



Phytochemical, Proximate, *In-vitro* Antimalarial and Antimicrobial screening of leaf Extracts of *Justicia carnea* Lindl

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study the phytochemical, proximate, antimicrobial and anti-malarial activities of leaf extracts of *Justicia carnea*

Methods: The leaf sample of *Justicia carnea* which has been used in Nigeria folklore in treating malaria, cancer and many other diseases was extracted with methanol, ethyl acetate and n-hexane. The extracts were subjected to qualitative and quantitative phytochemical screening, proximate, anti-malarial and antimicrobial screening.

Results: The results showed that alkaloids, flavonoids, glycosides, phenolic compounds were present. Ethyl acetate extract also exhibited the highest suppression against malaria parasite *in-vitro*, more than chloroquine. This inhibition and high abundance of alkaloids and glycosides gave credence to the therapeutic use of the plant as anti-malaria drug. The three extracts showed a concentration-dependent inhibition against *S. aureus*, *Aspergillus spp* and *Bacillus spp*. *S. typhi*, *K. pneumonia*, *P. aerogenosa* and *C. albicans* were resistant in all concentrations of the extracts.

Conclusion: The therapeutic use of the leaves of *J. carnea* in treatment of malaria was validated. Its antimicrobial activity was also affirmed in some of the tested organisms.

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Keywords: *Justicia carnea*; anti-plasmodium; phytochemical; proximate; antimicrobial.

1. INTRODUCTION

Malaria remains a major threat to public health and economic development in the tropical and subtropical regions of the world, causing high morbidity and mortality [1-4]. It is a leading cause of death and disease in many developing countries, where young children and pregnant women are the groups most affected [5-7]. It is believed that nearly 1 in every 5 deaths among kids in Africa is as a result of malaria. In Nigeria, malaria is a major public health problem where it accounts for more cases and deaths than any country world over. About 97% of Nigeria's populations are at risk for malaria because of their location. It is only 3% of Nigeria's populations live in the malaria free zones. Malaria alone accounts for more than 300,000 deaths each year in Nigeria [3,8]. Apart from burdens from malaria affecting the developing countries, they, especially sub-Saharan African countries, are also faced with the challenges of infectious tropical diseases such as HIV/AIDS, cholera, and tuberculosis, which claim millions of lives annually [9]. Medicinal plants have been used against various diseases for thousands of years, and 80% of the worldwide population still depends on herbal medicines [10-11]. Various plants have their antimalarial efficacies scientifically demonstrated and the active compounds isolated with their probable mechanisms of action studied [12]. Spread of multidrug-resistant strains of Plasmodium and the adverse side effects of the existing anti-malarial drugs have necessitated the search for novel, well tolerated and more efficient antimalarial drugs [6,13,14]. *Justicia carnea* Lindl, a species of *Justicia* which is the largest genus of Acanthaceae family [15-16] has been reported to be used ethnomedicinally in treatment of malaria and many infectious diseases. The plant is known as blood root, hospital too far, oso-afia, ogwu obara in Nigeria. *J. carnea* has been reported to be used in treatment of malaria, cancer, HIV, epilepsy, inflammation, sickle cell disease, arthritis, whooping cough, anemia, tumor, liver disease, hepatitis, diabetes, typhoid and bronchitis cold [17-25]. Hypocholesterolemic, anti microbial, antioxidant, analgesic and anti allergic properties of the plant have also been reported [20,22]. There is no available literature on the pharmacological activity of the plant against malaria and some infectious diseases.

2. METHODOLOGY

2.1 Materials and Methods

2.1.1 Collection and identification of the plant

The leaves of *Justicia carnea* Lindl were collected within the surroundings of the Zik Avenue, Enugu State, Nigeria. Mr. Alfred Ozioko (The Chief Taxonomist) of International Center for Ethnomedicine and Drug Development identified and authenticated the leaves. Herbarium specimens were deposited in the herbarium of the International Center for Ethnomedicine and Drug Development (Voucher number: Intercedd/26058).

2.1.2 Extraction procedure

The *Justicia carnea* leaves were air-dried and ground. 1 kg of the sample was macerated with 6 liters each of methanol, ethyl acetate and n-hexane for 48 hours in an air-tight container with constant stirring. 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 JcLM (methanol extract), JcLE (ethyl acetate extract) and JcLH (n-hexane extract).

2.2 Proximate Analysis

2.2.1 Moisture content determination

Moisture content was determined according to the standard method of Association of official analytical chemists (AOAC) 2010 [26]. 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

2.2.2 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.

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

Where,

W3 = weight of crucible and ash
W2 = weight of crucible and sample
W1 = weight of crucible

2.2.3 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

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

2.2.4 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 kjehdal 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:

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

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

2.2.5 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:

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

2.2.6 Carbohydrate content determination

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

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

2.3 Phytochemical Analysis

The methanol, ethyl acetate and n-hexane leaf extracts of *J. carnea* were used for qualitative

phytochemical screening. To identify the chemical constituents of plant extract, standard procedures were followed [7].

2.4 Test for Flavonoids

The presence of flavonoids was done using standard method as described by Yadavalli *et al.*, 2018 [27].

About 5 ml of dilute ammonia solution was added to the extracts of *J. carnea* followed by addition of concentrated H₂SO₄. A yellow color in the extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

2.5 Test for Alkaloids

Alkaloids determination was done using Mayer's test;

In a test tube, 1ml of each of the extracts was added, followed by addition of 1ml of Meyer's reagent (Mixture of 13.5 of HgCl₂ in 20 ml of distilled H₂O and 49.8 g of KI in 20 ml distilled water). The solution was mixed properly and absence of cream colour /precipitates indicated absence of alkaloids [28].

2.6 Test for Terpenoids (Salkowski test)

Salkowski test was used in the determination of presence of terpenoids. 5 ml of each of the extracts was mixed in 2 ml of chloroform, and 3 ml concentrated H₂SO₄ was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids [29].

2.7 Test for Saponins

Foam test was used in the determination of saponins. 2 ml of each of the extracts was added to 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent stable froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion, which indicated the presence of saponins [30].

2.8 Test for Carbohydrates (Molish's Test)

To 2 ml of each of the extracts, two drops of alcoholic solution of α -naphthol were added. The mixture was shaken vigorously and 1ml of

concentrated H₂SO₄ was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates [31].

2.9 Test for Steroids

About 2 ml of chloroform and concentrated H₂SO₄ were added with the 5 ml aqueous plant crude extracts. In the lower chloroform layer red color appeared that indicated the presence of steroids [32].

2.10 Test for Proteins (Millon's test)

To 2ml of extracts, few drops of Millon's reagent (1g of Hg was dissolved in 9 ml of fuming HNO₃. Equal volume of distilled H₂O was added when the reaction was completed) were added. A white precipitate indicated the presence of proteins [33].

2.11 Test for Cardiac Glycosides

To 0.5 ml of the extracts, 2 ml of glacial acetic acid and few drops of ferric chloride were added. This was under layered with 1 ml of conc. H₂SO₄. Formation of brown ring at the interface indicated the presence of cardiac glycosides [34].

2.12 Test for Tannins (Ferric chloride Test)

To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicated the presence of tannins [35].

2.13 Test for resins (Turbidity Test)

About 10 ml of distilled water were added to 1 g of dried plants, and ultrasonicated for 15 minutes at 300°C. The mixture was filtered. Occurrence of turbidity showed the presence of resins [36].

2.14 Test for Reducing Sugars (Fehling's test)

About 0.5 g of the extracts was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars [37].

