

Phytochemical screening and larvicidal efficacy of *Elephantopus scaber* and *Portulaca oleracea* leaf extracts against mosquito vector *Aedes aegypti*

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Abstract

With an aim to develop chemical free and environment friendly mosquito biocontrol agent, two plant species, *Elephantopus scaber* and *Portulaca oleracea* were selected as these plants show high degree anti microbial and mosquito larvicidal efficacy rates. The soxhlet extracts of above-mentioned plants were extracted in polar solvents such as n-propanol and methanol. Different concentrations were prepared to test the efficacy on late 3rd and early 4th instar larvae of *Aedes aegypti*. n-propanol extract of *E. scaber* showed LC₅₀ value of 49.351 ppm at 24 hrs exposure whereas the methanolic extract is of 31.517 ppm. Similarly, methanolic extract of the *P. oleracea* had less LC₅₀ value of 56.285 ppm than that of n-propanol extract (74.862 ppm) at 24 hrs exposure. The maximum larvicidal efficacy is shown by the methanolic extracts as compared to n-propanolic extracts in both the plants at 24 and 48 hrs of exposure. All the samples were also subjected to phyto-chemical screening for secondary metabolites and other chemicals like terpenoids, flavonoids, phenols, triterpenoids, fatty acids, alcohols, proteins etc. The GC-MS analysis of different leaf extracts have shown various active constituents, n-Propanol and methanol extracts of *E. scaber* showed 18 and 19 compounds respectively while *P. oleracea* leaf extract showed 10 compounds in n-propanol and 11 in methanol extracts.

Keywords: Mosquito, *Aedes aegypti*, N-propanol, methanol, plant extract, phytochemical screening, larvicidal efficacy

Introduction

Mosquitoes are carriers of diseases that may harm both humans and domestic animals. Most often transmitted diseases by mosquitoes are Japanese encephalitis, Chikungunya, Malaria and Yellow fever etc. According to Lija-Escaline *et al.* (2015), *Aedes aegypti* L. is the main vector for the transmission of many diseases that are endemic to Southeast Asia and Africa, including Dengue fever, Yellow fever, Chikungunya and Dengue hemorrhagic fever [1]. Almost four billion people, spread across 128 countries, are now at risk of contracting dengue viruses. This one is more deadly and causes greater human morbidity as compared to other arthropod-borne viral diseases [2, 3]. According to Thanigaivel *et al.* (2012), over 2/3 of the world's population reside in areas where dengue mosquito vectors are present. According to the estimates, 34% of dengue-related cases take place in India only, where the disease is prevalent [4, 5].

Currently, mosquito populations are becoming resistant to the heavy synthetic chemical pesticides, this allow mosquito populations to resurge and may have unintended impacts on beneficial non-target animals, including natural rivals in the terrestrial environment. Therefore, new alternatives are required as a substitute to help with mosquito control so as to protect themselves against insects and other herbivores [6]. Although, only a small portion has been thoroughly researched in this regard, more than 2000 plant species are considered to contain insecticidal properties. Secondary metabolites produced by the plants that are more concentrated or effective on mosquito larval stages that have been found in the last three decades [7]. Worldwide, plant-based chemicals are being utilized more and more so as to control insect pests including mosquitoes. Larvicides, ovipositional attractants, insect growth regulators,

repellents, and deterrents are few of the proven uses for phytochemicals. Plant-based products have zero negative environmental impacts, like alkaloids, saponins, essential oils, steroids, isoflavonoids, and tannins are some of the plant-based compounds that have performed admirably as mosquito larvicides in recent research. Mosquito repellents may also be made from synthetic analogues of secondary plant metabolites [8].

The goal of the current study was to evaluate the larvicidal efficacy of *Elephantopus scaber* and *Portulaca oleracea* plant leaf extracts on the mosquito *Aedes aegypti* along with understanding their phyto-chemical constituents.

Material and Methods

1. Collection of plant

Fresh leaves of *E. scaber* and *P. oleracea* plants were collected from a farm with plus code address (CX3C+5VM Mansur, Karnataka) village near Dharwad city of Karnataka (15.4029627° N, 74.9721790°E). The collected plants were washed thoroughly with distilled water to avoid contaminations and are shade dried for twenty days at room temperature.

2. Preparation of plant extracts

Using an electric blender, the dried plant leaves were grounded into fine powder and then sieved. The Soxhlet extractor was used to extract phyto-constituents, thimbles were prepared by Whatman No. 1 cellulose chromatography paper, and 100 grams of each plant powder was filled in thimble and extracted over a 10-hrs period in polar solvents n-propanol and methanol. All the extracts were concentrated using a rotary evaporator and stored in refrigerator at 5° C in sealed bottles until use.

3. Phyto-chemical screening

The phyto-chemical screening of the extracts was carried out through the method of Ugochukwu *et al.*, 2013 [9] to detect the presence of secondary metabolites in each sample. GC-MS analyses of all the samples were carried out in DST-SAIF (Sophisticated Analytical Instrument Facility) at Karnatak University, Dharwad with Reference number INT.SAIF-Dharwad.REF.4171.

