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Evaluation of the antioxidant activity of the dried leaves of *Phragmanthera Incana* (LORANTHACEAE) parasitic on *Elaeis Guineensis*

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ABSTRACT

***Background*:** *Phragmanthera incana* Schum belongs to the family Loranthaceae. This specie of mistletoe is mostly found on trees in the South-western part of Nigeria. This study evaluated the chemical composition and antioxidant potential of the leaves of *Phragmanthera incana*.

***Methods*:** The pulverized dried leaf was extracted using dichloromethane and methanol (1:1) and partitioned into respective fractions using n-hexane and methanol. Proximate analysis was done in accordance with the recommendation of the Association of Official Analytical Chemists (AOAC). Standard methods were used to qualitatively detect phytochemical compounds while 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to quantitatively assay for antioxidant potential. Bioactive compounds were detected using GC-MS analysis.

***Results*:** Proximate analysis showed 16.56% moisture, 14% ash, 13.13% crude protein, 1.80% crude fat, 44.29% crude fiber and 10.22% carbohydrate. The phytochemical assay revealed the presence of alkaloid, saponin, carbohydrate, sterol/triterpenoid, protein and cardiac glycoside. DPPH scavenging activity of the extract, methanol and n-hexane fractions showed dose-dependent activities. N-hexane fraction demonstrated potent anti-oxidant activity. Data analysis of the scavenging activity revealed that n-hexane at concentrations of 1250 and 2500µg/mL significantly (p<0.001) scavenged DPPH free radicals in comparison with dichloromethane-methanol extract and methanol fraction. GC-MS analysis revealed three important antioxidant bioactive compounds; phytol, tetradecanoic acid, ethyl ester and hexadecanoic acid, ethyl ester; associated with antioxidant activities.

Conclusion: The efficient antioxidant activity displayed by the extracts in this study has highlighted the potentials of this plant in scavenging free radicals and underscores the need to harness the plant for development of new therapeutics.

Keywords:- Antioxidant activity, *Elaeis Guineensis*, *Phragmanthera incana*, Proximate analysis

1.0 INTRODUCTION

Historically, plants have played a pivotal role as important sources of new compounds exploited in the design of novel drugs. Within the last 30 years, phytochemicals have been extensively used to develop potent novel therapeutics [1].

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The ethno-medical knowledge of mistletoe had, for long, been in the hands of a limited number of herbalists who asserted a range of applications, which ranges from countering sorcery and magical powers, ability to treat mental disorders, rheumatism, pain, infertility problems, and health issues connected to the urino-genital system. According to Burkill (1985) [2] and Adodo (2004) [3], mistletoes are hemi-parasitic plants, of the Loranthaceae and *Viscaceae genuses* that are widely utilized in many cultures on practically nearly all continents in treating a variety of illnesses, such as diabetes, cancer, and hypertension, or to act as a diuretic [4]. For instance, bone fracture and body discomfort are supposedly treated by a tea produced from Loranthaceae spp [5]. According to Yineger and Yewhalaw (2007), the freshly plucked leaves of *Tapinanthus globiferus* (A.Rich.) Tiegh were crushed and with the addition of cold water is used as a treatment for tumors (Tanachaa) in southwest Ethiopia [6]. In the Ebolowa section of Cameroon, one glass of a mixture made from one handful of fresh *Tapinanthus globiferus* leaves and one handful of *Boswella odorata* root bark is typically taken two times in a day for two weeks was in the treatment of syphilis [7]. More than twenty different health issues have been successfully treated with mistletoe, which is now referred to as the "cure all"[3]. The leaves of just one species of mistletoe are typically prepared for use in medicine alone, but more frequently, the leaves of at least two different species that are similar except with respect to the characteristics of their hosts are harvested and prepared for use as a single therapy. This is due to the fact that mistletoe harvested from specific hosts is suitable for the treatment of a specific health issue and that the efficiency of medicinal formulations is assumed to depend on the host plant(s). The African mistletoe species native to western Nigeria is called *Phragmanthera incana (P. incana*), and it belongs to the family: Loranthaceae. *Phragmanthera incana* has been utilized historically in traditional medicine as an antimicrobial, antihypertensive, anticancer, and anti-diabetic agent, as well as a treatment for epilepsy, infertility, cardiovascular disease, headaches, rheumatism, menopausal syndrome and agglutination, as well as other conditions that typically call for immune-modulatory. Several organizations and individuals have already endorsed and praised some of these ethno-medical uses [8]. Oxidative stress is a condition brought on by an abundance of oxidants and the production of free radicals. This is an unfavorable process that damages a few biological components, including deoxyribonucleic acid (DNA), proteins, lipids, and cell membranes [9]. Unchecked oxidative stress can cause acute pathologies, speed up the aging process of the body, and cause a variety of chronic and degenerative disorders. Although it is difficult to give a precise assessment of the burden of oxidative stress globally, however, it is well known that physiological changes arising from oxidative stress may further yield mild to severe disorders such as, atherosclerosis, ageing, degradation of essential fatty acids, epilepsy, ischemic heart disease, diabetes mellitus, rheumatoid arthritis, cancer, immunosuppression and neurodegenerative diseases [10]. A worrisome dimension is that oxidative stress is enormously implicated in various disease conditions as well as emerging health challenges, so the need for more potent antioxidants to mitigate the causes of oxidative stress is widely coveted. It has been found that some plants that grow on other plants (parasitic plants) are enriched with bioactive compounds that possess antioxidant properties [11]. Given that there is no study on the *P. incana* parasitic on *Elaeis guineensis* (oil palmtree) host, this has necessitated our research to profile the bioactive compounds and the antioxidant potentials of *Phragmanthera incana* parasitic on *Elaeis guineensis.*

