Aqueous Extracts of Seed and Peel 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. Therefore, researchers have diverted their attention towards natural products of plant origin with insecticidal properties for control of insects pests and vectors. Papaya (*Carica papaya*), an herbaceous fruit crop belonging to the family Caricaceae, has garnered popularity among researchers due to its nutritional and pharmaceutical value. Our study aimed to determined the larvicidal effect of aqueus extracts of seed and peel of *Carica papaya*. The results shows that the seed and peel extracts has a larvicidal activity against *Aedes aegypti*. The seed extracts has a higher larvicidal activity than peel extracts. This migh be de due the effect of pyhtochemical constituent in extract suc as flavonoid, alkaloid and tannin. From this study indicate that the aqueuous extracts of seed and peel of *Carica papaya* showed potential larvicidal activity for *Aedes aegypti*. Further studies might be needed for the used of this extracts for eco-friendly vector control programs especially for *Aedes aegypti.

***Index Terms*—aedes aegypti, carica papaya, larvicidal activity, phytochemical constituent**

I. Introduction

Dengue fever is considered as a serious public health problem in the world, mainly in tropical countries [1], [2]. Dengue viruses, mosquito-borne members of the Flaviviridae family, are the causative agent of dengue fever [3]. The symptomps of dengue fever include high fever, rash, and a severe headache. Addtional sypmtoms include severe joint and muscular pain (breakbone fever), nausea, vomiting, and eye pain. Dengue fever is usually a self-limited illness, and only supportive care is required [3].

However, Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever, and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement. A recent estimate shows that more than 50 million people are 13 at risk of dengue virus exposure worldwide. Annually, there 14 are 2 million infections, 500,000 cases of dengue haemorrhagic 15 fever, and 12,000 deaths [4].

Dengue viruses are born by the mosquito A. aegypti. Aedes aegypti is generally known as a vector for an arbo-virus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito also acts as a vector of yellow fever in Central and South America and West Africa [4]. Since Aedes aegypti is a fresh water breeding mosquito it is very difficult to control it during rainy season [1].

Synthetic chemical larvicides continue to be applied for controlling mosquitoes in most parts of the world. But many of these chemicals are toxic to human, plant and animal life and insecticides resistance can be problematic in maintaining control, especially with organophosphate and pyrethroid larvicide. Therefore, a more efficient approach to reduce the population of mosquitoes would be to target the larvae [5], [6].

The use of different parts of locally available plants and their various products in the control of mosquitoes has been well established globally by numerous researchers [4]. Many herbal products have been used as insecticides before the discovery of synthetic insecticides. The effects of botanical derivatives against mosquito have been reviewed by Sukumar *et al.* Extracts from leaves, flowers and roots of plants and oils were found to have mosquito larvicidal activity [7].

In this study, Aedes aegypti larvicidal activity was investigated using seed and peel of *Carica papaya.* Carica papaya, belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases [8], [9]. Carica papaya is a widely grown, perennial tropical tree, which grows up to 5 to 10 m tall with an erect and branchless trunk. Its leaves are large, 50-70 cm in diameter, deeply palmately lobed with 7 lobes. Its melon-like fruit (*papaya*) is known by different names in different parts of the world and these include *fruta bomba* (in Cuba), *lechoza* (in Venezuela, Puerto Rico, the Philippines and the Dominican Republic) and *papaw* (Sri Lanka) [10].

Ripe papaya is consumed fresh as a dessert fruit while green (unripe) papaya is added into fresh salads. Fairly easy to grow, the plant is commonly planted in home gardens using seeds. Papaya fruit can be harvested in approximately 9-12 months after sowing seeds and

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constantly bears fruit all year round. It is herbaceous and polygamous growing from a single stem bearing a crown of large palmate-shaped leaves. Flowers are produced at the axils of the leaf petiole [11].

Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber. In addition to all this, it contains a digestive enzyme-papain effectively treats causes of trauma, allergies and sports injuries [12].

Different parts of the plant are attributed with different medicinal values. For example, in African traditional medicine, the boiled green leaves of papaya combined with leaves of *Azadirachta indica*, *Cymbopogon citratus*, *Psidium guajava* and stem bark of *Alstonia boonei* boiled together and the hot infusion is drunk as one wine glass full thrice daily in the treatment of malaria. Its fresh leaves are also efficacious in the treatment of gonorrhea, syphilis and amoebic dysentery. The seeds are also effective as a vermifuge and in the treatment of hypertension, diabetes mellitus and hypercholesterolemia [10].

The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, à-tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates. Fruit and seed extracts have pronounced bactericidal activities. Leaves have been poulticed into nervous pains, elephantoid growths and it has been smoked for asthma relief amongst tropical tribal communities. The hypoglycemic effect of ethanolic extract of unripe, mature fruits has been reported [13].