4. Selection of mosquito species and culture

A. aegypti, a laboratory-raised vector mosquito free of diseases and chemical exposure were procured from Vector Biology Research Laboratory, Department of Studies in Zoology, University of Mysore, Mysuru, India and used in all the experiments. Cyclical generations of vector mosquitoes were kept alive by culturing them at controlled temperature of 24°C ± 2°C and at relative humidity of 75% ± 5% in laboratory, larvae were reared in plastic trays till the pupal stage and then transferred to mosquito cages of dimension 30x30x30cm for adult emergence. Dog biscuit powder mixed with yeast in a 3:1 ratio was used as larval diet, adults were fed with 10% sugar solution soaked in cotton, and females were given blood meal every third day. The blood was collected from chicken slaughter house in a cavity block covered with thin parafilm. A bowl with distilled water and lined with filter paper was introduced in cages so as to collect eggs for future use.

5. Larvicidal bioassay

The bioassay was performed according to the standard procedure of WHO (WHO, 2005a) on late 3rd and early 4th instar larvae of *A. aegypti*. Different concentrations of plant leaf extracts were prepared and used in the experiment. Stock solution was made by combining 100 mg of crude extract with 1 ml of DMSO (Dimethyl Sulfoxide), then by adding distilled water to bring the volume to 100 ml. Several dilutions of the stock solution were made at

concentrations of 20, 40, 60, 80, 100, 120 and 140 ppm. In a 500ml capacity plastic container, 1ml of plant constituent with different concentration was mixed thoroughly in 249ml of distilled water and 25 healthy larvae were introduced. Simultaneously, a control was maintained by adding 1ml of DMSO solvent instead of the plant leaf extract to 249ml of distilled water. The mortality rate of larvae was recorded after 24 and 48 hours. For each test, four replicates were done. In control if mortality varied between 5% - 20%, then it was noted and corrected using Abbott's formula.

6. Statistical analysis

The recorded mortality rates were analysed for a probit regression to obtain LC₅₀, LC₉₀ at the 95% confidence levels and other statistics like chi square and p-value analysis was also run in the IBM-SPSS (Statistical Package for Social Sciences) version 20.0 software. We regarded a result to be statistically significant, if the P value was ≤ 0.05.

Results

As per the findings of the current study, the leaf extracts of *E. scaber* were much more harmful than that of *P. oleracea* to the mosquito species (*Ae. aegypti*). The methanol extracts of *E. scaber* showed 100% death rate at 80 ppm, while the n-propanol's 100% mortality was seen at 120ppm at 24 hrs exposure. Similarly, the 24 hrs exposure of *P. oleracea* methanol extract showed 100% mortality at 120 ppm and about 99.25% mortality at 120 ppm. Overall methanol extracts of both plant leaves were found to be causing high mortality (Table 1). Forty parts per million (40ppm) of methanol extracts were sufficient to kill about 50% of the larval population soon after exposure for 24 hrs. Hence, *E. scaber*'s methanol extract was determined to be a powerful larvicidal agent compared to *P. oleracea* extract. The 24 hrs and 48hrs exposure mortality percentage with their standard deviation values of various concentration of plant extract used against *A. aegypti* larvae are shown in Table-1.

Table 1: Mortality percentage of *A. Aegypti* larvae exposed for 24 hrs and 48 hrs to different concentrations of the leaf extract *E. scaber* and *P. oleracea*

Plant	Extracts	Exposure (hrs)	Mortality (%) ± SD						
			Control	20 ppm	40 ppm	60 ppm	80 ppm	100 ppm	120 ppm
<i>E. scaber</i>	n-propanol	24	0	17.00 ± 0.81	33.75 ± 0.95	50.00 ± 0.81	68.25 ± 0.95	86.00 ± 2.58	100 ± 0.00
		48	0	19.75 ± 1.70	39.75 ± 2.21	56.00 ± 1.63	76.50 ± 2.38	95.50 ± 2.08	100 ± 0.00
	Methanol	24	0	29.50 ± 2.64	52.75 ± 0.95	81.75 ± 2.75	100 ± 0.00	100 ± 0.00	100 ± 0.00
		48	0	31.25 ± 1.70	58.25 ± 0.95	89.50 ± 1.91	100 ± 0.00	100 ± 0.00	100 ± 0.00
<i>P. oleracea</i>	n-propanol	24	0	17.75 ± 0.95	29.00 ± 0.81	42.25 ± 1.70	66.00 ± 1.82	83.75 ± 2.98	99.25 ± 0.50
		48	0	18.50 ± 1.29	37.25 ± 1.70	53.00 ± 2.16	70.25 ± 0.50	90.75 ± 0.95	100 ± 0.00
	Methanol	24	0	20.00 ± 0.81	39.00 ± 0.81	60.00 ± 1.63	78.50 ± 1.29	95.50 ± 1.29	100 ± 0.00
		48	0	23.00 ± 1.41	45.00 ± 0.81	68.25 ± 1.25	84.00 ± 0.81	100 ± 0.00	100 ± 0.00

SD = standard Deviation, ppm = parts per million.

