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Larvicidal activity assessment of *Artemisia pallens* leaves and *Crossandra infundibuliformis* flowers based synergistic formulation against Dengue fever causing *Aedes aegypti*

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Abstract

The most current methods for controlling diseases spread by mosquitoes involve methods that kill larvae at vector-rearing facilities on a big scale. Therefore, the current research was to carry out the phytochemical analysis of plant extracts from *Artemisia pallens* leaves and *Crossandra infundibuliformis* flowers in both aqueous and organic solvents to assess the larvicidal properties of these extracts on third-instar larvae of the dengue fever-causing mosquito *Aedes aegypti*. According to the phytochemical study, flavonoids, alkaloids, anthraquinones, and tannins were present in both extracts. The mortality percentage of larvae and larvicidal efficiency assay revealed that the acetone fraction of the *A. pallens* and *C. infundibuliformis* plant extract (500 ppm concentration) was the most active, with LC50 and LC90 values of 187.54 ppm and 871.22 ppm; respectively which was in contrast to the hexane fraction (LC_{50} = 307.65 and LC_{90} = 2472 ppm respectively). The plant extracts of *A. pallens* and *C. infundibuliformis* in synergistic combination had the greatest larvicidal potential in the following order acetone > hexane > aqueous extract. The findings imply that the extract of the herb *A. pallens* and the extract of *C. infundibuliformis* may be effective larvicidal agents against *Ae. aegypti* and demand further investigation as mosquito control agents.

Introduction

Mosquitoes have been observed in the environment as a principal vectors that spreads diseases such as filariasis, malaria, dengue, yellow fever and others; being the world's leading killer of humans. Mosquito-borne diseases are ubiquitous in over 100 countries, afflicting each year approximately 7 billion people worldwide, including 4 billion Indians (Ghosh *et al*., 2011). There are around 3,500 mosquito species worldwide. Only 120 species propagate the disease among them. The most frequent vectors in the globe are the three mosquito genera *Aedes, Anopheles, and Culex*. *Aedes aegypti* is the major vector of dengue fever, hemorrhagic fever, and chikungunya, affecting over 100 million people in over 110 tropical nations each year (Halstead, 2008). Therefore, since human infections are alarmingly rising, controlling mosquitoes is of utmost importance.

These mosquito borne diseases are controlled by either killing, preventing using repellents or triggering larval mortality to keep mosquitos from biting humans (Mohan *et al*., 2007). Commercial chemical insecticides such as carbamates, organochlorine, pyrethrins, organophosphorous are widely employed to manage the ever-increasing mosquito population (Ali *et al*., 2013). Due to the source of environmental risk and non-target organisms, which has led to the development of resistance, this is not safer. As a solution to these problems, various plant species with larvicidal effects are traditionally used to create environmentally friendly pesticides that target specific pests.

Larvicide will be an effective method of mosquito control rather than taking steps to kill adult free-flying mosquitoes (Ruchi Yadav *et al*., 2014). A survey of literature on mosquito larvicidal effects of plant products indicates that most of the studies included well-known horticultural and commonly grown plants (Mohan *et al*., 2007). The potential of *Artemisia nilagirica* leaf extract as an environmentally benign method for eradicating the target mosquito species has been substantiated. The efficacy of this method has been well established, and it is considered to be the most effective approach for the purpose. Consequently, the use of this extract for mosquito

control has become an important area of research in recent times. The results of these studies have demonstrated the effectiveness of the extract in eliminating vector mosquitoes, and its potential use as a safe and sustainable solution for mosquito control (Panneerselvam *et al*., 2007). *Artemisia pallens* (Dhavanam), (Order:Asterales, Family:Asteracea) is an aromatic herb which is xerophytic in nature. It is found in Andhra Pradesh, Karnataka, Maharashtra and Tamilnadu states in India. It was verified with the authentication number from the Botanical Survey of India in Pune (BSI/WC/Tech/2008/1059). It is grown for the essential oil known as Davana oil as well as for its aromatic leaves and blooms, which are used as floral decorations, offerings to deities, and for oil extraction. This oil is mostly used to flavour tobacco, cakes, pastries, and some pricey beverages. According to Ambasta (2000), the plants are regarded as good fodder and have anthelmintic, tonic, and antipyretic properties (Wickens *et al*., 1988).

