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Bioactive Constituents, Anti-Dengue and Insecticidal Potency of *Ricinodendron Heudelotii* (Baill.) Seed Oil

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Abstract:

Background: *Ricinodendron heudelotii (Baill.) seeds are among plants with least explored potentials. The deleterious effects of synthetic insecticides and larvicides have posed various health challenges and adverse effect on the eco-system, hence the need to explore safe and effective sources of insecticides and larvicides. Oil of Ricinodendron heudelotii seeds was extracted and analyzed for its chemical composition, larvicidal and insecticidal activities. Gas Chromatography-Mass spectrometry was employed for identification of bioactive compounds in the oil.*

Results: *The oil comprised of 14 bioactive natural compounds; (E,E)-2,4-nonadienal, 2,3-nonadiene, (E,E)-2,4-decadienal, hexanoic acid, 1-methylethylidene cyclohexane, 4-heptanal, n-hexadecanoic acid, (Z,Z)-9,12-octadecadienoic acid, 17-octadecynoic acid, oleic acid, methyl γ-linolenate, (Z,Z,Z)-9,12,15-octadecatrienoic acid, γ-tocopherol, and γ-sitosterol which made up 99.9% of the total components of the oil. At 200 ppm, the oil showed 81% larvicidal activity against Aedes aegypti with LC_{50} of 83.18 ppm and was active against Rhyzopertha dominica with 50% mortality.*

Conclusions: *The results showed that Ricinodendron heudelotii seed oil contained appreciable and promising bioactive constituents that can be explored further as insecticides and larvicides. The study therefore suggests the potential relevance of Ricinodendron heudelotii for incorporation in the production of eco-friendly and cost effective larvicides and insecticides.*

Keywords: *Ricinodendron heudelotii seed oil, bioactive compounds, insecticidal activity, larvicidal activity, Natural products*

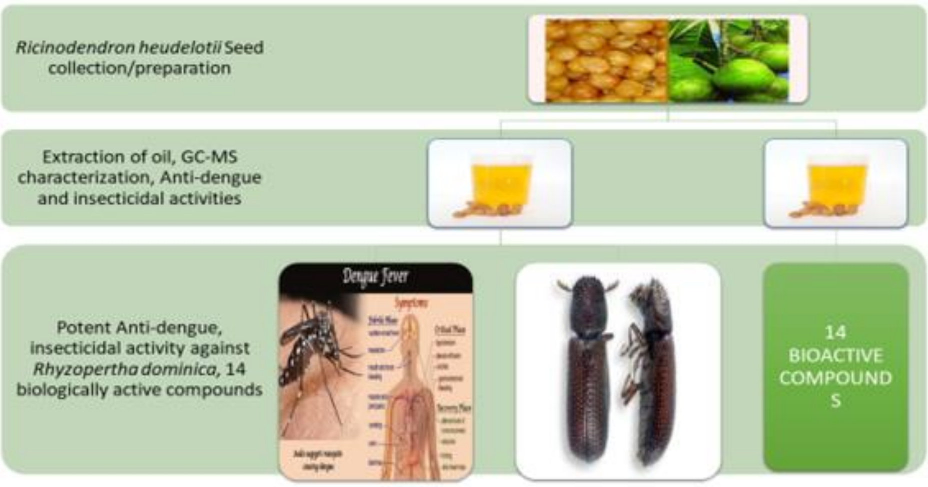
1. Background

The health and adverse effects on humans and the eco-system at large, arising via the use of synthetic insecticides and larvicides have posed great concern, thus, a need for safe and non-toxic insecticides and larvicides from natural sources. Plants have been reported to contain substances that could be used for various purposes, ranging from health, industrial and therapeutic uses. One of such plants is *Ricinodendron heudelotii*. *Ricinodendron heudelotii* (Baill.) commonly called Njangsa is an oily plant in Nigeria; it is widely used for various purposes ranging from food, medicines and a wide range of commodities for the local population. The plant is not much explored for its potential activities. Some of the activities such as anti-inflammatory, antidiabetic, hepato/nephroprotective potencies of the seed extract have been reported (Salehi et al., 2019; Ali et al., 2018). The presence of bioactive compounds in a plant confers on it some biological activities (Odinga et al., 2016; Elango and Jadhay, 2010). Some traditional and reported uses of the plant include treatment of sexual and fertility disorders, ulcer, hypercholesterol and coughs (Kumari et al., 2016; Odinga et al., 2018; Momeni et al., 2005).

