Original Research Article

PHARMACOGNOSTIC SCREENING AND ANTIMALARIA ACTIVITY OF METHANOL BARK EXTRACT OF *DANIELLIA OLIVERI* (ROLFE) HUTCH. & DALZ. [FABACEAE] EXTRACT ON *Plasmodium berghei* INFECTED MICE.

ABSTRACT

Introduction: Malaria parasite infection has remained a global leading cause of death and disability in which about 50% of the world population is estimated to be at risk, especially in low and middle income countries. **Aim:** This research is designed to evaluate the pharmacognostic, phytochemical profile, and investigate its antimalarial activity by analysing different hematological indices of the methanolic bark extract of *Daniellia oliveri,* a plant belonging to the family of fabaceae. **Methods:** The barks of this plants were collected, cleared, dried, pulverized and sequentially extracted with petroleum ether, n-hexane, ethyl acetate, methanol and aqueous using the soxhlet extractor. Acute toxicity studies (LD50) for the methanol bark extract was studied using standard method. Phytochemical and pharmacognostic screening was carried out using standard methods, its hematological analysis were investigated using standard methods. The antimalarial activity of the methanol bark extract *Daniellia oliveri,* was evaluated at different doses of 100, 200 and 400mg/kg using in-vivo models. **Result**: The plant is a tall slender tree with a dark grey colour. The following extractive values were obtained petroleum ether (0.600±0.10), n-hexane(0.667±0.88), ethyl acetate(1.600±0.10), methanol(8.400±0.10) and aqueous(6.200±0.10).The methanol extract had the hightest extractive value and was found to be non-toxic at dose 5000mg/kg. The qualitative and quantitative phytochemical analyses reveals the presence of alkaloids (10.179±0.61), saponins (1.674±0.43), tannins (10.738±0.61), flavonoids (3.923±0.15), steroids (2.665±0.07), phenols (134.604±14.83), terpenoids (22.436±4.87), glycosides (14.485±0.08), reducing sugars (4.138±1.36), soluble carbohydrates but absence of cyanogenic glycosides The pharmacognostic parameters values were obtained as follows, total ash value (5.600±0.10), acid insoluble ash value (2.800±0.88), water soluble ash value (0.500±0.10), moisture content (13.933±0.12), bitterness value , foaming index (less than 100), swelling index (2.867±0.99). **Conclusion**: This result has shown that the hematological analysis carried out exhibited significant improvement in PCV, RBC and Hb when administered the plant extract compared to the standard group. It exhibited significant increase in platelet and lymphocyte while reduction in neutrophil compared to the standard group.The increased hematological indices indicate a better transportation capacity of the red blood cells and this should be attributed to the antimalarial properties of the extract. Also, the white blood differential count indicates a boost in the immune system of the treated P. berghei infected mice. This study justifies the ethno-medicinal use of D. oliveri in the management of malaria.

Key words: *Daniellia oliveri, Plasmodium berghei, Hematological, Artemether, Malaria, Methanolic.*

Abbreviations

ACT-Artemisinin-based Combination Therapy, ANOVA-Analysis of variance, AQ-Amodiaqunine, CQ-Chloroquinine, DPPH -1, 1-Diphenyl-2-picrythydrazyl, EDTA-Ethylene diamine tetra acetic acid, Hb-Hemoglobin , HCL-Hydrochloric acid, H_2SO_4Hydrogen

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Tetraoxosulphate (vi) acid, $LD_{50,}$ Lethal Median Dose, PCR-Polymerase Chain Reaction, PCV-Packed Cell Volume, QBC-Quantitative Buffy Coat, RBC-Red Blood Cell, RDTs - Rapid Diagnosis Tests, SP-Sulphadoxine Pyrimethamine, WBC-White Blood Cell, WHO-World Health Organization.

Introduction

Malaria is a common and life-threatening disease in many tropical and subtropical areas. There are currently over 100 countries and territories where there is a risk of malaria transmission, and these are visited by more than 125 million international travelers every year. Each year many international travelers' fall ill with malaria while visiting countries/territories where malaria is endemic, and well over 10 000 are reported to become ill with malaria after returning home. Malaria is caused by parasitic protozoan Plasmodium. It is a vector-borne disease which is transmitted from person to person through a bite, the parasite multiply in the liver and subsequently infect red blood cells. (World malaria report, 2019).

Human malaria is caused by five different species of Plasmodium: P. falciparum, P. malariae, P. ovale, P. vivax and P. knowlesi (Greenwood et al., 2005).

The female Anopheles mosquito is the primary vector which introduces the parasite organism from its saliva into the human blood and circulatory system (Ukaegbu CO, Nnachi AU, Mawak JD, 2014). When an individual has been inoculated with a plasmodium parasite, a variety of clinical effects may follow which may lead to death. Many factors influence the disease manifestations of the infection and the likelihood of progression. These factors include the species of the infecting parasite, the levels of innate and acquired immunity of the host, and the timing and efficacy of treatment, if any (Greenwood et al., 2005).

In the human body, the parasites travel to the liver where they mature and multiply and then infect red blood cells causing fever and related symptoms. The mosquitoes which act as vector for this disease are female *Anopheles funestus, Anopheles moucheti, Anopheles gambiae, Anopheles arabiensis*(World Health Organization, 2015). Malaria is the major tropical pathology in the tropical world. A dramatic recrudescence of this disease is ongoing due to the increasing resistance of parasites.

Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today. Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated.

Traditional medicines have been used to treat malaria for thousands of years and are the source of the two main groups (artemisinin and quinine derivatives) of modern antimalarial drugs. With the problems of increasing levels of drug resistance and difficulties in poor areas of being able to afford and access effective antimalarial drugs, traditional medicines could be an important and sustainable source of treatment(Willcox & Gilbert, 2004)

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Malaria is one of the most important public health problems in term of morbidity and mortality, causing more than 200 million cases and 655,000 deaths every year.

According to the World Health Organization (WHO) Malaria Report 2011, a total of 106 countries in the world are at risk of transmission of malaria infection drugs, subsequently, SP was introduced to replace chloroquine as first line treatment for uncomplicated malaria but the parasite soon developed further resistance to the SP, which occurred at high frequency in major malarious regions (Laxminarayan, 2006). This development further led to the discovery of the artemisinin compounds known for their rapid efficacy. To enhance the effectiveness and reduce the risk of developing resistance, the artemisinin compounds were to be combined with other known antimalarial drugs in order to leverage the rapid properties of the

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artemisinin and longer duration of the partner drugs. The genus Daniellia was first named in 1854 by W. F. Daniell with the first species collected from Sierra Leone namely '*Daniellia thurifera'*. According to de la Estrella et al. (2010) thegenus is comprises of ten different species. Keay, (1958) recognized five species including Daniellia *oliveri, Daniellia thurifera, Daniellia ogea, Daniellia pynaertii* and *Daniella oblonga*.Among the genus Daniellia, *Daniellia oliveri* is the most common species that belongs to sub-family Caesalpinioideae and family Fabaceae . .*D. oliveri* is commonly known as African copoiba balsam in English, while in Nigeria, it is traditionally known by the three major languages in the country as 'Maje' in Hausa,'iya/ozabwa/agba' in Igbo, 'Emi iya' in Yoruba, 'Oda' in Igala, 'Ukpilla' in Igede and Ubakwa inIdoma. *Daniella oliveri* is a deciduous tree growing abundantly in several parts of African, and in amazon region of South America (Meggers et al.,1973; Balogun & Adebayo, 2009).

In Africa it is found abundantly in deciduous forest (starting from Senegal expanded to south Sudan and Sahel and Uganda) and in wooded savannah (de la Estrella, 2010). It was also reported to be found in Burkina Faso, in Gambia, in Ghana and in Benin Republic (Kabore, 2007; Dassou et al.,2014; & Boye et al.,2013).

MATERIALS AND METHODS

Collection of the stem barks of *Daniellia oliveri (Rolfe) Hutch. & Dalziel* was from the botanical garden University of Nigeria Nsukka ,Enugu Sate, identified by a taxonomist by name Mr.Felix Nwafor, from the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. Herbarium specimens were deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka(voucher number).

ANIMALS

Swiss albino mice weighing 15 - 40g, of about 12weeks of age were used. The animals were bred at the animal house of the Department of Pharmacology, University of Nigeria Nsukka, under standard conditions. They were housed in aluminium cages in a 12 hour light/dark cycle with litter changed every two days. They had free access to food and water. The rats were acclimatized to normal Laboratory condition prior to study.

