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Larvicidal Potential of *Persea americana* Seed Extract against *Aedes vittatus* Mosquito

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Authors' contributions

This work was carried out in collaboration between the two authors HCN and SUA. Author HCN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Author SUA managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate larvicidal activity of solvents (Ethanol, Ethyl Acetate, n-Hexane) extracts of *Persea americana* seed against *Aedes vittatus* and partially purify the most potent solvent extract and characterise most effective fraction isolated.
Place and Duration of Study: Entomology Research Laboratory and Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Between August-October, 2013 and June-August, 2014.
Methodology: The mosquito larvae (15 per replicate) were exposed to test concentrations. The number of dead larvae was recorded after six hours interval for 24 hours and the percentage mortality was calculated. The most potent solvent (n-hexane) extract was fractionated using column chromatography and most effective fraction isolated, was identified using gc/ms and ftir techniques.
Results: N-hexane extract is the most potent solvent extract, with an lc50 value of 0.827 ppm showing 100% mortality at 50 ppm, six hours post exposure. While ethanol and ethyl acetate extracts with an lc50 values of 1.79 ppm and 2.732 ppm, show 100% mortality at 200 ppm and 600 ppm twelve hours post exposure, respectively. The n-hexane chromatographic fractions of

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P. americana (nhp) tested on the larvae, showed increased activity of some fractions, nhp6 and nhp7 with lc¬50 values of 0.486 ppm and 0.727 ppm, having 100% mortality at 50 ppm and 25 ppm, at 24 and 12 hours post exposure respectively. The gc/ms s identification of the most effective fraction, nhp6, revealed eighteen peaks, dominant four of which include fatty acid and fatty acid methyl esters, which correspond to oleic acid (22%), 3-hydroxy-2,2,4-trimethyl pentyl ester of isobutanoic acid (12.26%), methyl-2-(acetyloxy) hexadecanoate (10.28%) and 3,4-dimethyl-1-decene (10.02%). The ftir analysis identified functional groups present which include; alkane, alkene, alkyl halide, aldehyde, carboxylic acid and carbonyl ester.

Conclusion: Based on these findings of all the solvents used, the n-hexane, ethylacetate and ethanol extracts of *Persea americana* seed have shown good larvicidal activity. The oleic acid content of this plant should be explored and optimised to serve as source of larvicide that is effective and environmentally friendly.

Keywords: Larvicidal; Aedes vittatus; Persea americana.

1. INTRODUCTION

Mosquito species belonging to genera; Anopheles, Aedes and Culex, are vectors [1] for the transmission of vector-borne diseases such as malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE). These mosquito-borne diseases remain a major cause of morbidity and mortality worldwide. Consequently, World Health Organization (WHO) has recommended vector control as an important component of the global strategy for preventing insect-transmitted diseases. The conventional method for control of mosquito-borne diseases involve the use of chemical-based insecticide, though it is not without numerous challenges, such as human and environmental toxicity (persistence and accumulation), resistance, affordability and availability, especially in the endemic regions of the world, such as Africa and Asia [2]. Thus, plant-derived products are being exploited as an alternative and abundant source of bioactive (larvicide) compounds that are easily biodegradable into non-toxic products and no resistance has been reported for these products because of their complex chemistry [3]. They act as larvicides, insect growth regulators, repellents, and oviposition attractants [4-6]. However, the main reasons for the failure in laboratory to field utilisation of bioactive phytochemicals are poor characterization and inability to determine the active toxic components responsible for larvicidal activity [2]. Thus this study has been able to demonstrate the larvicidal potential of extracts of *P. americana* seed and characterise the most effective fraction using GC/MS and FTIR techniques.

P. americana is an ever green tree belonging to Lauraceae family and its fruits are commonly known as avocado pear or alligator pear. The plant is widely cultivated in tropical and subtropical regions. The seed extracts of *P. americana* has many vital application in traditional medicine, for the treatment of diarrhoea, dysentery, tooth ache, intestinal parasites, skin infection (mycoses) and management of hypertension and the leaves have been reported to have anti-inflammatory and analgesic activities [7,8]. Previous phytochemical screening of avocado seed indicates the presence of fatty acids, flavonoids, triterpenes, anthocyanin, and abcissic acids [9]. Some of these phytochemicals have been reported to have larvicidal activities [10].