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection of Plant Materials

The leaves of *Phragmanthera incana* was obtained from stock at the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port-Harcourt. *Phragmanthera incana* was previously harvested from a palm tree (Arecaceae) in Igbokoda, Ondo State, Nigeria in June 2022 and authenticated by Taxonomist with voucher specimen deposited in the herbarium in the Department Plant Science and Biotechnology,

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Faculty of Sciences, University of Port-Harcourt. The sample was air-dried at room temperature. The dried sample was pulverized using an industrial blender and stored in an air-tight container for further use.

2.1.2 Glasswares and Apparatus

Grinding machine, analytical weighing balance, measuring cylinders, wide mouth bottles, spatula, foils, rotary evaporator, water bath, desiccator, porcelain crucible, airtight container, separating funnel etc.

2.1.3 Drugs, Chemicals and Reagents

Dichloromethane, Dimethyl sulfoxide and methanol (Sigma-Aldrich brand), water, n-hexane, Diphenyl picryl hydralazine, hydrochloric acid, sulphuric acid, sodium hydroxide, potassium hydroxide, acetone, ferric chloride, dragendorff's reagent, wagner's reagent, olive oil, hydrochloride acid, chloroform, acetic anhydride, sulphuric acid, Fehling's solution A + B, molisch reagent, million's reagent, glacial acetic acid, picric acid solution, distilled water.

2.2 Methods

2.2.1 Proximate Analysis

2.2.1.1 Moisture Content Determination (AOAC 925.10 Method - Air Oven)

1g of the sample was accurately weighed into a clean, dried porcelain evaporating dish. The dish, containing the sample, was then positioned in an oven set precisely at 105°C for duration of 6 hours. The evaporating dish, now holding the dried sample, was allowed to cool in a desiccator at room temperature. Subsequently, the dish, along with the dried sample, was re-weighed, and this new weight was recorded accurately.

The moisture content was calculated using the formula:

% Moisture = [(Weight of fresh sample - Weight of dried sample) /Weight of sample used] × 100.

2.2.1.2 Crude Fiber Determination (AOAC 962.09 Method)

A 2 g of pulverized sample was weighed (W_1) and 150 ml heated 0.128M H_2SO_4 was added and heated to boil for 30 minutes and then filtered. The residue was washed 3 times with hot distilled water. 150 ml of pre-heated 0.233M KOH was added and then heated to boiling, some drops of antifoaming agent (polyethylene glycol) was added and then boiled slowly for 30 minutes. This was then filtered and washed 3 times with hot water. Further washing was done with acetone. The residue was oven-dried at 130°C for 1-2 hours in an air oven. This was cooled, weighed (W_2) and asked at 500°C. it was cooled and weighed (W_3). The percentage crude fibre was calculated using the expression below.

