International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2023; 10(4): 37-42

https://www.dipterajournal.com
Received: 05-05-2023
Accepted: 11-06-2023

Manjunath Basappa Channi
Assistant Professor Department of Zoology, DRM Science College Davangere, Karnataka, India.

Pulikeshi M Biradar
Department of Zoology, Karnataka University Dharwad, Karnataka, India

Corresponding Author:
Pulikeshi M Biradar
Department of Zoology, Karnatak University Dharwad, Karnataka, India

Larvicidal efficacy of some plant extracts on *Culex quinquefasciatus* with characterization of their bioactive compounds

Manjunath Basappa Channi and Pulikeshi M Biradar

DOI: https://doi.org/10.22271/23487941.2023.v10.i4a.685

Abstract
Annually millions of people get affected by mosquito borne diseases worldwide. Lymphatic filariasis, West Nile Virus (WNV) and St. Louis encephalitis are major diseases transmitted by *Culex quinquefasciatus*. Three plants *Oxalis corniculata, Maytenus senegalensis*, and *Cassine glauca* were selected for biocontrol of Cx. quinquefasciatus and leaf extracts of the same plants in different solvents have shown good larvicidal activity on 4th instar larvae of Cx. quinquefasciatus. *Oxalis corniculata* extracts in Ethyl acetate, Chloroform, and Hexane were reported to have LC50 values of 33.23 ppm, 82.20 ppm, and 215.19 ppm against *C. quinquefasciatus*, LC50 values for *Maytenus senegalensis* extract in Ethyl acetate, Chloroform, and Hexane against *C. quinquefasciatus* were determined to be 87.87 ppm, 177.20 ppm, and 164.53 ppm and the LC50 values for *Oxalis corniculata* extract in Ethyl acetate, Chloroform, and Hexane against *C. quinquefasciatus* were 79.65 ppm, 177.20 ppm, and 133.80 ppm. The *M. senegalensis* ethyl acetate extracts showed greatest mortality rate and the *Cassine glauca* chloroform extract showed the least amount of activity (1%).

Keywords: Culex quinquefasciatus, bio-control, *oxalis corniculata*, *Maytenus senegalensis*, *Cassine glauca*

1. Introduction
More than 700 million individuals affected each year, by mosquito-transmitted illnesses continue to be a significant cause of death in humans worldwide [1]. Mosquito-borne illnesses have a negative influence on the economy, especially in nations with tropical and subtropical climates, and may also result in decreased labour and commercial outputs [2]. *Lymphatic filariasis* is a tropical illness that affects many individuals, affecting 120 million people globally and 44 million of them have a common chronic manifestation caused by *C. quinquefasciatus* [3].
Programs for controlling mosquitoes mostly rely on phytochemicals. By extracting the bioactive plant ingredient(s) using various polar and nonpolar solvents, such as benzene, petroleum ether, methanol, chloroform, acetone, absolute alcohol, etc., the complete plant or a particular part may be used [4]. Using botanicals that are easily biodegradable, nontoxic, and have wide range target specific action is one such option [5]. One strategy that offers a less expensive and more environmentally friendly means of controlling mosquito larvae is the use of phytochemicals found in the oils, leaves, and roots of plants [6].
The most efficient method of controlling this mosquito infestation is to use larvicides to inhibit mosquito reproduction. During the last 30 years in various countries, synthetic pesticides like organophosphates have been employed as larvicides [7]. The fact that these chemical pesticides are non-selective and may damage other creatures in the natural ecosystem, is a significant disadvantage to their usage [8]. The issue with toxicity and the rise in insect resistance serve as reminders of the need for efficient pesticides that are eco-friendly, target-specific, and biodegradable. The secondary metabolites of plants are considered as a potentially viable alternative approach against various life stages of different species of mosquitoes due to their excellent properties such as cheap availability, environmental safety, and the presence of a rich source of bioactive compounds [9-12]

The goal of the current research work was to characterize the bioactive chemical compound and investigate the larvicidal activity of the leaves of *Oxalis corniculata, Maytenus senegalensis,* and *Cassine glauca* against the larvae of *Cx. quinquefasciatus* mosquitoes.

