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Larvicidal activity of *Rivea hypocrateriformis* (Convolvulaceae) leaf extracts against *Aedes aegypti* (Diptera: Culicidae)

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Abstract
The deadly human disease dengue is spread by the *Aedes aegypti* vector. Scientists have focused more attention to plant-derived products because they are a grate source of new natural compounds with insecticidal capabilities that are safe for humans and the environment. Many studies conducted over the past decade years on natural plant products against vector mosquitoes suggest they could replace chemical and synthetic pesticides as a new approach of mosquito control. In this present investigation, larvicidal activity against the *A. aegypti* vector was observed for crude extracts of *Rivea hypocrateriformis* leaf prepared in hexane, ethanol, Ethyl acetate, petroleum ether, and acetone. For a 24hrs, mortality was recorded. The larvicidal activity of petroleum ether leaf extract had highest mortality, with LC_{50} and LC_{90} values of 106.63 ppm and 399.94 ppm, respectively, after 24 hours. Plant phytochemicals can be utilized as efficient of vector control agents alone or in combination.

Keywords: larvicidal activity, *Rivea hypocrateriformis*, Phytochemicals, *Aedes aegypti*

Introduction

Worldwide, diseases spread by insects continue to be a main source of illness and demise (Pavela *et al*.2009) [29]. Due to their social and economic effects, particularly in subtropical and tropical nations, vector and vector-borne diseases have grown to be a difficult concern for public health (Klempner *et al*. 2007) [23] (Rahuman *et al*. 2008) [31]. Millions of people die each year from mosquito-borne diseases such malaria, dengue fever, chikungunya, filariasis, and Japanese encephalitis that afflict both humans and domestic animals worldwide (James *et al*.1992) [21]. In many regions of the world, the mosquito *Aedes aegypti* is a significant carrier of dengue fever and dengue hemorrhagic fever. Programs for preventing and controlling dengue have relied on vector control in the absence of an effective vaccine and medications (Scott *et al*.1993) [37]. The development of pesticide tolerance in *Aedes* has made it difficult to control this disease vector with synthetic organic chemical insecticides. *A. aegypti*, the dengue virus's vector, is widely distributed throughout tropical and subtropical areas. *A. aegypti* and other dengue vectors are common in areas where two-thirds of the world's population lives. Asia, the Americas, and a number of Pacific islands are currently afflicted by epidemic dengue hemorrhagic fever, and all continents aside from Europe-have endemic dengue populations. Dengue viruses, which are the main cause of dengue fever and more dangerous dengue hemorrhagic fever/dengue shock syndrome, infect almost 100 million individuals each year (Hahn *et al*.2001) [18]. The alphavirus genus's chikungunya virus poses major public health risks in countries throughout Southeast Asia and Africa continents. (Pastorino *et al*.2005) [27]. Scientists have been working diligently to find solutions to these issues on a global scale. Recently, green chemistry, which uses natural plant phytocompounds to control mosquitoes, has received a lot of attention. It is generally known that natural substances made from plants, especially in the field of infectious diseases, are efficient, secure, and often utilized (Cragg *et al*.1997). Numerous studies (Rahuman *et al*.2000, Consoli *et al*.1988, Sakthivadive *et al*.2008, Das *et al*.2007, Arivoli and Samuel *et al*. 2011, Arivoli *et al*.2011, Arivoli and Tennyson *et al*. 2012, Arivoli *et al*.2012, Raveen *et al*.2011, Tennyson *et al*.2012) [5, 6, 7, 8, 13, 14, 15, 30, 33, 35, 41] have concentrated on plant products as efficient insecticides and larvicides for controlling various mosquito species. Since they include a variety of bioactive compounds that are selective and don't affect non-target creatures or the environment, natural products from plants provide an alternative source of pest control agents (Arnason *et al*.1989 and Hedin *et al*.1997) [9, 20]. Natural pesticides derived from plants are valuable sources of new pesticide research leads (Newman *et al*.2000) [26]. Numerous studies on natural plant products against mosquito vectors conducted over the past ten years suggest they may be an alternative to synthetic and chemical insecticides. To make these eco-friendly compounds practical for field use and for large-scale vector control activities, more focused efforts must be put into these investigations.

