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Constituents and Anthelmintic Activity Evaluation of Albizia Adiantifolia (Schumach) W.F. Wright Essential Oils FromNigeria

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Abstract

Albizia adiantifolia (Schumach) W.F. Wright (Fabaceae) is a plant used traditionally in treating different health ailments which includes worm infections. The essential oils (EOs) were obtained by hydrodistillation in an all glass Clevenger apparatus, and characterized by gas chromatography (GC) and gas chromatography-mass spectrometry analysis (GC-MS). *In vitro* petri-dish anthelmintic activity was carried out using adult earthworm, *Eudrilus eugeniae*. The leaves, stem bark and root bark EOs afforded a total of 40, 26 and 20 constituents representing 90.9%, 94.1% and 90.9% of the total oil fractions respectively. The classes of compounds identified in the leaves, stem bark and root bark are oxygenated monoterpenes (4.1%, 1.7% and 4.0% respectively), sesquiterpene hydrocarbons (39.5%, 67.3% and 42.6% respectively), oxygenated sesquiterpenes (18.7%, 22.3% and 30.1% respectively), non-terpene derivatives (12.1%, 2.6% and 14.2% respectively) and apocarotenoids (16.5% and 0.2% in the leaves and stem-bark). β-caryophyllene (23.0%), E-geranylacetone (7.4%), acorenone (6.4%), viridiflorol (6.4%), α-zingiberene (6.3%) and *ar*-curcumene (4.6%) were the major constituents in the leaves oil, β-caryophyllene (39.3%), selin-11-en-4-α-ol (10.4%), α-zingiberene (9.6%), *ar*-curcumene (7.2%), caryophyllene oxide (6.4%) and α-humulene (5.6%) were the major constituents in the stem bark oil, while β-caryophyllene (32.1%), selin-11-en-4-α-ol (13.1%), caryophyllene oxide (8.4%), pentadecanal (6.1%) and α -humulene (4.4%) were the major constituents in the root bark oil. β -caryophyllene dominated the oils. The leaf EO was the most active against *E. eugeniae* worm. All the oils showed a relatively higher activity compared to Albendazole, in a concentration dependent manner. There was significant difference ($p<0.001$) in activity.

Keywords: *Albizia adiantifolia*, Clevenger apparatus, β -caryophyllene, Eudrilus eugeniae, Albendazole

1. Introduction

Albizia adiantifolia (Schumach) W.F. Wight is a large deciduous tree commonly known as "the West African *Albizia* or rough-bark flat-crown" in English (Orwa *et al.*, 2009) and locally referred to as Ayinreta, igbabo (Yoruba) and kawo (Hausa) in Nigeria (Lawal *et al.*, 2010). *A. adiantifolia* is a tree in the Fabaceae family distributed majorly from Senegal, Kenya, Angola, South Africa, Swaziland and extending to Eastern Madagascar. Domestically it has found use as firewood, furniture, as well as in vehicle body, cabinet works, and locally valued as a shade tree for some crops such as cocoa and coffee. It can also be used for soil improvement and conservation while the gum from the bark is used in cosmetics (Lemmens, 2007). In traditional medicine, various parts are used in treating different ailments such as toothache, bronchitis, diarrhea, tapeworm infection (anthelmintic), abdominal pains, typhoid fever, urinary and respiratory tracts infections, Alzheimer's disease and as an antidote against poison. It is also used as vermifuge, purgative, irregular menstruation and even administered to women in child labour (Orwa *et al.*, 2009, Lawal *et al.*, 2010, Lemmens, 2007, Tamokou *et al.*, 2012, Beppe *et al.*, 2014, Abubakar and Majinda, 2015). *A. adiantifolia* administered alone or in combination with *Trichilia dregeana* Sond., can be used to treat Gonorrhea and Syphilis (De Wet *et al.*, 2012).

Extracts from different parts of the plant possess activities such as antioxidant (Tamokou *et al.*, 2012 and Beppe *et al.*, 2014) anxiety, depression and oxidative stress activities (Beppe*et al.*, 2015) antimicrobial, haemolytic, *in vitro* immunomodulatory, anti-inflammatory and anticholinesterase activities (Tamokou *et al.*, 2012 and Abubakar and Majinda, 2015). Lupeol, aurantiamide, D-pinitol, protocatechuic acid as well as triterpenoidal saponins and several flavonoids which are reported to be contained in different parts, have been isolated from the plant (Tamokou *et al.*, 2012 and Abubakar and Majinda, 2015).

