

Effects of *Psidium guajava* (Guava) Extracts on Immature Stage of Mosquito

Abstract

Psidium guajava is a shrub whose fruits are eaten by man and its leaves used locally in Nigeria for the management of some ailments. Following the negative effects of chemically synthesized insecticides in malaria control, plant natural products are now being screened all over the world for malaria vectors control. This study aimed to investigate the activity of aqueous and ethanolic extract from *Psidium guajava* (guava) leaves. The leaves were air-dried, ground into powder and extraction of the volatile compounds was done by soxhlet method at temperatures between 60°C and 80°C. The extracts were qualitatively and quantitatively analysed for active ingredients. Different concentrations (5%, 10%, 15%, 20%, and 25%) of the extract were prepared volume for volume and tested against equal numbers of live and active mosquito larvae collected from mosquito breeding sites. Results showed that only high concentrations of the aqueous extract gave positive larvicidal activity against the larvae at 20 minutes interval (15%, 20%, and 25%) while the ethanolic extract killed mosquito larvae at all the concentrations (5%, 10%, 15%, 20%, and 25%). This result implies that guava leaf extract can be effective in sustainable control of malaria vector.

Keywords: *Psidium guajava*; Mosquito; Larvicide; Phytochemical; Extract; Plasmodium parasites

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Introduction

Malaria caused by species of Plasmodium parasites and transmitted by infected female Anopheles mosquito remains the most important parasite disease, with a worldwide prevalence of over 200 Million cases and over 600 thousand deaths as reported by the world malaria report [1]. Over 90% of all malaria deaths still occur in Sub-Saharan Africa especially among children under the age of 5 years and pregnant mothers. According to world malaria report [2], Nigeria accounts for 25 percent of the global malaria burden. Malaria is still a major public health problem in Nigeria with a devastating effect on the nations' economy [3]. According to statistics from Nigeria [4], malaria accounts for nearly 110 million clinically diagnosed cases per year, and an estimated 300,000 children die of malaria each year. Also, 11% of material related mortality is related to malaria in pregnant women. It also leads to 25% of infant mortality and 30% of childhood mortality. Malaria is also known to contribute as much as 45% of the low birth weight babies in Nigeria [5]. The economic burden of malaria in Nigeria is estimated at about 132 billion naira lost in the form of treatment costs, prevention and loss of work time among other [4]. Malaria is a disease that is both preventable and treatable; however, it remains a burden. Part of the reasons malaria remain a major health and economic challenge has been traced to problems associated with human behaviors and local beliefs associated with the disease [6].

According to the latest estimates released in December, 2014, there were about 198 million cases of malaria in 2013 (with an uncertainty range of 124 million to 283 million) and an estimated 584,000 deaths (with an uncertainty range of 367,000 to 755,000). Malaria mortality rates have fallen by 47% globally

since 2000, and by 54% in the WHO African Region. Most deaths occur among children living in Africa where a child dies every minute from malaria. Malaria mortality rates among children in Africa have been reduced by an estimated 58% since 2000 [7].

Guava tree

According to [8], guava tree is described as a low evergreen tree or shrub 6 to 25 feet high, with wide spreading branches and downy twigs. The branches are very strong and highly tolerant to high winds. The leaves are oblong or oval and blunt, 3 to 6 inches long, and feather-veined. The flowers are an inch or more across, the calyx bell-shaped and splitting irregularly, the four to six petals are white, and the stamens are white with yellow anthers [8]. The fruit is yellow and lemon-shaped. Some fruits may be brownish yellow. The inside of the fruits has pink or cream-colored pulp and small hard seeds.

The control of mosquito-borne disease can be achieved either by killing, preventing mosquitoes to bite human being (by using repellents) or by causing larval mortality in a large scale at the breeding centers of the vectors in the environment. However, the extensive use of synthetic organic insecticides during the last five decades has resulted in environmental hazards and the development of physiological resistance in the major vector species. This has necessitated the need for search and development of environmentally safe, bio-degradable, low cost, and indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situations [9].

A large number of plant extracts have been reported to possess mosquitocidal or repellent activities against mosquito vectors, but very few plant products have shown practical utility for mosquito

control. The plant products can be obtained either from the whole plant or from a specific part by extraction with different types of solvents such as aqueous, methanol, chloroform, hexane, ethanol, petroleum ether, etc [10].

Methodology

Collection and identification of plant materials

Psidium guajava leaves were collected from the university farm.

Procedure for preparation of the guava leaves for extraction

The leaves were plucked from the stems and rinsed in clean water, then allowed to air dry on a wooden board for a period of three weeks at room temperature. After three weeks, the dried leaves were grinded into powder from with a mortar and a pestle. The powder was sent to the laboratory for extraction of their volatile oils.

