

Nutritional, Phytochemical and Biological Profile of *Pavetta corymbosa* (Rubiaceae)

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Abstract

P. corymbosa is traditionally known for its effectiveness in combating microbes, healing wounds, reducing fever, and treating respiratory disorders. This study investigated the bioactive constituents of *P. corymbosa* leaf hexane-acetone (PLHA) extract and assessed its nutritional and biological potentials. Proximate compositions were determined using standard methods. Gas Chromatography-Mass Spectrometry (GC-MS) was employed to identify and characterise the bioactive compounds present. FRAP and DPPH radical scavenging assays were employed for antioxidant activity. Brine shrimps lethality test using *Artemia salina* was utilised for cytotoxicity determination. Antimicrobial activity was assessed using agar well diffusion and broth micro-dilution techniques. Proximate analysis revealed that PLHA extract contains moisture, crude protein, crude fibre, fat, carbohydrate, and ash contents of 7.98±0.17, 13.13±0.05, 19.28±0.37, 2.46±0.06, 45.96±0.22, and 11.12±0.14%, respectively. The major constituents were Ethanone, 1-cyclopentyl- (28.23%); Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (21.08%); and n-Hexadecanoic acid (10.43%). PLHA extract (194.7346±0.575 µg/mL) had potent antioxidant activity comparable with ascorbic acid (137.6409±0.378 µg/mL). PLHA extract was active against *S. aureus*, *K. pneumonia*, *C. albicans* and *A. salina*. This study suggests that *P. corymbosa* is rich in bioactive compounds with nutritional benefits, and revealed promising antioxidant, antimicrobial and cytotoxicity potentials which can be leveraged for health-promoting applications.

Keywords: DPPH; FRAP; GC-MS; Medicinal plant; *Pavetta corymbosa*.

Abbreviations: PLHA: *P. corymbosa* leaf hexane-acetone; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric ion reducing antioxidant power; GC-MS: Gas chromatography-Mass spectrometry; RT: Retention time; *S. aureus*: *Staphylococcus aureus*; *B. subtilis*: *Bacillus subtilis*; *E. coli*: *Escherichia coli*; *P. mirabilis*: *Proteus mirabilis*; *V. cholerae*: *Vibrio cholerae*; *S. typhi*: *Salmonella typhi*; *K. pneumoniae*: *Klebsiella Pneumoniae*; *C. albicans*: *Candida albicans*; *T. rubum*: *Trichophyton rubrum*; *A. niger*: *Aspergillus niger*; NA: NOT Applicable; Cipro: Ciprofloxacin; Ketoco: Ketoconazole; *A. salina*: *Artemia salina*; p-INT: p-iodonitrotetrazolium violet.

INTRODUCTION

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Chaachouay & Zidane, 2024). *Pavetta corymbosa* (DC.) F. N. Williams, is a medicinal plant that belongs to the Rubiaceae family. It is typically a shrub or tree that thrives predominantly in the seasonally dry tropical biome. Its native habitat extends across West and West Central Tropical Africa: Nigeria, Benin, Burkina, Cameroon, Central African Republic, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Liberia, Mali, Nigeria, Senegal, Sierra Leone and Togo (POWO, 2026). *P. corymbosa* leaves have slightly recurved margins, with lateral nerves that may be inconspicuous, and they can be quite glabrous, with broad, cuspidate stipules; the corymbs are densely flowered, and the calyx

teeth are setaceous; branches are terete and whitish, fruit is black, shiny, roughly the size of a pea, and topped with the calyx.

The genus *Pavetta* L. have been used in ethnomedicine in western Africa as hypotensive, antimalarial, and as remedy for respiratory infections and wound healing. *Pavetta* L. encompasses a diverse array of natural product classes, including flavonoids, phenolic compounds, terpenoids, and proanthocyanins, as well as alkaloids and other non-polar substances like sterols and fatty acids (Al-Madhagy et al., 2022)

P. corymbosa is traditionally known for its effectiveness in combating microbes, healing wounds, reducing fever, and treating respiratory disorders (Anago et al., 2011; Nvau et al., 2011). Prior studies have documented the antibacterial effects of leaf ethanol extracts and the antimycobacterial properties of aqueous extracts (Anago et al., 2011; Nvau et al., 2011).