The objective of this study is to extract and characterize the essential oils (EOs) from *A. adiantifolia* leaves, stem bark and root bark, and further determine their *in vitro* anthelmintic activity using *Eudrilus eugeniae* adult earthworm.

2. Materials and Methods

2.1 Sample Collection and Essential Oil Isolation

Samples from the leaves, stem bark and root bark of the plant were collected fresh from a forest vegetation at Awotan area in Ibadan, Oyo state, Nigeria in July 2013 and identified at the herbarium of Forest Research Institute of Nigeria (FRIN), Jericho Ibadan where voucher specimens were deposited with herbarium number FHI 109922. Pulverized leaves (300 g), stem bark (350 g) and root back (350 g) samples were used in an all-glass Clevenger apparatus designed according to British Pharmacopoeia specifications (MHRA, 1980) to obtain the essential oils by hydrodistillation method in 4 hours. The oils were dried over anhydrous sodium sulphate (Na_2SO_4) and stored inside the refrigerator at 4^oC prior to use.

2.2 Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

Gas Chromatographic (GC) analyses of the essential oils were performed on a HP-5890 Gas chromatograph equipped with a HP-Wax and HP-5 capillary columns (30 m x 0.25 mm, film thickness of 0.25 mm). The GC oven temperature which was programmed at 60 °C was held for 10 min and heated to 220 °C at 5 °C/min. The temperature for both the injector and the detector was maintained at 250 °C. The carrier gas used was Helium at a flow rate of 2 mL/min. The Gas Chromatographic-Mass Spectrometry (GC-MS) analyses were carried out on a Varian CP-3800 gas chromatograph interfaced to a Varian Saturn 2000 ion trap Mass Detector operated at 70 eV. The injector and transfer line temperatures were 220 °C and 240 °C, respectively. The GC oven temperature was programmed from 60 °C to 240 °C at 3 °C/min. Helium was used as a carrier gas at a flow rate of 1 mL/min. The constituents of the oils were identified on the basis of comparison of the retention times with those of the authentic samples, comparing their retention indices relative to the series of n-hydrocarbons, and by comparison of their mass spectra with published spectra and those of reference compounds from NIST, 2002. The relative concentration of each constituent was calculated by integration of GC peak areas (Adams, 2007).

2.3 Anthelmintic Assay

Preliminary evaluation of *in vitro* anthelmintic activity was carried out according to the method by Priya *et al.*, 2012 with slight modifications. Indigenous in Africa, the adult earthworm (*Eudrilus eugeniae*) commonly known as the West African night crawler (Monebi and Ugwumba, 2013, Obo*het al.*, 2007 and Dominguez *et al.*, 2001) was used owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Lakshananam *et al.*, 2011, Thomas and Devi, 2013 and Pueblos *et al.*, 2015). These adult earthworms are common species in our region of study. Average sizes of the earthworms, 6 – 8 cm in length and 0.2-0.3 cm in width, were collected from moist soils and identified in the department of Zoology, University of Ibadan, Ibadan. They were washed with distilled water to remove any faecal matter.Albendazole, (ALBZ) (brand name – ZENTEL, by ©SmithKline Beecham Lab. Pharm.), often extensively administered clinically as an anthelmintic drug, was the test standard. EOs are relatively insoluble in water but were made soluble in Tween-80 (10% v/v in distilled water). The EOs and ALBZ were dissolved in 10 mL Tween-80 and diluted up to 30 mL to prepare five concentrations (1, 2, 3, 4 and 5% v/v for each of EO and ALBZ) in five petri-dishes. Five worms each were introduced into each concentration. 10% v/v Tween-80 in distilled water was used as negative control. Observations were made to determine the time for paralysis (when no movement could be observed except when shaken vigorously) and death (when worms neither wriggled when shaken nor moved when pinched with a needle, followed with fading away of body colour) of worms to have taken place. Activity was achieved by comparing results obtained for the EO extracts with that of the reference standard drug Albendazole.

Values for time of paralysis and death were expressed as mean ± standard error of mean (SEM). Analysis of variance (ANOVA), followed by student's t test was carried out using Graph Pad Prism, version 5.01 statistical software to determine the significance of differences between experimental and control groups. At 95% confidence interval, p values < 0.001 were considered statistically significant.