Procedure for the extraction of the volatile oil from the powdered guava leaves

The extraction was carried out in the analytical chemistry laboratory of the University.

Qualitative analysis methods

Alkaloids: The method described by [11]. Was used to determine the presence of alkaloids. 2ml of aqueous plant extract was put in a test tube and treated with 10ml of 1% HCl and kept in a water bath for 10minutes.

1ml of the filtrate was treated with a few drops of Mayers' reagent and a second 2ml portion was treated with Dvagenhoff's reagent. Turbidity or precipitation with either of these reagents was taken as a presence of alkaloids.

Saponins: The method described by [11] was used. 2ml of aqueous plant extract was shaken with water in a test tube. Frothing which persist on warming was taken as preliminary evidence for the presence of saponins.

Tannins: The method described by [11] was used 5ml of aqueous extract was stirred with 10ml of distilled water, filtered, and 1% of ferric chloride was added to the filtrate. Blackish, blue precipitate indicates the presence of hydrolysis of tannins (callic) while blackish green precipitate indicates the presence of tannins (cothecol).

Flavonoids: The method described by Gutierrez was used. 3ml of extract was added to a few pieces of manganese metal or aluminium metal and concentrated HCL was added. The formation of orange, red, crimson or magnetia was taken as an evidence for the presence of flavonoids.

Glycosides: 0.5g of sample was dissolved in 2ml of pyridine. 5 drops of 2% sodium nitropouside and 3 drops of 20% sodium hydroxide was added. Production of deep red colors which fades to brownish yellow is a positive test for cardenolide glycine of a cardiac glycoside.

Oxalates: 0.5g of sample was extracted with dilute HCL. 1ml of ammonium hydroxide was added to 5ml of the extract. It was then made acidic with acetic acid in the presence of phenolphthalein (2 to 3 drops). 1ml of 5% calcium chloride was added to the mixture and allowed to stand for 3hours.

Phytate (phytic acid): 2ml of 0.5N HCL was added to 5ml of the sample and shaken for 10 minutes. 2ml of ferric chloride was added to the extract to form precipitate (ferric phytate). 2ml of sodium hydroxide was added to form sodium phytate precipitate.

Phytochemical Analysis

Alkaloids

5g of sample was weighed into a beaker. 100cm³ of 100% acetic acid in ethanol (1:1) ratio was measured into the sample container and covered to stand for 4hours. The extracted sample was filtered after four hours. It was then concentrated using water bath to a quantity of the original volume. Ammonia solution was added to the concentrated sample (extract) drop wise until the precipitate was completed. The precipitate was allowed to settle, then was filtered and washed with dilute ammonium hydroxide. The residue left was taken as the crude alkaloid. It was then dried in an oven and weighed.

Flavonoid determination

5g of the sample was weighed into a beaker and extracted with 50cm³ of 80% methanol at room temperature for 1hour. The solution was filtered through filter paper. The filtered was evaporated to dryness over water bath and oven. The weight of the dried extract was weighed and the results recorded

Saponins determination

5g of the sample was dispersed in 50cm³ ethanol in a beaker. The suspension was heated over a hot water bath for 4hours with a continous stirring at about 60°C. The mixture was filtered after 4hour and the residue was re-extracted with another 25cm³ of the 20% ethanol. The combined extract was concentrated then reduced to 40cm³ over water bath at 90°C. The extract was transferred into a separatory funnel and 20 cm³ of diethyl ether was added and shaken thoroughly. Aqueous layer of the extract was recovered while the ether layer was discarded.

The purification process was repeated and 60cm³ of n-butanol was added and the extract was washed twice with 10cm³ of 5% aqueous sodium chloride. The remaining extract was evaporated in a water bath and dried in an oven to a constant weight. The saponin content was then calculated in percentage.

Tannin determination

0.5g of the sample was weighed into a plastic bottle and 50cm³ of water was added and shaken for 1hour in a shaker. It was then filtered and 5cm³ of the extract was measured in a test tube and mixed with 3cm³ of 0.1NHCL and 3 drops of ferrocyanide. It was allowed to stand for 10minutes then measured in the UV-vis spectrophotometer at 605nm. The liberated phosphorus was quantitated colorimetrically at 620nm after color development with molybdate reagent.

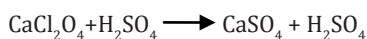
Determination of phytic acid

The method described by [13] was used. 2g of the sample was weighed into 25cm³ portion of 0.5N HCl and was shaken for 30 minutes. 2cm³ of ferric chloride solution was added to the extract, ferric phytate precipitate was formed. This precipitate was converted to sodium phytate by the addition of 3cm³ sodium hydroxide solution. The precipitate was digested with acid mixture of equal portions of concentrated sulphuric acid and perchloric acid in a digestion set. The liberated phosphorus was quantitated colourimetrically at 620nm after color development with molybdate reagent.

