Clinical Complementary Medicine and Pharmacology 2 (2022) 100053

Contents lists available at ScienceDirect

Clinical Complementary Medicine and Pharmacology

journal homepage: www.elsevier.com/locate/ccmp

Short Communication

The Influence of *Portulaca Oleracea* L. Leaves Extracts on the Histoarchitecture of *Culex Quinquefasciatus* and *Anopheles Stephensi* Larvae

Parthasarathy Sudharsan[a], Durairaj Siva[a], Kamaraj Prabhu[b], Chandran Janani[a,*]

[a] PG and Research Department of Biotechnology, Srimad Andavan Arts and Science College (Autonomous), Thiruvanaikovil, Tiruchirappalli 620 005, Tamil Nadu, India
[b] PG and Research Department of Microbiology, Srimad Andavan Arts and Science College (Autonomous). Thiruvanaikovil, Tiruchirappalli 620 005, Tamil Nadu, India

ARTICLE INFO

Keywords:
Portulaca oleracea L. leaves
Aqueous extract
Ethanol extract
Fatty acids
Culex quinquefasciatus larvae
Anopheles stephensi larvae

ABSTRACT

Background: Mosquito is a big threat to the human health. Mosquito-borne diseases cause millions of death to the human beings. Hence, a permanent solution is eagerly to be established to control its excessive growth in stagnant water. *Portulaca oleracea* Linn is a natural larvicidal agent, which contains active ingredients such as linolenic acid, linoleic acid (omega-3 fatty acids). These bioactive compounds may be responsible for its larvicidal properties on mosquito.

Objective: The present study is focussed on identifying the bioactive compounds, such as linolenic acid, through GC–MS, and analyzing the larvicidal efficacy of *Portulaca oleracea* L. against *Culex quinquefasciatus* and *Anopheles stephensi* larvae.

Methods: Preliminary phytochemical analysis, total protein content, total carbohydrate content, total phenol content, total flavonoid content and GC–MS analysis exhibited the presence of rich phytoconstituents in *Portulaca oleracea*. DPPH analysis was carried out to analyze the antioxidant potential of plant extract. Larvicidal activity and histological change were detected to evaluate the efficacy of *Portulaca oleracea L*. on the *Culex quinquefasciatus* and *Anopheles stephensi* larvae.

Results: Aqueous and ethanol leaf extracts of *Portulaca oleracea* L. against both *Culex quinquefasciatus and Anopheles stephensi* larvae, showed a very good larvicidal activity at 500 µg/mL among the various concentrations. Histological damages of mosquito larvae were observed when treated with the *Portulaca oleracea* L. extract, and provided further evidence for its larvicidal activity.

Conclusion: This study concluded that the plant *Portulaca oleracea* L. contained many useful bioactive compounds, can be a strong larvicidal agent against both *Culex quinquefasciatus and Anopheles stephensi* larvae. The molecular mechanism for the larvicidal activity will be identified in future studies.

1. Introduction

The deadliest diseases caused by mosquitoes are a challenging issue to the human being. Dangerous diseases such as dengue, malaria and yellow fever are caused by *Culex quinquefasciatus* and *Anopheles stephensi*. The human white blood cells and immune system were disrupted due to yellow fever and malarial fever, which finally lead to death without proper treatment to the patients. In a survey, it was reported that, around 725,000 humans were affected by mosquitoes (Gates, 2014). The scientific community is still expecting a better solution to eradicate the deadliest diseases caused by mosquitoes. Many plants are naturally having the ability to repel mosquito species (Dhossaradhan et al., 2021). In this study, *Portulaca oleracea* L. was selected to evaluate its larvicidal efficacy against *Culex quinquefasciatus* and *Anopheles stephensi*, because this plant is rich in omega-3 fatty acid such as alpha-linolenic acid, linoleic acid and docosahexaenoic acid. Also, some flavonoids, such as karanjin and karanjachromene are present in the *Portulaca oleracea* L. (Sodeifian et al., 2018). The compounds present in the plant may act against the larvae residing in the water (Ezeabara et al., 2014). *Portulaca oleracea* Linn. is commonly called Purslane, which is one of the native plants of India. Naturally, it is found in the environment as a weed plant. In the *Portulacaceae* family, 115 species are present in the single Portulaca genus. Among them only four species are found in India, but two species are exotics in nature, namely *Portulaca oleracea* L. and *Portulaca quadrifida* (Abdelhamid et al., 2020). The plant *Portulaca oleracea* L. has a green and purple stem up to 50 cm long, and is found throughout India as a weed plant, with a sessile base, long fleshy, flowers bright yellow in the terminal, sometime axillary clusters and black seeds (Harris, 2020). It has been popularly used in Siddha and Ayurveda for its higher medicinal impacts. From the earlier literature, it was recorded that *Portulaca oleracea* L. has been used as feed for rabbits, chicken, fish and humans for consuming this plant leading to a healthy life. It is a nutrition-rich source of plant-like iron, magnesium, calcium, protein and a high amount of fats and lipids presenting in

* Corresponding author.
E-mail address: janabio.net@gmail.com (C. Janani).

https://doi.org/10.1016/j.ccmp.2022.100053
Received 25 December 2021; Received in revised form 14 June 2022; Accepted 14 June 2022

the plant (Uddin et al., 2010). The plant has been reported for various medicinal properties, such as treatment for burns, headache, diseases related to the intestine, cough, shortness of breath, arthritis, cardiovascular disease; purgative, cardiac tonic, emollient, larvicidal activity, and a muscle relaxant. (Okafor and Ezejindu, 2014).

2. Materials and Methods

2.1. Sample collections

The *Portulaca oleracea* L. leaves were collected from Srirangam, Tiruchirappalli, Tamil Nadu, India, and the plant species were authenticated (Specimen number P.S.001) by Dr. S. John Britto, Director, The Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The collected healthy leaves were washed and extracted.

2.2. Preparation of extracts

Twenty-five grams of fresh *Portulaca oleracea* L. leaves were washed and crushed using a mortar–pestle, and 100 mL of distilled water and ethanol was added separately to it, incubated for 24 h, filtered using gauze cloth and placed it into the water bath at 60°C for 5–6 days to evaporate the water, and the remaining extract was used for further activities assay (Huda-Faujan et al., 2009).

2.3. Yield of extract

$$\text{Percentage Yield}\,(\%) = \frac{W\,a}{W\,b} \times 100$$

Where, W_a= weight of the obtained extract and W_b= weight of the extracted leaves (Osonwa et al., 2012).