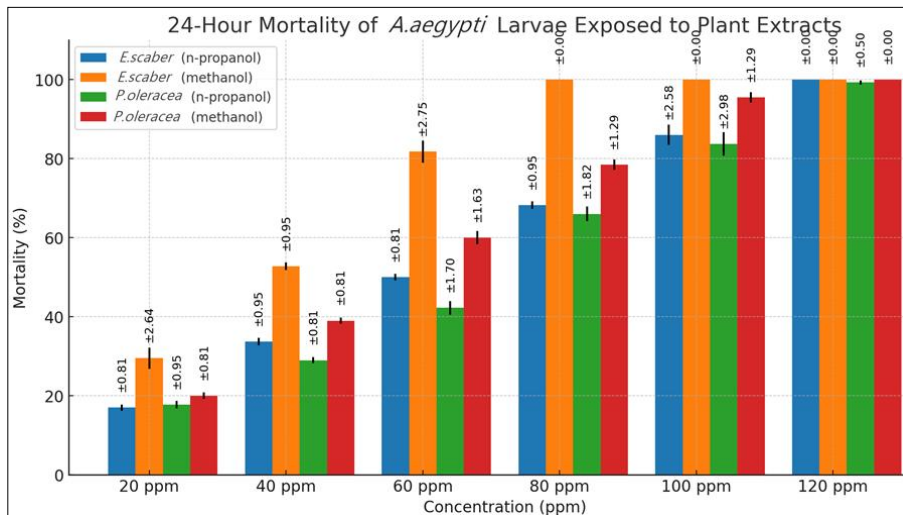


Fig 1: Bar graph showing %Mortality rates of *A. aegypti* larvae at 24 hrs exposure in different extracts.

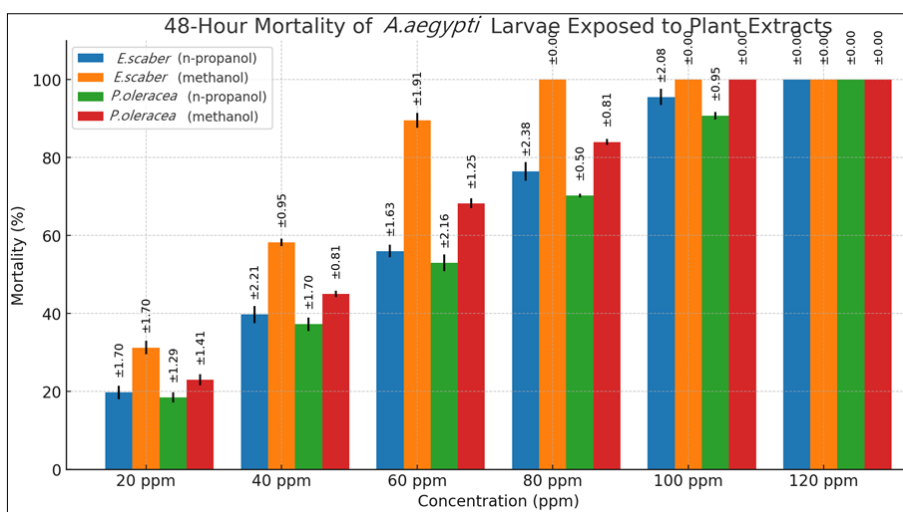


Fig 2: Bar graph showing %Mortality rates of *A. aegypti* larvae at 48 hrs exposure in different extracts.

Table-2 shows LC₅₀ and LC₉₀ values with 95% confidence limit, chi square value and respective significant values. At 24 hrs exposure, n-propanol extract of *E. scaber* showed LC₅₀ value of 49.351 ppm, where as methanol extract showed LC₅₀ value of 31.517 ppm. Similarly, n-propanolic extract of *P. oleracea* showed 74.862 ppm, where as methanolic extract of same plant at same exposure showed LC₅₀ value of 56.285 ppm. This shows that the methanolic extracts in both plants has higher efficacy. The 48 hrs exposure also showed high efficacy rates of methanolic

extracts of *E. scaber* and *P. oleracea* at 30.190 and 37.770 ppm respectively. The regression equations are also given for each sample with their p-value. The p-value showed high to moderate significance in both extracts of *E. scaber* plant where as low to no significance in *P. oleracea*. Considering the values of LC₅₀ and LC₉₀ at 24h and 48h exposure, it is evident that *E. scaber* show higher mortality rate as compared with *P. oleracea* plant in both ethanolic and methanolic extracts as mentioned in table-2.