Phytochemical analyses of ethyl acetate and dichloromethane leaf extracts have led to the isolation of certain flavonoids and triterpenoids, respectively (Ahmadu et al., 2011). The lipophilic extract demonstrated notable antimicrobial efficacy against *Bacillus subtilis* and *Streptococcus faecalis* (Aliyu et al., 2017). Phytochemical screening revealed the presence of polyphenols, saponins, quinones, flavonoids, polyterpenes, and sterols; also, an acute toxicity study involving the intraperitoneal administration of aqueous and 70% hydroethanolic extracts of *P. corymbosa* leaves to Wistar rats showed no fatalities, indicating that the *P. corymbosa* leaves extracts are non-toxic (Patrice et al., 2024).

The increasing interest in natural products and their potential health benefits led to the investigation of *P. corymbosa* which could lead to new dietary applications that enhance health and wellness aligning with Sustainable Development Goal 3 (Good Health and Well-Being). Although, there have been documented a few chemical and biological studies on *P. corymbosa*, however, there are still limited information available regarding its phytochemical, biological potentials and nutritional compositions. Therefore, we conducted an investigation to evaluate the nutritional potential, bioactive compounds present, antimicrobial, antioxidant and cytotoxicity potentials of PLHA extract for the first time.

MATERIALS AND METHODS

Plant material and Extraction

Leaves of *P. corymbosa* were obtained from Agbegi village near a farmland, Ikire, Osun state. The plant was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) herbarium in Ibadan (FHI), voucher number FHI 113439 was issued for reference use. The leaves were then air-dried for about 2 weeks to remove moisture and subsequently pulverized.

Air-dried ground leaves of *P. corymbosa* (500 g) was measured and placed into a 5 L aspirator bottle with a lid. Next, 2 litres of a 1:1 mixture of hexane and acetone was added to the sample in aspirator bottle. The cold extraction method was utilized and maceration was done for 72 hours. After which, was decanted into a whatman No. 1 filter paper. The filtrate obtained was then concentrated in vacuo using a rotary evaporator to obtain *P. corymbosa* hexane-acetone (PLHA) extract.

Proximate Analysis

The moisture content, fat, crude protein, crude fibre, ash and carbohydrate of the flour blends and the control were determined using the method according to AOAC (2006).

GC-MS Analysis

The GC-MS analysis of PLHA extract was carried out using an Agilent 7820A gas chromatograph fixed to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies Capillary column (HP-5) coated with 5% Phenyl Methyl Siloxane (30 m length x 0.32 mm diameter x 0.25 μ m film thickness) was the stationary phase of separation of the compounds. Helium was used as the carrier gas at constant flow of 1.4871 mL/min at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm/sec. At an injection temperature of 300 °C, an injection volume of 1 μ L of the sample was introduced in splitless mode. While the gas saver mode was turned off, the purge flow to split vent was 15 mL/min at 0.75 min with a total flow of 16.654 mL/min. Oven was initially auto regulated at 40 °C for (1 min) then ramped at 12 °C/min to 300 °C (10 min). The run time was 32.667 min with a 5 min solvent delay. The mass spectrometer was utilized in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Acquisition of ion was through Scan mode (scanning from m/z 45 to 550 amu at 2.0s/scan rate). The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. National Institute Standard and Technology (NIST) 14.L library (2018) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C: \ Database \ NIST14.L).

Antimicrobial Activity

Test microorganisms were two Gram positive bacteria: *S. aureus*, *B. subtilis*; five Gram negative bacteria: *E. coli*; *P. mirabilis*, *V. cholerae*, *S. typhi*, *K. Pneumoniae*; and three fungi namely *C. albicans*, *T. rubrum* and *A. niger*. Microorganisms were obtained from the stock cultures of the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria. Four concentrations (100, 50, 25 and 12.5 mg/mL) of PLHA extract were used in this study.

Susceptibility Testing of Microorganisms

Antibacterial assay - pour plate method: An overnight culture of each organism was prepared by taking two loopful of organism from the stock and inoculating each into sterile nutrient broth (5 mL) for 18-24 h at 37°C. From overnight culture, 0.1 mL of each organism was added into 9.9 mL of sterile distilled water to get 1:100 (10^{-2}) dilution of each organism. Sterilized Mueller Hinton Agar [Oxoid] was aseptically dispensed into sterile petri dishes. From the diluted organism, 0.2 mL was taken into prepared sterilized mueller hinton. Using the spread plate method, 10^{-5} diluted of an 18-hour broth

was spread on the surface of the agar using sterile cotton swab. After this, a cork borer of 6 mm in diameter was flamed, allowed to cool and used to bore wells into the inoculated agar. The already prepared different concentrations (100, 50, 25, 12.5 mg/mL) of PLHA and ciprofloxacin were added to the bored wells and labelled appropriately. The plates were left for about an hour to allow for the proper diffusion of the extracts and then incubated at 37°C for 24 hours.