Determination of oxalate

The method described by [14] was used. 2g of sample was weighed and extracted with dilute HCL. The oxalate in the extracted sample was precipitated with calcium chloride as calcium salts. The precipitated extract was washed with 50cm³ of 25% H₂SO₄ and dissolved in hot water. It was then titrated with 0.05N KMnO₄.

Note: 1cm³ of 0.05N KMnO₄ = 2.2mg oxalate



Collection of mosquito larvae

Mosquito larvae were collected from mosquito breeding sites in the University campus.

Preparation of extract concentrations

Five different concentration grades (5%, 10%, 15%, 20%, and 25%) were prepared (volume for volume) from the stock extract. 5ml of aqueous stock extract were measured with a clean glass measuring cylinder and then made up to 100ml with distilled water. The readings were taken from the lower meniscus. The procedure was repeated with 10ml, 15ml, 20ml, and 25ml of the aqueous stock extract to get the 10%, 15%, 20% and 25% concentrations respectively. The whole exercise was repeated with the ethanol extract to obtain 5%, 10%, 15%, 20% and 25% as in aqueous extract. The preparations were labeled and preserved at 4°C in separate conical flasks until required for further analysis.

Test of larvicidal activity of extract against mosquito larvae

The various aqueous and ethanolic extract concentrations were tested against mosquito larvae obtained from mosquito breeding sites on Vertas campus and observed for larvicidal activity as follows:

Two sets of 250ml beakers (i.e. 5 in each set) were filled with 100ml of clear stagnant water from the breeding sites and placed

on the laboratory bench, a set of beakers for aqueous extract and another set for ethanolic extract. With the aid of a pipette, 20 live and active mosquito larvae were carefully transferred from the mosquito culture container into each of the 10 beakers of water. Ten (10ml) of the different extract concentration grades were sucked with clean pipettes (1 pipette per concentration) and poured into the respective beakers containing the mosquito larvae. A control was set up with the same number of larvae both without adding any extract. The time of addition of extracts to the beaker was noted. After an interval of twenty minutes for each, the beakers were observed for mosquito larva death. The number of dead larvae in each beaker were counted and recorded.

Results

The result of the qualitative determination of phytochemicals in the aqueous guava leaf extract showed the presence of alkaloids, flavonoids, glycosides, oxalates and saponins (Table 1).

The result of the quantitative determination of phytochemicals present in the ethanolic guava leaf extract showed the presence of alkaloids and flavonoids (Table 2).

The quantitative determination of the phytochemicals present in both ethanol and aqueous extracts of the leaf shown in (Table 3).

Result of the extract assay

Aqueous dilution

The laboratory work revealed that aqueous guava leaf extracted was able to kill mosquito larvae as shown in (Table 4).

At 5% and 10% concentration of the extract, no effect was observed in the test. 15% concentration of the aqueous extract showed three larvae death. At 20% concentration of the aqueous extract, six larvae were killed and at 25% concentration of the aqueous extract, twelve larvae were killed all together (Table 5).

Ethanolic dilution

The laboratory work revealed that ethanol dilutions killed mosquito larvae. At 5% concentration of the extract, three larvae death was observed. At 10% concentration, three mosquito larvae death was observed. In the 15% concentration of the ethanol guava extract, three larvae death each was observed. While in the 20% concentration of the ethanol extract, six larvae were killed and at 25% concentration of the aqueous extract, fifteen larvae died (Table 6).

The average larvae death per ethanol extract concentration showed that 5%, 10% and 15%, an average of 1 larva each, while at 20% and 25% concentrations, averages of 2 and 5 larvae were killed (Table 7).

Table 1: Qualitative determination of phytochemicals present in aqueous extract of guava leaf.

Phytochemicals	Alkaloids	Flavonoids	Glycosides	Oxalate	Phytate	Saponins	Tannins
	+	+	+	+	-	+	-

+ = present

- = absent

Table 2: qualitative determination of phytochemicals in ethanol extract of guava leaf.

Phytochemicals	Alkaloids	Flavonoids	Glycosides	Oxalate	Phytate	Saponins	Tannins
	+	+	-	-	-	-	-

+ = present

- = absent

Table 3: quantitative determination of phytochemicals present in both ethanol and aqueous extracts of guava leaf.