2.4. Qualitative phytochemical tests

The phytocompounds such as alkaloids (Mayer's Test), glycosides (Keller Kilianin Test), saponins (Foam Test), terpenoids (Salkowski Test), steroids (Salkowski Test), protein (Biuret test), carbohydrate (Benedict's test), tannins (lead acetate), flavonoids (Alkaline Reagent Test) and phenol (Lead Acetate test), were analyzed using the aqueous and ethanol extract of *Portulaca oleracea* L (Aly and Khamis, 2018).

2.5. Total protein content

0.2 mL to 1.0 mL of the standard protein (BSA) was added in a test tube and made into 1 mL with distilled water. One milliliter of *Portulaca oleracea* L. aqueous and ethanol extract was added to a new tube. The blank was prepared by adding 1 mL of distilled water. Then, 4.5 mL of Lowry's reagent was added to all the tubes and incubated for 10 min. Then, 0.5 mL of Folin's reagent was added to all the test tubes and incubated for 20 min in dark room. The color change was observed, and the optical density was noted at 660 nm under a UV spectrophotometer (Lowry et al., 1951).

2.6. Total carbohydrate content

Hundred milligrams of *Portulaca oleracea* L. extract was hydrolyzed in a boiling tube with 5 mL of 2.5 mol/L HCl in boiling water bath for a period of 3 h. It was cooled to room temperature, then solid sodium carbonate was added until effervescence ceases. The contents were centrifuged, and the supernatant was made to 100 mL using distilled water. From this, 1 mL of sample was pipetted out and the volume was made up to 1 mL with distilled water. Then, 1.0 mL of phenol reagent was added, followed by 5.0 mL of sulphuric acid. The tubes were kept at 25–30°C for 20 min. The absorbance was read at 490 nm, and the values were noted (Geetha and Geetha, 2014).

2.7. Total phenol content

The total phenolic content of the crude ext was estimated with the Folin–Ciocalteu reagent method with modifications (Spanos and Wrolstad, 1990; Zahin et al.,2010). Gallic acid standard was prepared at different concentrations (Zahin et al., 2010). To 0.5 mL of each extract, 2.5 mL of 0.2 mol/L Folin–Ciocalteu reagent was added, mixed by gentle shaking and kept for 5 min. Then, 2 mL of Na_2CO_3 (7.5%, w/v) was added to the mixtures and incubated at 30°C for 20 min. Three replicates were maintained per each experimental procedure. The absorbance of the sample was recorded at 415 nm (Zahin et al., 2017) using a UV–Vis spectrophotometer. The phenolic content of the extract was estimated from the standard curve of gallic acid, and the results were expressed in gallic acid equivalent (GAE)/g of extract (Javanmardi et al., 2003).

2.8. Total flavonoid content

The content of the total flavonoid was evaluated by the $AlCl_3$ method. Rutin equivalent was used to prepare the standard curve in different concentrations. The extract (1 mL) was mixed with 1 mL of 2% aluminum chloride ($AlCl_3$) methanolic solution. The mixtures were incubated at room temperature for 15 min, and the absorbance was recorded at 510 nm in a spectrophotometer. The content of total flavonoid was reported in *A* per gram of extract (Qiu et al., 2010).

2.9. DPPH free radical scavenging activity

The free radical scavenging activity of the fractions was measured *in vitro* by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and stored at 20°C till required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98 ± 0.02 at 517 nm using the spectrophotometer. A 3 mL aliquot of this solution was mixed with 100 µL of the sample at various concentrations (10 µg/mL – 500 µg/mL). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then, the absorbance was taken at 517 nm. The control was prepared as above without any sample (Saeed et al., 2012).

2.10. GC–MS analysis

The aqueous and ethanol leaf extract of *Portulaca oleracea* L. were subjected to GC–MS analysis to identify the presence of unknown compounds. The extract was mixed with GC-grade methanol at 1 mg/mL concentration. The extract was purified through a syringe filter to ensure that the sample was devoid of impurities that may block the GC–MS column. One microlitre of the purified extract was injected into the GC–MS (Agilent GC 7890A/MS5975C). The column used was Agilent DB 5 ms with a dimension of 30 *m* × 250 µm × 0. 25 µm. The carrier gas used was helium at a column temperature of 325°C. The mode was set as split less. The oven was programmed at 50°C for 1 min and then increased at a rate of 10°C/min to 300°C for 2 min with a total runtime of 28 min. The pressure was maintained at 20.7 kPa. The MS acquisition was performed in scan mode, and the *m/z* ratio ranged between 50 and 550 amu. The compounds were identified using NIST library.

2.11. Larvicidal activity

The *Culex quinquefasciatus* and *Anopheles stephensi* larvae were collected from ICMR Institute, Madurai. The larvicidal activity was conducted at room temperature**.** The twenty third and fourth instar larvae species were placed in a 200 mL water-added beaker with the control set-up and kept in the environment chamber at 27°C with a period of

16:8-hour light and dark cycle. 100 µL, 200 µL, 300 µL, 400 µL, 500 µL of *Portulaca oleracea* L. extract was added to the beaker. The larvae species was observed for 24 h (WHO, 1996).

2.12. Histological examination

The tested mosquito larvae (*Culex quinquefasciatus* and *Anopheles stephensi*) were fixed in 10% formalin. Paraffin sections, 5 µm thick, were obtained and stained with haematoxylin and eosin. The histological changes were observed under a light microscope and recorded using the trinocular microscope with LED display (Deepak et al., 2019).

3. Results

The *Portulaca oleracea* L. leaves were collected, then washed and shade-dried. The aqueous and ethanol extract were prepared.

3.1. Yield of extract

$$\text{Aqueous extract: } \frac{2.5}{25} \times 100 = 10\%$$

$$\text{Ethanol extract: } \frac{2.8}{25} \times 100 = 11.2\%.$$

3.2. Qualitative phytochemical analysis

In Table 1, the phytocompounds such as alkaloids, glycosides, saponins, terpenoids, proteins, carbohydrates, tannins, flavonoids and phenol were present. The only compound steroid was absent in this *Portulaca oleracea* L. extract.

Table 1
Qualitative Phytochemical result for aqueous and ethanol extracts of *Portulaca Oleracea* L.

Compound name	Aqueous extract of *Portulaca oleracea* L.	Ethanol extract of *Portulaca oleracea* L.
Alkaloids	+	+
Phenols	+	+
Carbohydrate	+	+
Saponins	+	+
Glycosides	+	+
Terpenoids	+	+
Flavonoids	+	+
Proteins	+	+
Steroid	–	–
Tannins	+	+

3.3. Total protein content

Fig. 1A showed that 1000 µL of aqueous of the *Portulaca oleracea* L. contained 200 µg of protein while the ethanol extract of the *Portulaca oleracea* L. contained 220 µg of protein. A higher amount of protein was detected in the ethanol extract than that in the aqueous extract.

