Original Research Article

PHYTOCHEMICAL, PROXIMATE, *IN VITRO* ANTIMICROBIAL, ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTI-HYPERGLYCEMIC ACTIVITIES OF *Combretum platypterum* (Welw) ROOT EXTRACTS

Abstract

Aim: This work was to determine the phytochemical, proximate, *in vitro* antimicrobial, antioxidant, anti-inflammatory, and anti-hyperglycemic activities of root extracts of *Combretum platypterum.*

Methods: Roots of *C. platypterum* were cold extracted with methanol, ethyl acetate, and n-hexane. The extracts were subjected to proximate, phytochemical analyses, antimicrobial, antioxidant, anti-inflammatory, and anti-hyperglycemic assays.

Results: The result of proximate analysis revealed that the roots contained 73.90±0.10% carbohydrates, the mean % of fibre and ash contents were >7.00, whereas the average protein and moisture contents were >4.00. The result of qualitative phytochemical analysis revealed that alkaloids, saponins, tannins, phenolic compounds and carbohydrates were present in the three extracts, whereas steroids were sparingly present only in n-hexane extract. The result of antimicrobial screening indicated that *B. subtilis*, *S. aureus, E. coli, C. albicans* and *S. typhi* were susceptible to the inhibitions of the three extracts in concentration-dependent manner, whereas only the n-hexane extract showed a dose-dependent inhibition against *P. aeruginosa***.** Methanol and ethyl acetate extracts showed good anti-inflammatory as well as antioxidant activity. The inhibition of α-amylase was greater with n-hexane extract.

Conclusion: the extracts showed good antimicrobial, anti-inflammatory, antioxidant and anti-hyperglycemic activities and can be a potential antimicrobial, anti-inflammatory, antioxidant and anti-hyperglycemic agent.

Key words: *Combretum platypterum*, anti-hyperglycemic, anti-inflammatory, antioxidant, antimicrobial, phytochemical, proximate

Introduction

Medicinal plants have been used in healthcare since time immemorial. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines [1]. In the beginning, the trial and error method was used to treat illnesses or even simply to feel better, and in this way, to distinguish useful plants with beneficial effects [2] (Salmerón-Manzano *et al.,* 2020).

Combretum platypterum has been used in Nigeria traditional medicine in treating various forms of diseases such as helminthiasis, sexually transmitted diseases, conjunctivitis, malaria, lumps, fever, eye problems, diarrhea, lower backache, coughs and swellings [3] (Anarado *et al.,* 2021).

Despite the ethnomedicinal uses of *C. platypterum,* not much scientific works have been done on the plant to validate the traditional uses.

Materials And Methods

Collection and Identification of the Plant: The roots of *Combretum platypterum* were collected within the surroundings of the Adada River in Nsukka Local Government Area, Enugu State, Nigeria. The collected plant roots were identified and authenticated by Mr. Alfred Ozioko (The Chief Taxonomist) at the International Center for Ethno medicine and Drug Development. Herbarium specimens were deposited in the herbarium of the International Center for Ethno medicine and Drug Development (Voucher number: Intercedd/260510).

Preparation of Plant Extract: The extraction was carried out using cold extraction method. The *Combretum platypterum* roots were washed, air-dried at room temperature and ground into powder. 1 kg of dry root powder was then macerated with 6 liters, each of methanol, ethyl acetate and n-hexane for 48 hours in an air-tight container at room temperature. The mixtures were filtered with a glass funnel embedded with cotton wool into a beaker and evaporated to dryness using a rotary evaporator at 40 °C and labeled CpRM (methanol extract), CpRE (ethyl acetate extract), and CpRH (n-hexane extract). The extracts obtained were kept at 4 ^{0}C until further use.

Phytochemical Analysis: Freshly prepared extracts were subjected to quantitative and qualitative phytochemical analysis to determine the presence or absence of flavonoids, alkaloids, terpenoids, saponins, carbohydrates, resins, tannins, reducing sugars, glycosides, and proteins according to the methods described by [4-8] with slight modifications.

Proximate Analysis: Proximate analysis of the powdered root for moisture, ash, fiber, protein, oil, and carbohydrate content was determined using standard methods AOAC (2010) [9] as described by [10].

