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The Essential Oil Constituents of the Fresh and Air-dried *Phragmanthera incana* (Schum.) Balle and Its Bioactivities

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Abstract

Phragmanthera incana Schum (Loranthaceae), used in ethnomedicine as a worm expeller and for treating diseases such as cancer and inflammation, had its essential oil constituents characterized and assessed for certain pharmacological activities. Gas Chromatography-Mass Spectrometry was used to analyze the essential oils extracted from fresh and dried *P. incana* leaves, flowers, and stems via hydrodistillation. Standard bioassays were employed to determine the antibacterial activity, cytotoxicity, anthelmintic effects on *Pheritima posthuma*, and antioxidant activity of the essential oils. Thirty-six and forty-five essential oil constituents were detected in fresh and dried *P. incana*, respectively. The percentage constituent in fresh *P. incana* follows the trend; flower > stem > leaf, a trend of stem > leaf > flower was observed in dried *P. incana*. The major volatile oils constituents of leaf, stems and flowers were fluoranthene (37.30%), hentriacontane (76.11%), and 3-methyl phenol (24.20%) respectively. All essential oils showed cytotoxicity, with fresh leaf oil having the lowest LC_{50} of 0.49 µg/mL. The essential oils of air-dried and fresh stem antibacterial activities were 20 mm for *Bacillus aureus* and 16 mm for *Klebsiella pneumoniae* compared to 10 mm by Gentamycin for both organisms. Compared to Menbendazole (63.55±1.12 to 90.55±0.97) at 100 mg/mL, all essential oils caused considerable worm paralysis and death (12.58±2.62 to 24.19±7.84 min). They also demonstrated moderate antioxidant potency, ranging from 41.67 to 85.98%. The essential oil components and biological activities are described for the first time. Bioassays' results and presence of hentriacontane, phytol, γ-sitosterol, stigmasterol, and tocopherol in the oils, support its traditional applications as anti-cancer, anti-inflammatory, anti-oxidant, and anthelmintic.

Keywords: hydro distillation; Loranthaceae; *Phragmanthera incana*; volatile oils, ethnomedicinal, African mistletoe.

INTRODUCTION

Essential oils also regarded as volatile compounds or liquid aroma compounds are obtained from natural sources, most especially from plants, and can be found in different parts such as leaves, stems, flowers, roots, seeds, berries, bark, petals, and resin (Hyldgaard *et al.,* 2012; Sánchez-González *et al.,* 2011). The quantity of essential oil obtained from a plant varies from 0.01% to 10% of the weight of the plant, depending largely on many factors, such as climate, geographical location, season, harvest time, and extraction methods (Pannizi *et al.,* 1993, Hamid *et al.,* 2011a). While essential oils are mostly used as a food flavour, fragrance, and for aromatherapy (Finar 2003, Burt, 2004), their healing potency varies from antimicrobial, and antioxidant to insecticidal, as has been reported (Federspil and Zimmermann 1997, Rajesh and Howard 2003).

Phragmanthera incana is a woody parasitic shrub (with a stem 2 cm long) commonly found in secondary jungles and bush savannahs such as in Sierra Leone, West Cameroon and extending to the Congo basin, Zaire, and Angola. The plant is found to appear in different forms and is widely distributed as a common mistletoe (Dutta 2005, Ogunmefun *et al.,* 2013). *Phragmanthera incana* is found on such plant trees as *Anacardium occidentale, Bauhinia monandra, Aleurites molluccana, Bombax sessile, Cola acuminata, Cola nitida,* and *Alchornea castaneifolia* (F.W.T.A 1956; Ogunmefun *et al.,* 2013). Ethnomedicinally, *Phragmanthera incana* is useful for treating various ailments, including diabetes, high blood pressure, yellow fever, nervous system disorder, insomnia, cancer, and convulsions (Wade, 1999, Kafaru, 2000; Adodo, 2002; Fasanu and Oyedapo, 2008). *Phragmanthera incana* leaf harvested from kolanut (*Cola spp*) display better antioxidant properties than other host plants. Oxidative stress connected with diabetes and other complications could be managed or prevented by using *Phragmanthera incana* leaf as cheap nutraceuticals (Ogunmefun *et al.,* 2015).

