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Persea americana (Mill.) seed extracts: Potential herbal larvicide control measure against *Anopheles gambiae* Giles, 1902 (Diptera: Culicidae) Malaria vector

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Abstract
The prevalence of mosquito-borne diseases is one of the world's most important health problems. Therefore, it is necessary to prevent mosquito-borne diseases and improve public health by controlling mosquitoes. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, which makes it easy to deal with them in this habitat. Nowadays, the control of mosquitoes at larval stage is focused with plant extracts. The present study explored potential larvicidal activity of *Persea americana* seed using different solvents viz. chloroform, ethyl acetate and acetone for the crude extracts as alternatives to potentially harmful synthetic insecticides. Ten 3rd and 4th instar larvae of *Anopheles gambiae*, were subjected to different concentration (5µl, 10µl, 15µl, 20µl, 30µl and 40µl) of *P. americana* seed chloroform, ethyl acetate and acetone crude extracts under WHO laboratory procedure. The percentage mortality of the mosquito species was tested after 24 hrs of exposure to different concentration of the seed extracts. Mortality was dose dependent; ethyl acetate extract recorded higher mortalities after 24 hours at 40µl and similar trend was equally observed in other extracts. LC50 value was lowest for chloroform extract thus suggested to be more toxic than other extracts evaluated.

Keywords: *Anopheles gambiae,* larval mortality, mosquito-borne, potential, seed extracts, stagnant water bodies.

Introduction
World Health Organisation has declared mosquito "public enemy number one" [1] causing millions of death every year [2]. To prevent the epidemics caused by mosquito and to improve quality of environment and public health, mosquito control is essential as many species of mosquitoes are vectors of several life threatening diseases affecting humans and they constitute an intolerable biting nuisance [3, 4].

Various measures have been taken to control mosquito menace and one such approach is by killing the mosquitoes in the larval stages [5]. Larviciding is successful way of reducing mosquito densities in their breeding places before they emerge into adults, because during the immature stages, mosquitoes are immobile [6]. The use of synthetic insecticides in the vector control is not advisable due to lack of novel insecticides, high cost, concern for environmental sustainability, harmful effect on human health and increasing insecticide resistance on a global scale [3], which 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 [7].

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 [8-10]. Many plants have been reported about their potential insecticidal actions on different stages of *Anopheles stephensi*, via crude extracts or extracted active compounds, since they constitute a rich source of bioactive compounds that are biodegradable [11-12]. Indeed, the rational application of exceptional phytochemicals may not only lead to new IPM strategies but may inhibit the development of insect resistance to existing synthetic insecticides [13]. *Persea americana* (Lauraceae) commonly known as Avocado pear is widely cultivated in subtropical regions for its large, edible fruit. *P. Americana* has been reported to be effective against hepatotoxicity, inflammation, cancer, hypertension, etc [14]. In light of the concerted effort to develop plant products based insecticides as a veritable alternative to synthetic insecticides, and due to the

importance of this plant in traditional medicine, hence, this study was undertaken to assess the credible use of seed extracts of *P. americana* for its larvicidal potential against *An. gambiae*. The assessment of the mosquito control potential of this plant product may be useful in formulating an appropriate strategy for the management of malaria vector and a new mosquito control agent.

2. Materials and Methods

2.1 *Anopheles gambiae* culture

An. Gambiae mosquito larvae were obtained from successfully maintaining laboratory colonies at Environmental Biology Department, Adekunle Ajasin University, Akungba- Akoko, Ondo State, Nigeria (Latitude 7° 28' N and Longitude 5° 44' E). The larvae were kept in plastic bucket containing rain water, and were maintained in Environmental Biology Laboratory, Department Science Laboratory Technology Department, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria (Latitude 7° 11' N and Longitude 5° 35' E) and all the experiments were carried out at 30±2 °C, 75±5% relative humidity and 12:12 light and dark photo period cycle. Larvae were fed with a mixture of finely ground dog biscuit and yeast powder in the ratio of 3:1 by weight. Care was taken to prevent the formation of any scum on the surface of water.