% Crude Fiber = $(W_2-W_3) / W_1 \times 100$
Where:
W_1 = original weight of sample
W_2= weight after drying
W_3=weight after ignition

2.2.1.3 Crude Protein Determination (AOAC 984.13 Method - KJELDAHL)

A 1g of oven-dried minced sample was placed into a 30 ml Kjeldahl flask in a such way that the sample was prevented from touching the walls of the flask. 15 ml of conc. sulphuric acid was added and was followed by 1g of catalyst mixture (zinc metal). This mixture was heated slowly on the digestion rack under fumehood until a greenish-clear solution was visualized. After the digestion was cleared (about 30 minutes) it was heated for more 30 minutes and allowed to cool. About 10ml distilled water was added, to avoid caking. The resultant solution was transferred into the Kjeldahl distillation apparatus. 50 ml receiver flask into which 5 ml boric acid indicator solution has been added

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was placed under the condenser of the distillation apparatus in such a way that the tip was just 2 cm inside the solution. 10 ml of 40% NaOH solution was added slowly to the digested sample in the apparatus. The distillation process commenced with the cooling of the steam bypass and opening the inlet stop cork on the steam jet arm of the distillation apparatus. When the distillate obtained was at 35 ml mark on the receiver flask; the distillation process was stopped by closing the cooling inlet stop cork first, followed by the opening of the steam bypass. The condenser tip was titrated to the first pink color with 0.01M HCl. The % crude protein was calculated thus:

% Crude Protein = (100 x titre value x 0.0014 x 6.25) / sample weight
Where;
100= conversion to percentage
6.25= protein content according to kjeldahl method

2.2.1.4 Carbohydrate Content Determination (AOAC 920.39 Method - Cleg Anthrone)
0.1g of the sample was precisely weighed and placed into a 25ml volumetric flask. To this, 1ml of distilled water and 1.3ml of 62% perchloric acid were added. The mixture was vigorously shaken for close to 20 minutes to ensure complete homogenization. The volumetric flask was made up to the 25ml mark with distilled water and securely stoppered. The resultant solution was then allowed to settle for decanting purposes. From the filtrate, 1ml was carefully decanted into a 10 ml test tube and diluted to volume with distilled water. Subsequently, 1ml of the working solution was pipetted into a clean test tube, to which 5 ml of Anthrone reagent was added. A mixture of 1ml distilled water and 5ml Anthrone reagent was also prepared. Both mixtures were read at a wavelength of 630nm using the prepared blank (1ml distilled water and 5ml Anthrone reagent for calibration purposes. Additionally, a solution of glucose (0.1ml) was treated in the sample manner as the sample with Anthrone reagent, and its absorbance was measured.

The % Carbohydrate as glucose was calculated using the formula:
% CHO as glucose = (25 x absorbance of sample) / Absorbance of standard glucose.

2.2.1.5 Crude Lipid Content Determination (AOAC 963.15 Method - Soxhlet Extraction)
Soxhlet extractor was used. 250 ml extraction flask was carefully washed and placed in a hot oven for 45 minutes to dry. It was placed in a desiccator to cool. 2 g of minced plant sample was accurately weighted and placed into a rolled ash-less filter paper and kept in the extraction thimble. The thimble was inserted into Soxhlet extractor. 250 ml of n-hexane was poured into the flask for the extraction. Then the Soxhlet was connected to the flask at the bottom and also to the condenser. The heater was then switched on and the extract was allowed to reflux for about 3-6 hours. After complete extraction, the n-hexane was recovered before the thimble was removed. Finally, the oil detected in the flask was dried at 100°C inside an oven. The extracted oil was weighed and calculated by the difference in the weight of the empty flask and the flask containing the oil to yield the oil content of the sample. The % fat content was thus calculated using the formula below.

% Crude Fat= (C- A)/ B × 100
Where.
A = weight of empty flask
B= weight of the sample
C= weight of flask and oil after drying

2.2.1.6 Ash Content Determination (AOAC 942.05 Method - Furnace)
1g of the pre-dried sample was weighed into a preheated and pre-weighed porcelain crucible. The crucible, bearing the sample, was carefully placed into a muffle furnace, where it was exposed to a regulated temperature of 630°C for

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a period of three hours. Subsequently, the crucible was allowed to cool down to room temperature before being re-weighed.

The calculation for % Ash was performed using the formula:
% ASH = [(Weight of crucible + Ash sample - Weight of crucible and sample after ash) / Weight of sample] × 100.