Table 2: Larvicidal activity of n-propanol and methanol leaf extracts of *E. scaber* and *P. oleracea* against *A. Aegypti*

Sl. No	Solvent	Exposure (h)	LC ₅₀ (ppm)	95% Confidence limit for LC ₅₀		LC ₉₀ (ppm)	95% Confidence limit for LC ₉₀		Regression	Chi-Square (df=4)	P-value
				LCL (ppm)	UCL (ppm)		LCL (ppm)	UCL (ppm)			
<i>E. scaber</i>											
1	n-propanol	24	49.351	31.896	66.654	101.775	85.221	121.221	Y= -11.982 + 6.526X	11.110	0.011
		48	47.051	33.249	57.579	92.883	79.414	118.112	Y= -12.637 + 7.023X	9.807	0.020
2	Methanol	24	31.517	19.680	41.482	64.834	48.754	116.295	Y= -6.131 + 4.091X	8.488	0.048
		48	30.190	16.495	46.642	71.673	50.627	108.651	Y= -5.830 + 3.833X	13.465	0.000
<i>P. oleracea</i>											
1	n-propanol	24	74.862	66.549	80.539	108.155	101.085	232.773	Y= -15.033	7.637	0.054

									+ 8.021X		
		48	66.511	32.687	73.846	103.324	81.868	191.956	Y= -12.211 + 6.699X	11.174	0.011
2	Methanol	24	56.285	27.959	70.097	93.404	75.897	164.264	Y= -10.198 + 5.826X	8.081	0.044
		48	37.770	23.886	49.803	85.359	64.633	146.716	Y= -11.653 + 6.698X	10.330	0.016

LC₅₀ = Lethal Concentration at 50% mortality, LC₉₀ = Lethal Concentration at 90% mortality, LCL = Lower Confidence Limit, UCL = Upper Confidence Limit

The preliminary phyto-chemical screening of the leaf extracts showed phenol, tannins, coumarins, acids, alkaloids, flavonoids, quinones, terpenoids, carbohydrates, proteins, triterpenoids, and saponins (Table-3).

Table 3: Phytochemical screening of leaf extracts of *E. scaber* and *P. oleracea*

Sl. No.	Secondary metabolites	<i>E. scaber</i> Extracts in		<i>P. oleracea</i> Extracts in	
		n-propanol	Methanol	n-propanol	Methanol
1	Phenols	+	+	+	+
2	Tannins	+	+	+	+
3	Coumarins	-	+	-	+
4	Acids	+	+	+	+
5	Alkaloids	+	+	+	+
6	Flavonoids	+	+	+	+
7	Quinones	-	+	-	+
8	Terpenoids	+	+	+	+
9	Glycosides	-	-	-	-
10	Carbohydrates	+	+	+	+
11	Proteins	+	-	+	-
12	Triterpenoids	+	+	-	-
13	Saponins	+	+	+	+

The chromatogram of n-propanol extract of *E. scaber* (Fig.-3) showed 18 peaks representing 18 chemical compounds. Allene constitutes highest (68.86%) of all the compounds with molecular weight 40 and molecular formula C₃H₄,

followed by Propyne (14.88%) and 3- Butynoic acid (4.65%), where as all other chemical components are present in traces, whose details are provided in table-4.

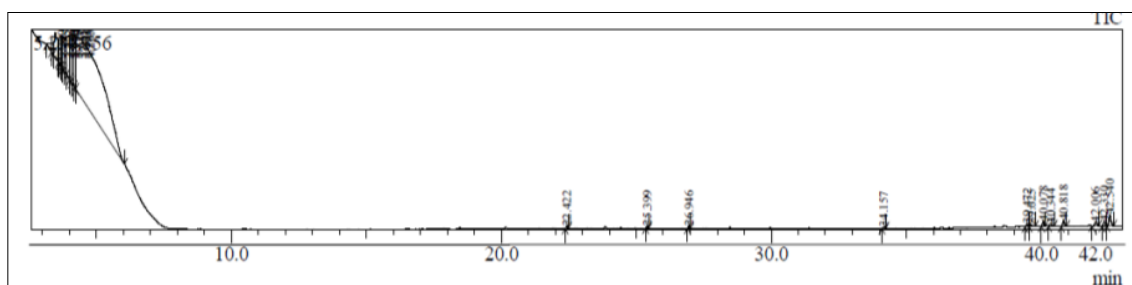


Fig 3: GC-MS chromatogram of n-propanolic leaf extract of *E. scaber*.

Table 4: Chemical constituents identified in n-propanol extract of *E. scaber* by GC-MS analysis.

Sl. No.	Retention Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area in %
1	3.200	Argon	Ar	40	0.69
2	3.365	3-Butyn-1-ol	C ₄ H ₆ O	70	3.38
3	3.470	Formamide, N, N-dimethyl-	C ₃ H ₇ NO	73	3.53
4	3.620	Cyclopropene	C ₃ H ₄	40	1.03
5	3.665	Propyne	C ₃ H ₄	40	14.88
6	3.770	3-Butynoic acid	C ₄ H ₄ O ₂	84	4.65
7	4.265	Allene	C ₃ H ₄	40	68.86
8	22.422	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	0.08
9	25.399	Tetratetracontane	C ₄₄ H ₉₀	618	0.12
10	26.946	Heptatriacontane	C ₃₇ H ₇₆	520	0.10
11	34.157	Octacosanol	C ₂₈ H ₅₈ O	410	0.08
12	39.472	Cholest-5-en-3-ol (3. beta.)-, carbonochloridate	C ₂₈ H ₄₅ ClO ₂	448	0.16
13	39.625	beta. Amyrone	C ₃₀ H ₄₈ O	424	0.17
14	40.078	24-Norursa-3,12-diene	C ₂₉ H ₄₆	394	0.29
15	40.344	Lup-20(29)-en-3-ol, acetate, (3. beta.)-	C ₃₂ H ₅₂ O ₂	468	0.17