Dengue fever is a mosquito-borne disease caused by the dengue virus, transmitted by female mosquitoes of the *Aedes* species particularly *Aedes aegypti* and to a lesser extent *Aedes albopictus*. These mosquitoes are also responsible for transmitting Chikungunya, Yellow fever and Zika infections (Paixao et al., 2017). Infection with dengue virus is a major cause of morbidity in the tropical and sub-tropical parts of the world. The World health organization (WHO) estimated there may be 50 million cases of Dengue fever infection worldwide every year (WHO, 2012). Vaccine development against dengue virus is still at an early stage and therefore the only method available for reducing incidence of the disease is the control of its mosquito vector (Rajasekaran and Duraikannan, 2012). One of the approaches for control of these mosquito borne diseases is the interruption of disease transmission, either by killing, preventing mosquitoes from biting human beings or by causing larval mortality in a large scale at breeding centers of the vectors.

Despite the explorative study on the composition and use of various plant oils, *Ricinodendron heudelotii* seed oil has had a rare investigation. This present study employed Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR) in the characterization of *Ricinodendron heudelotii* seed oil and investigated the larvicidal and insecticidal potentials of the oil against *Aedes aegypti* and *Rhyzopertha dominica* respectively.

This study investigated the larvicidal and insecticidal potentials of *Ricinodendron heudelotii* seed oil against *Aedes aegypti;* and *Tribolium castaneum, Sitophilus oryzea, and Rhyzopertha dominica* respectively.



2. METHODS

Materials

Economically important insect pests were used; test insects (*Tribolium castaneum, Sitophilus oryzea, and Rhyzopertha dominica*). Materials used were organic solvents (ethanol, methanol and acetone), standard insecticide (Permethrin), petri plates (9 cm diameter), micropipette (1000 µl), growth chamber, filter paper, glass vials and brush.

Extraction of oil

Dry seeds of *Ricinodendron heudelotii* were crushed to powder in order to increase the surface area. Seed powder (300 g) was soaked in 1200 ml of *n*-hexane with intermittent shaking. After 72 hours, the mixture was filtered. This process was repeated thrice so as to exhaustively extract oil from the seeds. The combined filtrate was concentrated under reduced pressure using a rotavapor. An oily extract obtained. The oil was stored in sealed glass bottle at 4 °C for further analysis.

Characterization of Oil

Triple quadrupole acquisition method was employed for the characterization of the oil using Agilent technologies 7000 GC/MS triple quad (MS-7000, GC-7890A). The ZEBRONZB-5HT Column at 400

°C: 30 m× 320 μm × 0.25 μm, In: Front SS inlet He and Out: vacuum was used. The oven was equilibrated for 5 minutes, at 60 °C, run time was 56.5 minutes (8 °C/min to 240 °C for 20 minutes and 15 °C/minute to 300 °C for 5 minutes). The volume of injected sample was 1.5 µL. Computer matching of mass spectra was performed using the NIST Mass Spectrometry Data Center and WILEY7.0 library and the retention times of known species injected in the chromatographic column were used for identification of the peaks.

Bruker Vector 22 FTIR Spectrometer was used for the Fourier Transform Infrared Spectroscopy (FTIR) analysis of *Ricinodendron heudelotii* seed oil. The analysis was conducted according to manufacturer's instruction.

Larvicidal assay

Stock solution of 200 ppm was prepared using 100 mg of *Ricinodendron heudelotii* seed oil. From the stock solution, 100, 50, 25 and 12.5 ppm were prepared. Afterwards batches of ten larvae (3rd instar of *Aedes aegypti*) were transferred into the flask containing test solution. Permethrin was used as positive control while negative control was ethanol. Percentage mortality was determined after 24 hours using the equation:

$$\textbf{\% Mortality} = \frac{\%\,\textit{Survival in the untreated control} - \%\,\textit{survival in the treated sample}}{\%\,\textit{survival in the untreated control}} \times \mathbf{100}$$

Insecticidal assay - Impregnated Filter Paper Method

Oil sample was prepared by dissolving 20 mg of *Ricinodendron heudelotii* seed oil in 3 ml of ethanol. The stored grain pests (*Tribolium castaneum*, *Sitophilus oryzea*, *and Rhyzopertha dominica*) were reared in the laboratory under controlled conditions (30 ± 2 °C and 65 ± 2 %RH) in plastic bottles containing sterile breeding media. Insects of uniform age and size were used for the experiment. Whatman filter paper (Ahlstrom Filter Paper, Grade 222, catalogue number 2228-1416) was cut according to the size of petri plate (9 cm) and put in the plates. The filter paper was impregnated with the dispersion of the oil sample with the aid of a micropipette at 2038.20 µg/cm$^{2.}$ The impregnated filter papers in the plates were left for 24 hours to evaporate the solvent completely. Ten (10) healthy and active insects of each specie were put in each plate (test and control) with the help of a clean brush. The plates were incubated at 27 °C for 24 hours with 50% relative humidity in growth chamber. Survival of the insects was assessed by counting the number of survivals of each specie. The Percentage Inhibition or Percentage Mortality was calculated using the equation:

$$\text{Percentage Mortality} = 100 - \left(\frac{\textit{No of insects alive in test}}{\textit{No of insects alive in control}}\right) \times 100$$

