

1 LARVICIDAL POTENTIAL OF *P. americana* SEED EXTRACT AGAINST *Aedes vittatus*
2 MOSQUITO

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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 A WIDE RANGE OF 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 GCMS AND FTIR TECHNIQUES.

RESULTS: N-HEXANE EXTRACT IS THE MOST POTENT SOLVENT EXTRACT, WITH AN LC_{50} VALUE OF 0.827PPM SHOWING 100% MORTALITY AT 50PPM, SIX HOURS POST EXPOSURE. WHILE ETHANOL AND ETHYL ACETATE EXTRACTS WITH AN LC_{50} VALUES OF 1.79PPM AND 2.732PPM, SHOW 100% MORTALITY AT 200PPM AND 600PPM TWELVE HOURS POST EXPOSURE, RESPECTIVELY. THE N-HEXANE CHROMATOGRAPHIC FRACTIONS OF *P. AMERICANA* (NHP) TESTED ON THE LARVAE, SHOWED INCREASED ACTIVITY OF SOME FRACTIONS, NHP6 AND NHP7 WITH LC_{50} VALUES OF 0.486PPM AND 0.727PPM, HAVING 100% MORTALITY AT 50PPM AND 25PPM, AT 24 AND 12 HOURS POST EXPOSURE RESPECTIVELY. THE GCMS IDENTIFICATION OF THE MOST EFFECTIVE FRACTION, NHP6, REVEALS 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.

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Keywords: [Larvicidal, *Aedes vittatus*, *Persea americana*]

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1. INTRODUCTION

11 Mosquito species belonging to genera *Anopheles*, *Aedes* and *Culex*, are vectors [1] for the transmission
12 of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE)
13 respectively. These mosquito-borne diseases remain a major cause of morbidity and mortality worldwide.
14 Consequently, World Health Organization (W.H.O.) 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 [4,5,6]. 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 GCMS and FTIR techniques.

Persea 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 *Persea 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, flavonoids and abscissic acids [9]. Some of these phytochemicals have been reported to have larvicidal activities, [10].

2. MATERIAL AND METHODS

Silica gel, 60-230 mesh, Silica gel TLC plates (60 F₂₅₄ Merck), pipette delivering 100-1000µl. Disposable tips (100µl, 500µl) for measuring aliquots of dilute solutions, 1ml pipettes, five droppers with rubber suction bulbs, wire loops, nylon netting cage 75x35x35cm, 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.

Collection and Identification of Plant Material

The *Persea americana* fruits were obtained from Samaru Area. The scientific identification and authentication was done in Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria.

Extract Preparation

The seeds of *Persea americana* were extracted from the ripened fruits and chopped into pieces. The dried plant material was ground into powder using laboratory milling machine in the Feed Science and Technology Programme Dept. of the Institute of Agricultural Research, Ahmadu Bello University Zaria, Nigeria. Each powdered plant material (100g) was weighed and put in separate beakers and 1000ml 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 Whatman No.1 filter paper. The filtrates were concentrated using rotary evaporator set at 45°C. The extracts obtained were labelled and stored at room temperature in amber coloured air tight bottle until required for use.

Mosquito Larvae culture

Aedes 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 tap water (previously boiled and cooled). 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 tap water and placed in screened cages where adults emerged. Adults of *Aedes 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 instars larvae of the second generation were used for the Bioassay [11].

Larvicidal Bioassay

Bioassay was performed according to WHO guidelines [12]. In each test concentrations, twenty five 3rd and 4th instars larvae were introduced into each plastic bowl (100 ml capacity). The mosquito larvae were exposed to a wide range of 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 were 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 12hrs light followed by 12 hours dark (12L: 12D).

Determination of Percentage Mortality and Lethal Concentrations

The number of dead larvae at various concentrations recorded from the triplicate readings were used to calculate percentage mortality and corrections for mortality where necessary were done by using Abbot's (1925) formula as shown below. The lethal concentrations (LC₅₀ and LC₉₀) were determined by Probit analysis with IBM SPSS (Finney, 1971).