2. MATERIALS AND METHODS

Silica gel, 60-230 mesh, Silica gel TLC plates (60 F254 Merck), pipette delivering 100-1000 µl, Disposable tips (100 µl, 500 µl) for measuring aliquots of dilute solutions, 1ml pipettes, five droppers with rubber suction bulbs, wire loops, nylon netting cage 75 x 35 x 35 cm, 100 ml disposable bowls, measuring cylinder and a strainer used for transfer of test larvae into test vessels. The chemicals used, include; DMSO, n-hexane, ethyl acetate, ethanol solvents and distilled water. These were of analytical grades, purchased from Sigma-Aldrich Company, U.S.A.

2.1 Collection and Identification of Plant Material

The *P. americana* fruits were obtained from Samaru Area and identified and with the help of Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria.

2.2 Extract Preparation

The seeds of *P. americana* were collectedted from the ripened fruits, chopped into pieces and

shade dried. The dried plant material was ground into powder using laboratory milling machine in the Food Science and Technology Programme Dept. of the Institute of Agricultural Research, Ahmadu Bello University Zaria, Nigeria. Each powdered plant material (100 g) was weighed and put in separate beakers and 1000 ml of each solvent (ethanol, ethyl acetate and n-hexane,) were added to each of the beakers with periodic shaking for 24 hours followed by filtration using What man No. 1 filter paper. The filtrates were concentrated using rotary evaporator set at 45ºC. The extracts obtained were labelled and stored at room temperature in amber coloured air tight bottle untill required for use.

2.3 Mosquito Larvae Culture

A. vittatus mosquito larvae were collected from Kufena Rock in Zaria. The larvae were identified and authenticated at the Entomology Research Laboratory of Department of Biological Sciences A.B.U. Zaria. The larvae were reared in plastic and enamel trays in dechlorinated tap water. They were maintained at room temperature and fed a diet of brewer's yeast and biscuits in a ratio of 1:1. Pupae were transferred from the trays to a cup containing dechlorinated tap water and placed in screened cages where adults emerged. Adults of *A. vittatus* were reared in wooden cages. Adults were continuously provided with 10% sucrose solution soaked on a cotton pad, placed at the middle of the cage. They were provided with a Guinea pig placed in restrained position overnight for blood feeding. A petri dish with moisten filter paper was provided in the cage for oviposition and was maintained at the same environmental condition. The third and fourth instar larvae of the second generation were used for the Bioassay [11].

2.4 Larvicidal Bioassay

Bioassay was performed according to WHO guidelines [12]. In each test concentrations, fifteen 3rd and 4th in star larvae were introduced into each plastic bowl (100 ml capacity). The mosquito larvae were exposed to test concentrations (12.5, 25, 50, 100, 200, 300 and 600 ppm) to find out the larval mortality. The numbers of dead larvae were recorded after six hours interval for 24 hours and the percentage mortality was calculated. Batches of 25 third and fourth instars larvae were transferred by means of strainers, screen loops or droppers to small disposable test cups. The depth of the water in the bowls was maintained between 5 cm and 10 cm to avoid undue mortality. The experiment was set up in triplicates for each concentration. The test containers were maintained at room temperature with a photoperiod of 12 hours light followed by 12hours dark (12L:12D).

2.5 Determination of Percentage Mortality and Lethal Concentrations

The number of dead larvae at various concentrations recorded from the triplicates readings were used to calculate percentage mortality and corrections for mortality were made by using Abbots formula [13] formula as shown below. The lethal concentrations (LC50 and LC90) were determined by Probit analysis with IBM SPSS [14].

$$\text{Mortality}\,(\%) = \frac{\text{Number of dead larvae}}{\text{Number of larvae exposed}} \times 100$$

$$\textit{Corrected mortality}\ (\%) = \frac{X-Y}{X} \times 100,$$

Where

X=Percentage survival in the untreated control.