2. Materials and Method

2.1 Plants materials

Plants of *Oxalis corniculata, Maytenus senegalensis,* and *Cassine glauca* were gathered from the city's local market. These plants were dried in the shade at room temperature for about 20 days.

2.2 Preparation of plant extract

The shade dried leaves of each plant (600 g) were mechanically ground into a powder using a commercial electric stainless-steel blender before being extracted for 6 hours in a Soxhlet apparatus with hexane (2,200 ml), chloroform (1,000 ml), and ethyl acetate (2,500 ml). The extracts were filtered by using Whatman (number 1) filter paper and a Buchner funnel. The residue recovered was held at 4ºC after the extract was concentrated at 45ºC under decreased pressure (22–26 mm Hg). The leftovers were then turned into an acetone-based 1% stock solution. Dilutions were created from the stock solution, 1000- 4.69 mg/l, using dechlorinated tap water. In the final test solution, Polysorbate 80 (Qualigens) was utilized as an emulsifier at a concentration of 0.05% [13].

2.3 Phytochemical screening

As per the procedures outlined by Senthil Kumar and Reetha (2009) [14] and Nazar *et al*. (2009) [15], the phytochemical analysis of all extracts was completed.

2.4 GC-MS analysis

GC-MS analysis of the crude extracts of shade dried leaves was carried out, which featured an auto sampler and GC-MS instrument, on an Agilent technology (6890 N). With a constant flow of 1.491 ml min^{-1}, an injection volume of 1 ml, an injector with 140^0C, and an ion source of temperature 200^0C, helium (99.999%) was used as the carrier gas. 624 ms capillary column operating in an electron mode at 70 eV. A 45°C setting was made for the oven. Mass spectra were captured at 70 eV.

2.5 Mosquito culture

Cx. quinquefasciatus mosquitoes used for all the experiments are raised in laboratories without exposure to diseases or pesticides. At a temperature of 25 to 29ºC, cyclical generations of this mosquitoe were kept alive. Dog biscuit powder and yeast were used as larval feed in a ratio 3:1, and 10% glucose solution was given to adult mosquitoes [16].

2.6 Larvicidal bioassay

The modified version of the WHO (1996) and the Rahuman *et al*. (2000) [17] procedure was used to assess the larvicidal activity. Larvae were collected for the bioassay test and placed in 249 ml of aqua with one ml of the selected plant extract. Polysorbate 80 and petroleum ether were used to create the control. After 24 hours of exposure, counting the quantity of dead larvae and the % mortality was calculated by using the average of five repeats.

2.7 Larval susceptibility tests

The WHO procedure was followed for conducting the test on larval susceptibility. The following approach was used to test each solution on an *Cx. quinquefasciatus* fourth instar larva to determine if it had any larvicidal properties or not. In 400 ml of the extract solution, 25 fourth instar larvae were released, and parallel control trials without extract were conducted. The average mortality rate from each experiment was determined by counting the number of larvae that had died after being exposed to each solution for 24 hours. When control mortality varied between 5% - 20%, mortality was noted and adjusted using Abbott's formula. The Abbott's (1925) formula was used to adjust the observed percentage mortality during the observation of the plant extract's larvicidal ability.

2.8 Statistical analysis

To determine the significance of relationship between the components and the death rate, a three-way factorial ANOVA with variables of concentrations, instars, and hours was performed. We performed statistical analysis on the experimental data using SPSS 25 and MS Excel so as to determine the fatal concentrations, regression equations, and regression co-efficient.

3. Results and Discussion

3.1 Phytochemical screening

Plant extracts were subjected to a preliminary phytochemical screening, which identified the presence of phenol, tannins, coumarins, alkaloids, flavonoids, quinones, terpenoids, carbohydrates, triterpenoids, saponins, vitamin E and ascorbic acid (Table-1).