Antifungal assay - surface plate method: A sterile sabouraud dextrose agar (62 g/L) was prepared accordingly and aseptically poured into the sterile plates and allowed to set. Then 0.2 mL of the 10^{-2} of each organism was spread on the surface of set agar using sterile cotton swab. The wells were made using a cork borer of 6 mm diameter. The already prepared different concentrations (100, 50, 25 and 12.5 mg/mL) of PLHA extract and ketoconazole were added to the bored wells and labelled appropriately. All the plates were left on the bench for an hour to diffuse properly into the agar. Plates were incubated for 48 h at 26-28°C.

Ciprofloxacin (10 µg/mL) and Ketoconazole (70%) were used as positive control and dimethyl sulphoxide was used as a negative control. The diameter of zones of inhibition were measured in mm. Assays were done in duplicates and the activity was reported by measuring the diameter (mm) of inhibition zone, greater than 6 mm, around each disk (Olaoluwa et al., 2021).

Minimum Inhibitory Concentration (MIC)

The MIC was done using broth micro dilution method and 96 well plates were used. PLHA extract was dissolved in double strength Tryptone Soya Broth (MERK) to obtain a solution of 100 mg/mL. This was then diluted serially in sterile 96 well plate to obtain concentration range of 50 – 0.39 mg/mL. The drug used as the reference were ciprofloxacin (10 µg/mL) and Ketoconazole (70%) for the anti-bacterial and antifungal assay, respectively. Each of the micro plate wells were inoculated with 10 µL of the microorganism and incubated at 37°C for 24 hours and 26-28°C for 48 hours for antibacterial and antifungal assay, respectively. The least concentrations which showed no growth or turbidity after hours of incubations were streaked on nutrient agar. The least concentration with no trace of growth was taken as the minimum inhibitory concentration (MIC)

Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC)

After checking for growth or turbidity in the test plates [MIC determination]. 10 µL of 0.2 mg/mL of p-INT solution was added to the wells. The plates were further incubated at 37°C and 26-28°C for 30 mins for antibacterial and antifungal assay, respectively. Wells with colour change from yellow to pinkish red was an indication of bacterial /microbial growth. The least concentration which showed no trace of growth or colour

change was taken as the minimum bactericidal/fungicidal concentrations (MBC/MFC).

Antioxidant Activity

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH*) method

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH*) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in methanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. Free radical scavenging activities of PLHA extract was determined using standard procedures (Olaoluwa et al., 2022). DPPH (0.1 M) was prepared by dissolving 3.94 mg in 100 mL of methanol. Each sample (2 mg) was dissolved in 2 mL of methanol to prepare 1.0 mg/mL concentration of each of them. These were vortexed and serially diluted with methanol to obtain sample solutions of various concentrations ranging from 1000 to 62.5 µg/mL. The five serially diluted concentrations (1000, 500, 250, 125, 62.5 µg/mL) of the samples and standards were prepared in triplicate. Standards used was ascorbic acid (vitamin C). A volume of 0.5 mL from each of these concentrations were added to 3 mL of methanol solution of DPPH (0.1 M) separately. Absorbance of each sample concentration against methanol DPPH blank was measured at 517 nm in UV-Vis spectrometer. All readings were taken after 30 min of reaction time at room temperature. Decrease in absorbance of DPPH on addition of test samples in relation to the blank was used to calculate the percentage inhibition (I %) using the following equation;

$$I \% = \frac{\text{Absorbance (Blank)} - \text{Absorbance (Sample)}}{\text{Absorbance (Blank)}} \times 100$$

Ferric ion reducing antioxidant power assay (FRAP)

Ferric ions reducing power was measured according to the method of Oyaizu (1987) with a slightest modification PLHA extract was mixed with 2.5 mL of 20 mM phosphate buffer and 2.5 mL 1%, w/v potassium hexacyanoferrate (III), and then the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 mL of 10%, w/v Trichloroacetic Acid (TCA) and 0.5 mL 0.1%, w/v Ferric chloride were added to the mixture, which was kept aside for 10 min. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard. All assays were run in triplicate.

