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Chemical constituents and insecticidal activity of essential oil of *Paullinia pinnata* L (Sapindaceae)

[Compuestos químicos y actividad insecticida del aceite esencial de *Paullinia pinnata* L (Sapindaceae)]Isiaka A. Ogunwande¹, Adesola A. Osunsami², Sunkanmi E. Sotubo² & Oladipupo A. Lawal¹¹Natural Products Research Unit, Department of Chemistry, Faculty of Science, Lagos State University, Badagry Expressway Ojo, Ojo, Lagos, Nigeria²Faculty of Science, Department of Chemical Sciences, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria
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Abstract: The chemical constituents and insecticidal activity of essential oil obtained by hydrodistillation of the leaves of *Paullinia pinnata* Linn (Sapindaceae) are being reported. The essential oil were analysed by using gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS). Different concentrations (50, 100, 150, 200 and 250 mg/mL) of *P. pinnata* essential oil prepared separately and diluted in dimethyl sulfoxide (DMSO) were tested on the maize weevil, *Sitophilus zeamais*. The main constituents of the oil were pentadecanoic acid (17.9%), isoaromadendrene epoxide (11.5%) and wine lactone (11.2%). Other significant compounds of the essential oil were eremophilene (6.9%) and phytol (6.2%). The essential oil displayed 100% mortality (fumigant toxicity) against *S. zeamais* adults at tested concentration of 150 mg/mL with lethal concentrations (LC₅₀) of 51.87 mg/mL air. This is the first report on the chemical constituents and insecticidal activity of essential oil of *P. pinnata* and may be explore as a potential natural herbal plant for the control of insect pest.

Keywords: *Paullinia pinnata*, hydrodistillation, essential oils, terpenes, fatty acids, *Sitophilus zeamais*, insecticidal activity

Resumen: Se informan los componentes químicos y la actividad insecticida del aceite esencial obtenido por hidrodestilación de las hojas de *Paullinia pinnata* Linn (Sapindaceae). El aceite esencial se analizó mediante cromatografía de gases (GC) y cromatografía de gases acoplada con espectrometría de masas (GC-MS). Se ensayaron diferentes concentraciones (50, 100, 150, 200 y 250 mg/ml) de aceite esencial de *P. pinnata* preparado separadamente y diluido en dimetilsulfóxido (DMSO) en el gorgojo de maíz, *Sitophilus zeamais*. Los componentes principales del aceite fueron ácido pentadecanoico (17,9%), isoaromadendreno epóxido (11,5%) y vino lactona (11,2%). Otros compuestos significativos del aceite esencial fueron eremophilene (6,9%) y phytol (6,2%). El aceite esencial mostró una mortalidad del 100% (toxicidad fumigante) contra los adultos de *S. zeamais* a una concentración de 150 mg/ml con concentraciones letales (CL₅₀) de 51,87 mg/ml de aire. Este es el primer informe sobre la composición del aceite esencial de *P. pinnata* y su actividad insecticida. Puede ser explorado como una potencial planta herbácea natural para el control de la plaga de insectos.

Palabras clave: *Paullinia pinnata*, hidrodestilación, aceite esencial, terpenos, *Sitophilus zeamais*, actividad insecticida

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Abbreviation List: v/w- volume by weight; GC Gas chromatography; GC-MS- Gas chromatography coupled to mass spectrometry; DMSO Dimethyl sulfoxide; LC₅₀ Lethal concentration.

INTRODUCTION

The present research report the chemical composition and insecticidal activity of essential oil obtained by hydrodistillation of the leaves of *Paullinia pinnata* L. (Sapindaceae) grown in Nigeria. This is part of an extensive research aimed at the characterization of the chemical composition and biological potentials of Nigerian medicinal plants and herbs (Lawal *et al.*, 2106). *Paullinia pinnata* (syn. *Cururu pinnata* House) is popularly known as “Five finger” (English), “Yaatsa biyar” or “Goron dorina” (Hausa), “Kakansela” (Yoruba), “Enu-kakanshela” (Nupe) and “Okpanam” (Igbo) in Nigeria. The leaves of the plant *P. pinnata* were used by the natives in Nigeria for the treatment of wounds, fever and as insect repellent. In other part of Africa, the plant is reported to be used in the treatment of gonorrhoea, wounds and microbial infections. The various extracts of *P. pinnata* were used to treat erectile dysfunction in mice (Zamble *et al.*, 2006), possess antioxidative (Jimoh *et al.*, 2007), antimicrobial (Annan *et al.*, 2005; Ikhane *et al.*, 2015), anticonvulsant (Maiha *et al.*, 2009) and anxiolytic (Aliyu *et al.*, 2014) activities. The wound healing, cytoprotective activity (Annan *et al.*, 2010b), antioxidant (Annan *et al.*, 2009a), anthelmintic (Spiegler *et al.*, 2016), cardiotoxicity (Mariame *et al.*, 2016), analgesic and anti-inflammatory (Ior *et al.*, 2011), hepatoprotective (Lunga *et al.*, 2015) and molluscicidal (Melendez *et al.*, 2002) actions of *P. pinnata* have been documented.