Table 1: Phytochemical screening of plant extracts of *Oxalis corniculata, Maytenus senegalensis* and *Cassine glauca.*

Sl. No.	Secondary metabolites	*Oxalis corniculata,*	*Maytenus senegalensis*	*Cassine glauca*
1.	Phenol	+ + +	+ + +	+ + +
2.	Tannins	+ + +	+ + +	+ + +
3.	Coumarins	+ + +	+ + +	+ + +
4.	Alkaloids	+ + +	+ + +	+ + +
5.	Flavonoids	+ + +	+ + +	-
6	Quinones	+ + +	+ + +	+ +
7.	Terpenoids	+ + +	+ +	+ + +
8.	Carbohydrates	+ + +	+ + +	+ + +
9.	Triterpenoids	+ + +	+ + +	+ + +
10.	Saponins	-	+ + +	+ + +
11.	Vitamin E	+ + +	-	-
12.	Ascorbic acid	+ + +	-	-

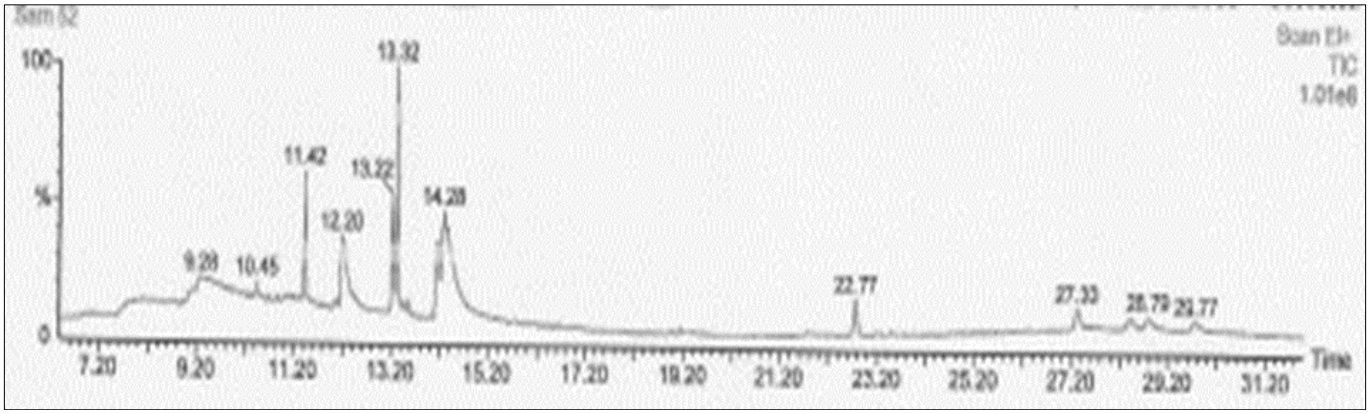
The composition and identification of the main compounds present in the ethyl acetate extracts of plants (*Oxalis corniculata*, *Maytenus senegalensis* and *Cassine glauca*) are shown in Table-2.

By using GC–MS, 12 compounds were identified in *Oxalis corniculata and* in *Maytenus senegalensis*, *Cassine glauca*, 7 and 5 compounds were identified respectively. The main compounds were "α-D-glucopyranoside, O-α-D-glucopyranosyl (1. fwdarw.3) -α- D fructofuranosyl, 9,12-Octadecadienoic acid, methyl ester, hexadecanoic acid, 12-hydroxy- 9- octadecenoic acid, 9,12,15-Octadecatrienoic acid, 9-octadecenoic acid, 1- dimethoxyacetone and 13-hexyloxacyclotridec-10-en-2-on" (Fig. 1)

