.19 (24.48/39.54)	85.26 (78.54/95.98)	y=2.39104x+1.62134	13.1765[a]
Fr-6	25.80 (22.08/34.56)	68.45 (62.16/88.54)	y=3.40732x+1.05426	12.5120[a]

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; Regression; Chi-square. * Fraction with strongest larvicidal effect

Table 2 Pupicidal activity of *Pn*-MLE tested against pupae of *An. stephensi.*

Fractions	Conc.	Stage at death					Stage of non-mortality	
		NDP	TDP	DP	DA	TM (%)	NAE	AE %
Control	0	-	-	-	-	-	-	100
Fr-1	1	22.85	76.1	1.2	0.7	78.00	6.6	22
	3	26.85	89.43	3.4	1.6	94.43	1.67	5.57

	5	30.0	100	-	-	100	-	-
Fr-2	1	20.47	68.19	1.5	2.1	71.79	8.47	28.21
	3	25.61	85.31	2.1	0.3	87.71	3.69	12.29
	5	28.45	94.77	1.7	0.4	96.87	0.93	3.13
Fr-3	1	19.14	63.73	2.3	0.8	66.83	9.96	33.17
	3	23.13	77.05	2.6	1.3	80.95	5.72	19.05
	5	25.64	85.41	3.6	0.2	89.21	3.24	10.79
Fr-4	1	17.44	58.1	1.2	0.9	60.2	11.95	39.8
	3	19.85	66.13	2.5	1.6	70.23	8.93	29.77
	5	23.74	79.08	0.4	1.8	81.28	6.22	20.72
Fr-5	1	14.57	48.55	0.9	1.7	51.15	14.66	48.85
	3	16.8	55.97	1.6	0.6	58.17	12.56	41.83
	5	20.13	67.06	3.1	0.3	70.46	8.87	29.54
Fr-6	1	11.55	38.49	3.4	1.3	43.19	17.06	56.81
	3	14.57	48.55	2.6	1.2	52.35	14.3	47.65
	5	17.2	57.3	2.2	0.4	59.9	12.04	40.1

NDP = normal death pupa, TDP = total death pupa, DP = deformed pupa,
DA = deformed adults, TM = Total mortality, NAE = normal adult emerge

3. 3. Chromatography analysis

Figure 2 is showing that, *Pn*-MLE was analyzed by using thin layer chromatography (TLC) and Column chromatography (CC) with varying solvent systems. Ethyl acetate: ethanol (2:8) gave 2 & 4 fraction, two fractions have been obtained in ethyl acetate: ethanol (1.5:8.5), four fractions have been obtained in ethyl acetate: ethanol (1:9) and the maximum of five fractions have been obtained in ethyl acetate: ethanol (0.5:9.5).

Figure 2. Column chromatography: separation of fraction from *Pn*-MLEFr

3. 4. FTIR analysis

Figure 3 is showing that, FT-IR analysis was carried out to identify the functional active groups of the *Pn*-MLE, and indicated the clear peaks value they corresponded to functional groups like, 1*, 2* amines, amides group (medium, N-H stretching 3456.16 cm^{-1}), alkanes group (medium, C-H stretching 2927.15, 2860.78 cm^{-1}), esters, saturated aliphatic groups (strong, C=O stretching 1742.53 cm^{-1}), 1* amines group (medium, N-H bend 1640.75 cm^{-1}), alkanes group (medium, C-H bend 1457.61 cm^{-1}), aliphatic amines group (medium, C-N stretching 1106.78 cm^{-1}) and alkyl halides group (medium, C-Cl stretching 704.32 and 662.76 cm^{-1}) confirmed their presence in MLE.

