Elytrigia Repens Extracts Control Urban Mosquito Vectors with a Larvicidal Potential

Prakash D [1*], Mohideen Abdulkader M [1], Sakthivel D, Manju I [1], Sreenivasan K S [1], Manikandan S [2]

Abstract

There is over 17% of global illnesses due to Vector-borne diseases and more than a million fatalities annually found as a significant public health challenge globally. Traditional methods of mosquito control are relied heavily on chemical insecticides, and led to ecological disruptions, pesticide resistance, and adverse effects on human health and the environment. Consequently, there is a persistent need for alternative and eco-friendly strategies mosquito control. Method: In this study, we investigated the efficacy of extracts from Eragrostis repens (E. repens) against the larvae of Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus. Plant extracts were prepared using different solvents, and their phytochemical profiles were analyzed. The larvicidal activity of these extracts was evaluated through susceptibility tests over a 24-hour period. Results: Methanol extracts exhibited significant larvicidal activity against Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus, with LC50 values ranging from 43,173 ppm to 58,234 ppm. GC-MS analysis identified 11 bioactive compounds in methanol extracts, highlighting their potential insecticidal activity. Conclusion: Our findings suggest that E. repens holds promise as a natural larvicide against mosquito vectors. The study highlights the importance of exploring botanical alternatives for mosquito control, offering a sustainable and environmentally friendly approach to combatting mosquito-borne diseases.

Keywords: *Elytrigia repens,* phytochemical screening, *Anopheles stephensi, Culex quinquefasciatus.*

Significance | This study demonstrated larvicidal activity to prevent mosquito disease and its spread, which might be due to insecticidal phytocompounds in the extract.

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Introduction

More than 17% of illnesses are attributed to vector-borne pathogens, resulting in over 1 million fatalities annually (Rueda, 2008). Diseases such as dengue fever, malaria, Japanese encephalitis, and filariasis are predominantly transferred by the three genera: Aedes, Anopheles, and Culex (Rueda, 2008). While there are over 3000 mosquito species across 34 genera worldwide, only approximately 300 species are capable of spreading diseases to humans and other vertebrates (Rueda, 2008). In India alone, approximately 40 million people are affected annually by mosquito-borne diseases (Benelli, 2015a).

The global burden of illness, mortality, hunger, and social vulnerability, particularly in tropical countries, is largely attributable to mosquito-borne diseases (Mittal, 2003). Water disruption and chemical insecticide application targeting adult mosquitoes have been primary strategies for combating these illnesses (Tolle, 2009). Control measures involving organophosphate compounds such as chlorpyrifos, temephos, and fenthion, as well as insect growth regulators like diflubenzuron and methoprene, heavily rely on larval mosquito control (Tolle, 2009).

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However, the widespread use of chemical insecticides has raised concerns regarding their adverse effects on human populations (Karunamoorthi & Tsehaye, 2012).

The efficient use of control agents has disrupted natural ecological processes, leading to the development of insecticide resistance, resurgence of pests, and environmental pollution (Govindarajan et al., 2012; Ifeanyi et al., 2014). The indiscriminate use of pesticides has resulted in various inconveniences, including industrial, animal, and wildlife toxicity, as highlighted by the World Health Organization (WHO) (2010).

Furthermore, the ecological balance has been adversely affected by the residues of certain chemical compounds (Govindarajan et al., 2012; Ifeanyi et al., 2014). To address these challenges associated with traditional mosquito control, the development of new or complementary management methods is imperative (Amiya Kumar Prusty et al., 2016). This necessity has spurred the search for eco-friendly, cost-effective, biodegradable, and selective insecticides targeting mosquitoes.

In light of the aforementioned pesticide drawbacks, researchers worldwide are actively seeking alternative methods to protect the environment. These challenges underscore the urgency of developing novel mosquito control techniques. Plant extracts have emerged as promising sources of natural biocidal products in this regard (Osorio et al., 2014).

Alternatives to conventional pesticides, such as phytochemicals, have demonstrated efficacy in mosquito control (Lee et al., 2001). Many plant species contain a complex array of chemicals with pest control properties, with over forty thousand plant species identified as possessing such chemicals (Rutledge et al., 2003; Kobayashi et al., 2008).