2.15 Test for Resins (Turbidity Test)

Distilled water (10 ml) was added to dried plants (1 g), and ultrasonicated for 15 minutes at 30 °C.

The mixture was filtered. Occurrence of turbidity showed the presence of resins [36].

2.16 Test for Oils (Saponification Test)

Few drops of 0.5 N alcoholic potassium hydroxide solution were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 2 hours. Formation of soap indicated the presence of oils [38].

Test for anthocyanins:

i) To the substance, 10% sodium hydroxide was added; blue color showed the presence of anthocyanins. ii) To the substance conc. sulphuric acid was added, yellowish orange color confirmed the presence of anthocyanins [39].

2.17 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 [38, 40-43] with slight modifications

2.17.1 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 alkaloids precipitate was recovered by filtration using previously weighed filter paper. After filtrate was dried in the oven at 80°C, the alkaloids content was calculated and expressed as a percentage of the weight of the sample analyzed.

$$\% \text{ weight of Alkaloids} = [(W_2 - W_1) / Wt] \times 100/1 \text{ equation 7}$$

Where,

W1 = weight of filter paper
W2 = weight of filter paper + alkaloids precipitate (residue)
Wt= weight of the sample

2.18 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 (125mm). The filtrate was transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

$$\% \text{ Flavonoids} = [(W_2 - W_1) / Wt] \times 100/1 : \text{equation 8}$$

Where,

W1= Weight of crucible
W2= Weight of crucible + Flavonoid extract (residue)
Wt= weight of the sample

2.19 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 sliming 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.

$$\% \text{ Saponins} = [(W_2 - W_1) / Wt] \times 100/1 : \text{Equation 9}$$

Where,

W1= Weight of filter paper
W2= Weight of filter paper + Saponin extract (residue)
Wt= weight of the sample

2.20 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 0.1M FeCl₃ in 0.1M HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

% Tannins = (An/As) X C X (100/W) X (Vf/Vn):
equation 10

Where:

An= Absorbance of test sample

As= Absorbance of standard solution

C= Concentration of standard solution

W= Weight of sample

Vf= Total volume of extract

Vn= Volume of extract analyzed

2.20.1 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 determined using gallic acid as standard.

2.20.2 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) [44]. Each extract was screened for an 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 red blood cells (RBC) were 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 (the experiments were done in three replicates).

2.21 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.

2.22 Antimicrobial Analysis

2.22.1 Samples

Three samples of plant extracts were analyzed. These include: JcLM, JcLH, and JcLE.

The antimicrobial sensitivity of both the crude and prepared concentrations of the plant extract was determined using the agar-well diffusion method [45-46].

2.22.2 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.

2.22.3 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.

2.22.4 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 the 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.

2.22.5 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, 1.5 and 0.60 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 (20µl) 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 (6mm) 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.

2.22.6 Determination of minimum inhibitory concentration (mic) of the samples on test isolates

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibits the bacterial growth. 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, 1.5 and 0.60mg/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 24-72days, 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.

3. RESULTS AND DISCUSSIONS

3.1 Proximate Composition of leaf Sample of *J. carnea*

The proximate composition of leaf sample of *J. carnea* was calculated based on dry weight and the results were presented in Table 1 and means plot showed in Fig 1. The results showed that leaf sample of *J. carnea* had high contents of crude fiber and carbohydrates (13.63% and 52.09%, respectively), but low contents of moisture, crude proteins, crude lipids, and ash (10.72%, 6.00%, 8.16% and 8.37% respectively).

3.2 Qualitative Phytochemical Screening of JCL

The results of the qualitative phytochemical screening of different extracts of *J. carnea* were shown in Table 2. The phytochemical studies of the JcLM extract revealed the presence of nine (9) out of the thirteen (13) phytochemicals determined with presence of alkaloids, flavonoids, glycosides, saponins, tannins, steroids, proteins, anthracynins and phenol compounds while terpenoids, carbohydrates, resins and sugars were absent. The JcLE extract showed the presence of seven (7) out of the thirteen (13) phytochemicals determined with presence of alkaloids, flavonoids, glycosides, tannins, steroids, anthracynins and phenolic compounds while terpenoids, carbohydrates, protein, saponins, resins and sugars were absent. Finally the result also showed that JcLH extract revealed the presence of six (6) out of the thirteen (13) phytochemicals determined with presence of alkaloids, flavonoids, steroids, glycosides, anthracynins and phenolic compounds while saponins, tannins, terpenoids, proteins, carbohydrates, resins and sugars were absent. It was observed that the abundance of steroids was most in the methanol extract which was against the report of Ghasemzadeh et al.,

2011 and Makin et al., 2010 [47-48] that steroids which are lipophilic compounds would dissolve more in non polar solvents. The presence of alkaloids in all the extracts gave credence to the folkloric use of the plant in treating cancer [49-52], malaria [50,52], HIV [50]. Flavonoids and glycosides were also reported to be anti cancer agents and could also be responsible in

ethnomedicinal use of the plant as anticancer drug [53-57]. It was not surprising that increasing in polarity of the solvents increased the abundance of phenolic compounds, since they are polar compounds [47, 58]. The high abundance of alkaloids [59-61] and flavonoids [1] in the samples also lends credence to the local use of the plant in treating malaria.

Table 1. Proximate composition of *J. carnea* leaf sample

Parameters	(%) Composition
MOISTURE	10.7233+ 0.68821 ^{cd}
ASH	8.3667+ 0.65064 ^{cd}
PROTEINS	6.0000+ 0.15875 ^d
FIBER	13.6333+ 1.10151 ^b
OILS	8.1667+ 1.04083 ^c
CARBOHYDRATES	52.0933+ 1.46131 ^a

*Values are mean compositions ± Standard deviation of three (3) replicates; *Data in the same column bearing different superscript differ significantly (p < 0.05).

Table 2. Qualitative Phytochemical Of JcL Samples

Parameters	JcLM	JcLE	JcLH
Saponins	+++	-	-
Flavonoids	+++	++	+
Alkaloids	+++	++	+
Tannins	+++	++	—
Steroids	+++	+	++
Terpenoids	-	-	-
Glycosides	++	+++	+
Carbohydrates	-	-	-
Proteins	+	-	—
Anthracynins	+	+	++
Phenolics	+++	+++	++
Oils And Resins	-	-	-
Reducing Sugars	-	-	-

Key: +++ = Present in high concentration; ++ = Present in moderate concentration; + = Slightly or sparingly present; - = Absent.

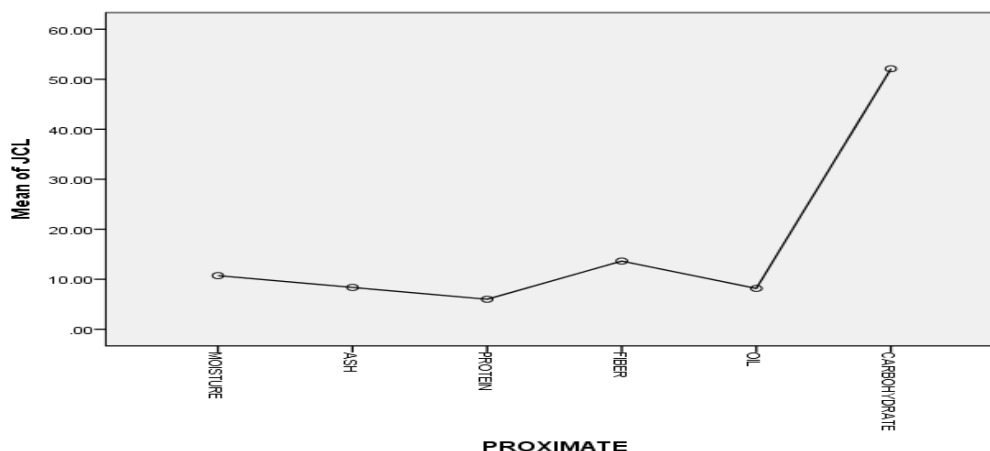


Fig. 1. Means plots for the proximate composition of JcL sample

3.3 Quantitative Phytochemical Screening of Leaf Sample of *J. carnea*

The results for the quantitative phytochemical compositions of *J. carnea* sample were shown in Table 3 while the means plot is also shown in Fig 2. The result showed flavonoids with the highest concentration (11.83mg/100g), followed by steroids (11.67mg/100g), saponins (4.58mg/100g), alkaloids (3.63mg/100g), phenolics (3.50mg/100g) while tannins have the lowest value (0.51mg/100g).