3. 5. GC-MS analysis

Table 3 provides the CCs of *Pn*-MLE were the retention indices and the percentage of the individual components (Fig. 4). The CLE was extracted by hydrodistilled in a clavenger apparatus and was analypzed by GC-MS. A total of 23 CCs were detected representing to 100%. The MPCs of MLE were Ergosta-5,22-dien-3-ol, acetate, (3á,22E)- (24.76%) ($C_{30}H_{48}O_2$) (Fig. 5), Octadecanoic acid, ethyl ester (9.93%) ($C_{20}H_{40}O_2$), 3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-7-one (9.83%) ($C_{26}H_{36}O_3$), n-Hexadecanoic acid (8.71%) ($C_{16}H_{32}O_2$), Cholestan-3-ol, 2-methylene-, (3á,5à)- (7.98%) ($C_{28}H_{48}O$) and

Strophanthidin (5.75%) ($C_{23}H_{32}O_6$). The percentage compositions of remaining 23 CCs ranged from 0.22% to 9.93% (Figure 5).

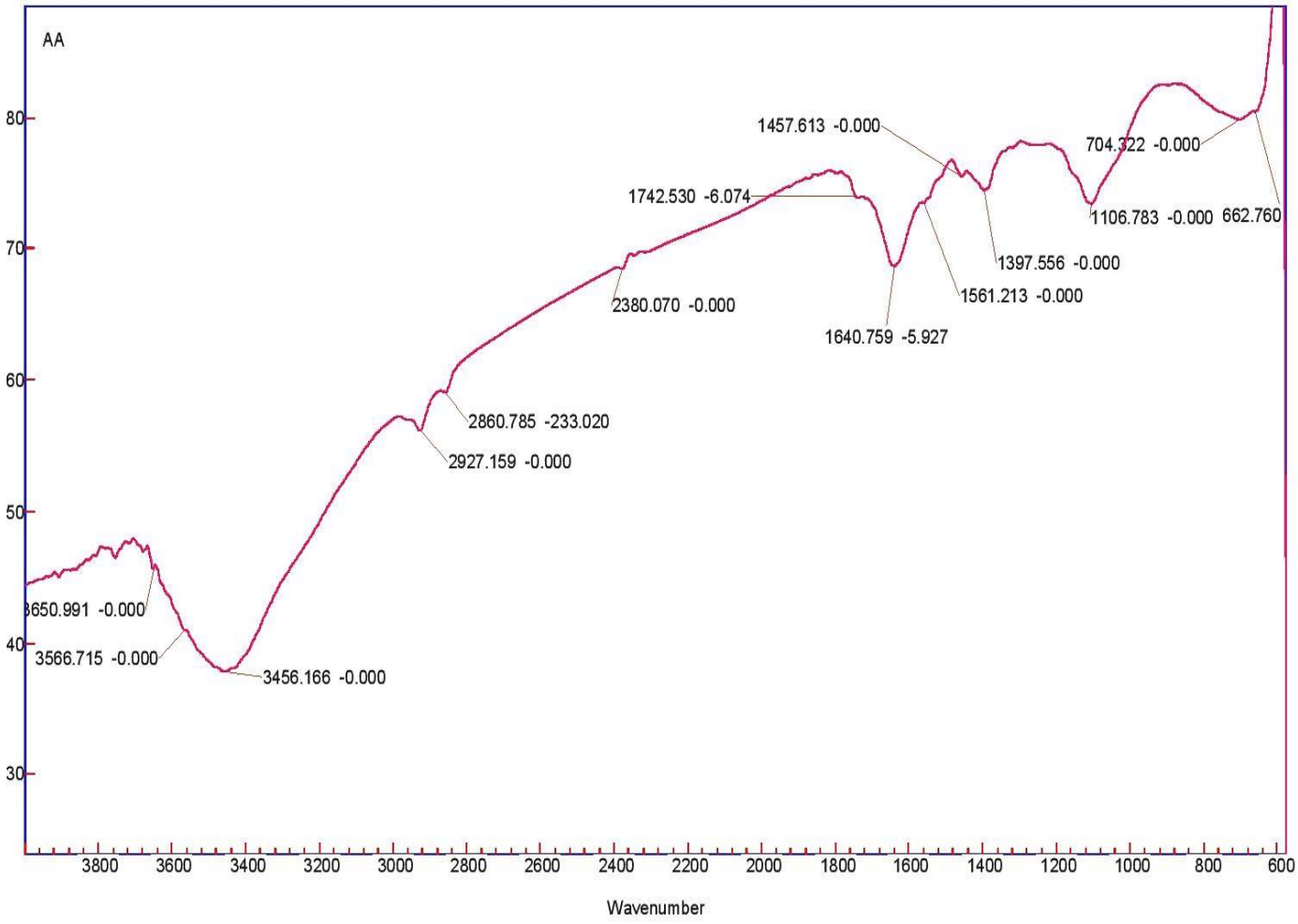

Figure 3. Fourier transfer-infra red (FT-IR) spectrum of *Pn*-MLE

Table 3. Components identified from *Pn*-MLE by GC-MS (Code No. 365)