Phytochemical examination of *P. pinnata* has led to the identification of paullinoside A and paullinimide A (Tsakala *et al.*, 2006; Dongo *et al.*, 2009), 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthren-6-one that showed moderate algicidal activity against the alga *Chlorella fusca*, β -amyirin, 5- α -poriferastane-3 β ,6 α -diol, β -sitosterol, *l*-quebrachitol and β -sitosterol glucopyranoside (Dongo *et al.*, 2009). In addition, 1, 3', 4', 5, 5'-tetraacetoxy-3-oxo-dimethyl-2H-pyranylacetate flavones, 3,4',5-triacetoxy-6,7-dioxoallylidenedimethylflavone, 4',5,7-triacetoxy-3-diacetoxycyclohexadienyloxy flavones were also described previously (Elfaki *et al.*, 2015). The triterpene, 6a-(3'-methoxy-4'-hydroxybenzoyl)-lup-20(29)-ene-3-one, was recently isolated from the

plant (Jackson *et al.*, 2015). Friedelin a compound of *P. pinnata* displayed antibacterial action against strains of *Staphylococcus aureus* (Annan *et al.*, 2009b) while 6 β -(3'-methoxy-4'-hydroxybenzoyl)-lup-20(29)-ene-one and 6 β -(3'-methoxy-4'-hydroxybenzoyl)-lup-20(29)-ene-ol showed a dose-dependent increase in proliferation of 142BR cells, an indication of fibroblast stimulatory activity (Annan *et al.*, 2010a). Lupeol-3-isovanilloyl ester isolated from the plant demonstrated significant antibacterial activity on the tested strains (MIC 15.2-30.20 μ g/mL) (Lasisi *et al.*, 2015). An investigation of the leaves of *P. pinnata* resulted in the isolation of diosmetin-7-*O*-(2'-*O*- β -D-apiofuranosyl-6'-*O*-acetyl- β -D-glucopyranoside) and tricetin-4'-*O*-methyl-7-*O*-(2'-*O*- β -D-apiofuranosyl-6'-*O*-acetyl- β -D-glucopyranoside) (Abourashed *et al.*, 1999). The antibacterial activity of (3 β)-3-*O*-(2'-acetamido-2'-deoxy- β -D-glucopyranosyl) oleanolic acid and DPPH radical scavenging action of methylinositol have been reported (Lunga *et al.*, 2014a). Other compounds present in *P. pinnata* were 3 β -(β -D-glucopyranosyloxy)stigmast-5-ene, (3 β ,16 α -hydroxy)-3-*O*-(2'-acetamido-2'-deoxy- β -D-glucopyranosyl) echinocystic acid and (3 β)-3-*O*-[β -D-glucopyranosyl-(1''-3')-2'-acetamido-2'-deoxy- β -D-galactopyranosyl]oleanolic (Lunga *et al.*, 2014b) and 2-*O*-methyl-L-chiro-inositol (Lunga *et al.*, 2014b). Both (3 β)-3-*O*-(2'-acetamido-2'-deoxy- β -D-glucopyranosyl) oleanolic acid and (3 β)-3-*O*-[β -D-glucopyranosyl-(1''-3')-2'-acetamido-2'-deoxy- β -D-galactopyranosyl]oleanolic acid displayed antibacterial and anti-yeast activities respectively (MIC 0.78-6.25 and 1.56-6.25 μ g/mL respectively), while (3 β ,16 α -hydroxy)-3-*O*-(2'-acetamido-2'-deoxy- β -D-glucopyranosyl) echinocystic acid exhibited anti-dermatophytic activity (MIC 6.25-25 μ g/mL) (Lunga *et al.*, 2014b). Chemical study on the fruits of *P. pinnata* produced cyclopinnatol, paulliniester, cycloart-22(*E*)-ene-3 β ,25-diol, cycloartenol, β -sitosterol, betulonic acid and oleanonic acid (Awouafack *et al.*, 2016). Cyclopinnatol exhibited significant antibacterial activity against *Staphylococcus aureus*, with MICs of 32 μ g/mL (Awouafack *et al.*, 2016).