3.4. Total carbohydrate content

Fig. 1B showed the total carbohydrate content that was analyzed. The aqueous extract of the *Portulaca oleracea* L. contained 112 µg of carbohydrate while the ethanol extract of the *Portulaca oleracea* L. contained 142 µg of carbohydrate.

3.5. Total phenolic content

In Fig. 1C the aqueous extract contained 174 µg of phenol while the ethanol extract contained 365 µg of phenol presenting in the plant *Portulaca oleracea* L., which were identified using the standard graph. The standard used was gallic acid.

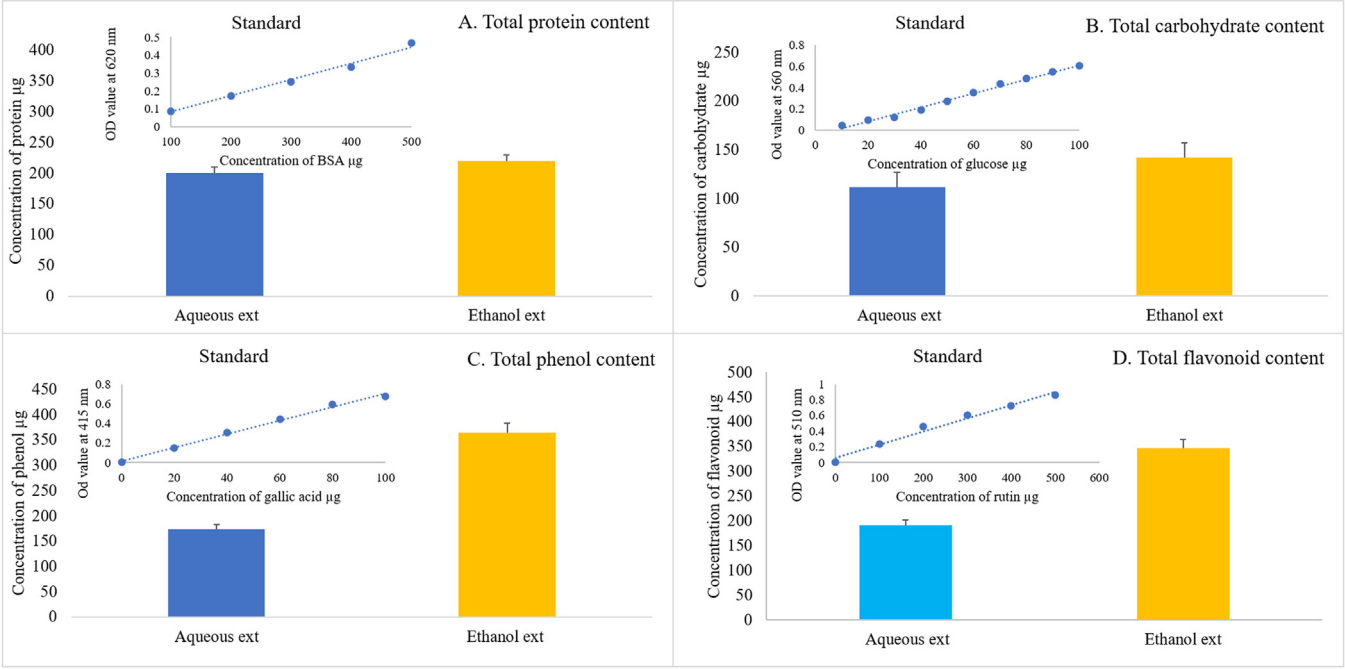

Fig. 1. A) Total protein content of aqueous and ethanol extracts of *Partulaca oleracea* L. B) Total carbohydrate content of aqueous and ethanol extracts of *Partulaca oleracea* L. C) Total phenolic content of aqueous and ethanol extracts of *Partulaca oleracea* L. D) Total flavonoid content of aqueous and ethanol extracts of *Partulaca oleracea* L.

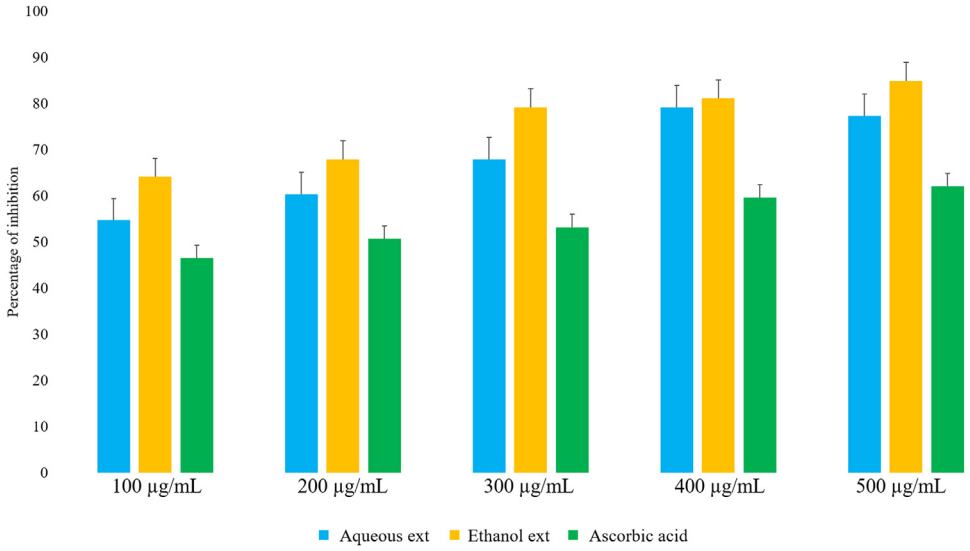

Fig. 2. DPPH activity of aqueous and ethanol extracts of *Partulaca oleracea* L.

3.6. Total flavonoid content

In Fig. 1D the aqueous extract contained 192 µg of flavonoid content while the ethanol extract contained 347 µg of flavonoid content presenting in the plant *Portulaca oleracea* L., which were identified using the standard graph. Rutin was used as a standard.

3.7. DPPH free radical scavenging activity

Fig. 2 showed the scavenging activity of the samples aqueous and ethanol extract of the plant. Though, the antioxidant potential of fractions was found lower than that of ascorbic acid. The study revealed that aqueous and ethanol extract had a prominent antioxidant activity and the presence of phenolic compounds was mainly found in these two extracts, which could be attributed to this activity (Chen et al., 2013).