MF	Compounds	MW (g/mol)	RT	PA%	MI
$C_{13}H_{16}O_3$	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	220	9.49	1.07	*RI, MS*
$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	10.89	1.20	*RI, MS*
$C_{17}H_{34}O_2$	Pentadecanoic acid, 14-methyl-, methyl ester	270	11.73	3.82	*RI, MS*

$C_{19}H_{25}NO_5$	Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester	347	12.23	1.44	*RI, MS*
$C_{16}H_{32}O_2$	n-Hexadecanoic acid	256	12.44	8.71	*RI, MS*
$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester	296	13.68	0.31	*RI, MS*
$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-	280	14.48	3.11	*RI, MS*
$C_{20}H_{40}O_2$	Octadecanoic acid, ethyl ester	312	14.81	9.93	*RI, MS*
$C_{17}H_{32}O_2$	7-Methyl-Z-tetradecen-1-ol acetate	268	17.44	0.22	*RI, MS*
$C_{17}H_{31}F_3O_2$	3-Trifluoroacetoxypentadecane	324	18.79	0.31	*RI, MS*
$C_{24}H_{38}O_4$	1,2-Benzenedicarboxylic acid, diisooctyl ester	390	19.45	2.59	*RI, MS*
$C_{21}H_{32}O_2$	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	316	20.16	0.38	*RI, MS*
$C_{23}H_{34}O_5$	Gitoxigenin	390	21.90	0.42	*RI, MS*
$C_{30}H_{48}O_2$	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-	440	22.73	24.76	*RI, MS*
$C_{22}H_{22}O_3$	2,6-Bis(4-methoxybenzylidene)cyclohexanone	334	24.15	2.49	*RI, MS*
$C_{26}H_{36}O_3$	3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-7-one	396	25.19	9.83	*RI, MS*
$C_{19}H_{18}O_3S$	Propenone, 1-[5-(3-hydroxy-3-methyl-1-butynyl)-2-thienyl]-3-(4-methoxyphenyl)-	326	26.50	3.24	*RI, MS*
$C_{23}H_{32}O_6$	Strophanthidin	404	27.88	5.75	*RI, MS*
$C_{30}H_{44}O_9$	Cymarin	548	29.19	2.76	*RI, MS*
$C_{22}H_{40}O_2$	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	336	29.93	2.99	*RI, MS*
$C_{28}H_{48}O$	Cholestan-3-ol, 2-methylene-, (3á,5à)-	400	30.84	7.98	*RI, MS*
$C_{28}H_{46}O$	Cholesta-8,24-dien-3-ol, 4-methyl-, (3á,4à)-	398	32.46	3.98	*RI, MS*
$C_{26}H_{44}O_5$	Ethyl iso-allocholate	436	33.86	2.70	*RI, MS*
Total percentage of chemical compositions					100.00

*RT = Retention time (min)