The main fatty acids of *P. pinnata* (Fabianska *et al.*, 2004) were 9-octadecenoic acid (oleic acid; 30.8%), hexadecanoic acid (palmitic acid; 30.0%) and octadecanoic acid (stearic acid; 11.8%). Azelaic acid (a dicarboxylic acid) from *P. pinnata* showed antibacterial activity against tested organisms tested with MIC of 32-256 μ g/mL (Annan *et al.*, 2009a).

Till moment there are no reports on the chemical constituents of essential oil and biological activity from *P. pinnata*. This paper reports for the first time the components of volatile of *P. pinnata* and its insecticidal activity.

MATERIALS AND METHOD

Plants collection

Mature leaves of *P. pinnata* were collected from Oke-Odo village in Ago-iwoye, Ogun-state, Nigeria, in May 2016. Botanical identification was carried out by Mr. SA Odewo and Mr. A Adeyemi at Herbarium, Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen FHI 110421 was deposited. The leaves were air-dried under laboratory shed for two weeks prior to hydrodistillation.

Hydrodistillation of the essential oils

Briefly, 500 g of each of the pulverized sample were carefully introduced into a 5 L flask and distilled water was added until it covers the sample completely. Hydrodistillation was carried out in an all glass Clevenger-type distillation unit for 3 h, according to established procedure (British Pharmacopoeia, 1980), at normal pressure. The volatile oils distilled over water and were collected separately in the receiver arm of the apparatus into a clean and previously weighed sample bottles. The oils were kept under refrigeration until the moment of analyses.

Gas chromatography (GC) analysis of essential oil

Gas chromatography analyses of the oils were carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and HP-5MS column (60 m x 0.25 mm id), film thickness was 0.25 μ L and the split ratio was 1:25. The oven temperature was programmed from 50° C (after 2 min) to 240° C at 5° C/min and the final temperature was held for 10 min. Injection and detector temperatures were 200° C and 240° C, respectively. Hydrogen was the carrier gas. An aliquot (0.5 μ L of the diluted oil) was injected into the GC. Peaks were measured by electronic integration. A homologous series of n-alkanes were run under the same conditions for determination of retention indices.

Gas chromatography-mass spectrometry (GC-MS) analysis of essential oil

Gas chromatography mass spectrometer analyses of the oils were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a HP 5-MS capillary column (30 m x 0.25 mm id, film thickness 0.25 μ m). The oven temperature was programmed from 70-240° C at the rate of 5° C/min. The ion source was set at 240° C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu. Diluted oil in n-hexane (1.0 μ L) was injected into the GC/MS.

Identification of the constituents

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of n-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST (NIST, 2011) and the home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature (Adams, 2007).

Determination of insecticidal activity of the essential oil

Adult insects of mixed sex, 7-14 days old of *Sitophilus zeamais* reared on maize at 25 \pm 1° C and 65 \pm 5% relative humidity (R.H.) were used for the bioassay. The insecticidal activity was evaluated as described previously (Lawal et al., 2014; Lawal et al., 2016) using the maize weevil (*Sitophilus zeamais*). Different concentrations (50, 100, 150, 200 and 250 mg/mL) of *P. pinnata* essential oil prepared separately and diluted in DMSO were tested on *S. zeamais*. The appropriate concentrations were applied to filter paper (Whatman number 1, cut into 7 cm diameter) and immediately introduced into Petri dish and sealed. For the control group, the insects were placed in the Petri dish under the same conditions but without the essential oil. Each concentration and control was replicated three times. Insect mortality was determined by observing the recovery of immobilized insects in 12 h intervals up to 72 h. When no movements were observed, insects were considered dead.

Table 1
Components of the essential oil of *P. pinnata*

Compound ^a	RI ^b	RI ^c	Percent Composition
Methyl salicylate	1187	1187	1.8
7-Methy-3-methylene-6-octene-1-ol	1219	1221	0.4
β -Cyclocitral	1225	1226	0.2
α -Citral	1270	1273	0.3
α -Cubebene	1351	1354	0.2
Cyclosativene	1363	1363	0.7
α -Copaene	1377	1374	1.9
β -Elemene	1387	1391	3.9
Longipinocarvone	1397	1398	2.4
Caryophyllene ^d	1419	1417	2.8
<i>trans</i> - α -Bergamotene	1435	1431	2.2
α -Humulene	1454	1452	0.7
Wine lactone	1457	1455	11.2
β -Chamigrene	1480	1478	1.2
Germacrene D	1483	1484	1.8
(<i>E</i>)- β -Ionone	1484	1485	2.5
Eremophilene	1500	1502	6.9
α -Cuparene	1512	1511	1.4
α -Calacorene	1545	1542	1.6
β -Vatirenene	1560	1554	1.0
(<i>E</i>)-Nerolidol	1573	1573	2.1
Caryophyllene oxide	1581	1583	3.1
Viridflorol	1583	1585	0.9
<i>trans,trans</i> -Pseudoionone	1587	1589	2.0
Isoaromadendrene epoxide	1592	1594	11.5
9-Cedranone	1640	1638	1.1
β -Eudesmol	1646	1648	3.1
Longiverbenone	1680	1678	4.9
Pentadecanoic acid	1873	1870	17.9
Phytol	2125	2119	6.2
Andrographolide ^e	2650	2634	1.8
Total			98.7
Monoterpene hydrocarbons			-
Oxygenated monoterpenes			16.0
Sesquiterpene hydrocarbons			25.3
Oxygenated sesquiterpenes			31.1
Diterpenes			8.0
Fatty acids			17.9
Others			0.4