PREPARATION OF THE PLANT EXTRACT

The Bark of *D. oliveri* was collected cleaned from unwanted deribs and air-dried for 14 days under normal room temperature (25±0.5ºC) and then pulverized using a milling machine to coarse uniform powder and to increase the surface area. The grinded bark, *D. oliveri* were properly packed in a bag.

EXTRACTION

A 200g of the grinded *D. oliveri* bark, were extracted using the solvents N- hexane and methanol. The 200g of the grinded bark, of *D. oliveri* were immersed in a 1000ml of N - heaxane and left for 72hours, with the use of mechanical shaker continuous agitation was maintained. Then it was filtered and allowed to dry under room temperature. Afterwards same dried sample 200g of the grinded bark, of *D. oliveri* were immersed in a 1000ml of methanol and left for 72hours, with the use of mechanical shaker continuous agitation was maintained. Then it was filtered and allowed to dry under room temperature, heated in a water bath at 50º C for 3-5 days to concentrate the extract. The extract was placed in a labeled container and stored in the refrigerator.

EXTRACTIVE VALUE

PETROLEUM ETHER SOLUBLE EXTRACTIVE VALUE:

A 5 g of the powdered sample was added into a beaker then 50ml of petroleum ether was poured into the beaker which was stirred and surface of the beaker was firmly covered with an aluminum foil and allowed to stand for 24 hours. The mixture was stirred at intervals at

the end of 24 hours the mixture was separated by filtration through filter paper, the residual was discarded. The filtrate was evaporated in a tarred dish at 105°C in an oven. The constant weight was gotten. Petroleum - soluble extractive value was calculated.

% Petroluem ether -soluble extractive value

= [(weight of residue ÷ weight of powdered drug) × 100%]…….1

N-HEXANE SOLUBLE EXTRACTIVE VALUE:

A 5 g of the powdered sample was added into a beaker then 50ml of N-hexane was poured into the beaker which was stirred and surface of the beaker was firmly covered with an aluminum foil and allowed to stand for 24 hours. The mixture was stirred at intervals at the end of 24 hours the mixture was separated by filtration through filter paper, the residual was discarded. The filtrate was evaporated in a tarred dish at 105°C in an oven. The constant weight was gotten. N-hexane - soluble extractive value was calculated.

% N-hexane -soluble extractive value

= [(weight of residue ÷ weight of powdered drug) × 100%]…….2

ETHYL ACETATE SOLUBLE EXTRACTIVE VALUE:

A 5 g of the powdered sample was added into a beaker then 50ml of ethyl acetate was poured into the beaker which was stirred and surface of the beaker was firmly covered with an aluminum foil and allowed to stand for 24 hours. The mixture was stirred at intervals at the end of 24 hours the mixture was separated by filtration through filter paper, the residual was discarded. The filtrate was evaporated in a tarred dish at 105°C in an oven. The constant weight was gotten. Ethyl acetate soluble extractive value was calculated.

% Ethyl acetate - soluble extractive value

= [(weight of residue ÷ weight of powdered drug) × 100%]…….3

METHANOL SOLUBLE EXTRACTIVE VALUE:

A 5 g of the powdered sample was added into a beaker then 50ml of methanol was poured into the beaker which was stirred and surface of the beaker was firmly covered with an aluminum foil and allowed to stand for 24 hours. The mixture was stirred at intervals at the end of 24 hours the mixture was separated by filtration through filter paper, the residual was discarded. The filtrate was evaporated in a tarred dish at 105°C in an oven. The constant weight was gotten. Alcohol soluble extractive value was calculated.

% Methanol - soluble extractive value

= [(weight of residue ÷ weight of powdered drug) × 100%]…….4

AQUEOUS SOLUBLE EXTRACTIVE VALUE:

A 5 g of the powdered drug samples were treated with 50 ml of water with frequent shaking during first 6 hours using electrical shaker (Stuart Scientific UK, Great Britain) and allowed to stand for 24 hours. The temperature was maintained at 45°C during the entire process. The extracts were filtered and filtrate was evaporated in a tarred dish at 10°C and weighed.

% Aqueous - soluble extractive Value

= [(weight of residue ÷ weight of powdered drug) × 100%]…….5

TOXICITY STUDIES

ACUTE TOXICITY

Swiss albino mice weighing 15 - 40kg, bred in the Department of Pharmacology and Toxicology of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka were used for the study. They were kept in clean metal cages in a 12 hour light/dark cycle with litter changed every two days. They had free access to food and water. Eighteen male and female Swiss albino mice of average weight 24.2kg were acclimatized for a week in cleaned cages and randomly divided into groups of animals three each. The animals were fasted prior to dosing, feed but not water was withheld over-night. Following the period of fasting, the animals were weighed and the test substance administered. Groups 1, 2 and 3 were orally

administered 10, 100 and 1000 mg/kg body weight, respectively, of methanol extract reconstituted in 3% tween80 following the method of Lorke (1983). The animals were observed individually after dosing at least once during the first 30 minutes, with special attention given during the first 4 hours thereafter, the nature and time of any adverse effect was noted for a total of 24 hours. Then experiment terminated. All animals were weighed and euthanized in a chloroform chamber.

DETERMINATION OF MEDIAN LETHAL DOSE (LD_{50})

Based on the result of the acute toxicity test, nine white Swiss albino mice of average weight 25.3g divided into 3 groups of three animals per group were orally administered 1600, 2900 and 5000 mg/kg body weight, methanol extract reconstituted in tween80. Death was monitored over a period of 24 h. LD_{50} was then determined using the method of Lorke (1983). The acute toxicity LD_{50} was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no death.

PHYTOCHEMICAL ANALYSIS OF *Daniellia Oliveri* BARK

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The following analysis was carried out using standard methods as described by Trease and Evans, 2009; Brain and Turner, 1975;
Sofowora, (2008).

TEST FOR ALKALOIDS

An extract of the powdered sample was prepared by macerating 3g of the powdered sample in 50ml of methanol. The extract was evaporated to dryness, 0.5g of the extract was mixed with 5ml of 1% aqueous hydrochloric acid and 1ml of the filtrate is treated with a few drops of Hagar's reagent. Turbidity or precipitation with the reagent is taken as evidence for the presence of alkaloids in the extract. 1ml of the filtrate is treated with a few drops of Dragendorff's reagent; occurrence of orange-red precipitate was taken as positive. 1ml of the filtrate is treated with a few drops of Mayer's reagent; appearance of buff-coloured precipitate was an indication for the presence of alkaloids.

TEST FOR FLAVONOIDS

A few quantity of the sample was dissolved in water and filtered. To this 2 ml of the filtrate, few drops 10% ferric chloride solution was added to produce a green-blue or violet colouration. A change in colour from green-blue or violet on addition of dilute ferric chloride was an indication of the presence of flavonoids.

TEST FOR GLYCOSIDES

A few sample was dissolved in glacial acetic acid. 1 ml of the filtrated was pipetted into the test tube and 10 ml water was added. This was boiled for 30 minutes and 2 ml of dilute ammonia was added. Then, 0.4 ml Fehling's solution A and B was added and boiled again for 5 minutes and a colour change to brick red showed the presence of glycoside.

TEST FOR HYDROGEN CYANIDE (HCN)

The sample (0.5 g) was dissolved in 2 ml of glacial acetic acid, containing 1 drop of 1 % ferric chloride. Few drops of conc. H_2SO_4 was added. Formation of a brown ring at the interface indicated the presence of hydrogen cyanide.

TESTS FOR TANNINS

Sample (0.5 g) was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. The occurrence of a blue-black, green or blue-green precipitate indicated the presence of tannins.

TESTS FOR SAPONINS

Sample (1 g) was boiled with 5 ml of distilled water and filtered. To the filtrate, 3 ml of distilled water was added and shaken vigorously for 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins.

TEST FOR TERPENOIDS

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A little of the sample was dissolved in ethanol. A 1 ml of acetic anhydride was added followed by the addition of conc. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids.

TEST FOR PHENOLS

Ferric chloride test: 10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.
Lead acetate test: 10mg extracts was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of phenol.

TEST FOR STEROIDS

To 0.2 g of the samples, 2 ml of acetic acid was added; the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e. aglycone portion of cardiac glycoside

TEST FOR REDUCING SUGARS

Each sample (0.5 g) was hydrolysed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralised with sodium hydroxide solution. To this, few drops of Fehling's solution was added and then heated on a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicated the presence of reducing sugars.

