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Evaluation of mosquito larvicidal effect of *Carica Papaya* against *Aedes Aegypti*

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Abstract

The development of resistance to chemical insecticides among mosquito species has been considered as a setback in vector control. This has necessitated the need for a research and development of eco-friendly, biodegradable indigenous method for vector control. Mosquito larvicidal efficacy and phytochemical screening of crude aqueous and ethanol extracts of the leaf, bark, root and seed of *Carica papaya* of family Caricaceae a common traditional medicinal plant were evaluated in the present study. Phytochemical screening of the selected parts' crude aqueous and ethanol extracts indicated the presence of alkaloids, carbohydrates, saponin, phenol, tannin, flavones, coumarins, anthocyanin and flavanoids which are known to possess medicinal and insect larvicidal properties. Larvicidal effect of 2nd 4th instar larvae of mosquito species *Aedesaegypti* have been investigated for 24hrs with 1mg/ml concentration of extracts of selected parts of *Carica papaya*. The results clearly indicated that there is a significant variation among the aqueous and ethanol extracts of the selected parts of the plant. The seed extracts are observed as effective larvicide than other selected parts of *Carica papaya*.

Keywords: *Carica papaya*, Phytochemicals, Larvicide, *Aedesaegypti*, Mosquito

1. Introduction

Mosquitoes can transmit a number of diseases than any other group of arthropods and affect more than 700 million people worldwide annually, including arboviruses responsible for yellow fever, dengue hemorrhagic fever, epidemic polyarthritis, several forms of encephalitis and bancroftianfilariasis (Kazembe and makusha, 2012) [6] and pathogens which continue to have devastating effect on human beings (Maheswaran *et al.*, 2008) [5].

Personal protection from mosquito bites is currently the most important way to prevent transmission of these disease (Fradin, 1998) [15]. To prevent proliferation of this mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential.

Larviciding is successful way of reducing mosquito densities in their breeding places before they emerge into adults. Pesticides are indeed very effective in its use. However, the use of chemical insecticides are often toxic to both human and non-target animals. The intensive use of chemical insecticides led to the development of resistant insect populations, resulting in reduced control, environmental pollution resulting in bio-amplification in food chain and contamination (Hag *et al.*, 1999) [14].

Plants have the major advantage of still being the most effective and cheaper alternative green measure for the control of arthropods of public health importance (Rawani *et al.*, 2009; Halder *et al.*, 2011; Banerjee *et al.*, 2012) [11, 8, 7]. Natural products of plant origin are safe to use than the synthetic insecticides (Kishore *et al.*, 2011) [10].

Carica papaya, the sole species in the genus *Carica* of the plant family Caricaceae is widely cultivated. Papaya is a large tree-like plant, with a single stem growing from 5 to 10 meters tall with spiral leaves. It is used as remedy against a variety of diseases (Mello *et al.*, 2008 and Munoz *et al.*, 2000) [2, 3]. The stem, bark and seed extracts have bactericidal activities (Emeruwa, 1982). The root infusion is used for syphilis in Africa and also used as analgesic. Leaf of papaya smoked for asthma relief in various remote areas.

The main objective of the study was to test the larvicidal ability of *Carica papaya* (Papaw) leaf, bark, root and seed of crude aqueous and ethanolic extracts against *Aedesaegypti* mosquito larvae.

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2. Materials and methods

2.1. Period and Location of the study

The study was carried out during Nov 2014. This research was conducted at the Laboratory of P.G and Research Department of Zoology, Periyar E.V.R College, Tiruchirappalli.

2.2. Plant material

The fresh leaves, bark, root and seed *Carica papaya* were collected from pudukkottai district, Tamilnadu, India. The selected plant parts were separated, washed, dried, powdered and the extracts were filtered using whatman filter paper. Chemical test were carried out on the crude aqueous and ethanolic extracts using standard procedures to identify the phytochemical constituents like alkaloids, carbohydrates, saponins, phenols, tannins, flavonoids, flavones and anthocyanins described by (Horborne 1998: Sofowora, 1982: Trease and Evans, 1989) [4, 22, 23].