2.2.2 Phytochemical analysis
2.2.2.1 Sample Preparation
A 119 g of the pulverized plant *Phragmanthera incana* was macerated with 600 ml each of methanol and dichloromethane (DCM) in a ratio of 1:1 at room temperature in a macerating jar for 72 hours. This was followed by filtration with Whatman filter paper no 1. Then the residue was further macerated for 24 hours only with 600ml of methanol. Then, it was filtered. The filtrate was obtained after being evaporated to dryness on a rotary evaporator. The extract was kept in a pre-weighed crucible and covered with a foil which was perforated and transferred to a desiccator for further evaporation of residual solvents. The dried extracts were weighed and preserved in an air-tight container inside a refrigerator until needed.

2.2.2.2 Phytochemical screening
Phytochemical screening was carried out on the dichloromethane-methanol extract obtained according to Trease and Evans [12]. The analysis was carried out to detect the phyto-constituents such as alkaloids, tannins, flavonoids, steroids, saponins, terpenoids, glycosides, etc. from the sample.

2.2.3 Partitioning of Extract
A 1.2 g of the methanol-dichloromethane extract was transferred into a beaker and dissolved with 100 ml of 90% methanol. The obtained solution was decanted into a separatory funnel carefully mounted on a retort stand. To this solution, 100 ml of n-hexane was added upon continuous stirring with a glass rod, two distinct layers were obtained. The n-hexane layer which was above was separated from the aqueous methanol layer below. This process was carried out severally, until the n-hexane fraction was fully separated from the aqueous methanol fraction. The two fractions were evaporated to dryness on a rotary evaporator. The fractions were further dried in a desiccator for 72 h and their weights determined.

2.2.4 Anti-Oxidant Studies With DPPH
A 0.05 g of the dried Methanol and Dichloromethane, non-polar and polar extract fraction each were weighed into a clean 50 ml beaker using a weighing balance. A 10 ml volume of methanol was measured into the beaker using a measuring cylinder. A glass rod stirrer was used to stir the mixture until a clear solution was obtained. The solution was carefully transferred into a 10 ml volumetric flask and was further diluted into seven (7) different concentrations which ranges from 78.125 ug/ml to5000 ug/ml. The negative control sample was also prepared. A standard solution of vitamin C was prepared and was diluted into seven (7) different concentrations which range from 78.125 ug/ml to 5000 ug/ml. The DPPH radical solution was prepared by the dissolution of 2.4 mg DPPH in 100 ml volume of methanol and was allowed to dissolve until a complete solution was obtained. A 2.5 ml of the methanol DPPH solution was measured and transferred into each of the diluted concentration of the sample solution. The mass spectrophotometer was set with an absorption maximum of 517 nm. The diluted concentration mixtures were shaken vigorously and were carefully transferred into the spectrophotometer and were maintained at room temperature in the dark for 30 minutes. Absorbance of the reaction mixture was obtained at 517 nm spectro-photometrically, also the absorbance of the negative control was measured and recorded. The absorbance of the standard solution of vitamin C (ascorbic acid) was also measured using the spectrophotometer and recorded. The DPPH scavenging capacity of the

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sample was further calculated. A graph of the average percentage inhibition was plotted against individual concentrations.

2.2.5 GC-MS Analysis

GC-MS evaluation of the n-hexane fraction was determined by an Agilent 7890B GC system and an Agilent 5977A MSD coupled to a Zebron-5MS column (ZB-5MS 30 m × 0.25 mm × 0.025 μm) (5%-phenylmethylpolysiloxane), the crude n-hexane extract of the leaves of *Phragmanthera incana* parasitic on *E. guineensis* was quantitatively evaluated through gas chromatography mass spectrometry (GC-MS) analysis. The carrier gas was GC-grade helium, flowing at a steady 2 mL/min rate. Before being used, the crude extract was filtered and dissolved in ethanol. An ultimate temperature of 300°C was attained by progressively raising the column temperature from 60°C to 10°C each minute. The GC-MS analysis ran for thirty minutes to complete each analysis. The obtained spectra were compared with the NIST 11 MS library (National Institute of Standards and Technology library), and the compounds were identified.

2.3 Statistical Analysis

The antioxidant activities were done in triplicates. The data were presented as mean ± standard error of mean (SEM). The significant differences between each column and the control were found using the Dunnett one-way analysis (ANOVA), with a *P* value of less than 0.05 being deemed significant. The program Graph Pad Prism version 8.0 (GraphPad Software, LA Jolla, CA, USA) was used for all statistical analysis.

