INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

*** Corresponding author**.

krishnappaprofessor@gmail.com

Phyto-Constituents of *Manihot esculenta* Crantz. (Euphorbiaceae): A Novel Bio-Weapon Against Human Threats Ecto -Parasitic Vectors and Lesser Environmental Risk

Thangarasu Mohan[1], Jeganathan Pandiyan[2], Govindaraju Chandru[2], Samidurai Jayakumar[2], Kuppusamy Elumalai[3], Sabapathy Vijayakumar[4], Karuvi Sivalingam Subasri[5], Kaliyamoorthy Krishnappa[2]*, Murugaiyan Annathurai[3], Joothi Pillai Paramanandham[2]

1 PG & Research Department of Zoology, Guru Nanak College, Velachery, Chennai, 600042, Tamil Nadu, India
2 Department of Zoology and Wildlife Biology, A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai, 609305, Tamil Nadu, India
3 Department of Advanced Zoology and Biotechnology, Govt. Arts College (Autonomous), Chennai, 600035, Tamil Nadu, India
4 PG & Research Department of Zoology, A.V.V.M Sri Pushpam College (Autonomous) (Affiliated to Bharathidasan University), Thiruchirappalli - 24), Poondi, Thanjavur, 613 503, Tamil Nadu, India
5 Department of Mathematics, Vivekananda College of Arts and Science for Women, Sirkali, 610003, Tamil Nadu, India

Abstract

Objectives: To identify the phyto-compounds from *Manihot esculenta* medicinal flora as well as leaf methanolic extract and major phyto-compounds were tested against larvae of medical pests. **Methods:** The various phytocompounds were isolated through GC-MS analysis, the selected phytocompounds and extract were tested by various concentrations against larvae of medical pests *Aedes vittatus*, *Anopheles subpictus* and *Culex vishnui*. **Findings:** A total of 65 PCs acquiring 100% and the Me-MPCs: 1,7-Dimethyl-4-(1-methylethyl)cyclodecane, Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters and Dibutyl phthalate were identified which strongly confirmed through GC-MS studies. Me-LME and Me-MPCs: 1,7-Dimethyl-4-(1-methylethyl)cyclodecane, Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters and Dibutyl phthalate were tested against 3rd instars larvae of vector species *Aedes vittatus, Anopheles subpictus* and *Culex vishnui* with their LC_{50}/ LC_{90} values were 64.17/125.03, 9.363/17.17, 11.88/22.12, 14.33/26.82 and 89.65/177.62, 9.47/19.80, 11.94/24.90, 15.41/31.55 and 115.62/221.16, 11.51/23.82, 14.05/29.26 and 17.88/36.16 μg/mL respectively. **Novelty:** The selected phyto-products (Me-LME and Me-MPCs) were noticeably harmless tool on non-target fauna and very outstanding expression towards vector control approaches. In meticulously, the pure Me-MPCs were found multifold toxicity

than the Me-LME in larval toxicity on selected mosquitoes which showed statically significant at $p \leq 0.05$ level.

Keywords: Phytoconstituents; Biotoxicity; Ecosafety; GCMS analysis; Vector mosquitoes

1 Introduction

Among the arthropods dipterans the mosquitoes are predominant disease spreader which are very deadliest enemies against blood yielding higher fauna including Mammals, Aves, Reptiles and Fishes[1]. Across the globe, around 100 countries have been heavily struggled with mosquito borne diseases (MBDs) especially in tropical and sub-tropical terrains which are more preferable climatic conditions for massively proliferation of vectors[2]. *Aedes vittatus* are mostly exhibited the diurnal behavior, perhaps more abundance at crepuscular peak, which has wide ranges of hosts, and they adopted to survive even in man-made locations, they known to breed in both natural and artificial containers. *Ae. vittatus* play a momentous role in medical field and also witnessed the successful transmission of life-threatening many human diseases[3]. *Anopheles subpictus* is a common blood sucking vector widely distributed in many topographies of India, Pakistan, Myanmar, Thailand, Saudi Arabia and Iran[4] and it is highly abundance in rural and urban areas of Asian continent and effectively transmitted malaria disease to public[5]. *Culex vishnui* breeds extensively in the different habitats of freshwater bodies especially in agro-ecosystems. In addition, with favorable ecological condition, other coherent activities like poor awareness about vectors and its borne diseases, high abundance of thrown containers, the hyper resistance with modern synthetic insecticides (SIs) and higher availability of hosts driven the exponential growth of mosquito vectors[6] and thereby control measures are bit challenging.