Table 3. Quantitative Phytochemical Of Jcl Samples

Phytochemicals	Abundance(mg/100g)
Alkaloids	3.6333+ 0.56862 ^b
Tannins	0.5167+ 0.09452 ^c
Saponins	4.5833+ 0.38188 ^b
Flavonoids	11.8333+ 0.28868 ^a
Steroids	11.6667+ 0.57735 ^a
Phenolics	3.500+ 0.38223 ^b

*Values are mean scores ± Standard deviation of three (3) replicates; *Data in the same column bearing different superscript differ significantly (p < 0.05)

3.4 In-vitro Antiplasmodial Efficacy of Leaf Extracts of *J. carnea* (JcL)

The anti-malaria efficacy of the three extract of JcL and control (chloroquine) were presented in Table 4. JcLH extract displayed no chemo-

suppressive activity against malaria parasite. Percentage inhibition/suppression analysis showed that the JcLE showed very high inhibition against the malaria parasite when compared with the suppression by the chloroquine (>63%). JcLM also showed small inhibition against the malaria parasite when compared with the activity of the control. The very activity of the JcLE validated the claim that *J. carnea* is being used in treating malaria.

Table 4. in-vitro antiplasmodial efficacy of JcL

Extract	Efficacy
JcLH (IC50 mg/ml)	0.0000+ 0.00
JcLE (IC50 mg/ml)	7.2667+ 0.25
JcLM (IC50 mg/ml)	2.3933+ 0.35
Chloroquine(IC50 µg/ml)	4.5833+ 0.38

3.5 Antibacterial Activity of JCL

Three JCL 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, it was found a statistically significant result. Since the result was significant, a Turkey HSD test was computed to compare the compound pair wise. From Table 1

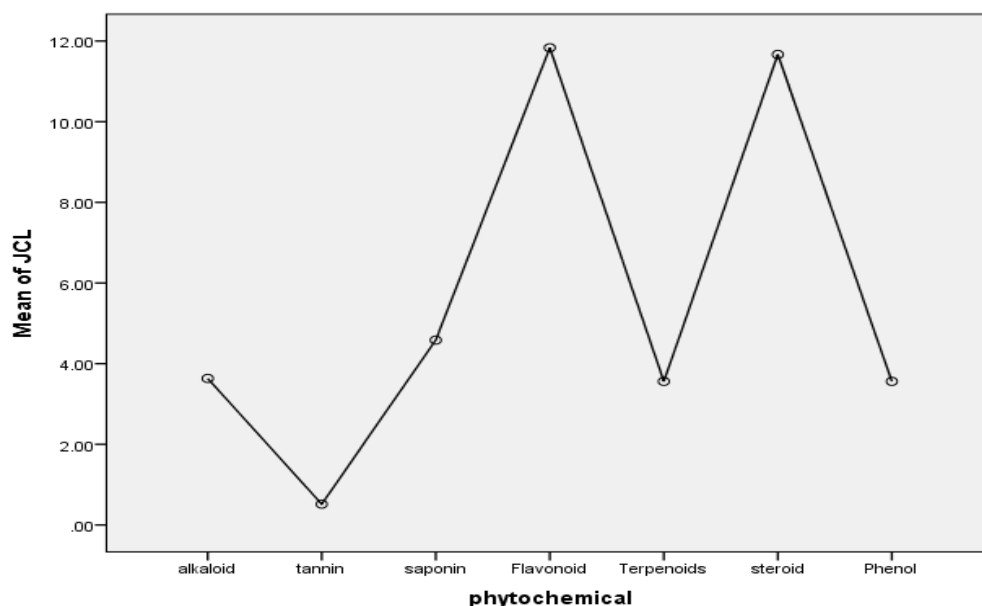


Fig 2. Means plot for the Quantitative Phytochemical of *J. carnea* leaf sample

it was observed that the in-vitro antibacterial activity of control (Ciprofloxacin), JcLE5.00 and JcLH5 significantly different from all other compounds. But Ciprofloxacin was used as standard to correlates the lead compound from the series. It was evident from the ANOVA that the antimicrobial efficacies of JCL samples were

dose dependent as 5.0 concentration exhibit higher inhibition zones than lower concentrations. The three extracts showed a concentration-dependent inhibition against *S. aureus* and *Bacillus spp.* *S. typhi*, *K. pneumonia*, and *P. aerogenosa* were resistant in all concentrations of the extracts.