Y=Percentage survival in the treated sample.

2.6 Isolation of Most Potent fraction by Chromatographic Techniques

TLC was carried out to determine the best solvent system for column development. The plate was developed with a mixture of hexane/Ethylacetate (9:1 to 1:9) ratio and then removed from the TLC tank, air dried and visualised with 20% sulpuric acid in alcohol under UV light. The chromatogaraphic column was packed with (80 g) silica gel 60-120 and n-hexane (crude) extracts (4 g) of *P. americana* seed mixed with silica gel were loaded on to the column. The components of the crude n-hexane extracts were separated by gradient elution on a silica packed column using an eluting mixture of n-hexane : ethyl acetate (starting from 100% n-hexane; n-hexane 9.8: Ethyl acetate 0.8, 9.5:0.5, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6 and then 100% ethyl acetate) obtained from TLC. Fractions of 30 ml aliquots were collected into different beakers. The solvent was allowed to evaporate and the contents were profiled on TLC plate [15].

Fractions with similar TLC profiles were pooled together. The pooled fractions obtained were (0.06 g, 0.079 g, 0.18 g, 0.16 g, 0.17 g, 0.08 g and 0.29 g) labelled as NHP1, NHP2, NHP3, NHP4, NHP5, NHP6 and NHP7 respectively. Larvicidal activities of these Fractions were tested (at 12.5, 25, 50, 100, 200, and 300 ppm refer to Line 71) against *Aedes vittatus* mosquito larvae. The fraction with highest larvicidal activity was selected for spectroscopic analysis.

2.7 Identification of Most Potent Fraction by GC/MS and FTIR Techniques

GC/MS (model GC-MS-QP-2010 plus, Shimadzu) was used. Helium was used as carrier gas at a constant column flow rate 1.58 ml/min at 108 kpa inlet pressure. Temperature programming was maintained from 100ºC to 200˚C with constant rise of 5ºC/min and then held isothermal at 200ºC for 6 min; further the temperature was increased by 10ºC/min up to 290ºC and again held isothermal at 290ºC for 10 min. The injector and ion source temperatures were 270ºC and 250ºC, respectively. Mass spectra were taken at 70eV; a scan interval of 0.5 s and fragments from 40 to 800 Dalton. The final confirmation of constituents was made by computer matching of the mass spectra of peaks with the National Institute Science and Technology (NIST) libraries 2005 mass spectral database at the National Research Institute for chemical Technology (NARICT), Zaria Kaduna State, Nigeria. The functional group(s) present, were identified using FTIR spectrophotometer (Shidmazu model), available at the National Research Institute for Chemical Technology (NARICT) Laboratory, Zaria, Kaduna State, Nigeria.

2.8 Statistical Analysis

IBM SPSS statistics version 20 was used to compute percentage mortality and lethal concentrations using Log-Probit Analysis.

3. RESULTS AND DISCUSSION

The result of the larvicidal bioassay presented in Table 1 reveals that n-hexane extract is most toxic, showing 100% mortality at 50 ppm with LC50 value of 0.827 ppm. This was followed by ethanol extract, showing 100% mortality at 200 ppm, after 12 hours of exposure with LC50 value of 1.799 ppm, while ethyl acetate is least toxic showing 100% mortality and having the highest LC50 value of 2.732 ppm. These results demonstrated that the larvicidal activity of plant materials vary due to change of solvents used for the extraction [2]. This variation arises due to difference in polarity of each solvent. Thus, each solvent, extracts chemical constituents of varying degree of toxicity. This was also reported by some researchers [2]. The relatively, rapid action of n-hexane extract could be due to the oily and lipophilic nature of its constituent chemicals which could lead to tracheal flooding and oxygen depletion [16]. Furthermore, some plants essential oils were reported to interfere with basic biochemical and physiological functions of insects, through their action on the octopaminergic system of these organisms [17].