Recently, vector control/eradication is an importance challenge as the results large scale of population/livestock getting more threats by mosquito borne diseases[7]. The plenty of unadvisable SIs using in many countries for eliminating mosquitoes and its diseases consequently, we receiving countless health troubles and high level disabling of non-target fauna and flora, poor soil viability, decaling beneficial microbes and decomposer counts, negative impacts on food chain/ food web and the above half of natural predators, parasites, pollinators destroyed due to the extensive use of SIs. As an alternative to SIs, the use of phyto-pesticides having multipurpose and dynamic activities on vector control mechanism. India has a very long history of the use of phyto-products as traditional medicine as well as pest control tool as it has least/ zero toxicity to public, livestock and other non-target fauna (NTF)[8,9]. *Manihot esculenta* Crantz. (Euphorbiaceae) otherwise called cassava, they are the woody shrub extensively cultivated in tropical and subtropical terrains. Cassava roots mostly consumed in boiled form by human but raw food used for animals as feed. Cassava has been reported to serve as good source of nutrients, several sets of vitamins and acting as a good source of phyto-chemicals. The available literature indicate the use of *M. esculenta* and its derived phyto-constituents' vital agents for prohibiting vector population and friendlier tool on NTF. Therefore, this work is a novel and could be considered as a modern eco-friendly bio-weapon against human threats- ecto-parasitic vectors.

2 Methodology

2.1 Leaves collection and extraction method

The well cleaned and fresh, fully matured and diseases free Cassava green leaves were collected from Karuvi Village, Poompuhar East Coastal Zone of Tamil Nadu, India. The leaves were thoroughly washed using distilled H_2O and shade dried at optimal

temperature ($28\pm2^\circ$C) for minimum 10 days. Nearly 250 g of well dried leaf were made into powder by using high speed electrical blender. The fine powder carefully loaded in a Soxhlet apparatus and extract was prepared by methanol. The yielded raw extract was evaporated to dryness in Rotary Vacuum Evaporator and the dried residues obtained were stored in airtight bottles in a refrigerator for further use [10].

2.2 Gas chromatography-mass spectrum (GC-MS) analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m x 0.25mm ID x 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1μL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10°C min^{-1}; and 300°C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec as well as the fragments from 40 to 600 Da [11].

2.3 Medical vectors

The medical vectors (*Ae. albopictus, An. maculates* and *Cx. mimulus*) primary life aquatic stages (Eggs/larvae/pupae) were collected from Agronomic field, Cauvery Delta Zone, Mayiladuthurai District, Tamil Nadu, India. The collected primary stages of vectors were identified by ICMR-Centre, Madurai, Tamil Nadu and carried to laboratory for incessant rearing. The larval feed was prepared with the composition of biscuits, yeast powder and *Apis florea* honey 3:1:1 ratio and adults feed were prepared sucrose, *Apis florea* honey and one-week age old chick for blood meal 1:1:1 ratio. Mosquitoes were maintained at $28 \pm 4^\circ$C, $75\pm4\%$ RH, with a photo period of 10L: 14D.

2.4 Larval toxicity on target fauna

The larval toxic effects of *Manihot esculenta* leaf methanol extract (Me-LME) and major phyto-compounds (Me-MPCs) were estimated using standard operating protocol [12]. The entire bioassay was investigated between 04 - 250 μg/mL and the selected phyto-concentrations were applied on earlier stage of 3rd instars larvae (0-5h age old). The Me-LME and Me-MPCs were diluted in 1-2 mL of DMSO then thoroughly mixed with 445 mL chlorine free H$_2$O. Each target fauna, in average 20 larvae used for every concentration were replicated five times constantly larval death rates were observed every 3 hrs. and up to 24 hrs. of post treatment. The death rates of control and treatment were corrected with applying following formula [13]. The LC$_{50}$/LC$_{90,}$ chi-square, regression and other valuable statistics were manipulated by using probit analysis [14].

2.5 Statistical analysis

The larval and NTF average death rates were calculated into LC$_{50}$/LC$_{90,}$ 95% confidence limit by applying IBM –SPSS- 25.0, results were p $\le$ 0.05 considered to be statistically significant.

3 Results and discussion

3.1 Identification of PCCs by GC-MS analysis

M. esculenta LME (Me-LME) was subjected into GC-MS spectral analysis for finding the valuable functional PCs is displayed in Figure 1 and their various parameters were evidently shown in Table 1. A total of 65 PCs acquiring 100% and the MPCs of Me-LME were (1). 1,7-Dimethyl-4-(1-methylethyl)cyclodecane (Peak- 10, retention time- 16.327, area- 2901542, area%- 14.21, height- 54696, height%- 0.46 and compound formula-C$_{15}$H$_{30}$) (2). Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters (Peak- 14, retention time- 18.364, area- 1616700, area%- 7.92, height- 350812, height%- 0.24 and compound formula-C$_{19}$H$_{30}$O) and (3). Dibutyl phthalate (Peak- 35, Retention time- 26.521, Area- 1611579, Area%- 7.89, Height- 599589, Height%- 3.52 and Compound formula- C$_{16}$H$_{22}$O$_4$) as well as the identified MPCs were strongly confirmed through MS studies they shown in Figures 2, 3 and 4. Earlier, many research works found on different floral origin, and they were potential vector controlling tool on egg, juvenile and adult stages of life [11–15]. The selected phyto-products showed outstanding output toward the control of vector mosquitoes. Previously, similar type of observations were noticed by using *C. limetta* Cl-LME under visualized into GC-MS analysis as results *C. limetta* major phyto-compound: Corynan-17-0l,18,19-didehydro-10-methoxy-,acelate (ester) identified and it was applied with against 3rd instars larvae *Ae. albopictus, An. maculatus* and *Cx mimulus. C.*