3.8. GC–MS chromatogram of aqueous extract

The above-mentioned GC–MS chromatogram showed the aqueous extract of *Portulaca oleracea* L. In this chromatogram, all the compounds' retention time and peak values were listed in Table 2.

In the above chromatogram (Fig. 3A), the retention time of 11.742 may be in the case of the alpha-linolenic acid ($C_{18}H_{30}O_2$), which is one of the omega-3 fatty acids. This peak compound might play a major role in this larvicidal activity. And then, the retention time 20.196 denoted in the case of linoleic acid ($C_{18}H_{32}O_2$), and which is also an omega-3 fatty acid. Another retention time 22.240 may denote an omega-3 fatty acid named docosahexaenoic acid ($C_{22}H_{32}O_2$). The above-mentioned three omega-3 fatty acid compounds are the main for this study.

Table 2
GC–MS result for aqueous extract of *Portulaca oleracea* L.

Peak no.	Retention time	Peak area	Compound name
1	6.298	2663	Pyrazine, Cyclobutanone
2	6.887	7587	Borane amine, Hexanoic acid
3	8.842	4110	Butanoic acid, Pteridine-8-oxide
4	11.742	2225	Benzo[*e*]pyrene, linolenic acid
5	12.741	1778	1,2-Cinnolinedicarboxylic acid
6	14.352	99,023	Diethyl Phthalate
7	15.319	1886	Distannoxane, Phosphine
8	15.930	1824	2-Amino-4,5-dimethoxybenzoic acid
9	16.374	1746	Erythro-Pentonic acid
10	17.030	3103	3,6-Acridinediamine
11	17.819	2044	Ethanone
12	17.963	12,434	Hexadecanoic acid, Pentadecanoic acid
13	18.518	1703	Cyclopropanecarboxamide
14	18.630	8786	Octadecanoic acid, Hexadecanoic acid
15	19.585	4273	Bicyclononane
16	19.641	9384	9,12,15-Octadecatrienoic acid
17	19.729	18,302	3,4-Dimethylcyclohexanol
18	19.874	4929	Decanoic acid
19	19.974	2080	Formic acid
20	20.196	2633	Linoleic acid
21	20.252	5475	Octadecatrienoic acid
22	20.485	1865	Octadecanoic acid, Decanoic acid
23	21.140	2737	Dimethyl hydrastate
24	22.240	1678	Ethane
25	22.718	2305	1,2,4-Benzenetricarboxylic acid
26	23.040	2233	1,2-Benzenediol
27	23.729	2882	Cyclotrisiloxane
28	24.107	2149	Tetrasiloxane
29	24.251	2330	Anthracene, 2-Ethylacridine
30	25.151	3101	Ethane, Cyclotrisiloxane

3.9. GC–MS chromatogram of ethanol extract

The above-mentioned GC–MS chromatogram (Fig. 3B) showed the ethanol extract of *Portulaca oleracea* L.

In Table 3, all retention time peaks were checked and analyzed where compounds retention time peaks were given. In the chromatogram, the retention time peak value 20.185 mostly denoted in the case of that relevant to the linoleic acid ($C_{18}H_{32}O_2$) was present in the *Portulaca oleracea* L. In this chromatogram, another retention time peak value of 22.607 denoted the docosahexaenoic acid ($C_{22}H_{32}O_2$).

The above-mentioned peak values were caused by the compounds which are majorly fatty acids. These fatty acids play a key role in larvicidal activity.

3.10. Larvicidal activity

3.10.1. Culex quinquefasciatus

The *Portulaca oleracea* L. aqueous and ethanol ext was added at different concentrations noted in Table 4. The LC_{50} values of the aqueous extract against the *Culex quinquefasciatus* were 508.20 for 24 h and 7.10 for 48 h, respectively. In this aqueous extract, 40% of the mosquito larvae species have died at 24 h in 500 µL of sample when 90% of the mosquito species have died at 48 h in 500 µL of sample.

In the *Portulaca oleracea* L. ethanol extract against the *Culex quinquefasciatus*, the LC_{50} values were 361.90 for 24 h and 3.31 for 48 h, respectively. Then, dead mosquito larvae species levels for the ethanol extract were 40% of the mosquito larvae died at 500 µL for 24 h.