In the other hand there is no literature shows the used of Carica papaya seed and peel especially for larvacides sources against Aedes aegypti. Because of that in this research we determined the larvacidal activity of Carica papaya seed and peel against Aedes aegypti larvae.

II. Material and Methods

A. Plant Materials and Preparation of Plant Extracts

Fully matured papaya was purchased from one of the selected markets in Sekumpul, Martapura, South Kalimantan, Indonesia. The maturity of papaya was determined through the fully matured colour of the peel. The papayas were washed under tap water and the seed and peel of papaya were separated from the pulp. Papayas peels were cut into small pieces of about 1 cm^2. After that the papaya peel and seed completely air dried at room temperature for 3 days. The dried peel and seed then pulverized using domestic mixer grinder.

40 g of the powdered Carica papaya seeds was boiled in 500 mL of distilled water for 30 minutes after which it was filtered using a piece of clean white cotton gauze. The filtrate was evaporated to complete dryness at 40 ℃, producing a fine sweet smelling and chocolate color solid residues. The extraction process was repeated 4 times and the solid residue was weighed after extraction and pooled together in an air and water proof container kept in a refrigerator at 4 ℃. From this stock, fresh preparations were made whenever required.

B. Alkaloid Content

Alkaloid determination using Harborne (1973) method [14] : 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a waterbath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed .

C. Tannin Content

Tannin measured according to the method of Van-Burden and Robinson (1981) [14] 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

D. Flavonoid Content

Flavonoid determination by the method of Bohm and Kocipai- Abyazan (1994) [14]. 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

E. Mosquito Culture

The larvae used to test for the larvicidal activity were obtained from colonies of *Aedes aegypti* mosquitoes cultured and maintained in the laboratory at a temperature of 28 ℃ ± 2 ℃ and 80 - 90% relative humidity. The larvae were fed with mice feed and yeast powder in the ratio of 3:1. They were transferred to another clean bowl for three days at 24hrs interval and the water was aerated with the aid of an air pump.

F. Evaluation Larvicidal Activity of Extracts

The procedure protocol of Ibeh (2004) [15] for test for toxicity was adapted with some modified.

Ten (10) grams each of the extract was added to 100ml of Dimethylsulphoxide (DMSO) to make the stock solution. It is expressed in weight per volume (w/v). The standard solution was made by serial dilutions. The concentrations are shown in Table I.

Three (3) replicates of each dose are made per aqueous extract of seed and peel Carica papaya at different dilutions. To make the first dose which was in ratio of 1:1, 25ml of extract was added to 25ml of tap water in a 50ml container and 20 mosquito larvae were introduced. The

second dose (ratio of 1:2), 12.5ml of extract was added to 37.5ml of tap water in a 50ml container and 20 mosquito larvae were added. The third dose (ratio of 1:3), 6.25ml of extract was added to 43.75ml of tap water and 20 mosquito larvae were added. The fourth dose (ratio 1:4), 3.13ml of extract was added to 46.87ml of tap water and 20 mosquito larvae were added. The fifth dose (ratio 1:5), 1.56ml of extract was added to 48.44ml of tap water and 20 mosquito larvae species were introduced, all are covered with a perforated cover to allow constant circulation of air and observed after intervals of 3hrs each for 24hrs to observe their mortality.

TABLE I. PREPARATION OF STOCK AND STANDARD SOLUTIONS ACCORDING TO DILUTION RATIOS

Dilution (v/v)	Concentration (mg/l)	Required Amount (ml)
1 : 1	500	25
1 : 2	250	12,5
1 : 3	125	6,25
1 : 4	62,5	3,13
1 : 5	31,3	1,56

TABLE II. LARVICIDAL ACTIVITY OF AQUEOUS EXTRACT OF CARICA PAPAYA SEED AND PEEL

Sample	Concentration (%)	Mortality Rate (%)
Seed	500	100
	250	100
	125	55
	62,5	38
	31,3	15
Peel	500	100
	250	63
	125	43
	62,5	32
	31,3	12

III. RESULTS

The quantitative phytochemical study of each extracts prepared from seed and peel of *Carica papaya* revealed that both seed and peel contain flavonoid, alkaloid and tannin (Fig. 1)

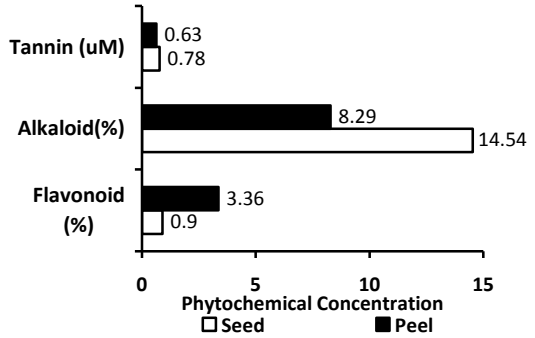

Figure 1. Phytochemical screening of aqueous extract of carica papaya seed and peel.