Cytotoxicity assay (Brine Shrimp lethality Assay)

PLHA extract was evaluated in a test for lethality to brine shrimp larvae. Toxicities of PLHA was tested at 1, 10, 100, and 1000 ppm in 10 mL sea-water solutions with 1% DMSO (v/v). Three replications were used for each concentration. The blank control is conducted with Distilled water. Ten, nauplii were used in each test and survivors counted after 24 h. After 24 h the number of survival of nauplii was counted and percentage of mortality was determined using the equation:

$$\% \text{ mortality} = \left(\frac{\text{no. of dead nauplii}}{\text{initial no. of live nauplii}} \right) \times 100$$

The lethal concentration at 50% (LC₅₀) was determined using a linear regression plot of percentage mortality and concentration using Microsoft Excel software ($y = 0.0689 + 33.356x$, $R^2 = 0.7796$). The LC₅₀ of the extract. LC₅₀ values greater than 1000 ppm for plant extracts were considered inactive; low potential for acute cytotoxic or antitumor activity (Meyer et al., 1982).

RESULTS AND DISCUSSION

Proximate composition of PLHA extract

The proximate analysis revealed the compositions of moisture, crude protein, crude fibre, fat, carbohydrate and ash contents in PLHA extract. The decreasing order of proximate content of PLHA extract was carbohydrate > crude fibre > protein > ash > moisture > fat (Figure 1). Hence, *P. corymbosa* leaves was found to be rich in carbohydrate ($45.96 \pm 0.22\%$), crude fibre ($19.28 \pm 0.37\%$) and crude protein ($13.13 \pm 0.05\%$). The moisture, fat and ash contents found in *P. corymbosa* were $7.98 \pm 0.17\%$; $2.46 \pm 0.06\%$ and $11.12 \pm 0.14\%$, respectively (Figure 1). The purity and safety of a plant is useful for quality control purposes, and are determined by the ash value and moisture content. The moisture content determines the plant's susceptibility to microbial or hydrolytic spoilage, the ash content demonstrates the content of physiological and non-physiological ash (Ibekwe et al., 2022).

In a study by Ibekwe et al. (2022) on *Pavetta crassipes*, the physicochemical properties were observed to range as follows: moisture content varied between 4.89% and 6.03%, total ash ranged from 3.67% to 13.55%, and acid insoluble ash contents were between 1.42% and 1.95% across different plant parts, including leaves, roots, stem bark, and fruits. In another study, *Pavetta indica* leaves have also been found to be rich in nutrients such as crude protein, carbohydrate and crude fibre (12.87, 13.36 and 39.94%) (Prasad & Bisht, 2011). Also, the flower of *Pavetta crassicaulis* have been reported to have high contents of crude protein, fat and carbohydrate along with appreciable macro and micronutrients (Rao et al., 2020). Hence, PLHA extract

showed appreciable amounts of carbohydrate, crude fibre, protein, ash, moisture and fat when compared with *P. crassipes*, *P. indica* and *P. crassicaulis*.

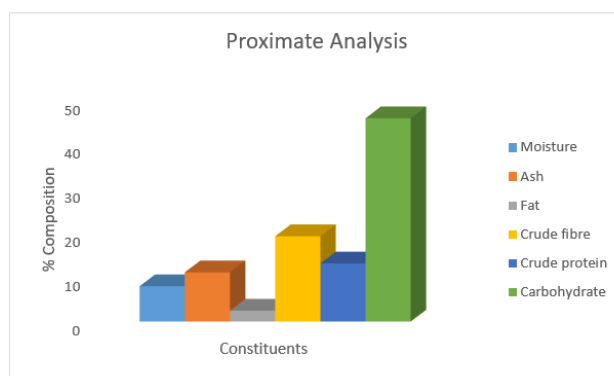


Figure 1. Comparison of various proximate constituents in PLHA extract.

GC-MS analysis PLHA extract

Twenty-eight compounds were identified PLHA extract. Compounds were identified from NIST database Library of GC-MS instrument. The GC-MS chromatogram of the identified compounds is shown in Figure 2. Among these, three major compounds with percentage peak area were found to be Ethanone, 1-cyclopentyl- (28.23%), Hexadecanoic acid, 2- hydroxy-1-(hydroxymethyl) ethyl ester (21.08%) and n-Hexadecanoic acid (10.43%).