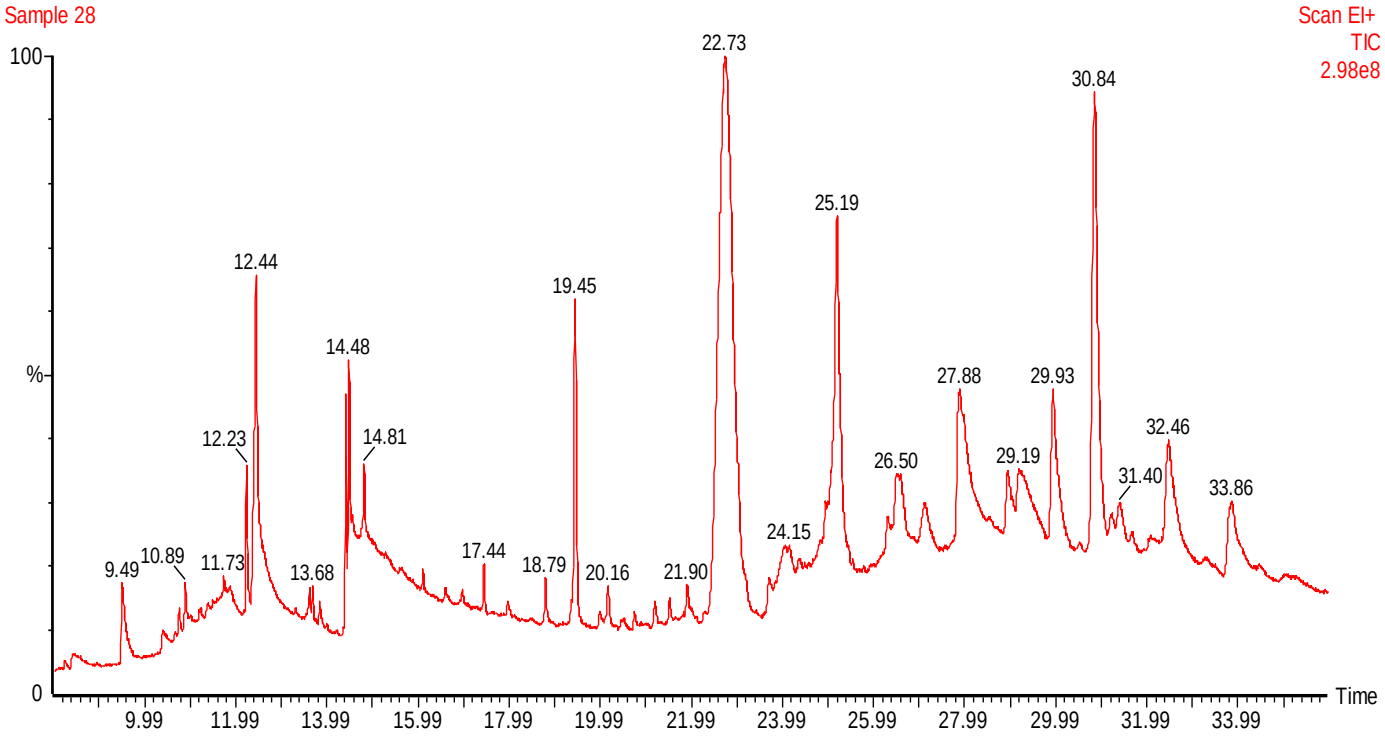

Figure 4. GC-MS Chromatogram of *Pn*-MLE

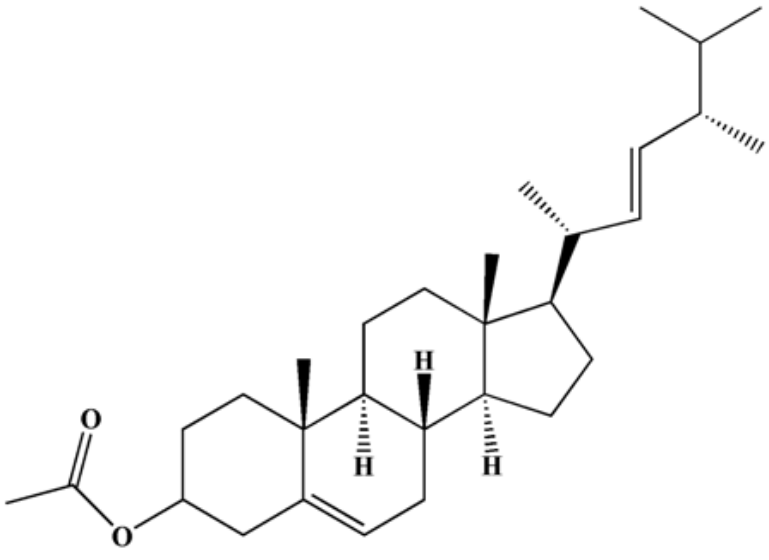

Figure 5. 2D structure of compound, Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-