An essential plant in horticulture is *Crossandra infundibuliformis* (Family:Acanthaceae). Tropical regions like South India and Sri Lanka are abounding with it. The blossoms of *Crossandra infundibuliformis* are also known as Tropical flame / Firecracker and the Puliyars tribe's women have used them to style their hair. *Crossandra infundibuliformis* leaf extracts have aphrodisiac, analgestic, and anti-inflammatory effects. Additionally, the extracts were found to have larvicidal, antibacterial, antioxidant, and wound-healing properties. This plant is used to cure a variety of illnesses because of its therapeutic potential. A phytochemical analysis of several solvent extracts of the *C. infundibuliformis* flower found the existence of terpenoids, steroids, alkaloid, saponins, tannin,and flavonoids (Vadivel *et al*., 2016).

The plant extracts of *Artemisia pallens* is reported to have antimicrobial properties (Ruikar *et al*., 2009). Also, the leaf extract of *C. infundibuliformis* possess a good antibacterial, antifungal and anticandidal activities[17]. With the support from the above-mentioned previous work reports the current investigation is conducted to perform the phytochemical analysis of three different extracts of *A. pallens* and *C. infundibuliformis* analyze the larvicidal efficacy of the extracts in both water and organic solvents, against third-instar larvae of *Ae. aegypti*, measured by the percentage of mortality, as well as by probit evaluation and regression estimation.

1. Materials and methods

2.1 Plant selection, collection and extraction:

Leaves of *A. pallens* and flowers of *C. infundibuliformis* were collected from the local market in Chennai city, Tamil Nadu, India, and brought to the laboratory at the Department of Biotechnology, Rajalakshmi Engineering College Thandalam, Tamil Nadu, India. *A. pallens* leaves and *C. infundibuliformis* flowers were shade dried, then crushed and pulverized using a planetary mixer. A total of 500g of dried *A. Pallens* powdered leaves and 500g of *C. infundibuliformis* powdered flower were extracted sequentially over 72 hours using 1.5 l of each solvent hexane (low polarity), acetone (medium polarity), and distilled water (high polarity) to obtain the crude extracts. The extract was concentrated by "Rotavapor" at 45^{o} C and a lowered pressure of 22 to 26 mmHg, and the resulting residue was kept at 4 ^{o} C until testing for further bioassays.

2.2 Phytochemical analysis:

Using standard procedures, phytochemical analysis was conducted on extracts of three types of A. pallens and C. infundibuliformis plants (hexane, acetone and water) (Evans *et al*., 2002).

1.2.1 Test for tannins

The sample of *A. pallens* and *C. infundibuliformis* contained 500 mg of sample from three different solvents (hexane, acetone, and water). The mixture was then filtered through a 0.2-m filter with 10 mL of distilled water and the aliquot was treated with 5 drops of 1% ferric chloride solution for blue-green precipitation.

1.2.2 Test for flavonoids

The *A. pallens* and *C. infundibuliformis* sample of 500 mg from three different solvents were dissolved in distilled water and 5 mL of 2N NaOH. The production of an orange colour confirmed the presence of flavonoids.

1.2.3 Testing of quinones

4-5 drops of concentrated H_2SO_4 were placed to the walls of test tubes containing 500 mg of sample from three different solvents of the *A. pallens* and *C. infundibuliformis* sample dissolved in distilled water. The production of a reddish colour demonstrated the existence of quinones.