16	40.818	Lupeol, trifluoroacetate	C ₃₂ H ₄₉ F ₃ O ₂	522	0.37
17	42.006	Lup-20(29)-en-3-one	C ₃₀ H ₄₈ O	424	0.29
18	42.330	Lupeol, trifluoroacetate	C ₃₂ H ₄₉ F ₃ O ₂	522	1.15
Total					100%

Fig.-4 represents chromatogram of methanol extract of *E. scaber* with 19 peaks representing 19 chemical compounds. Allene constitutes highest (60.93%) of all the compounds with followed by Propyne (29.54%) and 3-Butynoic acid

(3.88%). Table-5 shows the retention time, compound name, molecular formula, molecular weight and peak area (in %) of all the compounds analysed through GC-MS.

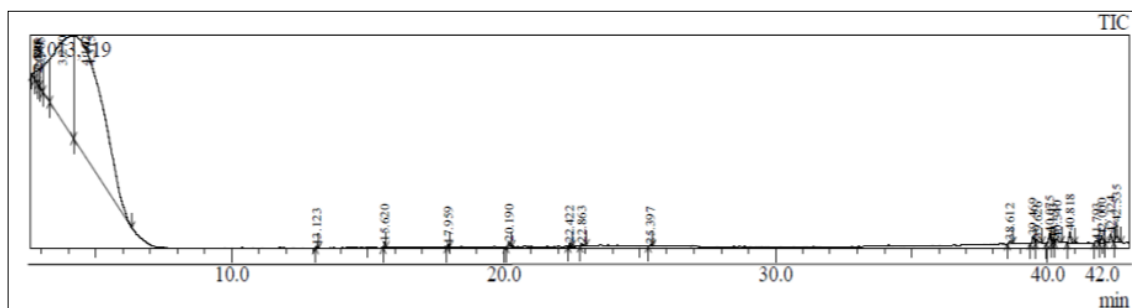


Fig 4: GC-MS chromatogram of methanolic leaf extract of *E. scaber*

Table- 5: Chemical constituents identified in methanol extract of *E. scaber* by GC-MS analysis.

Sl. No.	Retention Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area in %
1	2.670	Cyclopropene	C ₃ H ₄	40	2.31
2	3.270	3-Butynoic acid	C ₄ H ₄ O ₂	84	3.88
3	4.102	Propyne	C ₃ H ₄	40	29.54
4	4.315	Allene	C ₃ H ₄	40	60.93
5	13.123	Heneicosane	C ₂₁ H ₄₄	296	0.17
6	20.190	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	242	0.14
7	22.422	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	0.07
8	22.863	Z, Z-8,10-Hexadecadien-1-ol	C ₁₆ H ₃₀ O	238	0.08
9	25.397	Eicosane	C ₂₀ H ₄₂	282	0.03
10	38.612	Stigmasterol	C ₂₉ H ₄₈ O	412	0.09
11	39.469	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methylheptan-2-yl)-3-methoxy-10,13-dimethyl-2,3,4,7	C ₃₀ H ₅₂ O	428	0.23
12	39.626	24-Noroleana-3,12-diene	C ₂₉ H ₄₆	394	0.14
13	40.075	24-Norursa-3,12-diene	C ₂₉ H ₄₆	394	0.30
14	40.193	Betulin	C ₃₀ H ₅₀ O ₂	442	0.08
15	40.340	Lup-20(29)-en-3-ol, acetate, (3. beta.)-	C ₃₂ H ₅₂ O ₂	468	0.17
16	40.818	Lupeol, trifluoroacetate	C ₃₂ H ₄₉ F ₃ O ₂	522	1.15
17	41.793	Betulinaldehyde	C ₃₀ H ₄₈ O ₂	440	0.09
18	42.000	Lup-20(29)-en-3-one	C ₃₀ H ₄₈ O	424	0.25
19	42.324	Epilupeol	C ₃₂ H ₅₂ O ₂	468	0.35
Total					100%

The chromatogram of n-propanol extract of *P. oleracea* (Fig.-5) showed 10 peaks with different retention times, identifying 10 different chemical compounds. Of all the compounds Heptatriacontane constitutes highest percent

(32.93%) with molecular weight 522 and molecular formula C₃₇H₇₆, followed by Formamide, N, N-dimethyl- (24.76%) and Allene (17.99%). Remaining other components present in traces are represented in Table-6.