3. Results and Discussion

The hydrodistilled leaves, stem bark and root bark essential oils (EOs) of *Albizia adiantifolia* obtained by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses yielded 0.26%, 0.23% and 0.25% (v/w) respectively. As presented in table 1, a total of 40, 26 and 20 constituents representing 90.9%, 94.1% and 90.9% of the total oil fractions in the leaves, stem bark and root bark were identified. Based on the class of compounds present, complex mixtures in the leaves, stem-bark and root-bark were identified, such as oxygenated monoterpenes (4.1%, 1.7% and 4.0% respectively), sesquiterpene hydrocarbons – the most dominant class (39.5%, 67.3% and 42.6% respectively), oxygenated sesquiterpenes (18.7%, 22.3% and 30.1% respectively), non-terpene derivatives (12.1%, 2.6% and 14.2% respectively) and apocarotenoids (67.3% and 42.6%) in the leaves and stem-bark. Interestingly, none of the constituents present are in the class of monoterpene hydrocarbons.

The leaves essential oil constitutes majorly β-caryophyllene (23.0%), E-geranylacetone (7.4%), acorenone (6.4%), viridiflorol (6.4%), α-zingiberene (6.3%) and *ar*-curcumene (4.6%), while the stem bark oil is dominated by β-caryophyllene (39.3%), selin-11-en-4-α-ol (10.4%), -zingiberene (9.6%), *ar*-curcumene (7.2%), caryophyllene oxide (6.4%) and -humulene (5.6%). Also, the root bark oil contained majorly β-caryophyllene (32.1%), selin-11-en-4-α-ol (13.1%), caryophyllene oxide (8.4%), pentadecanal (6.1%) and α-humulene (4.4%). In the pattern of oil composition, -zingiberene and *ar*-curcumene were the dominant compounds in the leaves and stem bark, while selin-11-en-4-α-ol, caryophyllene oxide and α-humulene altogether dominated the stem bark and root bark oils. β-caryophyllene is the most abundant constituent in the three EOs. Other significant constituents include 1,8-cineole (2.2%, 1.5%, 1.9% in the leaves, stem bark and root bark respectively), β-sesquiphellandrene (2.5% and 2.8% in the leaves and stem bark respectively), valerianol (2.9% in the root bark) and δ-cadinene (3.8% in the root bark).

β-caryophyllene, the dominant constituent of *Commiphora gileadensis* EO (which is also one of the major oil constituents identified in this study), possesses many pharmacological properties such as anti-inflammatory, antifungal, local anesthetic and has been reported that the EO exhibited antiproliferative pro-apoptotic effects in tumor cells (Eitan *et al.*, 2012). Also, it was identified as a dominant component of *Garcinia mangostana* Linn. leaves and stem bark EOs. *G. mangostana* oils exhibited high toxicity (LC_{50} values of 1.70 and 5.15 µg/mL, leaves and stem bark respectively) against brine shrimp (*Artemia salina*) and also showed antibacterial activities against some clinical isolates (Aboaba *et al.*, 2014). Furthermore, β-caryophyllene and β-caryophyllene oxide are documented to occur in a large number of plants and possess significant anticancer activities by affecting growth and proliferation of numerous cancer cells (Fidyt *et al.*, 2016).

Murraya paniculata (L.) Jack, is a traditional medicinal plant for the treatment of abdominal pain, diarrhea, stomach ache, headache, edema, thrombosis, and blood stasis. Three of the major constituents (β-caryophyllene, α-zingiberene and selin-6-en-4-ol) identified in the EOs of *M. Paniculata* leaves are similar to the major constituents found in this study. The oil was reported to show moderate activity in the brine shrimp lethality test (LC_{50} = 41µg/mL) and a high nematicidal activity against *Caenorhabditi selegans* (LC_{50} = 37µg/mL) (Dosoky *et al.*, 2016). Eugenol and β-caryophyllene dominated *Ocimum sanctum* Linn. In addition, the essential oil showed potent *in vitro* anthelmintic activity against the nematode *C. elegans* according to Mali and Mehta, 2008. Zingiberene is the dominant constituent of ginger. As a medicine, ginger essential oil and its oleoresin are used to aid digestion, as an expectorant and as a cure for stomach ache, toothache, diarrhea and asthmatic respiratory disorders (Kamaliroosta *et al.*, 2013). Zingiberene has also been suggested as an important ingredient in stomachic medications (Malhotra and Singh 2003).

The result in table 2 shows the anthelmintic activity of *A. adiantifolia* essential oils. It was observed that the time of paralysis and death of worms decreases as concentration increases. The leaf essential oil shows activity higher than both the stem bark and root bark oils with time of paralysis and death at 12.60 and 60.20 minutes, respectively for the highest concentration. At all concentrations, the essential oils were more active than the standard drug (Albendazole) used. The effect of varying the concentrations of *A. adiantifolia* essential oils and albendazole were significantly different from one another. All values were also significantly different from the reference standard ($p<0.001$).