Environmental safety of an insecticide is of paramount importance while employing against vectors. An insecticide need not cause high mortality on target organisms in order to be acceptable (Baranitharan *et al.*, 2016). Due to the resistance developed by the mosquito against

chemical pesticides, scientists need to identify new larvicides compounds from the natural products (Benelli, 2016). In results showed that, *Pn*-MLEs have significant larval and pupal activity against *An. stephensi*. Table 1 shows that, Fr-1, Fr-2, Fr-3, Fr-4, Fr-5 and Fr-6 showed the LC_{50} values were 98.21 ppm, 86.49 ppm, 39.72 ppm, 31.46 ppm, 27.19 ppm, 25.80 ppm, and Fr-6 was found to be most effective for this activity provided pupa stage at death (NDP, TDP, DP, DA and TM%), and stage of non-mortality (NAE and AE%) (Figures 6 &7).

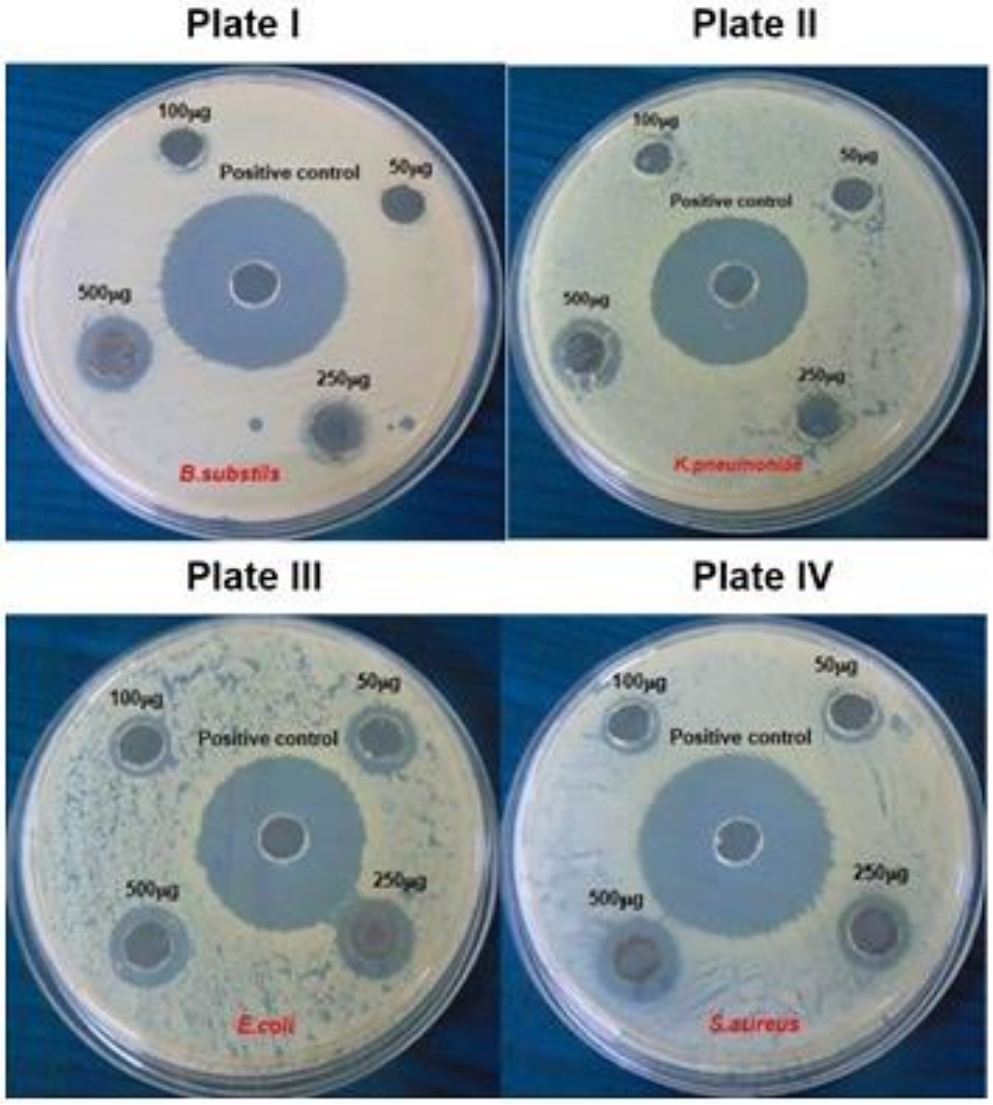

Figure 6. Disc diffusion assay of *Fm*-MLE

The consequence of present study are comparable with earlier reports to study the *Spondias mombin* dichloromethane fractions was the most effective fraction with LC_{50} value of 2172.815 µg/ml. Compounds identified were mainly ellagic acid and 1-*O*-Galloyl-6-*O*-luteoyl-α-D-glucose (Elijah *et al.*, 2016), and the most effective larvicidal activity with concentrations 0.4% *Cassia tora* extracts gave 80% mortality (Swati and Mansi, 2015), further LC_{50} 338.27