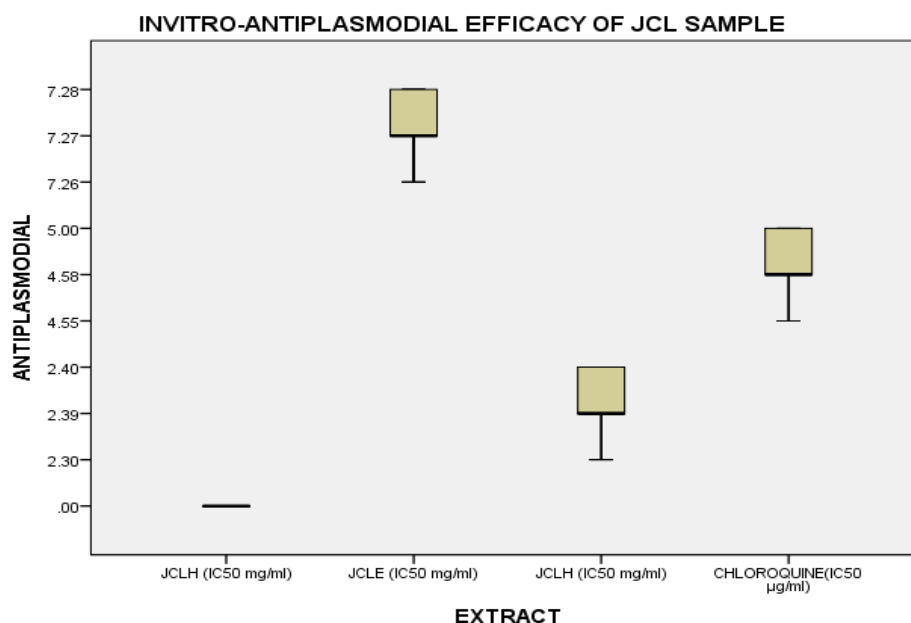


Fig 3. Boxplot For The *In-vitro* Antiplasmodial Efficacy Of Jcl

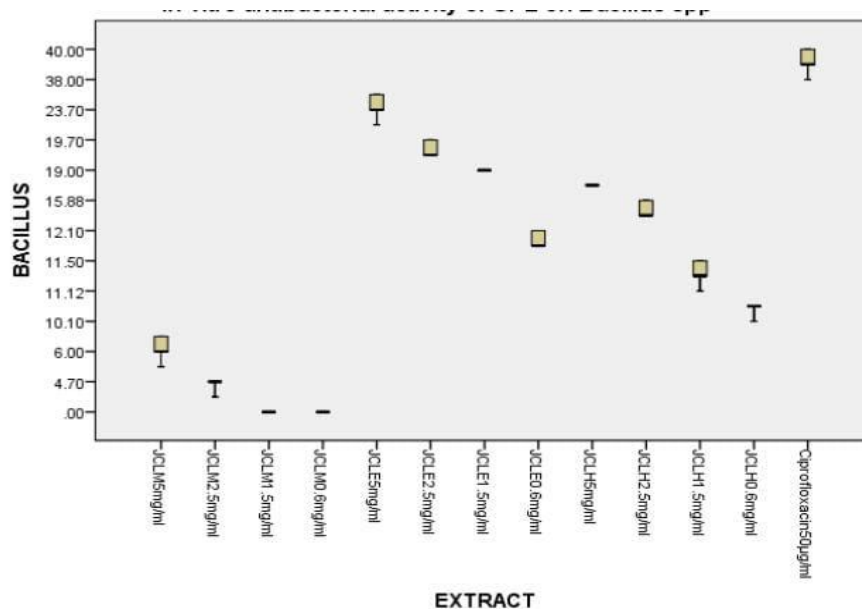


Fig. 4. Boxplot For The *In-vitro* Antibacterial Activity Of Jcl Against *B. subtilis*

Table 5. In-vitro antibacterial activity of *J. carnea* extracts

EXTRACT	<i>Bacillus spp</i>	<i>K. pneumonia</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>
JcLM5mg/ml	5.93+ .08 ^f	0.00+ 0.00 ^b	0.00+ 0.00 ^b	21.50+ 0.43 ^a	0.00+ 0.00 ^b	0.00+ 0.00 ^b
JcLM2.5mg/ml	4.63+ .011 ^f	0.00+ 0.00 ^b	0.00+ 0.00 ^b	20.00+ 0.00 ^b	0.00+ 0.00 ^b	0.00+ 0.00 ^b
JcLM1.5mg/ml	0.00+ .00 ^g	0.00+ 0.00 ^b	0.00+ 0.00 ^b	14.76+ 0.68 ^d	0.00+ 0.00 ^b	0.00+ 0.00 ^b
JcLM0.6mg/ml	0.00+ .00 ^g	0.00+ 0.00 ^b	0.00+ 0.00 ^b	14.00+ 0.00 ^d	0.00+ 0.00 ^b	0.00+ 0.00 ^b
JcLE5mg/ml	23.561.50 ^b	0.00+ 0.00 ^b	0.00+ 0.00 ^b	10.00+ 0.00 ^f	18.00+ 0.00 ^b	0.00+ 0.00 ^b
JcLE2.5mg/ml	19.56+ 0.11 ^c	0.00+ 0.00 ^b	0.00+ 0.00 ^b	6.13+ 0.23 ^g	16.26+ 0.51 ^c	0.00+ 0.00 ^b
JcLE1.5mg/ml	19.00+ 0.00 ^c	0.00+ 0.00 ^b	0.00+ 0.00 ^b	0.00+ 0.00 ^h	12.43+ 0.40 ^e	0.00+ 0.00 ^b
JcLE0.6mg/ml	12.0333+ 0.05 ^e	0.00+ 0.00 ^b	0.00+ 0.00 ^b	0.00+ 0.00 ^h	10.23+ 0.40 ^f	0.00+ 0.00 ^b
JcLH5mg/ml	17.00+ 0.00 ^d	0.00+ 0.00 ^b	0.00+ 0.00 ^b	22.00+ 0.00 ^a	14.36+ 0.32 ^d	0.00+ 0.00 ^b
JcLH2.5mg/ml	15.82+ 0.04 ^d	0.00+ 0.00 ^b	0.00+ 0.00 ^b	16.50+ 0.10 ^c	7.13+ 0.32 ^d	0.00+ 0.00 ^b
JcLH1.5mg/ml	11.30+ 0.19 ^e	0.00+ 0.00 ^b	0.00+ 0.00 ^b	14.66+ 0.57 ^d	4.76+ 0.25 ^g	0.00+ 0.00 ^b
JcLH0.6mg/ml	10.70+ 0.51 ^e	0.00+ 0.00 ^b	0.00+ 0.00 ^b	11.66+ 0.15 ^e	0.00+ 0.00 ^h	0.00+ 0.00 ^b
Ciprofloxacin50µg	38.931.00 ^a	23.661.15 ^a	41.66+ 1.15 ^a	22.33+ 0.57 ^a	19.16+ 0.28 ^a	34.83+ 1.75 ^a

*Values are mean scores ± Standard deviation of three (3) replicates; *Data in the same column bearing different superscript differ significantly (p < 0.05)

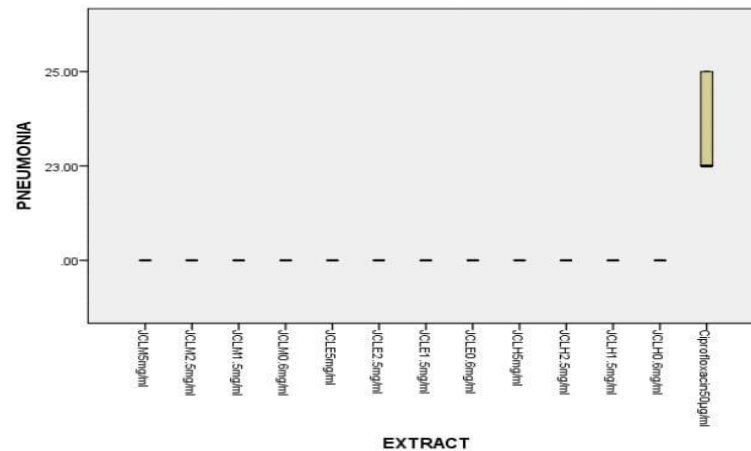


Fig. 5. Boxplot For The *In-vitro* Antibacterial Activity Of JcL Against *K. pneumonia*

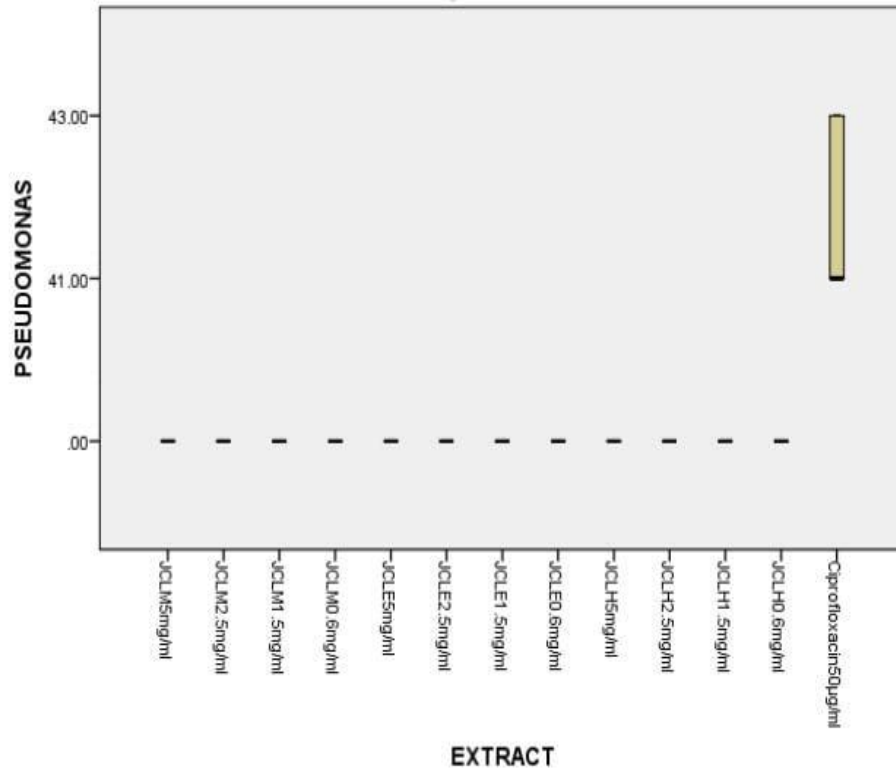


Fig. 6. Boxplot For The *In-vitro* Antibacterial Activity Of JcL Against *P. aeruginosa*

