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Phytochemical profiling and larvicidal activity of *Elytrigia repens* extracts against the urban mosquito vectors

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Abstract
Mosquito - transmitted infections by vectors are one of the reasons that most contribute to human mortality and morality in tropical and subtropical areas. Vector management seems to be the most powerful measure and, at the very least, the best way to deter infections, ensuring that there are no vacancies for many vector-borne infections. The most synthetic insecticides are the first line of action, because of their rapid activity, but their continuous use has led to the creation of tolerance and lasting residual environmental consequences which can be judicial in animals, including people. The biologically active processes in the Mosque control programme have so far drawn significant attention at the very latest. In the present report, the efficacy against the larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were tested for 24hrs after treatment by chloroform, ethanol, methanol, and aqueous extracts of *E. repens*. The phytochemical preliminary study showed that certain secondary metabolites are high and responsible for mosquito larvae death. GC-GC-MS analysis of *E. repens* Methanol extract repens showed various insecticidal phytocompounds.

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

Introduction
More than 17 percent of illnesses are caused by vector-borne pathogens and more than 1 million fatalities a year [1]. The three Aedes, Anopheles and Culex genera are transferred to pathogenesis of diseases such as dengue fever, malaria, Japanese encephalitis, and filariasis. More than 3000 mosquito species from 34 genera in the world can spread disease to people and other vertebrates, of which only about 300 species can. In India about 40 million people are affected annually by mosquito-borne diseases [2].
The burden of illness, mortality, hunger and social vulnerability worldwide, in particular in tropical countries, are major causes of mosquitoborne disease [3]. A major way of fighting mosquito-borne illnesses has been by water disruption or chemical insecticide destruction of the adult mosquitoes. Continuing organophosphate control (Chlorpyrifos, Temephos and Fenthion) and insect growth regulators (Divlubenzuron, and Methoprene) are also dependent upon the control of mosquito larvae. The population is widespread in the adverse effects of chemical insecticides used to combat mosquitos [4].
Efficient use of control agents interrupted natural ecological processes, resulting in resistant insecticides, return of pests and pollution [5]. However, the systematic and indiscriminate use of pesticides has led to significant inconvenience such as industrial, animal and wildlife toxicity. The ecology was consequently affected by the residues of certain residual chemicals [6]. The development of new or complementary management methods for mosquitoes is essential to address these challenges associated with traditional mosquito control [7]. This has contributed to the hunt for eco-, cheap, biodegradable and selective insecticides against mosquitoes.
Based on the above and many other pesticides drawbacks, researchers worldwide struggle to find alternative ways of protecting the environment. These issues have shown that new mosquito control techniques must be developed. Plant extracts were again used as efficient sources of natural biocidal products [8].
Alternatives to conventional pesticides have been shown to be phytochemicals and many are effectively regulated by mosquitoes. Plants or portions of plants have special biological function in a complex of chemicals. More than forty thousand plant species possess pest control chemicals [9]. One solution to combating mosquito-borne diseases is the management of mosquitoes and preventing mosquito bites to stop the spread of the disease. The transmission of mosquito-borne diseases into societies by plant products like possible insecticides or repellents is important [10].
Plant stockpiles are noted for their many biological properties and for secondary metabolites, such as tannins, glycosides, alkaloids and flavonoids. Herbal compounds are known as an essential natural resource of newly developed chemical insecticides, interfering with the larvae's cuticle membrane, destroying eventually the mucosa, which is the most possible cause of larval death [11].
Currently, mosquito protection is aimed primarily against larvae and, if possible, against adults. This is due to the temporary and unsatisfactory battle against adults and the more local handling of larvae in time and time, resulting in less harmful effects. Larval management can be an efficient control mechanism, since the major breeding habitats are man-made and are easy to identify, leading to low mobility of larval mosquitoes [12]. In this research, *E. repens* extracts against the fourth instar larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* are examined in larvicidal activity.

Materials and Methods
Selection of Plant
Natural and disease-free plants of the *E. repens* family was collected from the natural population in and around Chennai, Tamilnadu, 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.

