

Phytochemical, Proximate, *in-vitro* Anti-malarial, Anti-inflammatory and Antimicrobial Screening of Leaf Extracts of *Combretum platypterum* (Welw) Hutch & Dalziel

By

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Abstract

Background: *Combretum platypterum* is used ethno medically in the treatment many diseases in Africa, such as; helminthiasis, sexually transmitted diseases, conjunctivitis, malaria, lumps, fever, eye problems, diarrhea, lower backache, coughs, swellings, as febrifuge, tonic, and to stop post-partum bleeding.

Aim: This work was aimed at investigating the phytochemical, proximate, anti malarial, anti inflammatory, anti bacterial and anti fungal activities of leaf extracts of *Combretum platypterum*.

Methods: Leaves of *C. platypterum* were cold extracted with methanol, ethyl acetate and n-hexane. The extracts were subjected to preliminary phytochemical analyses, proximate, anti malarial, anti inflammatory and antimicrobial tests.

Results: The results revealed that, alkaloids, terpenoids, flavonoids, cardiac glycosides, carbohydrates, resins and reducing sugars were found in all the extracts. It was also observed that the presence of the metabolites in each extract was in polarity-dependent manner. The percentage composition of carbohydrates was also the highest followed by the composition of proteins. The suppressions of the extracts against *P. falciparum* at half-maximal inhibitory concentration (IC₅₀ mg/ml) were found to be higher than that of chloroquine at IC₅₀ µg/ml. It was also found that methanol extract has the highest inhibition against Gram positive, Gram negative bacteria and *C. albicans* in a dose-dependent manner. *K. pneumonia* was resistant to the inhibition of the extracts. The anti inflammatory assays showed that methanol extracts at 500 mg/ml exhibited higher lipoxxygenase Inhibition than diclofenac at 500 µg/ml. Ethyl acetate

extract showed highest inhibition heat-induced hemolysis, protein denaturation and proteinase among all the extracts.

Conclusion: The therapeutic use of the leaves of *C. platypterum* against malaria and swelling was confirmed by the activities of the extracts against inflammation and *P. falciparum*. It is also necessary that the bioactive compounds responsible for these activities be isolated.

Keywords: Anti-inflammatory, Anti-malarial, Antimicrobial, *Combretum platypterum*, Phytochemicals, Proximate

Introduction

In developing countries, the use of plants as alternative therapy for the treatment of microbial infections is significant in the maintenance of good health. About 85% of the world population still depends on the medicinal plants as their source of primary health care. These plants have been reported to be a resource of drug discovery and 80% of synthetic drugs were derived from the plants (1). *Combretum platypterum* (Welw.) Hutch. & Dalziel belongs to the family Combretaceae which is known to contain many secondary metabolites and have many pharmacological activities (2). *Combretum platypterum* is used ethno medically in treating helminthiasis, sexually transmitted diseases, conjunctivitis, malaria, lumps, fever, eye problems, diarrhea, lower backache, coughs, swellings, as febrifuge, tonic, and to stop post-partum bleeding (3). The Nigeria, paste made from the leaves and leaves of other plants is used in treating eye problems, the decoction of the root is used in the treatment of malaria (3-5). Some traditional herbal medicine practitioners claim that decoction of the leaves of *Combretum platypterum*, *Justicia carnea* and *Boerhavia diffusa* is used in the treatment and management of malaria, inflammation and spontaneous abortion. Some members of the genus *Combretum* have been indicated to possess antibacterial, antioxidant, anti-fungal, antiviral, analgesic, diuretic and nephroprotective properties (2-5). However, no much scientific investigation has been carried out for *Combretum platypterum* to validate its medicinal uses. To address this gap in knowledge, the present study was carried out to investigate the phytochemical, proximate and medicinal properties of leaf extracts of *Combretum platypterum*.

MATERIALS AND METHODS

Collection and identification of the plant

The leaves of *Combretum platypterum* were collected within the surroundings of the Adada River in Nsukka Local Government Area, Enugu State, Nigeria. Mr. Alfred Ozioko (The Chief

Taxonomist) of International Center for Ethno medicine and Drug Development identified and authenticated the leaves. Herbarium specimens were deposited in the herbarium of the International Center for Ethno medicine and Drug Development (Voucher number: Intercedd/260510).

Extraction procedure

The extraction was carried out using a cold extraction method. The *Combretum platypterum* leaves were air-dried at room temperature and ground. 1 kg of the sample was then macerated with 6 liters each of methanol, ethyl acetate and n-hexane for 48 hours in an air-tight container. The mixtures were filtered with a glass funnel embedded with cotton wool into a beaker. The filtrates were concentrated by evaporating in a rotary evaporator at 40°C to remove the solvents and labeled CpLM (methanol extract), CpLE (ethyl acetate extract) and CpLH (n-hexane extract).

Proximate Analysis

Moisture content determination

Moisture content was determined according to the standard method of Association of official analytical chemists (AOAC) 2010 [6]. A petri-dish was washed and dried in the oven. Exactly 2 g of the sample was weighed into petri dish. The weight of the petri dish and sample is noted before drying. The petri-dish containing the sample was put in the oven and heated at 100 °C for 1 hour, the result was noted and then heated another 1 hour until a steady result was obtained and the weight noted. The drying procedure was continued until a constant weight was obtained.

$$\% \text{ moisture content} = \frac{(W1 - W2) \times 100}{Wt} \quad \text{Equation 1}$$

Where: W1 = weight of petri dish and sample after drying

W2 = weight of petri dish

Wt = weight of sample

Ash content determination

Ash content was determined according to the standard method of AOAC (2010). Empty platinum crucible was washed, dried and the weight noted, exactly 2 g of wet sample was weighed into the platinum crucible and placed in a muffle furnace at 500 °C for 3 hours. The sample was cooled in desiccators after burning and weighed.

$$\% \text{ Ash content} = \frac{W3 - W1}{W2 - W1} \times \frac{100}{1} \text{ :..... Equation 2}$$

Where: W3 = weight of crucible and ash

W2= weight of crucible and sample

W1 = weight of crucible

Fiber content determination

Fiber content was determined according to the standard method of AOAC (2010). About 2 g of the sample was defatted with petroleum ether (if the fat content was more than 10 %). The sample was boiled under reflux for 30 minutes with 100 ml of a solution containing 1.25% of H₂SO₄ per 100 ml of solution. The solution was filtered through several layers of cheese cloth on a fluted funnel, washed with boiling water until the washings were no longer acid. The residue was transferred into a beaker and boiled for 30 minutes with 100 ml of a solution containing 1.25 NaOH per 100 ml. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, dried in an electric oven and weighed, incinerated, cooled and weighed.

The loss in weight after incineration x 100 is the percentage of crude fiber

$$\% \text{ crude fiber} = \frac{\text{weight of fiber}}{\text{Weight of sample}} \times 100 \text{ : Equation 3}$$

Weight of sample

Protein content determination

Protein content was determined according to the standard method of AOAC (2010). Exactly 0.5 g of sample was weighed into a 30 ml kjedahl flask (gently to prevent the sample from touching the walls of the side of each and then the flasks was stoppered and shaken). Then 0.5 g of the kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling, about 100 ml of distilled water was added to avoid caking, 50 ml was transferred to the Kjedahl distillation apparatus. A 100 ml receiver flask containing 5 ml of 2 % boric acid and indicator mixture containing 5 drops of bromocresol blue and 1 drop of methylene blue was placed under a condenser of the distillation apparatus so that the tap was about 20 cm inside the solution. The 5 ml of 40 % sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until 50 drops got into the receiver flask, after which it was titrated to pink colour using 0.01 N Hydrochloric acid.

Calculations:

$$\% \text{ Nitrogen} = \frac{\text{Titre value} \times 0.01 \times 14 \times 100}{\text{Weight of sample}} : \dots\dots\dots \text{Equation 4}$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25$$

Fat content determination

Fat content determination was determined according to the standard method of AOAC (2010). 250 ml clean flask was dried in oven at 105 to 110 °C for about 30 minutes. It was transferred into a desiccator, allowed to cool and the weight noted. The flask was filled with about 300 ml of petroleum ether (boiling point 60 °C), the extraction thimble was plugged lightly with cotton wool and soxhlet apparatus assembled. The set-up was allowed to reflux for about 6 hours. The thimble was removed with care and petroleum ether collected in the top container of the set-up, drained into a container for re-use. When flask was almost free of petroleum ether, It was removed and dried at 105 to 110 °C for 1 hour, transferred from the oven into a desiccator and allowed to cool. The % of the fat was calculated as:

$$\% \text{ fat} = \frac{\text{weight of fat}}{\text{Weight of sample}} \times 100 : \dots\dots\dots \text{Equation 5}$$

Carbohydrate content determination

Carbohydrate content was determined according to the standard method of AOAC (2010).

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Protein} + \% \text{ Moisture} + \% \text{ Ash} + \% \text{ Fat} + \% \text{ Fibre}) : \dots\dots \text{Equation 6}$$

Phytochemical Analysis

The preliminary qualitative phytochemical analysis for the presence of flavonoids, alkaloids, terpenoids, saponins, carbohydrates, resins, tannins, phenolic compounds, reducing sugars, cardiac glycosides, proteins, was done using standard method as described by Ayoola et al., 2008; Yadavalli *et al.*, 2018; Khlif et al., 2015; Edeoga et al, 2005; Fayaz et al, 2017; Mera et al., 2019; Gul et al, 2017; Banu et al., 2015; Roghini and Vijayalakshmi, 2018; Keo et al., 2017; Usman et al, 2009 (7-17), with little modifications. .

Quantitative Phytochemical Analysis

The quantitative analysis was carried out according to the methods described by Raaman, 2006; Ukoha et al., 2011; Senguttuvan et al, 2014; Sankhalkar and Vernekar, 2016; Anarado et al, 2020 (18-22) with slight modifications

Alkaloids determination

Into a 250 ml beaker, 5 g of the powdered sample and 200 ml of 10% acetic acid in ethanol were added. The mixture was stood for four (4) hours at room temperature (25°C). Thereafter, the mixture was filtered through Whatmann filter paper No. 42. The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonium hydroxide solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the residue on the filter paper is the alkaloid, which is dried in the oven at 80°C. The alkaloids content was calculated and expressed as a percentage of the weight of the sample analyzed (20, 22).

% weight of Alkaloid = $(W_2 - W_1) / \text{weight of the sample} \times 100/1$: Equation 7

Where,

W1 = weight of filter paper

W2 = weight of filter paper + alkaloid precipitate
(residue)

Flavonoids determination

With 100 ml of 80% aqueous methanol, 10 g of the plant sample was extracted at room temperature. The whole solution was filtered through Whatmann filter paper No. 42 (125 mm). The filtrate was transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (22).