limetta major phyto-compound at lower concentration itself showed topper mortality apart from that the same *C. limetta* major phyto-compound showed considerably countable/lesser mortality were showed on non-target fauna[10]. *J. repens* leaf ethanol extract assessed by GC-MS analysis found *J. repens* major phyto-compound: 4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl]-1H-indol-2-yl]-á-methyl-, methyl ester the both natural compositions were showed predominant aquatic juvenile (larvae) toxicity on medical vectors: *Ae. albopictus, An. stephensi* and *Cx. quinquefasciatus*[9]. GC-MS spectral analysis is a basic/fundamental assessment for finding the naturally available functional groups from various floral communities[16].

Table 1. GC-MS analysis of Manihot esculenta Me -LME

PE	RT	ST	ET	AR	AR %	HE	HE %	A/H	CN
1	6.45	6.415	6.485	37541	0.18	18624	0.22	2.02	([(1Z)-1,3-DIPHENYL-1-PENTENYL]OXY)(TRIMETHYL)SILANE #
2	10.046	9.985	10.09	840096	4.12	429599	5.14	1.96	CYCLOPENTASILOXANE, DECAMETHYL-
3	11.4	11.345	11.445	83029	0.41	33585	0.4	2.47	1-UNDECANOL
4	11.602	11.575	11.64	34665	0.17	19307	0.23	1.8	OCTADECYL FLUORIDE
5	12.569	12.53	12.615	84649	0.41	43154	0.52	1.96	CYCLOHEXANE, HEXYL-
6	13.976	13.895	14.035	688265	3.37	798039	9.56	2.03	Cyclohexasiloxane, dodecamethyl-
7	15.398	15.36	15.43	58605	0.29	32912	0.39	1.78	CYCLOHEXANE, (3-METHYLPENTYL)-
8	15.962	15.92	16.005	344770	1.69	172150	2.06	2	1-TRIDECENE
9	16.139	16.09	16.19	42310	0.21	19689	4.2	1.96	TETRADECANE
10	16.327	16.29	16.37	2901542	14.21	54696	0.46	1.9	1,7-Dimethyl-4-(1-methylethyl)cyclodecane
11	17.206	17.165	17.245	83947	0.41	42047	0.5	2	CYCLOHEXANE, HEXYL-
12	17.593	17.545	17.645	756818	3.71	372361	4.46	2.03	Cycloheptasiloxane, tetradecamethyl-
13	18.246	18.195	18.3	416838	2.04	212399	2.54	1.96	HEPTADECANE
14	18.364	18.3	18.405	1616700	7.92	350812	0.24	2.15	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters
15	19.957	19.915	20.005	66902	0.33	27485	0.33	2.43	1-UNDECENE, 9-METHYL-
16	20.093	20.005	20.165	696432	3.41	322145	3.86	2.16	1-Heptadecene
17	20.242	20.165	20.29	416927	2.04	209064	2.5	1.99	NONADECANE
18	20.834	20.785	20.88	371011	1.82	181836	2.18	2.04	Cyclooctasiloxane, hexadecamethyl-
19	21.375	21.335	21.415	64971	0.32	32468	0.39	2	CYCLOHEXANE, UNDECYL-
20	21.589	21.55	21.63	69628	0.34	36574	0.44	1.9	8-Pentadecanone
21	22.137	22.095	22.185	173629	0.85	89093	1.07	1.95	NONADECANE
22	22.823	22.785	22.85	44135	0.22	23068	0.28	1.91	2(4H)-BENZOFURANONE, 5,6,7,7A-TETRAHYDRO-6-HYDROXY-4,4,7A-TRIMETHYL-, (6S-CIS)-
23	23.634	23.59	23.665	235352	1.15	115985	1.39	2.03	Cyclononasiloxane, octadecamethyl-
24	23.692	23.665	23.75	151255	0.74	64405	0.77	2.35	BICYCLO[2.2.1]HEPTAN-2-ONE, 5-HYDROXY-4,7,7-TRIMETHYL-, EXO-
25	23.814	23.75	23.865	812702	3.98	375356	4.49	2.17	1-Nonadecene
26	23.938	23.865	23.98	113833	0.56	47363	0.57	2.4	DECANE, 2,3,7-TRIMETHYL-
27	24.566	24.52	24.62	1396164	6.84	631249	7.56	2.21	Neophytadiene
28	24.68	24.62	24.72	137587	0.67	36482	0.44	3.77	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-
29	24.985	24.94	25.04	286093	1.4	134920	1.62	2.12	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
30	25.113	25.08	25.145	42825	0.21	21238	0.25	2.02	Cyclohexane, (1-methylethyl)-
31	25.185	25.15	25.235	107623	0.53	55530	0.66	1.94	8-Octadecanone
32	25.298	25.245	25.38	424635	2.08	187426	2.24	2.27	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
33	26.127	26.095	26.165	117052	0.57	59203	0.71	1.98	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-ICOSAMETHYLCYCLODECASILOXANE #
34	26.275	26.24	26.305	50657	0.25	22584	0.27	2.24	2-(1,3-BENZOTHIAZOL-2-YLSULFANYL)ETHANOL #
35	26.521	26.47	26.575	1611579	7.89	599589	3.52	2.09	Dibutyl phthalate
36	27.075	27.045	27.14	59369	0.29	18815	0.23	3.16	2,3,4-Trimethyl-1-pentanol
37	27.187	27.14	27.235	633394	3.1	278280	3.33	2.28	1-Nonadecene
38	27.289	27.235	27.325	87303	0.43	40786	0.49	2.14	DECANE, 2,3,7-TRIMETHYL-
39	28.4	28.35	28.44	134899	0.66	55803	0.67	2.42	CYCLODODECASILOXANE, TETRACOSAMETHYL-
40	28.5	28.44	28.54	73686	0.36	19284	0.23	3.82	Cyclohexane, octadecyl-