^a Elution order on HP-5MS column; ^b Retention indices on HP-5MS column; ^c Literature retention indices (see Experimental); ^d Correct isomer not identified; ^e Tentative identification

Statistical analysis

The mean and standard deviation of three experiments were determined. Statistical analysis of the differences between mean values obtained for experimental groups were calculated using Microsoft excel program, 2003. Data were subjected to one way analysis of variance (ANOVA). P values ≤ 0.05 were regarded as significant and P values ≤ 0.01 as very significant. Mortality percentages were calculated by the correction formula for natural mortality in the untreated control (Lawal et al., 2014; Lawal et al., 2016). The Lethal concentrations (LC₅₀) values for the insecticidal activity were calculated using probit analysis program, version 1.5

RESULTS AND DISCUSSION

Chemical constituents of the essential oil

The percentage yield of the light yellow essential oil of *P. pinnata* was 0.08% (v/w) calculated on a dry weight basis. Table 1 indicated the percentages and identities of compounds present in the oil. A total of 31 compounds representing 98.7% of the total oil contents were identified. Monoterpene hydrocarbon compounds were conspicuously absent in the oil sample. The main classes of compounds identified in the oil were sesquiterpene hydrocarbons (25.3%), oxygen containing sesquiterpenes (42.3%) and fatty acids (17.9%). The main constituents of the essential oil were pentadecanoic acid (17.9%), isoaromadendrene epoxide (11.5%) and wine lactone (11.2%), with significant amounts of eremophilene (6.9%) and phytol (6.2%). Wine lactone which was previously isolated from different wine varieties (Guth, 1996) possesses sweet smelling odour. The authors are not aware of any literature report on the chemical constituents of essential oil of *P. pinnata*, hence the present study may represent the first of its kind.

A comparison of literature data revealed that the essential oil of *Paullinia* species has not been the subject of literature discussion. The authors are aware of one report on volatile constituents of *P. cupana* (Benoni et al., 1996). Except for identification of α -copaene and caryophyllene, all other compounds such as limonene, estragole, anethole, carvacrol, 1,4-dimethylbenzene, trimethylbenzene and 4-(dimethylpropyl)-phenol that were present in *P. cupana* were conspicuously absent in *P. pinnata*. However, the presence of fatty acids has been reported in other *Paullinia* plants. For example, *cis*-13-eicosenoic acid

(paullinic acid; 44.4%) and *cis*-11-octadecenoic acid (*cis*-vaccenic acid; 19.8%) were present in *P. elegans* (Spitzer, 1995), while *cis*-11-octadecenoic (*cis*-vaccenic acid; 30.4%), *cis*-11-eicosenoic acids (38.7%) and oleic acid (37.4%) were identified in *P. cupana* var. *sorbilis* (Avato et al., 2003).

Insecticidal activity of the essential oil

Figure 1 (percentage mortality) and Table 2 (lethal concentrations, LC₅₀) displayed the fumigant insecticidal effects of essential oil of *P. pinnata* against the adults of *S. zeamais*. The results showed the essential oils to be concentration dependent with some inhibitory action on adults of *S. zeamais* after 72 h of treatment. At concentrations of 50 and 100 mg/mL, the essential oil of *P. pinnata* did not possess reasonable contact toxicity towards *S. zeamais*. However, at concentration of 150 mg/mL and after 48 h, the mortality rate was 80% while at 72 h the maximum mortality of 100% was achieved against *S. zeamais*. The same mortality of 100% was exhibited by at concentrations of 200 and 250 mg/mL at 48 and 72 h. From Table 2, the essential oil of *P. pinnata* after 24 h at all tested concentrations displayed weak lethal concentrations (LC₅₀) of 155.64 mg/mL air towards *S. zeamais*. Also, the fumigant toxicity of the essential improved significantly after 48 h with a total LC₅₀ value of 68.69 mg/mL air. Finally, the essential oil showed marked and improved fumigant toxicity against *S. zeamais* adults with 100% mortality at concentration (150 mg/mL). The lethal concentrations LC₅₀, was calculated to be 51.87 mg/mL air. A comparison of the result with standard and pure insecticidal compounds allethrin (LC₅₀ 7.40 mg/mL air) and permethrin (LC₅₀ 11.10 mg/mL air) revealed that the essential oil of *P. pinnata* to possess some toxicity against *S. zeamais* adults.