% Flavonoids = $(W_2 - W_1) / \text{weight of the sample} \times 100/1$: Equation 8

Where,

W1= Weight of crucible

W2= Weight of crucible + Flavonoid extract
(residue)

Determination of Saponins

Into a conical flask, 20 g of the sample and 100 cm³ of 20 % aqueous ethanol were added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in water bath. After evaporation, the sample was dried in oven to a constant weight. The saponins content was calculated in percentage (22).

$$\% \text{ Saponins} = (W_2 - W_1) / \text{weight of the sample} \times 100/1: \dots\dots\dots \text{Equation 9}$$

Where,

W₁= Weight of filter paper

W₂= Weight of filter paper + Saponin extract
(residue)

Tannins Determination

Into a 50 ml plastic bottle, 500 mg of the sample was added. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml of volumetric flask and made up the mark. Then 5 ml of the filtrate was added into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes (19-20).

$$\% \text{ Tannin} = (A_n/A_s) \times C \times (100/W) \times (V_f/V_n): \dots\dots\dots \text{Equation 10}$$

Where:

A_n= Absorbance of test sample

A_s= Absorbance of standard solution

C= Concentration of standard solution

W= Weight of sample

V_f= Total volume of extract

V_n= Volume of extract analyzed

Total phenolics content

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent. The plant extract (0.5 mL) was mixed with 0.5 mL of FC reagent (1:1 diluted with distilled water) and incubated for 5 min at 22 °C followed by addition of 2 mL of 20 % Na₂CO₃. The mixture was then incubated further at 22 °C for 90 min and the absorbance was measured at 650 nm. The total phenolic content (mg/mL) was calculated using gallic acid as standard (21).

In vitro antiplasmodial assay against *P. falciparum* strains

The method used to measure parasite viability was the parasite lactate dehydrogenase activity (pLDH) method of Makler et al. 2013 (23). Each extract was screened for in vitro antiplasmodial activity using the chloroquine sensitive (CQS) D10 strain. Antiplasmodial activities of extracts were investigated against the chloroquine sensitive (CQS) and chloroquine resistant (CQR) strain of *P. falciparum*.

Extracts were serially diluted two-fold in complete medium up to a concentration of 0.5 µg/ml using a flat-bottomed 96-well microtitre plate. CQ was taken as a positive control drug and was tested at a starting concentration of 0.5 µg/ml. Unparasitised erythrocyte (RBC) was added to column 1 (blank) which had no drugs, while parasitized red blood cells (pRBC) were added to columns 2 to 12. The plate was gassed for 2 min and incubated for 48 h. A final hematocrit and parasitemia of 2 % was used for all experiments. Parasite growth in the wells containing different extract was compared to control wells. The IC₅₀ recorded in this study is the mean of 3 independent experiments.

Statistical analysis and data evaluation

To ascertain the absorbance of each well from the in vitro antiplasmodial experiment, plates were read when the colour changes from yellow to purple, using a microplate reader at 590 nm. The percentage parasite survival and the concentration that inhibits the growth of parasites by 50 % were determined by measuring the conversion of NBT by *P. falciparum*. This was achieved by analyzing the readings from the microplate reader using Microsoft Excel® 2002, and the IC₅₀ value was determined using a non-linear dose response curve fitting analysis in Graph.

Antimicrobial Analysis

Samples

Three samples of plant extracts were analyzed. These include: CpLM, CpLH, and CpLE.

The antimicrobial sensitivity of both the crude and prepared concentrations of the plant extract was determined using the agar-well diffusion method (24-25)

Test organisms

Strains of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus pneumonia*, *Candida albicans* and *Aspergillus niger* were used in this study. These were obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

Culture Media and Other Reagents used in the Microbiological Analyses

Culture media used were Nutrient agar, Nutrient Broth, Mueller Hinton Agar, Sabouraud Dextrose Agar and Sabouraud Dextrose Broth (Oxoid Limited, England). Culture media were prepared according to the instructions of the manufacturers.

Preparation of Stock/Working Solutions

For the primary antimicrobial screening of the samples, stock solutions were prepared by dissolving 15 mg of each sample in 3 mL of DMSO to obtain 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 attain a working concentration of 20 mg/mL. These were transferred to a screw capped bottle and stored at 4 °C.

Primary Screening of the Samples for Antibacterial and Antifungal Activity

The antibacterial and antifungal activities of the samples were determined by the agar well diffusion method. Dilutions of 2.5, 1.25, and 0.625 mg/mL were prepared from the 5 mg/mL stock solution of each sample in a 2-fold dilution process. Twenty (20) mL of molten Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (for bacterial and fungal isolates respectively) were poured into sterile Petri dishes (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 made in the agar plates using a sterile metal cork-borer. Twenty (20 µl) of the various dilutions of the samples and controls were put in each hole under aseptic condition, kept at room temperature for 1 hour to allow 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 at 37 °C for 24 hours, and the SDA plates were incubated at room temperature (25-27 °C) for 24-72 hours. The inhibition zones 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 calculated and recorded.

Determination of Minimum Inhibitory Concentration (MIC) of the Samples on Test Isolates

The MICs of the samples on the test isolates were determined by the agar dilution method. The stock solution (20 mg/mL) of each sample was further diluted in a 2-fold serial dilution to obtain the following concentrations: 10, 5, 2.5, 1.25, and 0.625 mg/mL. Agar plates were prepared by pouring 4 mL 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 grown 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 °C for 24 hours and the SDA plates were incubated at room temperature (25-27 °C) for 24-72 days, after which all plates were observed for growth. The minimum dilution (concentration) of the samples completely inhibiting the growth of each organism was taken as the MIC.

IN VITRO ANTI-INFLAMMATORY ACTIVITY

a. Heat-Induced Hemolysis

This test was carried out as described by Okoli et al. (2008) (26). Briefly, the isotonic buffer solution (5 ml) containing 100, 200 and 500 µg/ml of the aqueous extracts were put in 4 sets (per concentration) of centrifuge tubes. Control tubes contained 5 ml of the vehicle or 5 ml of 100 µg/ml of hydro-cortisone. Erythrocyte suspension (0.05 ml) was added to each tube and gently mixed. A pair of the tubes was incubated at 54 °C for 20 min in a regulated water bath. The other pair was maintained at 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 min and the absorbance (OD) of the supernatant measured at 540 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan).

The level of hemolysis was calculated using the following equation Inhibition of hemolysis (%) = $100 [1 - \{(OD2 - OD1) / (OD3 - OD1)\}]$ Where OD1 = absorbance of test sample unheated; OD2 = absorbance of test sample heated; OD3 = absorbance of control sample heated.

b. Effect on Protein Denaturation

Protein denaturation assay of the CpLH, CpLE and CpLM was performed according to the method described by Banerjee et al, 2014 (27). 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 CpLH, CpLE, and CpLM 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, SP-3000, Tokyo, Japan). Diclofenac sodium at the final concentrations ($\mu\text{g}/\text{ml}$) 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:

$$\% \text{ inhibition of denaturation} = 100 \times (1 - A2/A1): \dots\dots\dots \text{Equation 11}$$

where A1 = absorption of the control sample, and A2 = absorption of the test sample.

c. Proteinase Inhibitory Activity

Protease inhibition activity was analyzed by Dabhade et al, 2013 (28). 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 °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 °C. After 10 minutes of incubation, reaction was arrested 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 content was mixed thoroughly by swirling and incubated at room temperature for 30 minutes. The reaction mixture was filtered through Whatman filter paper no.1 and the absorbance of the filtrate recorded at 280 nm. Similarly, an assay set without protease inhibitor was analyzed. Each assay was conducted in triplicate. One unit of protease activity was defined as the amount of enzyme that liberated 1 μg of tyrosine per mL of the reaction mixture per min under the assay conditions. 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 per cent inhibition

d. Lipoxygenase Inhibition Assay

Lipoxygenase inhibition activity of the extracts of leafy vegetables was assayed according to the method of Anosike et al. (2012) (29). Briefly, a mixture of a solution of sodium borate buffer (1 mL, 0.1 M, pH 8.8) and lipoxygenase (10 μL , final concentration 8000 U/mL) was incubated different concentrations of the extracts in a 1 mL cuvette at room temperature (30 ± 2 °C) for 5 min. The reaction was initiated by the addition of 10 μL linoleic acid substrate (10 mmol). The absorbance of the reaction solution was measured at 234 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). Phosphate buffer solution was used as the control, and the percentage inhibition of lipoxygenase was calculated using the following equation:

% inhibition = $100 \times (\text{absorbance of the control} - \text{absorbance of the sample}) / \text{absorbance of the control}$:..... Equation 12

Results and Discussions

Table 1: Results of Qualitative Analysis of Leaf Extracts of *C. platypterum*

Phytochemical Constituents	CpLH	CpLE	CpLM
Alkaloids	+	++	+++
Saponins	-	+++	+
Tannins	-	++	+++
Flavonoids	+	+++	+
Steroids	+++	+	-
Terpenoids	+	+	+
Cardiac Glycosides	+	+	+
Carbohydrates	+	+	+
Resins	+	++	+
Phenolics	-	++	+++
Reducing Sugars	+	+	+
Proteins	+	+	+

+++ : highly present, ++ : moderately present, + : low, - : absent

Table 2: Results of Quantitative Analysis of Leaf Sample of *C. platypterum*

Phytochemical Constituents	Quantity (%)
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Alkaloids	8.22
Flavonoids	8.62
Saponins	12.25
Tannins	15.70
Phenolics	17.44
Steroids	6.53

Discussions

The results of preliminary qualitative phytochemical analysis showed that alkaloids, flavonoids, cardiac glycosides, carbohydrate, resins were found in all the extracts (table 1). It was also observed that the polarity of the solvents used played a role in the metabolites found in some extracts. Example was seen in the presence of tannins and phenolic compounds where the abundances of the metabolites increased with increase in polarity of the solvent used. The absence of tannins and phenolics in the n-hexane leaf extract and their presence in the methanol extract is in line with the previous work done by Thavamoney et al., 2018 (30), that the solubility of phenolic compounds increased with increasing solvent polarity. Also same was also observed where the abundance of the steroids decreased with increase in polarity of the solvents, methanol extract contained all the secondary metabolites except steroids, which was not surprising since steroids are known to be hydrophobic (31-32). The ethno-medicinal use of the plant in treating malaria could be as result of the presence of alkaloids, saponins, terpenoids and flavonoids (33-39) found in the plant. The use was also confirmed by the research of Wande and Babatunde, 2017 (40).

Proximate Composition of *C. platypterum* Leaf sample

The results for the proximate composition of CPL are presented in table 3 and means plot showed in fig 1. The results showed that carbohydrate (66.32 %) and protein (14.62 %) were the

highest concentrations in CPL, followed by moisture (6.16 %), crude fiber (5.51 %), ash (4.69 %) while oil was the least content (2.68 %).