Consequent upon the result obtained in Table 1, the n-hexane extract, considered as most potent, was chosen for partial purification using column chromatography and some of the fractions obtained showed increased potency against the larvae (Table 2).

Table 1. Percentage mortality and lethal concentration of extracts of *P. americana* seed against *A. vittatus* mosquito larvae at 6, 12 and 24 hours post exposure

Solvents extract	Mortality (%)							Lethal conc. (ppm)	
	Conc./ Time	25 ppm	50 ppm	100 ppm	200 ppm	300 ppm	600 ppm	LC_{50}	LC_{90}
Ethanol	6 hr	7	20	20	33	27	40	1.799	10.242
	12 hr	40	86	73	100	100	100		
	24 hr	40	86	73	100	100	100		
Ethylacatate	6 hr	0	0	20	20	33	40	2.732	9.016
	12 hr	27	13	87	87	93	100		
	24 hr	33	40	93	93	93	100		
n-hexane	6 hr	40	40	100	100	100	100	0.827	1.972
	12 hr	60	100	100	100	100	100		
	24 hr	100	100	100	100	100	100		

Values represent mean of three replicates at n=15

Chromatographic fractions that are more potent than the n-hexane (crude) extracts are NHP6 and NHP7 with LC50 values of 0.486 and 0.727ppm, respectively. The increased potency observed (Table 2) could be as a result of change in chemical constituents of the aliquots due to differences in the polarity of the eluents [2].

The chemical components of (most potent fraction (NHP6) fraction were identified by GC/MS shown in Fig. 1 and supported with functional groups analysis by FTIR spectroscopy shown in Fig. 2. The GC/MS result, Table 3 reveals eighteen components, four of which are dominant.

The dominant peaks include; oleic acid, 22%, 3-Hydroxy-2,2,4-trimethyl pentylester of isobutanoic (12.26%), Methyl 2-(acetyloxy) hexadecanoate (10.28%), and 3,4-Dimethyl-1-decene (10.02%).

Table 2. Percentage mortality and lethal concentrations of n-Hexane extract of *P. americana* chromatographic fractions against *Aedes vittatus* mosquito larvae at 6, 12 and 24 hours post exposure

Fractions	Mortality (%)							Lethal conc. (ppm)	
	Conc/TIME	12.5 ppm	25 ppm	50 ppm	100 ppm	200 ppm	300 ppm	LC_{50}	LC_{90}
NHP1	6 hr	2	0	2	7	20	31	8.024	32.076
	12 hr	4	9	16	29	40	33		
	24 hr	11	11	22	36	51	64		
NHP2	6 hr	0	2	13	24	33	36	4.674	9.993
	12 hr	0	16	18	42	62	82		
	24 hr	4	18	22	49	71	91		
NHP3	6 hr	0	18	4	9	18	29	4.622	21.867
	12 hr	4	31	38	29	64	78		
	24 hr	27	40	56	60	89	98		
NHP4	6 hr	20	29	40	47	47	53	1.509	8.337
	12 hr	36	51	78	91	100	100		
	24 hr	71	78	98	100	100	100		
NHP5	6 hr	0	0	7	13	20	47	3.679	18.577
	12 hr	0	20	27	40	33	100		
	24 hr	60	63	80	100	87	100		
NHP6	6 hr	24	36	58	56	64	84	0.486	5.130
	12 hr	84	93	96	100	100	100		
	24 hr	91	93	100	100	100	100		
NHP7	6 hr	9	7	7	38	56	82	0.727	8.875
	12 hr	89	100	100	100	100	100		
	24 hr	91	100	100	100	100	100		

