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RESEARCH ARTICLE

Chemical Constituents and Biological Activities of *Phragmanthera incana* (Schum.) Balle Harvested from *Cola acuminata* Schott and Endl. Tree

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Abstract

Plants serve as the major source of medicine for many diseases in developing countries. *Phragmanthera incana* (Loranthaceae) is used in traditional medicine to treat diabetes, inflammation, and cancer. However, there is a dearth of information on its chemical composition. The study aims to isolate and characterise the chemical constituents of *Phragmanthera incana* and assess its pharmacological activities. The air-dried leaves and stem of the plant were pulverised and macerated with ethyl acetate and methanol, which yielded respective crude extracts. The extracts were tested for cytotoxicity and antioxidant activity using brine shrimp lethality test and 1,1-diphenyl-2-picrylhydrazyl, respectively. The anti-diabetic activity was assessed using alloxan-induced rats with glibenclamide as reference drug. Data were subjected to descriptive statistics. The ethyl acetate extract of the plant was subjected to chromatographic techniques. The isolated compounds' structures were determined using Mass Spectrometry, Fourier-transform Infrared Spectroscopy, and NMR Spectroscopy. The crude extracts' percentage yield ranged from 0.43 to 1.63 % while the crude extracts' weight ranged from 6.0 to 22.0 g. The extracts exhibited cytotoxicity against brine shrimps with LC_{50} 0.49-188.8 µg/mL except for the ethyl acetate extract of leaves and stem of *P. incana* that were non-toxic with LC_{50} > 1000 µg/mL. All extracts and isolated compounds exhibited antioxidant potency with radical scavenging activity ranging from 57.99-92.62%, and 73.15-87.68% respectively. There was reduction in the percentage of blood glucose (71.8%) of alloxan-induced diabetic rats treated by the extracts compared to that of glibenclamide's reduction (53.0%). Friedelin, canophyllol, and eudesmic acid were isolated from *P. incana* leaves. *Phragmanthera incana* contained some bioactive constituents that are potential candidates for the treatment of diabetes and oxidative stress-induced pathology.

Keywords *Phragmanthera incana,* Antimicrobial, Brine shrimps, Cytotoxicity, Canophyllol

Introduction

Loranthaceae is a family of flowering plants consisting of 75 genera and 1,000 species, which are woody plants, many are hemiparasites, and all have mistletoe features. They are known to be evergreen plants [1,2]. Loranthaceae are employed worldwide in traditional medicine to treat numerous diseases, like diabetes, condyloma,

inflammation, arthritis, breathing and nervous disorders, haemorrhoids, and some kinds of cancer [3, 4, 5].

Phragmanthera is one of the genera in the Loranthaceae family. There are about thirty-four species of *Phragmanthera,* which are distributed in tropical Africa and Arabia particularly from Nigeria to Angola. These species are found to be common locally and are likely to become pests of plantation crops. Some of the species are *P. batangae, P. baumii, P. exellii, P. longiflora, P. seretii, P. nigritana, P. vignei, P. glaucocarpa, P. cinerea, P. raynaliana, P. macrosolen, and P. zygiarum* [1]. Most of these species are found in or near forest areas, while few are found in dry habitats, especially in South Central and Southern Africa. *Phragmanthera incana* has been reported to show antimicrobial potentials and antioxidative properties [2, 6]. *Phragmanthera incana* is a woody shrub with a stem that is 2 cm long which grows mainly on trees like *cola acuminata*, *Cola nitida*, *citrus spp*, and *Psidium guajava*. *Phragmanthera incana* is widely spread in secondary jungles, bush savannah, and the Nigerian savannah [1,2]. Ethnomedicinally the plant is used to treat ailments like high blood pressure, yellow fever, sleeplessness, cancer, and convulsions [7]. The plant contains tannins, steroids, cardiac glycosides [2] and a peptide called *phragmanthin* [7]. It has been reported that *P. incana* leaves harvested from *Cola* species display better antioxidant activities than those harvested from other hosts. The leaves could be used as a low-cost nutraceutical to treat or prevent oxidative stress associated with diabetes and other diseases [6]. The main purpose of this investigation is to isolate and characterise the chemical constituents of *Phragmanthera incana* and to assess its pharmacological activities, thereby improving the available information on the plant.