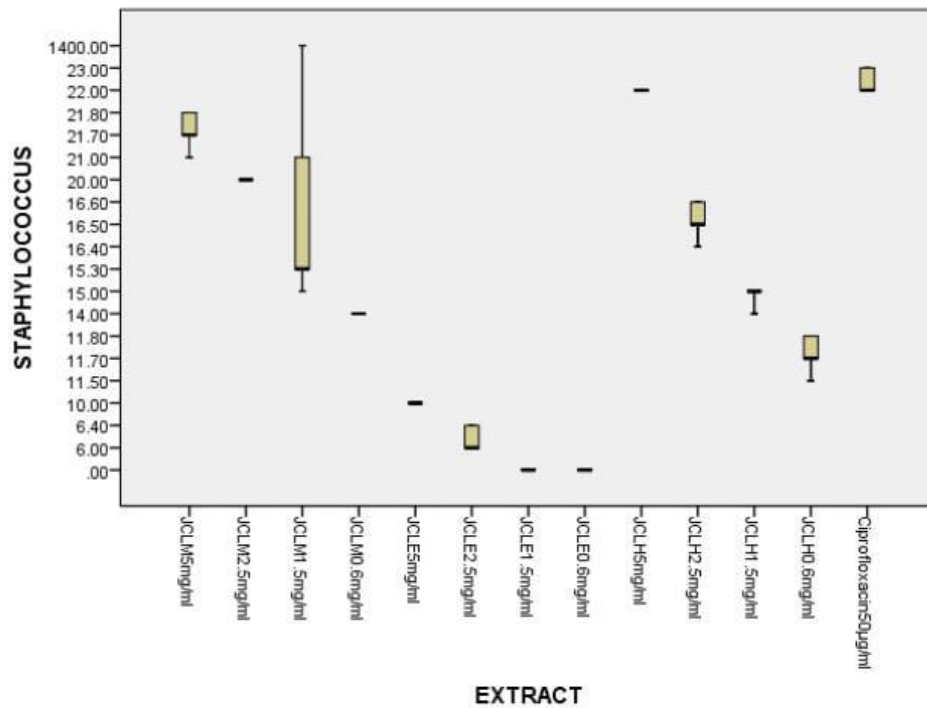


Fig. 7. Boxplot for the *In-vitro* Antibacterial Activity Of JcL Against *S. aureus*

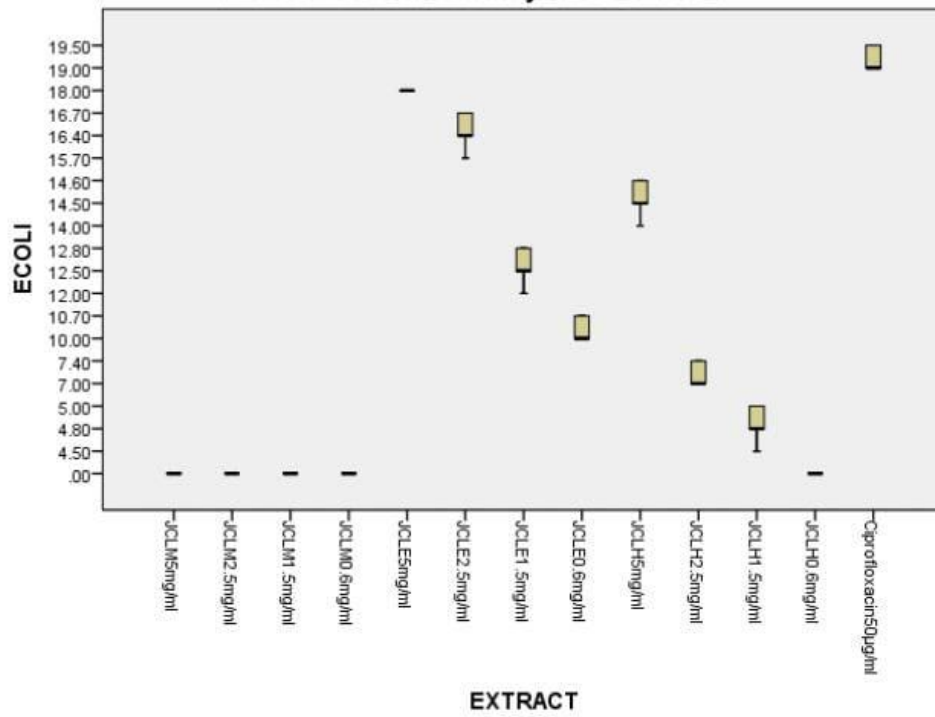


Fig. 8. Boxplot for the *In-vitro* Antibacterial Activity Of JcL Against *E. coli*

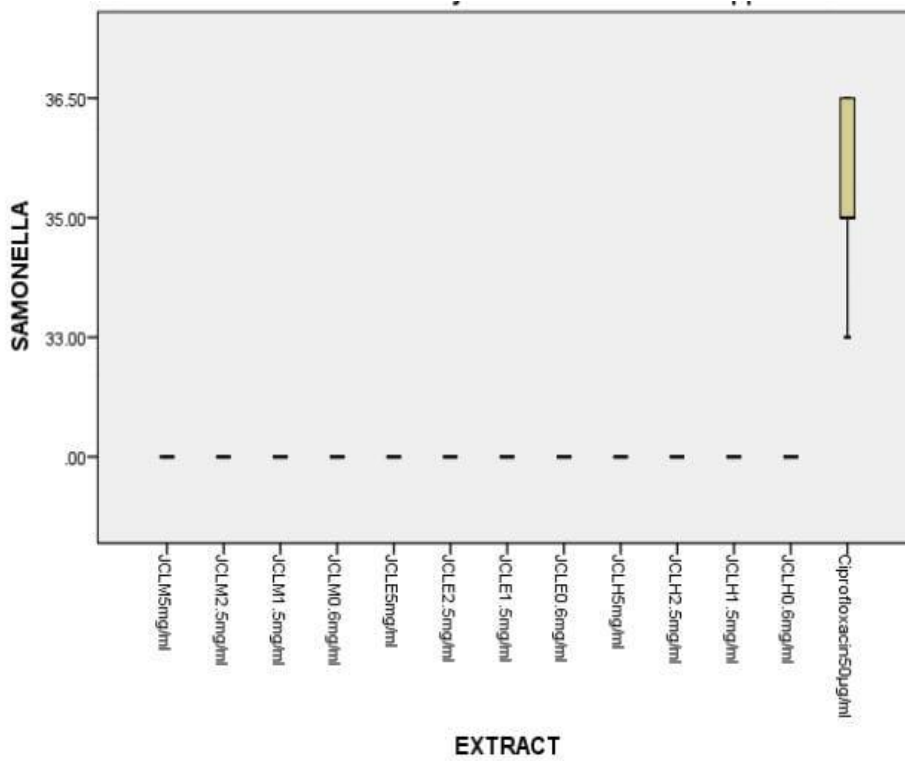


Fig. 9. Boxplot For The *In-vitro* Antibacterial Activity Of JcL Against *S. typhi*

3.6 Antifungal Activity

Three JcL extracts- JcLM, JcLE and JcLH, along with standard Fluconazole were studied with different concentration levels on two different fungi. From Table 6, it was observed that there was significant difference between *in-vitro* antifungal activities of the three samples of JcL. A one-way ANOVA tool was applied to compare and compute the effect of in-vitro antifungal activity of compounds. From Table 6, it was found that the results were statistically significant. Since the results were significant, a

Turkey HSD test was computed to compare the compound pair wise. Table 6 showed that the JcLE 5µg/ml was more significantly different from all other compounds on *in vitro* antifungal activity of the *J. carnea* extracts along with standard. Furthermore, it was also evident that JcLH sample exhibited no antifungal activity in all the four concentrations used. JcLM and JcLE showed dose-dependent inhibition against *Aspergillus spp.* At 5µg/ml, both exhibited greater activities more than fluconazole used as positive control. *C. albicans* was resistant to all the extracts.