Antimicrobial Assay

Samples: The antimicrobial sensitivity of the three samples from the plant extracts was determined using the agar-well diffusion method [11-12].

Test Organisms: The test organisms were obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria. Bacteria strains of Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pneumonia, Candida albicans, and Aspergillus niger were used in the study.

Culture Media and Other Reagents Used in Microbiological Analyses: Nutrient agar, Nutrient Broth, Mueller Hinton Agar, Sabouraud Dextrose Agar, and Sabouraud Dextrose Broth (Oxoid Limited, England) were the culture media used. The culture media were prepared according to the direction of the manufacturers.

Preparation of Stock/Working Solutions: Stock solutions for primary antimicrobial screening of the samples were made by dissolving 15 mg of each sample in 3 mL of DMSO to achieve a working concentration of 5 mg/mL. For determining the MICs, stock solutions of the samples were prepared by dissolving 60 mg of each sample in 3 mL of DMSO to obtain a working

concentration of 20 mg/mL. These were transferred to a screw-capped bottle and stored at a temperature of 4 °C.

Primary Screening of The Samples for Antibacterial and Antifungal Activities: The agar well diffusion technique was used to assess the antibacterial and antifungal activities of the samples. A two-fold dilution process was used to prepare dilutions of 2.5, 1.25, and 0.625 mg/mL from the stock solutions of 5 mg/mL for each sample. For bacterial and fungal isolates, respectively, twenty (20) mL of molten Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) were poured onto sterile Petri plates (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates and holes (6 mm) were drilled into the agar plates using a sterile metal cork borer. Each hole received Twenty (20 µl) of the different dilutions of the samples and controls, which were placed under aseptic condition, kept at room temperature for an hour to enable the agents to diffuse into the agar medium, and incubated accordingly. Ciprofloxacin (50 µg/mL) and fluconazole (30 µg/mL) were used as positive controls in the antibacterial and antifungal evaluations respectively; while DMSO was used as the negative control. The MHA plates were then incubated for 24 hours at 37^0C, and the SDA plates for 24 to 72 hours at room temperature (25 to 27 ^{0}C). The inhibition zone diameters (IZDs) were measured and recorded. The size of the cork borer (6 mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in triplicate and the mean IZDs were calculated and recorded.

Minimum Inhibitory Concentration (MIC) Assay: The maximum dilution of a plant extract that still has a growth-inhibiting impact on microorganisms is referred to as MIC. The agar dilution technique was used to determine the MIC of the samples on the test isolates. The following concentrations 10, 5, 2.5, 1.25, and 0.625 mg/mL were made from the stock solution (20 mg/mL) of each sample using a 2-fold serial dilution. Agar plates were prepared by pouring 4mL of molten double strength MHA and SDA (for bacterial and fungal isolates respectively) into sterile Petri plates containing 1mL of the various dilutions of the sample making the final plate concentrations to become 4, 2, 1, 0.5, 0.25, and 0.125 mg/mL. The test isolates which were cultured overnight in broth were adjusted to McFarland 0.5 standard and streaked onto the

surface of the agar plates containing dilutions of the sample. The MHA plates were then incubated at 37 ^{0}C for 24 hours and the SDA plates were incubated at room temperature (25 - 27^{0}C) for 24-72 days, after which all plates were observed for growth. The MIC was determined to be the lowest dilution (concentration) of the samples that completely prevented the growth of each organism.

In vitro Antioxidant Assay

DPPH Radical Scavenging Assay: The free radical scavenging activity was determined using DPPH as previously described by Shimada *et al*., 1992[13]. DPPH was initially dissolved in EtOH (ethanol) to a concentration of 0.1 mM and a solution of DPPH (1 mL) was added to an EtOH solution (3 mL) of the tested samples at different concentrations (200, 150, 100, 50, and 25 µg/mL). An equal volume of EtOH was added in the control test. The mixture was shaken vigorously and let to stand at room temperature for 30 minutes. A UV-Vis spectrophotometer was then used to detect the absorbance at 517 nm. The percentage of inhibition (I%) of DPPH radical was calculated as follows:

$$I\% = \left[\frac{A_{blank} - A_{sample}}{A_{blank}}\right]100 \quad \text{..... equ 1}$$

Where;

$A_{blank}$is the absorbance value of the control reaction (containing all reagents except the sample) and A_{sample} is the absorbance value of the test sample. The sample concentration providing 50 % inhibition (IC_{50} value) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as mean ± SD of triplicates.