Continued on next page

							Table 1 continued			
41	28.986	28.93	29.095	838969	4.11	297746	3.57	2.82	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R*,R*-(E)]]-	
42	30.264	30.215	30.315	351364	1.72	154201	1.85	2.28	1-Nonadecene	
43	30.349	30.315	30.395	51056	0.25	20797	0.25	2.45	Undecane, 2,4-dimethyl-	
44	30.464	30.395	30.505	114835	0.56	48642	0.58	2.36	CYCLONONASILOXANE, OCTADECAMETHYL-	
45	30.629	30.595	30.67	44610	0.22	18987	0.23	2.35	Oxalic acid, monomorpholine, undecyl ester	
46	32.372	32.325	32.425	91210	0.45	38290	0.46	2.38	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	
47	33.093	33.05	33.14	156185	0.77	77133	0.92	2.02	1-Nonadecene	
48	34.12	34.085	34.15	79224	0.39	32090	0.38	2.47	BENZOFLEX	
49	34.176	34.15	34.245	110727	0.54	40087	0.48	2.76	CYCLONONASILOXANE, OCTADECAMETHYL-	
50	34.875	34.81	34.97	613138	3	293782	7.18	2.69	Bis(2-ethylhexyl) phthalate	
51	35.193	35.075	35.335	187768	0.92	544728	6.52	5.33	Cholesterol	
52	35.705	35.665	35.74	77687	0.38	32025	0.38	2.43	(CIS)-2-NONADECENE	
53	35.875	35.82	35.91	79318	0.39	28457	0.34	2.79	CYCLONONASILOXANE, OCTADECAMETHYL-	
54	37.484	37.405	37.535	207299	1.02	58261	0.7	3.56	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	
55	38.145	38.05	38.16	211475	1.04	61178	0.73	3.46	DESACYL-KONDURANGOGENINS A	
56	38.177	38.16	38.225	147485	0.72	53023	0.63	2.78	OCTADECANOIC ACID, 16-OXO-, METHYL ESTER	
57	38.334	38.225	38.39	72352	0.35	38140	0.66	3.43	Squalene	
58	38.855	38.835	38.87	20103	0.1	19272	0.23	1.04	Cycloheptasiloxane, tetradecamethyl-	
59	38.961	38.935	38.98	72147	0.35	36226	0.43	1.99	SILICONE GREASE, SILICONFETT	
60	38.995	38.98	39.01	35686	0.17	26068	0.31	1.37	SILANE, 1,4-PHENYLENEBIS[TRIMETHYL-	
61	39.085	39.01	39.105	102746	0.5	26036	0.31	3.95	Benzoic acid, 3-(2,3-dimethoxy-4,5-methylenedioxyphenyl)propyl ester	
62	39.12	39.105	39.205	85406	0.42	24565	0.29	3.48	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane	
63	39.345	39.33	39.36	26575	0.13	19689	0.24	1.35	2,6-Lutidine 3,5-dichloro-4-dodecylthio-	
64	39.425	39.36	39.44	132184	0.65	35451	0.42	3.73	Ethyl 1-thio-.alpha.-d-arabinofuranoside	
65	39.993	39.94	40.05	134801	0.66	40249	0.48	3.35	.delta.-Tocopherol	

PE: Peak; RT: Retention time; ST: Start time; ET: End Time; AR: Area; AR%: Area %; HE: Height; HE%: Height %; A/H: Area /Hight; CN: Compound Name