Many of the *in vitro* investigations on anthelmintic efficacy of plant extracts and their essential oils have been based on the effects they pose on organisms such as earthworms (Sutar *et al.*, 2010; Akhtar *et al.*, 2000; Bairagi *et al.*, 2011; Mali and Mehta, 2008) as well as various gastro-intestinal nematodes (roundworms), cestodes (tapeworms) and trematodes (flukes) of human and livestock (Akhtar *et al.*, 2000; Mali and Mehta, 2008; Ferreira *et al.*, 2013). Due to the availability and easy access to earthworms, suitable models for anthelmintic drug screening have been established (Subash *et al.*, 2012; Mali and Mehta, 2008). Many substances toxic to earthworms tend to cause irritation which eventually often lead to withdrawal of the worm from the environment of such substance, or cause flaccid paralysis. However, by virtue of these effects, very probably, anthelmintics would act by a way of expelling parasitic worms from their host's gastrointestinal tract through peristalsis (Subash *et al.*, 2012; Akhtar *et al.*, 2000).

4. Conclusions

The compositional patterns and components of *A. adiantifolia* essential oils of Nigeria origin are reported for the first time. Sesquiterpene hydrocarbons dominated the leaves, stem bark and root bark essential oils. Result from the anthelmintic assay displayed the activity of *A. adiantifolia* oils against the worm used and their potency were inversely proportional to the time taken for paralysis and death of the worms to occur. The three essential oils show a relatively higher activity compared to Albendazole in concentration dependent manner which could be attributed to one or more components of the oil. This result could be a preliminary inference to the traditional medicinal usage of the plant in treating tapeworm infection, abdominal pains and as purgative. Also, owing to known activities demonstrated by some of the compounds identified in the oils, further suggests the potential pharmacological activities of the plant. Since the discovery and treatment of diseases with herbs has been on the increase, and the attention which their active principles have attracted as sources for new drugs, *A. adiantifolia* essential oils could be a good natural product for many pharmacological activities.