2.2 Plant collection

Seeds of *Persea americana* was collected from the fruit seller stall at Emure-Ile, Owo Local Government Area, Ondo State, Nigeria (Latitude 7° 11' N and Longitude 5° 35' E). The seeds were carefully separated from the fruits and shade dried at room temperature of 30±2 °C for about 20 days. The dried seeds epicarps were properly removed and the dried seeds crushed with mortar and pestle, powdered using hammer mill and sieved thoroughly to get fine powder.

2.3 Preparation of the extract

The powdered material was weighed and 200 g of the material was extracted in 1000 ml of ethyl acetate, chloroform and acetone separately using soxhlet extraction apparatus with boiling temperature of 40 °C for 8 hrs [15]. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper and concentrated; the residue obtained was stored at 4 °C for further use. The stock solution was used to prepare the desired concentrations of the extracts for investigating their larvicidal potential against *An. gambiae* [16-17].

2.4 Larvicidal bioassay

The laboratory colonies of *An. gambiae* were used for the larvicidal activity. Different concentrations of extract (5µl, 10µl, 15 µl, 20 µl, 30 µl and 40 µl) were prepared using methanol to dissolve the crude extracts. Ten (10) healthy larvae of 3rd/4th instar obtained from the culture were introduced into each 125 ml capacity plastic container containing 120 ml of distilled water in different test concentration of both plant extracts along with a set of control containing distilled water without any test solution. All the experiments were carried out at room temperature of 30±2 °C and relative humidity of 75±5 per cent. Mortality was recorded after 24 h of exposure during which no nutritional supplement was added. The number of dead larvae at the end of 24 hr was recorded. Dead larvae were removed as soon as possible in order to prevent decomposition which may cause rapid death of the remaining larvae. Dead larvae were identified when they failed to move after touching with tip of thin brush. By counting the number of dead larvae at 24 hrs of exposure, the mortality rate and the median lethal concentration were obtained. Three replications were maintained for each treatment [18].

2.5 Statistical analysis

The percent mortalities were corrected using Abbott's formula [19] whenever required. The percentage larval mortality data were subjected to ANOVA procedure using SPSS 16.0. Probit analysis [20] was applied to determine lethal dosages causing 50% (LC50) mortality of *An. gambiae* larvae 24 h after treatment application and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL). All the results were expressed as mean ± SD of three replicates in each treatment, results with $p<0.05$ were considered to be statistically significant and Tukey HSD test (P=0.05) was applied for mean separation.

3. Results

3.1 Larvicidal activity of *P. americana* seed extracts against *An. gambiae*

The larvicidal activities of the extracts against mosquito larvae under laboratory conditions are given in tables 1. All *P. americana* seed extracts acetate exhibited significant larvicidal activity against *An. gambiae* (Table 1). Mortality of larvae in different concentration during the experiment ranges from 28.13 – 64.20%. The result indicated that increase in concentration of the plant extracts resulted increase in the mortality of the larvae. Significant differences among different treatments were recorded at each concentration. Among the seed extracts, it can be observed that, chloroform extract showed moderate larvae mortality closely followed by acetone extract having record lowest LC_{50} of 40.56 and 49.09 respectively and ethyl acetate extract recorded lowest larvae mortality activity based on the highest value of 60.50 LC_{50} recorded (Table 2).

Table 1: Larvicidal activity of *Persea americana* extracts against *Anopheles gambiae*

Concentration	Ethyl Acetate	Chloroform	Acetone
5µl	54.33±17.08	28.13±5.88	31.00±8.16
10 µl	64.20±0.00	29.23±2.19	36.86±2.36
15 µl	58.03±10.68	34.30±10.05	54.33±17.08
20 µl	58.03±10.68	37.80±2.77	58.03±10.68
30 µl	64.20±0.00	49.73±12.93	55.93±14.31
40 µl	64.20±0.00	64.20±0.00	64.20±0.00
LSD	7.6028	5.9196	6.1137

Values represent mean ± S.D of 3 replications

3.2 Lethal Concentration Effects of *P. americana* Extracts on *Anopheles gambiae* Larvae

Table 2 shows the lethal concentration of *P. americana* seed extracts against third/fourth stage larvae *An. gambiae*. Results showed that the LC50 values of *P. Americana* were 60.15, 40.56 and 49.09 for ethyl acetate, chloroform and acetone extracts respectively. The slope was highest of 15.813 for ethyl acetate extract, followed by acetone extract (11.212) and lowest of 9.472 for chloroform extract. The chi-square values were significant at $p<0.05$ level. The results indicated that chloroform extract is more toxic to the larvae and followed by acetone extract and the least toxic been ethyl acetate.