Managing mosquito populations and preventing mosquito bites are key strategies in combating mosquito-borne diseases. The utilization of plant-derived products, such as insecticides or repellents, holds significance in this regard (Sylla el, H.K. et al., 2000).

Plants are recognized for their diverse biological properties and secondary metabolites, including tannins, glycosides, alkaloids, and flavonoids. Herbal compounds serve as a valuable natural resource for the development of chemical insecticides that act by disrupting larvae's cuticle membrane, ultimately leading to larval mortality (Moghadamtousi et al., 2015; Zuharah et al., 2016).

Presently, mosquito control efforts primarily target larvae, with additional measures directed towards adult mosquitoes whenever feasible. This strategy stems from the transient and less effective nature of adult mosquito control, contrasting with the localized and timely management of larvae, which results in fewer adverse effects. Larval management proves to be an efficient control mechanism, given that major breeding habitats are often man-made and easily identifiable, leading to limited larval mosquito mobility (Harborne & Hall, 1964; Howard & Zhou, 2007).

In this study, the larvicidal activity of E. repens extracts against fourth instar larvae of A. aegypti, A. stephensi, and C. quinquefasciatus is investigated.

Materials and methods

Selection of Plant:

Natural and disease-free specimens of the E. repens family were gathered from the natural population in and around Chennai, Tamil Nadu, India. The plant was identified and authenticated by Prof. P. Jayaraman and deposited at the Research Center for Plant Anatomy, West Tambaram, Chennai-45, Tamil Nadu, India.

Plant Extract Preparation:

The dried plants were pulverized using an electrical blender to obtain a fine powder. Subsequently, the plant powder was sequentially extracted with aqueous, chloroform, ethanol, and methanol solvents. Each solvent was used in a ratio of 50g of powder soaked in 500ml of solvent. The collected solvents were concentrated using a Rotary Evaporator and stored in airtight bottles at 40°C.

Selection of Mosquito Species:

The fourth instar larvae of A. aegypti, A. stephensi, and C. quinquefasciatus were selected for this study. A. aegypti is well-domesticated and anthropophilic, while Aedes and Anopheles species are responsible for transmitting arboviruses and malaria parasites, respectively. C. quinquefasciatus is a vector for lymphatic filariasis and is prevalent in tropical regions.

Preparation of Plant Solvent Extracts:

The plants were thoroughly washed and dried in the shade for approximately 20 days at room temperature. The dried plants were then powdered and extracted using a Soxhlet extractor with solvents including hexane, chloroform, ethanol, and methanol. The resulting extracts were concentrated using a rotary flash evaporator and stored in airtight bottles at 4°C for further use.

Phytochemical Profiling:

The phytochemical profiling of the samples was conducted according to methods described by Abubakar & Abdurrahman (1998) and Masfria & Permata (2018). The plant extracts were tested for various compounds including carbohydrates, alkaloids, flavonoids, phytosterols, anthocyanins, betacyanins, phenols, tannins, saponins, glycosides, and proteins.

GC-MS Analysis:

GC-MS analysis of the whole plant crude extracts was performed using Agilent Technologies (6890 N) and JEOL GCMATE II.

Mosquito Larvicidal Activity:

All experiments were conducted using laboratory-reared mosquitoes that were not exposed to insecticides or pathogens,

Table 1. Phytochemical profiling of E. repens plant extracts.

S. No	Secondary metabolites	Aqueous	Chloroform	Ethanol	Methanol
1	Carbohydrates	++	+++	++	+++
2	Tannins	+	+++	+	+++
3	Saponin	+++	++	+++	++
4	Flavonoids	+++	+++	+++	+++
5	Alkaloids	-	+++	-	+++
6	Quinones	-	+++	-	+++
7	Glycosides	-	-	-	-
8	Terpenoids	-	++	+	+++
9	Triterpenoids	-	-	+	+
10	Phenols	-	+++	-	+++
11	Coumarins	++	++	++	++
12	Acids	-	++	+++	-
13	Proteins	_	-	-	-
14	Anthocyanin	++	+++	-	+++
15	Cardiac glycosides	-	+	++	++
16	Steroids	-	+++	++	+++