Ferric Reducing/Antioxidant Power (FRAP) Assay: The FRAP test is performed according to the method described by Benzie & Strain,[14]. The stock solutions included 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh

working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ, and 2.5 mL $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37 °C prior to use. The essential oil (150 µL) was allowed to react with 2850 µL of the FRAP solution for 30 min in the dark condition. After incubation, the absorbance was read at 593 nm using a UV–vis spectrophotometer. The results were calculated by standard curves prepared with known concentrations of ascorbic acid (AA) and were expressed as mg AA/g.

ABTS Radical Scavenging Assay: To evaluate the radical scavenging capacity of crude extracts, the $ABTS^+$ decolorization test was conducted in accordance with the method outlined by **[**15] with a minor modification made by [16]. In order to generate $ABTS^+$, 2.45 mmol/L potassium persulfate was added to 7 mmol/L ABTS and the mixture was left to sit at room temperature for 12 to 16 hours in the dark. This stock solution of $ABTS^{\bullet+}$ was diluted with ethanol to give an absorbance of (0.70 ± 0.02) at 734 nm, which acted as a positive control. Ten microliters of crude extract (prepared in ethanol) were mixed with 1.0 mL diluted $ABTS^{\bullet+}$ solution and incubated at 30 °C for 30 minutes. A UV-vis spectrophotometer was used to detect the absorbance at 734 nm. The Trolox standard was also prepared (in ethanol: 0–1.5 mmol/L) to get the concentration-response curve. The unit of Trolox equivalent antioxidant activity (TEA) was defined as the concentration of Trolox with theequivalent antioxidant activity expressed as mmol/g of extracts. The TEA of BHA, rutin, and tannic acid was also measured by $ABTS^{\bullet+}$ method for comparison.

Nitric oxide Radical Scavenging Activity: Nitric oxide radical scavenging potential of the plant extracts were examined according to [17]. All sample extracts or standards (ascorbic acid and quercetin) (500 µg) were mixed with sodium nitroprusside (final concentration 5 mmol/L) in phosphate buffered saline, pH 7.4 to the final volume of 1 mL and incubated at 25 °C for 150 min. After incubation, the reaction mixture was mixed with Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride in 5% ortho phosphoric acid). The absorbance was measured at 540 nm with UV–vis spectrophotometer. The degree of nitric oxide radical scavenging activity (NRSA) was calculated as follows:

$NRSA/\% = A_c - A_s A_c \times 100$ …… equ 2

Where;

A_c is the absorbance of the control;

A_s is the absorbance of the sample.

In vitro Anti-Inflammatory Activity

Heat-Induced Hemolysis: This test followed the guidelines provided by [18]; 4 sets (per concentration) of centrifuge tubes were filled with the isotonic buffer solution (5 ml) containing 100, 200, and 500 g/ml of the aqueous extracts. 5 ml of the vechicle or 5 ml of hydrocortisone 100 ug/ml were included in the control tubes. Erythrocyte suspension (0.05 ml) was added to each tube and gently mixed. A pair of the tubes was incubated in a controlled water bath at 54 °C for 20 minutes. The other pair was maintained at a temperature of 0–4 °C in a freezer for 20 min. At the end of the incubation, the reaction mixture was centrifuged at 1300 g for 3 minutes and the absorbance OD) of the supernatant measured at 540 nm using a UV/VIS spectrophotometer (Optima, SP-3000, Tokyo, Japan). The level of hemolysis was calculated using the following equation:

Inhibition of hemolysis (%) = 100 [1-{(OD2-OD1)/ (OD3-OD1)}] …… equ 3

Where;

OD1 = absorbance of test sample unheated.

OD2 =absorbance of test sample heated.

OD3 = absorbance of control sample heated.