TEST FOR SOLUBLE CARBOHYDRATES.

The sample (1 g) was dissolved with 20 ml of distilled water and filtered. The filtrate (1 ml) was pipetted into the test tube. H_2S0_4 (1 ml) was added and allowed to stand for 5 minutes. This was diluted with 5 ml of distilled water, red or dull violet colour at the interphase of the two layers indicated a positive result.

A 5 g of the powdered sample was added into a beaker then 50ml of petroleum ether was poured into the beaker which was stirred and surface of the beaker was firmly covered with an aluminum foil and allowed to stand for 24 hours. The mixture was stirred at intervals at the end of 24 hours the mixture was separated by filtration through filter paper, the residual was discarded. The filtrate was evaporated in a tarred dish at 105°C in an oven. The constant weight was gotten. Petroleum - soluble extractive value was calculated.

% Petroluem ether -soluble extractive value

= [(weight of residue ÷ weight of powdered drug) × 100%]……1

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

A 5 g of powdered *Daniella oliveri*bark was macerated over night with 95% methanol with occasional shaking. It was filtered and the filtrate was used for the analysis

ESTIMATION FOR ALKALOIDS

This was determined according to the method of El-Olemyl (1994). A 0.1ml of the extract was macerated with 20 ml of ethanol 20 % sulphuric acid and filtered. Each sample (1 ml) of the filtrate and 5 ml of 60 % H_2SO_4, mix and allow to stand for 3 hrs. Measurement of absorbance was at 490 nm.The alkaloid content was calculated from the regression equation for the standard.

ESTIMATION FOR SAPONIN

Vanillin–Sulfuric acid assay was used to determine saponin content of the extract. 0.5 ml of aqueous sample solution, 0.5 ml vanillin solution of 8% (w/v) and lastly 5.0 ml of sulfuric acid of 72% (w/v) were added and mixed in an ice water bath. After the mixture was then warmed in a bath at 60 ˚C for 10 minutes then cooled in ice–cold water. Calibration curve was obtained for measured absorbance values at 527 nm.

DETERMINATION OF TANNINS

A 5ml of the filtrate was pipetted out into a tube and mixed with 3ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 530nm wave length, within 10 minutes. A blank sample was measured at the same wave length (Van Burden and Robinson, 1981). The blank sample was used to bring the spectrophotometer to zero for direct measurement of the sample extract absorbance percentage weight of tannins. The tannin content was calculated from the regression equation of the tannic acid standard.

DETERMINATION OF FLAVONOIDS

Total flavonoid content was measured by the aluminum chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminum chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as rutin equivalent mg RE/g extract on a dry weight basis using the standard curve.

ESTIMATION OF STEROIDS

1ml of Methanolic extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyano ferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2^0C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

ESTIMATION OF CYANOGENIC GLYCOSIDES

A1.0 ml of filtrate was pipette into a test tube; 4ml of alkaline picrate solution was added and incubated for 5 minutes in a water bath at 90℃. The test tube was cooled to room temperature and absorbance of the solution was recorded at 490nm.

ESTIMATION OF TOTAL PHENOLIC CONTENT

Folin-ciocalteu method was used for analysis of total phenol the plant extracts. A 0.1 ml of organic extract, 10 ml of water (deionized) and 2 ml of Folin-ciocalteu reagent were mixed in a test tube, 20% sodium carbonate solution (2 ml) added to reaction mixture and kept in dark at room temperature for 1 hour of incubation. Absorbance was measured at 640 nm. The total phenolics concentration was calculated from a calibrated curve of standard phenolic compound Gallic acid and phenolic contents of plant extracts were expressed as mg GAE/g, Gallic acid equivalent.(Gawron-Gzella *et al.*,2012).

ESTIMATION OF TERPENOIDS

To 0.1 ml of the plant extract, 1ml of phosphomolybdic acid was added and mixed well. Then 1ml of conc. $H_2SO_4$was added. The sample mixture was thoroughly vortexed and left for 3 min and then 200 µl of concentrated sulfuric acid (H_2SO_4) was added. Then it was incubated at room temperature and the absorbance was read at 700 nm using UV/visible spectrophotometer. The total terpenoid content was calculated by calibration curve of Linalool.

ESTIMATION OF GLYCOSIEDS

This was determined according to the method of El-Olemyl *et al.*, (1994).

A quantity, 1 g of the extract was macerated with 20 ml of distilled water and filter. Add 2.5 ml of 15% lead acetate and we filter and add 2.5 ml of chloroform, Shake vigorously. Collect the lower layer and evaporate to dryness. The residue was dissolve with 3 ml of glacial acetic acid and 0.1 ml of 5% ferric chloride was added. Concentrated H_2S0_4 (0.25 ml) was added and the container kept in the dark for 2 hours. Absorbance measured at 530 nm.

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ESTIMATION OF REDUCING SUGARS

This was determined according to the method of El-Olemyl *et al*., (1994). A quantity, 1 g of the extract was macerated with 50 ml of distilled water and filter. Each sample (1 ml) of filtrate was pipette inside the test tube. The alkaline copper reagent (1 ml) was added and boiled for 5 minutes. After that, allow to cool at room temperature. Add I ml of phosphomolybdic acid reagent. The distilled water (7 ml) was added inside the test tube. Measure the absorbance at 700 nm.

PHARMACOGNOSTIC PARAMETERS

TOTAL ASH VALUE

About 10g of accurately weighed *D. oliveri* grounded leaves was placed into a nickel crucible that has been heated, cooled and stored in desiccators (the grounded bark was spread in an even layer). This was heated gently in the fume cupboard until all the moisture has been driven off and the material has been completely charred. The flame (450º C) was gradually increased until the residue became white, an indication that it is free from carbon; it was then cooled and weighed. Heating and cooling was continued until a constant weight was achieved. Experiment was carried out in triplicate. The percentage total ash value was calculated with relevance to air dried drug.

% Total ash value

= [(weight of residual ash ÷ weight of initial powdered drug) × 100%]……6

ACID INSOLUBLE ASH VALUE

The ash obtained from method 1 above was boiled in a crucible with 25 m of dilute hydrochloric acid (2M) for 5 minutes; the crucible was covered with a watch glass. Then filtration was done to collect the insoluble matter on an ashless filter paper. The wash glass and the crucible were washed with hot water and the washings passed through the filter paper. Washing of the insoluble matter was continued until it was free from acid (i.e until the filtrate was neutral) and the solid was washed into the tip of the edge of the filter paper. The filter containing the insoluble matter was transferred into the original crucible, then dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a desiccator for 30minutes and then weighed.. Experiment was carried out in triplicates. The percentage acid –insoluble ash was calculated with reference to the air dried drug.

% Acid-insoluble ash value

= [(weight of residual acid-insoluble ash ÷ weight of initial powdered drug) × 100%]……7

WATER SOLUBLE ASH VALUE

To the crucible containing total ash, 25ml of distilled water was added and boiled for 5minutes. The insoluble matter was collected on an ashless filter paper. Then it was washed with hot water and ignited in a crucible for 15minutes at a temperature not exceeding 450º C. The weight of this residue was subtracted from the weight of the total ash. The content of water soluble ash in mg/kg of the air dried material was calculated.

MOISTURE CONTENT

LOSS ON DRYING

To an evaporating dish which has been heated to constant weight and stored in a desiccator, 3g of *Danielle oliveri* grounded bark was accurately weighed into the dish. Then it was placed in an oven at 100 – 105º C for 5 hours and the sample was weighed. The drying and weighing was continued at 1 hour intervals till the difference between two successive weighing corresponded to not more than 0.25 %. The mixture content is the total weight lost expressed as percentage of the initial weight of sample.

FOAMING INDEX

The Foaming Index of the powdered bark sample was evaluated following the method prescribed in WHO (2011). A 1.0 g of coarsely powdered drug was placed in a 500 ml

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conical flask containing 100 ml of boiling water maintained at moderate boiling at 900C for 30 minutes. It was cooled and filtered into a volumetric flask, sufficient water was passed through the filter paper to make the volume up to 100 ml. Ten clean and stoppered test tubes were marked 1 to 10 and successive portions of 1 ml, 2 ml … up to 10 ml of the filtrate was placed in each separate tubes and the volume was made up to 10ml in each. The test tubes were shaken for 15 seconds and allowed to stand for 15 minutes and the height of the foam was measured.