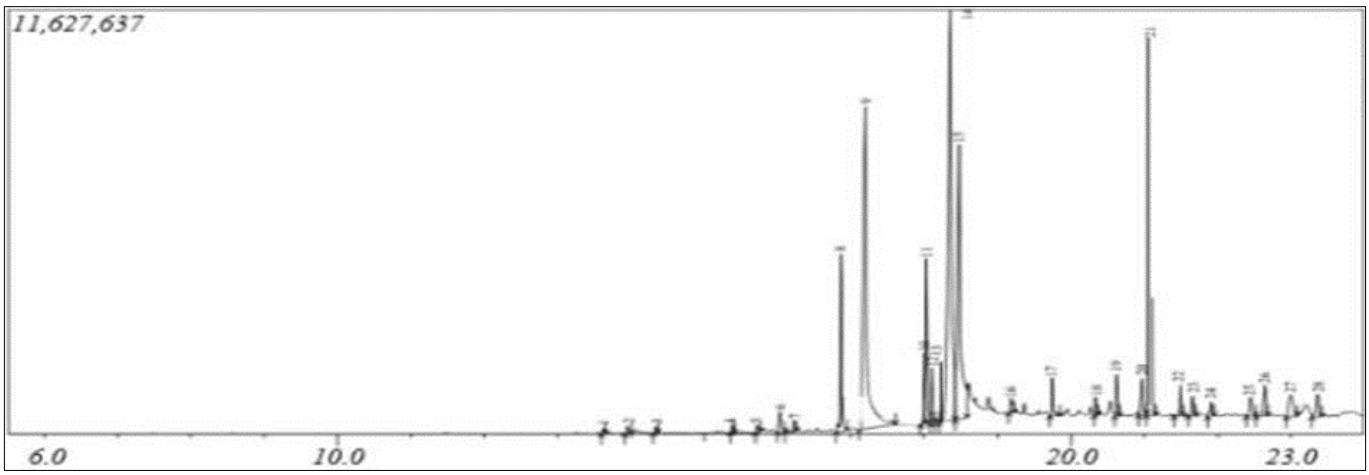
3.2 GC-MS Analysis

Table 2: Phytocompounds identified from the whole plant extract of *Oxalis corniculata*, *Maytenus senegalensis* and *Cassine glauca* by GC–MS

Oxalis corniculata,	***Maytenus senegalensis***	***Cassine glauca***
"D-Glucose, 4-o-α-D-glucopyranosyl"	Hexadecanoic acid	Dibutyl phthalate
"α-D-glucopyranoside, O-α-D-glucopyranosyl (1. fwdarw.3) -α D fructofuranosyl"	9-octadecenoic acid (Z)	Phthalic acid
"Hexadecanoic acid, Methyl ester"	"12-hydroxy-9- octadecenoic acid"	Butyl ester
"n-Hexadecanoic acid"	Methyl-1-cyclopentene-1-carbxylate	Hexadecenoic acid
"9,12-Octadecadienoic acid, Methyl ester"	12-hydroxy-9-octadecenoic acid	Ethyl ester
"9,12-Octadecadienoic acid"	Methyl ester	
"9,12,15-Octadecatrienoic acid, Methyl ester"	1, 1-dimethoxyacetone and 13 hexyloxacyclotridec-10-en-2-on	
Squalene		
Vitamin E		
"9,12-Octadecadienoic acid (Z, Z)-, phenyl, Methyl ester"		
"1b, 5, 5, 6a Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one"		
"7-Oxabicyclo [4.1.0] heptanes,1-methyl-4-(2-methyloxiranyl)"		



a)



b)