Materials and Methods

Plant material

Phragmanthera incana leaves and stem collected at Olabisi Onabanjo University, in Ago-Iwoye, Ogun State, Nigeria, were identified and authenticated at the Herbarium of Forestry Research Institute of Nigeria (FRIN) by Mr. K. A. Adeniji. Its voucher specimen with Herbarium number FHI 108423 was deposited at FRIN,

Extraction of plant materials

The air-dried and pulverized *Phragmanthera incana*: leaves (1300 g) and stem (1200 g), were extracted in a successive order with ethyl acetate and methanol by maceration for at least 72 hours. The extracts obtained were concentrated using a rotary evaporator (Buchi R215) at 40°C and then stored in a vacuum desiccator.

Isolation of compound Canophyllol (F1) Friedelin (F2), and Methyl-3,4,5-trimethoxybenzoic acid (Eudesmic acid) (F3)

The ethyl acetate extract (10 g) of *Phragmanthera incana* leaves was subjected to open column chromatography packed with silica gel 70-230 mesh (200 g) and fractionated using gradient elution of n-hexane: ethyl acetate and ethyl acetate: methanol, **F1** (33 mg) eluted with hexane: ethyl acetate (8:2) gave a white crystalline solid on recrystallization and **F2** (12 mg) eluted with hexane: ethyl acetate (7:3) gave a white crystalline solid while **F3** (11 mg) eluted with hexane: ethyl acetate (4:6) also gave a white solid. The structural elucidation of the three compounds was determined through their Proton, Carbon and 2D- Nuclear Magnetic Resonance (NMR). The chemical shift for the three compounds is given below, and the ^{1}HNMR and ^{13}CNMR data are presented in **Tables 1, 2, and 3.**

Friedelin (F1)

^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.97, 1.73 (2 H, m-H1); 2.40, 2.32 (2 H, m-H2); 2.25 (1 H, q-H4); 1.75, 1.27 (2 H, m- H6); 1.49, 1.37 (2 H, m-H7); 1.38 (1 H, m-H8); 1.52 (1 H, m-H10); 1.46, 1.27 (2 H, m-H11); 1.35,1.35 (2 H, m-H12); 1.52, 1.31 (2 H, m-H15); 1.57, 1.36 (2 H, m-H16); 1.56 (1 H,m-H18); 1.37, 1.21 (2 H, m-H19); 1.46, 1.27 (2 H, m-H21); 1.50, 0.95 (2 H, m-H22), 0.89 (3 H, d-H23); 0.73 (3 H, s-H24);

0.87 (3 H, s-H25); 1.01 (3 H, s-H26), 1.05 (3 H, s-H27); 1.17 (3 H, s-H28); 0.95 (6 H, s-H29); 1.00 (6 H, s-H30).

^{13}C-NMR ($CDCl_3$ 150 MHz) δ: 213.2 (C-3), 59.5 (C-10), 58.2 (C-4), 53.1 (C-8),42.8 (C-18), 42.2 (C-5), 41.5(C-2), 41.3 (C-6) 39.7 (C-13), 39.3 (C-22), 38.3 (C-14), 37.5 (C-9), 36.1 (C-16) 35.6 (C-11) 35.4 (C-19), 35.0 (C-29), 32.8 (C-21), 32.4 (C-15), 32.1 (C-28), 31.8 (C-30), 30.5 (C-12), 30.0 (C-17), 28.2 (C-20), 22.3 (C-1), 20.3 (C-26), 18.7 (C-27), 18.3 (C-7), 17.9 (C-25), 14.7 (C-24) and 6.8 (C-23).

Canophyllol (F2)

^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.95, 1.70 (2 H, m-H1); 2.40, 2.25 (2 H, m-H2); 2.25 (1 H, q-H4); 1.75, 1.26 (2 H, m- H6); 1.49, 1.38 (2 H, m-H7); 1.45 (1 H, m-H8); 1.53 (1 H, m-H10); 1.46, 1.30 (2 H, m-H11); 1.34,1.34 (2 H, m-H12); 1.52, 1.31 (2 H, m-H15); 1.57, 1.36 (2 H, m-H16); 1.56 (1 H,m-H18); 1.37, 1.21 (2 H, m-H19); 1.46, 1.27 (2 H, m-H21); 1.50, 0.95 (2 H, m-H22), 0.88 (3 H, d-H23); 0.72 (3 H, s-H24); 0.86 (3 H, s-H25); 0.98 (3 H, s-H26), 0.91 (3 H, s-H27); 0.99 (3 H, s-H28); 0.87 (6 H, s-H29); 1.13 (6 H, s-H30).