Effect on Protein Denaturation: Protein denaturation assay of the CpRH, CpRE and CpRM was performed according to the method described by [19], 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of the CpRH, CpRE, and CpRM extracts by which the concentrations (µg/ml) became 100, 200 and 500. Then the mixtures were incubated at 37 °C ± 2 °C in a biological oxygen demand incubator for 15 minutes and then heated at 70 °C for 5 minutes. After cooling, their absorbance was measured at 660 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). The reaction mixture (5 mL) consisted of 0.2 mL of 1 % bovine albumin, 4.78 mL of phosphate buffered saline (PBS, pH 6.4), and different concentrations of the extracts, and the mixture was

mixed, and was incubated in a water bath (37 °C) for 15 min, and then the reaction mixture was heated at 70 °C for 5 min. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer (Optima, SP3000, Tokyo, Japan). Diclofenac sodium at the final concentrations (µg/ml) of 100, 200 and 500 were used as reference and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition of denaturation = 100 × (1 − A2/A1) …… equ 4

Where;

A1 = absorption of the control sample, and

A2 = absorption of the test sample.

Proteinase Inhibitory Activity: [20] analyzed the activity of protease inhibition. Aliquots of trypsin and LPI were prepared in 0.2 mol/L phosphate buffer (pH 7.2). One mL aliquot of trypsin (400 U/mL) was pre-incubated with 1 mL of a suitable dilution of the protease inhibitor (containing 0.18 mg/mL protein concentration) at 37 ^{o} C for 1 h. One mL of 0.65 % casein was added to the trypsin-LPI mixture. The reaction mixture was immediately swirled and incubated at 37 ^{o} C. After 10 minutes of incubation, reaction was stopped by addition of 3.0 mL chilled TCA mixture (0.11 M trichloroacetic acid, 0.22 M sodium acetate and 0.33 M acetic acid). The mixture was thoroughly swirled before being let to incubate at room temperature for 30 minutes. Whatman filter paper no. 1 was used to filter the reaction mixture, and the absorbance of the filtrate was recorded at 280 nm. Equally, an assay set without protease inhibitor was analyzed. Each assay was conducted in triplicate. The quantity of enzyme that released 1 µg of tyrosine per mL of the reaction mixture per minute under the assay conditions was defined as one unit of protease activity. Protease inhibitor activity was defined as the difference between the proteolytic activities measured in the absence and presence of inhibitor. The protease inhibitor activity was expressed in terms of percent inhibition.

Lipoxygenase Inhibition Assay: The root extracts of *Combretum platypterum* were tested for their ability to inhibit lipoxygenase using the technique described by [21]. In a 1 mL cuvette, a combination of sodium borate buffer (1 mL, 0.1 M, pH 8.8) and lipoxygenase (10 µL, final

concentration 8000 U/mL) was incubated with varied concentrations of the extracts for 5 minutes at room temperature (30 ± 2 °C). The reaction was initiated by the addition of 10 µL linoleic acid substrate (10 mmol). Using a UV/VIS spectrophotometer, the reaction solution's absorbance was determined at 234 nm (Optima, SP-3000, Tokyo, Japan). Phosphate buffer solution served as the control, and the percentage inhibition of lipoxygenase was calculated using the following equation:

% inhibition = 100 × (absorbance of the control − absorbance of the sample)/absorbance of the control. ……. Equ 5

In vitro Antihyperglycemic Activity

Alpha-amylase Inhibitory Assay: According to a technique previously described by [22], the alpha-amylase inhibitory assay of methanol, ethyl acetate, and n-hexane extracts of *Combretum platypterum* was assessed. In summary, 0.5 ml of extracts was combined with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl).

The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated using 1 ml of dinitro salicylic acid color reagent. The test tubes were then immersed in a water bath (100 °C for 5 minutes) and cooled until room temperature was reached. The mixture was then diluted with 10 ml of deionized water, and absorbance was determined at 540 nm. The absorbance of blank (buffer instead of extract and amylase solution) and control were also determined. Acarbose was used as standard drug. The inhibition of α-amylase was calculated using the following equation:

%inhibition of α−Amylase = Abs (control) – Abs (Sample) / Abs (control) x 100…. equ 6

Where;

$Abs_{control}$ corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α -amylase solution, and

Abs_{sample} corresponds to the solution with extract and α -amylase solution.