3. RESULTS

3.1 Result of proximate analysis of the pulverized plant of *Phragmanthera incana*

The proximate analysis of the dried leaves of *Phragmanthera incana* showed that moisture, crude protein, carbohydrate, ash contents were 16.56%, 13.13%, 10.22%, 14.00% respectively with Crude fibre having the highest percentage content of 44.29% as shown in fig 1.

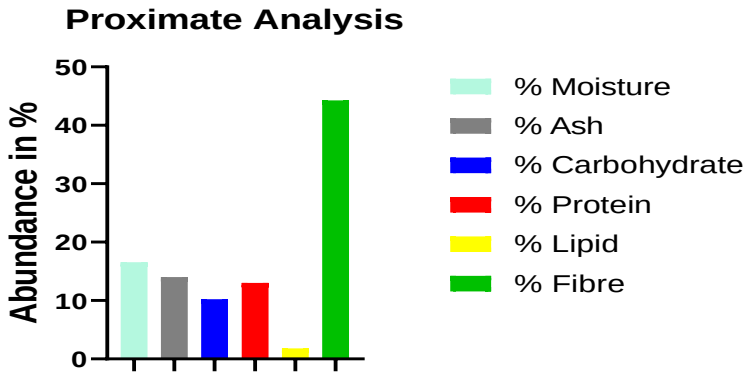

Figure 1. Proximate analysis of dried leaves of *Phragmanthera incana*

3.2 Phytochemical screening of the Crude Methanol and Dichloromethane (1:1) extract of the dried leaves of *Phragmanthera incana* (Loranthaceae).

Phytochemical analysis of the extract is shown in table 1. The methanol extracts yielded several phytochemical compounds that were well distinguishable. Phytochemical compounds such as alkaloid, saponin, carbohydrate, sterol/ triterpenoid, protein and cardiac glycoside were detected. However, phenolic compound, tannins, flavonoids and phlobatannin were not observed in the extract.

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Table 1: Phytochemical screening of Crude Methanol and Dichloromethane (1:1) extract of the dried leaves of *Phragmanthera incana*

S/N	Phytochemicals	Result
1	Alkaloid	+
2	Saponin	+
3	Carbohydrate	+
4	Phenolic compound	-
5	sterol/ triterpenoid	+
6	Tannins	-
7	Flavonoids	-
8	Protein	+
9	Phlobatannin	-
10	Cardiac glycoside	+

Key: - : Absent; +: Present

3.3 DPPH radical scavenging activity of Methanol and Dichloromethane, n-hexane and methanol extract of the dried leaves of *Phragmanthera incana*.

The scavenging activity on DPPH free radical by total extract, methanol and n-hexane fractions of the dried leaves of *Phragmanthera incana* were measured spectrophotometrically at 517 nm. The percentage inhibition of DPPH radical scavenging by the methanol:dichloromethane extract at varying concentration ranges of 78.125, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/mL. Although a moderately high (44.631 %) DPPH free radical scavenging potential was observed for polar (methanol) extract at the concentration of 5000 µg/mL, a very low inhibitory potential was observed at the concentration ranges of 78.00-2500 µg/mL (figure 2). At 156.25 µg/mL of the non-polar extract (n-hexane) fraction, negative (-2.679%) inhibitory effect was observed. However, as the concentrations increased from 312.5µg/mL, the fraction demonstrated increased antioxidant activity with 1250µg/mL having the highest (56.711 %) antioxidant activity for n-hexane fraction (figure 2). Data analysis of the scavenging activity of the three extract, showed all extracts/fractions scavenged DPPH free radicals.