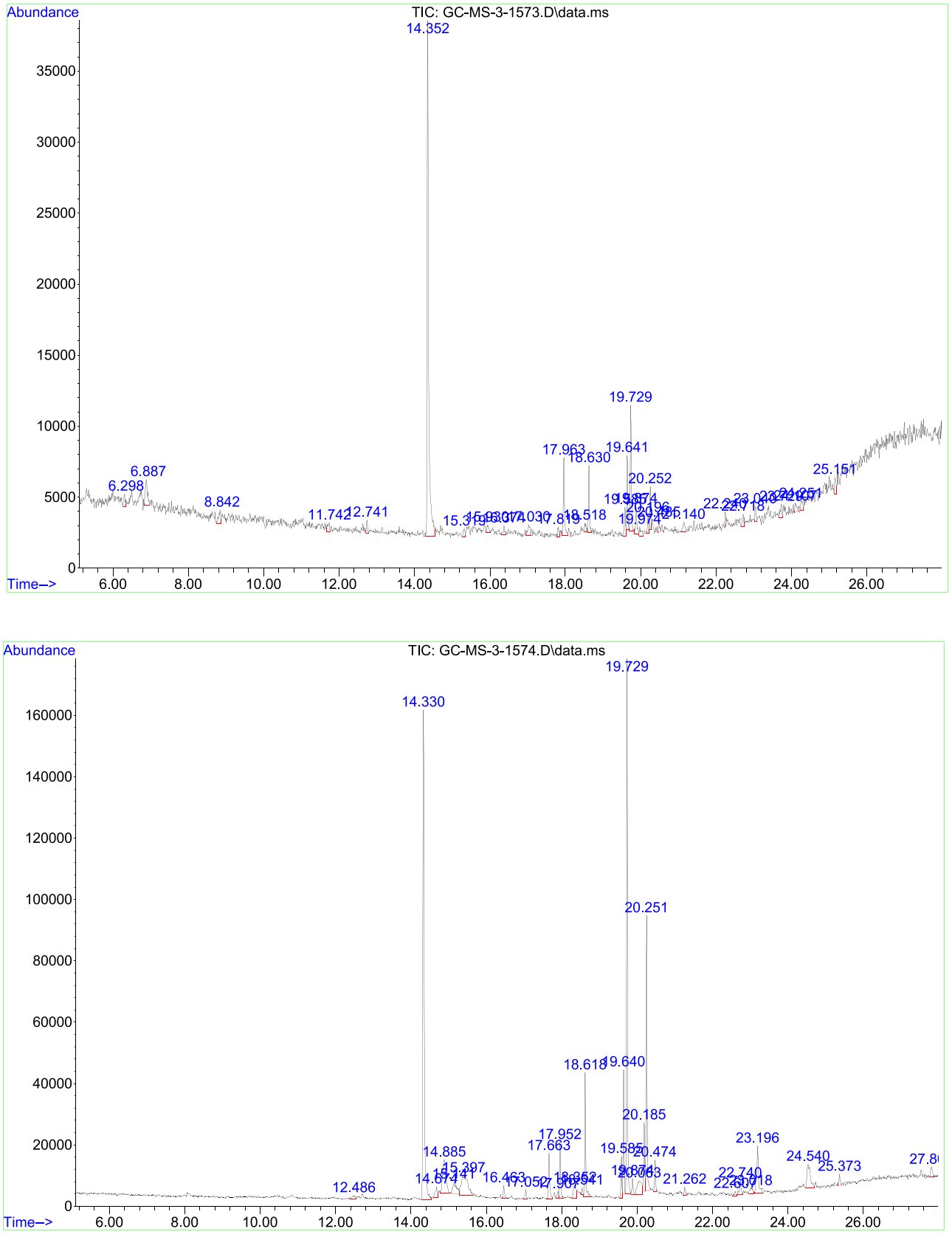

Fig. 3. top panel) GC–MS chromatogram for aqueous extract of *Portulaca oleracea* L. bottom panel) GC–MS chromatogram for ethanol extract of *Portulaca oleracea* L.

Table 3
GC–MS result for ethanol extract of *Portulaca oleracea* L.

Peak no.	Retention time	Peak area	Compound name
1	14.330	412,897	Diethyl Phthalate
2	14.674	13,764	Glucose
3	14.885	63,535	Pentanoic acid, Phospholane, alpha-D-glucopyranoside
4	15.141	26,638	Octanoic Acid
5	15.397	77,318	4-Methyl-2-pentyl acetate
6	16.463	9834	1-Cyclopenteneacetic acid
7	17.052	7856	3-Fluorobenzoic acid, 16-Octadecadien-1-ol acetate
8	17.663	33,399	Acetamide, Propanamide
9	17.907	5231	Amino pyrazine, Pyridinone
10	17.952	31,926	Hexadecanoic acid, Pentadecanoic acid
11	18.352	21,423	Nonadecanoic acid, Tricosanoic acid
12	18.541	4819	Cyclohexanebutanoic acid, 11-Bromoundecanoic acid
13	18.618	74,180	Hexadecanoic acid, Ethyl tridecanoate
14	19.585	22,153	7-Hexadecyne, 5-Dodecyne
15	19.640	60,456	9,12,15-Octadecatrienoic acid, Methyl 8,11,14-heptadecatrienoate
16	19.729	282,644	Phytol, Cyclohexanol
17	19.874	11,309	Octadecanoic acid, Decanoic acid
18	20.063	37,053	9,12,15-Octadecatrienoic acid
19	20.185	44,686	Octadecadienoic acid, Linoleic acid
20	20.251	155,419	Octadecatrienoic acid
21	20.474	18,278	Octadecanoic acid, Heptadecanoic acid
22	21.262	5575	Ethanamine, 5-[[7-Chloro-4-quinolinyl] amino]
23	22.607	5565	DL-Phenylalanine
24	22.740	12,456	Fumaric acid, 2-Propanamine
25	23.018	9689	Cyclopropanecarboxylic acid, Propanenitrile
26	23.196	50,536	3-Quinolinecarboxamide, Benzaldehyde
27	24.540	49,671	Cyclobarbital
28	25.373	7736	Cyclotrisiloxane, Pyridine
29	27.806	9921	Cyclotrisiloxane, Silicic acid

Table 4
Larvicidal activity of *Portulaca oleracea* L. aqueous and ethanol extracts against *Culex quinquefasciatus*.

Samples			Dosage (µg/mL)					LC_{50} (µg/mL)	95% of Fiducial limits		χ^2 (df=3)
			100	200	300	400	500		LCL	UCL	
Portulaca oleracea L. (Aqueous extract)	No. of dead larvae and mortality rate	24h	06 30%	06 30%	08 40%	07 35%	08 40%	508.2 ± 2.55	61.98	4166.5	0.987
		48h	14 70%	16 80%	17 85%	18 90%	18 90%	7.10 ± 0.87	3.06	16.49	1.000
Portulaca oleracea L. (Ethanol extract)		24h	05 25%	05 25%	06 30%	07 35%	08 40%	361.90± 1.67	88.81	1474.7	0.986
		48h	16 80%	17 85%	18 90%	18 90%	19 95%	3.31 ± 0.971	1.18	9.26	1.000

Table 5
Larvicidal activity of *Portulaca oleracea* L. aqueous and ethanol extracts against *Anopheles stephensi*.

Samples			Dosage (µg/mL)					LC_{50} (µg/mL)	95% of Fiducial limits		χ^2 (df=3)
			100	200	300	400	500		LCL	UCL	
Portulaca oleracea L. (Aqueous extract)	No. of dead larvae and mortality rate	24h	05 25%	06 30%	06 30%	07 35%	08 40%	386.23 ± 1.84	82.60	1805.8	0.996
		48h	15 75%	15 75%	16 80%	17 85%	17 85%	1.915 ± 1.692	0.406	9.034	1.000
Portulaca oleracea L. (Ethanol extract)		24h	04 20%	05 25%	07 35%	07 35%	08 40%	204.70 ± 1.17	76.21	549.7	0.996
		48h	15 75%	15 75%	16 80%	17 85%	18 90%	4.133 ± 1.22	1.322	12.92	0.999

Ninety-five percentage of the mosquito larvae species died at the highest concentration of 500 µL for 48 h. In this activity, finally, 90% of the mosquito larvae species have died at the end of the activity. The Chi-square test for 48 h was shown the same value of 1.000.

3.10.2. Anopheles stephensi

The *Portulaca oleracea* L. aqueous and ethanol extract was added at different concentrations noted in Table 5. LC_{50} values were tabulated after the extract against the *Anopheles stephensi* was tested. The LC_{50} values for the aqueous extract were 386.23 for 24 h and then 1.915 for 48 h, respectively. For the aqueous extract, 40% of the *Anopheles* mosquito larvae species have died at 24 h in 500 µL and 90% of the *Anopheles* species have died at 48 h in 500 µL of sample.