***In vitro* Evaluation of Yeast Cell Uptake of Glucose:** Commercial baker's yeast was wash by repeated centrifugation (3,000×g, 5 min) in distilled water until the supernatant fluid was clear. Then a 10 % (v/v) suspension was prepared in distilled water, and different concentrations of extracts (1-5 mg /mM) were added to 1mL of glucose solution, which was then further incubated for 10min at 37^0 C. The reaction was initiated by adding 100 µl of yeast suspension, vortex and further at 37 °C at 60 minutes. Afterward, the tubes were centrifuged (2,500×g, 5 min) and glucose was estimated in the supernatant, metformin was taken as the standard anti-diabetic drug used. All tests were carried out in triplicates and absorbance was measured at 540nm. The formula below was used to determine the percentage increase in glucose uptake by yeast cells.

$$\text{Activity \%} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \quad \text{.....equ7.}$$

Increase in glucose uptake

Where,

$Abs_{control}$ is the absorbance of the control reaction (containing all reagents except the test sample), and

Abs_{sample} is the absorbance of the test sample.

Proximate Composition of *C. platypterum* Whole Root Sample

The result for the proximate composition of *C. platypterum* whole root sample was presented in Table 1.

Table 1: Proximate Composition of *C. platypterum* Whole Root Sample

Parameter	Root Composition (%)
Moisture	4.33± 0.11
Ash	7.55± 0.16

Protein	4.20± 0.40
Fiber	7.63± 0.35
Oil	2.26± 0.05
Carbohydrate	73.90± 0.10

The result whole root sample of *C. platypterum* showed that carbohydrates (73.9000 %) and crude fibre (7.6333%) were the highest concentrations in *C. platypterum* root, followed by ash (7.550 %), moisture (4.3267%), proteins (4.2000%) while oil was the least content (2.260 %).

Qualitative and Quantitative Phytochemical Composition of Whole Root extracts of *C. platypterum*

The results of preliminary qualitative and quantitative phytochemical analyses of whole root extracts of *C. platypterum* were shown in Tables 2 and 3.

Table 2: Qualitative Phytochemical Composition of Whole Root extracts of *C. platypterum*

Phytochemical Constituents	CpRH	CpRE	CpRM
Alkaloids	+	++	++
Saponins	+++	++	+++
Tannins	++	+++	+
Flavonoids	+++	-	-
Steroids	+	-	-

Terpenoids	-	++	-
Cardiac Glycosides	-	+	+
Carbohydrates	+	+	+
Resins	-	-	-
Phenolics	+	+++	+++
Reducing Sugars	-	+	-
Proteins	-	-	-
Anthocyanins	-	+++	+++

Table 3: Results of Quantitative Analyses of Whole Root Sample of *C. platypterum*

Phytochemical Constituents	**Root Quantity (mg/100g)**
Alkaloids	8.82±0.30
Flavonoids	1.06±0.08
Saponins	34.17±0.29
Tannins	18.37±0.55
Phenolics	100.53±3.58

Steroids	0.13±0.02
Terpenoids	3.30±0.03

The result of qualitative phytochemical screening of *C. platypterum* root extracts showed that alkaloids, saponins, carbohydrates, phenolic compounds and tannins were present in all the extracts. Steroids were sparingly present only in n-hexane extract, which was not surprising since "like dissolves like"- hydrophobic steroids would only be soluble in non- polar solvent such as n-hexane. The high abundance of flavonoids only in n-hexane extract showed an absence of hydrophilic flavonoid glycosides such as quercetin [23]. The solubility of the flavonoids in the n-hexane extracts was also in line with the report of Chaves *et al.,* 2020[24] that less hydrophilic flavonoids such as aglycones of isoflavones, flavanones, methylated flavones could be extracted with n-hexane. Tannins were found more in ethyl acetate and n-hexane extracts, and slightly in methanol which was surprising, considering that tannins which are polyphenolic compounds were expected to be most in polar extracts. The polarity of the solvents also played role in the extraction of polyphenolic compounds and anthocyanins [25]. The amphiphilic nature of saponins was also observed in their high abundance in both methanol and n-hexane extracts [26]. Proteins, oils and resins were absent in all the extracts.