Foaming Index was calculated by using this formula

Foaming Index=$1000a$

Where, a = Volume (ml) of decoction used for preparing the dilution in the tube where 1 cm or more foam.

BITTERNESS VALUE

The method adopted was based on (WHO, 2011) with slight modifications. 0.1 g of quinine hydrochloride was carefully weighed and dissolved in enough distilled water to get 100 ml solution, 5 ml of this solution was placed in a 500 ml flask and made up to the 500 ml mark with distilled water. A 3 g portion of the powdered leaf material was mixed with 100 ml of distilled water, shaken and filtered.

Nine test tubes were washed and rinsed with distilled water, allowed to dry and labeled 1 to 9 and various amounts of the standard quinine solution were put in them in an increasing order-1ml, 2ml, 3ml up to 10ml) and made up to 10 ml with distilled water. The same was done with the plant extract.

Table 1a; Serial dilution for bitterness value determination

Tube no	1	2	3	4	5	6	7	8	9
Std.quinine(ml)	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
Distilled water(ml)	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2
Quinine HCLin 10ml of sol.	.042	.044	.046	.048	.050	.052	.054	.056	.058

The same was done with the test solution as shown below.

Table 1b: Serial dilution for bitterness value determination

Tube no	1	2	3	4	5	6	7	8	9
Test solution(ml)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0
Distilledwater(ml)	9.0	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0

The tester rinsed his mouth with distilled water and swirled 10 ml of the test tube number 1 of the serially diluted quinine solution for 30 minutes, the solution was withdrawn and the mouth was rinsed after 1 minute and the presence or absence of a bitter taste was noted.

This was repeated for the test tubes in ascending order till a bitter taste was observed; the test tube number was noted. The mouth was rinsed repeated till no bitter sensation was observed. After ten minutes the above procedure was performed using the test solution. The bitterness value was calculated using the following equation;

Bitterness value =2000 x c axb IU/g Where, c is the threshold concentration of quinine hydrochloride in mg/ 10 ml of solution, a is the quantity of test substance in mg per ml, and b is the volume of the test substance stock solution per 10ml of dilution.

SWELLING INDEX

Procedure: The swelling Index of the powdered bark sample was evaluated following the method prescribed in WHO (2011). 1g of plant material was placed in a 25 ml glass-stopper measuring 36 cylinder. The internal diameter of the cylinder was 16 mm, the length of the

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graduated portion was 125 mm, marked in 0.2 ml divisions from 0-25 ml in an upwards direction. 25 ml of water was added, the mixture was shaken thoroughly every 10 minutes for 1 hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material, including any sticky mucilage was measured. This was carried out simultaneously for three determinations.

EXPERIMENTAL DESIGN

Swiss albino mice weighing 15 - 40kg, bred in the Department of Pharmacology and Toxicology of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka were used for the study. They were kept in clean metal cages in a 12 hour light/dark cycle with litter changed every two days; they had free access to food and water. Twenty-fiveSwiss albino mice mixed up of males and females with an average weight 27. 54kg were acclimatized for a week in cleaned cages and randomly divided into 5 groups of five animals each.

Each mouse in the treatment group was infected with a standard inoculum of 107 parasitized erythrocytes in phosphate buffered saline (0.2 ml) prepared from the donor mouse erythrocytes. A set of 20Swiss albino mice randomized into 4 groups (n = 4) were intra-peritoneally infected with107 parasitized erythrocytes on the 1st day of the experiment (Day 0) with oral treatments commencing on Day 3 post inoculation (i.e.72 hours later) until Day 6.

To ascertain effect of the extract compared to the standard drug and the untreated group blood was collected, on the third day of experiment, thin blood smears were made and stained with 10% giemsa in phosphate buffer, pH 7.2 for 20 min. A blood sample for the slide preparation was taken using tail bleeding method. The slide was examined under a microscope at 100×.

The experiment had a positive control group and a negative control group.

Animals in Group 1 (Toxic group) received neither extract nor standard drug but received parasitized blood only.

Animals in Group 2 were given feed and water (normal group) and served as the negative control non-parasitized blood.

Animals in Group 3 were given Artemether 80mg/kg throughout the treatment period and served as the positive control.

Animals in Group 4 received the methanol extract 200mg/kg.

Animals in Groups 5 received the methanol extract 400mg/kg.

Stained (10% Giemsa) tail blood films of the infected animals were examined microscopically with 100 × magnification under oil immersion on Day 4 post-inoculation.

HAEMATOLOGICAL INDICES EVALUATION.

Whole blood samples were collected in EDTA tube for determination of hematological parameters including Packed Cell Volume (PCV), Red Blood Cell Count (RBC), Hemoglobin (Hb), White Blood Cell (WBC), Platelets, Differential White Blood Count - Neutrophils, Lymphocytes, Monocytes, Erythrocytes and basophils.

PCV Count: The blood was collected into a capillary tube and spinned in a centrifuge for 5 minutes and then read using a PCV or hematocrit reader.

PLATELET Count: 20 µl of blood + 380µl of ammonium oxalate solution were prepared and drops of the solution mixture was added to a counting chamber and mounted on a microscopic then counted.

RBC Count: 20µl of blood + 3.98ml of 0.85% of NaCl solution. Drops were added on the counting chamber, mounted on the microscope and then counted.

WBC Count: 20µl of blood + Tucks reagent then drops added to on the counting chamber, mounted on a microscope then read.

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Hb Count: 20µl of blood + 4ml of Drapkins solution then read in a spectrophotometer at 540nm wavelength.

DIFFERENTIAL COUNT: A thin smear of the blood sample was made, allowed to air dry, stained with eosin stain and counted using the microscope under immersion oil which helps to increase the refractive index.

- **Erythrocytes count**: pink to red-orange biconcave discoid forms (usually).
- **Lymphocytes count:** dark violet nucleus with medium blue cytoplasm.
- **Monocytes count:** lobated nucleus, medium purple with light blue cytoplasm.
- **Neutrophils count:** dark blue to purple nucleus (3 or more lobes), pale pink to almost colorless cytoplasm, red to lavender small granules.
- **Eosinophils count**: bright red or reddish orange granules in pale pink cytoplasm, blue to blue-purple nucleus (multilobed).
- **Basophils count**: deep purple and violet black granules in pale blue or neutral cytoplasm, dark blue to purple nucleus (often bilobed).

STATISTICAL ANALYSIS

Results of the study were expressed as mean±standard deviation (M ± SD) except in the hematological indices in which ± standard error of mean (M ± SEM) was used. All the tests were performed at the 95% confidence interval using SPSS version 20. Comparison of hematological parameters among groups and statistical significance was determined by one-way ANOVA and post hoc test (Duncan).The results were considered significant when $P < 0.05$.

RESULTS

EXTRACTIVE VALUES

The result of the extractive values of *D. oliveri* bark is represented in table 2 and it reveals that methanol solvent has the highest extract value of 8.4%w/w.

Table 2: The extractive values of D. oliveri bark

Extraction solvent	Extractive value (%w/w)
Petroleum ether	0.600±0.10
N- hexane soluble	0.667±0.88
Ethyl acetate soluble	1.600±0.10
Methanol soluble	8.400±0.10
Aqueous soluble	6.200±0.10

Data are presented as means of 3 determinations ± SD

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Table 3: Qualitative phytochemical analysison *D. oliveri bark*

Phytochemical Test	Inference
Alkaloids	+
Flavonoids	+
Glycosides	+
Cyanogenetic compound	-
Tannins	+
Saponins	+
Terpenoids	+
Phenols	+
steroids	+

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Reducing sugar	+
Soluble Carbohydrates	+

Key:+ = **Present** - = **Absent**

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Table 4: Quantitative phytochemical analysison *D. oliveri bark*

Phytochemical	**Quantity (mg/g)**
Alkaloids	10.179±0.61
Saponin	1.674±0.43
Tannis	10.738±0.61
Flavonoids	3.923±0.15
Steriods	2.665±0.07
Cyanogenic glycosides	NIL
Phenols	134.604±14.83
Terpenoids	22.436±4.87
Glycosides	14.485±0.08
Reducing sugars	4.138±1.36

Data are presented as means of 3 determinations ± SD

PHARMACOGNOSTIC PARAMETERS

ASH VALUES

Table 5: Ash values of *D. oliveri bark*

Ash contents	**Composition(%w/w)**
Total ash	5.600±0.10
Acid insoluble ash	2.800±0.88
Water soluble ash	0.500±0.10

Data are presented as means of 3 determinations ± SD

MOISTURE CONTENT

Table 6: Moisture content of *D. oliveri bark.*

Moisture content	**Composition(%w/w)**
Loss on drying	13.933 ±.12

FOAMING INDEX

Determination of the foaming index on *D. oliveri bark* was insignificant because length of the foam obtained was below 100cm.