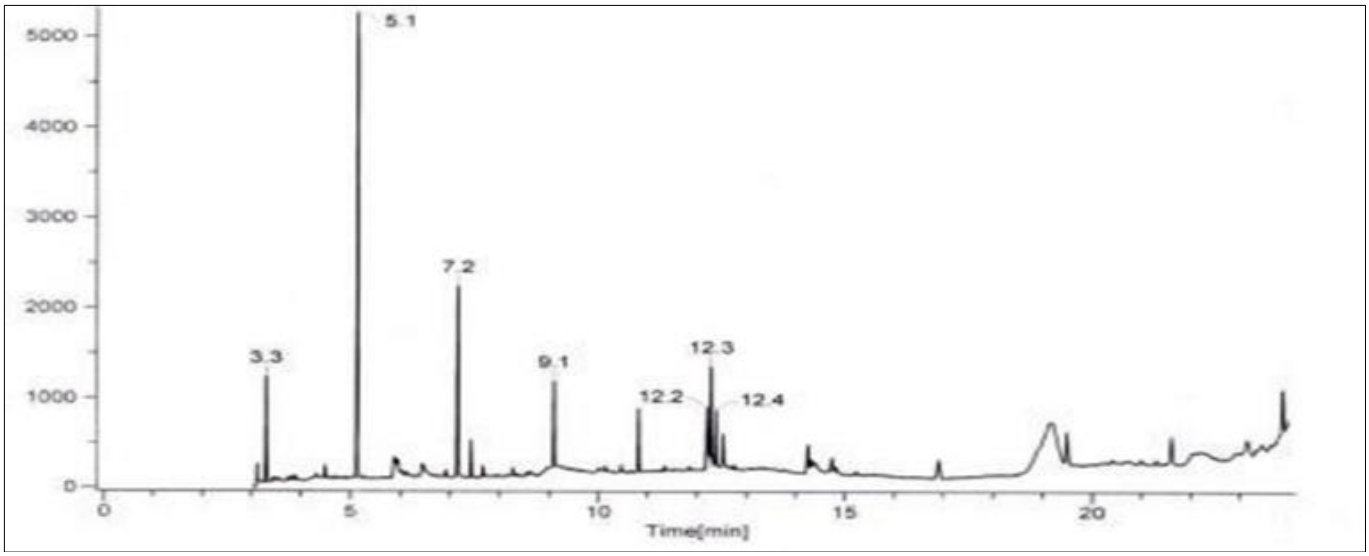


Fig 1: GC–MS chromatogram of a) *Oxalis corniculate* b) *Maytenus senegalensis* and c) *Cassine glauca*

3.3 Larvicidal Activity

Table 3: Larvicidal activity of different solvent leaf extracts of selected plants against 4th instar larvae of *C. quinquefasciatus* at 400 ppm (0.04%)

Sr. No	Plant names	Ethyl acetate	Hexane	Chloroform
1.	*Oxalis corniculata*,	90.6±0.5	26.6±2.3	10.6±1.1
2.	*Maytenus senegalensis*	97.3±0.5	21.3±0.5	34.6±1.1
3.	*Cassine glauca*	14.6±1.5	85.3±1.5	6.6±3.0

Table 4: Larvicidal efficacy of *Oxalis corniculata* against *C. quinquefasciatus*

Solvent	Mosquito species	LC50 (ppm)	95% confidence interval		LC90 (ppm)	95% confidence interval		Chi-Square
			Lower Bound	Upper Bound		Lower Bound	Upper Bound	
Hexane	*C. quinquefasciatus*	215.19	24.453	39.977	71.64	62.020	88.831	0.454
Chloroform	*C. quinquefasciatus*	82.20	0.544	168.247	201.57	134.641	655.708	8.919
Ethyl acetate	*C. quinquefasciatus*	33.23	136.617	315.137	527.86	399.508	844.975	8.133

Table-3 displays the findings on the larvicidal effectiveness of various solvent extracts of the chosen plants. After 24 hours of exposure at a 400 ppm (0.04%) concentration, all plant extracts had a fair to moderate impact on *Cx. quinquefasciatus* fourth instar larvae. The *M. senegalensis* ethyl acetate extracts showed greatest mortality (100%) rate. The activity was shown to be significant (P>0.05) in ethyl acetate extracts. The remaining extracts of the chosen plants exhibited the least larvicidal activity, while other extracts showed significant (45-82%) larvicidal activity. The *Cassine glauca* chloroform extract showed the least amount of activity (1%).

Plant extracts in Hexane, Chloroform, and Ethyl acetate exhibit mediocre larvicidal action against mosquito vectors. *Oxalis corniculata* extracts in Ethyl acetate, Chloroform, and Hexane were reported to have LC50 values of 33.23 ppm, 82.20 ppm, and 215.19 ppm against *C. quinquefasciatus* in Table 4. With an LC50 value of 33.23 against *C. quinquefasciatus*, the extract made of ethyl acetate showed relatively high potency. Nevertheless, hexane and chloroform extract were less effective against *C. quinquefasciatus*

Table 5: Larvicidal efficacy of *Maytenus senegalensis* against *C. quinquefasciatus*.