Meanwhile, it is important to know that the pharmacological activities of most medicinal plants have been attributed, in part, to nature and quantity of their essential oils' constituents (De Sousa *et al.,* 2024). For instance, studies have attributed some essential oils such as carvacrol, cinnamaldehyde, limonene, caryophyllene, thymol, phytol, linalool and humulene to the antibacterial, anti-inflammatory, and antioxidant activity of the plants (Atewolara-Odule and Oladosu, 2016; Atewolara-Odule *et al.,* 2018; Atewolara-Odule *et al.,* 2020; De Sousa *et al.,* 2024). When considering the ethnomedicinal properties possessed by *Phragmanthera incana*, the need to characterise its essential oil constituents, for the first time, becomes imperative. It is also important to understand the likely essential oils responsible for some reported pharmacological activities of the plant. We thus set out to extract and characterise the constituents of the essential oils from fresh and air-dried flower, leaf and stem of *P. incana* and attribute them to the antibacterial, anthelmintic, cytotoxicity, and antioxidant properties of the plant.

MATERIALS AND METHODS

Plant materials

Phragmanthera incana on *Cola acumulata* was collected from the main campus of Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. Identification and authentication were done at the Forest Herbarium of the Forestry Research Institute of Nigeria (FRIN) where a voucher specimen was deposited with Herbarium number FHI 108423.

Extraction of Essential Oils

The essential oils from the fresh and air-dried samples (leaf, flowers, and stem) of the *Phragmanthera incana* were obtained by hydrodistillation using a Clevenger-type apparatus (Clevenger 1928), for 3 hours (Gavahian et al., 2012). The oils were dried over anhydrous sodium sulphate (Na_2SO_4), kept inside vials, and refrigerated until analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The gas chromatography-mass spectrometry (GC–MS) analysis was performed on an Agilent technology 7890 gas chromatograph with a splitless injector interfaced to a 5973-mass selective detector operated at 70 eV with a mass range of m/z 40–420 (Atewolara-Odule *et al.,* 2018; Essein *et al.,* 2018). The GC column was HP-5MS fused silica capillary with a 30 m x 0.32 mm x 0.25 µm. Helium was used as the carrier gas with a flow rate of 3 mL/min the column temperature was kept initially at 80 ^oC and then to 280 ^oC at 10^o C min-1, held for 6 min, 1.0 µL was injected in the splitless mode. The essential oil components were identified based on the retention indices and mass spectra, by comparison with published data using the NIST11 library database/Chemstation data system and Adam, (2001).

Cytotoxicity test

This was carried out using the Brine shrimp lethality test method**.** The method used was according to (Meyer et al., 1982; Aboaba et al., 2010) with slight modifications. The brine shrimp eggs (Hobby Artemix) were hatched for about 48 h. The essential oil samples were prepared in concentrations of 1000 µg/mL, 100 µg/mL, and 10 µg/mL. 2 mL and 0.5 mL of the samples at each concentration of the essential oils were put into test tubes and 5 mL of distilled water was added. To these solutions, 10 shrimp nauplii were added and made up to 10 mL with distilled water. 24 h later, the test tubes were observed and the number of shrimps nauplii survivors (lethality estimate) was counted and the number of dead shrimps nauplii was subtracted.

Antibacterial Assay of *P. incana* Essential Oils

An overnight culture of each organism was prepared and then inoculated individually into 5 mL sterile nutrient broth, which was incubated for bacteria growth at 37 ^oC for 24 h. From each cultured organism, 0.1 mL was taken and put into 9.9 mL of distilled water to obtain (1:100) of the diluted organism. From the dilution, 0.2 mL was taken into the prepared Nutrient Agar (Oxoid). These were poured on the plate and allowed to solidify for almost 1 h and 8 mm cork borer was employed to make wells on the plates. Essential oils of different concentrations were prepared (between 6.25 mg/mL and 100 mg/mL) and put into the well. Standard antibiotic disc – Gentamycin (10 µg) (Oxoid) was placed on each agar plate with sterile forceps and gently pressed onto the agar surface. The plates stayed on the bench for about two hours before they were incubated at 37 ^oC for 24 h. The assays were carried out in duplicates (Hamid *et al.,* 2011b) and the results were recorded as the average of the duplicate test. The diameter of zones of inhibition around each well was measured and used to evaluate the antibacterial activity of the essential oils. The microorganisms used are *Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus,* and *Klebsiella pneumoniae.*