In the *Portulaca oleracea* L., ethanol extract was added at various concentrations. The LC_{50} value for the ethanol extract was 204.70 for 24 h and 4.133 for 48 h, respectively. 40% of the mosquito larvae species

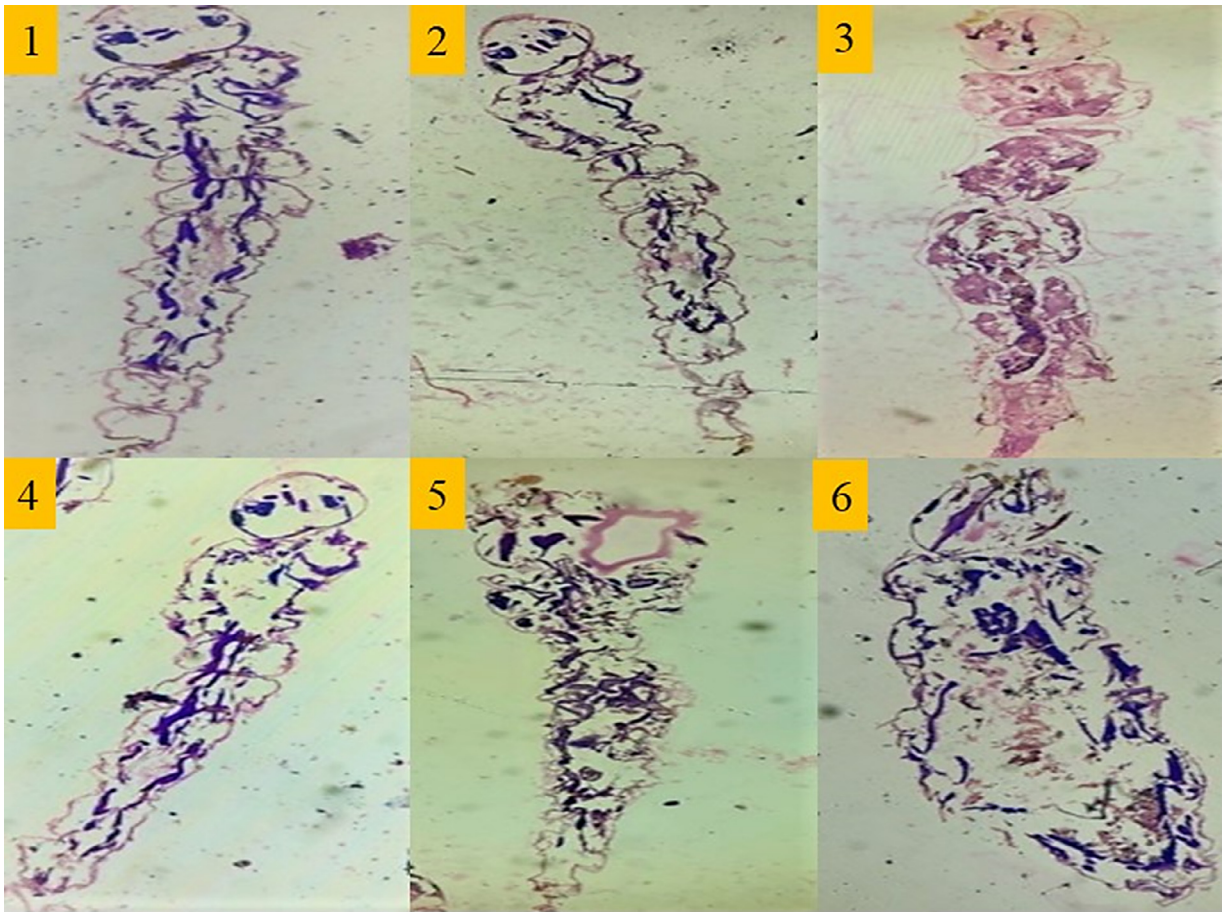

Fig. 4. Histology of different mosquito larvae with different treatment. 1. Control of *Anopheles stephensi*; 2. Aqueous extract against *Anopheles stephensi*; 3. Ethanol extract against *Anopheles stephensi*; 4. Control of *Culex quinquefasciatus*; 5. Aqueous extract against *Culex quinquefasciatus*; 6. Ethanol extract against *Culex quinquefasciatus*

have died at 24 h at the concentration of 500 µL. Then, 90% of the Anopheles larvae were dead at 48 h at the highest concentration of 500 µL. The Chi-square test was used for the aqueous extract of *Portulaca oleracea* L. Anopheles larvae species showed the value of 1.000 for 48 h and 0.999 for 48 h, respectively.

3.11. Histological examination

After the larvicidal activity of *Culex quinquefasciatus and Anopheles stephensi* larvae species were tested, the larvae were subjected to histoarchitecture studies.

In Fig. 4 histoarchitectures 1 and 4 showed no damage in the larvae species while histoarchitectures 2, 3, 5 and 6 showed some damages in the gut and stomach region. By consuming the aqueous and ethanol extract, the larvae were damaged and died (Dey et al., 2020).

4. Discussion

In this study, the *Portulaca oleracea* L. plants leaves were used. The results were explained and tabulated under a couple of assays as followed, phytochemical analysis, total protein content, total carbohydrate content, GC–MS (gas chromatography–mass spectroscopy) and the larvicidal activity. The plant naturally has many medicinal properties, such as for diuretic, treatment for cardiovascular diseases and live related diseases as well, which were reported by Okafor and Ezejindu (Okafor and Ezejindu, 2014). It is a succulent plant that has a high amount of fats and lipids presenting in it. It is one of the richest sources of omega-3 fatty acids, which is an essential fatty acid that cannot be synthesized by the human body. So, the fatty acid must be taken by a dietary source (Sodeifian et al., 2018).

In the phytochemical analysis, the result shows the presence of alkaloids, steroids, phenol, terpenoids, tannins, protein, carbohydrate, glycosides and saponins which was reported in the study (Xiang et al., 2005). In this phytochemical analysis, some of the compounds were present in the trace amount. Other compounds were present in moderate levels and reported in the study (Ezeabara et al., 2014). In total protein content, the aqueous and ethanol extracts of the plant were analyzed. Then the values were shown in Fig. 1A. As noted in the figure, 1 mL plant aqueous and ethanol extract contained 200 µg and 220 µg of protein, respectively. In the aqueous extract, the protein range was slightly lower compared with the ethanol extract. In the fresh plant, it has a higher amount of proteins presenting in the plant (Mohamed and Hussein, 1994). Carbohydrates such as starch, sucrose, cellulose, glucose and fructose are present in the trace amount. They go through the metabolic pathways and generate the energy to the plant and the humans. In this analysis, 1 mL of aqueous and ethanol extract containing 112 µg and 142 µg of carbohydrate is present in it. The test was conducted with the fresh *Portulaca oleracea* L. plant (Nemzer et al., 2020).