2.3. Mosquito species

Eggs of *Aedes aegypti* was collected from ICMR (Indian Council for Medical Research) Madurai, Tamilnadu, India. *Aedes aegypti* was obtained as egg rafts on the filter paper and were reared in trays containing tap water and maintained at $28 \pm 2^\circ\text{C}$. When the eggs were hatched out into first instar larvae, they were fed with a mixture of yeast powder and dog biscuits in the ratio of (1:3). On the third day after hatching the first instar larvae moulted into second instar larvae on the fifth day, third instar larvae observed, which moulted into fourth instar larvae on the seventh day (Rajmohan and Ramaswamy, 2007) [12]. The 2nd and 4th instar of *Aedes aegypti* was experimented for the present study.

2.4. Larvicidal Assay

The larvicidal assay were conducted according to (WHO, 1981) [17]. In the Larvicidal assay, 2nd and 4th instar larvae of *Aedes aegypti* were exposed to test concentration of 1% extract of crude aqueous and ethanol of selected parts of *Caricapapaya* separately. Stock solution was prepared by dissolving 10g of powder in 100 ml of distilled water for aqueous and 100ml of 30% ethanol for ethanol solvent. From the stock 1ml was taken in separate bowl made up to 100 ml with distilled water and respective solvent separately for crude aqueous and ethanol extracts. 10 numbers of 2nd and 4th instar larvae were transferred gently to the test medium separately and simultaneously a control was maintained for aqueous and ethanol. The larval mortality in both treated and control were recorded after 24hrs. Dead larvae were identified when they failed to move after touching with tip of thin brush. This experiment was repeated three times.

The percentage mortality was calculated by

$$\text{No of larvae Dead} / \text{No. of larvae} \times 100$$

3. Results and Discussion

As per the preliminary phytochemical investigation, the constituents like flavanoids, tannins, alkaloids, saponins, phenolic compounds, coumarins and Carbohydrates are equally present in leaf, root, bark and seed of aqueous and ethanolic extracts as mentioned in Table 1. These findings were in agreement of similar nature of study conducted by (Okaye, 2011) [13]. The presence of phenolic compounds in seed of *Carica papaya* showed that the seed may have antimicrobial potential. This explains its use in treating

typhoid fever and some other intestinal problem (Oakenful, 1981) [18]. Alkaloids are the most efficient and therapeutically significant plant substance.

Moreover, flavanoids are very important constituent of natural product and have got apart antioxidant activity an ability to combat tumour growth (Okwu, 2004) [16]. *Carica papaya* have been reported to possess potent mosquito larvicidal activity. The Mosquito larvicidal activity reported in the present study could be due to any of the bioactive compounds mentioned above.

The larvicidal activity of selected parts of *Carica papaya* extracts against *Aedes aegypti* are given in table 2. It is evident that the results on mortality of *Aedes aegypti* Show extract dependents activity.

The findings of the present investigation revealed that the leaf, bark, root and seed of *Carica papaya* possess larvicidal and repellent activity against mosquito *Aedes aegypti*. In addition crude ethanol extract even at a very low concentration is toxic against mosquito larva with all parts of the plant selected for the present study when compared to crude aqueous extract. All the tested parts of the plant are very efficient and effective in its larvicidal action when compared to that of their respective control. Eventhough seed ethanol has the highest mortality rate of 93% in 2nd stage and 100% in 4th stage *Aedes* treated larvae. Seed aqueous has 83% in 2nd stage and 86 % in 4th stage *Aedes* treated larvae. The mortality rate was significantly higher seed extract in both aqueous 2nd and 4th stage *Aedes* treated larvae when compared with other parts of the plant extracts. The remaining parts of leaves, bark and root has below 50% mortality in both 2nd and 4th stage *Aedes* treated larvae.

The result of the present study is similar to that of (Sesanti *et al.*, 2014). Papaya seed extract is more effective in killing larvae of *Anopheles* species. The content of secondary metabolites in the leaves and seeds of papaya in the form in which the principle works alkaloids inhibit the body's metabolic processes in larvae, interfere with growth hormones, and digest the protein in the larval body and turn it into peptone derivatives that will host larvae as food shortages and eventually die (Utomo and Margo, 2013).