Anthelmintic Assay

The anthelmintic activity assay was conducted following the methods of (Ajaiyeoba et al., 2001); and (Deore *et al.,* 2009) with slight amendments. *Pheretima posthuma* worm was used for this assay due to its anatomical and physiological resemblance to the human intestinal roundworm parasite. Mebendazole (25 mg/mL) was used as a standard reference drug. 10 mL of each extract with a specific concentration was taken into petri dishes where three worms were placed. Times of paralysis and death of the worms were taken and recorded using a stopwatch. The time of paralysis is when there is no movement, that is, the worms are very weak and can only be noticed to

be alive by the way they shake. At the time of death, there is no movement at all, which means the worms are dead. The death of the worms was confirmed by dipping them in hot water at 50 °C, which caused movements if the worm was alive. In the control group, 10 mL distilled water was used. Concentrations of 100-10 mg/mL were employed. All solutions were prepared in distilled water. The experiments were in duplicate.

Antioxidant Assay

The antioxidant assay of the essential oils was determined in terms of free radicals scavenging abilities, using the methods of (Saleh et al., 2010) and (Khelifa et al., 2012) with some modifications. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.1 Mm) was prepared in methanol and four concentrations of 12.5, 25, 50 and 100 µg/mL of the essential oils were also prepared in methanol. To each concentration, 1 mL of freshly prepared DPPH was added, thoroughly mixed, and incubated for half an hour inside a dark cupboard at room temperature. After incubation, the absorbance of each oil was measured at 517 nm and recorded as A_{sample} against $A_{control}$ using the spectrophotometer. Each test was carried out in triplicate. The DPPH free scavenging ability of each sample was determined using the equation below:

$$\%\ inhibition = 100 \times \frac{A_{control} - A_{sample}}{A_{blank}}$$

Ascorbic acid was used as a standard and its antioxidant activity was measured using the same method for comparison.

Data Analysis

Values were expressed as means and standard errors of means. The significant difference between the means of the control and the treated groups were determined by the standard error of the mean (SEM). The significance levels at $P< 0.05$ were measured using a student t-test.

RESULTS

The percentage yield of each essential oil obtained was found to be 0.25, 0.22, 0.27, 0.23, 0.88, and 0.76 v/w for fresh leaf, air-dried leaf, fresh stem, air-dried stem, fresh flower, and air-dried flower respectively. The essential oils obtained from all the samples were colourless, with an herbal smell. The GC-MS analysis of the essential oils revealed a total of forty-eight, fifteen, and thirty constituents from the flower, leaf, and stem respectively. Thirty-six (36) constituents were identified in the three parts of fresh *Phragmanthera incana* accounting for 25, 9, and 7 representing 99.98%, 96.97%, and 98.91% of essential oils for the fresh flower, leaf, and stem respectively as shown in Table 1. The main constituents of the fresh flower essential oil were Phytol (17.48%), α -Selinene (11.28%), while other constituents were Caryophyllene (7.40%), Hexahydrofarnesyl acetone (6.52%), Thymol (5.55%), β-Elemene (5.03%), Nonacosane (4.74%), Tetracosane (4.12%), Humulene (4.02%), β-sesquihellandrene (3.81%), Nerolidol (3.47%) and Myristaldehyde (3.72%). The fresh leaf of *P. incana* consisted mainly of Phytol (26.65%), Fumaric acid, 2-decyl tridecyl ester, (17.54%), and 2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-acetate (E, E) (15.50%). The main constituents of the fresh stem oil was Hentriacontane (76.11%); while the other constituents were Eicosane (8.86%), Octasiloxane (4.93%), Tetracosane (3.91%) and Estra-1,3,5(10)-trien-17beta-ol (2.09%). Phytol which is the major constituent of the fresh flower and fresh leaf essential oils is conspicuously missing in the fresh stem oil. Caryophyllene is found in all the plant parts while α-Cubebene and Phytol are present in the flower and leaf oils. Also, Tetracosane was present in the flower and stem oils.

A total number of forty-five constituents were found in the air-dried flower, leaf, and stem essential oils accounting for 22, 6, and 24 constituents of flower, leaf, and stem representing 91.80%, 93.67% 94.40% of essential oils respectively as presented in Table 2. The main constituents of the air-dried-flower essential oil were 3-Methyl phenol (24.20%), Squalene (12.34%), 3-Pentadecyl-phenol (11.51%), and δ-Tocopherol (11.33%). The major constituents of the air-dried leaf were Fluoranthene (37.30%), Pyrene (23.78%), Bis (2-ethyl hexyl) phthalate (16.50%), and fluorene (13.04%), while the major constituents of the air-dried stem oil were γ-Sitosterol (26.33%), stigmasterol (12.59%), 3-β-Ergost-5-en-3-ol (13.21%), and Ledol (10.02%).