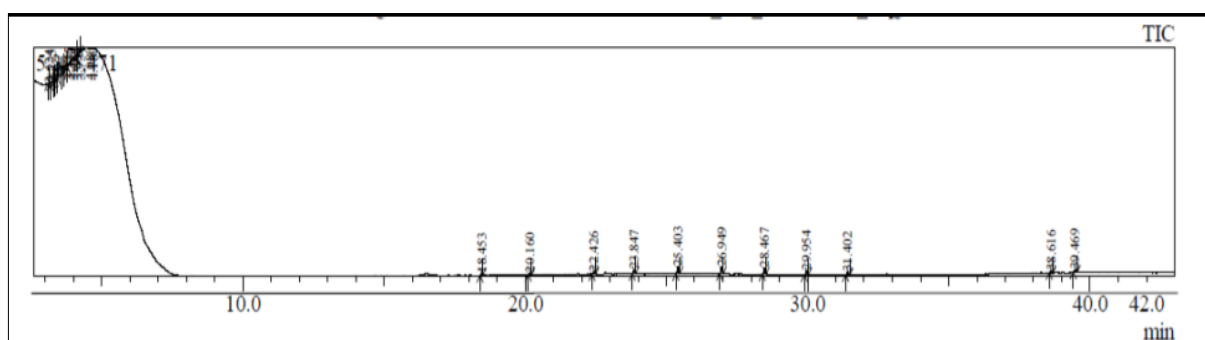


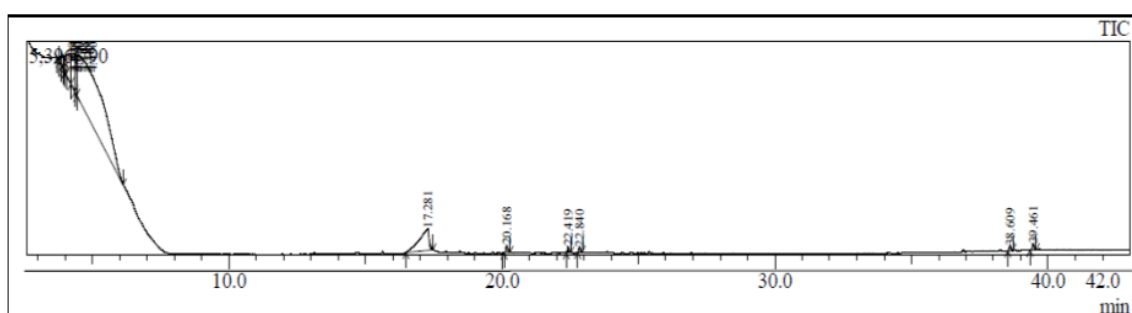
Fig 5: GC-MS chromatogram of n-propanolic leaf extract *P. oleracea*

Table 6: Chemical constituents identified in n-propanol extract of *P. oleracea* by GC-MS analysis

Sl. No.	Retention Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area in %
1	3.124	Allene	C ₃ H ₄	40	17.99
2	3.370	3-Butynoicacid	C ₄ H ₄ O ₂	84	0.26
3	3.484	Formamide, N, N-dimethyl-	C ₃ H ₇ NO	73	24.76
4	4.080	3(2H)-Furanone, dihydro-5-methyl-	C ₅ H ₈ O ₂	100	12.94
5	18.453	Neophytadiene	C ₂₀ H ₃₈	278	1.43
6	20.160	Pentadecanoicacid	C ₁₅ H ₃₀ O ₂	242	1.96
7	22.426	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	2.89
8	23.847	Heptatriacontane	C ₃₇ H ₇₆	520	32.92
9	38.616	Stigmasterol	C ₂₉ H ₄₈ O	412	1.98
10	39.469	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methylheptan-2-yl)-3-methoxy-10,13-dimethyl-2,3,4,7,	C ₃₀ H ₅₂ O	428	2.87
Total					100

Fig.-6 represents chromatogram of methanol extract of *P. oleracea* with 11 peaks representing 11 different chemical compounds. Of all the compounds Allene constitutes highest percent (76.17%) followed by 3-O-Methyl-d-

glucose (10.22%) and 3-Butyn-1-ol (6.48%). Table-7 shows the retention time, compound name, molecular formula, molecular weight and peak area (in %) of all the compounds.

**Fig 6:** GC-MS chromatogram of methanolic leaf extract *P. oleracea***Table 7:** Chemical constituents identified in methanol extract *P. oleracea* by GC-MS analysis.

Sl. No.	Retention Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area in %
1	3.739	3-Butynoicacid	C ₄ H ₄ O ₂	84	0.77
2	3.825	Boranecarbonyl	CH ₃ BO	42	0.75
3	3.950	3-Butyn-1-ol	C ₄ H ₆ O	70	6.48
4	4.000	Allene	C ₃ H ₄	40	76.17
5	4.385	1,2-Propadiene-1,3-dione	C ₃ O ₂	68	3.39
6	17.281	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	194	10.22
7	20.168	n-Hexadecanoicacid	C ₁₆ H ₃₂ O ₂	256	0.59
8	22.419	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	0.38
9	22.840	cis, cis, cis-7,10,13-Hexadecatrienal	C ₁₆ H ₂₆ O	234	0.36
10	38.609	Stigmasterol	C ₂₉ H ₄₈ O	412	0.34
11	39.461	Cholest-5-en-3-ol(3. beta.)-, carbonochloridate	C ₂₈ H ₄₅ ClO ₂	448	0.55
Total					100