Controls: Positive and negative controls were run with test insects.

Positive control contained standard insecticide (Permethrin - 239.5 µg/cm^2 - The concentration which is effective against all test insects) and test insects.

Negative control contained solvent and the test insects only.

3. RESULTS

Constituents of *Ricinodendron heudelotii* seed oil: Fourteen compounds were identified from the oil via GC-MS analysis (Figure 1 and Table 1), they include; (E,E)-2,4-nonadienal, 2,3-nonadiene, (E,E)-2,4-decadienal, hexanoic acid, 1-methylethylidene cyclohexane, 4-heptanal, *n*-hexadecanoic acid, (Z,Z)-9,12-octadecadienoic acid, 17-octadecynoic acid, oleic acid, methyl γ-linolenate, (Z,Z,Z)-9,12,15-octadecatrienoic acid, γ-tocopherol, and γ-sitosterol.

Functional groups present in *Ricinodendron heudelotii* seed oil: Results obtained from Fourier Transform Infrared Spectroscopy revealed that the prominent functional groups present included -OH, C=C, C=O, C=N, NH_2, C-H. The prominent absorption bands ranged from 3326.79 cm^{-1} to 2852.78 cm^{-1}. The peaks contained single bond areas (2500-4000 cm^{-1}), sharp peaks between 2500-3500 cm^{-1}. No absorption peak was observed within 2000-2500 cm^{-1} region, thus, depicted the absence of any compound with C≡C bond. IR absorption at 2922.70 cm^{-1} infers the presence of methylene (CH_2).