Four constituents were found to be common in both the fresh and air-dried flower oil: Caryophyllene, Humulene, Phytol, and Nonacosane with varying percentages. Caryophyllene and Eicosane were found in both the fresh and dried stem oils. The classes of compounds found in fresh samples were hydrocarbons (12.77%), esters (2.75%), and sesquiterpenes (1.35%); while those of air-dried-sample oils were triterpenes (52.13), oxygenated sesquiterpenes (15.33), sesquiterpenes (11.84%), esters (6.23%), ketones (2.11%), and diterpenes (1.06%).

Caryophyllene, Hexahydrofarnesyl acetone, and Phytol were the major constituents found in all the parts (leaf, stem, and flower) of *P. incana* essential oils in varying percentages. The constituents found in the stem and flower oils were Humulene, Nerolidol, Trans-β-ionone, Caryophyllene Oxide, Tetracosane, Eicosane, and Hexahydrofarnesyl acetone in various compositions. Bis (2-ethyl hexyl) phthalate was found in both the leaf and stem oil while α-Farnesene was common to the leaf and flowers.

Table 1. Constituents of Essential Oils of Fresh Samples of *P. incana.*

S/NO	Compound Name	Retention Index	Chemical Formula	% Composition Flower	% Composition Leaf	% Composition Stem
1	1,4-dimethyladamantane	1183	$C_{12}H_{20}$		4.86	
2	Thymol	1294	$C_{10}H_{14}O$	5.55	-	
3	α-Cubebene	1342	$C_{15}H_{24}$	1.00	5.14	-
4	Cis-Pinane	1382	$C_{10}H_{18}$	1.94		
5	β-Bourbonene	1384	$C_{15}H_{24}$	1.25		
6	β-Elemene	1390	$C_{15}H_{24}$	5.03		
7	2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-acetate (E,E)	1398	$C_{17}H_{28}O_2$		15.50	-
8	Caryophyllene	1418	$C_{15}H_{24}$	7.40	6.44	1.35
9	Humulene	1451	$C_{15}H_{24}$	4.02		
10	α-Curcumene	1482	$C_{15}H_{24}$	1.19		
11	α-Selinene	1488	$C_{15}H_{24}$	11.28		
12	β-sesquihellandrene	1507	$C_{15}H_{24}$	3.81		
13	α-Farnesene	1511	$C_{15}H_{24}$		8.50	-
14	Nerolidol	1557	$C_{15}H_{26}O$	3.47		
15	Caryophyllene Oxide	1579	$C_{15}H_{26}O$	2.64		
16	Myristaldehyde	1614	$C_{14}H_{28}O$	3.72		
17	Tetradecanal	1621	$C_{14}H_{28}O$	3.76		
16	Hentriacontane	1714*	$C_{31}H_{64}$			76.11
19	Phytane	1810	$C_{20}H_{42}$	1.40		
20	Hexahydrofarnesyl acetone	1846	$C_{18}H_{36}O$	6.52	-	
21	Tricosane	1894*	$C_{23}H_{48}$	3.07		
22	Farnesyl acetone	1915	$C_{18}H_{36}O$	1.23		
23	Heneicosane	1972*	$C_{21}H_{44}$	1.02		
24	(z)-7-Tetradecenoic acid	1989	$C_{14}H_{26}O_2$		3.86	-
25	Eicosane	2050	$C_{20}H_{42}$			8.86
26	Phytol	2119	$C_{20}H_{40}O$	17.48	26.65	-
27	Linolenic acid	2143	$C_{18}H_{30}O_2$	1.01		
28	Pentacosane	2222*	$C_{25}H_{52}$	1.89		
29	(Z)-9- tricosene	2298	$C_{23}H_{46}$	1.44		
30	4-heptyltridecyl ester, fumaric acid	2330*	$C_{24}H_{44}O_4$			1.66
31	Tetracosane	2405	$C_{24}H_{50}$	4.12		3.91
32	Estra-1,3,5(10)-trien-17beta-ol	2622	$C_{18}H_{24}O$			2.09
33	Octasiloxane	2810	O_7Si_8			4.93
34	4-phenyl-pyrido-[2,3-d] pyrimidine	2820*	$C_{13}H_9N_3$		8.48	-
35	Nonacosane	2964	$C_{29}H_{60}$	4.74		
36	Fumaric acid, 2-decyl tridecyl ester	2990	$C_{27}H_{50}O_4$		17.54	-
	TOTAL			99.98	96.97	98.91