The total phenol and flavonoid contents were also analyzed from both the aqueous and ethanol extract of the plant. As shown in Figs. 1C and 1 D, gallic acid was used as the standard for the phenol content while rutin was used as a standard for the flavonoid content.

The electron donation ability of natural products can be measured by 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH

solution. In the present study, aqueous and ethanol extract showed significantly higher inhibition percentage and positively correlated with total phenolic content. Results of this study suggest that the plant extract contains phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage (Deng et al., 2011; Müller et al., 2011).

In the GC–MS analysis, the compounds presenting in the aqueous and ethanol extract of the *Portulaca oleracea* L. were analyzed and listed in Tables 2 and 3. The result from the aqueous and ethanol extracts showed that the presence of the linolenic acid and docosahexaenoic acid was omega-3 fatty acid. It is the main compound of the study that plays the role in producing toxins to kill the mosquito larvae species and is useful for human growth and development (Zhu et al., 2010).

The mosquito larvae species such as *Culex quinquefasciatus* and *Anopheles stephensi* were killed by this activity. Mostly, the larvae are more dangerous compared with the mosquito (Egunjobi et al., 2020). The mosquito ranges would be decreased if the larvae species were killed. The larvae are converted into the mosquito and fly at 10 days, and mostly, it will lay eggs in other water bodies. It was reported in that the World Health Organization processed a standard procedure to kill the mosquito larvae species (WHO, 1996). In the world, the majority of people have died from the dangerous mosquito. So, this study will provide evidence to protect humans from the mosquito and mosquito larvae species (Perumalsamy et al., 2015; Senthilkumar et al., 2009). Compared with the results of the larvicidal activity on *Culex quinquefasciatus* and *Anopheles stephensi*, showing that the *Portulaca oleracea* L. extract was effective against the *Culex quinquefasciatus* mosquito larvae species as reported in the study (Sirivanakarn and White, 1978). While comparing with the other report, it can be used as the larvicidal plant in the world. The extract can be used as mosquito-repellent coils.

Culex quinquefasciatus larvae species were killed by the decanoic acid, hexadecanoic acid and octadecanoic acid which have the larvicidal activity (Hemalatha et al., 2015). The compounds were analyzed by the GC–MS analysis. The artificial oils may be used for asthma patients by some side effects. Thus, this plant also has the anti-asthma property. The *Portulaca oleracea* L. aqueous and ethanol leaves extract can kill the mosquito larvae species, which has been proved by the above larvicidal activity and histoarchitecture. There are many effective compounds presenting in the aqueous and ethanol extract of *Portulaca oleracea* L. Various concentrations were analyzed for the larvicidal activity. 500 μL of extract has a higher activity to damage the larvae. Histoarchitecture also showed the damages in the mosquito larvae species, as shown in Fig. 4 (Vivekanandhan et al., 2018).

The above study showed that the *Portulaca oleracea* L. plant had highly medicinal values. The phytochemical constituents, protein and carbohydrate were present in the plant. This plant also could kill mosquito larvae of *Culex quinquefasciatus* and *Anopheles stephensi* which had the larvicidal activity (Hill and Connelly, 2009).

5. Conclusion

The above study concluded that the *Portulaca oleracea* L. plant had many medicinal values, and some of which were required for protection human beings. The plant has many phytochemical analyses, saponins, tannins, terpenoids and some other chemical compounds presenting in this plant which were analysed. The *Portulaca oleracea* L. plant has various bioactive compounds such as a high amount of protein, a high amount of carbohydrate, a rich source of fatty acids like omega-3 fatty acids such as linolenic acid, linoleic acid and flavonoids. Presences of these compounds were analyzed through GC–MS. From the GC–MS analysis of aqueous and ethanol extracts of the *Portulaca oleracea* L., it was identified that the presence of omega-3 fatty acids and other important components was responsible for the larvicidal activity of *Culex quinquefasciatus larvae* and *Anopheles stephensi.* 80% to 90% of the mortality was recorded in the study. Although it is a weed plant, which contains various medicinal ingredients and be used as larvicidal plants. Application of the bioactive compounds presenting in the plant extract may be very useful for the preparation of mosquito repellent kit in future.

Ethical Approval

Not applicable.

Data Availability

Nil.

Funding

Nil.

Declaration of Competing Interest

There is no conflict of interest among the authors.

CRediT authorship contribution statement

Parthasarathy Sudharsan: conceptualization, methodology, investigation and writing - original draft; **Durairaj Siva:** visualization of larvicidal activity; **Kamaraj Prabhu**: GC-MS; and **Chandran Janani**: supervision.

Acknowledgement

The authors sincerely thank Srimad Andavan Arts and Science College, Tiruchirappalli, Tamil Nadu, India, to carry out the research. The authors also thank ICMR Institute, Madurai for providing the *Culex quinquefasciatus* and *Anopheles stephensi* larvae.

ORCID

Janani Chandran, https://orcid.org/0000-0003-1416-501X.

Supplementary Materials

Nil.