Table 2: Lethal Concentration Effects

Solvent tested	Lc50	95% confidence Limit		Slope	Chi-square
		LCL	UCL		
Ethyl acetate	60.50	56.128	64.872	4.028	15.813
chloroform	40.56	33.382	47.751	3.279	9.472
Acetone	49.09	41.240	57.215	3.830	11.212

Lc50 = lethal concentration brings out 50% mortality, LCL = lower confidence Limit; UCL = Upper confidence limit, Slope, chi-square.

Discussion

Mosquitoes are the major public health problem contributing significantly to disease burden, death, poverty and social debility throughout tropical and sub-tropical countries of the world [21]. The resistance to chemical insecticides among mosquito species has been considered as a setback in vector control. In recent years, the emphasis to control the mosquito populations has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials. For this purpose, a lot of plant extracts from various plant species have been tested for their larvicidal and mosquitocidal actions against mosquitoes [22-24].

The present study is focused on natural products of plant origin with insecticidal properties for control of insect vectors. Ethyl acetate, acetone and chloroform extracts of *P. americana* were evaluated against the fourth instar larvae of *An. Gambiae* In the present study, the three extracts exhibited good larvicidal activities on mosquito with varying susceptibility. The larvicidal activity was significant at all the concentrations and the activity was concentration dependent; as the concentration of the plant extracts increases the total larval mortality were also found to be increased. Although none of the tested plant powder was able to exert 100% larval mortality. The high mortality rate recorded in the present study could be due to the presence of secondary metabolites present in the extract that inhibits the developmental stages of insects [25-27] by interacting with cuticle membrane or larvae ultimately disarranging the membrane, which is probable reason for larval death. The moderate larvicidal activity in the present study may be due to the mixture of chemical constituents present in the plant seed which may act in combination or independently. Leite *et al* [28] reported the presence of fatty acids, flavonoids, triterpenes, anthocyanin, and abcissic acids in *P. Americana* seed. Some of these phytochemicals have also been reported to possess larvicidal activities [29]. The present results correlate with previous findings of Pushpanathan *et al*., [30]; Govindarajan [11]; Sathya Narayanan *et al*. [31].

Findings from this study show that chloroform, ethyl acetate and acetone, extracts of *P. Americana* exhibited larvicidal activity against *An. gambiae* with LC50 values of 40.56, 60.50 and 49.09 respectively. Extract with the lowest LC50 proved to be more toxicant compare to others. The lower the LC50 the higher the toxic effect and vice versa. The lowest toxicity observed from this study as reflected by the LC50 confirms the findings of Nzelibe and Albaba [32] who reported that ethyl acetate is least toxic extract having the highest LC50 value against *Aedes vittatus* and Rudi *et al* [33] who opined that *Lantana Camara* chloroform extract shows the very good anti-mosquito activity with very low LC50 value. The LC50 values recorded in this study correlates with Sharma *et al*., [34] who reported that petroleum ether extract of *Ageratum conyzoides* leaves exhibited larvicidal activity with LC50 value of 42.60**.** These results demonstrated that the solvent used can also contribute to the variation in larvicidal activity, since it has been shown that the extraction of active biochemical from plants depends upon the polarity of the solvents [35]. Thus, each solvent, extracts chemical constituents of varying degree of toxicity as well as larviciding potency [36].

Conclusion

The results suggested the plant extracts were found effective in controlling *An. gambiae* larvae under lab conditions and has contribute to use of botanical derivatives in mosquito control instead of synthetic insecticides; thus could be used in stagnant water bodies which are known to be the breeding grounds for the mosquitoes, while in turn will increase the opportunity for natural control of various medically important pests by botanical pesticides. As *P. Americana seed* are readily available in Nigeria their formulation might prove to be an effective and eco-friendly larvicide, and seeds will be better utilized rather than the wastage associated it during the season.

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