* = Calculated Retention Index

Table 2. Constituents of Essential Oils of Air-dried Samples of *P. incana.*

S/NO	Compound Name	Retention Index	Chemical Formula	% Composition Flower	% Composition Leaf	% Composition Stem
1	Supraene	892	$C_{30}H_{50}$	0.37		
2	2-Thiophene acetic acid, 2-isopropoxyphenyl ester	1037*	$C_{15}H_{18}SO_3$			0.94
3	3-methyl phenol	1105	C_7H_8O	24.20		
4	Alloaromadendrene	1342	$C_{15}H_{24}$			3.15
5	Caryophyllene	1418	$C_{15}H_{24}$	1.50		1.46
6	Trans-α-Bergamotene	1438	$C_{15}H_{24}$	0.45		
7	1-methylene-2-vinylcyclopentane	1442	C_8H_{12}			1.00
8	Humulene	1451	$C_{15}H_{24}$	0.45		1.48
9	Germacrene D	1487	$C_{15}H_{24}$			4.37
10	Trans-β-ionone	1489	$C_{13}H_{24}$	0.60		0.74
11	α-Farnesene	1511	$C_{15}H_{24}$	1.13		
12	β-Sesquiphellandrene	1525	$C_{15}H_{24}$			1.38
13	Nerolidol	1557	$C_{15}H_{26}O$	-		0.74

S/NO	Compound Name	Retention Index	Chemical Formula	% Composition Flower	% Composition Leaf	% Composition Stem
14	Ledol	1563	$C_{15}H_{26}O$			10.02
15	Caryophyllene Oxide	1579	$C_{15}H_{26}O$	-		2.05
16	Fluorene	1583	$C_{13}H_{10}$		13.04	
17	2-methyl-3-Decen-5-one	1618	$C_{11}H_{20}O$			0.98
18	α-Cadinol	1651	$C_{15}H_{26}O$			2.52
19	Isopropyl myristate	1835	$C_{17}H_{34}O_2$			0.53
20	Linoleic acid	2134	$C_{18}H_{32}O_2$	0.92		
21	2-(3-methyl phenoxy)- ethanol	1800*	$C_{19}H_{12}O_2$	0.27		
22	Hexahydrofarnesyl acetone	1896	$C_{18}H_{36}O$	-	1.46	1.13
23	Squalene	1853	$C_{30}H_{50}$	12.34		
24	Octadecamethyl, cyclononasiloxane	1858	$C_{18}H_{54}O_9Si_9$	9.00		
25	2-methyl-7-nonadecene	2000*	$C_{20}H_{40}$	0.54		
26	(E,E,E)-3,7,11,15- tetramethyl hexadeca1,3,6,10-14-pentaene	2019	$C_{20}H_{32}$	1.23		
27	Eicosane	2050	$C_{20}H_{42}$	0.85		0.34
28	Fluoranthene	2061	$C_{16}H_{10}$		37.30	
29	Phytol	2119	$C_{20}H_{40}O$	4.08		1.06
30	Pyrene	2134	$C_{16}H_{20}$		23.78	
31	n-Nonadecanol-1	2181	$C_{19}H_{40}O$			1.09
32	2-(octadecyoxy)-ethanol	2361	$C_{20}H_{42}O_2$	1.17		
33	(Z)-9-Octadecanamide	2375	$C_{18}H_{35}NO$		1.59	
34	3-pentadecyl-phenol	2499	$C_{21}H_{36}O$	11.51		
35	Bis (2-ethyl hexyl) phthalate	2550	$C_{24}H_{38}$		16.50	4.54
36	1-mono linoleoylglyceroltrimethylsilyl ether	2742	$C_{27}H_{54}O_4Si_2$			0.87
37	13-undecyl-pentacosane	2852	$C_{36}H_{74}$	2.17		
38	2,6,10,15,19,23-hexamethyl-tetra cosapentaene	2900				1.88
39	hexahydro-5-methyl-4-methylene 1(2H)-pentalenone	2960*	$C_{10}H_{14}O$	0.48		
40	Nonacosane	2964	$C_{29}H_{60}$	4.13		
41	δ-Tocopherol	2968	$C_{27}H_{46}O_2$	11.33		
42	γ-Tocopherol	3074	$C_{28}H_{48}O_2$	3.08		
43	3-β-Ergost-5-en-3-ol	3131	$C_{28}H_{48}O$			13.21
44	Stigmasterol	3170	$C_{29}H_{48}O$			12.59
45	γ-Sitosterol	3351	$C_{29}H_{50}O$			26.33
	TOTAL			91.80	93.67	94.40