Solvent	Mosquito Species	LC50 (ppm)	95% confidence interval		LC90 (ppm)	95% confidence interval		Chi-Square
			Lower Bound	Upper Bound		Lower Bound	Upper Bound	
Hexane	*C. quinquefasciatus*	164.53	47.775	287.333	478.42	335.375	994.149	2.639
Chloroform	*C. quinquefasciatus*	177.20	61.397	300.813	434.67	352.246	1011.943	2.187
Ethyl acetate	*C. quinquefasciatus*	87.87	62.326	112.006	268.25	225.219	340.198	2.036

According to Table-5, the LC50 values for *Maytenus senegalensis* extract in Ethyl acetate, Chloroform, and Hexane against *C. quinquefasciatus* were determined to be 87.87 ppm, 177.20 ppm, and 164.53 ppm, respectively. Ethyl acetate extract showed relatively high efficacy against *C. quinquefasciatus*, with an LC50 value of 87.87 ppm. Nevertheless, the effectiveness of hexane and chloroform extract against *C. quinquefasciatus* was noticed to be low

Table 6: Larvicidal efficacy of *Cassine glauca* against *C. quinquefasciatus*.

Solvent	Mosquito species	LC50 (ppm)	95% confidence interval		LC90 (ppm)	95% confidence interval		Chi -square
			Lower Bound	Upper Bound		Lower Bound	Upper Bound	
Hexane	*C. quinquefasciatus*	133.80	109.286	161.074	320.56	273.913	394.119	2.307
Chloroform	*C. quinquefasciatus*	177.20	145.097	212.275	433.67	374.651	525.196	4.637
Ethyl acetate	*C. quinquefasciatus*	79.65	2.262	133.040	499.34	376.800	596.013	2.439

According to Table-6, the LC50 values for *Oxalis corniculata* extract in Ethyl acetate, Chloroform, and Hexane against *C. quinquefasciatus* were 79.65 ppm, 177.20 ppm, and 133.80 ppm, respectively. Here also Ethyl acetate extract showed relatively high efficacy against *C. quinquefasciatus*, with an LC50 value of 79.65 ppm. Nevertheless, the effectiveness of Hexane and chloroform extract against *C. quinquefasciatus* was reduced here.

4. Discussion

The increasing use of synthetic pesticides has been connected to a number of contentious contamination incidents [18]. Several nations throughout the globe place a high focus on spreading knowledge about and using Integrated Pest Management (IPM), strategies as well as using biopesticides and less damaging chemicals. If more individuals had access to good training on how to utilize biopesticides, there may be a decrease in the wasteful use of chemicals that have been stored. For incorporation of Integrated Pest Management (IPM) systems, biopesticides made from non-toxic or low-toxic secondary plant metabolites provide new opportunities. The objective is to develop biopesticides that are efficient but also emit less hazardous by-products into the environment, as well as products that biodegrade swiftly and are advantageous to both humans and beneficial insects [19]. The search for the development of more eco-friendly biopesticides has been prompted by these issues. Insecticide resistance in certain populations is being decreased by new plant-based biopesticides [20]. Plant extracts and their secondary metabolites have been successfully used throughout the history to reduce insect infestations [21, 22].

Natural botanicals are increasingly being used as a safe substitute for synthetic pesticides, whose widespread availability and quick degradability make them a popular choice. A few botanicals, such as *Chrysanthemum cinerarifolium*, have transitioned from laboratory to field application; even though other plants from various families have been shown to have mosquitocidal properties [23, 24].

The large range of secondary metabolites produced by the plants participate in many biological functions. In the current study, phytochemicals like saponins, tannins, alkaloids, and flavanoids were found to be the main mosquito-inhibiting components in the leaf extracts of chosen plants. In most of the preliminary research, steroidal saponins were identified as the active ingredients with mosquito larvicidal properties. According to Wiesman and Chapagain [20], saponin taken from the *Balanites aegyptica* caused 100 percent inhibition of *Stegomyia aegypti* larvae. Ghosh and Chandra [25] assessed the larvicidal efficacy of a saponin combination derived from *Cestrum diurnum* against *A. stephensi* mosquito.