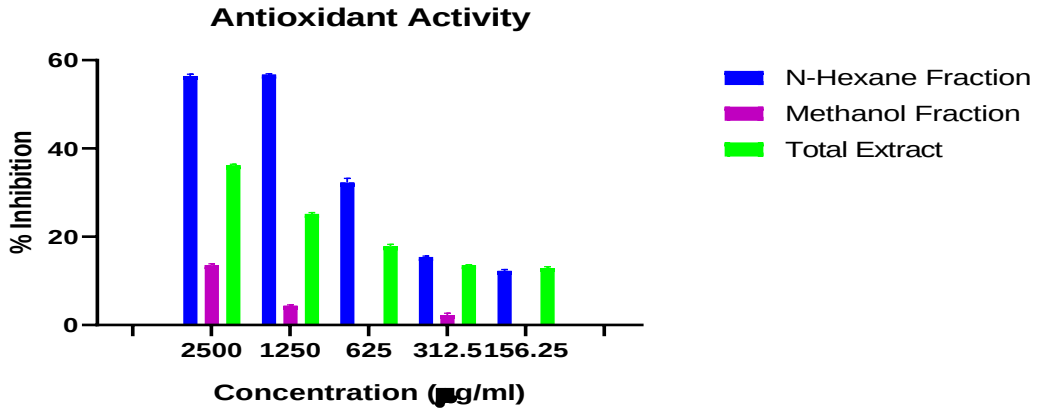

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Figure 2: Comparative percentage inhibition of n-hexane, methanol and total extract fraction

A comparison of the percentage radical scavenging potentials of the methanol:dichloromethane extract, methanol and n-hexane fractions, showed that n-hexane fraction displayed most effective antioxidant activity, significantly scavenged DPPH free radicals with a p-value of $p<0.001$, compared to methanol fraction and total extract. The IC_{50} of the antioxidant activities of the plant extract/fractions were determined and presented in figure 3.

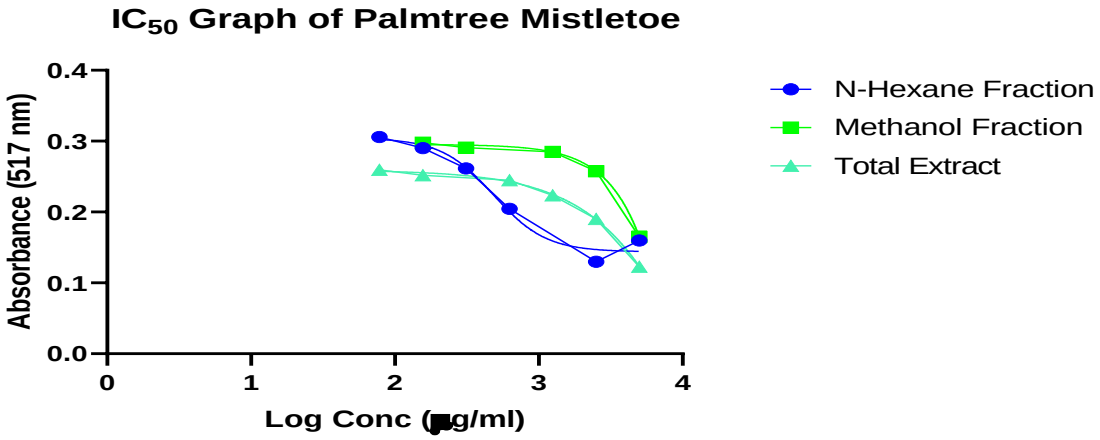

Figure 3: showing the comparative IC_{50} of n-hexane, methanol and methanol:dichloromethane extract.

The IC_{50}values of the extract and fractions were determined and the values presented in table 2.

Table 2. The half maximal inhibitory concentration (IC_{50}) values of the n-hexane, methanol and methanol: dichloromethane fractions of the leaves of *Phragmanthera incana*

Sample	IC_{50} (µg/mL)
n-hexane fraction	484.6
methanol fraction	~ 48770
methanol: dichloromethane fraction	~ 30108

The results in figure 3 and table 2 showed that only n-hexane fraction was moderately active. The total extract and the methanol fraction had very weak antioxidant activities, which is in line with the categorization of Moga and his co-workers that an extract of ≤ 10 µg/ml is highly active, 10-100 µg/ml active, 100-500 µg/ml moderately active and > 500µg/ml inactive [13].

3.4 GC-MS detection of n-hexane fraction of the dried leaves of *Phragmantheratincana.*

From the GC-MS analysis, a total of 7 bioactive compounds were identified as shown in table 3. Among the detected bioactive components of *Phragmanthera incana* included, 10-methylnonadecane ($C_{20}H_{42}$) which had the highest (24.0252%) peak area with the retention time of 36.5748 min, followed by 20.281%; undecanoic acid, ethyl ester ($C_{13}H_{26}O_2$), 12.5277%; tetradecanoic acid, ethyl ester ($C_{16}H_{32}O_2$), 8.4693%; hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$), 5.8186%; phytol ($C_{20}H_{40}O$). A very low percentage peak area of 1.8089% and 0.9893% was observed for bioactive compounds of Tetraethyl silicate, $(C_2H_5O)_4Si$) and 3,4-Octadiene, 7-methyl- (C_9H_{16}) respectively.