Values represent mean of three replicates at n=15

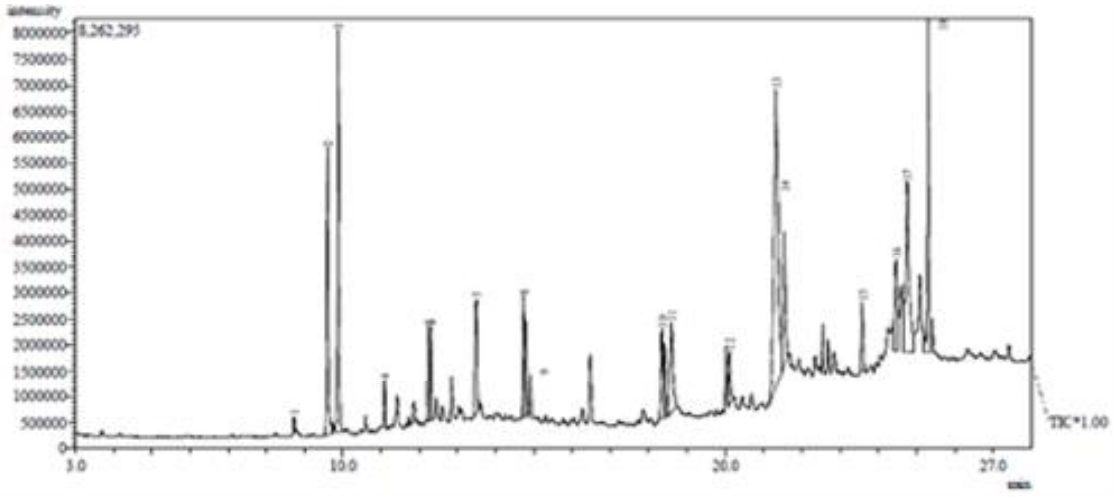
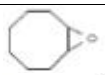
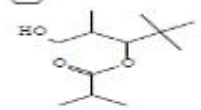
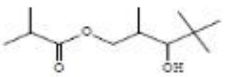
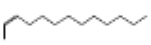
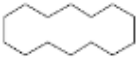
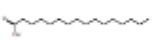
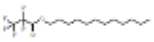
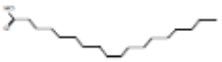
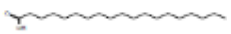
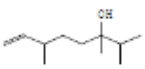
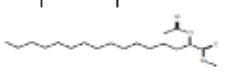
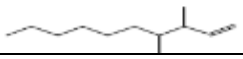


Fig. 1. GC/MS spectra of *P. americana* fraction six (NHP6)

The larvicidal activities of these components were also reported by researchers. In a related study, oleic acid was demonstrated to be the only fatty acid having larvicidal activity against A. aegypti larvae, with an LC50 of 47.9 ppm [10]. In another study [18] show that oleic and linoleic acids were quite toxic against the larvae of *A. aegypti*, *An. stephensi* and Culex quinquefasciatus. When the larvicidal properties of different fatty acid constituents were compared, oleic acid was demonstrated to be the most effective against A. aegypti [19]. While, Farag, et al. [20] found that fatty acids and their esters were mainly responsible for the insecticidal and growth inhibition activity against Spodoptera. Littoralis. Hexadecanoic acid detected was also reported to have larvicidal activity against *Anopheles* and *Aedes* species [21,22].

The FTIR spectra (Fig. 2.) show absorption bands corresponding to various functional groups present (Table 4). The result identified the functional groups present, which quite agree with the various components revealed in the GC/MS result. The functional groups include; carboxylic acid, ester, alkyl halide, alkane and alkenes, all of which were identified in the GC/MS.

Table 3. GC/MS spectral interpretation of *P. americana* fraction 6 (NHP6)

PN	RT (min)	Ar %	Compound name	SI	Structure	Molecular weigt
1	8.72	0.41	1,2-Epoxycyclooctane	90		126
2	9.60	9.23	2,2-Dimethyl-1-(2-hydroxy-1-isopropyl) propyl ester of isobutanoic	93		216
3	9.88	12.26	3-Hydroxy-2,2,4-trimethyl pentylester of isobutanoic acid	91		216
4	11.09	1.02	n-Tridecane	95		184
5	12.24	2.02	(2Z)-2-Tridecene	96		182
6	12.30	1.89	Hexadecane	96		226
7	13.50	4.77	2,6-Dimethylheptadecane	97		268
8	14.73	3.35	n-Hexadec-1-ene	95		224
9	14.90	1.43	n-Dodecane	95		170
10	18.35	2.82	Cyclotetradecane	94		196
11	18.59	4.72	Hexadecanoic acid	93		226
12	20.12	1.81	Pentafluoropropionic acid, dodecyl ester	94		332
13	21.35	22.23	Oleic Acid	92		282
14	21.55	5.70	Nonadecanoic acid	89		298
15	23.59	2.07	1-Iodo-2-methylundecane	94		296
16	24.48	3.94	2,3,6-Trimethyl-7-octen-3-ol	87		170
17	24.77	10.28	Methyl 2-(acetyloxy)hexadecanoate	83		328
18	25.32	10.02	3,4-Dimethyl-1-decene	85		168