Fig 1: *Elytrigia repens*

Plant extract preparation

The dried plants were tamed with electrical blender and powdered. The powder of the plant was successively extracted with watery, chloroform, ethanol, and methanol solvents, respectively. Every solvent had 50gm of powder soaked in 500ml. With the aid of Rotary Evaporator, the collected solvents were concentrated and deposited in airproof bottles under 40C.

Selection of mosquito species

The 4th instar larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* were the mosquito species chosen for this research (Fig. 2). It is very well domesticated and anthrophilic [21]. *A. Aegypti* has a cosmopolitan distribution. *Aedes* contains the arbovirus transmission species responsible for dengue and dengue fever *Aedes Anopheles* contains species that spread the four malaria parasites with the most violent type of malaria caused by *P. falciparum*. *Wuchereria* species vector that cause's lymph filariasis and is common in trophic regions, *C. quinquefasciatus* [22].

Fig 2: Mosquito species selected for the study

Preparation of plant solvent extracts

The plants were washed with tap water and rinsed with double distilled water. The whole washed plant was split into small parts and dried in the shadow for around 20 days at room temperature. The dried plant was pulverized and tamed with an electrical mixer for fine powder. In the thimble 100 g of weed powder has been extracted successively using a Soxhlet extractor for 14 hours by means of solvents, hexane, chloroform, ethanol and methanol. All the extracts have been concentrated using a rotary flash evaporator and stored for further use in airtight bottles at 4° C.

Phytochemical profiling

The phytochemical profiling of the sample was carried out as described by [23]. The plant extracts were tested for carbohydrates, alkaloids, flavonoids, phytosterols and steroids, anthocyanins and betacyanins, phenols, tannins, saponins, glycosides and proteins.

GC-MS analysis

GC-MS analysis of the whole plant crude extracts were carried out on Agilent technologies (6890 N), JEOL GCMATE II.

Mosquito Larvicidal activity

All the studies were performed with laboratory-raised, non-insecticide and pathogenic insect exposures, such as *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. Insectariums at 25-29°C were maintained for cyclic generations of vector mosquitos. The larvae were fed with a mixture of 3:1 larval feed, powdered dog biscuit, yeast, and 10 percent glucose solution for adult mosquitoes [24].

Larval susceptibility tests

The larval sensitivity tests were conducted according to normal methods [25]. Extract solutions with various concentrations were developed and larvae from *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were put in each solution for the following process. to monitor their larvicidal behaviour. Twenty larvae were grouped into 200 ml plant extract solution glass beakers. Extract-free control trials were conducted simultaneously. The larvae in each solution then remained 24 hours, the number of dead larvae was measured after 24 hours exposure, and mortality by Abbott method (1925) [26] was determined from the average of five replicates.

Statistical analysis

In order to quantify LC_{50} LC_{90} as stated in [27] and other figures, average results on larvel mortality had been tested at 95 percent of the reference limits for the upper and lower limits of trust and chi-square values using the software method described [28]. Statistically significant results with $p<0.05$ have been regarded (EPA probit analysis 1.4v).

Results and Discussion

Phytochemical profiling and separation of bioactive compound from *E. repens*

The phytochemical investigation of *E. repens* showed the strong presence of phytocompounds in all the plant extracts. Methanol extracts of *E. repens* showed tannins, saponins, flavanoids, coumarins, glycosides, triterpenoids and steroids were highly present. Whereas alkaloids, anthocyanins, cardiac glycosides were moderately present in the methanol extract and quinones, terpenoids, phenols, acid and proteins were absent. All the other extracts also revealed the strong presence of phytocompounds when compared to the methanol extract. (Table 1).