Table 3: Proximate Composition of *C.platypterum* Leaf

Parameters	Composition (%)
Moisture	6.1667± 0.28868 ^c
Ash	4.6900± 0.37723 ^d
Protein	14.6200± 0.10583 ^b
Fiber	5.5100± 0.49122 ^{cd}
Oil	2.6867± 0.38280 ^e
Carbohydrate	66.3267± 0.75963 ^a

*Values are mean scores ± Standard deviation of three (3) replicates

*Data in the same column bearing different superscript differ significantly (p < 0.05).

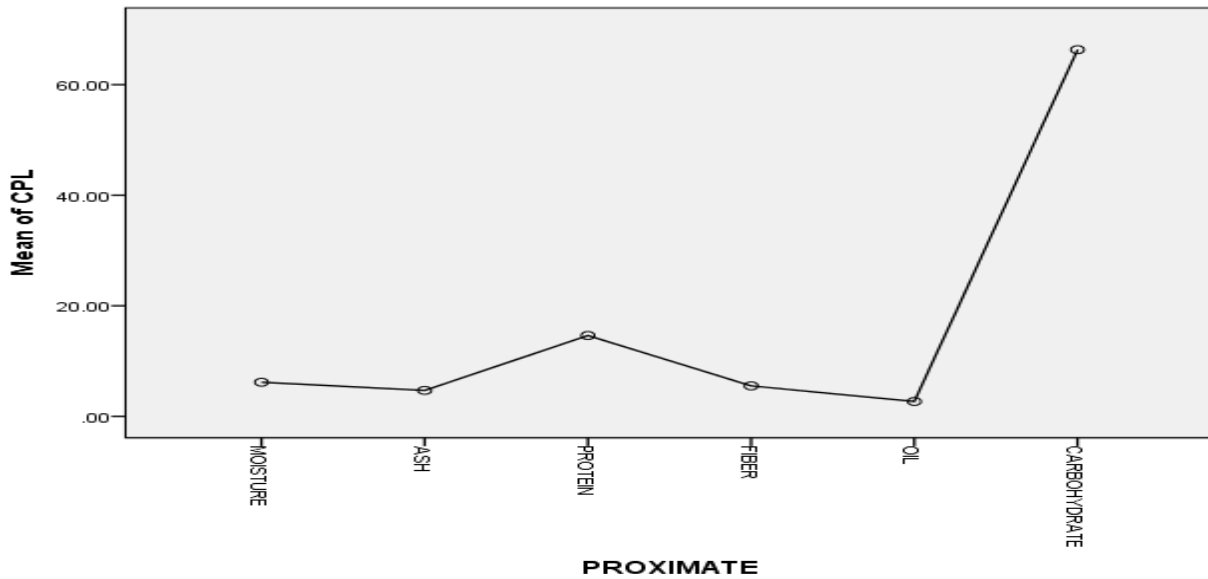


Fig 1: Means Plots for the proximate composition of *C.platypterum* Leaf sample

***In-vitro* Anti-Plasmodial Efficacy of *C. platypterum* Extracts**

The anti malaria efficacy of the three extracts of CPL and control (chloroquine) are presented in table 4. Percentage inhibition or suppression analysis showed that the observed percentage parasitemia reduction due to the extracts was not significantly different ($P > 0.05$) from the positive control with CPLE (IC_{50} mg/ml) having the highest efficacy. The percentage suppression observed for all the extracts did exceed the inhibition by the control drug. The inhibition *in-vitro* exhibited by the methanol extract in this study supported findings of Ogbole et al, 2019 (41) that the methanol extract of the plant also showed antiplasmodial activity *in-vitro*. Similarly, acetone extract of *C. platypterum* showed also a higher suppression against the parasite than the chloroquine (40). But contrary to the report of Wande and Babatunde, 2017 that methanol extract of *C. platypterum* showed no activity *in-vitro* at 25 mg/ml, methanol extract from this study showed a higher activity than chloroquine at (IC_{50} mg/ml). This result also validated the therapeutic use of the plant as anti malaria agent.

Table 4: *In-vitro* Antiplasmodial Efficacy of *C. platypterum* Extracts

Extract/Control	Efficacy
CPLH (IC_{50} mg/ml)	9.0967± 0.09 ^c
CPLM (IC_{50} mg/ml)	15.1667± 0.28 ^b
CPLE (IC_{50} mg/ml)	21.2333± 0.11 ^a
Chloroquine(IC_{50} µg/ml)	4.5833± 0.38 ^d

*Values are mean scores ± Standard deviation of three (3) replicates

*Data in the same column bearing different superscript differ significantly ($p < 0.05$).

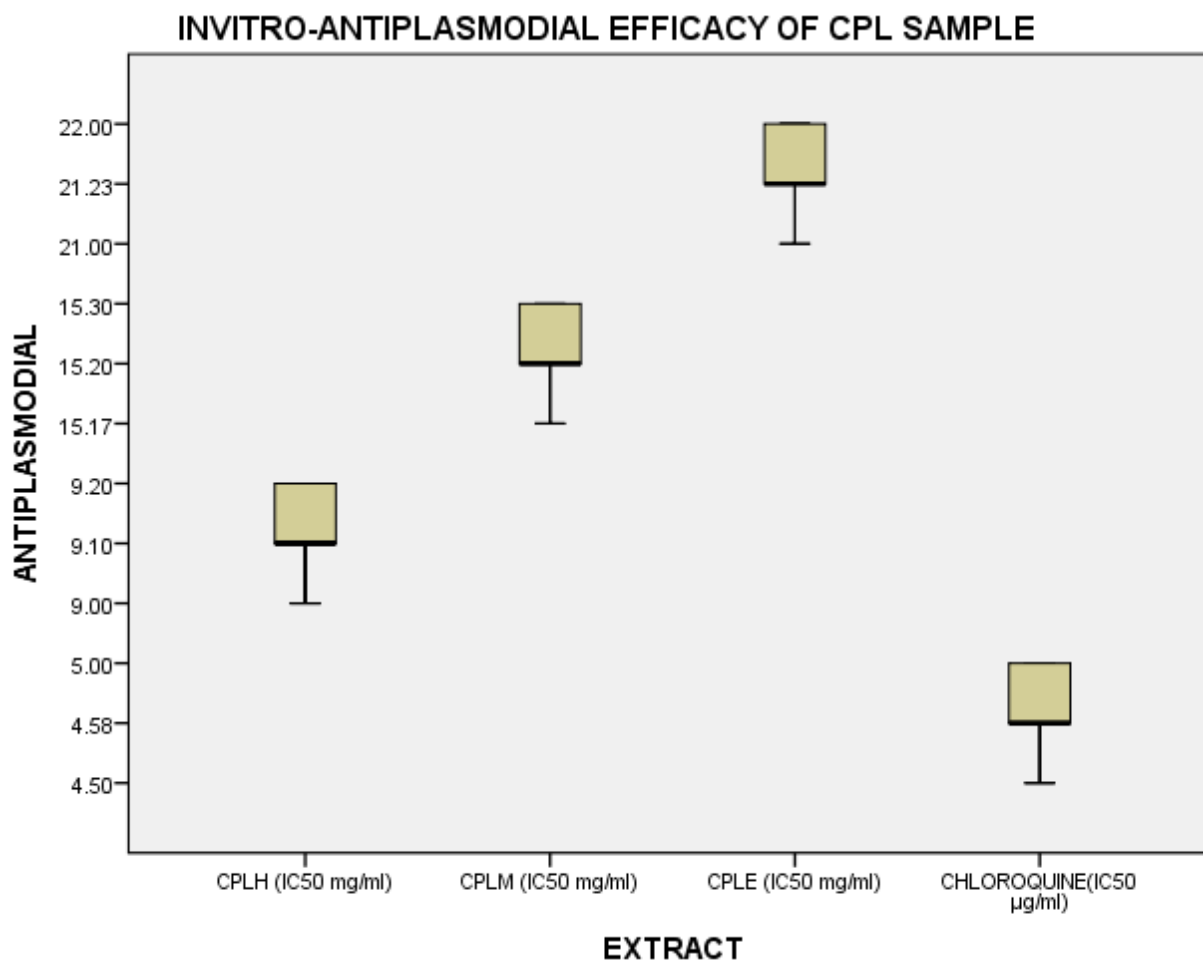


Fig 2: Mean Plots for the *In-vitro* Antiplasmodial Efficacy of *C. platyterum* Extracts

Antibacterial Activity of *C. platyterum* Extracts

Three CPL extracts studied with four different concentration levels on both gram positive and gram negative bacteria, formed four subsets in accordance with the zone of inhibition values. A one-way ANOVA was conducted to compare the effect of *In-vitro* antibacterial activity of compounds. From Table 5, we have found a statistically significant result. Since the result was

significant, we computed a Turkey HSD test to compare the compound pair wise. From Table 5 it was observed that the in-vitro antibacterial activity of control (Ciprofloxacin), was significantly different from all other compounds. It was evident from the ANOVA that the antimicrobial efficacy of CPL samples was dose dependent as at 5.0 concentrations, the extracts exhibited higher inhibition zones than at lower concentrations. The inhibition of growths of *B. subtilis*, *E.coli*, *P. aeruginosa* could be attributed to the presence of alkaloids which have been reported to inhibit the bacteria by perturbing bacterial FtsZ Z-ring formation and inhibiting bacterial cytokinesis (42). It was not surprising that methanol leaves extract showed inhibition against *S. typhi* since it also contained great amount of tannins which *S. typhi* was said to be sensitive to (43). The presence of reducing sugars in all the three extracts can also be said to be responsible for the antimicrobial activity of some of the extracts (44). The non sensitivity of Methicillin resistance *Staphylococcus aureus* against methanol leaves extract of *C. platypterum* (45) is against our findings, since our methanol extract showed inhibition against the *S. aureus*.