Others were 9,12,15- Octadecatrien-1-ol, (Z,Z,Z)- (6.76%), 4,6-Di-O-methyl- α -D-galactose (4.59%), Neophytadiene (3.97%), Octadecanoic acid (3.50%), 1,4-Anhydro-D-galactitol (2.49%), Pentyl tetraoctyl ether (2.01%), D- Allose (1.96%), 1,5- Anhydro-D-mannitol (1.90%), Decanoic acid, ethyl ester (1.25%), and 3-Octadecyne (1.00%). The minor compounds were considered to have compositions of less than 1% which include: Benzene, 1-ethyl-3-methyl (0.86%), Benzene, 1,2,4-trimethyl- (0.52%), N-carbobenzyloxy-DL-phenylalanylglycine (0.82%), Orcinol (0.81%), Octanoic acid, ethyl ester (0.98%), beta-Alanine, N,N-diethyl- (0.98%), 2-pyridinamine oxide (0.75%), 2-Methoxy-4-vinylphenol (0.75%), Hydroquinone (0.35%), Phenol, 4-ethenyl-2,6-dimethoxy- (0.33%), α -R-Tumerone (0.66%), 1-Octadecanulphonyl chloride (0.40%), 11-Tetradecyn-1-ol acetate (0.31%), Octadecane, (ethenyl-oxo)- (0.38%), 8-Hexadecanal, 14-methyl-, (Z)- (0.89%). Some of the identified compounds with their reported bioactivities are shown in Table 1.

Ethanone, 1-cyclopentyl-, commonly referred to as cyclopentyl methyl ketone, is a ketone known to exhibit certain biological activities. Although detailed documentation of its specific biological effects is limited, the presence of the cyclopentyl ring and the ketone functional group implies potential interactions with biological molecules, such as enzymes and receptors. Cyclopentyl methyl ketone plays a significant role in synthetic chemistry, particularly in reactions like Suzuki coupling, which are essential for constructing complex

organic molecules (Kocienski, 2016). This synthetic capability can have effects on biological systems by aiding in the development of drugs and other bioactive compounds.

Hexadecanoic acid, 2- hydroxy-1-(hydroxymethyl) ethyl ester (also known as palmitic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester) exhibits various biological activities such as antioxidant, hypocholesterolemic, nematocide, pesticide, antiandrogenic activities, haemolytic-5- α reductase inhibitor (Duke, 2016; Muzahid et al., 2023). n- Hexadecanoic acid can scavenge free radicals, reducing oxidative stress and also possess anti-inflammatory and antimicrobial activity. The anti-inflammatory effects of n- hexadecanoic acid may be related to its ability to inhibit phospholipase A2, which is involved in the production of inflammatory mediators. The antimicrobial activity may be due to the ability of the fatty acid to disrupt bacterial cell membranes. n- Hexadecanoic acid have hypocholesterolemic effects, potentially lowering blood

cholesterol levels. It has also been investigated as a potential nematocide and pesticide (Starlin et al., 2019; Nabi et al., 2022).

A similar study of the GC-MS analysis of *Pavetta corymbosa* lipophilic extract revealed that 9-methyl octadecanoic acid- ethyl ester (22.6%), cyclotetracosane (15.3%), 2,4,6-trimethyl hexacosanoic acid- methyl ester (12.7%) and 9,12,15-octadecatrienoic acid- methyl ester (12.1%) were present as the major chemical components. Other compounds including terpenes/terpenoids, steroids, aldehydes and ketones were identified in trace quantities as less than 1%. The antimicrobial potency of the lipophilic extract is attributed to fatty acids/esters chemo types present (Aliyu et al., 2017). It is worth noting that both the major and minor compounds found in PLHA extract exhibit a range of biological activities, as detailed in Table 1. Therefore, this study indicates that PLHA extract contains bioactive compounds that could be utilized as sources for health-promoting treatments.