BITTERNESS VALUE

Table 7: Bitterness value of *D. oliveri bark.*

Bitterness Value	4.57 ±.01

Data are presented as means of 3 determinations ± SD

SWELLING INDEX

Table 8: Swelling indexof*D. oliveri bark.*

Swelling index	2.867±.986

Data are presented as means of 3 determinations ± SD

HAEMATOLOGICAL INDICES

PACKED CELL VOLUME (PCV)

The bar chart below the results of the study showed that the un-infected mice (Group 2) had the highest %PCV (37.00±.70), followed by Group 3 which was treated with standard artemether (36.00±.89), Group 5 which was treated with 400mg/kg of the extract (35.00±.22), Group 4 which was treated with 200mg/kg of the extract (33.60±.22). Meanwhile Group 1 which was infected but untreated recorded the lowest PCV count (30.00±1.34) as shown in

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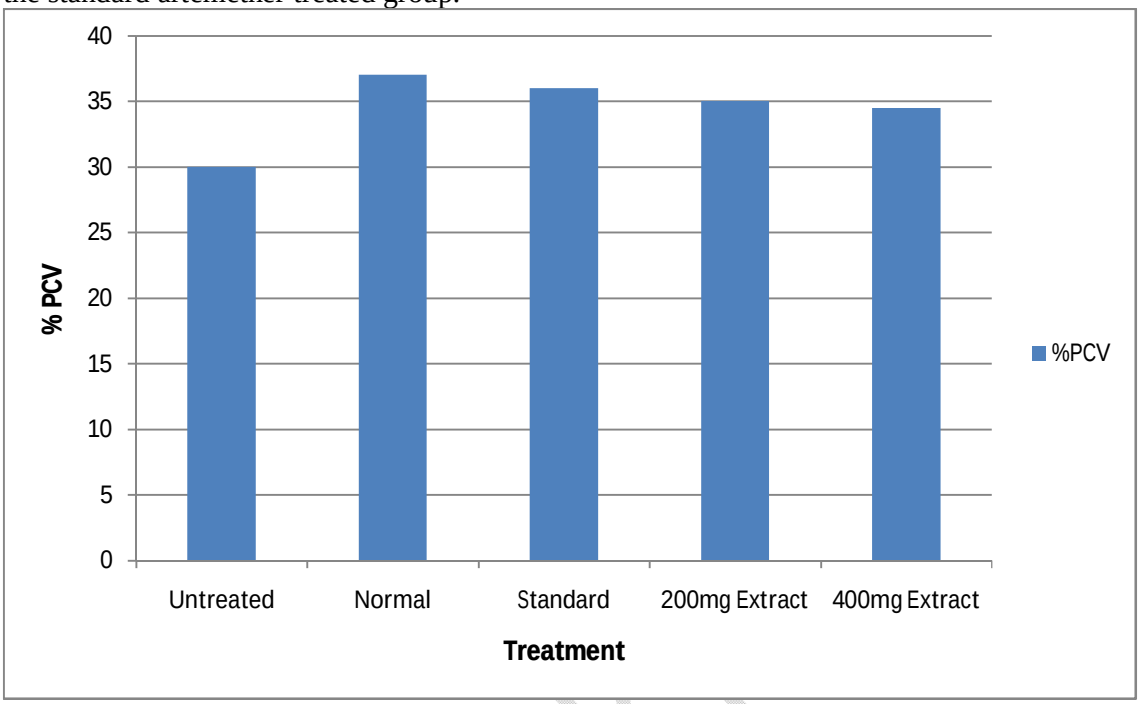
the Appendix i. It was also noted from the result obtained that there was no significant difference (P≤0.05) in the PCV count of the groups treated with the extract when compared to the standard artemether treated group.



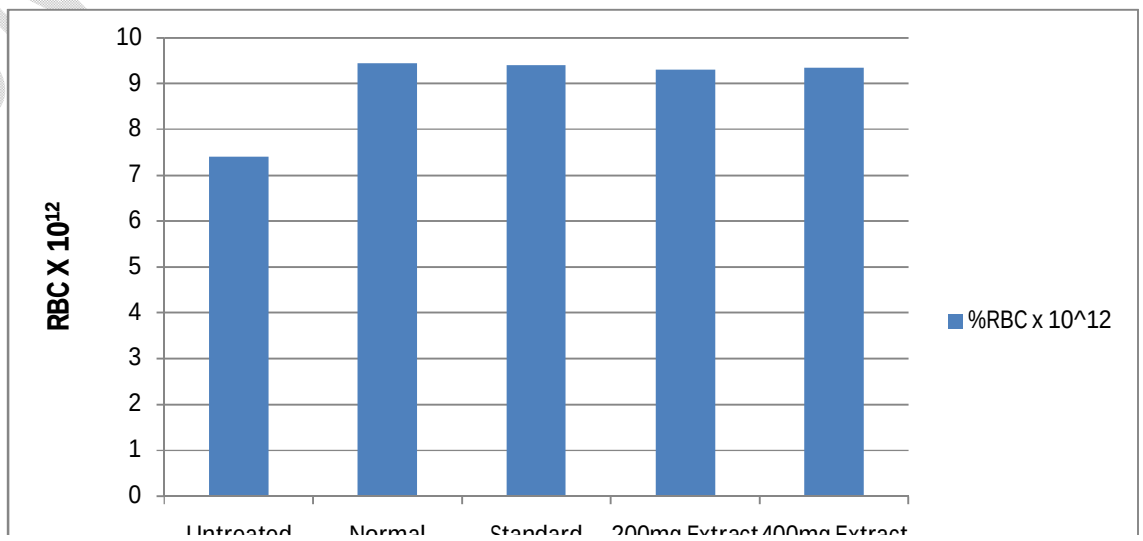
Significantly higher than infected untreated animals at P≤0.5

Figure 1: Effects of *D. oliveri* methanol extract and artemether on (PCV) of *P. berghei* infected mice.

Data are presented as means of 5 determinations ± SEM

RED BLOOD CELL COUNT (RBC)

The bar chart below the results of the study showed that the un-infected mice (Group 2) had the highest %RBC (9.44±.18), followed by Group 3 which was treated with standard artemether (9.40±.17), Group 5 which was treated with 400mg/kg of the extract (9.28±.14), Group 4 which was treated with 200mg/kg of the extract (9.14±.20). Meanwhile Group 1 which was infected but untreated recorded the lowest RBC count (7.40±.27) as shown in the Appendix i. It was also noted from the result obtained that there was no significant difference (P≤0.05) in the RBC count of the groups treated with the extract when compared to the standard artemether treated group.



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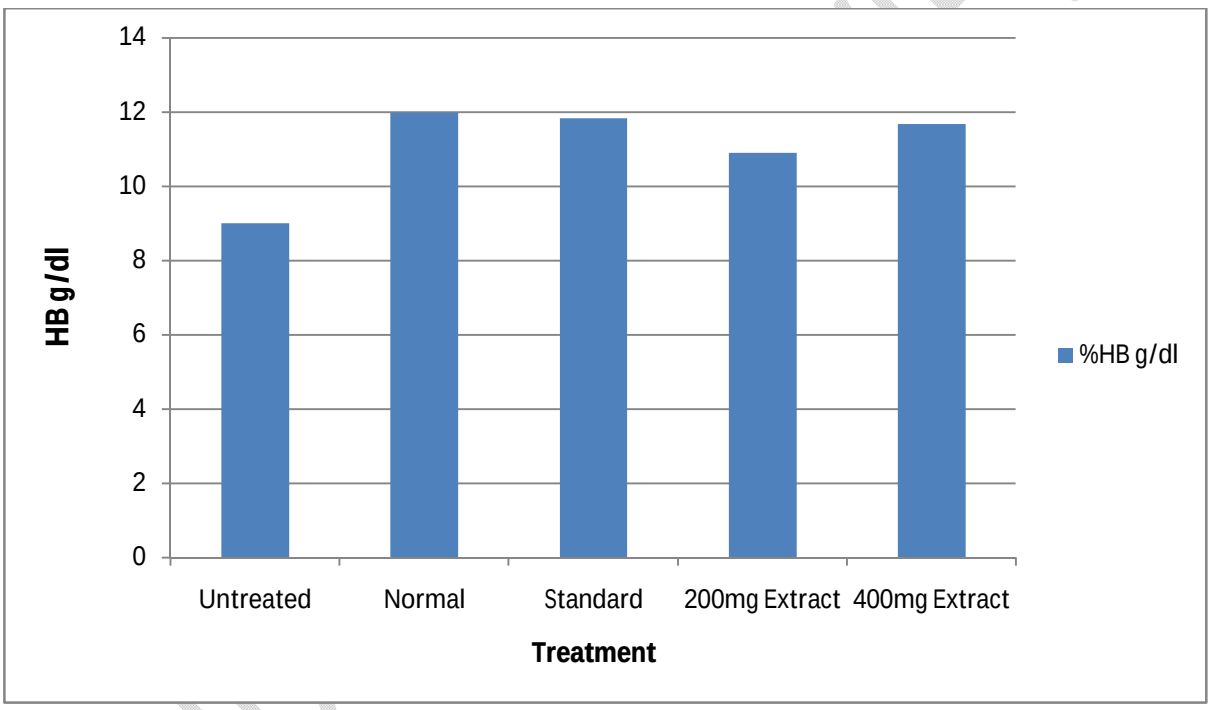
Significantly higher than infected untreated animals at P≤0.5