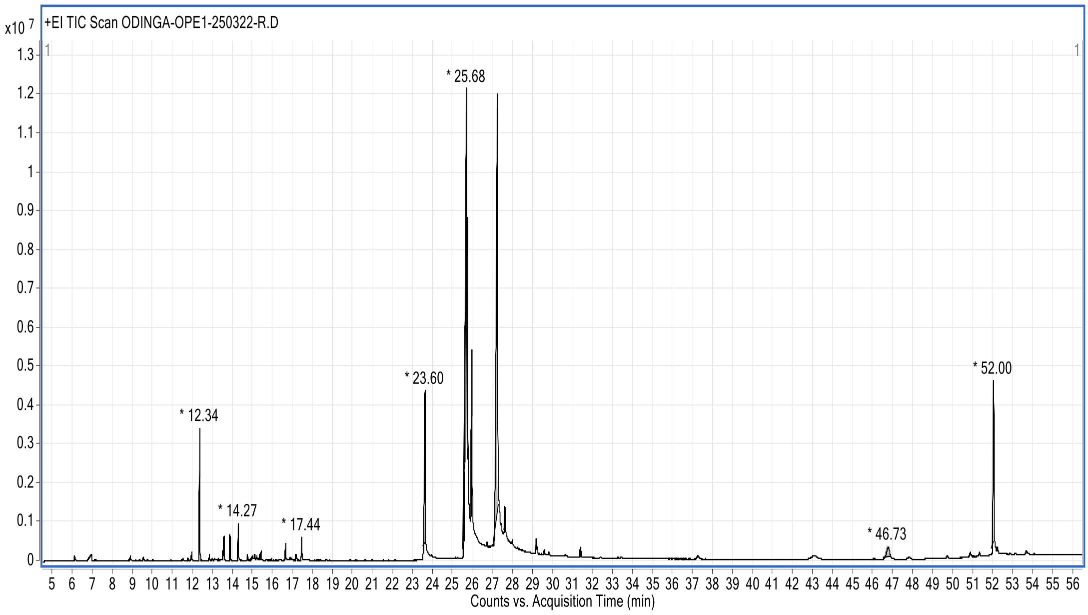


Figure 1. *GC-MS spectrum of Ricinodendron heudelotii seed oil*

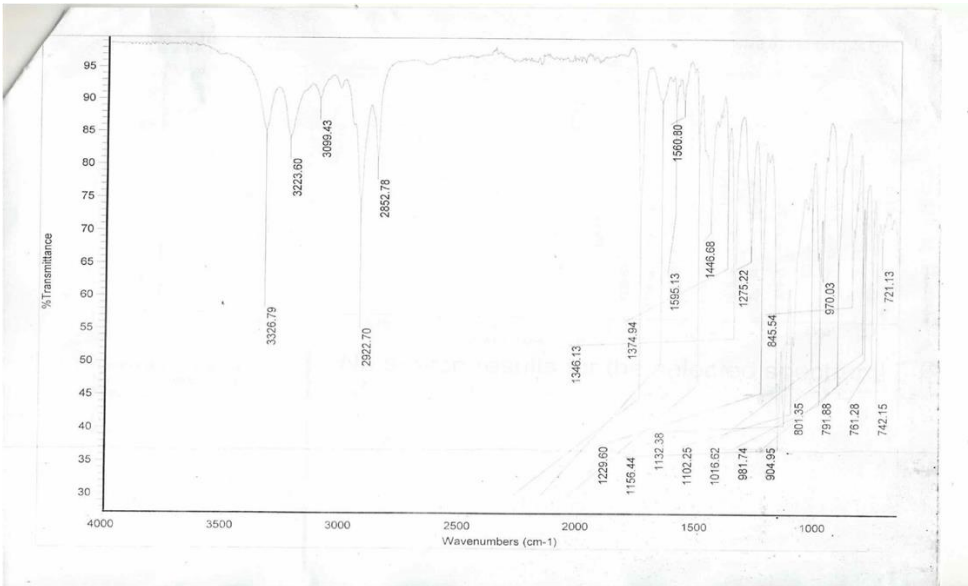


Figure 2. *FTIR Spectrum of Ricinodendron heudelotii seed oil*

Table 1. *Chemical composition of Ricinodendron heudelotii seed oil*

Peak No.	Compound Name	Molecular formula	Retention time (Minutes)	Molecular weight (g/mol)	Concentration (%)
1	(E,E)-2,4-Nonadienal	$C_9H_{14}O$	12.34	138	4.74
2	2,3-Nonadiene	C_9H_{16}	13.56	124	0.63
3	(E,E)-2,4-Decadienal	$C_{10}H_{16}O$	13.87	152	0.9
4	Hexanoic acid	$C_6H_{12}O_2$	6.94	116	1.4
5	*n*-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	23.56	256	8.51
6.	4-Heptenal	$C_7H_{12}O$	14.72	112	0.66
7	1-Methylethylidene cyclohexane	C_9H_{16}	16.64	124	0.51
8	(Z,Z)-9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	25.68	280	16.26
9	17-Octadecynoic acid	$C_{18}H_{32}O$	25.72	280	3.42

10	Oleic acid	$C_{18}H_{34}O_2$	25.94	282	7.18
11	Methyl γ-linolenate	$C_{19}H_{32}O_2$	27.22	292	42.32
12	(Z,Z,Z)-9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	27.59	278	1.01
13	γ-Tocopherol	$C_{28}H_{48}O_2$	46.73	416	2.55
14	γ-Sitosterol	$C_{29}H_{50}O$	52.00	414	9.81

Larvicidal and insecticidal activities of *Ricinodendron heudelotii* seed oil: The larvicidal activity of *R. heudelotii* seed oil against *Aedes aegypti* showed a dose dependent positive activity (Table 2). While for the insecticidal assay using impregnated filter method, 0%, 50% and 70% mortality were observed for *R. heudelotii* seed oil (at 2038.20 µg/cm^3) against *Tribolium castaneum, Sitophilus oryzae and Rhyzopertha dominica* respectively (Table 3).

Table 2. *Larvicidal activity of Ricinodendron heudelotii seed oil*

Larvicide	Mortality (%)	Concentration (ppm)	LC_{50} (ppm)
Ricinodendron heudelotii oil	81	200	83.18
Ricinodendron heudelotii oil	62	100	
Ricinodendron heudelotii oil	21	50	
Ricinodendron heudelotii oil	11	25	
Ricinodendron heudelotii oil	6	12.5	
Permethrin (Standard)	100	100	

Table3. *Insecticidal activity of Ricinodendron heudelotii seed oil*

Name of Insects	% Mortality		
	Positive Control (Permethrin)	Negative Control	*Ricinodendron heudelotii* seed oil
Tribolium castaneum	100	0	0
Sitophilus oryzae	100	0	50
Rhyzopertha dominica	100	0	70