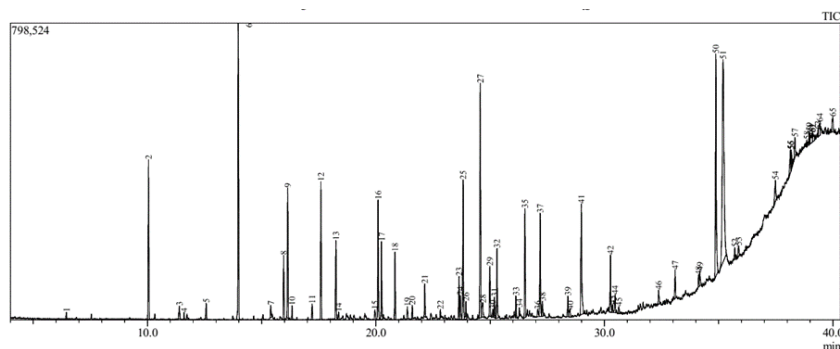

Fig 1. GC-MS chromatogram of *Manihot esculenta* floral Me -LME

3.2 Larval toxicity of Me-LME and Me-MPCs

Me-LME and Me-MPCs were tested against 3rd instars larvae of three different vector mosquitoes *Ae. vittatus, An. subpictus* and *Cx. vishnui*. The larval death (Maximum/ minimum) were directly propositional to the concentrations of Me-LME and Me-MPCs (1,7-Dimethyl-4-(1-methylethyl)cyclodecane, Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters and Dibutyl phthalate) LC$_{50}$/LC$_{90}$ values were 64.17/125.03, 9.36/17.17, 11.88/22.12 and 14.33/26.82 μg/mL respectively, against 3rd instar larvae of *Ae. vittatus*. The Me-LME and Me-MPCs (1,7-Dimethyl-4-(1-methylethyl)cyclodecane, Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters and Dibutyl phthalate) LC$_{50}$/LC$_{90}$ values were 89.65/177.62, 9.47/19.80, 11.94/24.90 and
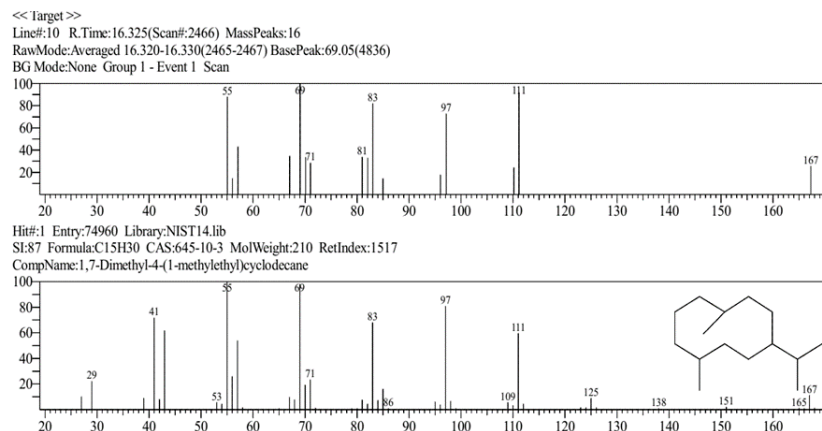
Fig 2. Mass spectrum and structure of Me-MPCs 1,7-Dimethyl-4-(1-methylethyl)cyclodecane identified through GC-MS in the *M. esculenta* Me -LME
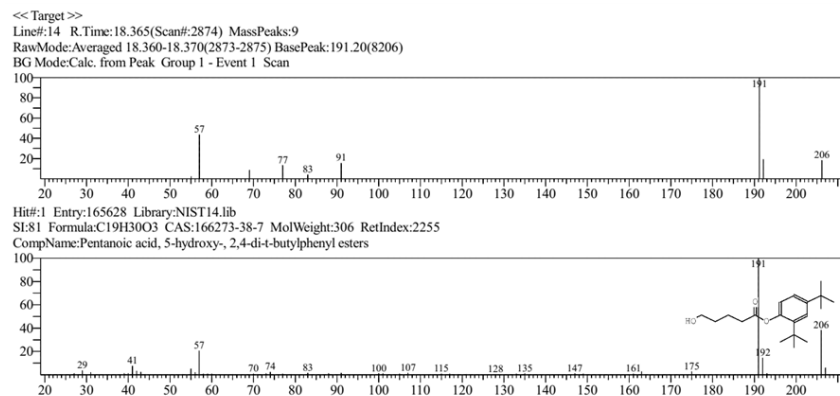