^{13}C-NMR ($CDCl_3$ 150 MHz) δ (ppm): 213.2 (C-3), 59.5 (C-10), 58.2 (C-4), 52.5 (C-8),42.8 (C-18), 42.1 (C-5), 41.5 (C-2), 41.2 (C-6) 39.5 (C-13), 33.4 (C-22), 38.2 (C-14), 37.5 (C-9), 29.1 (C-16) 35.4 (C-11) 34.5 (C-19), 32.8 (C-29), 31.4 (C-21), 31.2 (C-15), 68.1 (C-28), 34.3 (C-30), 30.1 (C-12), 35.2 (C-17), 28.2 (C-20), 22.3 (C-1), 19.1 (C-26), 19.2 (C-27), 18.2 (C-7), 18.1 (C-25), 14.7 (C-24) and 6.8 (C-23).

Isolation of Methyl-3,4,5-trimethoxybenzoic acid (Eudesmic acid) (F3)

^{1}HNMR (600 MHz, Acetone-d_6) revealed signals at δ (ppm): 7.34 (s, 2H), 3.90 (6H, s- OCH_3), 3.81 (3H, s- OCH_3)

^{13}CNMR (150 MHz, Acetone-d_6) δ (ppm): 60.19 (OCH_3), 56.05 (OCH_3), 142.90 (C-2/6), 126.03 (C-1), 107.49 (C-4), 153.68 (C-3/5), 166.93 (C = O).

Brine Shrimp Lethality Test

The seawater was poured into the partly covered soap case, and a slide was used to divide the case to make a dam. Into the dark side, brine shrimp eggs were added and this was put in a well-illuminated place. The eggs hatched in about 48 hours (2 days) and swam to the uncovered side of the partly-covered soap case. The samples were prepared in concentrations of 10,000 µg/mL, 1000 µg/mL and 100 µg/mL for the extracts.2 mL of the samples at each concentration for the extracts was put into test tubes and 5 mL of seawater was added. To these solutions, 10 shrimp nauplii were added and made up to 10 mL with seawater. The test tubes were observed 24 hours later, and the number of shrimps nauplii survivors (lethality estimate) was counted, and the number of dead shrimps nauplii was subtracted [8, 9].

Antidiabetic Assay

Experimental Animals

Swiss male albino rats were used for the study. The rats were bought from the Department of Pharmacology, University of Ibadan, Ibadan, Nigeria. The average weight of the rats was between 120-150 g. The rats were acclimatized for three weeks. The animals were fed with standard pelletized feed and allowed water *ad libitum.*

Animal Grouping

The animals were grouped into: (i) untreated control rats: (ii) alloxan-induced diabetic rats, not treated with the extracts nor the glibenclamide (this was the negative control), (iii) alloxan-induced diabetic rats treated with the 50 mg/kg extracts, (iv) alloxan-induced diabetic rats treated with the 100 mg/kg extracts, (v) alloxan-induced diabetic rats treated with the 200 mg/kg extracts, and (vi) alloxan-induced diabetic rats treated with 10 mg/kg glibenclamide (positive control)

Experimental Procedure

The rats were kept in different cages with six rats of about the same weight in each group. After the grouping, the fasting blood glucose

levels of the rats were measured with glucometer by collecting blood from the tail [10, 11]. Alloxan monohydrate was injected into the rats intraperitoneally for the induction of diabetes in the rats. The induction was carried out for seventy-two hours, after which another blood glucose level was taken, which revealed that the blood glucose level of the rats had already increased. The ethyl acetate and methanol extracts were prepared in three different concentrations – 200, 100 and 50 mg/kg – administered orally to the rats for 3 days. The rats were given 1 mL of the prepared extract concentration twice on the first day and once in the other days, with their blood glucose level measured daily. The anti-diabetic activity was determined in triplicates to ascertain the level of blood glucose released. Glibenclamide (10 mg/kg) served as reference standard drug. Blood sample was collected from the rats through retro-orbital plexus puncture method. The blood glucose levels were determined and estimated using AccuChek™ and glucose strips in AccuChek™ test meter.

Antioxidant Assay

The antioxidant assays of the extracts (ethyl acetate and methanol) and the pure compounds were determined in terms of free radicals scavenging abilities, using the methods of Atewolara-Odule *et al.* 2020 [12] and Khelifa *et al.* (2012) [13] with some modifications. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared in methanol and the concentration was kept as 0.1 mM. Five concentrations of 10, 20, 50, 80, and 100 mg/mL of both the extracts (ethyl acetate and methanol) and 0.005 µg/mL of each pure compound were prepared in methanol. To each sample concentration, 1 mL of 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 sample was measured at 517 nm and recorded as $L_{control}$ against sample L_{sample} using the spectrophotometer. Each test was done in triplicate. The free scavenging ability of each sample was determined using the equation below:

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

Where $L_{control}$ is the absorbance of control; L_{sample} is the absorbance of sample.