A higher flavonoid concentration found in peel (3,36 %) of Carica papaya compared to seed (0,90%). Otherwise, alkaloid is found higher in seed (14,54 %) than peel (8,29 %) extract of Carica papaya. Tannin levels are almost same between the two different parts extracts of carica papaya.

The present study on the seed and peel of *C. Papaya* aqueous extracts expressed the presence of larvicidal activity. Table II represnted the dose dependent effect of aqueous extracts of seed and peel of *C. Papaya* on the mortality percentage of *Aedes aegypti* larvae. Maximum seed extract effect on *Aedes aegypti* larvae is between 250 ppm and peel extract is between 500 ppm.

IV. DISCUSSION

Mosquito borne diseases are one of the most public health problems in the developing countries. It can be controlled by using repellent, causing larval mortality and killing mosquitos. Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides. However to familiar synthetic insecticides better alternative means are sought. An insecticide does not have to cause high mortality on target organisms in order to be acceptable. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily availables in many areas of the world [16]. Roark described approximately 1,200 plant species having potential insecticidal value, while Sukumar *et al* listed and discussed 344 plant species that only exhibited mosquitocidal activity [17].

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920s, but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. At present phytochemicals make upto 1 per cent of world's pesticide market [18].

Phytochemical constituent that produced in plants for its protection against microorganisms and predator insects are natural candidates for the discovery of new products to combat A. aegypti. Several studies have focused on natural products for controlling Aedes mosquitoes as insecticides and larvicides, but with varied results [1].

The present studies showed that the seed and peel of *C. papaya* has potential larvicidal effect on *Aedes aegypti* larvae. These may be due to the presence of phytochemical constituent in seed and peel of *C. papaya*. From phytochemical screening seed and peel of *C. papaya* contain flavonoid, alkaloid and tannin.

Gopieskhanna and kannabiran have observed the presence of carbohydrates, saponins, phystoterols, phenols, flavonoids and tannins in the plant extracts having mosquito larvicidal activity [18]. This agrees with reports by james *et al* who reported that mosquito larvicidal activity are mainly due tannis, phytosterols, flavonoids, phenols, saponins and carbohydrates [19].

From present study, the first phytochemical constituent in seed and peel of *C. papaya* is flavonoid. Flavonoid are widely distributed in plants and have many functions like producing pigmentation in flowers and protection from microbial and insect attack [20]. Keerti *et al*, analyzed the effect of flavonoid extracts from different parts of two selected plants which possessed larvicidal activity against two selected mosquito species, hence, could be utilized for developing flavonoid based, ecofriendly insectiside as an alternative to synthetic insecticitides [19].

Second is Tannins, have been found to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. It has been reported that the tannin from *Eclipta prostrate, hemidesmus indices and gymnema sylvestre* was responsible for the mortality in *C. quinquefasciatus* larvae [19].

The third phytochemical constituent is alkaloid. the long chain alcohol and alkaloids, protopine and sanguinarine in the whole plants extracts of *A. mexicana* have been reported to possess potent mosquito larvicidal activity [21]. From other studies showed that alkaloid and limonoids derived from the unripe fruits of *Evodia rutaecarpa* showed effective larvicidal activity against *Aedes albopictus*. This may be due to the effect of alkaloid that can affect protein kinase that is involved in signal transduction and development process of most cells and tissues [19].

Crude extracts or isolated bioactive phytochemicals from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitos. However, further studies on the identification of the active principals involved and their mode of action and field trials are usally needed to recommend any of these plant materials as an anti-mosquito product used to combat and protect from mosquitoes in a control program [1].

Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of syhthetic insecticides could be reduce the cost and environmental pollution. Further analysis is required to isolate the active priciples and its mode of action in inhibiting the developmental of *Aedes aegypti* larvae. The phytochemicals of seed and peel of *C. papaya* extratcs can be well utilized for preparing biocides or insecticidal formulation [1].

V. Conclusion

We can conclude from this study that the presence of phytochemical constituent suc ah flavonoid, alkaloid and tannin in seed and peel of *C. papaya* might be the reason for its larvicidal activity against *Aedes aegypti*. The result of this experiment indicate that the shrub could be studied further in detail and its beneficial effectivity to the control of vector borne diseases could be utilized for healthy environments.

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