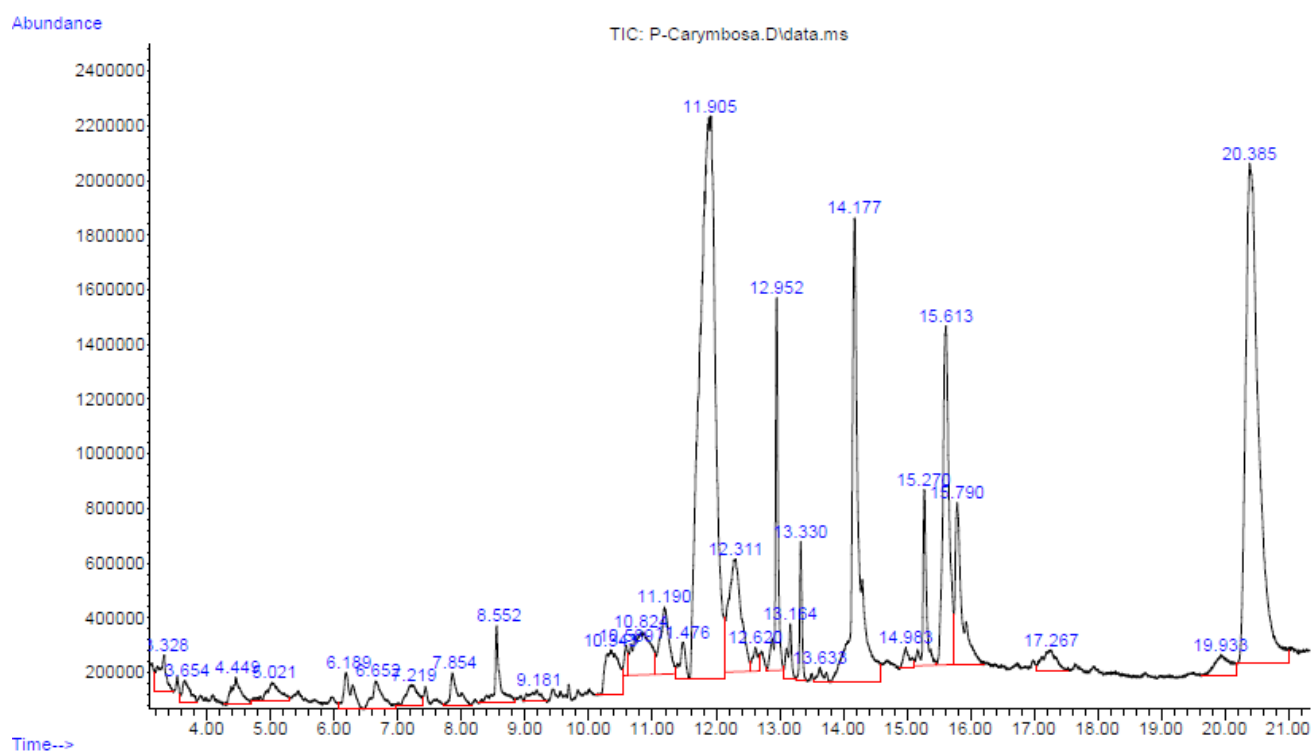
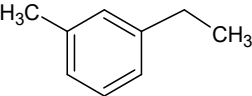
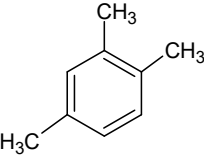
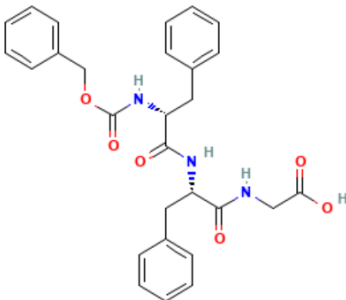
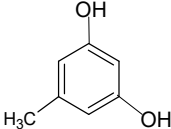
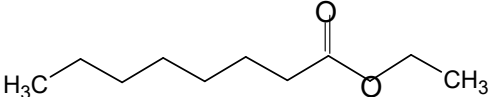
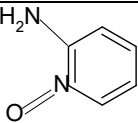
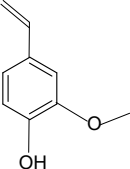
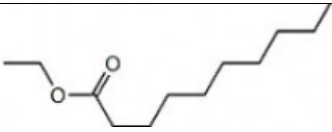
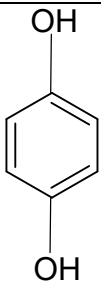
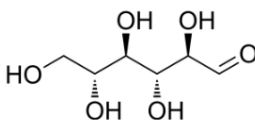
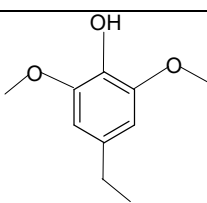
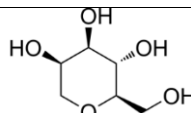
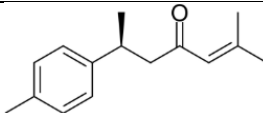
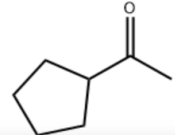
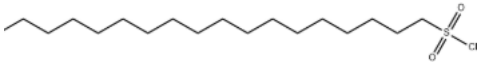
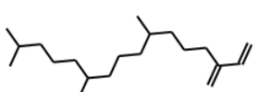


Figure 2. GC-MS Chromatogram of PLHA extract.

Table 1. Some compounds identified in PLHA extract and their reported activity/applications.