Note: - = Not detected; * = Calculated Retention Index.

Brine shrimp lethality test of essential oils of *Phragmanthera incana*

The cytotoxicity of the essential oils of *Phragmanthera incana* was evaluated by brine shrimp lethality assay with the results presented in Table 3. The brine shrimp assay revealed that the essential oils of *P. incana* were cytotoxic at LC_{50} ranging from 0.49 to 2.77 µg/mL.

Table 3. Cytotoxic activity of essential oils from *P. incana.*

Essential Oils	% Mortality at Different Concentrations*			LC_{50} µg/mL
	10 µg/mL	100 µg/mL	1,000 µg/mL	
PIALEO	70.0	73.3	96.7	1.84
PIFLEO	70.0	76.7	90.0	0.49
PIASEO	66.7	76.7	96.7	2.77
PIFSEO	76.7	83.3	100	0.88

* Mean of three determinations.

Key: PIALEO- *P. incana* air-dried leaf Essential oil
PIFLEO- *P. incana* fresh leaf Essential oil
PIASEO- *P. incana* air-dried stem Essential oil
PIFSEO- *P. incana* fresh stem Essential oil

Antibacterial result of essential oils of *Phragmanthera incana*

The essential oils of *Phragmanthera incana* leaf and stem were tested against *Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae*. The results of the antibacterial properties are shown in Table 4. All the essential oils displayed concentration-dependent antibacterial activities at 100 µg/mL against three of the microorganisms namely *Bacillus cereus, Escherichia coli,* and *Klebsiella pneumoniae*.

Table 4. Antibacterial activities of essential oil of *Phragmanthera incana.*

Essential oil	Concentration (µg/mL)	Organisms/Diameter of zone of inhibition (mm)			
		Bacillus cereus	*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebsiella pneumoniae*
PIALEO	100	13	12	8	13
	50	11	-	8	11
	25	-	-	-	11
	12.5	-	-	-	-
	6.25	-	-	-	-
PIFLEO	100	13	-	12	12
	50	-	-	11	12
	25	-	-	10	10
	12.5	-	-	8	8
	6.25	-	-	-	-
PIASEO	100	20	-	8	-
	50	-	-	-	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
PIFSEO	100	16	-	15	16
	50	13	-	8	13
	25	10	-	-	10
	12.5	-	-	-	12
	6.25	-	-	-	9
Gentamycin (10 µg)		10	-	8	10
Negative control		-	-	-	-

Key: PIALEO- *P. incana* air-dried leaf Essential oil
PIFLEO- *P. incana* fresh leaf Essential oil
PIASEO- *P. incana* air-dried stem Essential oil
PIFSEO- *P. incana* fresh stem Essential oil

Anthelmintic activities of essential oils from *P. incana*

Helminths affect both animals and human beings causing stunted growth and may lead to death because most helminths are devastating (Dewanjee et al., 2007). The anthelmintic assay was carried out using *Pheretima posthuma*. The time taken for the essential oils to cause paralysis and death of *Pheretima posthuma* ranged from 12.58±2.62 to 26.65±4.27 and 24.19±7.84 to 39.40±3.63 min respectively while the time taken for the Mebendazole to cause paralysis and death of *Pheretima posthuma* was 63.55±1.12 to 88.26±1.21 and 90.59±0.97 to 113.79±1.47 min at a concentration of 100 mg/mL, hence the essential oils are potent anthelmintic agents. All the essential oils demonstrated dose-dependent activities as presented in Table 5.