Table 5: In-vitro antibacterial activity of *C. platypterum* Extracts

EXTRACT	<i>S. typhi</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumonia</i>	<i>Bacillus spp</i>
CPLM5mg/ml	15.36± 0.77 ^b	14.46± 0.00 ^b	19.73± 0.77 ^b	28.13± 0.79 ^d	0.00± 0.00 ^b	28.13± 0.77 ^b
CPLM2.5mg/ml	15.03± 0.26 ^c	14.10± 0.00 ^b	18.00± 0.26 ^c	17.00± 0.21 ^e	0.00± 0.00 ^b	22.20± 0.26 ^c
CPLM1.5mg/ml	12.00± 1.02 ^d	11.00± 0.00 ^b	15.67± 1.02 ^c	13.87± 0.00 ^e	0.00± 0.00 ^b	20.86± 1.02 ^d
CPLM0.6mg/ml	10.01± 0.50 ^f	7.90± 0.00 ^b	15.03± 0.50 ^f	0.00± 0.30 ^d	0.00± 0.00 ^b	14.46± 0.50 ^f
CPLE5mg/ml	0.00± 0.00 ^d	17.27± 0.00 ^b	13.13± 0.23 ^d	0.00± 0.00 ^d	0.00± 0.00 ^b	28.10± 0.23 ^b
CPLE2.5mg/ml	0.00± 0.00 ^d	17.00± 0.00 ^b	10.13± 0.00 ^c	0.00± 0.00 ^d	0.00± 0.00 ^b	17.00± 0.00 ^b
CPLE1.5mg/ml	0.00± 0.00 ^d	15.17± 0.00 ^b	10.00± 0.00 ^f	0.00± 0.00 ^d	0.00± 0.00 ^b	13.87± 0.77 ^g
CPLE0.6mg/ml	0.00± 0.00 ^d	11.30± 0.00 ^b	8.53± 0.00 ^g	0.00± 0.00 ^d	0.00± 0.00 ^b	.00± 0.00 ^j
CPLH5mg/ml	0.00± 0.00 ^d	9.00± 0.00 ^b	8.40± 0.00 ^g	28.13± 0.42 ^c	0.00± 0.00 ^b	18.03± 0.05 ^e
CPLH2.5mg/ml	0.00± 0.00 ^d	7.20± 0.00 ^b	7.57± 0.00 ^g	22.20± 0.23 ^d	0.00± 0.00 ^b	14.37± 0.10 ^f
CPLH1.5mg/ml	0.00± 0.00 ^d	0.00± 0.00 ^b	7.00± 0.00 ^g	20.87± 0.00 ^d	0.00± 0.00 ^b	10.51± 0.20 ^h
CPLH0.6mg/ml	0.00± 0.00 ^d	0.00± 0.00 ^b	4.67± 0.00 ^g	14.46± 0.47 ^e	0.00± 0.00 ^b	8.03± 0.50 ⁱ
Ciprofloxacin50µg	34.83± 1.00 ^a	19.1± 1.15 ^a	22.33± 0.57 ^a	41.66± 0.57 ^a	23.66± 1.15 ^a	38.93± 1.00 ^a

*Values are mean scores ± Standard deviation of three (3) replicates

*Data in the same column bearing different superscript differ significantly (p < 0.05).

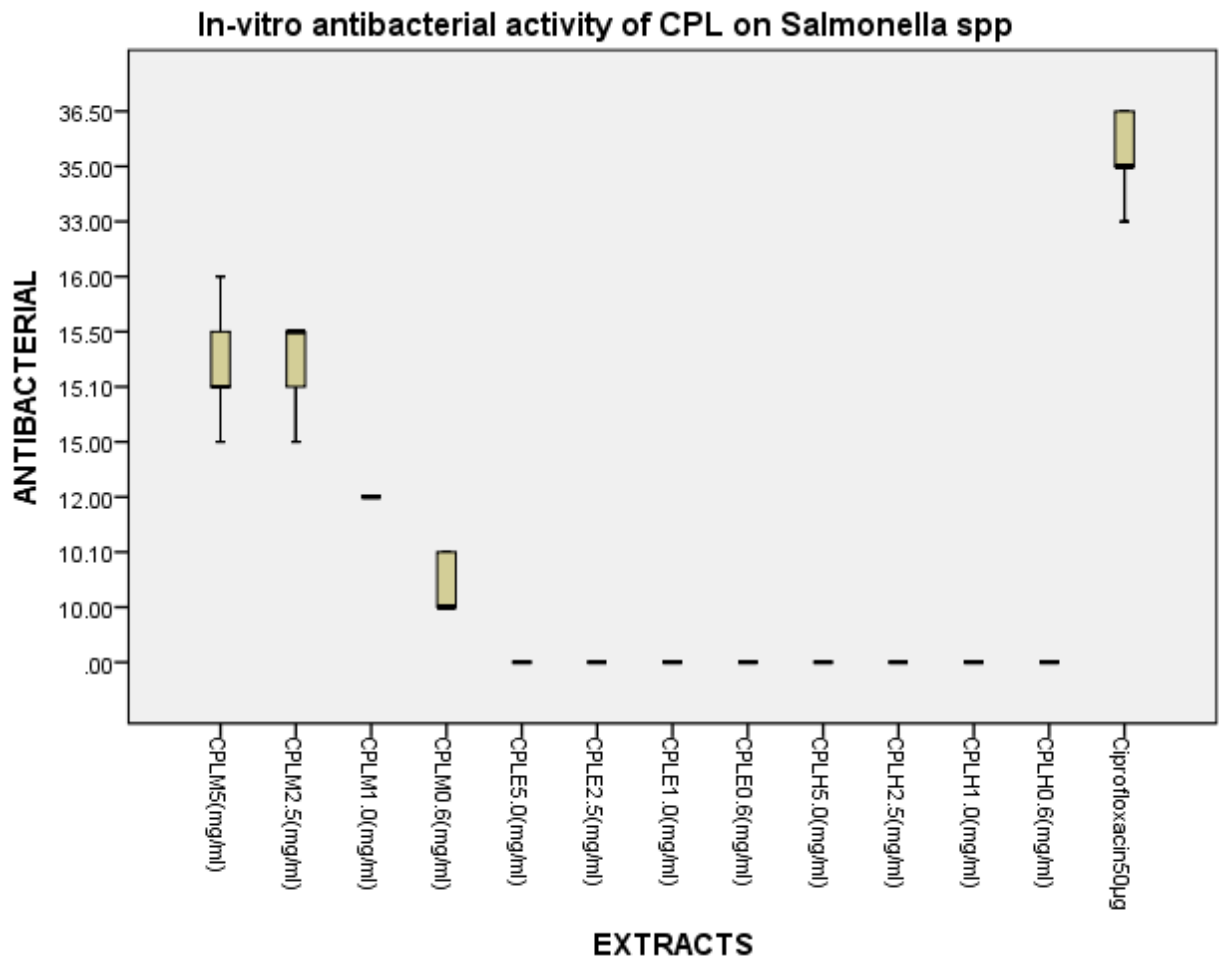


FIG 3: Box -plots represent Zone of inhibition in mm of the Extracts against *S. typhi*

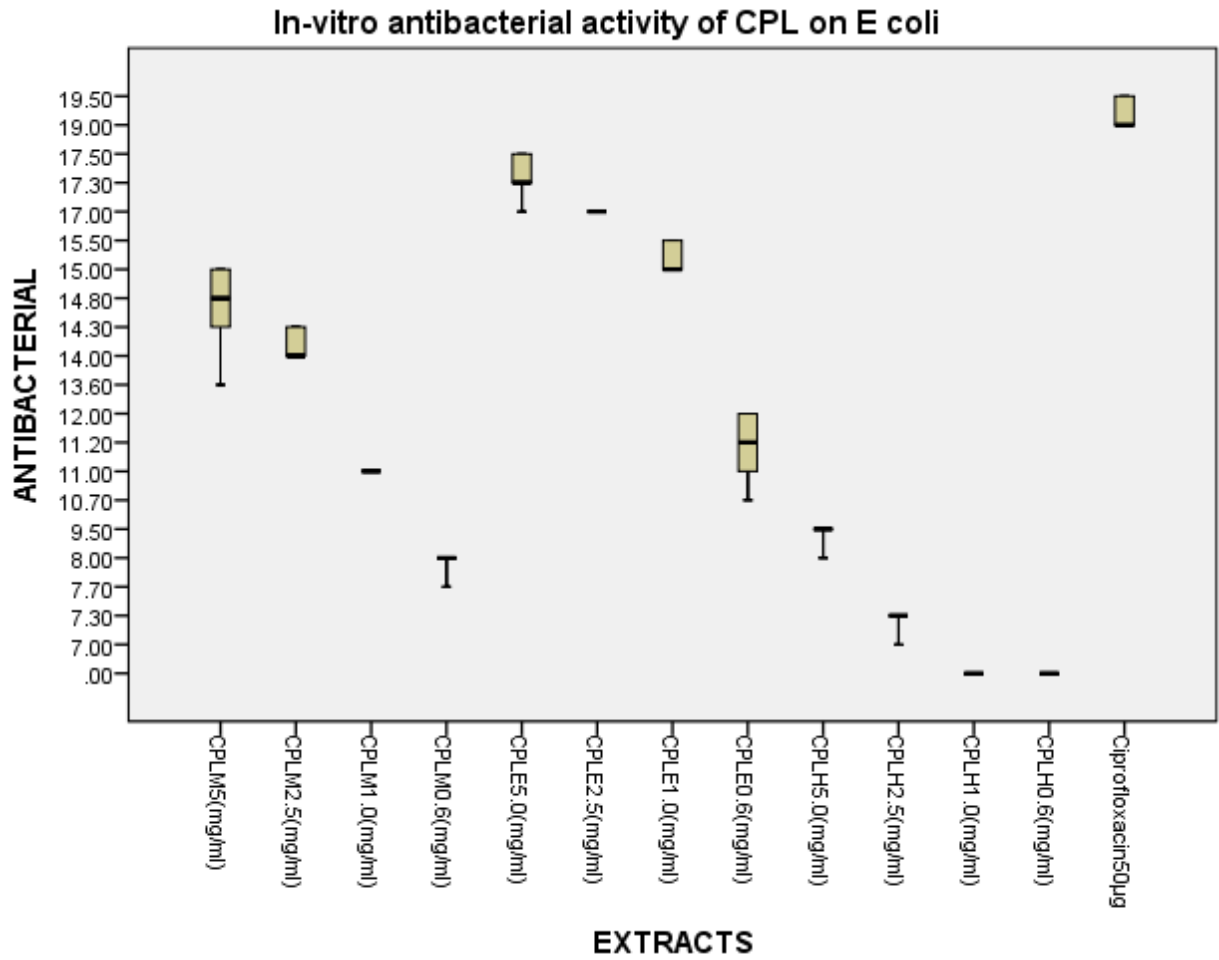


FIG 4: Box -plots represent Zone of inhibition in mm of the Extracts against *E. coli*