1.2.4 Testing of cardioglycosides

One ml of glacial acetic acid was added to 1 mL of sample from three different solvent extracts of *A. pallens* and *C. infundibuliformis* samples. Along the test tube's sides, 5 drops of ferric chloride and concentrated H_2SO_4 was added. The creation of a brown ring indicated the presence of cardioglycosides.

2.3 Test for terpenoids

The 500 mg of sample from three different solvent extracts of *A. pallens* and *C. infundibuliformis* sample were dissolved in 1 mL of chloroform and 4-5 drops of concentrated H_2SO_4 was added to it. The resulting reddish brown color confirmed the presence of terpenoids.

2.4 Test for phenol

500 mg of sample from three different solvents extracts were dissolved in 1 mL of distilled water, 1 mL of Na_2CO_3, and 1 mL of Folin's reagent. The appearance of a greenish or bluish colour indicated the presence of phenol.

2.5 Test for coumarins

500 mg of sample from three different solvent extracts were dissolved in 1 mL of 10% NaOH and distilled water. The development of yellow color confirmed the presence of coumarins.

2.6 Testing of steroids

500 mg of sample from three different solvent extracts were dissolved in 1 mL of distilled water, followed by 1 mL each of chloroform and H_2SO_4. The presence of steroids was indicated by the interface's appearance in reddish brown.

2.7 Testing of alkaloids

500 mg of sample from three different solvent extracts were disintegrated in distilled water and 1mL of concentrated HCl was added to the test tube. The presence of alkaloids resulted in a greenish-white precipitate formation with the addition of 1 mL of Mayer's reagent.

2. Test organisms:

The larvicidal assay was conducted using laboratory-raised *Ae. Aegypti* larvae that had not been exposed to diseases or pesticides. The larvae were kept at a temperature of 25 to 29° C and a relative humidity of 80% to 90% to maintain the cyclic generations. Larvae were fed on a 3:1 mixture of powdered dog biscuit and yeast as their larval meal.

3. Larvicidal action:

The study used the standard WHO protocol with minor adjustments. Concentrations of 62.5, 125, 250, and 500 ppm were made from the stock solution of three distinct solvent extracts in separate beakers. Five larvae in the early third instar were placed in a beaker with 200 mL of 4 different concentrations. Acetone and hexane alone were added to water to create a control. Mortality was noted 24 hours later. Five replicates were used in each of the three trials, which

were conducted. However, Abbott's formula (1987) was used to correct the observed percentage mortality when the control mortality was between 5 and 20% (Abbott *et al*., 1987).

4.1 Statistical investigation

The LC_{50} and LC_{90} values were calculated using the SPSS 11.5 software program. Analysis of variance was used to analyze data from mortality and the effect of concentrations. EPA probit analysis software was used to conduct a log prohibit and lethal concentration analysis of the larvicide efficacy of the three distinct solvent extracts of *A. pallens* and *C. infundibuliformis* on third-instar larvae of the *Ae. aegypti* mosquito during a period of 24-hour treatment.

RESULTS

Table 1

Analysis of phytochemical constituents of *A. pallens* leaves and C. *infundibuliformis* flowers were extracted using various solvents.

S.No	Constituents	Different types of extracts		
		Crude Methanolic extract	Acetone Extract	Residual Aqueous portion
1	Alkaloids	+	+	+
2	Anthraquinones	+	+	+
3	Carbohydrates			
	i. Free reducing sugar	+	+	+
	ii Starch	+	+	+
4	Flavonoids	+	+	-
5	Saponins	+	+	+

Key = + present; − = absent; CH = crude methanolic extract; CA= n-butanol portion; RA = Residual aqueous portion.