Table 5. Anthelmintic activities of essential oils from *P. incana.*

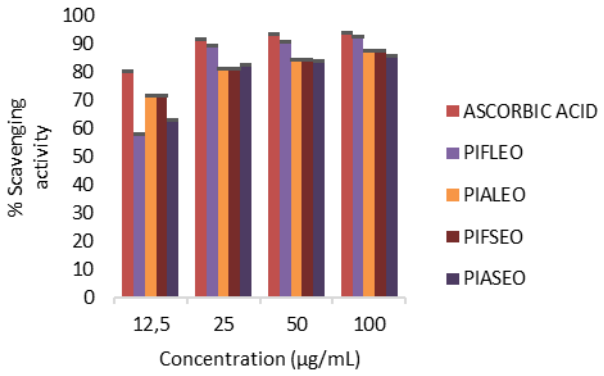
Conc. (mg/mL)	Essential Oils								Control	
	PIALEO		PIFLEO		PIASEO		PIFSEO		MEBENDAZOLE	
	Time of paralysis (P) and Death (D) of worms in minutes (±SEM) *									
	P	D	P	D	P	D	P	D	P	D
10	32.45±1.02	47.12±4.75	50.55±5.00	68.07±8.03	25.64±5.78	56.61±3.01	59.73±7.59	69.23±9.17	88.26±1.21	113.79±1.41
20	31.89±7.40	46.45±9.67	45.10±6.83	59.88±7.81	19.35±2.76	47.04±7.18	52.87±3.13	58.65±7.40	86.36±1.34	111.26±1.20
50	24.10±5.68	40.80±5.47	32.51±2.58	48.36±6.52	17.59±2.56	45.11±3.84	46.45±5.98	53.17±6.24	80.54±1.13	105.81±1.32
80	20.15±4.11	32.85±7.72	21.46±2.71	37.80±7.00	13.65±1.45	37.49±5.09	33.51±4.67	45.85±4.67	67.38±1.30	95.21±1.23
100	13.49±2.85	24.19±7.84	13.89±2.64	26.56±6.80	12.58±2.62	33.48±6.01	26.65±4.27	39.40±3.63	63.55±1.12	90.59±0.97

*SEM is the standard error of the mean

Key**:** PIALEO- *P. incana* air-dried leaf Essential oil
PIFLEO- *P. incana* fresh leaf Essential oil
PIASEO- *P. incana* air-dried stem Essential oil
PIFSEO- *P. incana* fresh stem Essential oil

Antioxidant activities of essential oils of Phragmanthera incana

The antioxidant activity of the essential oils was determined using DPPH and the results were presented in Figure 1. The DPPH scavenges free radicals which have been found to contribute majorly to different diseases such as hypertension (Cook and Samman 1996). The result revealed that the oils are potent antioxidants. The radical scavenging properties of the essential oils were comparable to ascorbic acid at all concentrations.



Key: PIALEO- *P. incana* air-dried leaf Essential oil
PIFLEO- *P. incana* fresh leaf Essential oil
PIASEO- *P. incana* air-dried stem Essential oil
PIFSEO- *P. incana* fresh stem Essential oil

Figure 1: Percentage scavenging activity of Essential oil *P. incana.*

DISCUSSION

The other constituents present in fresh leaf were α-Farnesene (8.50%), 4-phenyl-[2,3-d] pyrimidine, pyrido (8.48%), Caryophyllene (6.44%), α-Cubebene (5.14%), 1,4-dimethyl adamantane (4.86%) and (Z)-7-Tetradecenoic acid (3.86%). The constituents of the fresh leaf were classified as diterpenes (26.65%), sesquiterpenes (20.08%), esters (17.54%), and fatty acids (3.86%). Oher constituents of the fresh stem oil were eicosane (8.86%), Octasiloxane (4.93%), Tetracosane (3.91%) and Estra-1,3,5(10)-trien-17-beta-ol (2.09%).

Other constituents present in air-dried flower essential oil are Octadecamethyl, cyclononasiloxane (9.00%), Nonacosane (4.13%), Phytol (4.08%) and γ-Tocopherol (3.08%). Minor constituents of air-dried leaf were Hexahydrofarnesyl acetone (1.46%) and (Z)-9-Octadecanamide (1.59%). The constituents of the air-dried leaf oils were found to be mainly polyaromatic hydrocarbons, hydrocarbons and esters. The other constituents of the air-dried stem were Alloaromadendrene (3.15%), Caryophyllene (1.46%), Humulene (1.48%), Germacrene D (4.37%), phytol (1.06%), Caryophyllene oxide (2.05%), Eicosane (0.34%) and n-Nonadecanol-1(1.09%).