mg/L from *Sesamum indicum*-MLE in the larvae of *An. stephensi* (Baranitharan *et al.*, 2015). The highest larval and pupae of values LC_{50} = 137.40 ppm, 172.65 ppm, 217.81 ppm, 269.37 ppm and 332.39 ppm from *Euphorbia hirta*-MLE against *An. stephensi* (Panneerselvam *et al.*, 2013), other one plant *Cassia fistula*-MLE was tested larvicidal, with the LC_{50} value of 17.97 mg/L, respectively (Govindarajan *et al.*, 2008). The lethal activity values of *Commiphora caudata*-ELE were LC_{50} 96.04 mg/L (Baranitharan and Dhanasekaran, 2014), and *Croton sparciflorus*-ELE was reported with LC_{50} 28.88 mg/L, respectively (Baranitharan *et al.*, 2014). Phytochemicals primarily affect the midgut epithelium and secondary affect the gastric caeca and the malpighian tubules in mosquito larvae (David *et al.*, 2000). Furthermore, the highest larval mortality was noticed *P. zeylanica* against *An. stephensi* (LC_{50} = 222.34 mg/L), respectively (Patil *et al.*, 2010). *Annona reticulata*-LE was found more effective against *An. stephensi* larvae with LC_{50} values of 262.71 ppm, respectively (Mohankumar *et al.*, 2016).