Rivea hypocrateriformis The Convolvulaceae family of woody climbing shrubs, or choisy, is extensively found throughout India, Nepal, Sri Lanka, Pakistan, Bangladesh, Myanmar, and Thailand (Salehi *et al*.2020) [36]. Traditional remedies for this plant's bark, stems, and leaves include treatments for analgesia, malaria, cancer, and mental illnesses. For instance, this plant is used by the locals of Pakistan's Tharparkar region to treat malaria and relieve discomfort. The plant has been linked to a variety of biological potentials, including anti-inflammatory, anticancer, anti-pregnancy irruption, anti-implantation, and antioxidant characteristics (Choudhury *et al*.2013 and Unander *et al*.1995) [12, 43]. It is also a key component of the ayurvedic remedy "Rasa panchaka," which is used to treat asthma (Mishra *et al*.2003) [24]. Additionally, this plant is used as a psychoactive drug in Pakistan and as a hallucinogenic drug in India, much like other forms of a closely related genus, such as *Rivea corymbosa* Hall and Ipomea violacea L. found in Mexico (Mukim *et al*.2022) [25]. In the present study an attempt was made to explore the larvicidal activity of *R. hypocrateriformis* plant leaf extract.

Materials and methods

Collection of plant material

The plant, *Rivea hypocrateriformis* (Convolvulaceae), leaves were collected from the various places in Tirunelveli district Tamil Nadu, India. Then, their identification was confirmed with the help of an experienced botanist, Dr. A. Sarvanan Gandhi Department of Botany, Rani Anna Government College for Women. Then leaves were rinsed under running water thoroughly, blotted and shade dried for 7 to 14 days at room temperature (27 to 37 °C).

Extraction

The plant powder extract was derived with the help of a Soxhlet apparatus using hexane, ethanol, Ethyl acetate, petroleum ether and acetone as solvent (Boiling temperature (45-70 °C) for 8 hours (Kaushik *et al*.2008) [22]. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper and concentrated; the residue obtained and stored at 4 °C for further use (Patil *et al*.2010 and Suman *et al*.2012) [28, 40].

Phytochemical analysis of the plant

The phytochemicals studied were phenolic compounds, terpenoids, saponins, alkaloids, flavonoids, steroids, tannins, and glycerol and they were determined using Harborne's (1984) [19] and Stahl's (1989) [39] methods.

Raring of *Aedes aegypti* larvae

The *A. aegypti* eggs were purchased from ICMR-Vector Control Research Centre, Madurai, Tamil Nadu, India. The *A. aegypti* egg rafts were housed in a tray with tap water as the culture medium at a temperature of 29°C. The eggs were shown to hatch out into first instar larvae after one day of incubation. To promote the growth of the larvae, the right amount of nutrients (sterilized yeast powder and dog biscuit in a 3:1 ratio) were added. The study used larvae in their fourth instar.

Larvicidal bioassay

Larvicidal activity was assessed using the WHO 2005 [44] procedure. One gram of the raw plant extract was diluted in 100 ml of acetone (1% stock solution) to create the plant extracts. 25 early fourth instar larvae were housed in a 500 ml glass beaker with 249 ml decholorinated water and 1 ml of the desired plant extract concentrations. For the test larvae, larval food was provided. Larvae were treated with various quantities (ranging from 50 to 450 ppm) of dechlorinated water obtained from the stock solution. two to three replications at each test dosage. With 249 ml of decholorinated, 1 ml of acetone was kept as a control. The fourth instar of *A. aegypti* larval mortality was noted. At the end of 24 hours, the number of dead larvae was counted, and the percent mortality was computed. The mortality rate was determined as (Number. of dead larva / No. of introduced larvae)*100. Abbot's formula was used to account for the corrected mortality.

Statistical analysis

Statistical analysis is an essential tool for understanding and interpreting data. Probit analysis is a widely used statistical technique for examining mortality data. In this study, the larval mortality data were subjected to Probit analysis in order to calculate LC_{50} and LC_{90} values. Further, Chi-square values were calculated using SPSS 20 version for Windows to ensure accuracy of the results.

Results

Preliminary qualitative phytochemical examination of all plant extracts produced data that indicated the numerous bioactive compound that could be possibly responsible for biocontrol larval stage of mosquito and are included in (Table 3).

For usage as environmentally friendly insecticides against *A. aegypti*, the crude extracts of *R. hypocrateriformis* plant leaves in hexane, ethanol, ethyl acetate, acetone, and petroleum ether were investigated. The study's findings regarding the larvicidal properties of leaf extracts (Tables.1 and 2) confirm their important for reducing mosquito vector larval populations. Petroleum ether extract was determined to have the greatest 100%mortality rate and their LC_{50} values for early fourth instar *A. aegypti* larvae were 106.63 ppm and 399.94 ppm, respectively. The extract of ethyl acetate showed the lowest mortality rate its LC_{50} and LC_{90} values are 176.00 ppm and 449.32 ppm, respectively. After 24 hours of exposure, larval mortality was noted. The control group experienced no mortality. According to the larvicidal activity results, the percentage of mortality is inversely correlated with the extract concentration. *R. hypocrateriformis* plant extract solvents were utilized at various concentrations, ranging from 50 to 450 ppm, respectively At $P< 0.05$, values are significant. Furthermore determined were the LC_{50} (LCL-UCL) and LC_{90} (LCL-UCL) 95% confidence limits were analyzed.