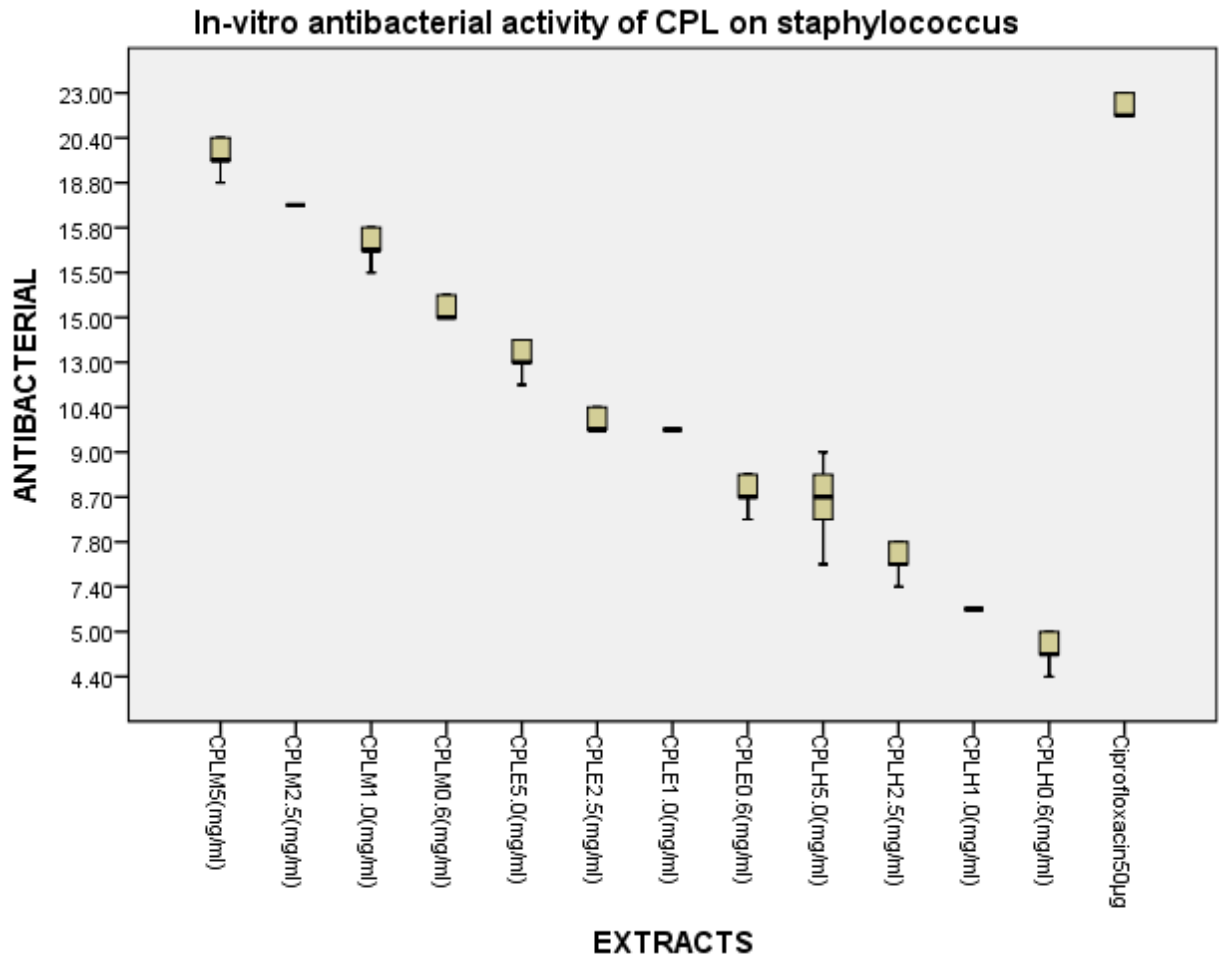


FIG 5: Box -plots represent Zone of inhibition in mm of the Extracts against *S. aureus*

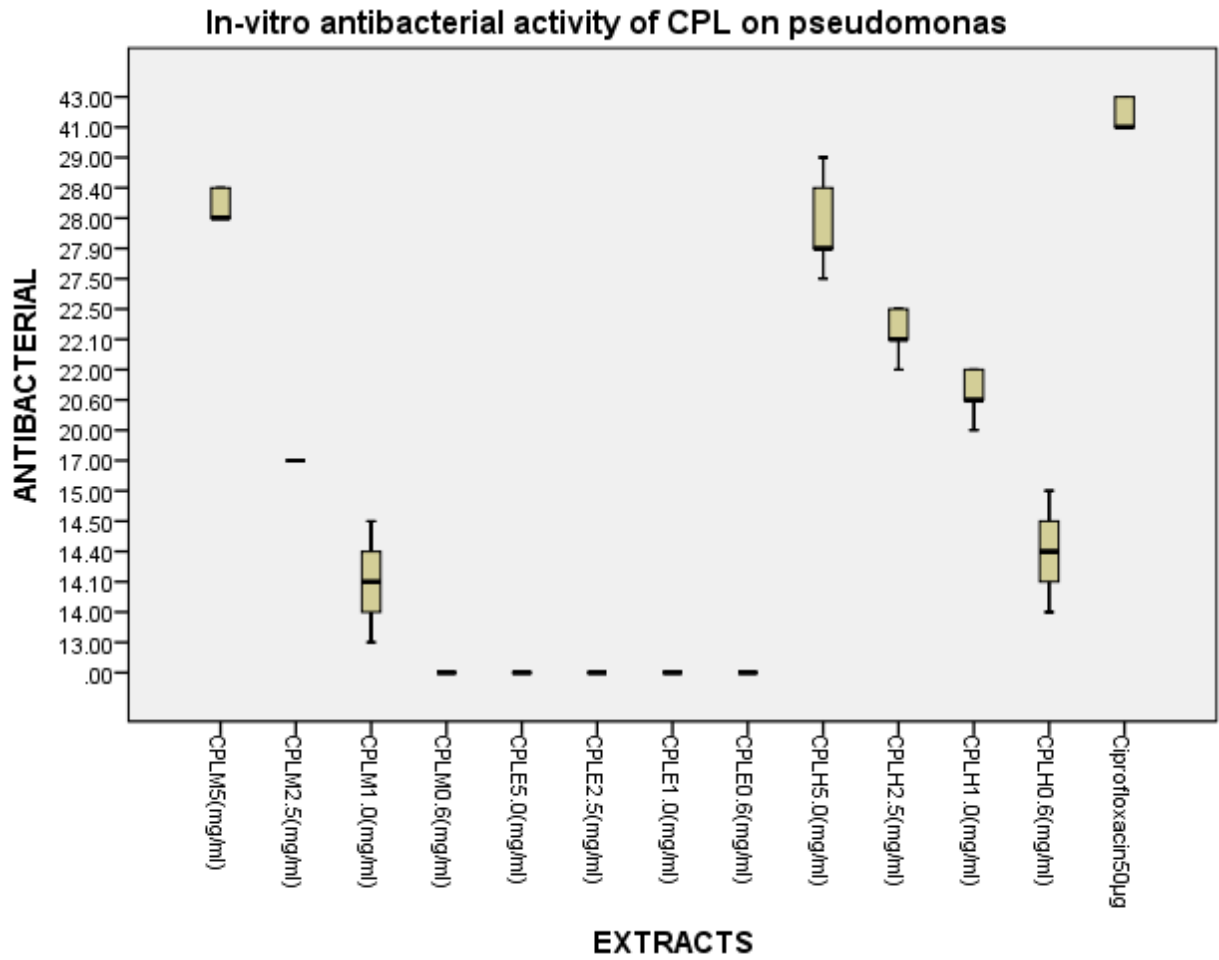


FIG 6: Box -plots represent Zone of inhibition in mm of the Extracts against *P. aeruginosa*

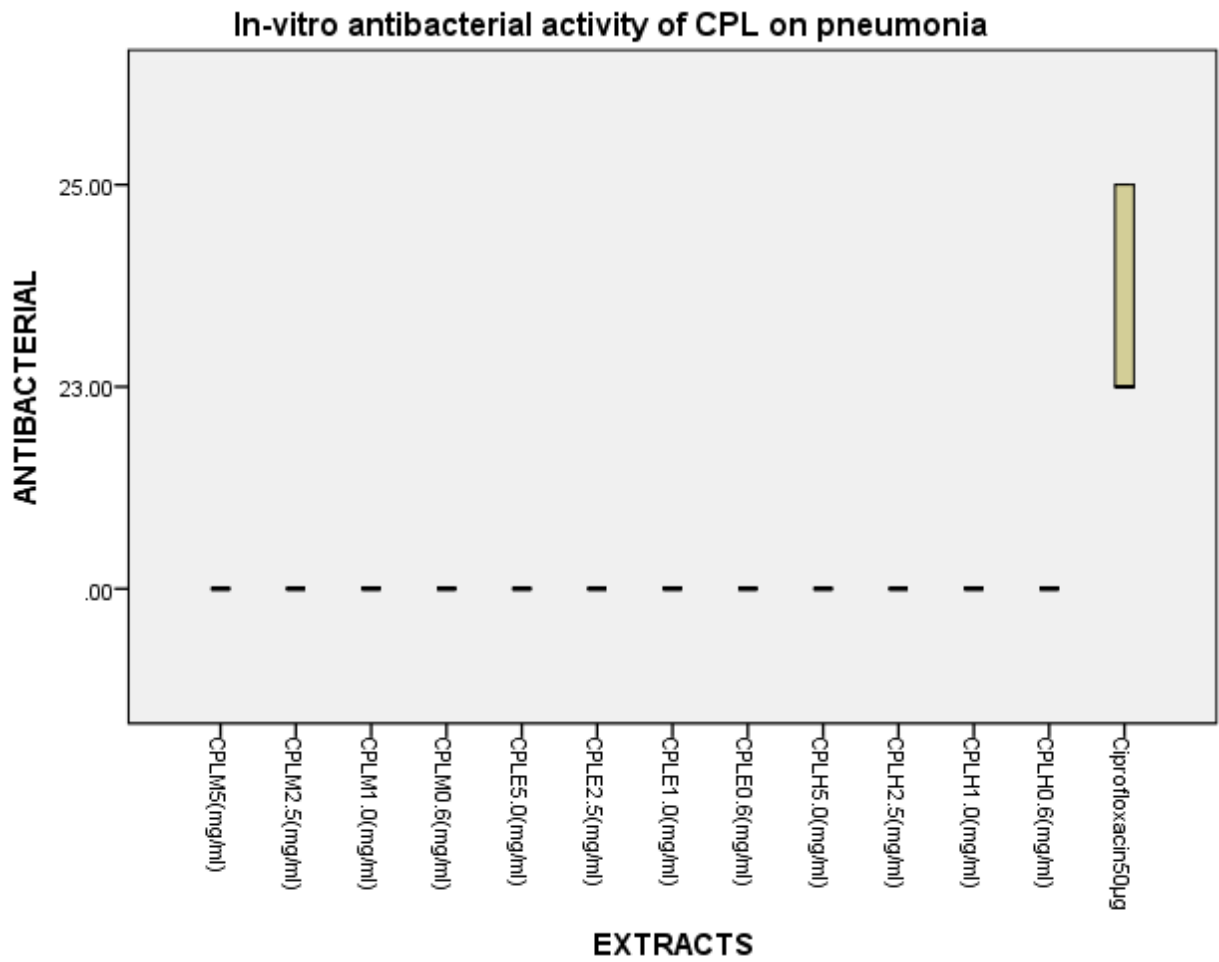


FIG 7: Box -plots represent Zone of inhibition in mm of the Extracts against *K. pneumonia*