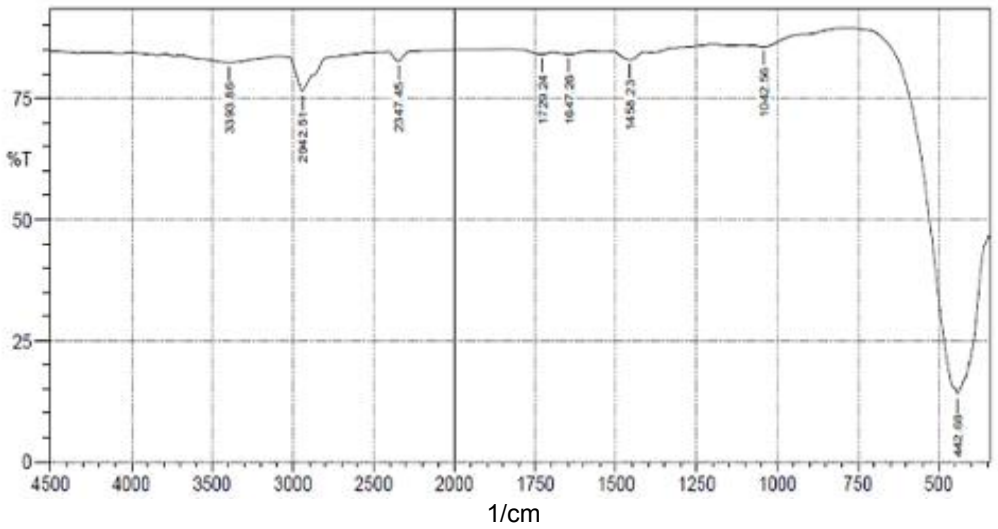


Fig. 2. FTIR spectra of *P. americana* fraction six (NHP6)

Table 4. Fourier transformed infrared spectroscopy (FTIR) analysis of *P. americana* NHP6 fraction six (NHP6)

Peak no	Frequency (CM^{-1})	Intensity area	Bond type	Functional groups	Vibration
1	442.68	15.29	C-Br stretch	Alkyl halides	M
2	1042.56	10.17	C-N stretch	Aliphatic amines	M
3	1458.23	7.17	C-H stretch	Alkenes	M
4	1647.26	0.87	-C=C- stretch	alkenes	M
5	1727.24	0.29	C=O stretch	Carbonyl ester, Sat Aliphatics	S
6	2347.45	11.41	C-N Triple bond	Nitrile	
7	2942.51	14.76	C-H	Alkanes	M
8	3393.86	1.87	O-H stretch	Carboxylic O-H	M

M-medium, S- strong vibrational mode

4. CONCLUSION

The result of this study demonstrated that various solvent extracts of *P. americana* seed have good larvicidal activity with n-hexane (LC50 of 0.827 ppm) extracts as the most toxic amongst three solvents extracts. The chromatographic techniques employed lead to isolation of most potent fraction in the n-hexane crude extract, with increased toxicity in some fractions (NHP6 and NHP7). The GC/MS identification of most toxic fraction revealed oleic acid and other as the dominant component which could synergistically, with other fatty acid methyl esters present, be responsible for the larvicidal activity of this fraction. The FTIR analysis identified carboxylic acids, esters and alkyl halide, thus, supporting the GC/MS findings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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