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

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

Where X = percentage survival in the untreated control

Y = percentage survival in the treated sample.

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% sulphuric acid in alcohol under UV light. The chromatographic column was packed with (80g) silica gel 60-120 and n-hexane (crude) extracts (4g) of *Persea 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 30ml aliquots were collected into different beakers. The solvent was allowed to evaporate and the contents were profiled on TLC plate [13]. Fractions with similar TLC profiles were pooled together. Larvicidal activities of the fractions were tested against *Aedes vittatus* mosquito larvae. The fraction with highest larvicidal activity was selected for spectroscopic analysis.

Identification of most potent fraction by GCMS and FTIR Techniques

GC-MS (model GC-MS-QP-2010 plus, Shimadzu) was used, the result of which was compared with National Institute Science and Technology (NIST) mass spectral database, 2005. The functional group(s) present, were identified using FTIR spectrophotometer (Shimadzu model), available at the National Research Institute for Chemical Technology (NARICT) Laboratory, Zaria, Kaduna State, Nigeria.

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 50ppm with LC₅₀ value of 0.827ppm. This was followed by ethanol extract, showing 100% mortality at 200ppm, after 12 hours of exposure with LC₅₀ value of 1.799ppm, while ethyl acetate is least toxic showing 100% mortality and having the highest LC₅₀ value of 2.732ppm. 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 [14]. 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, [15].

TABLE 1: Percentage Mortality and Lethal Concentration of Extracts of *P. americana* seed against *Aedes vittatus* Mosquito Larvae at 6, 12 and 24 hours post exposure.

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

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	Conc./ TIME	Mortality (%)						Lethal conc. (ppm)	
		12.5ppm	25ppm	50ppm	100ppm	200ppm	300ppm	LC ₅₀	LC ₉₀
NHP1	6hr	2	0	2	7	20	31	8.024	32.076
	12hr	4	9	16	29	40	33		
	24hr	11	11	22	36	51	64		

NHP2	6hr	0	2	13	24	33	36	4.674	9.993
	12hr	0	16	18	42	62	82		
	24hr	4	18	22	49	71	91		
NHP3	6hr	0	18	4	9	18	29	4.622	21.867
	12hr	4	31	38	29	64	78		
	24hr	27	40	56	60	89	98		
NHP4	6hr	20	29	40	47	47	53	1.509	8.337
	12hr	36	51	78	91	100	100		
	24hr	71	78	98	100	100	100		
NHP5	6hr	0	0	7	13	20	47	3.679	18.577
	12hr	0	20	27	40	33	100		
	24hr	60	63	80	100	87	100		
NHP6	6hr	24	36	58	56	64	84	0.486	5.130
	12hr	84	93	96	100	100	100		
	24hr	91	93	100	100	100	100		
NHP7	6hr	9	7	7	38	56	82	0.727	8.675
	12hr	89	100	100	100	100	100		
	24hr	91	100	100	100	100	100		

The chemical components of this fraction were identified by GCMS shown in Figure 1 and supported with functional groups analysis by FTIR spectroscopy shown in figure 2. The GCMS 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%). 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 *Aedes aegypti* larvae, with an LC_{50} of 47.9 ppm (Cantrel, 2011). In another study, [16] show that oleic and linoleic acids were quite toxic against the larvae of *Aedes aegypti*, *Anopheles 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 *Aedes aegypti* [17]. While, [18] 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 [19, 20]