Discussion

The widespread use of synthetic pesticides has been linked to many cases of contested contamination [10]. Increasing awareness and adoption of Integrated Pest Management (IPM) tactics, as well as the use of less harmful biopesticides, is a priority for many countries throughout the world. There may be less wasteful use of stored chemicals, if more people were properly educated on how to use biopesticides. Biopesticides derived from non-toxic or low-toxic secondary plant metabolites provide new possibilities for use into Integrated Pest Management (IPM) systems. The goal is to create biopesticides that are effective, which leave behind fewer toxic by-products in the environment, products that biodegrade quickly and benefit both humans and beneficial insects [11]. Because of all these problems, scientists have been looking for and creating more environmentally friendly biopesticides. New plant based

biopesticides are helping to reduce insecticide resistance in certain populations [12]. Plant extracts and their secondary metabolites have been used historically to control insect infestations with a track record of success [13].

Today, an insecticide's environmental safety is of the highest importance and shouldn't tolerate the killing of non-target organisms [14]. An important aspect of the prevention of vector-borne illnesses is the suppression of mosquito larvae by using larvicidal chemicals extracted naturally from plants. At present, using plant extracts as possible larvicides is seen as a feasible and preferable option to controlling deadly mosquito species at community level. While, certain phyto-chemicals functions as growth inhibitors, chemosterilants, repellents, or attractants, others operate as general toxicants against both adult and larval stages of mosquito vectors. Although, several plant extracts have been reported to have mosquitocidal or repellent properties

on various insect vectors, only a small fraction of plant products have shown effective application results in the field [15].

The virtue of focusing on mosquito larvae is that they are unable to leave their breeding grounds before emerging as adults. It also helps to minimize the amount of pesticides needed for the control adult mosquitoes via aerial application of adulticidal chemicals. As an alternative to the synthetic pesticides already in use, bioactive crude extracts or separated phyto-constituents from the plants may be employed against insect vectors. Numerous chemicals such as phenolics, terpenoids, and alkaloids found in plants may contribute a lot to the biological action of plant extracts [16]. In present study, the phyto-chemical components present in the leaf extract includes phenol, tannins, coumarins, alkaloids, flavonoids, quinones, terpenoids, carbohydrates and triterpenoids which might be contributed in larvicidal efficacy.

Table-2 displays the results of evaluation of the larvicidal efficiency of various solvent extracts of the two plants. After 24 hrs and 48 hrs of exposure, the leaf extracts of *E. scaber* and *P. oleracea* had a good to moderate impact on fourth instar larvae of *A. aegypti*. Methanol extracts of *E. scaber* had the greatest mortality rate (100%) of all the tested solvents. Similar research work was tested with silver nanoparticles of *E. scaber* for their larvicidal activity against *C. quinquefasciatus*, by Amita Hajra and Naba Kumar Mondal [17] which showed LC₅₀ at 1.43 mg/L and LC₉₀ value of 4.32 mg/L during 48 hrs of exposure. Previous research by Parthasarathy *et al.*, found that LC₅₀ value of 508.20 µg/mL at 24 hrs and 7.10 µg/mL at 48hrs from *P. oleracea* aqueous extract, while ethanol extract showed LC₅₀ value of 361.90 µg/mL at 24hrs and 3.31 µg/mL at 48hrs against *C. Quinquefasciatus*. Similarly, against *A. Stephensi*, *P. oleracea* aqueous extract showed LC₅₀ value of 386.23 µg/mL for 24hrs and 1.915 µg/mL for 48 hrs. The ethanolic extract had LC₅₀ value of 204.70 and 4.133 µg/mL at 24hrs and 48 hrs respectively. Our study investigation found that *P. oleracea* was only moderately effective at killing larvae, when used with either ethanol or methanol extract [18].

The phytochemical screening of *P. oleracea* methanol extract revealed the presence of Alkaloid, Carbohydrate, Tannin, Phenols, Flavonoides, Saponin, Protein and steroids [19]. Ethanol extracts of *E. scaber* showed presence of Alkaloid, Flavonoid, Saponin, Plifenols [20] Based on the above discussion, n-propanol and methanol extracts of *E. scaber* shown improved larvicidal efficacy against the mosquito species *A. aegypti* as compared to *P. oleracea* extracts. This activity may be due to the presence of triterpenoids in the extracts of the plants used in this experiment. Numerous studies have noted the diverse functions that phyto-chemicals derived from plant sources, including their ability to operate as larvicides, insect development regulators, repellents, and ovipositor attractants [21].

Conclusion

The findings of the study demonstrated that *E. scaber* crude extracts have the potential to be mosquito larvicidal, as compared to *P. oleracea*. Mosquitoes thrive in stagnant water; therefore, using the plant's crude extract or the bioactive compounds that can be derived from the plant might be much useful. There will undoubtedly be greater success in controlling mosquitoes, if useful chemicals found

in the plant species are screened, purified, and identified. The extract might be sprayed in still water areas, which are known to be mosquito breeding grounds. Based on the results of present work, more investigation is essential the isolation, purification, and screening of bioactive phyto-chemical constituents/compounds from plant extract is required to recognize the potential use of controlling the development of vector borne mosquitoes.