Table 1: Mortality (%) of different solvent extracts of *R. hypocrateriformis* at different concentrations against *A.aegypti* larava

Concentration (ppm)	Acetone	Hexane	Ethanol	Ethyl acetate	Petroleum ether
Control	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
50	33.2±1.14	26.2±1.97	36.8±1.78	23.2±2.1	39.6±1.98
150	49.0±1.56	46.6±1.45	53.2±1.36	42.8±1.5	58.2±1.65
250	70.2±1.25	59.8±1.56	63.2±1.25	54.0±1.35	70.6±1.38
350	80.6±1.78	78.2±1.23	79.2±1.68	73.6±1.85	88.6±1.63
450	96.4±1.63	93.6±1.31	98.2±1.63	91.2±1.25	100±1.74

Each value (mean ± SD) represents mean value of three replicates*Significant at P<0.05 level

Table2: Lethal concentration of *R. hypocrateriformis* leaf extracts after 24 hrs * According to Probit Analysis (Finney, 1971) [16]

Name of the Extract	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ2 (df=3)
Acetone	151.82 (111.91-278.35)	434.20 (310.66-562.66)	16.49*
Hexane	152.90 (128.82-255.34)	431.20 (237.65-557.12)	13.42*
Ethanol	116.88 (54.52-260.34)	411.26 (270.25-596.3)	12.45*
Ethyl acetate	176.00 (127.25-256.74)	449.32 (262.53-615.2)	15.19*
Petroleum ether	106.63 (69.77-298.63)	399.94 (199.65-650.25)	19.64*

Table 3: Results of phytochemical test leaf crude extract

Solvents	Acetone	Hexane	Ethyl acetate	Ethanol	Petroleum Ether
Alkaloids	+	-	+	-	-
Steroids	+	-	+	+	+
Tannins	-	+	-	+	+
Saponins	-	+	+	-	+
Flavanoids	-	+	+	+	+
Terpenoids	+	+	+	-	-
Glycerol	+	+	+	-	+
Phenolic Compounds	+	-	+	+	+

+present
-absent

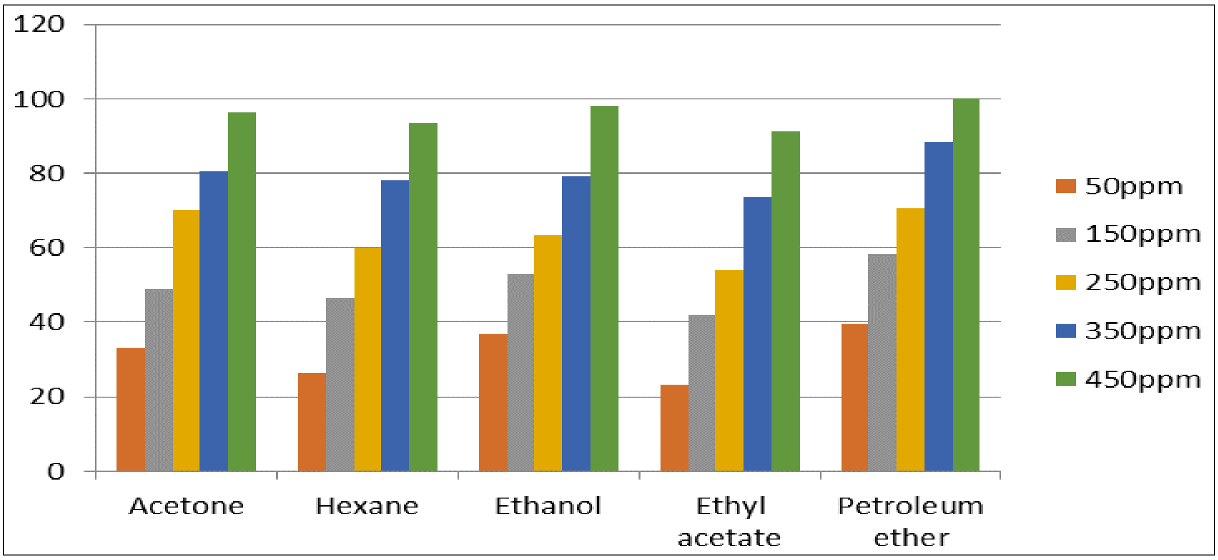

Fig 1: The mortality % of IV instar larvae of *A. agypti* treated with *R. hypocrateriformis* various extracts.