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

Results and Discussion

Compound **F1** (10 mg) was obtained as a white crystalline solid with a melting point of 266-268 °C. The IR spectrum exhibited a strong band at 1714 cm^{-1}, characteristic of saturated ketones [14,15]. Absorptions at 2925 and 2868 cm^{-1} are due to C-H stretching vibration of a methyl group, and bands at 1455 and 1075 cm^{-1} are due to C-H methyl bending vibrations from cyclohexane rings. The IR spectrum was in conformation with that reported for friedelin by Tanaka and Matsunaga, 1988 [15]. The ^{1}HNMR (600 MHz, $CDCl_3$) spectrum showed the signals for seven methyl singlets at δ (ppm) 1.17 (s, 3H-28), 1.05 (s, 3H-27), 1.01 (s, 3H-26), 1.00 (s, 3H-30), 0.99 (s, 3H-29), 0.87 (s, 3H-25), 0.73 (s, 3H-24), a methyl doublet at δ 0.88 (d, J = 6.9 Hz, 3H-23), a methine proton at δ 2.25 (q, J = 6.6 Hz, H-4) and methylene proton δ 2.40 (ddd, J = 13.8, 5.0, 1.9 Hz, 1H-2), 2.32 (dd, J = 26.1, 6.6 Hz, 2H-2). The signals at 2.40 and 2.32, 1.97 and 1.73, 1.75 and 1.27, 1.50 and 0.95 were as a result of unequivalent methylene protons at C-2, C-1, C-6, and C-22, respectively. The observation was by the presence of correlations between methylene proton signals in the COSY spectrum. Also, the signals at 0.99 ppm and 0.87 ppm were as a result of equivalent methyl protons at C-29 and C-25. The absence of an olefinic proton signal revealed that **F1** is a saturated skeleton. The ^{13}C-NMR (150 MHz, $CDCl_3$) spectrum showed signals for thirty carbon atoms. DEPT-135 spectrum revealed eight methyl, four methine, eleven methylene, six quaternary carbons, and

one carbonyl (213.2) carbon as shown in Table 1. This further confirms that **F1** is a saturated triterpene. The HREIMS spectrum m/z 427.3932 [M+H] confirmed **F1** to have molecular formula $C_{30}H_{50}O$. Comparing the ^{1}H and ^{13}C-NMR spectroscopic data of **F1** with the available data in literature, such as Atewolara-Odule *et al.,* 2020 [12], Ogunnusi *et al.,* 2010 [15]; Mahato and Kundo, 1994[17]; Mann *et al.,* 2011 [18], Omeje *et al.,* 2014 [19], F1 was identified as Friedelin **(Figure 1)**.

Table 1: ^{1}H and ^{13}C-NMR (600 and 150 MHz) of F1 in $CDCl_3$

Assignment	δc ppm PILE	Reported [a-c] δc ppm	DEPT	δH ppm	Reported [a-c] δH ppm
1	22.3	22.3	CH_2	1.97, 1.73 dd	1.95, 1.71 ddd
2	41.5	41.5	CH_2	2.40, 2.32 dd	2.36, 2.27 ddd
3	213.2	213.2	C	-	-
4	58.2	58.2	CH	2.25 q	2.26 q
5	42.2	42.1	C	-	-
6	41.3	41.3	CH_2	1.75, 1.27 m	1.72, 1.27 d
7	18.3	18.2	CH_2	1.49, 1.37 m	1.49, 1.37 m
8	53.1	53.1	CH	1.38 d	1.38 dd
9	37.5	37.4	C	-	-
10	59.5	59.4	CH	1.52 m	1.53 m
11	35.6	35.6	CH_2	1.46,1.27 m	1.45,1.27 m
12	30.5	30.5	CH_2	1.35,1.35 m	1.34,1.33 m
13	39.7	39.7	C	-	-
14	38.3	38.3	C	-	-
15	32.4	32.4	CH_2	1.52,1.31 m	1.47,1.27 m
16	36.1	36.0	CH_2	1.57, 1.36 m	1.58, 1.35 m
17	30.0	30.0	C	-	-
18	42.8	42.8	CH	1.56 m	1.56 m
19	35.4	35.3	CH_2	1.37, 1.21 m	1.37, 1.23 m
20	28.2	28.1	C	-	-
21	32.8	32.7	CH_2	1.46,1.27 m	1.50,1.32 m
22	39.3	39.2	CH_2	1.50,0.95 m	1.54,0.95 m
23	6.8	6.8	CH_3	0.89d	0.88 d
24	14.7	14.6	CH_3	0.73 s	0.73 s
25	17.9	17.9	CH_3	0.87 s	0.87 s
26	20.3	20.2	CH_3	1.01 s	1.01 s
27	18.7	18.6	CH_3	1.05 s	1.05 s
28	32.1	32.1	CH_3	1.17 s	1.18 s
29	35.0	35.0	CH_3	0.95 s	0.99 s
30	31.8	31.8	CH_3	1.00 s	0.93 s