The essential oils from the fresh samples of the leaf and stem exhibited higher cytotoxicity than their corresponding air-dried samples. The result obtained could be because mistletoes contain lectins that are cytotoxic to living organisms (Adodo, 2004). Also, the cytotoxicity potency of the essential oils may be due to constituents such as caryophyllene, germacrene, and sesquiterpenes which have been reported to be potent cytotoxic agents (Turkez *et al*., 2014). This result also supports the findings that African mistletoes are cytotoxic (Adodo, 2002; Ihegboro and Owolarafe, 2014). This implies that the essential oils from the fresh samples could be promising cytotoxic agents which means *Phragmanthera incana* essential oils could be promising antitumor agents.

All the essential oils did not display any antibacterial activity against *Pseudomonas aeruginosa,* except that of the air-dried leaf (PIALEO), which only displayed activity at 100 µg/mL. Essential oil of fresh stem of *P. incana* (PIFSEO) showed the best activity against *Klebsiella pneumoniae* and *Bacillus cereus*. Essential oil of air-dried stem of *P. incana* (PIASEO) was found to be resistant to *Klebsiella pneumoniae.* PIFSEO was found to be the most susceptible to *Bacillus cereus*, while PIFLEO was most susceptible to *Escherichia coli*. The antibacterial potency of PIALEO and PIFLEO were similar at 100 µg/mL whereas PIFSEO showed higher

activity than PIASEO. The antibacterial activities of the oils compared favourably with the standard drug–gentamycin. The presence of oxygenated terpenes in the essential oils of this plant could be responsible for the antibacterial activity because it has been reported that the oxygenated terpenes possessed antibacterial properties (Guleria *et al.,* 2012). The antimicrobial properties displayed by the essential oils of *P. incana* may also be due to the presence of tannins and saponins in the plant. These groups of compounds have been reported to be good antimicrobial agents (Abo *et al.,* 1999; Adeniyi *et al.,* 2013).

It was observed that all the essential oils demonstrated powerful anthelmintic properties better than mebendazole – the standard drug – at all concentrations. The essential oils from the leaf were found to be more effective than the ones from the stem, especially at 80 and 100 mg/mL. It was also observed from the results that the essential oils from the dried leaf (PIALEO) showed significant paralysis as well as death of the worms than the essential oil from the fresh leaf (PIFLEO) at all the concentrations. Also, essential oil from the air-dried stem (PIASEO) was more effective than the essential oils from the fresh stem (PIFSEO). PIALEO was equally found to be the most potent anthelmintic at 100 mg/mL of all the essential oils. Essential oils have been reported to contain compounds that have promising anthelmintic properties (Olounlade *et al.,* 2012). The anthelmintic properties displayed by the essential oils from *Phragmanthera incana* could have been a result of the chemical constituents of the oils. The class of compounds present in the essential oils of *Phragmanthera incana* was found to be mainly diterpenes, sesquiterpenes, and phenolic compounds. Terpenes such as caryophyllene, humulene, phytol, α-Farnesene, α-cubebene, germacrene, and thymol have been found to possess anthelmintic activities (Macedo *et al.,* 2010; Taur *et al.,* 2010; Katiki *et al.,* 2010; Turkez *et al.,* 2014).

The essential oil from the fresh leaf was found to be the most potent. The results were also found to be concentration-dependent. The antioxidant potency is attributed to the chemical constituents of the oils such as thymol, which has been reported to be a good antioxidant (Vazirian *et al.,* 2015). Thus, these essential oils can be used to treat ailments associated with oxidative stress such as diabetes, cardiovascular diseases, and cancer.

CONCLUSION

In conclusion, this study has provided information about the essential oil constituents of the different parts of *P. incana.* Also, the various bioassays they were subjected to have established the likely pharmacological activities of the oils and how they are related to distinct disease conditions. Thus, the traditional uses of the plant in managing disease conditions such as inflammation, worm and bacterial infestations, and cancer have been justified. Although the likely essential oil responsible for the antibacterial, anthelmintic, cytotoxicity, and antioxidant properties of *P. incana* has only been established in this study, addition study is required to isolate and determine the active oil constituent or ingredient of the plant against its specific medicinal property.

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Data Availability: All the data in this manuscript will be made available upon request.

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