Fig 3. Mass spectrum and structure of Me-MPCs Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters identified through GC-MS in the *M. esculenta* Me -LME
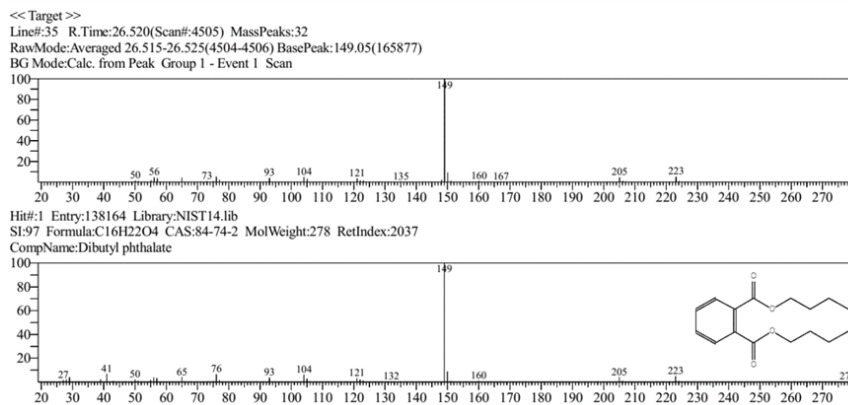

Fig 4. Mass spectrum and structure of Me-MPCs Dibutyl phthalate identified through GC-MS in the *M. esculenta* Me -LME

15.41/31.55 μg/mL respectively, against 3rd instar larvae of *An. subpictus*. The Me-LME and Me-MPCs (1,7-Dimethyl-4-(1-methylethyl)cyclodecane, Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters and Dibutyl phthalate) LC$_{50}$/ LC$_{90}$ values were 115.62/221.16, 11.51/23.82, 14.05/29.26 and 17.88/36.16 μg/mL respectively, against 3rd instar larvae of *Cx. vishnui*. The other important statistical values LCL, UCL, Regression, Chi-square values are statically significant at p$\leq$ 0.05 level and larval toxicity were clearly shown in Table 2. The current investigation outputs are compared with previously published similar reports, the various medicinal floral extracts and its major phyto-constituents showed a prime toxic effect on aquatic juvenile (various larval stage) of vectors: Corynan-17-0l,18,19-didehydro-10-methoxy-, acelate (ester) (*C. limetta*); 4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl]-1H-indol-2-yl]-á-methyl-, methyl ester (*J. repens*) [9,10]. Previously, several reported outcomes extremely supported with present research, the mosquito larval toxicity potential of various phyto-compositions against different mosquitoes [17–19]. Similarly, present investigation authentically supported by many of the previously published research explorations against pupae of vector mosquitoes, the various phyto-products (extract/essential oil/phyto-compound) of *Helicteres velutina* [20], *Lavandula latifolia* [7] showed hyper toxicity against pupae of various vectors mosquitoes. The major phyto-compounds of Heptasiloxane, hexadecamethyl- and 1,1-Dimethylethyl 3-Phenyl-2-Propenoate were derived from *I. tinctoria* Indian medicinal flora which tested against human vector mosquitoes: *Ae. aegypti* and *Cx. quinquefasciatus* earlier larval stage (3rd instar) and it's LC$_{50}$/LC$_{90}$ data were 10.93/18.65 μg/mL, 10.87/18.77 and 11.16/19.38 μg/mL and 10.43/18.51 μg/mL, respectively [21]. The major phyto-compounds of Fumaric acid, di(1-adamantylmethyl) ester and 2-Pentamethyldisilanyloxypentane were obtained from Indian medicinal flora *P. longifolia* were tested towards 3rd instar larvae of human vector mosquitoes: *Ae. aegypti* and *Cx. quinquefasciatus* and its LC$_{50}$/LC$_{90}$ data were 11.01/18.73 μg/mL, 10.56/17.90 μg/mL and 10.91/18.75 μg/mL and 10.70/18.26 μg/mL respectively [22].

Table 2. Larval toxicity of *Manihot esculenta* Me-LME and Me-MPCs against 3rd instar larvae of vector mosquitoes

Phyto-products	LC$_{50}$ (μg/mL)	LCL-UCL (μg/mL)	LC$_{90}$ (μg/mL)	LCL-UCL (μg/mL)	R-value	χ^2
Ae. vittatus						
Me-LME	64.17	57.19-70.40	125.03	115.97-136.94	y=1.8+0.02x	6.838
1,7-Dimethyl-4-(1-methylethyl)cyclodecane	9.36	5.89-12.00	17.17	14.05-25.24	y=1.34+0.14x	11.684
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	11.88	6.63-15.71	22.12	17.73-35.36	y=1.71+0.15x	14.046
Dibutyl phthalate	14.33	9.82-17.89	26.82	22.31-37.21	y=1.63+0.12x	8.933
An. subpictus						
Me-LME	89.65	50.38-116.44	177.62	144.46-265.97	y=1.08+0.01x	10.465
1,7-Dimethyl-4-(1-methylethyl)cyclodecane	9.47	8.18-10.57	19.80	18.30-21.79	y=1.04+0.11x	6.428
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	11.94	10.38-13.28	24.90	23.00-27.43	y=1.47+0.12x	6.328
Dibutyl phthalate	15.41	13.59-17.01	31.55	29.12-34.82	y=1.31+0.09x	4.743
Cx. vishnui						
Me-LME	115.62	59.13-154.14	221.16	176.54-359.20	y=1.17+9.89x	13.859
1,7-Dimethyl-4-(1-methylethyl)cyclodecane	11.51	10.00-12.82	23.82	22.03-26.19	y=1.07+0.09x	6.728
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	14.05	12.23-15.62	29.26	27.03-32.21	y=1.25+0.09x	3.893
Dibutyl phthalate	17.88	15.85-19.69	36.16	33.41-39.84	y=1.28+0.07x	2.719