(a) Mann *et al.,* 2011. (b) Omeje *et al.,* 2014. (C) Mahato and Kundo, 1994. (d) Tanaka and Matsunaga, 1988. (e) Atewolara-Odule, 2020

F2 (12 mg) was also obtained as a white crystalline solid. Its melting point was 281-282 °C. The IR spectrum indicated absorption at 3537.66 cm^{-1} (OH stretching), 2928.59 cm^{-1}/2860.63 cm^{-1} (CH stretching), 1705 cm^{-1} (C=O stretching), 1464.99 cm^{-1}/ 1388.71 cm^{-1} (CH bending) and 1053.84 cm^{-1} (C-O stretching) bonds. The ^{1}HNMR (600 MHz, $CDCl_3$, ppm) spectrum in revealed the presence of seven methyl singlets at δ 1.13 (s, 3H), 0.99 (s,6H), 0.98 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.72 (s, 3H), a methyl doublet at δ 0.87 (d, *J* = 6.9 Hz, 3H), and an oxymethylene at 3.63 (CH_2OH) (2H-28). It was also found that there were eight methyl, four methine, eleven methylene, six quaternary signals, and one carbonyl carbon signal (213.15) due to ketone group from the DEPT-135 experiment. No signal in the unsaturated region at (δ 5-7 ppm) and (100-160 ppm) suggest **F2** is also a saturated triterpene. The ^{13}C (150 MHz, $CDCl_3$) spectrum in showed signals for thirty carbons, and they are assigned as shown in Table 2. Based on the spectroscopic analysis and the comparison of ^{1}H and ^{13}C-NMR spectra of F2 with the published results [14, 17, 20, 21], the compound as identified as Canophyllol (Figure 1). The molecular mass was found to be $C_{30}H_{50}O_2$ based on the high-resolution electrospray ionization mass spectrum [HRESIMS] which gave (m/z 443.3882 $[M+H]^+$).

Table 2: ^{1}H-NMR (600 MHz), ^{13}C-NMR (150 MHz) of F2 in $CDCl_3$

Assignment	δc ppm	Reported [a-d] δc ppm	DEPT	δH ppm
1	22.26	22.1	CH_2	1.95, 1.70 dd
2	41.51	41.3	CH_2	2.40, 2.25 dd
3	213.16	212.6	C	-
4	58.22	57.8	CH	2.25 q
5	42.11	41.9	C	-
6	41.24	41.0	CH_2	1.75, 1.26 m
7	18.24	18.1	CH_2	1.49, 1.38 m
8	52.48	52.2	CH	1.45 d
9	37.46	37.3	C	-
10	59.48	59.1	CH	1.53 m
11	35.43	35.3	CH_2	1.46,1.30 m
12	30.09	29.9	CH_2	1.34,1.34 m
13	39.45	39.1	C	-
14	38.15	38.0	C	-
15	31.23	31.3	CH_2	1.52,1.31 m
16	29.13	29.0	CH_2	1.57, 1.36 m
17	35.16	35.1	C	-
18	39.37	39.2	CH	1.56 m
19	34.49	34.4	CH_2	1.37, 1.21 m
20	28.15	27.9	C	-
21	31.39	31.4	CH_2	1.46,1.27 m
22	33.36	33.2	CH_2	1.50,0.95 m
23	6.83	6.7	CH_3	0.88 s
24	14.67	14.5	CH_3	0.72 s
25	18.08	18.0	CH_3	0.86 s
26	19.07	18.9	CH_3	0.98 s
27	19.20	19.1	CH_3	0.91 s
28	68.05	67.0	CH_3	0.99 s
29	32.84	32.9	CH_3	0.87 s
30	34.28	34.2	CH_3	1.13 s