S/N	RT (min)	Area (%)	Compound	Structures	Bioactivities/Applications from Literature*
1	3.328	0.86	Benzene, 1-ethyl-3-methyl-		Antifungal and antibacterial activity.
2	3.654	0.52	Benzene, 1,2,4-trimethyl-		Found in various applications, including dyes, pharmaceuticals, and as a component of commercial organic solvents used in the chemical, plastics, and printing industries.
3	4.449	0.82	N-carbobenzyloxy-dl-phenylalanylglycine		Used in peptide synthesis. Antiviral activity
4	5.021	0.81	Orcinol		Exhibits antioxidant, antidepressant, and neuroprotective effects. It also plays a role in melanogenesis inhibition and has been linked to altered nitrogen balance in animals. Orcinol and its derivatives, found in lichens, are associated with anti-inflammatory, cytotoxic, and antimicrobial activities.
5	6.189	0.98	Octanoic acid, ethyl ester		Has slightly sweet aroma, commonly used in food and flavorings.
6	7.219	0.75	2-Pyridinamine, 1-oxide		Antihypertensive agent, anti-HIV agent
7	7.854	0.75	2-Methoxy-4-vinylphenol		Anti-inflammatory, antimicrobial, and antioxidant properties. It can induce cell cycle arrest and apoptosis in cancer cells and inhibits key enzymes like α -glucosidase and tyrosinase
8	8.552	1.25	Decanoic acid, ethyl ester		Used as flavouring agent and food additive. Also derivatives of Decanoic acid are known for their antimicrobial and antioxidant activity

S/N	RT (min)	Area (%)	Compound	Structures	Bioactivities/Applications from Literature*
9	9.181	0.35	Hydroquinone		Possesses anti-oxidative, neuroprotective, immunomodulatory, and anti-inflammatory functions.
10	10.343	1.96	D-Allose		Anti-cancer, anti-tumor, anti-inflammatory, anti-oxidative, anti-hypertensive, cryoprotective, and immunosuppressant activities.
11	10.589	0.33	Phenol, 4-ethenyl-2,6-dimethoxy-		Antioxidant, antimicrobial, and anti-inflammatory properties
12	11.190	1.90	1,5-Anhydro-d-mannitol		Anticancer
13	11.476	0.66	aR-Turmerone		Anti-inflammatory, anti-angiogenic, neuroprotective, and anti-aging effects
14	11.905	28.23	Ethanone, 1-cyclopentyl-		Used in the Synthesis of complex molecules
15	12.620	0.40	1-Octadecanesulphonyl chloride		Antioxidant, antifungal, pesticidal activity
16	12.952	3.97	Neophytadiene		Analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant activity

* Source of reference: Dr. Duke's Phytochemical and Ethnobotanical Databases, 1992-2016

Antimicrobial Activity

The antimicrobial activity PLHA extract showed excellent antimicrobial activity comparable with the standards. PLHA extract were more active against bacteria than fungi. Remarkable activity were observed at 100 mg/mL for *S. aureus*, *B. subtilis*, *E. coli* and *S. typhi* with zones of inhibition (ZI) of 20, 22, 22 and 18 mm, respectively. Among the fungi used, PLHA extract showed best activity against *C. albicans* (ZI = 20 mm), next was *T. rubrum* (ZI = 17 mm) and relatively low activity was observed against *A. niger* (ZI = 12 mm)

(Table 2). PLHA extract was active against *S. aureus* (MIC= 6.25 mg/mL; MBC=12.5 mg/mL), *K. pneumoniae* (MIC= 6.25 mg/mL; MBC= 50 mg/mL) and *C. albicans* (MIC=6.25 mg/mL; MFC=25 mg/mL) which was further confirmed due to their low MIC values depicting good antimicrobial activity (Table 3). The phytochemical compounds present in this extract could be responsible for the excellent antimicrobial activity displayed by PLHA extract. One of the major compound, n-Hexadecanoic acid have been reported to exhibit good antimicrobial activity. Also minor compounds present as

revealed by the GC-MS analysis could also have a synergetic effect. Some of the minor compounds includes Benzene, 1-ethyl-3-methyl-; Orcinol; 2-Methoxy-4-vinylphenol; Decanoic acid, ethyl ester; Phenol, 4-

ethenyl-2,6-dimethoxy-; 1-Octadecanesulphonyl chloride; Neophytadiene; 3-Octadecyne; and Nerol, methyl ether have all been reported in literature to possess good antimicrobial activity (Table 1).

Table 2. Zone of Inhibitions of PLHA extract and Standards.