Although, literature information is devoid of insecticidal potential of *P. pinnata* essential oils however, the insecticidal effects of some essential oils from other plants against the adults of *S. zeamais* have been reported (Lawal et al., 2014; Lawal et al., 2016). Thus, this study shows *P. pinnata* essential oil (LC₅₀ ca. 50.00 mg/mL air) to have a noticeable insecticidal action on *S. zeamais* adults and may be explored as a potential natural herbal plant. The biological activity of an essential oil may depend on the effect of the major constituents or synergy between the major constituents and some minor compounds. The various compounds present in the

essential oil may have contributed to the observed insecticidal property. For example, phytol has shown insecticidal potential (Lawal *et al.*, 2014). Lactones with the *p*-menthane system, in addition to interesting odoriferous attributes exhibit valuable biological

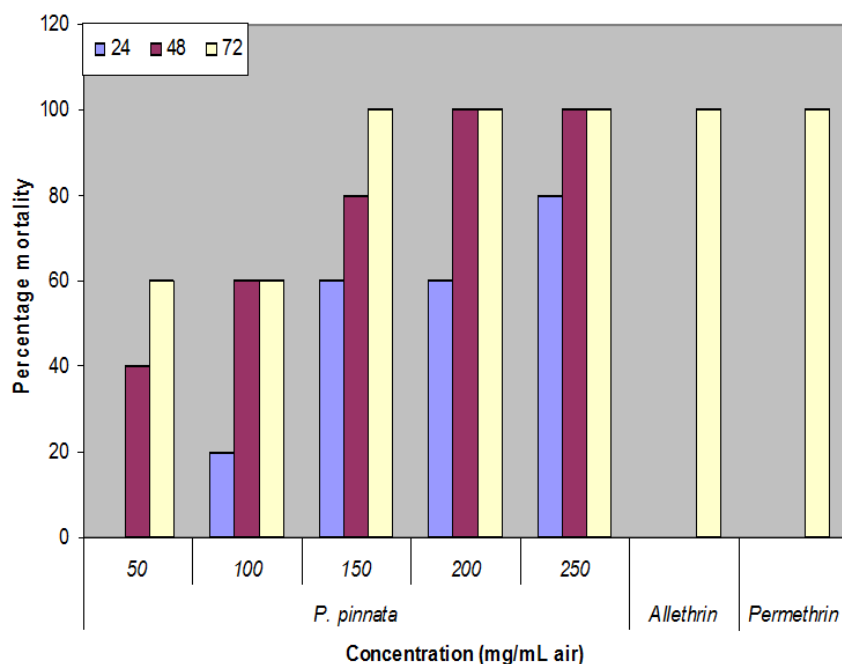
activities such as antifungal and antifeedant properties (Grudniewska *et al.*, 2015). Further chemical analysis aimed at the identification of the compound(s) is ongoing.

Table 2
Insecticidal activity of *P. pinnata* essential oil against *S. zeamais*^a

Sample	LC ₅₀ (95 CI) ^b		
	24 ^c	48	72
<i>P. pinnata</i>	155.64 (95.89 – 249.28)	68.69 (13.17 – 105.36)	51.87 (0.00 – 92.33)
Allethrin ^d	-	-	7.40 (2.01 – 14.65)
Permethrin ^d	-	-	11.10 (6.03 – 23.19)

^a(n= 3, X ± SEM; ^bLC₅₀ - Lethal concentrations with 50 % mortality; ^c- Time, h; ^d- Controls.

Figure 1
Percentage mortality of *S. zeamais* adults against different concentrations of *P. pinnata* essential oil after 72 h



CONCLUSION

In the present investigation of chemical constituents and insecticidal activity of essential oils from the leaves of *P. pinnata*, pentadecanoic acid, isoaromadendrene and wine lactone were found to be the major constituents. In addition, the essential oil demonstrated potential insecticidal activity and may be used for the control of insect pest. Due to the non-availability of literature citation, the results could not

compare with previous study on the essential oil of the plant.

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