Discussion

For the purpose of lowering resistance issues and environmental safety concerns, it can be helpful to identify several plant extracts that are effective larvicidal agents against mosquitoes. It's common practice to manage vectors, especially mosquitoes, in order to prevent sickness (Adewole *et al*.2013) [1]. By reducing the quantity of mosquitoes, controlling mosquito larvae may lessen the risk of disease. Phytochemicals may be a good substitute for synthetic pesticides due to their generally high levels of safety, accessibility, and affordability. It is obvious that finding plant-based insecticides that are efficient, appropriate for and adaptable to regional environmental conditions, biologically degradable, and possess the widespread insecticidal property will work as a new weapon in the insecticide arsenal and in the future may act as a suitable alternative product to combat mosquito-borne diseases. In the past ten years, a number of plant species have been discovered that contain mosquito-repelling properties. In contrast to Sukumar *et al*. (1991), catalogued and discussed 344 plant species that showed mosquitocidal activity, Roark (1947) [34] described more than 1,200 plant species. Shaalan *et al*. 2005 [38] listed the growth- and reproduction-inhibiting phytochemicals, botanical ovicides, synergistic, additive, and antagonistic joint action effects of botanical mixtures, residual capacity and effects on non-target organisms, and appearance of resistance in their review of the state-of-the-art regarding larvicidal plant species. Secondary metabolites of plants have often been shown to be the cause of both their chemical resistance and toxicity. In addition to phenolics (Tripathi and Rathore 2001) [42], steroids (Ghosh *et al*. 2008; Chowdhury *et al*. 2008; Rahuman *et al*. 2008a; Zolotar *et al*. 2002) [17, 31], and essential oils from plant extract (Carvalho *et al*. 2003., Amer and Mehlhorn 2006; Carvalho *et al*. 2003) [11] Although the majority of studies are limited to the laboratory bioassay, many plant-based biocides have been chemically synthesised and are frequently used in mosquito control programs, including Ocimenone, Rotenone, Capillin, Quassin, Neolignans, Arborine, Goniothalamin, etc. (Shaalan *et al*. 2005) [38]. Study of inexpensive medicinal plants for mosquito control is thought to benefit local public health initiatives, create jobs, and lessen reliance on high priced imports (Bowers *et al*.1995) [10]. As they can be easily controlled in this ecosystem because they breed in water, mosquito larvae make great pesticide targets. Yet, the use of traditional pesticides in water sources has a number of hazards for the environment and people. In this way, natural insecticides especially those made from plants hold more potential. Many chemicals that are employed in a variety of ways come from aromatic plants and their essential oils (Amer *et al*.2006) [4]. It is possible to employ the plant's raw extracts or extracted bioactive phytochemicals in stagnant water bodies, which are known to be mosquito breeding grounds. Before proposing any of these plant materials as an anti-mosquito product used to combat and defend against mosquitoes in a control program, additional research on the identification of the active components involved, their mechanism of action, and field testing are typically required.

The current study discovered that *R. hypocrateriformis* plant leaf extracts include bioactive phytocompounds that function either independently or in combination as larvicides. The majority of concentration dependent activities and virtually all plant extracts have a larvicidal action, which is what causes larval mortality in fourth instar *A. aegypti* larvae. When the *A. aegypti* mosquito larvae had been incubated for 24 hours, the petroleum ether extract had a 100% mortality rate. The outcomes were significantly with those earlier discoveries (Rajasekaran *et al*. 2012) [32]. The experiment group displays some behavioral abnormality movement after treating of *R. hypocrateriformis* plant extract , whereas the control group does not, suggesting that the plant extract may contain a neurotoxic phytocompound. Since they frequently have no side effects and have the potential to be sources of bioactive compounds, plants offer an alternative source for mosquito larvicides. By using these natural pesticides to control mosquitoes rather than synthetic ones, costs and environmental harm may be reduced. Formulations of biolarvaecides or insecticides can be successfully created using the phytochemicals contained in *R. hypocrateriformis* extracts.

Conclusion
The crude extracts of *R. hypocrateriformis* leaves to showing mortality of *A. aegypti* larvae. It has been used as larvicide compound in mosquito control programs. The crude element from this plants will aid in lowering mosquito populations and environmentally safe. Further studies need to understand chemical structure of specific compound.

Appendix
Ppm: Parts per million
hrs: Hours
LC_{50}: Lethal Concentration, 50% of the exposed larvae are killed
LC_{90}: Lethal Concentration, 50% of the exposed larvae are killed
UCL: Upper Confidence Limit
LCL: Lower Confidence Limit
⁰C: Degree Celsius
$\chi 2$: Chi-square

Declaration

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The researchers behind this study are not required to disclose any conflicts of interest

Data availability statement
The research outlined in the article didn't involve any data.

Ethical approval
There is no need for ethical approval because this study is observational.

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