According to Lee [26] and Francois *et al*. [27], alkaloids extracted from the *Piper longum* fruit and *Triphyophyllum pellatum* both displayed larvicidal efficacy against the larvae of *C. pipiens* and *A. stephensi*, respectively. Joseph *et al*. [28], discovered that the, iso-flavonoids found in the tubers of *Neorautanenia mitis* exerted larvicidal effects on the *Cx. quinquefaciatus* and *A. gambiae*. Mann *et al*. [29] discovered that the root of *M. senegalensis* include alkaloids, flavonoids, saponins, tannins, and steroidal substances that were efficient against mosquitoes.

In the current investigation, it was discovered that an extract from mature *M. senegalensis* leaves had significant larvicidal action against *Cx. quinquefasciatus* mosquitoes. The extract of ethyl acetate showed the strongest larvicidal effect against mosquito larvae. Many bioactive compounds were detected in the qualitative and chromatographic examination, and the bioassay experiment's probit analysis confirmed the bioactive chemicals' substantial LC50 values for various instars of *C. quinquefasciatus* larvae. Yet the IR spectra and GCMS analyses of the bioactive compounds used in the current investigation also revealed the existence of steroid compound(s) that might be too accountable for larval toxicity.

5. Conclusion

The present results showed that, compared to *Oxalis corniculata*, and *Cassine glauca*, the leaf extract of *Maytenus senegalensis* had the potential to be larvicidal. Screening, purifying, and identifying potentially beneficial compounds discovered in these species would surely increase the effectiveness in mosquito control. Stagnant water provides breeding habitat for mosquitoes, which may spread a wide range of ailments. In certain areas, the extract could be sprayed. Ultimately, we found that leaf extract of *Oxalis corniculata*, *Maytenus senegalensis*, and *Cassine glauca* has the potential to be developed as an ecofriendly larvicide. Also, our findings provide a foundation for future research into the larvicidal capabilities of natural plant product extracts.

6. Acknowledgment

I sincerely thank Bapuji Education Association and D.R.M. Science College Davanagere, Karnataka, India for permitting and providing necessary space to conduct the research work.

7. Reference

1. Taubes G. A mosquito bite back. New York Times Magazine. 1997 Aug 24;24:40-6.
2. Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. New England Journal of Medicine. 2002 Jul 4;347(1):13-8.
3. Bernhard L, Bernhard P, Magnussen P. Management of patients with lymphoedema caused by filariasis in North-eastern Tanzania: alternative approaches. Physiotherapy. 2003 Dec 1;89(12):743-9.
4. Rawani A, Ghosh A, Chandra G. Mosquito larvicidal activities of Solanum nigrum L. leaf extract against *Culex quinquefasciatus Say*. Parasitology Research. 2010 Oct; 107:1235- 40.
5. PreetiSharma LM, Srivastava CN. Larvicidal potential of *Nerium indicum* and *Thuja oriertelis* extracts against malaria and Japanese encephalitis vector. J Environ. Biol. 2005;26(4):657-60.
6. Mandal S. Effect of *Azadirachta indica A. Juss*