+++ Strongly Positive ++ Positive

\+ Trace - Not detected

Table 2. Mosquito larvicidal activity of *E. repens* plant extracts against 4th instar larvae of *A. aegypti*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Sq
Aqueous	20	14	51.665 31.475 ± 75.905	124.635 82.267 ± 649.810	16.856
	40	29			
	60	49			
	80	72			
	100	93			
Chloroform	20	14	53.471 33.322 ± 80.382	138.731 88.600 ± 874.306	15.235
	40	31			
	60	47			
	80	66			
	100	91			
Methanol	20	13	49.787 28.496 ± 72.773	109.888 74.549 ± 535.037	20.286
	40	27			
	60	56			
	80	74			
	100	97			
Ethanol	20	15	48.103 23.787 ± 74.218	112.621 73.333 ± 946.409	22.336
	40	34			
	60	52			
	80	74			
	100	98			

Control- nil mortality

Significant at $p < 0.05$ level

LC_{50} - Lethal concentration that kills 50% of the exposed larvae

LC_{90} – Lethal concentration that kills 90% of the exposed larvae

UCL- Upper confidence limit; LCL- Lower confidence limit

Table 3. Mosquito larvicidal activity of *E. repens* plant extracts against 4th instar larvae of *A. stephensi*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Sq
Aqueous	20	13	54.568 37.170± 77.342	138.520 91.714± 558.616	12.597
	40	29			
	60	46			
	80	68			
	100	89			
Chloroform	20	11	70.333 63.074 ± 79.879	224.756 173.358 ± 333.031	5.899
	40	25			
	60	37			
	80	52			
	100	73			
Methanol	20	18	43.173 20.366 ± 63.976	95.513 64.349 ± 506.183	23.350
	40	35			
	60	61			
	80	84			
	100	100			
Ethanol	20	13	58.234 52.775 ± 64.396	166.072 136.448 ± 219.883	5.380
	40	28			
	60	47			
	80	63			
	100	81			

Control- nil mortality

Significant at $p < 0.05$ level

LC_{50} - Lethal concentration that kills 50% of the exposed larvae

LC_{90} – Lethal concentration that kills 90% of the exposed larvae

UCL- Upper confidence limit; LCL- Lower confidence limit

Table 4. Mosquito larvicidal activity of *E. repens* plant extracts against 4th instar larvae of *C. quinquefasciatus*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Sq
Aqueous	20	15	46.656 20.085 ± 73.243	103.431 67.810 ± 1055.594	26.462
	40	34			
	60	53			
	80	79			
	100	100			
Chloroform	20	14	51.420 25.472 ± 83.922	122.497 77.746 ± 1726.041	22.959
	40	30			
	60	48			
	80	70			
	100	96			
Methanol	20	17	43.347 23.161 ± 62.021	97.359 66.741 ± 381.595	19.779
	40	38			
	60	61			
	80	81			
	100	100			
Ethanol	20	16	45.162 22.575 ± 66.903	101.455 68.105 ± 552.741	22.205
	40	35			
	60	59			
	80	78			
	100	100			