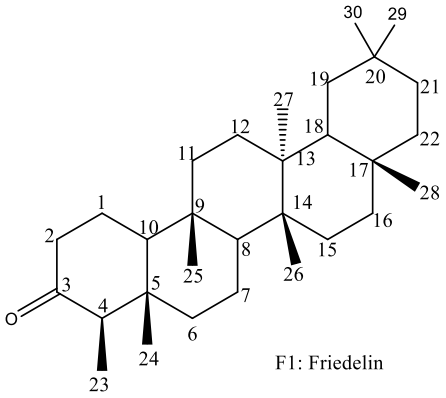
(a) Nozaki, *et al.,* 1986 (b) Abass, 2007 (C) Mahato and Kundo, 1994. (d) Joshi *et al.,* 2013

F3 (13.8 mg) was obtained as a crystalline solid. Its melting point was 172-174 °C and its IR showed absorption at 2927.23 cm^{-1} (C-H), 1679.53 cm^{-1} (C=O) bonds. The proton and carbon NMR chemical shift values are given in Table 3. The ^{1}HNMR (600 MHz, acetone) spectrum in revealed the presence of three methoxy groups at δ 3.81 (s, 3H), 3.90 (s, 6H), and aromatic proton at 7.34 (s, 2H). ^{13}CNMR (150 MHz, $CDCl_3$) spectrum revealed ten carbon atoms, comprising six aromatic, three methoxy carbons and one carbonyl carbon of a

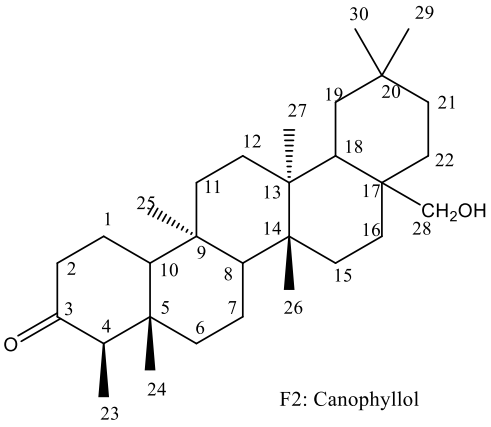
carboxylic acid at δ 166.93 (C-7). The HMBC spectrum assisted in assigning the position of the methoxy groups. The presence of five quaternary carbon atoms – four on the ring and the fifth being carbonyl of carboxylic acid was determined by HSQC. The comparison of ^{1}HNMR and ^{13}CNMR spectral data with the literature data [12, 19-22] confirmed that the compound is Eudesmic acid. This compound has been isolated from *Loranthus* [21] and *Tapinanthus bangwensis* [14]. The HRESIMS spectrum of F3 had a molecular ion at m/z 213.0759 $[M+H]^{+}$, which gave the molecular formula $C_{10}H_{12}O_5$.

Table 3: ^{1}HNMR (600 MHz), ^{13}CNMR (150 MHz) of F3 in Acetone (D_6)

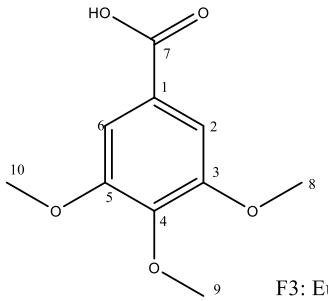
Assignment of C	**δC ppm**	**δH ppm**
C-8 & C-10	56.05	3.90 s, 6H
C-9	60.19	3.81 s, 3H
C-4	107.49	-
C-1	126.03	-
C-2 & C-6	142.90	7.34 s, 2H
C-3 & C-5	153.68	-
C-7	166.93	-



F1: Friedelin



F2: Canophyllol



F3: Eudesmic acid

Figure 1: Structures of Compound **F1**: Friedelin, **F2**: Canophyllol and **F3**: Eudesmic acid

Cytotoxicity Activity of *P. incana* Extracts

The test reveals a wide range of medicinal properties such as antitumor, antimicrobial, and pesticidal activities, and has led to the isolation of some pesticidal and anticancer agents [25]. The results obtained from brine shrimp lethality assay using *P. incana* is shown in **Table 4.** The extracts showed strong significant lethality against the brine shrimp. The percentage mortality observed was found to be concentration dependent. The variation observed in the results may be due to differences in the amount and kind of cytotoxic constituents of the crude extracts, such as tannins, flavonoids or steroids. Moreover, the strong lethality of the crude extracts (LC_{50} value < 1000 µg/mL) [8,], was an indication of the existence of powerful cytotoxic compounds in the extracts. The LC_{50} ranges from 10.03 to 189.84 µg/mL, hence all the extracts were good cytotoxic agents, except for PILE and PISE, whose LC_{50} was found to be greater than 1000 µg/mL. The positive results observed from the assay suggest that the extracts could be a promising source of antitumor, antibacterial or