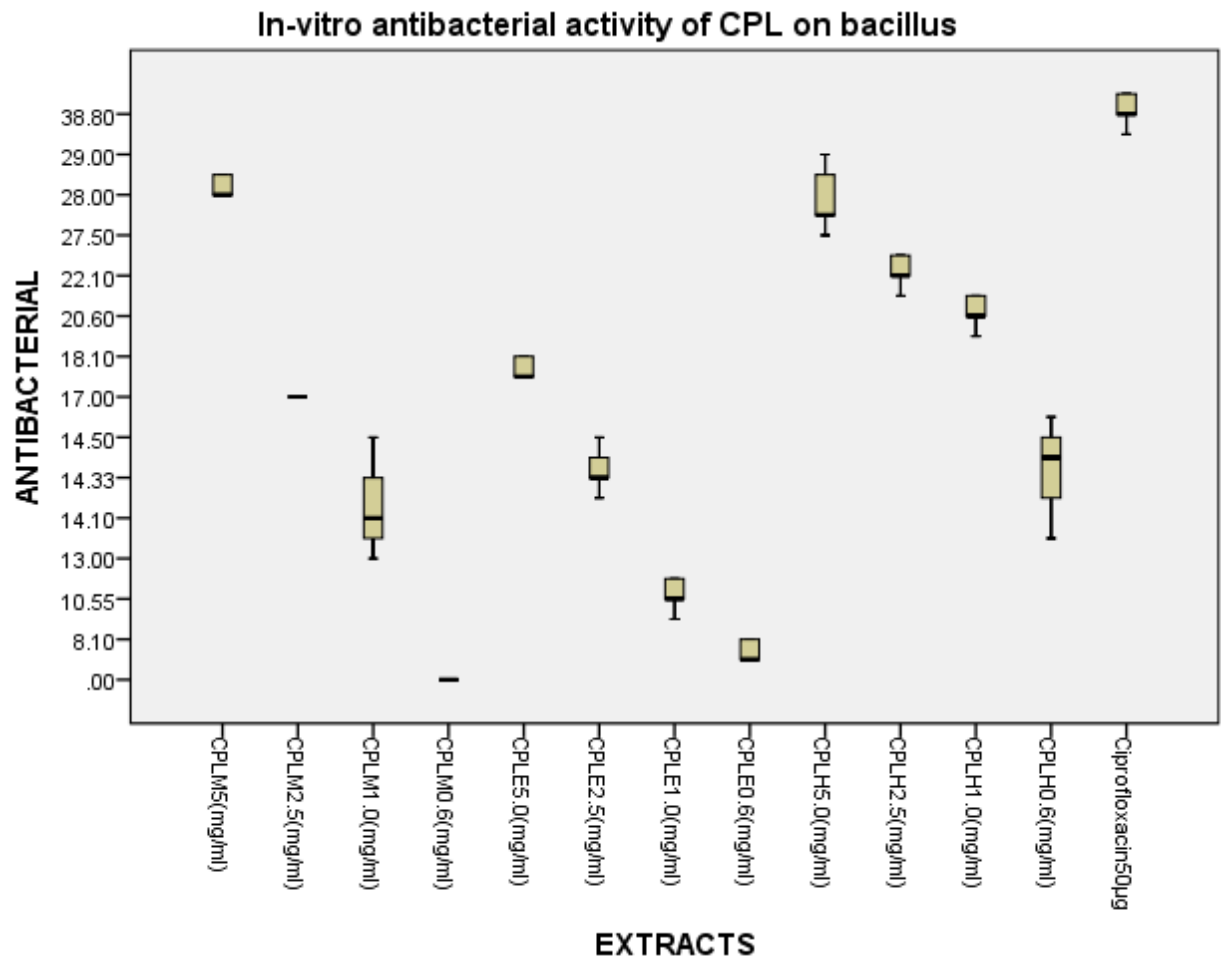


FIG 8: Box -plots represent Zone of inhibition in mm of the Extracts against *B. subtilis*

Minimum Inhibitory Concentration (Mg/mL)

Table 6: Minimum Inhibitory Concentration (Mg/mL)

Microorganism	CpLH	CpLM	CpLE	Control
<i>S. typhi</i>	5.30	0.30	7.30	0.30
<i>E. coli</i>	2.90	0.30	0.50	0.30
<i>S. aureus</i>	0.30	0.50	0.50	1.00
<i>P. aeruginosa</i>	0.30	0.30	5.30	0.30
<i>S. pneumonia</i>	10.20	10.60	15.50	0.30
<i>B. subtilis</i>	0.50	1.00	0.50	1.00
<i>C. albicans</i>	3.30	0.30	10.30	0.30
<i>A. niger</i>	10.20	0.60	2.50	0.30

Antifungal Activity

Three CPL extracts, along with standard Fluconazole were studied with different concentration levels on two different fungi. From Table 6 is observed that there is significant difference between on in-vitro antifungal activity of the three CPL extract. A one-way ANOVA tool was applied to compare and compute the effect of in-vitro antifungal activity of compounds. From Table 7, we have found that the result showing statistically significant. CpLM showed no inhibition against *Aspergillus spp* in all concentrations, while *C. albicans* was susceptible to inhibition of CpLM in a dose-dependent manner, but was resistant to inhibition of CpLH in all concentrations. CpLE was only activity against the two fungi at 5µg/ml. *Aspergillus spp* was also sensitive to inhibition of CpLH in a concentration-dependent manner.

Table 7: In-vitro antifungal activity of *C. platyterum* Extracts and Control

Extract	<i>Candida albicans</i>	<i>Aspergillus spp</i>
CPLM5mg/ml	12.00± 0.00 ^c	0.00± 0.00 ^d
CPLM2.5mg/ml	6.50± 0.00 ^e	0.00± 0.00 ^d
CPLM1.5mg/ml	0.00± 0.00 ^f	0.00± 0.00 ^d
CPLM0.6mg/ml	0.00± 0.00 ^f	0.00± 0.00 ^d
CPLE5mg/ml	18.00± 0.00 ^b	6.00± 0.11 ^{bc}
CPLE2.5mg/ml	10.00± 0.00 ^d	0.00± 0.00 ^d
CPLE1.5mg/ml	0.00± 0.00 ^f	0.00± 0.00 ^d

CPLE0.6mg/ml	0.00± 0.00 ^f	0.00± 0.00 ^d
CPLH5mg/ml	0.00± 0.00 ^f	7.80± 0.00 ^b
CPLH2.5mg/ml	0.00± 0.00 ^f	5.30± 0.00 ^c
CPLH1.5mg/ml	0.00± 0.00 ^f	5.00± 0.00 ^c
CPLH0.6mg/ml	0.00± 0.00 ^f	4.00± 0.00 ^d
Fluconazole 30µg/ml	32.27± 1.36 ^a	12.00± 0.00 ^a

*Values are mean scores ± Standard deviation of three (3) replicates

*Data in the same column bearing different superscript differ significantly (p < 0.05).

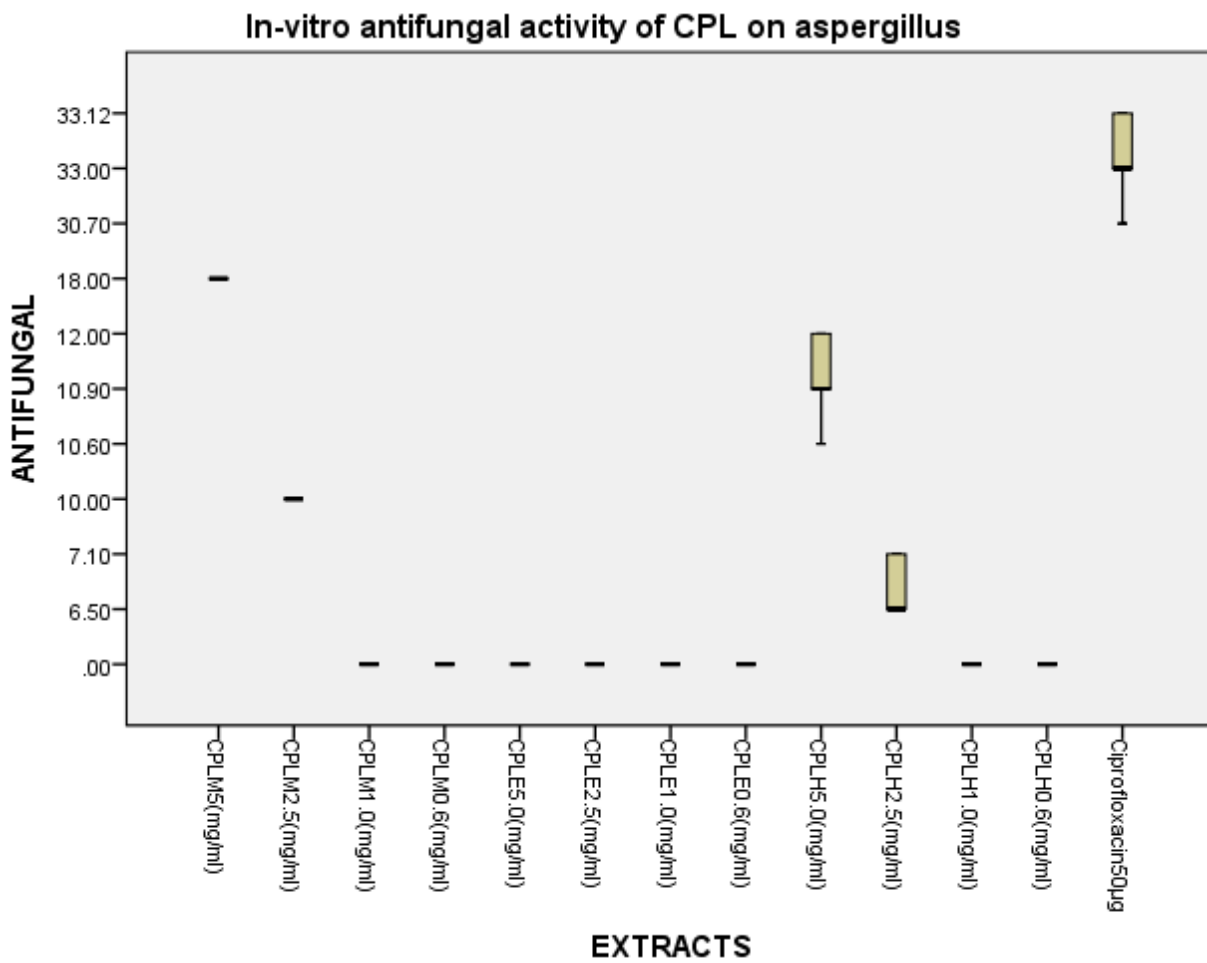


FIG 9: Box -plots represent Zone of inhibition in mm of the Extracts against *A. niger*