The result of quantitative phytochemical analysis of *C. platypterum* root sample showed that phenolics, saponins and tannins were found in high abundance, while flavonoids were least in abundance.

Results of *in vitro* Antimicrobial activities of *C. platypterum* Root Extracts

The results of antibacterial, antifungal and MIC analyses were shown in Tables 4, 5 and 6.

Table 4: Result of Antibacterial Screening of *C. platypterum* Whole Root Extracts (mm)

Extract	*B. subtilis*	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*E. coli*	*S. typhi*

CpRM-5.0 mg/mL	12.17±0.47	0.00±0.00	7.00±0.00	22.2±0.87	10.20±0.26	17.00±0.00
CpRM-2.5 mg/mL	10.00±0.00	0.00±0.00	5.53±0.06	20.00±0.00	6.1±0.17	15.00±0.00
CpRM-1.0 mg/mL	7.00±0.00	0.00±0.00	4.03±0.06	13.00±0.00	3.00±0.00	10.00±0.00
CpRM-0.6 mg/mL	0.00±0.00	0.00±0.00	0.00±0.00	9.53±0.50	0.00±0.00	0.00±0.00
CpRH-5.0 mg/mL	22.40±1.04	0.00±0.00	33.33±1.53	15.33±0.58	22.00±0.00	10.00±0.00
CpRH-2.5 mg/mL	15.73±0.70	0.00±0.00	30.00±0.00	10.00±0.00	13.27±0.23	5.03±0.25
CpRH-1.0 mg/mL	0.00±0.00	0.00±0.00	26.9±0.173	0.00±0.00	6.50±0.50	0.00±0.00
CpRH-0.6 mg/mL	0.00±0.00	0.00±0.00	20.00±0.00	0.00±0.00	3.37±0.40	0.00±0.00
CpRE-5.0 mg/mL	47.7±1.14	0.00±0.00	0.00±0.00	34.67±1.53	26.33±1.16	16.00±0.00
CpRE-2.5 mg/mL	41.00±0.00	0.00±0.00	0.00±0.00	30.00±0.00	19.23±0.21	7.00±0.00
CpRE-1.0 mg/mL	34.33±1.53	0.00±0.00	0.00±0.00	20.33±0.58	10.97±1.00	4.10±0.17
CpRE-0.6 mg/mL	16.07±0.81	0.00±0.00	0.00±0.00	13.4±0.3605	7.54±0.227	0.00±0.00

Ciprofloxacin 50 µg/mL	38.93±1.00	41.67±2.0816	41.67±1.15	22.33±0.57	19.16±0.28	35.57±0.30

Table 5: Result of *in vitro* Antifungal activities of *C. platypterum* Whole Root Extracts (mm)

Extract	*A. niger*	*C. albicans*
CPRM-5.0 mg/mL	5.70±0.26	15.87±0.46
CPRM-2.5 mg/mL	0.00±0.00	12.00±0.00
CPRM-1.0 mg/mL	0.00±0.00	10.00±0.00
CPRM-0.6 mg/mL	0.00±0.00	8.83±1.04
CPRH-5.0 mg/mL	0.00±0.00	32.67±2.08
CPRH-2.5 mg/mL	0.00±0.00	27.67±0.58
CPRH-1.0 mg/mL	0.00±0.00	21.33±0.58
CPRH-0.6 mg/mL	0.00±0.00	19.07±0.81
CPRE-5.0 mg/mL	0.00±0.00	26.33±1.15
CPRE-2.5 mg/mL	0.00±0.00	19.23±0.21
CPRE-1.0 mg/mL	0.00±0.00	10.97±1.00
CPRE-0.6 mg/mL	0.00±0.00	7.54±0.23
Fluconazole 50 µg/mL	12.00±0.00	32.27±1.36

Table 6: Result of Minimum Inhibitory Concentration (mg/mL) of *C. platypterum* Whole Root Extracts

Microorganism	CpRM	CpRE	CpRH	Control Drug
S. typhi	1.20	0.80	1.00	0.10

E. coli	1.00	0.10	0.10	0.50
S. aureus	0.50	0.50	2.00	0.10
P. aeruginosa	1.50	10.00	0.50	0.10
S. pneumonea	-	-	-	0.50
B. subtilis	2.50	0.50	0.50	0.50
C. albicans	0.50	0.50	0.50	0.10