4. DISCUSSION

This study characterized the chemical composition of *Ricinodendron heudelotii* seed oil, assessed its larvicidal activity against *Aedes aegypti* and its insecticidal potency against *Tribolium castaneum, Sitophilus oryzae and Rhyzopertha dominica*. Result of the Gas Chromatography-Mass Spectroscopy analysis (Table 1) revealed the presence of 14 compounds. Methyl γ-linolenate had the highest concentration (42.32%) while 1-methylethylidene cyclohexane had the least concentration (0.51%) in the oil. Other compounds present were (E,E)-2,4-nonadienal (4.74%), 2,3-nonadiene (0.63%), (E,E)-2,4-decadienal (0.9%), hexanoic acid (1.4%), *n*-hexadecanoic acid (palmitic acid) (8.51%), 4-heptenal (0.66%), (Z,Z)-9,12-octadecadienoic acid (16.26%), 17-octadecynoic acid (3.42%), oleic acid (7.18%), (Z,Z,Z)-9,12,15-octadecatrienoic acid (1.01%), γ-tocopherol (2.55%) and γ-sitosterol (9.81%). The total percentage of the compounds detected in the oil equals 99.9%. Oil being a mixture of various chemical compounds (Dhifi et al., 2016) has been characterized using Fourier Transform Infrared Spectroscopy to detect the functional groups present (Lievens et al., 2012). The difference in the chemical composition of oil can be deduced via the information on the functional groups (Le Zhang et al., 2014). Recorded absorption peaks (Figure 2) in the range 3500 - 2500 cm^{-1} and 1600 - 710 cm^{-1} indicates C=O, C=C, C-O, C-H and O-H bond stretches, confirming the presence of alcohols, phenols, aromatics and acids (Nandiyanto et al., 2019). GC-MS result as shown in Table 1 and Figure 1 revealed that the oil contained three aldehydes, fatty acids, steroids and esters.

Table 2 shows that the oil caused 80% mortality of *Aedes aegypti*. This observation is corroborated by the work of Araujo et al. (2016) who reported the larvicidal activity of *Syzygium aromaticum* and *Citrus sinensis* against *Aedes aegypti*. Essential oils from plants have been reported by numerous studies to have larvicidal activities against various insects (Jack et al., 2018). Also, the potency of Piper species essential oil as effective larvicide against mosquito strains resistant to several types of insecticides in Brazil has been confirmed (Pereira Filho et al., 2021). Thus, the findings of this study are in consonance with previous studies proving the larvicidal activities of some plant seed oils.

The insecticidal activity of oil from seeds of *Ricinodendron heudelotii* is as shown in Table 3. The result showed that the oil at 2038.20 µg/cm^3 exhibited the highest mortality percentage for *Rhyzopertha dominica* (70%) and *Sitosphilus oryzae* (50%). The oil showed 0% mortality for *Tribolium castaneum.*

Ricinodendron heudelotii seed oil contains methyl γ-linolenate and palmitic acid amongst others (Table 1). Palmitic acid is a 5-alpha-reductase inhibitor, has antialopecic, anti-androgenic, anti-fibrinolytic, antioxidant activities. Also it can enhance taste, act as lubricant and is a nematicide (Duke, 2004). This agrees with the observed insecticidal and larvicidal activities of the oil, hence, it is suggested that the presence of palmitic acid in the oil contributed to the insecticidal and larvicidal properties of *Ricinodendron heudelotii* seed oil. The insecticidal components of plants have been reported to be monoterpenoids, this is due to their high volatility and their inhibitory effects against acetylcholinesterase (Liu et al., 2021). The insecticidal mode of action of oils may be associated to fumigant action, made by penetrating the insect body via the respiratory system. The eco-friendliness of essential oil from plants for the management of storage insect pests has been reported (Chaudhari et al., 2021). Their study also supports the insecticidal activity of plant seed oil. In view of the above reports, this study has provided evidence for the larvicidal and insecticidal potency of *Ricinodendron heudelotii* seed oil.

5. Conclusion

This study is the first report on the anti-dengue and insecticidal potentials of *Ricinodendron heudelotii* seed oil. The larvicidal efficacy of *R. heudelotii* seed oil against *Aedes aegypti*, and insecticidal potency against *Rhyzopertha dominica* and *Sitosphilus oryzae* may be attributed to the presence of some of the fourteen biologically active compounds contained in the oil. Thus, the oil can be utilized as an eco-friendly larvicide and insecticide, thereby eliminating the toxicity risk of synthetic insecticides and larvicides.

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AUTHORS CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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