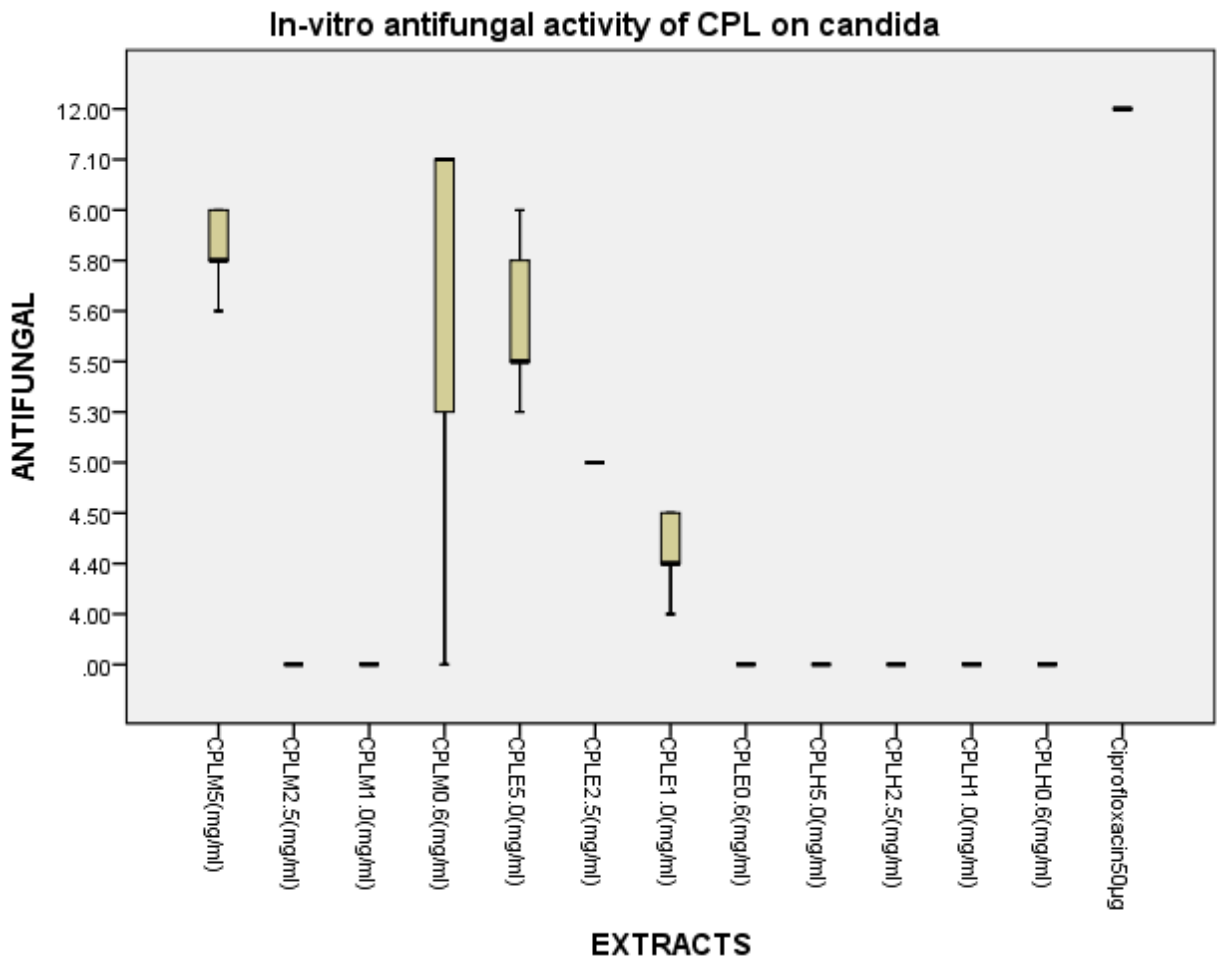


FIG 10: Box -plots represent Zone of inhibition in mm of the Extracts against *C. albicans*

Anti Inflammatory Activity

Inflammation, is a biological process that involves vascular and cellular events coordinated by mediators like prostaglandin, leukotrienes, cytokines and thromboxanes that include an essential and protective mechanism of the organism in response to injury, infection and trauma (46). Inflammation is considered the major contributor to rheumatic and heart diseases. Many plants such as *C. molle*, *C. zenkeri*, *C. micranthum* have shown anti-inflammatory property, which they do so by inhibition of phospholipase A2 (47). The anti-inflammatory activities of CPL extract were determined in terms of Effect on Protein Denaturation (EOPD), Heat-Induced

Anti-inflammatory activities	PLANT EXTRACT									Control		
	CPLH			CPLM			CPLE			Diclofenac		
Heat-Induced Hemolysis	3.8867± 0.040414 ^f	7.7067 ± 0.19604 ^f	20.7233 ± 0.62660 ^e	18.9 ± 0.18027 ^e	41.36667 ± 0.92915 ^d	51.7233 ± 1.13244 ^c	20.8433 ± 0.27135 ^e	41.29 ± 0.28513 ^d	64.6767 ± 0.33560 ^b	22.4 ± 4.351E-15 ^e	44.7233 ± 4.19185 ^d	73.0333 ± 1.7091 ^a
Effect on Protein Denaturation	45.7 ± 1.21243 ⁱ	49.8± 0.91651 ^h	54.3667 ± 1.09696 ^g	54.1567± 0.74272 ^g	55.6033 ± 0.39399 ^g	61.63 ± 0.64085 ^f	72.4633± 2.1083722 ^e	79.8667± 1.0016653 ^c	84.1333 ± 0.15275 ^b	75.8133± 1.4908163 ^d	84.3667 ± 0.55076 ^b	88.7667± 0.40415 ^a
Proteinase Inhibitory Activity	17.49333 333± 1.147228 ⁱ	27.386667± 0.24906 ^h	37.283333± 0.25658 ^f	42.416667 ± 1.45115 ^e	46.433333 ± 1.159022 ^d	53.6667± 0.66583 ^c	34.1667± 0.28868 ^g	40.8± 0.91652 ^e	55.7067± 1.13848 ^c	35.5333± 1.36137 ^{fg}	61.7667 ± 1.06926 ^b	84.8567 ± 0.76461 ^a
Lipoxygenase Inhibition Assay	4.7033± 0.81867 ^g	22.3667± 1.184624 ^f	25.3333± 0.351188 ^f	42.6± 2.193171 ^e	78± 0 ^b	84.7± 0.51961 ^a	42.713 ± 1.92523 ^e	63.8667 ± 0.7094 ^d	70.8± 0.34641 ^c	45± 0 ^e	83.6667 ± 5.50757 ^a	84.6667 ± 0.57735 ^a

*Values are mean scores ± Standard deviation of three (3) replicates

*Data in the same row bearing different superscript differ significantly (p < 0.05).

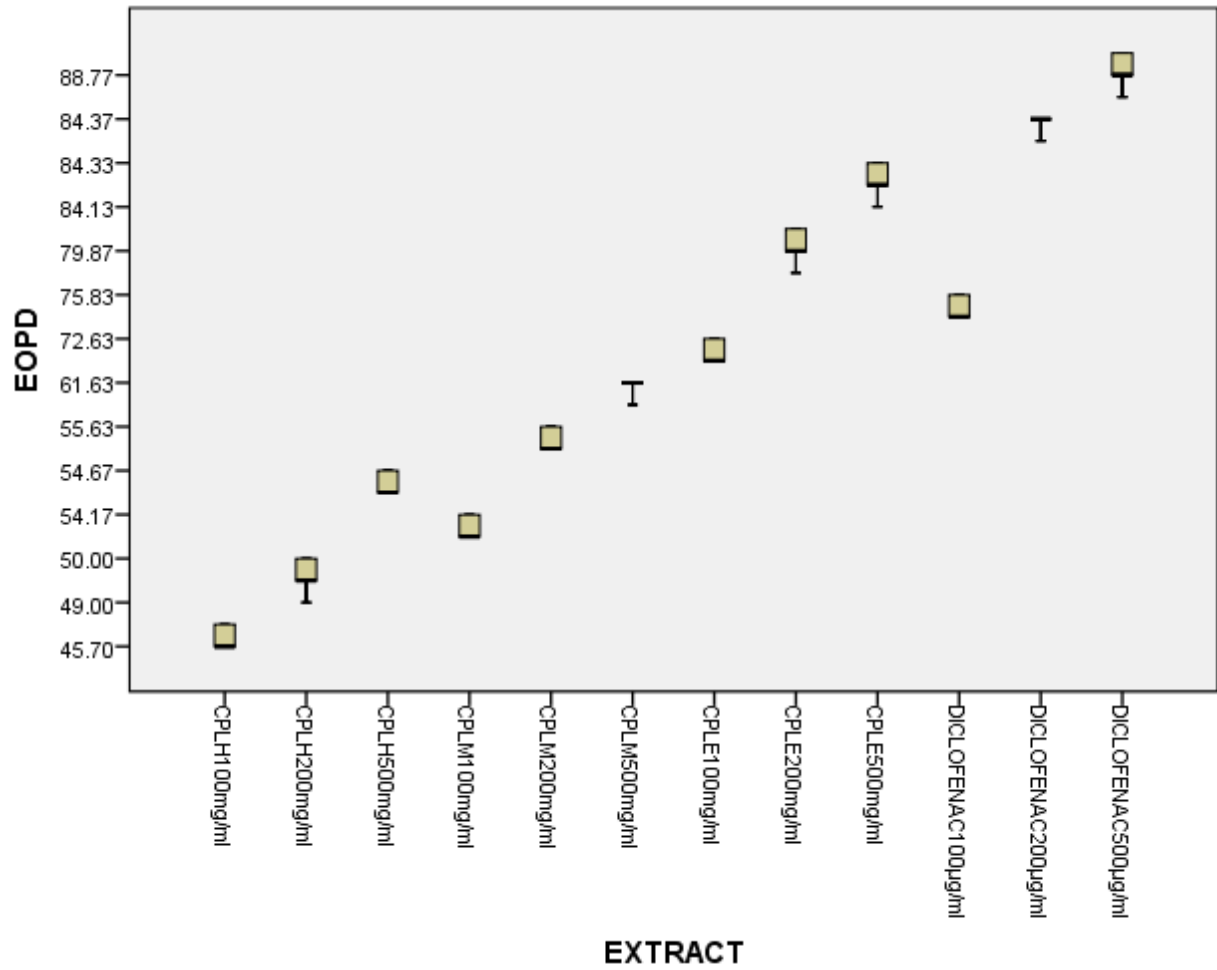


FIG 11: Means Plot of Effect On Protein Denaturation Of Different Concentrations Of CPL Samples