Table 2 *Ae aegypti* mosquito larvae mortality in percentage at different concentrations (ppm) of ppm of hexane, acetone, and aqueous extracts of *A. pallens* in 24 hrs of exposure period

Different solvent extracts of *A. pallens* and *C. infundibuliformis*	% mortality of *Ae. aeypti* larvae at different concentration			
	62.5 ppm	125 ppm	250 ppm	500 ppm
Hexane extract	40.6 ± 0.68	52.5±0.58	61.0±0.71	71.2±0.73[a]
Acetone extract	30.0 ±0.71	42.6±1.36	55.4±0.75	85.8±1.16[ab]
Aqueous extract	20.6±0.51	31.2±0.37	42.0 ± 0.32	78.4±0.75[a]

Values are mean ± SE from 5 observations. P Values: [a]<0.001 when compared between different concentrations in terms of ppm; [b]<0.001 when compared to three different extracts.

Table 3: Log probit and regression analysis of larvicidal efficiency of three different solvent extracts of *A. pallens* and *C. infundibuliform* on the third-instar larvae against the *Ae. aegypti* mosquito for the period of 24 hours of treatment

Treatment using different extracts of A pallens and C. infundibuliformis	LC50 (ppm)	95% confidence Limit		LC90 (ppm)	95% confidence Limit		Intercept ±SE	Slope ±SE	$\chi 2$
		LFL	UFL		LFL	UFL			
Hexane	307.65	226.48	498.68	2472.62	1143.8	14063.15	1.4 ± 0.6	1.4 ± 0.2	0.04*
Acetone	187.54	148.8	238.43	871.22	573.32	1828.7	0.6 ± 1.9	0.6 ± 0.2	1.7*
Water	499.29	350.97	979.38	3374.73	1478.54	22005.02	0.8 ± 0.7	1.5 ± 0.3	0.007*

LFL- Lower fiducidal limits UFL- Upper fiducidal limits χ^2 - Chi square analysis *P<0.05, significant level; each value (mean±SD) five replicates

3. Results and Discussion

The chemical-based pesticides represent a significant risk to humans (Abbott *et al*., 1987). Artemisinin, a chemical discovered in the *Artemisia* species plant, is effective against the parasite that causes malaria (Bhakuni *et al*., 2001). The insecticidal properties of many species of *Artemisia* are due to the presence of 1,8-cineole. There is evidence suggesting that *C. infundibuliformis* is effective in fighting mosquito-borne diseases due to its insecticidal properties (Murini *et al*., 2018). The presence of flavonoids and saponins may contribute to its activity. Phytonutrients derived from various *A. pallens* and *C. infundibuliformis* sources have provided numerous compounds with potential use as repellants. The present study endeavors to investigate the larvicidal efficacy of two plant species, namely, *A. pallens* and *C. infundibuliformis*, which are predominantly found in the plains of Tamil Nadu, India.

The primary objective of this research is to evaluate the potential of these plant species as an alternative to synthetic insecticides for controlling mosquito larvae. The findings of this study are expected to shed light on the larvicidal action of *A. pallens* and *C. infundibuliformis* and contribute to the development of eco-friendly and sustainable mosquito control strategies. The present study conducted a comprehensive phytochemical screening of aqueous, acetone, and hexane extracts of *A. pallens* and *C. infundibuliformis*. The analysis results revealed the presence of potentially bioactive phenolic compounds and flavonoids in both extracts. Additionally, a trace of saponin was detected in the extracts, while no alkaloids were found.

These findings, which are summarized in Table 1, suggest that both *A. pallens* and *C. infundibuliformis* could be potential sources of natural products with therapeutic properties. Further studies are needed to isolate and characterize the active compounds responsible for the observed biological activities and to evaluate their safety and efficacy in vivo. The larvicidal action of plant extracts of leaves against *Ae. aegypti* revealed in this study was attributed to the presence of different phytochemicals, suggesting their utility in mosquito population control (Harve *et al*., 2004).