Figure 2: Effects of *D. oliveri* methanol extract and artemether on (RBC) count of *P. berghei* infected mice.

Data are presented as means of 5 determinations ± SEM

HEMOGLOBIN CONCENTRATION

The bar chart below the results of the study showed that the un-infected mice (Group 2) had the highest Hb concentration (11.98±.14), followed by Group 3 which was treated with standard artemether (11.82±.74), Group 5 which was treated with 400mg/kg of the extract (11.68±.16), Group 4 which was treated with 200mg/kg of the extract (10.90±.04). Meanwhile Group 1 which was infected but untreated recorded the lowest Hb concentration (9.00±.00) as shown in the Appendix i. It was also noted from the result obtained that there was no significant difference (P≤0.05) in the Hb concentration of the groups treated with the extract when compared to the standard artemether treated group.



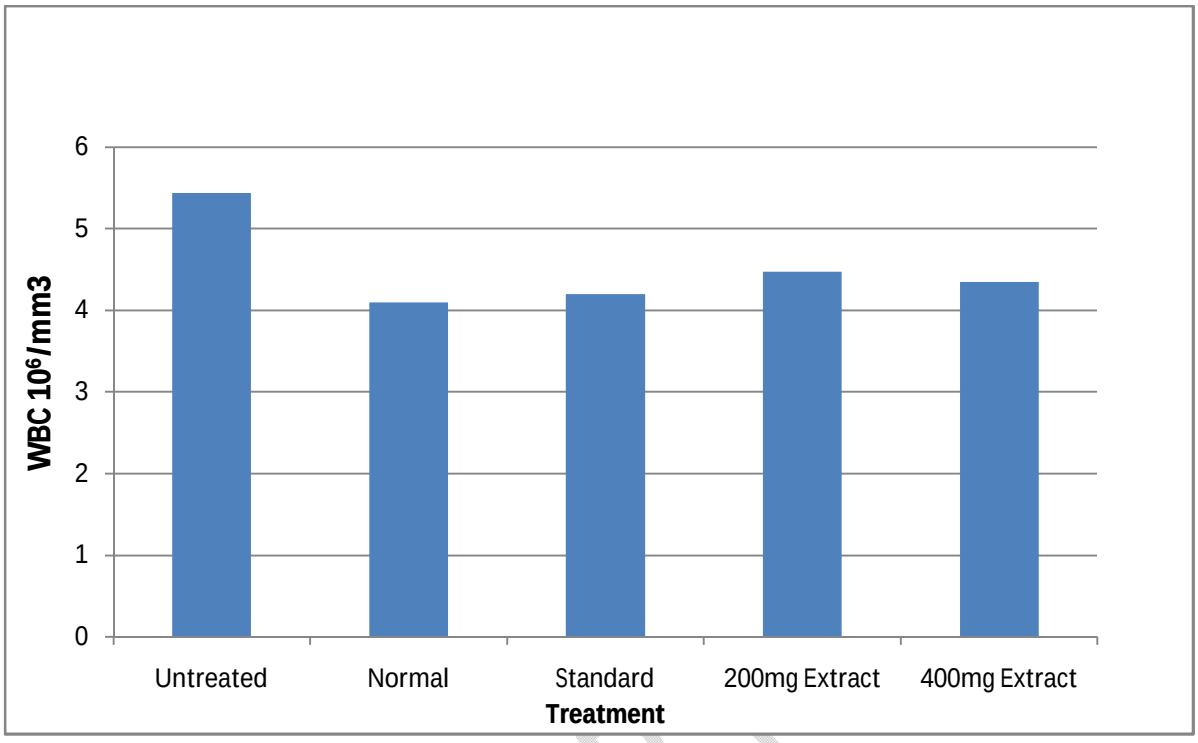
Significantly higher than infected untreated animals at P≤0.5

Figure 3: Effects of *D. oliveri* methanol extract and artemether on (Hb)concentration of *P. berghei* infected mice.

Data are presented as means of 5 determinations ± SEM

WHITE BLOOD CELL COUNT (WBC)

The bar chart below the results of the study showed that infected and untreated mice (Group 1) had the highest % WBC ($5.44x10^3$), followed by Group 4 which was treated with 200mg/kg of the extract ($4.48x10^3$), Group 5 which was treated with 400mg/kg of the extract ($4.22x10^3$), Group 3 which was treated with artemether standard drug($4.20x10^3$). Meanwhile Group 2 which was uninfected and untreated recorded the lowest WBC count ($4.10x10^3$) as shown in Appendix i. It was also noted from the result obtained that there was no significant difference (P≤0.05) in the WBC count of the various groups.



Significantly lower than infected untreated animals at P≤0.5

Figure 4: Effects of *D. oliveri* methanol extract and artemether on (WBC) count of *P. berghei* infected mice.

Data are presented as means of 5 determinations ± SEM

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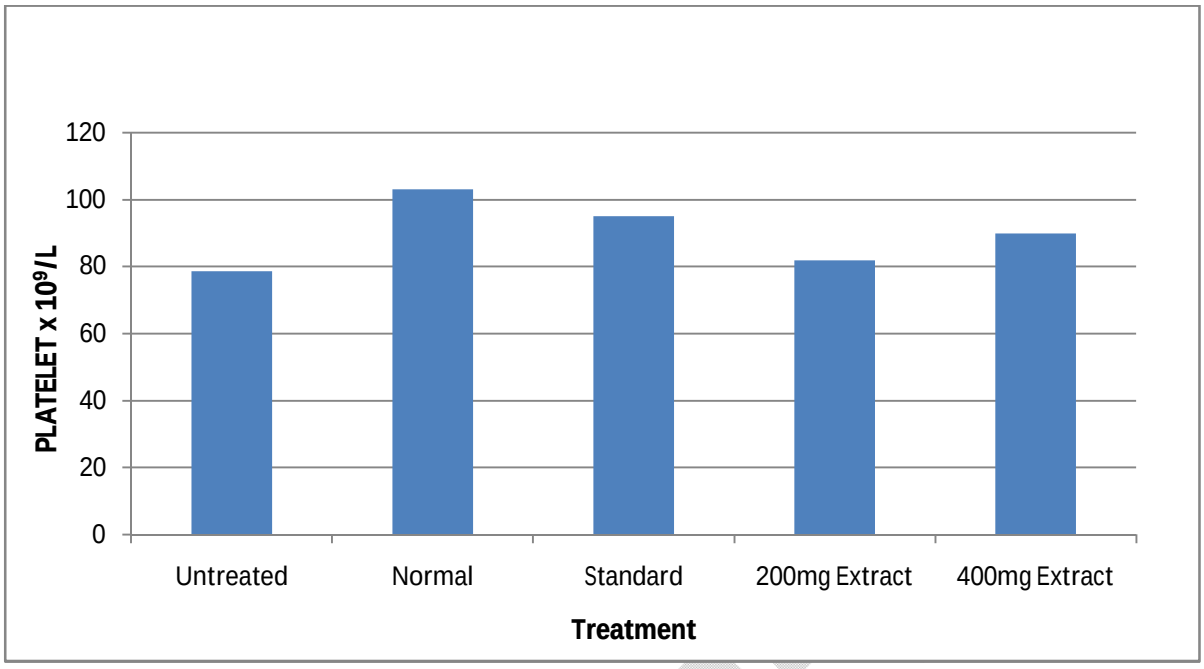
PLATELET COUNT

The bar chart below the results of the study showed that the un-infected mice (Group 2) had the highest %platelet (103.20±3.07), followed by Group 3 which was treated with standard artemether (95.20±2.51), Group 5 which was treated with 400mg/kg of the extract (90.00±.44), Group 4 which was treated with 200mg/kg of the extract (82.00±.89). Meanwhile Group 1 which was infected but untreated recorded the lowest %platelet (77.20±.96) as shown in the Appendix I.