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

S. No	Secondary metabolites	Aqueous	Chloroform	Ethanol	Methanol	Hexane
1	Carbohydrate	+++	++	++	+++	+
2	Tannins	-	+	++	+++	+
3	Saponins	+++	++	+++	+++	-
4	Flavonoids	++	++	+++	+++	++
5	Alkaloids	-	+	++	++	-
6	Anthocyanin	++	-	+++	++	-
7	Quinones	-	-	-	-	-
8	Glycosides	-	-	+++	+++	+++
9	Cardiac glycosides	++	-	+++	++	-
10	Terpenoids	+	-	-	-	-
11	Triterpenoids	+++	-	+++	+++	++
12	Phenols	+	-	-	-	-
13	Coumarins	-	-	++	+++	+
14	Fatty acids	-	-	-	-	-
15	Protein	-	-	-	-	-
16	Steroids	++	+	+++	+++	+

Mosquito Larvicidal activity of *E. repens*

Fourth instar larvae deaths are seen in Tables 2, 3, and 4, with various polar and nonpolar solvent extracts of *E. repens*. The results revealed that the *E. repens* extract had larvicidal activity against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* vectors. In whole plant methanol extracts, repens against *A. stephensi* were reported for 100% larvicidal action, while extracts of ethanol were recorded as 81% mortal compared with chloroform and acquired extracts. The *E. repens* methanol extract values LC_{50} and LC_{90} were 44.219 ppm and 94.614 ppm against *A. stephensi*. Ethanol extract against fourth instar larvae of *A. aegypti* species has been found to be successful and the LC_{50} and LC_{90} values were 46.102ppm and 110. 512 ppm respectively, Whereas 44.247ppm and LC90 were 98.418 ppm for *C. quinquefasciatus*, LC_{50} and LC_{90} and 59.199ppm and 168.095 ppm respectively for *A. stephensi*, In comparison to methanol and ethanol extracts of *E. repens* all the other extracts revealed moderate larvicidal activity.

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	52.443 31.475 ± 75.905	123.542 82.267 ± 649.810	16.856
	40	29			
	60	49			
	80	72			
	100	93			
Chloroform	20	14	52.342 33.322 ± 80.382	137.728 88.600 ± 874.306	15.235
	40	31			
	60	47			
	80	66			
	100	91			
Methanol	20	13	48.758 28.496 ± 72.773	108.795 74.549 ± 535.037	20.286
	40	27			
	60	56			
	80	74			
	100	97			
Ethanol	20	15	46.102 23.787 ± 74.218	110.512 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	53.468 37.170± 77.342	136.284 91.714± 558.616	12.597
	40	29			
	60	46			

	80	68			
	100	89			
Chloroform	20	11	71.299 63.074 ± 79.879	223.659 173.358 ± 333.031	5.899
	40	25			
	60	37			
	80	52			
	100	73			
Methanol	20	18	44.219 20.366 ± 63.976	94.614 64.349 ± 506.183	23.350
	40	35			
	60	61			
	80	84			
	100	100			
Ethanol	20	13	59.199 52.775 ± 64.396	168.095 136.448 ± 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	45.458 20.085 ± 73.243	104.578 67.810 ± 1055.594	26.462
	40	34			
	60	53			
	80	79			
	100	100			
Chloroform	20	14	52.419 25.472 ± 83.922	133.488 77.746 ± 1726.041	22.959
	40	30			
	60	48			
	80	70			
	100	96			
Methanol	20	17	44.247 23.161 ± 62.021	98.418 66.741 ± 381.595	19.779
	40	38			
	60	61			
	80	81			
	100	100			
Ethanol	20	16	46.172 22.575 ± 66.903	112.455 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

GC- MS Analysis

GC-MS chromatogram of the methanol extracts was shown in Figure 3 and the predicted constituents in the methanol extracts are listed in the Table 5. A total of 11 compounds were found namely 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.