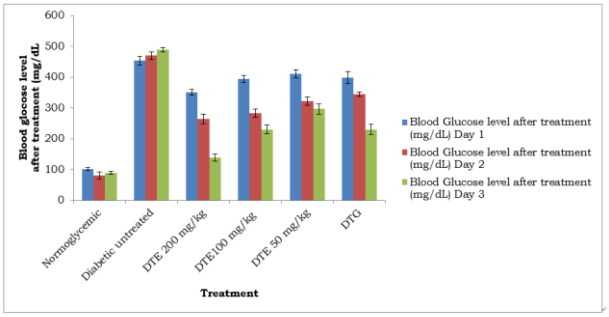
pesticidal compounds [27]. The result is in agreement with what has been reported in the literature, that African mistletoes are toxic [28].

Table 4: Cytotoxicity activity of *P. incana*

Plant extracts	% Mortality at Different Concentrations*			LC_{50}
	100 µg/mL	1,000 µg/mL	10,000 µg/mL	µg/mL
PILE	57.6	67.6	96.7	>1000
PILM	50.0	63.7	83.3	10.03
PISE	46.7	73.3	96.7	>1000
PISM	56.7	63.3	89.7	21.63

Antidiabetic Activity of *P. incana*

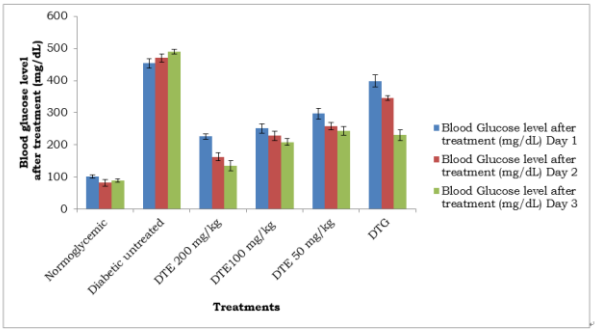
Diabetes mellitus is a common and chronic disease that could result in death if not managed properly [29, 30]. Based on this fact, it is imperative to continue to search for alternative therapies that will be affordable and available to combat or prevent the disease. The antidiabetic potentials of *P. incana* were assessed using alloxan-induced Swiss male albino rats and the results are captured in **Figures 2 and 3**. All the extracts of *P. incana* displayed significant reduction in the blood glucose level of the rats, except on Day 1 at concentration of 50 mg/kg, where no reduction was observed.



Key: DTE = Diabetic Treated with Extract; DTG = Diabetic Treated with Glibenclamide

Figure 2: Antihyperglyceamic effect of PILE extract

The highest reduction in the blood glucose level of rats was observed at a dosage of 200 mg/kg and the reduction was concentration-dependent. The result also revealed that, at 200 mg/kg of the extracts, there was a drastic reduction in the glucose level of the rats compared with the rats treated with the standard drug glibenclamide at 10 mg/kg. PILM showed stronger activity with reduction of 77.1% in the blood glucose level compares to PILE with 71.8% reduction on Day 3 at 200 mg/kg. However, glibenclamide (10 mg/kg) gave a reduction 53.0% on Day 3. It was also observed that all the plant extracts exhibited considerable reduction at $P < 0.05$ in the glucose level of hyperglyceamic alloxan-induced rats. The findings suggest that the extracts could be sources of antidiabetic agents The findings suggest that the extracts could be a source of antidiabetic agents. The result obtained is similar to that of *Tapinanthus butungil* in alloxan-induced Sprague-Dawley rats [12], antidiabetic effect of *Loranthus micranthus* [31], and *P. incana* harvested from *Cola nitida*[32]. This finding supports the folkloric usage of the plant as antidiabetic agents.