Significantly lower than infected and treated animals at P≤0.5

Figure 5: Effects of *D. oliveri* methanol extract and artemether on platelet count of *P. berghei* infected mice.

Data are presented as means of 5 determinations ± SEM

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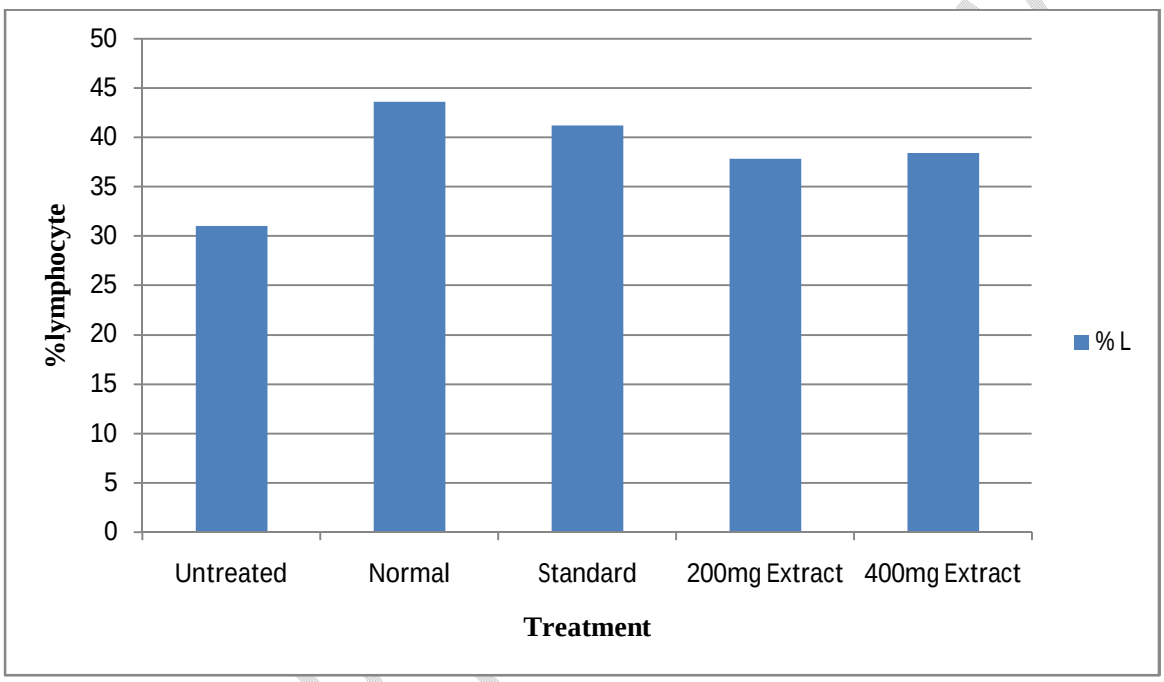
LYMPHOCYTE COUNT

The bar chart below the results of the study showed that unfected and untreated mice Group 2 had the highest % lymphocyte (43.60±.68), followed by Group 3which was treated with artemether standard drug (41.20±92),then Group 5 which was treated with 400mg/kg of the extract (38.40±.81) and Group 4 which was treated with 200mg/kg of the extract (37.80±.86).Meanwhile Group 1 which was infected and untreated recorded the lowest %lymphocyte (31.00±.00) as shown in (Appendix I). It was also noted from the result obtained that there was no significant difference (P≤0.05) in the lymphocyte count of the groups treated with the extract when compared to the standard artemether treated group.



Significantly higher than infected and treated animals at P≤0.5

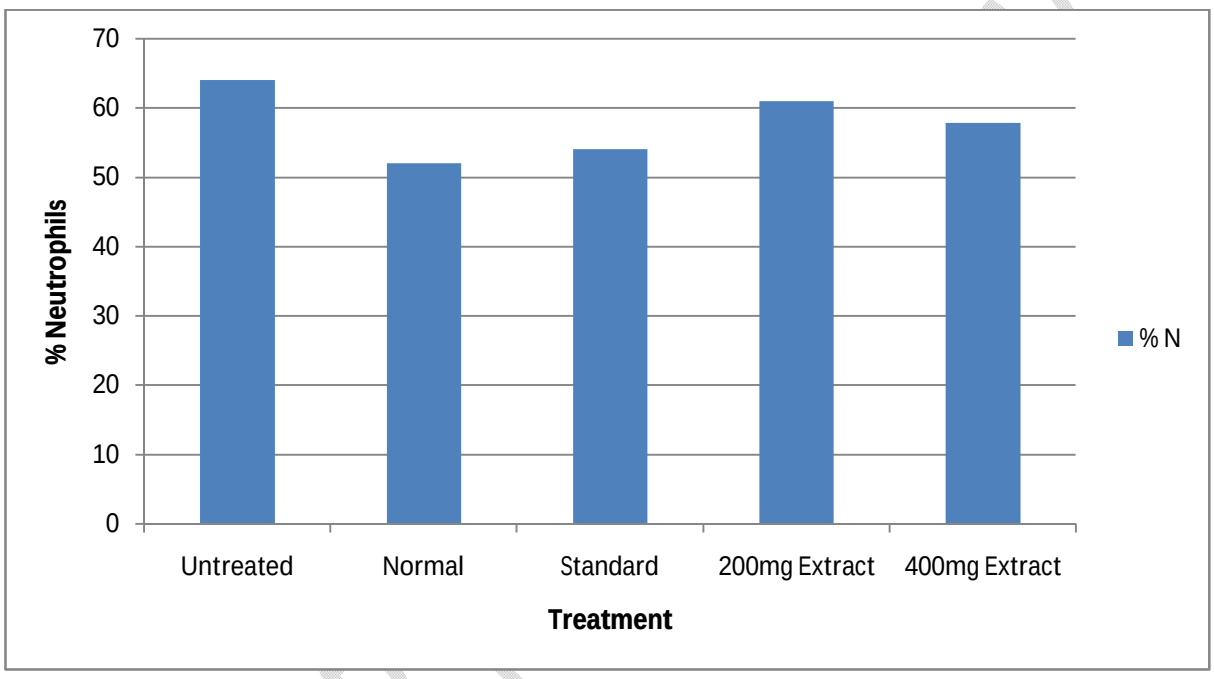
Figure 6: Effects of *D. oliveri* methanol extract and artemether on lymphocyte count of *P. berghei* infected mice.

Data are presented as means of 5 determinations ± SEM

UNDER

NEUTROPHIL COUNT

The bar chart below the results of the study showed that infected and untreated mice Group 1 had the highest %Neutrophils (64.00±6.02), followed by Group 4 which was treated with 200mg/kg of the extract (61±.44), Group 5 which was treated with 400mg/kg of the extract (57.80±1.11), Group 3 which was treated with artemether standard drug (54.00±.89). Meanwhile Group 2 which was uninfected and untreated recorded the lowest %Neutrophils (52.00±.89) as shown in Appendix i. It was also noted from the result obtained that there was no significant difference (P≤0.05) in the Neutrophils count of the groups treated with the extract when compared to the standard artemether treated group.