3.1 Determination of larvicidal action of various solvent extracts of *A. pallens* and *C. infundibuliformis* against *Aedes aegypti* mosquito larvae

The mortality rate of *Aedes aegypti* mosquito larvae at various concentrations (ppm) of -hexane, acetone, and aqueous extracts of *A. pallens* and *C. infundibuliformis* in 24 hrs of exposure period was tabulated and the various crude extracts using solvents of *A. pallens* and *C. infundibuliformis* showed promising larval mortality against *Aedes aegypti* mosquito species (Table-2). The findings of this study showed that the mortality rate of mosquito larvae at 500 ppm concentration of *A.pallens* and *C. infundibuliformis* was significantly higher than the percentage of mortality at other lower concentrations for all the solvent extracts ($p^a<0.001$ Table 2). On comparison between the extracts, the higher mortality percentage was significantly observed in the acetone extract (85.8 ± 1.16^{ab}, Table 2)

The data resulting from the determination of lethal concentration, analyzed using log probit and regression of larvicidal efficiency of three different solvent extracts of *A. pallens* and *C. infundibuliformis*, is shown in Table 3. After 24 hours of exposure, the LC_{50} was calculated as 187.54 for the acetone extract, 307.65 for the hexane extract, and 499.29 for the aqueous extract, respectively. In addition, the LC_{90} was calculated as 871.22 for the acetone extract, 2472.62 for the hexane extract, and 3374.73 for the aqueous extract, respectively. The larvicidal activity at 250 ppm and 500 ppm of the acetone extract was significantly higher compared to the larvicidal activities of the other two extracts (62% for 50 ppm and 74% for 90 ppm, respectively) than that of the aqueous extract and significantly higher (39% for 250 ppm and 65% for 500 ppm, respectively) than that of the hexane extracts.

The findings of the current study can be compared to similar studies from past researchers. *Ageratum conyzoides* leaves extract in petroleum ether demonstrated larvicidal activity with LC_{50} values of 425.60 and 267.90 ppm after 24 and 48 hours of exposure, according to a previous study (Sharma *et al*., 2009). The methanol extracts of *Cecropia obtusifolia, Cassia tora*, and *Vicia tetrasperma* showed greater than 90% larval mortality on *Ae. aegypti* in a study realized by (Jang *et al*., 2002). In earlier research, Eugeni Anitha Preethi (Preethi *et al*., 2014), looked at the effectiveness of larvicidal characteristics of crude chloroform extracts of *Jasminum grandifloruma* as a potential agent for controlling *Ae. Aegypti* (Preethi *et al*., 2014). The herbal

extract application to mosquitoes would cause pathological changes that might limit the larvae's metabolic capacity, resulting in their demise (Riat *et al*., 2017).

Hence in comparison with aqueous and ethanolic plant extracts, the larvicidal activity of acetone extracts of *A. pallens* and *C. infundibiliformis* was found to be significantly higher with low LC_{50} value for an exposure period of 24 hours. The complex combinations of phytocompounds found in the *A. pallens* and *C. infundibuliformis* extract could be used to create pest-controlling larvicidal products that are environmentally benign.

5. Conclusion

The phytoproducts have multiple bioactive components that can be utilized as all-purpose toxicants against different mosquito larval stages. The results of this study showed that *A. pallens* and *C. infundibuliformis* extracts have larvicidal action, which is shown to be the most potent activity in acetone extract against the *Ae. aegypti*. Hence, due to their larvicidal toxicity, plant-origin compounds from the leaf extracts of *A. pallens* and *C. infundibuliformis* revealed insecticidal and therapeutic properties that had higher effectiveness in reducing mosquito threat. The current study indicates that there is potential for using *Artemisia pallens* and *C. infundibuliformis* leaf extracts to control the immature stages of *Ae. aegypti*. However, more research is required, including screening, isolating, and purifying bioactive phytochemical constituents, as well as comprehensive laboratory and field bioassays. Additional research is required to understand this activity against a wide variety of mosquito species at all life stages as well as to pinpoint the active component(s) of the extract that is responsible for larvicidal activity. To sum up, this work aims to assess the importance of *A. pallens* and *C. infundibuliformis* in comparison to a different strategy for controlling *Ae. aegypti*.

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Conflicts of Interest-Nil

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