Table 6. *In-vitro* antifungal activity of JcL

Extract	<i>Candida albicans</i>	<i>Aspergillus spp</i>
JcLM 5µg/ml	0.00+ 0.00 ^b	12.33+ 0.28 ^{bc}
JcLM 2.5µg/ml	0.00+ 0.00 ^b	12.00+ 0.00 ^c
JcLM 1.5µg/ml	0.00+ 0.00 ^b	10.16+ 0.28 ^d
JcLM 0.6µg/ml	0.00+ 0.00 ^b	8.65+ 0.13 ^e
JcLE 5µg/ml	0.00+ 0.00 ^b	14.36+ 0.11 ^a
JcLE 2.5µg/ml	0.00+ 0.00 ^b	12.70+ 0.17 ^b
JcLE 1.5µg/ml	0.00+ 0.00 ^b	10.00+ 0.00 ^d
JcLE 0.6µg/ml	0.00+ 0.00 ^b	3.76+ 0.30 ^f
JcLH 5µg/ml	0.00+ 0.00 ^b	0.00+ 0.00 ^g
JcLH 2.5µg/ml	0.00+ 0.00 ^b	0.00+ 0.00 ^g
JcLH 1.5µg/ml	0.00+ 0.00 ^b	0.00+ 0.00 ^g
JcLH 0.6µg/ml	0.00+ 0.00 ^b	0.00+ 0.00 ^g
Fluconazole 30µg/ml	32.271.36 ^a	12.00+ 0.00 ^b

*Values are mean scores ± Standard deviation of three (3) replicates; *Data in the same column bearing different superscript differ significantly (p < 0.05).

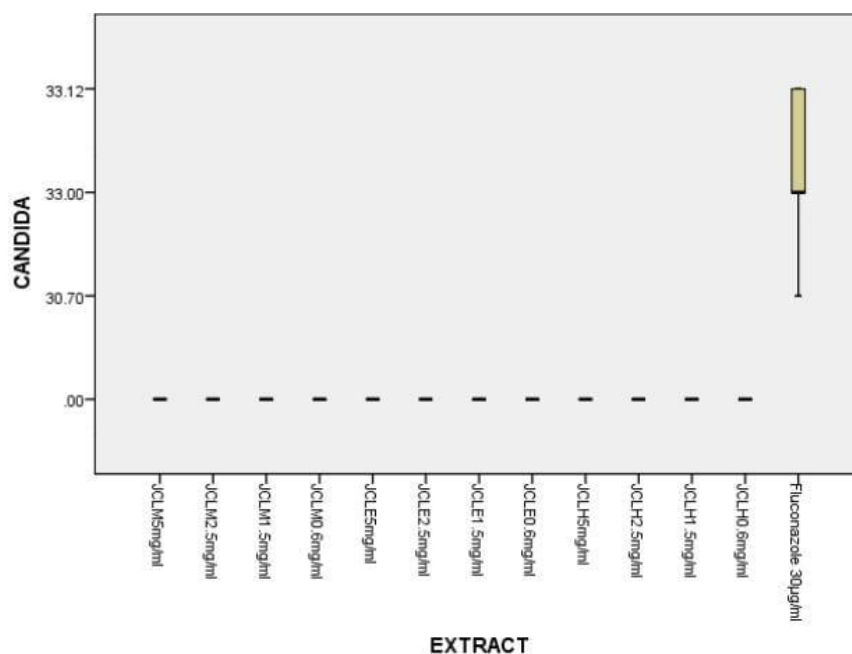


Fig 10. Boxplot for the *In-vitro* antifungal activity Of JcL Against *C. albicans*

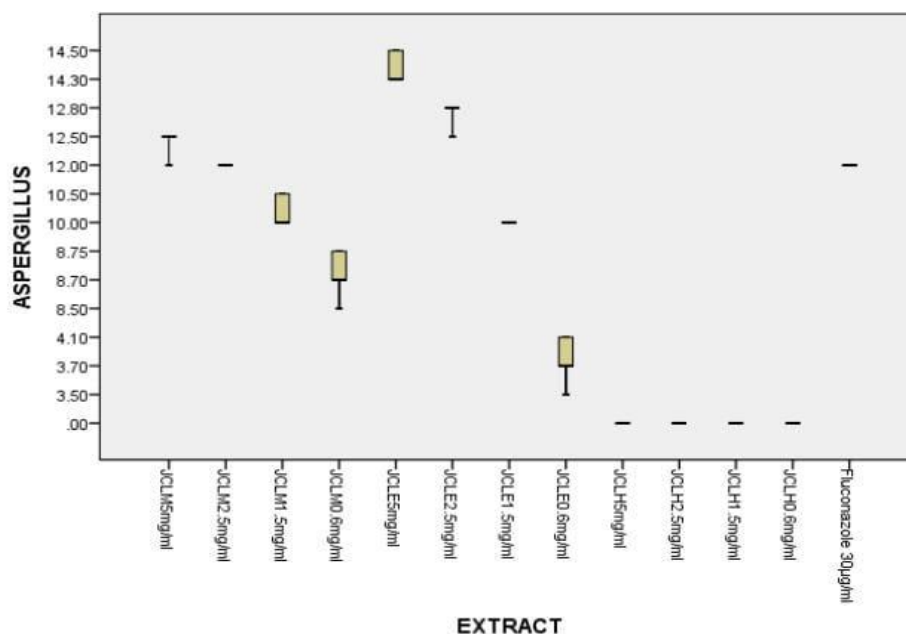


Fig 11. Boxplot for the *In-vitro* Antifungal Activity Of JcL Against *A. niger*

4. CONCLUSION

The results of phytochemical and anti-plasmodium screening showed that the leaves of *J. carnea* was very rich with many bioactive metabolites and an extract showed higher inhibition against the malaria parasite more than the positive control. This confirmed the therapeutic use of the leaves in treatment of malaria. Its antimicrobial activity was also affirmed in some of the tested organisms. There is need to further work on isolation and identification of the active metabolites responsible for the anti malaria and antimicrobial properties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Pan WH, Xu XY, Shi N, Tsang SW, Zhang HJ. Antimalarial activity of plant metabolites. *International Journal of Molecular Sciences*. 2018;19(5):1382. Available:https://doi.org/10.3390/ijms19051382
- Ajayi IO, Ughasoro MD, Ogunwale A, Odeyinka O, Babalola O, Sharafadeen S, Adamu AY, Ajumobi O, Orimogunje T, Nguku P. A qualitative exploration of malaria operational research situation in Nigeria. *PLOS ONE*. 2017;12(11):e0188128. Available:https://doi.org/10.1371/journal.pone.0188128
- Okeke EU. Nigerian malaria: the problems and the fight. *Malaria Journal*. 2012;11. DOI:10.1186/1475-2875-11-S1-P122.
- Samba EM. The burden of malaria in Africa. *Afr Health*. 1997;19(2):17. PMID: 12292299.
- Gebrehiwot S, Shumbahri M, Eyado A, Yohannes T. Phytochemical Screening and *In vivo* antimalarial activity of two traditionally used medicinal plants of afar region, Ethiopia, against plasmodium berghei in Swiss Albino Mice. *Journal of Parasitology Research*;2019. Available:https://doi.org/10.1155/2019/4519298
- Iyamah PC, Idu M. Ethnomedicinal survey of plants used in the treatment of malaria in Southern Nigeria. *Journal of ethnopharmacology*. 2015;173:287–302. Available:https://doi.org/10.1016/j.jep.2015.07.008
- Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. Phytochemical screening and antioxidant activities of some selected medicinal plants used for