Zero mortality observed under control (without phyto-products); LC$_{50}$ lethal concentration lookout 50% lethality exposed in mosquitoes; LC$_{90}$ = lethal concentration lookout 90% lethality exposed in mosquitoes; UCL 95 % Upper Confidence Limit of LC$_{50}$/LC$_{90}$; LCL 95 % Lower Confidence Limit of LC$_{50}$/LC$_{90}$; R-value = Regrasion value; χ^2 = Chi- square.

4 Conclusion

The outcome of the research revealed that, the *M. esculenta* Me-LME and Me-MPCs exhibited predominant toxicity on selected mosquitoes. In consequently, the present output should be evolved in mosquito control strategy since it shown scanty damaging

on ecologically important NTF. It is a noteworthy bio-weapon on controlling mosquitoes in the wild/human settlements zones.

5 Acknowledgements

References

1) Elumalai K, Mahboob S, Al-Ghanim KA, Al-Misned F, Pandiyan J, Baabu PMK, et al. Entomofaunal survey and larvicidal activity of greener silver nanoparticles: A perspective for novel eco-friendly mosquito control. *Saudi Journal of Biological Sciences*. 2020;27(11):2917–2928. Available from: https://doi.org/10.1016/j.sjbs.2020.08.046.

2) Baranitharan M, Alarifi S, Alkahtani S, Ali D, Elumalai K, Pandiyan J, et al. Phytochemical analysis and fabrication of silver nanoparticles using Acacia catechu: An efficacious and ecofriendly control tool against selected polyphagous insect pests. *Saudi Journal of Biological Sciences*. 2021;28(1):148–156. Available from: https://doi.org/10.1016/j.sjbs.2020.09.024.

3) Al-Ghanim KA, Krishnappa K, Pandiyan J, Nicoletti M, Gurunathan B, Govindarajan M. Insecticidal Potential of Matricaria chamomilla's Essential Oil and Its Components (E)-β-Farnesene, Germacrene D, and α-Bisabolol Oxide A against Agricultural Pests, Malaria, and Zika Virus Vectors. *Agriculture*. 2023;13(4):1–14. Available from: https://doi.org/10.3390/agriculture13040779.

4) Alsalhi MS, Elumalai K, Devanesan S, Govindarajan M, Krishnappa K, Maggi F. The aromatic ginger Kaempferia galanga L. (Zingiberaceae) essential oil and its main compounds are effective larvicidal agents against Aedes vittatus and Anopheles maculatus without toxicity on the non-target aquatic fauna. *Industrial Crops and Products*. 2020;158:113012. Available from: https://doi.org/10.1016/j.indcrop.2020.113012.

5) Wilai P, Ali RSM, Saingamsook J, Saeung A, Junkum A, Walton C, et al. Integrated systematics of Anopheles subpictus (Diptera: Culicidae) in the Oriental Region, with emphasis on forms in Thailand and Sulawesi, Indonesia. *Acta Tropica*. 2020;208:105503. Available from: https://doi.org/10.1016/j.actatropica.2020.105503.

6) Rani A, Nagpal BN, Singh H, Mehta SS, Srivastava A, Saxena R. Potential role of Anopheles subpictus as a malaria vector in Ghaziabad District, Uttar Pradesh, India. *International Journal of Tropical Insect Science*. 2021;41(2):1107–1117. Available from: https://doi.org/10.1007/s42690-020-00296-4.

7) Elumalai K, Krishnappa K, Pandiyan J, Alharbi NS, Kadaikunnan S, Khaled JM, et al. Characterization of secondary metabolites from Lamiaceae plant leaf essential oil: A novel perspective to combat medical and agricultural pests. *Physiological and Molecular Plant Pathology*. 2022;117:101752. Available from: https://doi.org/10.1016/j.pmpp.2021.101752.

8) Esan V, Elanchezhiyan C, Mahboob S, Al-Ghanim KA, Al-Misned F, Ahmed Z, et al. Toxicity of <i>Trewia nudiflora</i>-mediated silver nanoparticles on mosquito larvae and non-target aquatic fauna. *Toxin Reviews*. 2022;41(1):229–236. Available from: https://doi.org/10.1080/15569543.2020.1864648.

9) Krishnappa K, Baranitharan M, Elumalai K, Pandiyan J. Larvicidal and repellant effects of Jussiaea repens (L.) leaf ethanol extract and its major phyto-constituent against important human vector mosquitoes (Diptera: Culicidae). *Environmental Science and Pollution Research*. 2020;27(18):23054–23061. Available from: https://doi.org/10.1007/s11356-020-08917-8.