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Table 3: Bioactive compounds in n-hexane fraction of dried leaves of *Phragmanthera incana.*

S/N	Retention time	Peak Area (%)	Compound	Molecular formula	Molecular weight
1	5.8553	1.8089	Tetraethyl silicate	$(C_2H_5O)_4Si$	208.33
2	21.2772	12.5277	Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$	214.3443
3	25.8922	8.4693	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256.42
4	30.1059	5.8186	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4772
5	32.4754	4.527	Phytol	$C_{20}H_{40}O$	296.5
6	33.3947	0.9893	3,4-Octadiene, 7-methyl-	C_9H_{16}	124.2233
7	36.5748	24.0252	10-Methylnonadecane	$C_{20}H_{42}$	282.5475

4. DISCUSSION

Phragmanthera incana as well as other medicinal plants and fruits, produce secondary metabolites that have antioxidant properties as a result of their redox properties. In this study, the low amount of fat (1.80 %) detected from the proximate analysis, is in line with the study of Tabe and his co-workers [14] that reported 5.84%. This low content of lipid indicates a high level of medicinal value of the plant and its promising potential as antiobesity agent. Fibre, a non-digestible form of carbohydrate from plant origin has been linked with a plethora of health benefits which includes insulin sensitivity, haemorrhoid, constipation, and increased glycemia, as well as a variety of numerous pathological conditions of colonic health, reduction of the risk of colorectal carcinoma, cardiovascular disease and gut motility [15]. This study reports that a high percentage content of fibre was detected in *Phragmanthera incana*; this represents a therapeutic strategy for tackling these disease conditions if the plant is harnessed for novel drug development. The qualitative detection of bioactive compounds in this study showing detection of alkaloids correlates the previous studies of Ezema *et al.* (2016) [16] and Onoja *et al*. (2017) [17], which reported a moderate detection of alkaloids in methanol extract of mistletoe leaf. Contrary to this study, Osadebe *et al*. (2010) reported very high concentration of alkaloids in the crude methanol extract of mistletoe leaf in their study [18]. Aside from protecting the plants against predators, alkaloids have been documented to have potential health benefits to humans including, serving as an anti-inflammatory, antidiabetic, antioxidant and antibacterial agent. The discrepancies in the concentration of detected alkaloids in these studies could be as a result of the differences in the sample types and solvents used in extraction. Saponins are known to have numerous health potentials. This study detected moderate abundance of saponins. This is in line with the previous study of Hlophe and Bassey, (2023), which detected moderate abundance of saponins in DCM extracts of both Nigerian and South African mistletoe leaves [1]. The immense therapeutic potentials of saponins such as anti-microbial, anti-asthmatic, hypolipidemic, hypoglycaemic, antioxidant and anti-hypertensive activity etc. coupled with its detected abundance in this study, highlights the potential health benefits of *P. Incana* leaves and support the harnessing of the plants in treating the aforementioned diseases. Terpenoids, particularly volatile terpenoids, are well known to confer tolerance to plants against biotic stressors. Terpenoids protect plants against biological insults from pathogens and could release signals to carnivorous predator in the event of attack by