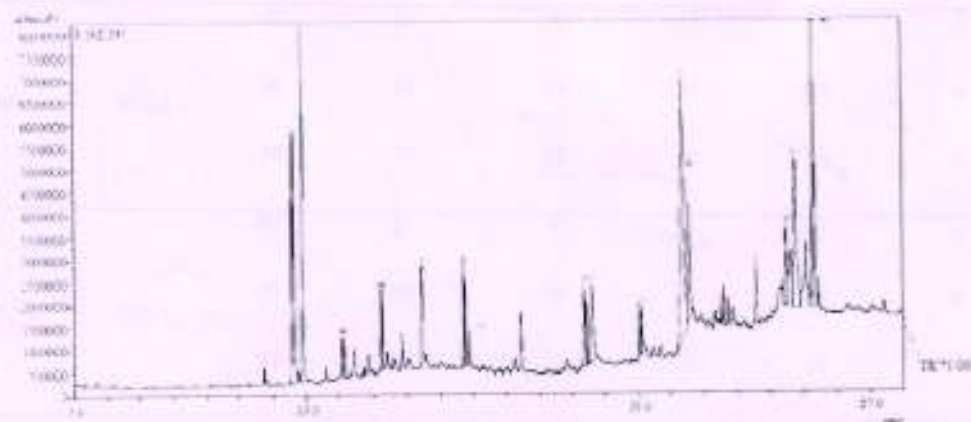


FIGURE 1: GC/MS SPECTRA OF *P.americana* FRACTION SIX (NHP6)

TABLE 3: GC/MS Spectral interpretation of *P.americana* Fraction 6 (NHP6)

P/N	RT (min)	Area %	Compound name	SI	Structure	Molecular weight
1	8.72	0.41	1,2-Epooxycyclooctane	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 (acetyloxy)hexadecanoate	2-83		328
18	25.32	10.02	3,4-Dimethyl-1-decene	85		168

The FTIR spectra (Figure 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 GCMS result. The functional groups include: carboxylic acid, ester, alkyl halide, alkane and alkenes, all of which were identified in the GCMS.

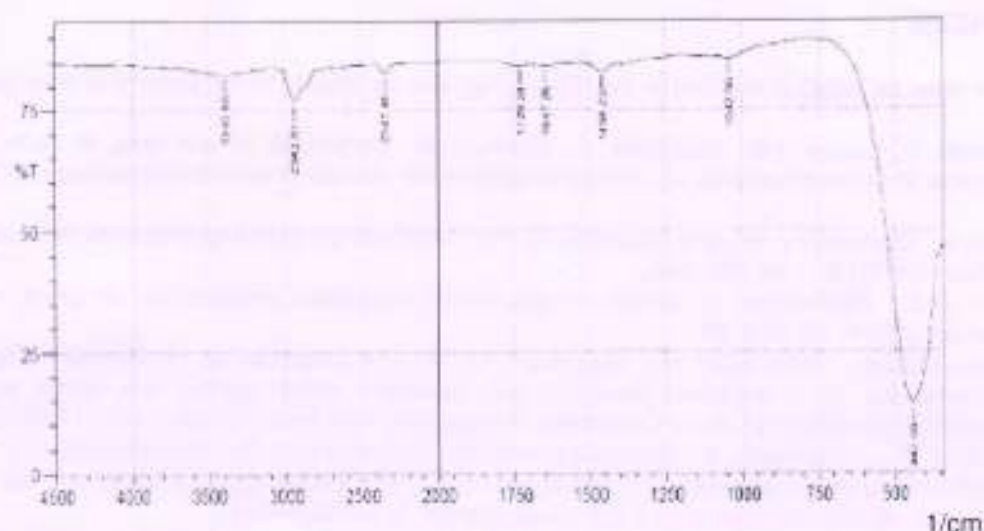


FIGURE 2: FTIR Spectra of *P. americana* fraction (NHP6)

TABLE 4: Fourier transformed infrared spectroscopy (FTIR) Analysis of *P. americana* NHP6 Fraction six (P6)

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, Aliphatics Sat	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 *Persea americana* seed have good larvicidal activity with n-hexane (LC₅₀ of 0.827ppm) 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 GCMS

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 GCMS findings.

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242 **DEFINITIONS, ACRONYMS, ABBREVIATIONS**

243 Here is the Definitions section. This is an optional section.

244 **Term:** Definition for the term