10) Baranitharan M, Krishnappa K, Elumalai K, Pandiyan J, Gokulakrishnan J, Kovendan K, et al. Citrus limetta (Risso) - borne compound as novel mosquitocides: Effectiveness against medical pest and acute toxicity on non-target fauna. *South African Journal of Botany*. 2020;128:218–224. Available from: https://doi.org/10.1016/j.sajb.2019.11.014.

11) Rajalakshmi R, Vaidehi J, Krishnappa K. Phyto-constituents of Croton bonplandianus as eco-friendly bio-weapon against human vector mosquitoes. *Uttar Pradesh Journal of Zoology*. 2022;43(24):101–109. Available from: https://doi.org/10.56557/upjoz/2022/v43i243300.

12) Krishnappa K, Elumalai K. Toxicity of Aristolochia bracteata methanol leaf extract against selected medically important vector mosquitoes (Diptera: Culicidae). *Asian Pacific Journal of Tropical Diseases*. 2012;2(Supplement 2):S553–S557. Available from: https://doi.org/10.1016/S2222-1808(12)60219-9.

13) Abbott WS. A Method of Computing the Effectiveness of an Insecticide. *Journal of Economic Entomology*. 1925;18(2):265–267. Available from: https://doi.org/10.1093/jee/18.2.265a.

14) Finney DJ. A statistical treatment of the sigmoid response curve. In: Probit analysis. London. Cambridge University Press. 1971;p. XV, 333. Available from: https://search.worldcat.org/title/probit-analysis-3d-ed/oclc/802882862?referer=di&ht=edition.

15) Krishnappa K, Pandiyan J, Elumalai K, Baranitharan M, Jayakumar S, Gokulakrishnan J. GC-MS analysis and mosquitocidal properties of Loranthes pentandrus Linn. (Loranthaceae) against human vector mosquitoes (Diptera: Culicidae). *Academia Journal of Medicinal Plants*. 2019;7(12):261–268. Available from: https://academiapublishing.org/journals/ajmp/abstract/2019/Dec/Krishnappa%20et%20al.htm.

16) Rajalakshmi R, Vaidehi J, Krishnappa K. The naturally available phyto-products of Indian medicinal plants against adulticidal activity of human vector mosquitoes. *Uttar Pradesh Journal of Zoology*. 2022;43(24):80–87. Available from: https://mbimph.com/index.php/UPJOZ/article/view/3298.

17) Elumalai K, Mahboob S, Al-Ghanim KA, Al-Misned F, Pandiyan J, Baabu PMK, et al. Entomofaunal survey and larvicidal activity of greener silver nanoparticles: A perspective for novel eco-friendly mosquito control. *Saudi Journal of Biological Sciences*. 2020;27(11):2917–2928. Available from: https://doi.org/10.1016/j.sjbs.2020.08.046.

18) Ma J, Ugya YA, Isiyakuc A, Hua X, Imam TS. Evaluation of Pistia stratiotes fractions as effective larvicide against Anopheles mosquitoes. *Artificial Cells, Nanomedicine, and Biotechnology*. 2019;47(1):945–950. Available from: https://doi.org/10.1080/21691401.2019.1582538.

19) Zuharah WF, Yousaf A, Ooi KL, Sulaiman SF. Larvicidal activities of family Anacardiaceae on Aedes mosquitoes (Diptera: Culicidae) and identification of phenolic compounds. *Journal of King Saud University - Science*. 2021;33(5):1–8. Available from: https://doi.org/10.1016/j.jksus.2021.101471.

20) Fernandes DA, Rique HL, De Oliveira LHG, Santos WGS, Vanderlei De Souza MDF, Nunes FDC. Ovicidal, pupicidal, adulticidal, and repellent activity of Helicteres velutina K. Schum against Aedes aegypti L. (Diptera: Culicidae). *Brazilian Journal of Veterinary Medicine*. 2021;43(1):1–15. Available from: https://doi.org/10.29374/2527-2179.bjvm112120.

21) Vasumathi D, Senguttuvan S. The Major Phyto-Compounds Heptasiloxane, hexadecamethyl- and 1,1-Dimethylethyl 3-Phenyl-2-Propenoate Derived from Indigofera tinctoria Medicinal Flora Tested Against Various Target Medical and Agronomic Pests. *Indian Journal Of Science And Technology*. 2023;16(16):1178–1186. Available from: https://doi.org/10.17485/IJST/v16i16.310.

22) Vasumathi D, Senguttuvan S. The phyto-compounds Fumaric acid, di(1-adamantylmethyl) ester and 2-Pentamethyldisilanyloxypentane Borne from Physalis longifolia Against Various Medical and Agronomic Pests. *Indian Journal Of Science And Technology*. 2023;16(15):1126–1134. Available from:

https://doi.org/10.17485/IJST/v16i15.306.