Control- nil mortality

Significant at $p < 0.05$ level

LC_{50} - Lethal concentration that kills 50% of the exposed larvae

LC_{90} – Lethal concentration that kills 90% of the exposed larvae

UCL- Upper confidence limit; LCL- Lower confidence limit

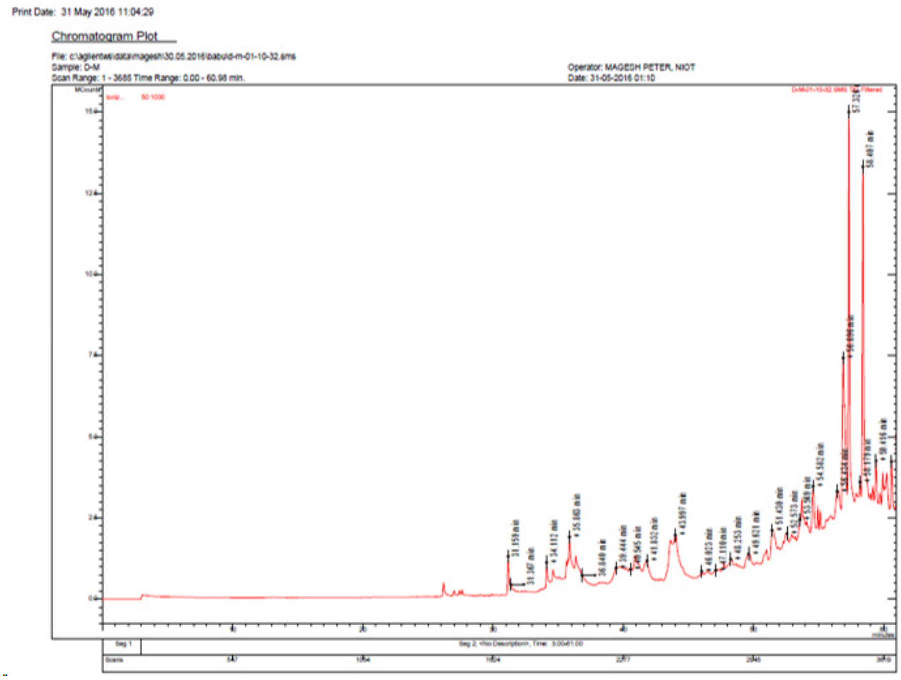

Figure 1. GC- MS analysis of methanol extract of *E. repens*

Table 5. GC- MS analysis of methanol extract of *E. repens.*

	RT	Name of the compound	Peak Area (%)	Amount
1.	31.159	n-Hexadecanoic acid	4.751e+6	0.561
2.	35.624	6-Octadecenoic acid	2.724e+6	0.322
3.	35.743	Oleic Acid	3.604e+6	0.426
4.	35.863	Ethyl 9,12-hexadecadienoate	1.136e+7	1.342
5.	36.496	3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1	4.575e+6	0.540
6.	39.503	Oleanolic acid	1.111e+6	0.131
7.	46.843	Rubrene	355429	0.042
8.	50.564	4,7-Benzofurandione, 3-acetyl-3a,7a-dihy	55653	0.007
9	54.086	Acetic acid, 17-(4-hydroxy-5-methoxy-1,5	5.828e+6	0.688
10	55.697	3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3-pheny	2.098e+6	0.248
11	60.065	5-Chloro-6beta-nitro-5alpha-cholestan-3-	5.319e+6	0.628

including A. aegypti, A. stephensi, and C. quinquefasciatus. Insectariums were maintained at temperatures between 25-29°C to support cyclic generations of vector mosquitoes. Larvae were provided with a diet consisting of a mixture of larval feed, powdered dog biscuit, yeast, and a 10% glucose solution for adult mosquitoes (Evans, 2005; Middleton et al., 2000).

Larval Susceptibility Tests:

Larval sensitivity tests were performed following standard methods outlined by the World Health Organization (WHO, 2005). Extract solutions of varying concentrations were prepared, and larvae from A. aegypti, A. stephensi, and C. quinquefasciatus were exposed to each solution to assess their larvicidal activity. Twenty larvae were placed in glass beakers containing 200 ml of plant extract solution, with concurrent control trials conducted without extracts. After 24 hours of exposure, the number of dead larvae was recorded, and mortality was determined using the Abbott method (1925) based on the average of five replicates.

Statistical Analysis:

To determine LC50 and LC90 values, as well as other statistical parameters, average larval mortality data were analyzed at 95% confidence intervals using the software method described by Singh et al. (2009) and Majumder (2013). Statistically significant results with $p < 0.05$ were considered, utilizing EPA probit analysis 1.4v.

Results and Discussion

Phytochemical Profiling and Separation of Bioactive Compounds from E. repens:

Phytochemical analysis of E. repens revealed the presence of various compounds in all ethanol extracts, including tannins, saponins, flavonoids, alkaloids, coumarins, anthocyanins, and phenols. Additionally, traces of quinines, terpenes, triterpenoids, phenols, cardiac glycosides, and steroids were detected (Table 1).