Key: DTE = Diabetic Treated with Extract; DTG = Diabetic Treated with Glibenclamide

Figure 3: Antihyperglyceamic effect of PILM extract

Antioxidant Activity of *P. incana* and Isolated Compounds

Antioxidants scavenging free radicals have been reported to have healing properties on cardiovascular diseases, inflammatory disorder, cancer and aging [33]. The scavenging capacity of free radicals by the extracts and the isolated compounds are determined by the use of DPPH [13]. The extracts of *P. incana* and isolated compounds exhibited good scavenging activities in vitro due to their inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The antioxidant activities of the extracts and the isolated compounds are represented by their percentage scavenging inhibition as shown in **Figure 4 and 5**. The results showed that the activities were concentration-dependent, because the activity increased with the increase in the concentration of the extracts. The activities are comparable to that of ascorbic acid- (the standard drug)- at all the

concentrations. The extracts of the leaves were found to be more potent than the stem extracts, especially at 80 mg/mL and 100 mg/mL. The extracts thus possess good antioxidant activity which is similar to the report of Fitrilia *et al.*, 2015 [34] on the antioxidant activity of clove mistletoe leaf extracts (*Dendrophthoe pentandra* (L.) Miq). The isolated compounds showed significant antioxidant activities which compared favourably with the ascorbic acid. The compound with the highest activity is Eudesmic acid. This is due to the fact that compounds that are derivatives of gallic acid are known to be good antioxidants [35]. The results obtained suggested that the plant extracts could be used as possible sources of natural antioxidants for healing and commercial sources of drugs for the treatment of oxidative stress.

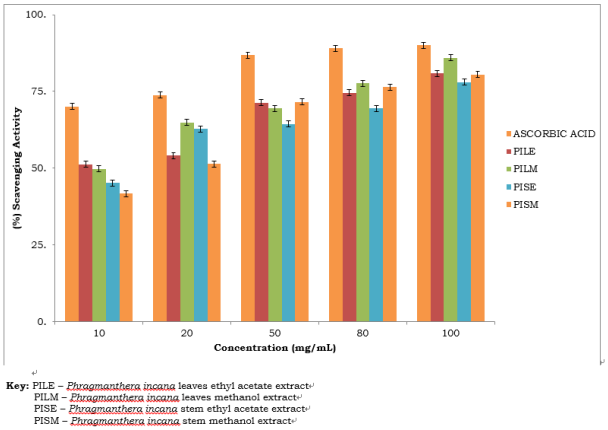


Figure 4: Antioxidant activity of *Phragmanthera incana* extracts

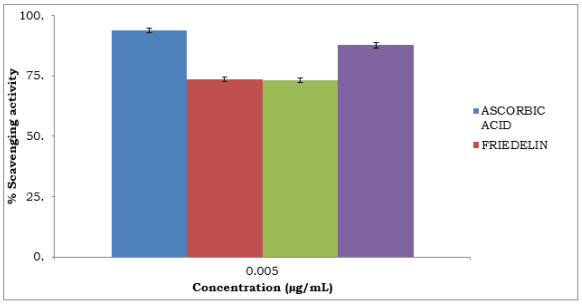


Figure 5: Antioxidant activity of isolated compounds

Conclusions

Phragmanthera incana is used traditionally to treat hypertension, diabetes, and other ailments. The isolated compounds from *P. incana* were friedelin, canophyllol, and eudesmic acid (Methyl- 3, 4, 5- trimethoxy-benzoic acid) which to the best of our knowledge are isolated for first time from this plant. The antidiabetic activities displayed by the plants at 200 mg/mL were higher than glibenclamide a known anti-diabetic agent. This is a validation of the ethnomedicinal uses of *P. incana*. Also, the plant has a potential for the development of drugs for the treatment of ailments connected to oxidative stress since some of the isolated compounds are gallic acid derivatives which are known as good antioxidants. Moreover, the antioxidant properties exhibited by the plant compared favourably to ascorbic acid. Pentacyclic triterpenoids like friedelin have been reported to have anti-diabetic, anti-malaria, antioxidant, anti-mycobacterial and anti-hypertensive activities; hence, the plant could serve as a source of remedies for diseases associated with them. The plant was found to be cytotoxic, suggesting it could be a promising anticancer agent.

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Author's contribution statement

Odunayo C. Atewolara-Odule: Conceptualization, methodology, Data analysis, Interpretation of spectra, Writing of manuscripts, **Olapeju O. Aiyelaagbe** and **Eleonora D. Goosen:** Methodology, Supervision, **Oseyemi O. Olubomehin**: Writing, review and editing, **Sunday. O. Ajibade**: Data analysis, Interpretation of spectra, **Abdulrazaq O. Ogunmoye:** Review, editing, spectra interpretation, **Seide M. Akoro:** Data Analysis, spectra interpretation, review and editing

Conflict of interest

The authors hereby disclose that there is no conflict of interest in this research work.

Data availability statement

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

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