Organisms	PLHA extract				Standards	
	Concentrations (mg/mL)				Cipro. (µg/mL)	Ketoco. (%)
	100	50	25	12.5		
<i>S. aureus</i>	20	14	13	10	18	NA
<i>B. subtilis</i>	22	18	15	14	20	NA
<i>E. coli</i>	22	20	16	12	18	NA
<i>S. typhi</i>	18	18	16	14	16	NA
<i>K. pneumoniae</i>	14	12	12	10	14	NA
<i>V. cholera</i>	14	13	10	10	14	NA
<i>P. mirabilis</i>	15	14	12	10	10	NA
<i>C. albicans</i>	20	16	15	12	NA	20
<i>T. rubrum</i>	17	15	14	12	NA	16
<i>A. niger</i>	12	10	0	0	NA	22

Table 3. MIC and MBC/MFC of PLHA extract.

Organisms	PLHA extract		Standards			
	Concentrations (mg/mL)		Cipro. (µg/mL)		Ketoco (%)	
	MIC	MMC	MIC	MMC	MIC	MMC
<i>S. aureus</i>	6.25	12.5	5	5	NA	NA
<i>B. subtilis</i>	25	12.5	10	10	NA	NA
<i>E. coli</i>	12.5	25	>10	>10	NA	NA
<i>S. typhi</i>	25	25	10	10	NA	NA
<i>K. pneumoniae</i>	6.25	50	>10	10	NA	NA
<i>V. cholera</i>	25	50	10	>10	NA	NA
<i>P. mirabilis</i>	50	50	5	10	NA	NA
<i>C. albicans</i>	6.25	25	NA	NA	0.25	5
<i>T. rubrum</i>	12.5	25	NA	NA	1	5
<i>A. niger</i>	>50	>50	NA	NA	1	1

Antioxidant activity

The antioxidant potential of PLHA extract was tested using the 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assays and ferric radical reducing power (FRAP). Both antioxidant studies were observed to be concentration dependent, increasing with increasing concentration. The antioxidant activity of PLHA extract using 2, 2-diphenyl picrylhydrazyl showed activity comparable to ascorbic acid (standard). PLHA extract had an IC₅₀ of 194.7346 ± 0.575 µg/mL and ascorbic acid standard had IC₅₀ of 137.6409 ± 0.378 µg/mL. This depicts that PLHA extract has a high antioxidant activity. The FRAP content of PLHA extract at ascorbic acid equivalent was between 34.402±0.045, 26.238±0.060, and 15.027±0.045 at concentrations of 500, 250 and 125 µg/mL, respectively, indicating that the plant has ability to reduce ferric ions to ferrous ions. The reducing capacity of PLHA extract pose a significant indicator of its potential antioxidant activity

Cytotoxicity

The brine shrimp lethality assay (BSLA) is a simple and inexpensive bioassay used for testing the efficacy of phytochemical present in the plant extracts. The extent of lethality was directly proportional to the concentration of the extract. PLHA extract killed 100% of the *A. salina* at the highest concentration (1000 µg/mL) and least mortality was observed at 1 µg/mL concentration. The lethality concentration (LC₅₀) at 50% concentration for PLHA extract was 241.57 µg/mL, which revealed that PLHA was active against *A. salina* depicting cytotoxicity potential. According to Meyer *et al* (1982), the principle of brine shrimp lethality assay is that lower LC₅₀ values indicate higher cytotoxicity (less than 1000 µg/mL).

CONCLUSIONS

The bioactive constituent and biological activities of PLHA extract and its nutritional properties were reported for the first time. *P. corymbosa*, long used in traditional

African medicine for a range of various ailments shows notable nutritional promise in addition to its medicinal uses. This research indicates that PLHA extract have a substantial proximate composition, including moisture, protein, fiber, fats, carbohydrates, and ash, highlighting its potential as a nutraceutical. Gas Chromatography-Mass Spectrometry analysis identified twenty-eight bioactive compounds, with three major compounds shown to be Ethanone, 1-cyclopentyl-, Hexadecanoic acid, 2- hydroxy-1-(hydroxymethyl) ethyl ester and n-Hexadecanoic, highlighting the plant's rich chemical profile. These compounds are associated with various reported biological activities from literature, suggesting that the presence of beneficial bioactive constituents in PLHA extract could serve as a valuable addition to dietary and health-promoting applications. The PLHA extract displayed notable antioxidant, antimicrobial and cytotoxicity potentials which is traceable to the various bioactive compounds identified PLHA extract. This study provided a biochemical justification of the ethnomeidical use of *P. corymbosa*. Bioassay-guided isolation of bioactive compounds is essential to further validate their pharmacological significance.

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