The result of antibacterial analysis of root extracts of *C. platypterum* showed that ethylacetate extract inhibited the growth of *B. subtilis* in a dose-dependent manner. At 5 mg/mL and 2.5 mg/mL, the inhibition of the extract against *B. subtilis* and *S. aureus* was higher than the inhibition of ciprofloxacin at 50 µg/mL, while the methanol and n-hexane extracts showed mild activity against both organisms especially at higher concentrations. At 5 mg/mL, n-hexane and ethylacetate extracts inhibited the growth of *E. coli* more than ciprofloxacin at 50 µg/mL.

K. pneumonia was resistant to the activities of all the extracts in all concentration, while *P. aeruginosa* and *S. typhi* were mildly susceptible to the inhibition of the extracts in a concentration-dependent manner. The inhibition of the *S. aureus* by the methanol extract was against the report of Ogbole *et al.*, 2016[27], who reported that strains of methiciline-resistant *S. aureus* were resistant to methanol extract of the plant.

The result of antifungal analysis of *C. platypterum* root extract showed that *A. niger* was only mildly susceptible to activity of methanol extract of the plant at 5 mg/mL, and was resistant to the inhibitions of other extracts in all concentrations. The resistance of *A. niger* to many concentrations of methanol extract was in line with what was obtained with the methanol leaf extract of the same plant. Ethyl acetate and n-hexane extracts showed no activity against *A. niger* in all concentrations. *C. albicans* was sensitive to the activity of the extracts, with n-hexane extract showing highest activity (32.67±2.08 mm) at 5 mg/mL, higher than the activity of fluconazole (32.67±0.58 mm) at 50 µg/mL. The high activity of the n-hexane extract of the root

against *C. albicans* was against the non-activity result obtained with the n-hexane leaf extract of the same plant.

in vitro Anti-Inflammatory Activities of *C. platypterum* Root Extracts

Table 7: Results of *in vitro* Anti-Inflammatory Activities of *C. platypterum* Root Extracts

Sample concentrations	Heat-Induced Hemolysis(%)	Effect on Protein Denaturation(%)	Proteinase Inhibitory Activity(%)	Lipoxygenase Inhibition Assay(%)
CpRM-250 mg/mL	3.20±0.26	12.27±0.25	24.00±0.0	3.63±0.12
CpRM-500 mg/mL	12.13±0.15	28.33±1.53	17.00±0.00	8.33±0.21
CpRM-1000 mg/mL	30.00±0.00	35.03±0.06	29.40±0.10	12.20±0.17
CpRE-250mg/ml	0.93±0.12	3.33±0.40	2.33±0.29	14.30±0.20
CpRE-500 mg/mL	2.01±0.53	22.50±0.78	11.77±0.40	20.40±0.10
CpRE1000 mg/mL	6.23±0.25	25.17±0.29	26.30±1.56	30.31±0.60
CpRH-250 mg/mL	3.29±0.26	1.157±0.16	2.16±0.30	5.17±0.29
CpRH-500 mg/mL	8.47±0.47	7.60±0.69	7.00±0.00	16.20±0.26
CpRH1000 mg/mL	14.10±0.78	15.33±0.06	20.3±0.2645	27.47±0.90
Diclofenac -250 mg/mL	24.93±0.50	75.88±1.53	35.33±1.1547	45.00±0.00
Diclofenac -500 mg/mL	49.57±0.75	84.37±0.55	63.50±0.71	75.67±1.15
Diclofenac -1000 mg/mL	75±0	88.40±1.55	85.40±0.66	86.33±2.31

The result of anti-inflammatory screening of the root extracts of *C. platypterum* indicated that methanol extract showed anti-inflammatory activity through heat-induced hemolysis, protein denaturation and proteinase inhibition in concentration-dependent manner. At 100 mg/mL, methanol extract showed greater percentage heat-induced hemolysis than diclofenac at 250 mg/mL. The anti-inflammatory activity of the methanolic extracts could be as a result of the

presence of phenolic compounds found in the extract [28]. The ethylacetate extract also inhibited lipoxygenase as well as proteinase in a dose-dependent manner. Lipoxygenase was inhibited by the hexane extract in concentration-dependent manner. The use of the plant in the treatment of inflammation is justified.