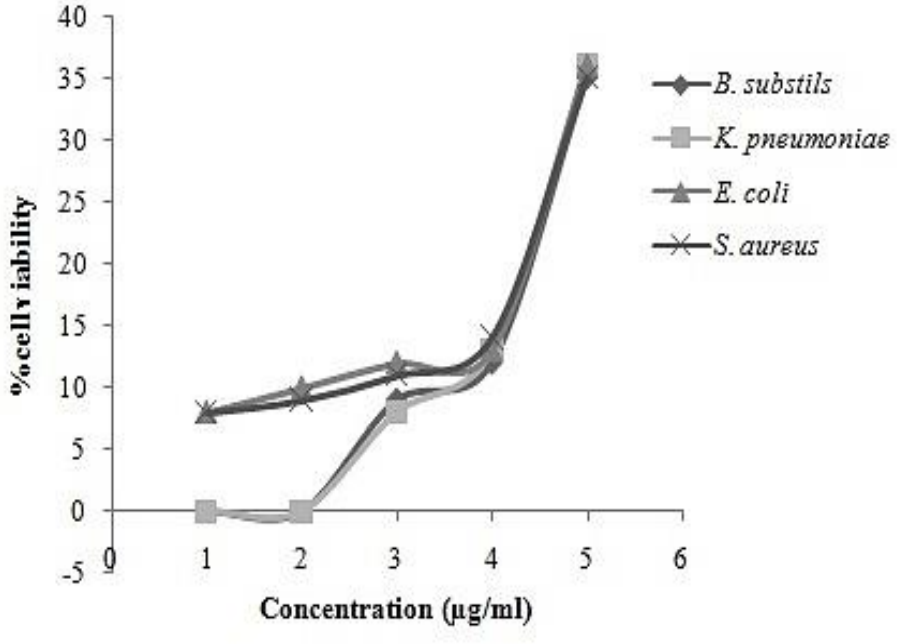

Figure 7. Antibacterial activity of *Pn*-MLE

The consequence of present study is comparable with earlier reports to study the GC-MS analysis of *Punica granatum*-ELE was identified seven MCCs, and the important Methyl 4-piperidineacetate (Baranitharan *et al.*, 2019), and ellagic acid and 1-*O*-Galloyl-6-*O*-luteoyl-α-D-glucose identified from *Spondias mombin* dichloromethane fraction (Elijah *et al.*, 2016). LC-MS analysis of the chloroform extract provided a hesitant identification of 13 components, and Bis-(3-oxaundecyl) tetrasulfide was known as important compound in A7 fraction (Ke-Xin *et al.*, 2015). *Citrus limetta*-MPCs presence of 6 compounds, Corynan-17-01,18,19-didehydro-10-methoxy-,acelate (ester) was found as MPC (39.01%) (Baranitharan *et al.*, 2020). The MCCs of 24 compounds was identified from *Melissa officinalis* compounds noticing to 98.73% (Baranitharan *et al.*, 2016), and totally MCCs 9 were identified in the *Coleus aromaticus*-MLE (Baranitharan *et al.*, 2017). *Punica granatum*-MLE was first identified using a GC-MS analysis and important was phenol, 2-methyl-5-(1-methylethyl) (Jebanesan *et al.*, 2020).

The MCCs of 24 compounds was identified Citronellal compound from *Melissa officinalis* noticing to 98.73%. (Baranitharan *et al.*, 2016). Systematic bioassay-guided fractionation of *Veratrum lobelianum*-E reported in the isolation of five compounds such as ethyl palmitate, ethyl linoleate, β-sitosterol, resveratrol and oxyresveratrol from mass spectral (Nurhayat *et al.*, 2018). Unsaturated fatty acids such as oleic acid, linoleic acid, linolenic acid, and elaidic acid were more toxic than the saturated fatty acids (Perumalsamy *et al.*, 2015).

The antibacterial assay showed, *E. coli* and *P. aeruginosa* were more sensitive to than *S. aureus*, which was resistant at all concentrations (Chaves *et al.*, 2020). Bacteria can also respond to adverse conditions in a transient way, through so-called stress tolerance responses, and it stress tolerance responses include structural and physiological modifications in the cell, complex genetic regulatory machines mediate them (Alvarez-Ordone *et al.*, 2015). *Origanum majorana* noticed the highest antimicrobial activity, particularly against *Candida albicans* and *Salmonella typhimurium* (Duru *et al.*, 2004), and Lamiaceae, which may also be active against *E. coli* and *Enterococcus faecalis* (Singh *et al.*, 2015, Thosar *et al.*, 2013). *Erythroxylum catuaba*-CLE have shown antibacterial activity in vivo against gram positive, *E. coli* and gram negative, *S. aureus* (Mahady, 2005), and shown by *Simarouba glauca* tested against some enterobacteria (Caceres *et al.*, 1990). Some studies have argued that monoterpenes can cross cell membranes and interact with intracellular sites critical for antibacterial activity (Turchi *et al.*, 2017). *Tephrosia purpurea* and *Bacillus sphaericus* materials could serve as a potential larvicidal agent (Ramesh *et al.*, 2017). *Bacillus sphaericus* isolated from soil sample and used to control the malarial vector *An. stephensi* (Kovendan *et al.*, 2012). Cytotoxicity assays revealed that the TNJ-Cr and TNJ-Bs ethanolic extracts reduced viability of CCA cell lines through induction of apoptosis by up-regulation of p53 and Bax proapoptotic proteins, and promoted ROS generation by activating the ERK1/2 signaling in well-differentiated CCA cells KKU-213B (Jeerati *et al.*, 2021).

The finding of the present investigation, Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-compound of *Pn*-CLEs was attempted. Further we demonstrated the possible application of *Pn*-MLE in medical field as it shows anti-mosquito larvicidal as well as its antibacterial activity against *An. stephensi*. The data represented in our study contributes to a novel and unexplored area of *Pn*-MLE as an alternative medicine for future.

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