Table 1. Essential oil constituents of *A. adiantifolia* from GC/GC-MS analysis

Constituents	L.R.I	L.R.I*	AAL	AASB	AARB
2-heptanone [f]	891	889	0.8	-	-
Benzaldehyde [f]	962	952	-	-	1.4
1-octen-3-one [f]	980	972	-	-	0.9
6-methyl-5-hepten-2-one [f]	987	981	1.0	-	-
2-octanone [f]	993	988	1.0	-	-
Mesitylene [f]	996	994	-	-	1.4
1,8-cineole [f]	1034	1026	2.2	1.5	1.9
Seudenone [f]	1063	-	0.4	-	-
Linalool [b]	1101	1095	0.8	-	2.1
Nonanal [f]	1104	1100	1.6	-	-
2,4-dimethylbenzaldehyde [f]	1179	-	0.4	-	-
Naphthalene [f]	1181	1178	0.6	-	1.1
(*Z,E*)-undeca-1,3,5-triene [f]	1182	-	0.5	-	-
α-terpineol [b]	1191	1186	0.4	-	-
Safranal [e]	1197	1196	0.7	-	-
Decanal [f]	1206	1201	0.4	-	-
β-cyclocitral [e]	1222	1217	1.4	-	-
p-menth-4-en-3-one [b]	1251	-	0.7	-	-
β-cyclohomocitral [e]	1256	-	0.7	-	-
5-methyltetralin [f]	1264	-	0.5	-	-
Thymol [b]	1292	1289	-	0.2	-
n-tridecane [f]	1300	1300	0.5	-	-
α-ionone [f]	1352	-	0.6	-	-
Dehydro-*ar*-ionene [f]	1353	-	0.5	-	-
(*E*)-β-damascenone [e]	1382	1383	0.7	-	-
1,4-dimethyltetralin [f]	1391	-	0.5	-	-
β-elemene [c]	1392	1389	-	0.5	-
Cyperene [c]	1398	1398	-	-	2.3
n-tetradecane [f]	1400	1400	1.3	-	-
Isocaryophyllene [c]	1405	1408	-	0.4	-
Italicene [c]	1405	1405	0.7	-	-
β-caryophyllene [c]	1419	1417	23.0	39.3	32.1
(*E*)-α-ionone [e]	1428	1428	0.5	0.2	-
cis-α-ambrinol [e]	1437	1439	0.4	-	-
2-phenylethyl butanoate [f]	1440	1439	-	-	0.9
α-humulene [c]	1455	1452	2.4	5.6	4.4
(*E*)-geranyl acetone [e]	1457	1453	7.4	-	-
Sesquisabinene [c]	1460	1457	-	0.8	-
γ-muurolene [c]	1478	1478	-	0.3	-
ar-curcumene [c]	1483	1479	4.6	7.2	-
(*E*)-β-ionone [e]	1487	1487	4.7	-	-
α-zingiberene [c]	1496	1493	6.3	9.6	-
α-bulnesene [c]	1507	1509	-	0.2	-
β-bisabolene [c]	1508	1505	-	0.3	-
trans-γ-cadinene [c]	1514	1513	-	0.3	-
δ-cadinene [c]	1524	1522	-	-	3.8
β-sesquiphellandrene [c]	1525	1521	2.5	2.8	-
Occidentalol [d]	1548	1550	-	-	1.7
(*E*)-nerolidol [d]	1564	1561	0.7	0.5	-
Caryophyllene oxide [d]	1582	1582	3.6	6.4	8.4
Viridiflorol [d]	1591	1592	6.4	2.7	3.0
trans-β-elemenone [d]	1601	1601	-	0.3	-
Humulene epoxide II [d]	1607	1608	-	0.5	1.0
Tetradecanal [f]	1614	1611	-	0.8	1.0
Caryophylla-4(14),8(15)-dien-5-ol [d]	1636	1639	0.8	0.8	-
T-cadinol [d]	1641	1638	0.8	0.5	-
Selin-11-en-4-α-ol [d]	1655	1658	-	10.4	13.1
Valerianol [d]	1656	1656	-	-	2.9
Acorenone [d]	1688	1692	6.4	0.2	-
2-pentadecanone [f]	1699	1697	-	-	1.4
Pentadecanal [f]	1716	-	1.5	1.8	6.1
[a]Monoterpene hydrocarbons			0.0	0.0	0.0
[b]Oxygenatedmonoterpenes			4.1	1.7	4.0
[c]Sesquiterpene hydrocarbons			39.5	67.3	42.6
[d]Oxygenatedsesquiterpenes			18.7	22.3	30.1
[e]Apocarotenoids			16.5	0.2	0.0
[f]Non-terpene derivatives			12.1	2.6	14.2
Total identified			**90.9**	**94.1**	**90.9**

Major constituents are represented in bold

LRI = Linear retention index; LRI* = Linear retention index values from Adams, 2007; AAL = *Albizia adiantifolia* leaves; AASB = *Albizia adiantifolia* stem bark; AARB = *Albizia adiantifolia* root bark.

Table 2. Anthelmintic activity of *Albizia adiantifolia* essential oils

	Time of Paralysis (Mins) expressed as Mean±SEM (N=5)			
EO Conc. (% v/v)	AAL	AASB	AARB	ALBZ
1.00	30.40±1.36	32.80±2.01	34.00±1.64	97.20±1.39
2.00	25.80±1.71	27.20±1.43	28.60±1.60	94.20±1.77
3.00	20.20±1.28	24.40±1.50	24.80±1.71	89.60±1.29
4.00	17.00±1.73	18.20±1.28	20.00±1.22	87.40±1.08
5.00	12.60±1.21	15.60±0.93	16.60±1.08	82.80±1.28
	Time of Death (Mins) expressed as Mean±SEM (N=5)			
EO Conc. (% v/v)	AAL	AASB	AARB	ALBZ
1.00	96.20±3.51	98.20±3.22	101.40±3.97	154.60±1.86
2.00	85.60±2.66	89.80±2.87	98.00±3.18	149.20±2.35
3.00	78.60±2.54	80.80±2.60	87.40±3.36	140.60±1.72
4.00	70.40±3.43	72.80±2.96	76.80±2.65	135.00±1.92
5.00	60.20±3.09	61.20±2.73	69.60±2.94	130.20±1.77

AAL = *Albizia adiantifolia* leaves; AASB = *Albizia adiantifolia* stem bark; AARB = *Albizia adiantifolia* root bark; ALBZ = Albendazole (Standard); SEM = Standard error of mean; N = number of worms in each petri-dish.

The time taken for paralysis and death of worms to occur in distilled water (negative control) was observed to be >> 200 minutes. This is due to the body cells eventually absorbing water by osmosis.

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