Result of *in vitro* Antioxidant Studies of *C. platypterum* Root Extracts

Table 8: Results of *in vitro* Antioxidant Activities of *C. platypterum* Root Extracts

Sample concentrations	Nitric Oxide (%)	DPPH (µg/mL)	FRAP (mgAA/g)	ABTS ASSAY (µmol/EAA/g)
CPRM-1000 mg/mL	0.15±0.00	1.64±0.12	10.68±0.10	1.16±0.10
CPRM-500 mg/mL	0.11±0.00	1.41±0.015	9.74±0.488	0.84±0.03605
CPRM-250 mg/mL	0.083±0.002	1.00±0.04	7.58±0.07	0.42±0.025
CPRE-1000 mg/mL	0.87±0.02	3.10±0.24	15.96±0.04	0.24±0.06
CPRE-500 mg/mL	0.55±0.00	2.06±0.10	9.79±0.05	0.15±0.00
CPRE250 mg/mL	0.18±0.01	1.00±0.00	9.00±0.00	0.00±0.00
CPRH-1000 mg/mL	1.97±0.00	4.40±0.15	23.50±0.7975	1.89±0.12
CPRH-500 mg/mL	1.36±0.02	3.30±0.14	20.06±0.10	1.05±0.06
CPRH250 mg/mL	1.00±0.00	2.95±0.09	19.27±0.25	0.00±0.00
Ascorbic acid-1000 mg/mL	5.37±0.15	45.97±0.96	85.88±0.29	16.20±0.18
Ascorbic acid-500 mg/mL	3.46±0.04	40.07±0.12	80.55±0.03	9.13±0.05
Ascorbic acid-250	2.14±0.16	37.43±0.1	68.15±0.26	5.03±0.06

mg/mL

The result of antioxidant screening showed that the three extracts showed good antioxidant activity through ferric reducing antioxidant power at the highest concentration (1000 mg/mL). The activity of the n-hexane through nitric oxide was comparable to the activity of ascorbic acid which was used as the standard drug.

Result of *in vitro* Antihyperglycemic Assay of *C. platypterum* Root Extracts

Table 9: Results of *in vitro* Anti-Hyperglycemic Activities of *C. platypterum* Root Extracts

sample concentrations	αAmylase inhibitory activities (%)	Glucose uptake (%)
CPRM-1000 mg/mL	23.38±0.78	28.93±0.05
CPRM-500 mg/mL	18.40±0.26	22.27±0.26
CPRM-250 mg/mL	8.26±0.28	17.64±0.16
CPRE-1000 mg/ml	27.54±0.24	31.26±0.96
CPRE-500 mg/mL	23.49±0.43	19.82±0.03
CPRE250 mg/mL	19.87±0.35	13.88±0.30
CPRH-1000 mg/mL	12.67±0.66	8.46±0.42
CPRH-500 mg/mL	7.073±0.34	4.45±0.03
CPRH250 mg/ml	2.39±0.18	1.71±0.45
Metformin -1000 mg/mL	53.27±0.46	68.00±0.00
Metformin -500 mg/mL	47.53±0.90	43.83±1.04
Metformin -250 mg/mL	40.40±0.66	27.00±2.00

The result of antidiabetic activity showed that methanol and ethyl acetate extracts showed good glucose uptake and also inhibit α-amylase in a dose-dependent manner. At 1000 mg/mL,

both extracts were uptake glucose more than the standard drug- metformin at 250 mg/mL with uptake percentage of 28.93±0.05 and 31.26±0.96 respectively. The n-hexane extract only exhibited moderate activity.

Conclusion

Methanol, ethyl acetate and n-hexane extracts showed very good antimicrobial, antioxidant, anti-inflammatory and antidiabetic activities, which may be as a result of the metabolites present in them. Methanol extract showed best activity among the three extracts. Thus, the plant whole root can act as anti-inflammatory, antioxidant and antidiabetic agent.

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