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herbivorous insects [19]. In this study, presence of sterol/ triterpenoid was detected. This finding is in concordance with the studies of Osadebe *et al*. (2010), [18] Ezema *et al.* (2016), [16] Hlophe and Bassey, (2023) [1] which reported abundance of terpenoid in methanol, crude methanol and DCM extracts of *L. micranthus* leaves. Several biological activities are known to be associated with terpenoids including cholesterols synthesis inhibitor, anticancer, antibacterial, anti-inflammatory, antiviral, antioxidant and antimalarial agent. Thus, the abundance of terpenoids detected further validates the leveraging on mistletoe plant to optimize treatment of these disease conditions. Polyphenolic compounds such as flavonoids, tannins and phenolic compounds are well-known phytochemicals with antioxidant potentials. Their antioxidants activities are attributed mainly, to their redox properties which plays vital roles such as absorption and neutralization of free radicals also, in the quenching of both singlet and triplet oxygen, and the decomposition of peroxides [20]. Surprisingly, this study detected no trace of these bioactive compounds in the *P. Incana* leaves. Although this finding does not correlate with the studies previously carried out by Hlophe and Bassey, (2023); who detected flavonoids [1], and Ikokoh *et al*. (2023); who found tannins and phenolics in Nigerian Mistletoe plants [21]. The absence of these bioactive compounds in this study could stems partly from the compounds being possibly below the detectable limit of the analyzed extracts. It could also be from the influence of the host plant. This is because *P. incana* as a parasite of the xylem tissue of the host plant depend on the host for nutrient, water and some carbon compounds through a series of chemical exchange [22]. This dependency of the parasite on the host, as well as the intimate and complex chemical exchange has been shown to facilitate possible phytochemical similarities between the host and the parasitic plants [23]. Considering that there is no previous study on *Phragmanthera incana* parasitic on *Elaeis guineensis* host, there is need for more studies to be conducted on the *Phragmanthera incana* from this host. Radical scavenging activities are vital to the mitigation of the deleterious effect of free radicals in various disease conditions. Hydrogen donating potentials are believed to be responsible for the effect of antioxidants on DPPH. Due to its simplicity and cost effectiveness, DPPH assay has been a widely used method for reducing chemical reactions as indicator of antioxidant potential of drugs [14]. In this study, DPPH radical at 517 nm, showed a deep violet coloration with a strong adsorption band and subsequently turned colourless or pale amber (yellow) when neutralized. This observed property enabled the visual observation and monitoring of the reaction process. In this study, all the fractions and the extract displayed scavenging activity of the DPPH free radicals indicating, their electron transfer or hydrogen donation ability. This finding aligns with the study of Hlophe and Bassey, (2023) which reported free radical scavenging capacity of 60-87% by the DCM extract of South African mistletoe leaves [1]. Although qualitative detection of phytochemicals in the mistletoe dried leaves showed absence of flavonoids and phenolic compounds, the efficient antioxidant potentials demonstrated by DPPH assay could be due to the presence of phenolic and flavonol content which might be below the detectable limit of the analyzed extracts as earlier suggested. The GC-MS analysis detected bioactive compounds of medicinal importance including carboxylic acids, saturated fatty acids, isoprenoid lipid, saturated alkanes. Important bioactive compounds with known antioxidant properties detected among the seven phytochemicals included, tetradecanoic acid, ethyl ester ($C_{16}H_{32}O_2$) and phytol which are important phytochemical compounds in plant with antioxidant capacity. Aside its antioxidant capacity, tetradecanoic acid is also being used in cancer preventive, production of lubricants, nematicide, in cosmetics and hypocholesterolemic drugs, [24, 25]. The abundant amount of this bioactive compound detected in this study validates the health benefits of mistletoe leaves and its use in optimizing treatments. Another important bioactive compound with antioxidant property; 1-Hexadecanol, 2-methyl ($C_{17}H_{36}O$) detected in this study and is widely present in plants known for antioxidant and anti-bacterial properties [26]. The detectable abundance of this compound in this study also indicates the immense health benefit of mistletoe leaves in scavenging free radicals. Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$) has myriads of benefits apart from serving as a good antioxidant agent including, the production of pesticide, lubricant, nematicide, antiandrogenic and hypocholesterolemic drugs, [25, 27]. The abundant presence of antioxidants and other

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bioactive compounds with known health benefits such as antihypertensive, anti-inflammatory, antifungal diuretic antiarthritic, improve insulin synthesis, antiasthma, antieczemic, in treatment of circulatory diseases etc., in this study underscores the myriads of health benefits of *Phragmanthera incana* and validates its use as antioxidant agent and in the optimization of treatment against disease conditions.

5. CONCLUSION

The substantial nutrient content of the dried leaves of mistletoe, as revealed by proximate analysis, makes it a viable option for use in nutraceuticals and functional foods. In phytochemical and antioxidant investigations, *Phragmanthera incana* demonstrated promising outcomes, identifying discernible bioactive components and eliminating DPPH free radicals. More research on *Phragmanthera incana* parasitic on *Elaeis guineensis* host is necessary. Meanwhile, moderate antioxidant potential demonstrated by the n-hexane fraction in this study highlight the potentials of the plant in scavenging free radicals.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Authors Contributions

Johnson-Ajinwo conceptualized/designed the study and wrote the manuscript.
Ozobu carried out the research under the supervision of Johnson-Ajinwo.

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