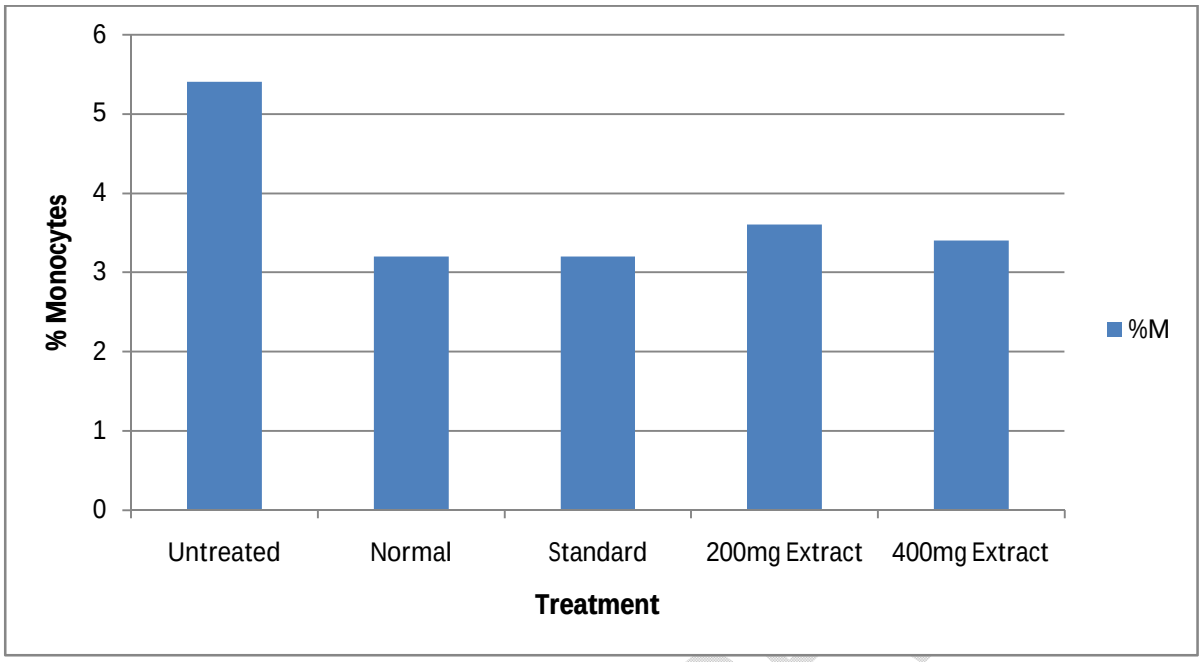
Significantly higher than infected and treated animals at P≤0.5

Figure 7: Effects of *D. oliveri* methanol extract and artemether on neutrophil count of *P. berghei* infected mice.

Data are presented as means of 5 determinations ± SEM

MONOCYTE COUNT

The bar chart below the results of the study showed that infected and untreated Group 1 recorded the highest % Monocyte (5.40±.24), followed by Group 4 which was treated with 200mg/kg of the extract (3.60±.25), Group 5 which was treated with 400mg/kg of the extract (3.40±.25). Meanwhile un-infected mice Group 2and Group 3 which was treated with artemether standard drug had the lowest % Monocyte (3.20±.37), as shown in Appendix i. It was also noted from the result obtained that there was no significant difference (P≤0.05) in the monocytes count of the groups treated with the extract when compared to the standard artemether treated group.



Significantly higher than infected and treated animals at P≤0.5

Figure 8: Effects of *D. oliveri* methanol extract and artemether on monocyte count of *P. berghei* infected mice.

Data are presented as means of 5 determinations ± SEM

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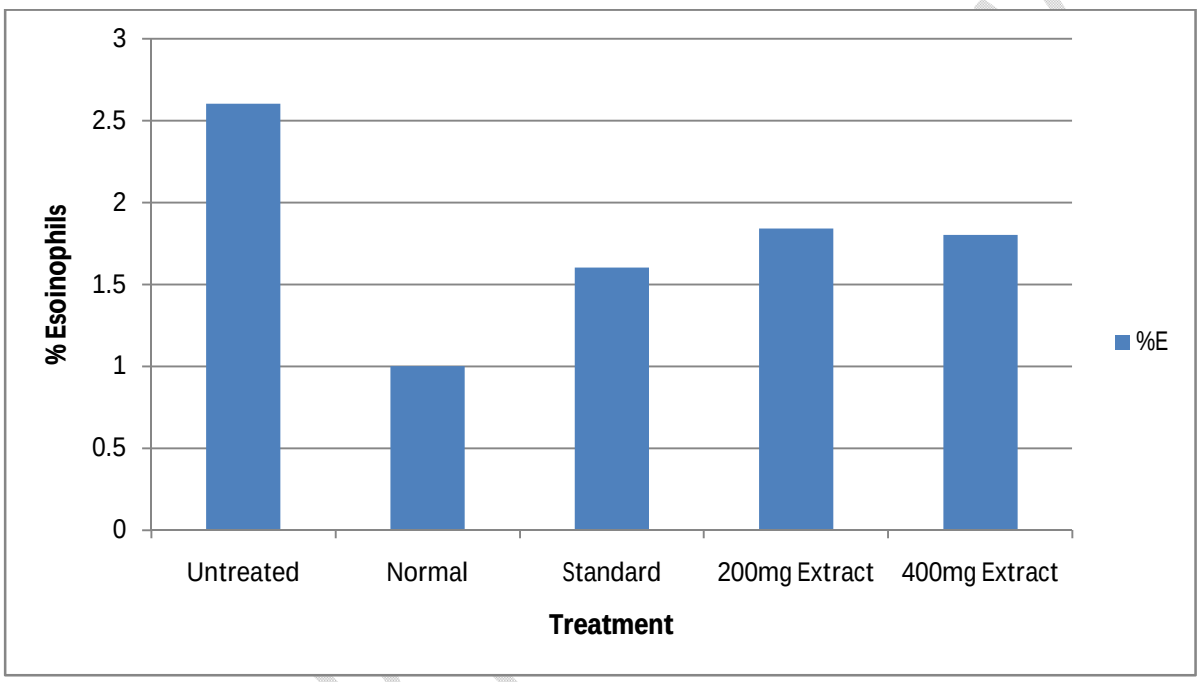
ESOINOPHIL COUNT

The bar chart below the results of the study showed that infected and untreated mice Group 1 had the highest %Eosinophil(2.60±.00), followed by Group 4 which was treated with 200mg/kg of the extract (1.84±.20), Group 5 which was treated with 400mg/kg of the extract (1.80±.20), Group 3 which was treated with artemether standard drug (1.60±.25). Meanwhile Group 2 which was uninfected and untreated recorded the lowest % Eosinophil(1.00±.60) as shown in Appendix i. It was also noted from the result obtained that there was no significant difference (P≤0.05) in the %Eosinophil count of the groups treated with the extract when compared to the standard artemether treated group.



Significantly higher than infected and treated animals at P≤0.5

Figure 9: Effects of *D. oliveri* methanol extract and artemether on eosinophil countof *P. berghei* infected mice.

Dataare presented as means of 5 determinations ± SEM

DISCUSSION

The content of herbal medicines has changed from crude mixture of material from plants to standardized materials, to extracts, and to isolates and chemically modified entities. Member of the Fabaceae family and *oliveri* species have been found through researches to contain many chemical compounds and possess many pharmacological activities. This research has attempted to provide phytochemical, physiochemical and pharmacological (antimalarial activity) information the plant studies *Daniellia oliveri.* This will go a long way in laying the foundation for any future work on the plant by providing this basic information. *Daniellia oliveri* is a tall (15cm – 20cm height) and slendertall tree. Its scaly bark is light grey in colour with a stripped deep red slash and the leaves are perpinnate (pink to red colour during flowering period). The qualitative phytochemical screening of *D. oliveri*stem bark in the research revealed the presence

of alkaloids, phenols, terpenoids, glycosides, and reducing sugars. Cyanogenic glycosides were absent in the qualitative analysis. This was in line with previous publications by (Hassan et al., 2008; Muanda 2009; Boye et al., 2013; Ahmadu et al., 2004; Alain et al., 2015
The result of the acute toxicity test *of D. oliveri* showed that the extract was not lethal even at the highest dosage (5000 mg/kg body weight) administered. There was no morphological,

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APPENDICES

Appendix I: Raw data obtained for hematological indices of the infected and untreated group.

	%PCV	RBC x 10^{12}	Hb g/dl	WBC $10^6/mm^3$	PLATELET x $10^9/L$	% N	% L	%M	%E
Un-treated Group									
Head	27.00	6.70	9.00	6000.00	100.00	88.00	31.00	6.00	2.00
Tail	33.00	8.30	9.00	4800.00	102.00	60.00	31.00	5.00	3.00
Trunk	30.00	7.50	9.00	5400.00	110.00	58.00	31.00	5.00	3.00
RH	27.00	7.50	9.00	6000.00	94.00	57.00	31.00	6.00	2.00
LH	33.00	7.00	9.00	5000.00	110.00	57.00	31.00	5.00	3.00

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