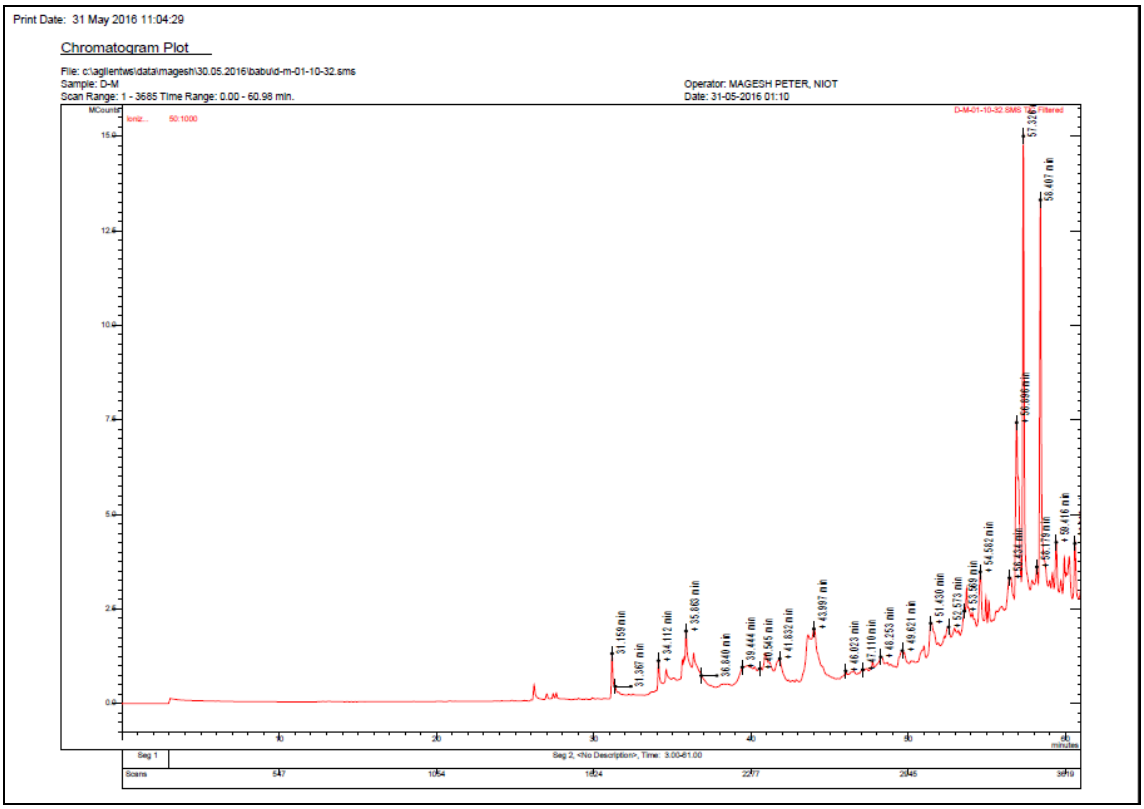

Fig 3: GC- MS analysis of methanol extract of *E. repens*

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

Effective vector control typically starts with the use of chemical pesticides to adults or larvae [29]. However, it has been established that mosquitoes are impervious to a wide range of chemical insecticides that pose a threat to vector control systems. An pesticide-targeted protein mutation or enhanced insecticide biodegradation could be the outcome of insecticide resistance [30]. This could lead to insecticide resistance.

Bioactive and bioactive agricultural compounds that repel insects and diseases are abundant in plants. They have the ability to function as attractants, young hormones, moulting hormones, repellents, insecticides, and developmental inhibitors [31]. The advantages of botanical pesticides over traditional ones are their toxicity, less susceptibility to resistance, and ease of degradation.

Worldwide, a variety of plant species have been employed to control mosquitoes [19]. The Poaceae family of plants has long been used in underdeveloped nations as a pesticide and to combat a variety of insect pests. In order to combat mosquito vectors, the current study aimed to determine the

larvicidal potential of the Poaceae family's locally accessible plant, *E. repens*. The methanol extract from *E. repens* shown enhanced larvicidal efficacy against all three tested mosquito species, according to the current study.

Conclusion

The goal of this study is to determine how effectively *E. repens* plant extracts work against *A. aegypti, A. stephensi,* and *C. quinquefasciatus* larvae in their fourth instar. The plant extracts from *E. repens* were found to have strong larvicidal effects on all three of the tested mosquito larvae.

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