- malaria therapy in Southwestern Nigeria. *Trop. J. Pharm. Res.* 2008;7: 1019–1024
8. Oladepo O, Oyeyemi AS, Titiloye, MA, Adeyemi AO, Burnett SM, Apera I, Oladunni O, Michael Alliu M. Malaria testing and treatment knowledge among selected rural patent and proprietary medicine vendors (PPMV) in Nigeria. *Malar J.* 2019;18:103. Available: <https://doi.org/10.1186/s12936-019-2732-z>
 9. Aishat A. Microbiology Education in Nigeria: Common Training Deficits Could Be Addressed with Modern Educational Tools[†]. *Journal of Microbiology & Biology Education.* 2019;20(1). Available: <https://doi.org/10.1128/jmbe.v20i1.1595>
 10. Parsaeimehr A, Martinez-Chapa SO, Parra-Saldívar R. Medicinal Plants Versus Skin Disorders: A Survey From Ancient to Modern Herbalism. *The Microbiology of Skin, Soft Tissue, Bone and Joint Infections.* 2017;2:205-221. Available: <https://doi.org/10.1016/B978-0-12-8111079-9.00013-6>
 11. Akinyemi O, Oyewole SO, Jimoh KA. Medicinal plants and sustainable human health: a review. *Horticult Int J.* 2018;2(4):194-195. DOI: 10.15406/hij.2018.02.00051
 12. Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: A review. *Journal of Ethnopharmacology.* 2011;133(2):289-302. Available: <https://doi.org/10.1016/j.jep.2010.11.024>
 13. Abera T, Ashebir R, Basha H, Debebe E, Abebe A, Meresa A, Woldekidan S. Phytochemical-constituents, safety and efficacy of commonly used medicinal plants for the treatment of malaria in Ethiopia-a review. *Pharm Pharmacol Int J.* 2019;7(6):284-295. DOI: 10.15406/ppij.2019.07.00266
 14. Bankole AE, Adekunle AA, Sowemimo AA, Umebese CE, Abiodun O, Gbotosho GO. Phytochemical screening and in vivo antimalarial activity of extracts from three medicinal plants used in malaria treatment in Nigeria. *Parasitology Research.* 2016;115(1):299–305. Available: <https://doi.org/10.1007/s00436-015-4747-x>
 15. Kitadi JM, Lengbiye EM, Gbolo BZ, Inkoto CL, Muanyishay CL, Lufuluabo GL, Tshibangu DST, Tshilanda DD, Mbala BM, Ngbolua K, Mpiana PT. *Justicia secunda* Vahl species : Phytochemistry, Pharmacology and Future Directions: A mini-re-view. *Discovery Phytomedicine.* 2019;6(4):157-171. DOI:10.15562/phytomedicine.2019.93.
 16. Corrêa GM, Alcântara AFD. Chemical constituents and biological activities of species of *Justicia* - a review. *Rev. Bras. Farmacogn. Braz. J. Pharmacogn.* 2012;22(1):220-238.
 17. Oladele AT and Elem JA. Medicinal Plants Used to Treat Sexual Diseases by Ogba/Egbema/Ndoni Ethnic of Rivers State, Nigeria. *World News of Natural Sciences (WNOFNS).* 2018;17:16-38
 18. Onyeabo C, Achi N, Ekeleme-Egedigwe CA, Ebere CU, Okoro CK. Haematological and biochemical studies on *Justicia carnea* leaves extract in phenylhydrazine induced-anemia in albino rats, *Acta Sci Pol Technol Aliment.* 2017;16(2):217-230. Available: <http://dx.doi.org/10.17306/J.AFS.2017.0492>
 19. Udedi SC, Ani ON, Asogwa KK, Maduji FC and Okafor CN. In-vitro and In-vivo Antioxidant Activity of Ethanol Leaf Extract of *Justicia carnea*. *International Journal of Biochemistry Research & Review.* 2020;29(4):48-60. Available: <https://doi.org/10.9734/ijbcrr/2020/v29i430185>
 20. Orjiakor CA, Uroko RI, Njoku OU, Ezeanyika LUS. Nutritive properties of aqueous extract *Justicia carnea* leaves and its effects on haematological and some biochemical indices of anaemia induced male wistar albino rats. *Biomedical Research.* 2019;30(4):645-654.
 21. Ani ON, Udedi SC, Akpata EI, Ezeigwe OC, Oguazu CE, Onyishi CK, Nwakaudu EN. Effects of Ethanol Leaf Extract of *Justicia Carnea* on Biochemical Indices of Alloxan-Induced Diabetic Rats. *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB).* 2020;6(2):39-46. DOI: 10.9790/264X-0602013946
 22. Ukpabi-Ugo JC, Ndukwe PAC, Iwuoha AG. Hepatoprotective Effect of Methanol Extract of *Justicia carnea* Leaves on Carbon Tetrachloride-Intoxicated Albino Rats. *Biochem Anal Biochem.* 2019;8:381. DOI: 10.35248/2161-1009.19.8.381
 23. Igbinaduwa PO, Kabari KM and Chikwue TC. Phytochemical and Anti-Anaemic Properties of Ethanol Leaf Extract of *Justiciacarnea* Vahl (Acantheceae).