References

Abdelhamid et al., 2020. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. doi:10.1002/14651858.CD003177.pub5.
Aly, A.A., Khamis, I.M., 2018. Antimicrobial and Preliminary Phytochemical investigation of Galeniaasiatica (Mustard bush). Sci 8 (03), 768–775.
Chen, Z., Bertin, R., &Froldi, G., 2013. EC50 estimation of antioxidant activity in DPPH assay using several statistical programs. Food Chem 138 (1), 414–420.
Deepak, T., Shashank, A.S., Shivaraj, Y., & Asiya Nuzhat, (2019) F. B. Toxic effect of Bacillus thuringiensis (Serotype 14) bacteria shows behavioural & histological changes on mosquito larvae.
Deng, J., Cheng, W., Yang, G., 2011. A novel antioxidant activity index (AAU) for natural products using the DPPH assay. Food Chem 125 (4), 1430–1435.
Dey, P., Goyary, D., Chattopadhyay, P., Kishor, S., Karmakar, S., Verma, A., 2020. Evaluation of larvicidal activity of Piper longum leaf against the dengue vector, Aedes aegypti, malarial vector, Anopheles stephensi and filariasis vector, Culex quinquefasciatus. S. Afr. J. Bot. 132, 482–490.
Dhossaradhan, P., Janani, C., Siva, D., 2021. Larvicidal Activity of Aqueous Extract of Solanum trilobatum L. Leaves and Stem against Aedes aegypti. IJONS 12, 65.
Egunjobi, F.B., Okoye, I.C., 2020. Ovicidal and Larvicidal Activities of Ethanolic Leaf Extracts of Three Botanicals Against the Malaria Vector-Anopheles Gambiae. Int. Ann. Sci 9 (1), 111–121.
Ezeabara, C.A., Ikeh, C.F., Ilodibia, C.V., Aziagba, B.O., Okanume, O.E., &Mbaekwe, E.I., 2014. Comparative determination of phytochemical, proximate and mineral compositions in various parts of Portulaca oleracea L. J. Plant Sci 2 (6), 294–298.
Gates, B., 2014. The deadliest animal in the world. Gates notes. com 25.
Geetha, T.S., Geetha, N., 2014. Phytochemical screening, quantitative analysis of primary and secondary metabolites of Cymbopogancitratus (DC) stapf. leaves from Kodaikanal hills, Tamilnadu. Int. J. Pharmtech. Res 6 (2), 521–529.
Harris, W.S., 2020. Nutrigenetics: omega-3 and Omega-6 Fatty Acids. In: Principles of Nutrigenetics and Nutrigenomics. Academic Press, pp. 223–226.

Hemalatha, P., Elumalai, D., Janaki, A., Babu, M., Velu, K., Velayutham, K., &Kaleena, P.K., 2015. Larvicidal activity of Lantana camara aculeata against three important mosquito species. J. EntomoL. ZooL. Stud. 3 (1), 174–181.

Hill, S., & Connelly, R. (2009). Features creatures: southern house mosquito. UF. EEN Y–457.

Huda-Faujan, N., Noriham, A., Norrakiah, A.S., &Babji, A.S., 2009. Antioxidant activity of plants methanolic extracts containing phenolic compounds. Afr. JOL Bt. 8 (3).

Javanmardi, J., Stushnoff, C., Locke, E., &Vivanco, J.M., 2003. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chem 83 (4), 547–550.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Bio. Chem. 193, 265–275.

Mohamed, A.I., Hussein, A.S., 1994. Chemical composition of purslane (Portulaca oleracea). Plant Food. Hum. Nutr. 45 (1), 1–9.

Müller, L., Fröhlich, K., &Böhm, V., 2011. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. Food Chem 129 (1), 139–148.

Nemzer, B., Al-Taher, F., &Abshiru, N., 2020. Phytochemical composition and nutritional value of different plant parts in two cultivated and wild purslane (Portulaca oleracea L.) genotypes. Food Chem, 126621.

Okafor, I.A., &Ezejindu, D.N., 2014. Phytochemical studies on Portulaca oleracea (purslane) plant. GJBAHS 3 (1), 132–136.

Osonwa, U.E., Umeyor, C.E., Okon, U.V., Uronnachi, E.M., &Nwakile, C.D., 2012. Stability studies on the aqueous extract of the fresh leaves of Combretummicranthum G. Don used as antibacterial agent. J. Chem. ChemEng. 6 (5).

Perumalsamy, H., Jang, M.J., Kim, J.R., Kadarkarai, M., &Ahn, Y.J., 2015. Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in Millettiapinnata seed toward three mosquito species. Par.. Vec. 8 (1), 237.

Qiu, M., Xie, R.S., Shi, Y., Zhang, H., Chen, H.M., 2010. Isolation and identification of two flavonoid-producing endophytic fungi from Ginkgo biloba L. Ann. Microbiol 60 (1), 143–150.

Saeed, N., Khan, M.R., Shabbir, M., 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilisleptophylla L. BMC Complement Altern. Med. 12 (1), 1–12.

Senthilkumar, N., Varma, P., &Gurusubramanian, G., 2009. Larvicidal and adulticidal activities of some medicinal plants against the malarial vector, Anopheles stephensi (Liston). Par. Res. 104 (2), 237–244.

Sirivanakarn, S., & White, G.B. (1978). Neotype designation of Culex quinquefasciatus say (Diptera: culicidae). SMITHSONIAN INSTITUTION WASHINGTON DC MEDICAL ENTOMOLOGY PROJECT.

Sodeifian, G., Ardestani, N.S., Sajadian, S.A., &Moghadamian, K., 2018. Properties of Portulaca oleracea seed oil via supercritical fluid extraction: experimental and optimization. J. Super Flu 135, 34–44.

Spanos, G.A., Wrolstad, R.E., 1990. Influence of Processing and Storage on the Phenolic Composition of Thompson Seedless Grape Juice. J. Agric. Food Chem. 38, 1565–1571. doi:10.1021/jf00097a030.

Uddin, M.K., Juraimi, A.S., Ismail, M.R., &Brosnan, J.T., 2010. Characterizing weed populations in different turfgrass sites throughout the Klang Valley of Western Peninsular Malaysia. Weed Technol. 24 (2), 173–181.

Vivekanandhan, P., Kavitha, T., Karthi, S., Senthil-Nathan, S., &Shivakumar, M.S., 2018. Toxicity of Beauveria bassiana-28 mycelial extracts on larvae of Culex quinquefasciatus mosquito (Diptera: culicidae). Inter. J. Envirres. Pub. 15 (3), 440.

World Health Organization. (1996). Report of the WHO Informal Consultation on the" Evalua TestInsect", WHO/HQ, Geneva, 7 to 11 October 1996 (No. CTD/WHOPES/IC/96.1). World Health Organization.

Xiang, L., Xing, D., Wang, W., Wang, R., Ding, Y., Du, L., 2005. Alkaloids from Portulaca oleracea L. Phytochem 66 (21), 2595–2601.

Zahin et al., 2010. Broad spectrum antimutagenic activity of antioxidant active fraction of Punica granatum L. peel extracts. doi:10.1016/j.mrgentox.2010.08.001.

Zahin et al., 2017. Identification of putative G-quadruplex forming sequences in three manatee papillomaviruses. doi:10.1101/138602.

Zhu, H., Wang, Y., Liang, H., Chen, Q., Zhao, P., Tao, J., 2010. Identification of Portulaca oleracea L. from different sources using GC–MS and FT-IR spectroscopy. Talanta 81 (1–2), 129–135.