Reference

1. Lija-Escaline J, Senthil-Nathan S, Thanigaivel A, Pradeepa V, Vasantha-Srinivasan P, Ponsankar A, Edwin ES, Selin-Rani S, Abdel-Megeed A. Physiological and biochemical effects of botanical extract from *Piper nigrum* Linn (Piperaceae) against the dengue vector *Aedes aegypti* Liston (Diptera: Culicidae). *Parasitology Research*,2015;114(11):4239-49.
2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF. The global distribution and burden of dengue. *Nature*,2013;496(7446):504-7.
3. Duong V, Lambrechts L, Paul RE, Ly S, Lay RS, Long KC, Huy R, Tarantola A, Scott TW, Sakuntabhai A, Buchy P. Asymptomatic humans transmit dengue virus to mosquitoes. *Proc Natl Acad Sci USA*,2015;112(47):14688-93.
4. Thanigaivel A, Chandrasekaran R, Revathi K, Nisha S, Sathish-Narayanan S, Kirubakaran SA, Senthil-Nathan S. Larvicidal efficacy of *Adhatoda vasica* (L.) Nees against the bancroftian filariasis vector *Culex quinquefasciatus* Say and dengue vector *Aedes aegypti* L. in *in-vitro* condition. *Parasitology Research*,2012;110(5):1993-9.
5. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. *Indian J Med Res*,2012;136(3):373.
6. Kalaivani K, Senthil-Nathan S, Murugesan AG. Biological activity of selected Lamiaceae and Zingiberaceae plant essential oils against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae). *Parasitology Research*,2012;110(3):1261-8.
7. Sutthanont N, Choochote W, Tuetun B, Junkum A, Jitpakdi A, Chaithong U, Riyong D, Pitasawat B. Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and-resistant strains of *Aedes aegypti* (Diptera: Culicidae). *J Vector Ecol*,2010;35(1):106-15.
8. Senthil-Nathan S. A review of biopesticides and their mode of action against insect pests. In: *Environmental Sustainability*, 2015, 49-63.
9. Ugochukwu SC, Uche A, Ifeanyi O. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala*. *Asian J Plant Sci Res*,2013;3(3):10-13.
10. Obermok VV, Laikova KV, Gninenko YI, Zaitsev AS, Nyadar PM, Adeyemi TA. A short history of insecticides. *J Plant Prot Res*,2015;55(3):221-6.
11. East R. Soil science comes to life. *Microbiome. Nature*,2013;501:18-9.
12. Senthil-Nathan S. The use of *Eucalyptus* leaf extract as a natural larvicidal agent against malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Bioresour Technol*,2007;98:1856-60.

13. Senthil-Nathan S. Physiological and biochemical effect of Neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. *Front Physiol*,2013;359(4):117.
14. Karthi S, Uthirarajan K, Manohar V, Venkatesan M, Chinnaperumal K, Vasantha-Srinivasan P, Krutmuang P. Larvicidal enzyme inhibition and repellent activity of red mangrove *Rhizophora mucronata* (Lam.) leaf extracts and their biomolecules against three medically challenging arthropod vectors. *Molecules*,2020;25(17):3844.
15. Elumalai D, Hemalatha P, Kaleena PK. Larvicidal activity and GC-MS analysis of *Leucas aspera* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. *J Saudi Soc Agric Sci*,2017;16(4):306-13.
16. Sarma R, Khanikor B, Mahanta S. Essential oil from *Citrus grandis* (Sapindales: Rutaceae) as insecticide against *Aedes aegypti* (L) (Diptera: Culicidae). *Int J Mosq Res*,2017;4(3):88-92.
17. Amita H, Naba KM. Phytofabrication of silver nanoparticles using *Elephantopus Scaber* and *Azadirachta indica* leaf extract and its effect on larval and pupal mortality of *Culex quinquefasciatus*. *Asian Pac J Trop Dis*,2016;6(12):979-86.
18. Parthasarathy S, Durairaj S, Kamaraj P, Chandran J. The influence of *Portulaca oleracea* L. leaves extracts on the histoarchitecture of *Culex quinquefasciatus* and *Anopheles stephensi* larvae. *Clin Complement Med Pharmacol*,2022;2(3):100053.
19. Shital D. *Portulaca oleracea* L.: A review of phytochemistry and pharmacological effects. *World J Pharm Res*,2020;9(3):285-94.
20. Riski S, Anggie P, Wisnu CP. Phytochemicals and anti-hemorrhoidal activities of *Tapak Liman* (*Elephantopus Scaber*) leaves. *J Adv Biotechnol Exp Ther*,2023;6(2):436-44.
21. Manikandan G, Rajalakshmi A, Suganya P, Kumari RM, Katerere DR. Chemical profiling, antimicrobial and antimalarial activity of leaf essential oil of *Eugenia rottleriana* Wt. & Arn. (Myrtaceae). *Arab J Med Aromat Plants*,2022;8(3):57-76.