- Nigerian Journal of Pharmaceutical and Applied Science Research. 2019;8(2):55-61
24. Komlaga G, Agyare C, Dickson RA, Mensah MLK, Annan K, Loiseau PM, Champy P. Medicinal plants and finished marketed herbal products used in the treatment of malaria in the Ashanti region, Ghana. *Journal of Ethnopharmacology*. 2015;172:333-342. Available: <https://doi.org/10.1016/j.jep.2015.06.041>
 25. Nduche MU, Offor IC. Anatomical Studies of *Sida Acuta* Burm, *Spigelia Anthelmia* Linn, *Centrosema Pubescens* Benth, *Pueraria Phaseoloides* (ROXB) Benth, *Justicia Carnea* Lindl and their Taxonomic Significance. *International Journal of Research in Pharmacy and Biosciences*. 2019;6(2):21-32
 26. Association of official analytical chemists (AOAC). *Official methods of Analysis*. Association of official chemists, 15th Edn., Association of official analytical chemists, Washington DC. 2010;10-30.
 27. Yadavalli R, Peasari JR, Mamindla P, Praveenkumar MS, Ganugapati J. Phytochemical screening and in silico studies of flavonoids from *Chlorella pyrenoidosa*, *Informatics in Medicine Unlocked*. 2018;10:89-99. Available: <https://doi.org/10.1016/j.imu.2017.12.009>
 28. Khlif I, Jellali K, Michel T, Halabalaki M, Skaltsounis AL and Allouche N. Characteristics, phytochemical analysis and biological activities of extracts from tunisian chetoui olea Europaea variety. *Journal of Chemistry*;2015. Available: <https://doi.org/10.1155/2015/418731>
 29. Edeoga HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian Medicinal plants. *Journal of Biotechnology*. 2005;4(7):685-688
 30. Fayaz M, Bhat MH, Kumar A and Jain AK. Comparative Studies on Different Solvents Used for the Extraction of Phytochemicals from the Plant Parts of *Arnebia benthamii*. (Wall Ex. G. Don) Johnston. *Journal of Chemical and Pharmaceutical Research*. 2017;9(1):220-224.
 31. Mera I, Falconí D, Morera V. Secondary metabolites in plants: main classes, phytochemical analysis and pharmacological activities. *Bionatura*. 2019;4(4). DOI: 10.21931/RB/2019.04.04.11
 32. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan. *The Scientific World Journal*;2017. Available: <https://doi.org/10.1155/2017/5873648>.
 33. Banu KS and Cathrine L. General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science (IJARCS)*. 2015;2(4):25-32.
 34. Roghini R and Vijayalakshmi K. Phytochemical Screening, Quantitative Analysis of Flavonoids and Minerals in Ethanolic Extract of *Citrus paradisi*. *Int J Pharm Sci & Res*. 2018;9(11):4859-64. DOI: 10.13040/IJPSR.0975-8232.9(11).4859-64
 35. Prabhavathi RM, Prasad MP and Jayaramu M. Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quadrangularis*. *Advances in Applied Science Research*, 2016;7(4):11-17.
 36. Keo S, Meng C, Oeung S, Nov V, Lon S, Vichet T, Va T, Sourn M, Chea S. Preliminary phytochemical screening of selected Medicinal Plants of Cambodia. *Asian Journal of Pharmacognosy*. 2017;1:16-23
 37. Usman H, Abdulrahman F, Usman A. Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *African Journal of Traditional, Complementary, and Alternative Medicines: AJTCAM*. 2009;6(3):289-295. Available: <https://doi.org/10.4314/ajtcam.v6i3.57178>
 38. Raaman N. *Phytochemical Techniques*. New India Publishing Agency, New Delhi. 2006;19-22.
 39. Firdouse S, Alam P. Phytochemical investigation of extract of *Amorphophallus campanulatus* tubers. *International Journal of Phytomedicine*. 2011;3:32-35.
 40. Anarado CE, Anarado CJO, Umedum NL, Ogbodo QM. Comparative phytochemical and anti-microbial studies of leaf, stem, root of *spathodea companulata*. *Asian Journal of Applied Chemistry Research*. 2020;6(1):10-20. DOI: 10.9734/AJACR/2020/v6i130148

41. Ukoha PO, Cemaluk EAC, Nnamdi OL and Madus EP. Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities. *African Journal of Pure and Applied Chemistry*. 2011;5(8):237-244
42. Senguttuvan J, Paulsamy S, Karthika K. phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochoeris radicata* L. for in vitro antioxidant activities. *Asian Pac J Trop Biomed*. 2014;4(Suppl 1):S359-S367
43. Sankhalkar S, Vernekar V. Quantitative and Qualitative Analysis of Phenolic and Flavonoid Content in *Moringa oleifera* Lam and *Ocimum tenuiflorum* L. *Pharmacognosy Research*. 2016;8(1):16-21
44. Makler MT, Ries JM, Williams JA, Bancroft JE, Piper RC, Gibbins B.L, Hinrichs DJ. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. *Am. Soc. Trop. Med. Hyg*. 2013;48:739-741
45. Srinivasan D, Nathan S, Suresh T, Perumalsamy LP. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. of Ethnopharm*. 2001;74: 217–220
46. Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN, Bakri MM. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*. 2018;25:361–366.
47. Ghasemzadeh A, Jaafar HZE, Rahmat A. Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts. *Journal of Medicinal Plants Research*. 2011;5(7):1147-1154.
48. Makin H, Honour J, Shackleton C, Griffiths W. General methods for the extraction, purification and measurement of steroids by chromatography and mass spectrometry. In: Makin H., Gower D. (eds) *Steroid Analysis*. Springer, Dordrecht;2010. Available:https://doi.org/10.1023/b135931_3
49. Patel K, Gadewar M, Tripathi R, Prasad SK, Patel DK. A review on medicinal importance, pharmacological activity and bioanalytical aspects of beta-carboline alkaloid “Harmine”. *Asian Pac J Trop Biomed*. 2012;2(8):660-664
50. Bribi N. Pharmacological activity of Alkaloids: A Review. *Asian Journal of Botany*. 2018;1. DOI:10.63019/ajb.v1i2.467
51. Jayakumar K, Murugan K. Solanum Alkaloids and their Pharmaceutical Roles: A Review. *Journal of Analytical & Pharmaceutical Research*. 2016;3(6). DOI: 10.15406/japlr.2016.03.00075
52. Ain Q, Khan H, Mubarak MS, Pervaiz A. Plant Alkaloids as Antiplatelet Agent: Drugs of the Future in the Light of Recent Developments, *Frontiers in Pharmacology*. 2016;7
53. Panche AN, Diwan AD, and Chandra SR. Flavonoids: an overview. *J Nutr Sci*. 2016;5:e47. DOI: 10.1017/jns.2016.41
54. Hayat M, Abbas M, Munir F, Hayat MQ, Keyani R and Amir R. Potential of plant flavonoids in pharmaceutics and nutraceutics. *J Biomol Biochem*. 2017;1(1):12-17.
55. Tungmunnithum D, Thongboonyou A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines (Basel)*. 2018;5(3):93.
56. Al-Snafi A. Phenolics and flavonoids contents of medicinal plants, as natural ingredients for many therapeutic purposes- A review. *IOSR Journal of Pharmacy (IOSRPHR)*. 2020;10:42-81.
57. Khan H, Saeedi M, Nabavi SM, Mubarak MS, Bishayee A. Glycosides from Medicinal Plants as Potential Anticancer Agents: Emerging Trends Towards Future Drugs. *Current medicinal chemistry*. 2019;26(13):2389–2406. Available:<https://doi.org/10.2174/0929867325666180403145137>
58. Thavamoney N, Sivanadian L, Tee LH, Khoo HE, Prasad KN, Kong KW. Extraction And Recovery Of Phytochemical Components And Antioxidative Properties In Fruit Parts Of *Dacryodes rostrata* Influenced By Different Solvents. *J Food Sci Technol*. 2018;55(7):2523–2532.
59. Bekono BD, Ntie-Kang F, Onguéné PA, Lifongo LL, Sippl W, Fester K, Owono LCO. The potential of anti-malarial compounds derived from African medicinal plants: a review of pharmacological evaluations from 2013 to 2019. *Malar J*. 2020; 19:183.

- Available:<https://doi.org/10.1186/s12936-020-03231-7>
60. Uzor PF. Alkaloids from plants with antimalarial activity: a review of recent studies. Evidence-Based Complementary and Alternative Medicine. 2020;2020 (Article ID 8749083):17. Available:<https://doi.org/10.1155/2020/8749083>
61. Amoa Onguéné P, Ntie-Kang F, Lifongo LL, Ndom JC, Sippl W, Mbaze LM. The potential of anti-malarial compounds derived from African medicinal plants. Part I: A pharmacological evaluation of alkaloids and terpenoids. Malar J. 2013;12:449. Available:<https://doi.org/10.1186/1475-2875-12-449>

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