Mosquito Larvicidal Activity of E. repens:

Tables 2, 3, and 4 depict the mortality of fourth instar larvae exposed to various polar and nonpolar solvent extracts of E. repens. Results indicated larvicidal activity against A. aegypti, A. stephensi, and C. quinquefasciatus vectors. Notably, methanol extracts of E. repens exhibited 100% larvicidal action against A. stephensi, while ethanol extracts demonstrated 81% mortality compared to chloroform and hexane extracts. The LC50 and LC90 values for methanol extract against A. stephensi were 43,173 ppm and 95,513 ppm, respectively. Ethanol extract was also effective against A. aegypti larvae, with LC50 and LC90 values of 48,103 ppm and 112,621 ppm, and 120,94 ppm, respectively. Similarly, for C. quinquefasciatus, LC50 and LC90 values were 45,162 ppm and 101,455 ppm, while for A. stephensi, LC50 and LC90 values were 58,234 ppm and 166,072 ppm. Both A. aegypti and A. stephensi larvae showed high mortality rates after 24-hour exposure to E. repens extracts, with LC50 and LC90 values of 46,656 ppm and 103,432 ppm for C. quinquefasciatus, and 54,568 ppm and 138,520 ppm for A. aegypti, respectively (Table 4). Furthermore, methanol extracts of D. repens revealed the presence of seven compounds through TLC, including tannins, coumarins, glycosides, phenols, alkaloids, flavonoids, and steroids (Table 5).

GC- MS Analysis

GC-MS Chromatogram and Predicted Constituents:

The GC-MS chromatogram of methanol extracts is depicted in Figure 1, while Table 5 lists the predicted constituents identified in the methanol extracts. A total of 11 compounds were detected, including n-hexadecanoic acid, 6-octadecenoic acid, oleic acid, ethyl 9,12-hexadecadienoate, 3-oxatricyclo[20.8.0.0(7,16)]triaconta-1, oleanolic acid, rubrene, 4,7-benzofurandione, 3-acetyl-3a,7a-dihy, acetic acid, 17-(4-hydroxy-5-methoxy-1,5, 3-hydroxy-1-(4-{13-[4-(3-hydroxy-3-pheny and 5-chloro-6beta-nitro-5alpha-cholestan-3.

Chemical Insecticides and Insecticide Resistance:

The use of chemical insecticides, either in adult or larval stages, is a cornerstone of effective vector control (Finney, 1971). However, various chemical insecticides employed in vector control have been found to induce mosquito resistance. Insecticide resistance can occur due to mutations in insecticide-targeted proteins or enhanced insecticide biodegradation (Kahkonen et al., 1999).

Botanical Pesticides:

Plants serve as a rich source of bioactive agricultural chemicals, exerting insect and disease repellent properties. They can act as insecticides, repellents, growth inhibitors, juvenile hormones, moulting hormones, and attractants (Rey et al., 1999; Clements, 1992; AlMehmadi & Khalaf, 2010). Botanical pesticides offer advantages over conventional pesticides, including reduced toxicity, lower susceptibility to resistance, and easier degradation.

Larvicidal Potential of E. repens:

Numerous plant species worldwide have been explored for mosquito control, particularly within the Poaceae family, which has historically demonstrated insecticidal and repellent effects against various insect pests in developing countries. The current study aimed to assess the larvicidal potential of the locally available E. repens plant from the Poaceae family against mosquito vectors. The findings revealed that methanol extracts of E. repens exhibited enhanced larvicidal activity against all three tested mosquito species.

Conclusion

In conclusion, this study assessed the effectiveness of E. repens plant extracts against fourth instar larvae of A. aegypti, A. stephensi, and C. quinquefasciatus. Our findings demonstrate that E. repens extracts possess potent larvicidal activity against all three tested mosquito larvae species. These results suggest the potential of E. repens as a natural and eco-friendly alternative for mosquito control strategies. Further research into the specific bioactive compounds responsible for the observed larvicidal effects and their mechanisms of action could provide valuable insights for the development of novel mosquito control interventions. Overall, the efficacy of E. repens highlights the importance of exploring plant-based solutions in the ongoing fight against mosquito-borne diseases.

Author contribution

P.D., M.A.M., S.D., M.I., S.K.S., M.S. conceptualized, reviewed the literature, and wrote the article.

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None declared

Competing financial interests

The authors have no conflict of interest.

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