In previous studies with powdered *Carica papaya* seeds, Figueroa-Brito (2002) [26] reported 100% larval mortality at concentrations of 10, 15 and 20%. In evaluating the insecticide effect of the four different parts of *Carica papaya* in this study, lower mortality rates than those reported for seed powder were obtained. This can be explained by the fact that the high toxic activity of the *Carica papaya* seeds in powder form may due to a possible synergy, in which the active components act against *Aedes aegypti*.

Aliphatic amide from seeds of *Carica papaya* as mosquito larvicide, Pupicide, Adulticide, Repellent an Smoke toxicity study was carried out by (Rawani *et al.*, 2012) [24]. Crude and solvent extracts of *Carica papaya* was investigated for anti-mosquito potential, against *Anopheles stephensi*. The result of the study was highest rate of larvicidal was obtained when compared to pupicidal and adulticidal activity. In another study benzyl isothiocyanate isolated from *Carica papaya* seed extract was tested for antihelminthic activity by viability assay using *Caenorhabditis elegans* and was claimed as a chief antihelminthic agent (Kermanshai *et al.*, 2001) [25].

A survey of literature on control of different phytochemicals obtained from various plants has been carried out by number of researchers in the field of vector control (Sukumar *et al.*,

1991) [21]. There are many studies of toxicity carried out with other plants that reflect a similar behaviour against *Aedes aegypti*. Plant could be an alternate source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Many of the defensive components of plants are biodegradable with non-residual effects on the biological environment. Hence an attempt has been made in the present

investigation to identify the larvicidal potential of the locally available plant *Carica papaya*.

We can conclude from this study that in totality, the data collected show that *Carica papaya* indeed has larvicidal potential when treated to larvae in low concentrations, and can be used as substitute for commercial insecticides. Though the presence of phytochemicals in *Carica papaya* could be studied further in detail and its beneficial effect to control mosquitoes.

Table 1: Preliminary Qualitative analysis of *Carica papaya*

Sl. No	Phytochemical Constituents	Name of the Test	Leaf		Bark		Root		Seed	
			A	E	A	E	A	E	A	E
1	Alkaloid	Mayer's test	-	+	-	+	-	+	+	-
		Dragendroff's test	-	+	-	+	+	-	+	++
		Wagner Test	+	+	+	+	-	+	+	+
2	Carbohydrate	Molisch Test	++	-	+	+	-	+	+	+
	Steroidal Glycosides	Liebermann's test	+	+	-	+	+	-	+	-
4	Saponin	H ₂ SO ₄ test	-	-	+	-	+	+	++	++
5	Tannin	Lead Acetate	+	+	+	+	+	+	+	+
6	Phenol	Phenol reagent	+	+	+	+	+	+	++	+
7	Chlorogenic acid	Ammonia test	-	+	-	+	-	-	+	++
8	Flavones	Shinoda's Test	+	+	+	++	-	-	+	+
9	Flavonoids	Ammonia test	+	-	-	-	+	-	++	++
10	Coumarin	Sodium chloride test	+	+	-	+	-	+	+	+
11	Anthocyanin	H ₂ SO ₄ test	+	-	-	+	-	+	+	++

A-Aqueous extract (+) Positive (++) Highly positive
 E-Ethanol extract (-) Negative

Table 2: Mortality of 2nd and 4th stage extract treated *Aedes* larvae

Larvae	Types of Extracts	Different parts of papaya	Percentage (%) Mortality
2 nd stage	Aqueous	Leaf	26
		Bark	26
		Root	33
		Seed	83
		Control	0
	Ethanol	Leaf	33
		Bark	40
Root		36	
Seed		93	
Control	0		
4 th stage	Aqueous	Leaf	20
		Bark	33
		Root	43
		Seed	86
		Control	0
	Ethanol	Leaf	36
		Bark	46
		Root	46
		Seed	100
		Control	0

Table 2 shows the percentage mortality of four different parts of *Carica papaya* and two types of extracts treated 2nd and 4th stage *Aedes aegypti* mosquito.

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