(Meliaceae) seed oil and extract against *Culex quinquefasciatus Say* (Diptera: Culicidae) larval susceptibility of Indian subcontinent. Macedonian Journal of Medical Sciences. 2011 Mar;4(1):5-11.
7. Borah R, Kalita MC, Kar A, Talukdar AK. Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus*. African Journal of Biotechnology. 2010;9(17):2527-30.
8. De Omena MC, Navarro DM, De Paula JE, Luna JS, De Lima MF, Sant' Ana AE. Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. Bioresource Technology. 2007 Sep 1;98(13):2549-56.
9. Rajkumar S, Jebanesan A. Scientific Note Oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract against the malarial vector *Anopheles stephensi.* Journal of Insect Science. 2005 Jan 1;5(1).
10. Kamaraj C, Rahuman AA, Bagavan A, Elango G, Zahir AA, Santhoshkumar T. Larvicidal and repellent activity of medicinal plant extracts from Eastern Ghats of South India against
11. Malaria and filariasis vectors. Asian Pacific Journal of Tropical Medicine. 2011 Sep 1;4(9):698-705.
12. Dohutia C, Bhattacharyya DR, Sharma SK, Mohapatra PK, Bhattacharjee K, Gogoi K, Gogoi P, Mahanta J, Prakash A. Larvicidal activity of few select indigenous plants of North East India against disease vector mosquitoes (Diptera: Culicidae). Trop Biomed. 2015 Mar 1;32(1):17-23.
13. Pavela R. Larvicidal effects of some Euro-Asiatic plants against *Culex quinquefasciatus Say* larvae (Diptera: Culicidae). Parasitology research. 2009 Aug;105:887-92.
14. Mehra BK, Hiradhar PK. Effect of crude acetone extract of seeds of *Annona squamosa* Linn. (Family: Annonaceae) on possible control potential against larvae of *Culex quinquefasciatus Say*. Journal of Entomological Research. 2000;24(2):141-6.
15. Senthilkumar PK, Reetha D. Screening of antimicrobial properties of certain Indian medicinal plants. Journal of Phytology. 2009 Sep 24;1(3).
16. Nazar S, Ravikumar S, Williams GP, Ali MS, Suganthi P. Screening of Indian coastal plant extracts for larvicidal activity of *Culex quinquefasciatus*. Indian journal of science and technology. 2009 Mar;2(3):24-7.
17. Arivoli S, Samuel T. Bioefficacy of *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) whole plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). International Journal of Current Research. 2011 Jan 27;3(4):296-304.
18. Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S, Himalayan B. Effect of *Feronia limonia* on mosquito larvae. Fitoterapia. 2000 Sep 1;71(5):553-5.
19. Obermok VV, Laikova KV, Gninenko YI, Zaitsev AS, Nyadar PM, Adeyemi TA. A short history of insecticides. J. Plant Prot. Res. 2015;55(3):221-226.
20. East R. Soil science comes to life. Microbiome. Nature. 2013;501:18-19.
21. Senthil-Nathan S. The use of Eucalyptus leaf exract as a natural larvicidal agent against malarial vector *Anopheles stephensi Liston* (Diptera: Culicidae). Bioresour. Technol. 2007;98:1856-1860.
22. Senthil-Nathan S. Physiological and biochemical effect of Neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. Front. Physiol. 2013;359(4):117.
23. Saktivadivel M, Thilagavathy D. Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone mexicana L*. seed. Biores Technol. 2003; 89: 213-216.
24. Sharma RS, Sharma GK, Dhilon GPS. National malaria eradication programme (DGHS). Delhi: Epidemiology and Control of Malaria in India, Shakun Enterprises; c1996, p.272.
25. Wiesman Z, Chapagain BP. Larvicidal effects of aqueous extracts of Balanites aegyptiaca (desert date) against the larvae of *Culex pipiens* mosquitoes. Afr J Biotechnol. 2005;4:1351-1354.
26. Ghosh A, Chandra G. Biocontrol efficacy of *Cestrum diurnum* (L.) (Solanales: Solanaceae) against the larval forms of *Anopheles stephensi*. Nat Pro Res. 2006;20: 371-379.
27. Lee SE. Mosquito larvicidal activity of pipernonaline, a piperidine alkaloid derived from long pepper, Piper longum. J Am Mos Cont Assoc. 2000;16:245-247.
28. Francois G, Looveren MV, Timperman G, Chimanuka B, Assi L A, Holenz J, *et al*. Larvicidal activity of the naphthylisoquinoline alkaloid dioncophylline-A against the malaria vector *Anopheles stephensi*. J Ethnopharmacol. 1996; 54:125-130.
29. Joseph CC, Ndoile MM, Malima RC, Nkunya MH. Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from Neorautanenia mitis. Trans Royal Soc Trop Med Hyg. 2004;98:451-455.