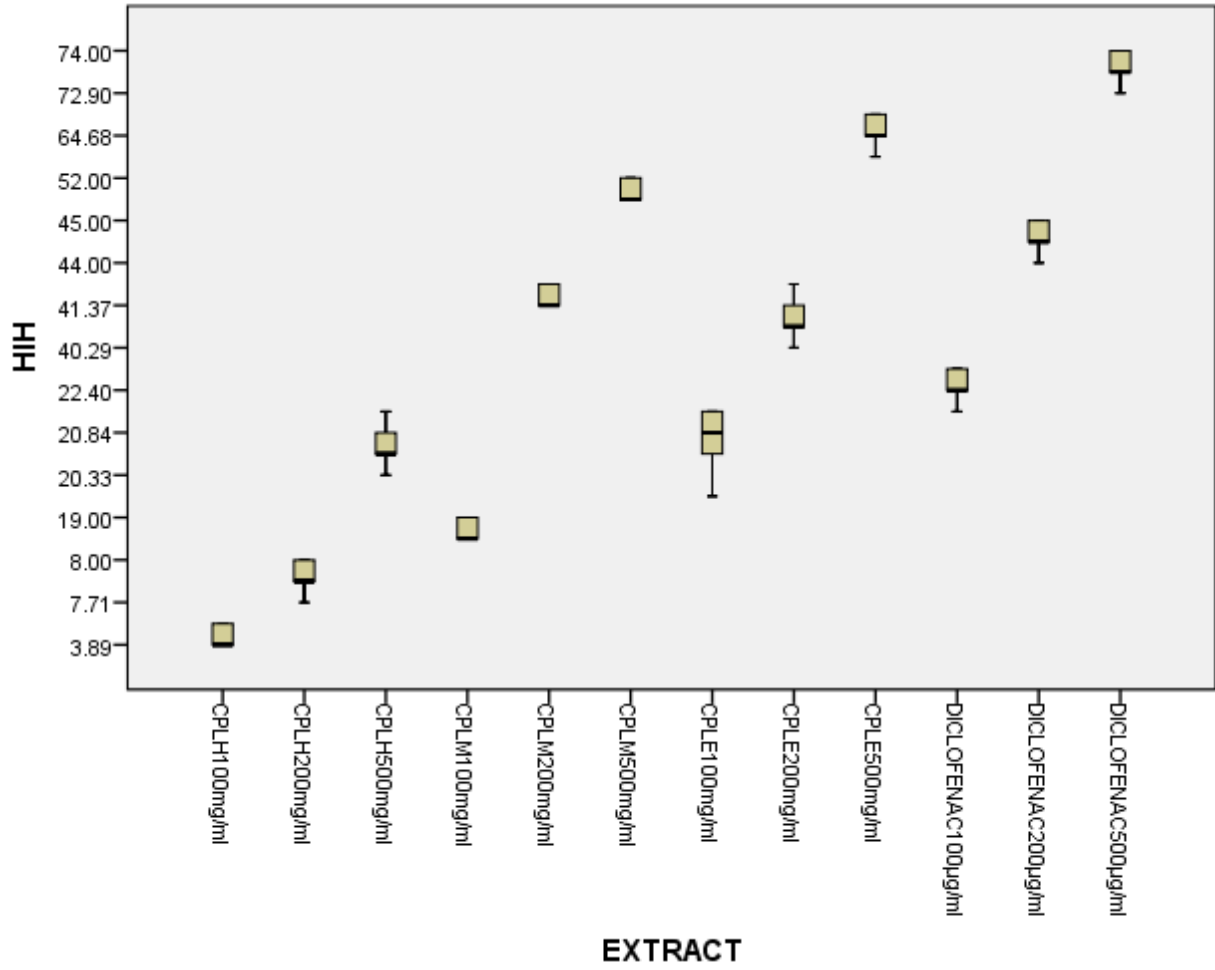


FIG 12: Means Plot of Heat-induced Hemolysis (HIH) of Different Concentrations of CPL Samples

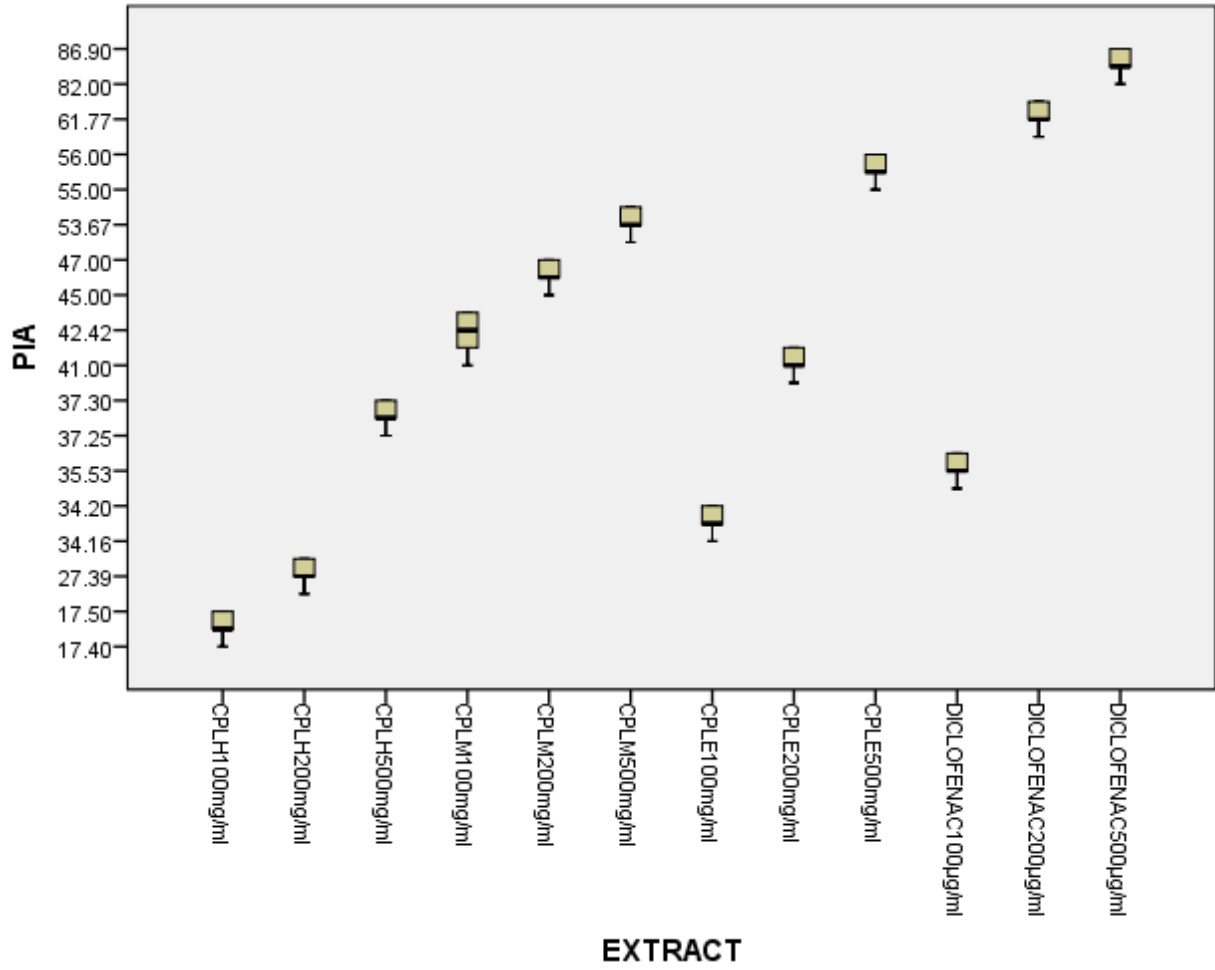


FIG 13: Means Plot of Proteinase Inhibitory Activity (PIA) of Different Concentrations of CPL Samples

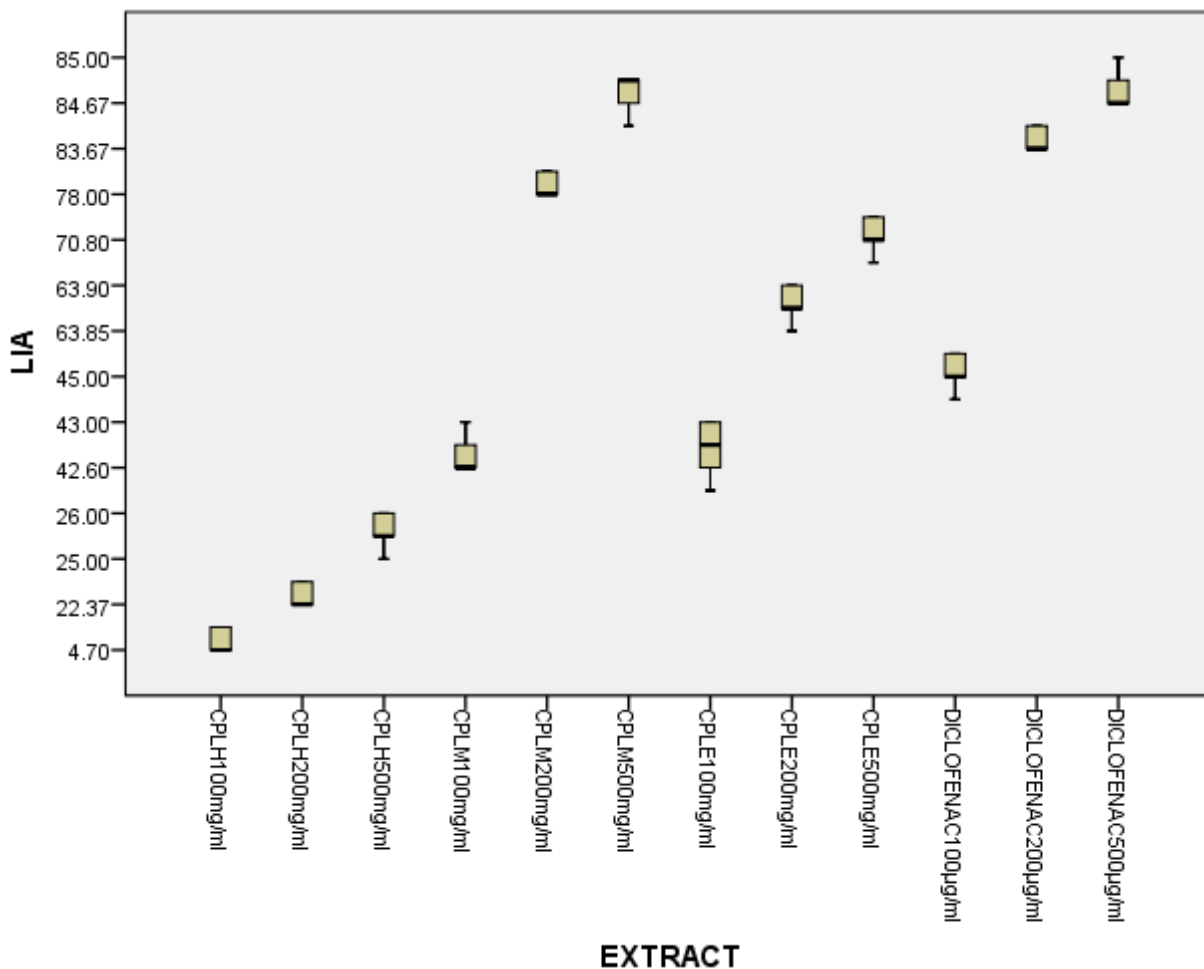


FIG 14: Means plot of Lipoxigenase Inhibition Assay (LIA) of Different Concentrations of CPL Samples

Conclusion: The therapeutic use of the leaves of *C. platypterum* against malaria and swelling was confirmed by the activities of the extracts against inflammation and *P. falciparum*. Isolation of the bioactive compounds responsible for these activities is recommended.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area

of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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