	Phytochemicals						
	Alkaloids	Flavonoids	Glycosides	Oxalate	Phytate	Saponins	Tannins
Ethanoic extract	6.24	18.50	25.34	15.40	0.01	19.94	0.67
Aqueous extract	6.22	18.52	27.65	15.40	0.01	19.94	0.67

UNITS = All parameters are in percentage (%).

Table 4: Result of larvicidal activity of aqueous guava leaf extract on mosquito larvae.

	Concentrations				
	5%	10%	15%	20%	25%
Experiment 1	-	-	1	2	4
Experiment 2	-	-	1	3	5
Experiment 3	-	-	1	1	3

Table 5: Average larvae death per aqueous extract concentration.

	Concentrations				
	5%	10%	15%	20%	25%
Average	0	0	1	2	3

Table 6: Result of larvicidal activity of ethanol guava leaf extract on mosquito larvae.

	Concentrations				
	5%	10%	15%	20%	25%
Experiment 1	1	2	-	2	6
Experiment 2	1	1	2	2	4
Experiment 3	1	-	1	2	5

Table 7: Average larvae death per ethanol extract concentration

	Concentrations				
	5%	10%	15%	20%	25%
Average	1	1	1	2	5

Discussion

Five percent (5%) concentration of the aqueous extract showed no effect throughout the repeated test on the larvae signifying that this concentration was too low to cause larval death, whereas in the ethanolic extract, three larvae were killed, thus signifying that the 5% concentration of the ethanol extract was more effective compared to the aqueous extract at this concentration.

At 10% concentration of the aqueous extract, no mosquito larva died whereas in the ethanolic extract at this concentration, three larvae died thus signifying that the larvae was slightly susceptible at this concentration and that the ethanolic extract had a more positive effect than the aqueous extract. Fifteen percent (15%) concentration of the aqueous extract revealed three larvae death while the ethanol extract showed three larvae death. Both aqueous and ethanolic extract showed high mortality. At 20% concentration

effected more kill while six larvae death was observed in the test with ethanolic extract at this concentration. Here, the effect of both aqueous extract, fifteen larvae death was observed while in the ethanol extract, twelve larvae death was observed. At this concentration, the ethanolic extract was more effective than the aqueous extract. The result of the control of the two experiments with both aqueous and ethanolic extracts was negative, that is, no mosquito larvae were killed. This further proved that the extracts were responsible for the larvicidal activity.

Therefore, the experiment with the aqueous and ethanol guava leaf extract revealed that as the concentration increased, more mosquito larvae were killed showing that the aqueous and ethanol guava leaf extracts are more effective at high concentration. However, comparing the two extracts, the ethanol extract seemed to be more active against mosquito larvae than the aqueous extract since it kills more larvae at low concentrations.

This result does not conform to the result carried out by other researchers with various plant extracts [15]. Reported that methanol extract of the leaves of *A. aspera* caused 50% mortality of *A. aegypti* larvae at 409ppm [16]. Found that the hexane extract of *Abutilon indicum* leaves caused 100% mortality at 1000ppm with LC_{50} value of 261.31ppm against the larvae of *A. aegypti* at 24h [17]. Carried our investigations with crude aqueous and hexane extract of *Momordica charantia* against larvae of *A. staphensi*, *C. quinquefasciatus*, and *A. aegypti* and revealed the LC_{50} values of 0.50, 1.29, and 1.45% respectively with aqueous extracts and 66.05, 96.11 and 122.45ppm, respectively with hexane extracts.

The results of the qualitative analysis of aqueous guava leaf extract revealed the presence of alkaloids, flavonoids, glycosides, oxalates and saponins while the qualitative analysis of ethanolic extract of guava leaf showed the presence of alkaloids and flavonoids. Flavonoids are widely distributed in plants and have many functions including producing pigmentation in flowers and protection from microbial and insect attack. Tannins and other polyphenols may be responsible for its broad spectrum antimicrobial activity against viruses, bacteria and fungi. The saponins are known to be anti-microbial and to inhibit moulds and protect plants from insect attacks. Some of the phytochemicals identified in the leaf extracts of guava like saponins, tannins, etc., have been reported to possess potent mosquito larvicidal activity [18].

The results of the aqueous and ethanolic guava extracts on mosquito larvae revealed that the extracts are larvicidal in nature. Larvicidal activity was more at high concentrations of the aqueous and ethanol extracts. The presence of several bioactive chemicals like alkaloids, saponins, tannins, flavonoids can be attributed to the susceptibility of the mosquito larvae to guava plant extracts as killing agent.

Conclusion

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friendly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. From the research on guava leaf extract, it has shown effectiveness in the control of mosquito larvae and this approach

is easy to perform and inexpensive and also does not pose any harm on man and the environment at large and as such should be adopted for use in the control of mosquito larvae.

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