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Phytochemical Composition and Antiplasmodial Potential of Fruit Extract of *Hunteria umbellata* on Chloroquine-Sensitive *Plasmodium berghei berghei* (NK65)-Infected Albino Mice

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ABSTRACT

Background and Objective: *Hunteria umbellata* plant is used in African traditional medicine for treating a variety of illnesses including malaria. This study evaluated the bioactive compounds, proximate composition and the antiplasmodial potentials of the fruit extract of *H. umbellata*. **Materials and Methods:** The fruits of *H. umbellata* were air-dried for 21 days. The dried fruits were pulverized into a fine powder and extracted with 20 mL of dichloromethane, concentrated on a steam bath to about 5 mL and air-dried to about 2 mL for GC-MS analyses. A total of 105 albino mice were divided into 3 sets of 35 mice used for the curative, chemo-suppressive and prophylactic assessment, respectively. Each set is comprised of 7 groups of 5 mice. Groups 1-7 were infected with *Plasmodium berghei*. Group 1 received distilled water only, Group 2 received 5 mg kg^{-1} b.wt., of chloroquine phosphate. Groups 3-7 received 500, 1000, 1500, 2000 and 2500 mg kg^{-1} b.wt., of aqueous fruit extract of *H. umbellata* respectively. **Results:** Proximate analysis showed the composition of moisture, total ash, crude protein, crude lipid, crude fibre and carbohydrate as: 7.12±0.20, 2.46±0.22, 5.84±0.58, 15.21±0.15, 22.86±0.19 and 46.81±0.50%, respectively. Quinoline, 2-methyl 13.27%, Hyoscyamine 9.42%, Benzyl Benzoate 7.62%, Beta-Caryophyllene 6.75% and Coumaric acid 6.48% were predominant among the 30 bioactive components identified. The extract, at different dosage, induced a significant decrease ($p<0.05$) in percentage parasitaemia with a corresponding increase in percentage suppression of the *Plasmodium* parasite. **Conclusion:** *Hunteria umbellata* fruit is high in crude fibre, carbohydrates and the bioactive compounds have antiplasmodial potentials.

KEYWORDS

Hunteria umbellata, bioactive compounds, proximate composition, fruit extract, *Plasmodium berghei*, antiplasmodial

INTRODUCTION

Since plants can produce a large number of secondary metabolites that act as their defensive systems against micro- and macro-organisms, plants are the primary source of medicines for humans. Flavonoids, alkaloids, tannins and phenols are a few examples of some of the most significant phytochemicals present

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in medicinal plants. Several studies have demonstrated that different medicinal plant extracts possess numerous biological properties such as antimicrobial, antioxidant, anti-inflammatory, anticancer and anti-diabetic activities. According to estimates from the World Health Organization, a sizable population relied on traditional medicinal plants to treat a variety of disorders[1]. In addition, many individuals have started using medicinal plants as an alternative therapy to contemporary medications. Since the majority of current medications are either from plants or partially synthesized from them, new antibacterial agents from plants are constantly being developed. Millions of people around the world continue to be affected by malaria, one of the most terrible diseases. It is an infectious sickness brought on by parasitic Plasmodium protozoans and transmitted by the female anopheles' mosquito. According to recent WHO data, 241 million cases and 627,000 deaths were reported in 2020 and Nigeria has the highest percentage of malaria infections (26.8%) and deaths (31.9%) worldwide[2]. Sub-Saharan African nations bear the bulk of the world's mortality burden, the Democratic Republic of the Congo and Nigeria account for more than 35% of all estimated malaria fatalities worldwide[1].

Treatment for people with malaria is becoming increasingly challenging due to the growing issue of drug resistance, this is because the parasite constantly modifies how the metabolic pathways interact with one another throughout its life cycle. Natural malaria treatments frequently focus on the use of plant-based combinations that can reduce the activities of the *Plasmodium* spp. According to Manach *et al*.[3], Africa is blessed with enormous biodiversity resources and it is estimated to contain between 40 and 45,000 species of plant with a potential for development and out of which 5,000 species are used medicinally. These herbs have been continuously used as an elixir in the treatment of a wide range of ailments due to their widespread availability, low cost and putative efficacy[4]. Modern conventional medicine has gone digital and unprocessed plant products can now be bought. It is occasionally preserved and packaged as tablets, capsules, powder and tonics. Knowing which plants are helpful is crucial for making use of all the advantages they have to offer. The Osu fruit, scientifically known as *Hunteria umbellata*, is one of these vitally important beneficial plants.

Hunteria umbellata popularly known as "Osu, Nkpokiri, Madaci and Abere or Erin" among the Edo, Igbo, Hausa and Yoruba people of Nigeria respectively, is a tropical rainforest tree widely used in traditional and folkloric systems of medicine. It is a member of the Apocynaceae family. The plant, a small glabrous tree with a girth of 2 to 5 feet and a height of 25 to 40 feet, thrives and is common throughout the tropical West African forest grove, particularly in Ghana and Southern Nigeria, as well as in Cameroun and Ubangi-Shari. The wood is yellow, very hard and fine-grained[5,6]. It is considered one of the best local woods for tool-handles. It is said to be termite-proof and durable. A number of alkaloids has been detected in the plant while the forked stems are used as hut-posts[7]. In Nigeria, the root and bark are used to make a bitter tonic and the powdered root and root-decoction are used to treat menorrhagia and prevent miscarriage[6]. Various plant components, especially the leaves, seeds, roots and barks, are highly prized in African traditional medicine for treating a variety of human illnesses, such as fever, pain, abdominal cramps, diabetes mellitus, obesity and as an immune booster[8,9]. Various medications manufactured from plant extracts are currently utilized to treat a variety of clinical disorders, although the therapeutic properties of the majority of plant species have not received much scientific support. However, the objective of the present study was to assess *H. umbellata's* identification, characterization, phytochemical analysis and antiplasmodial potential.

MATERIALS AND METHODS

Sample collection and identification: The study was carried out from January to November, 2022. The fruit of *H. umbellata* was purchased locally at the Rumuokoro market, Obio-Akpor Local Government Area in Rivers State, Nigeria. It was first classified and identified at the herbarium of the Plant science and Biotechnology Department, Faculty of Science, University of Port Harcourt by Dr. Chimezie Ekeke and the herbarium number UPH/P/211was assigned to it.

Proximate analysis of the fruit extract of *H. umbellate*: The proximate analysis of the fruit extract of *H. umbellata* was done to establish its moisture, total ash, crude protein, crude lipid, crude fibre and carbohydrate composition. The methods as described by Onyegeme-Okerenta *et al.*[10] were adopted.

Sample extraction: The unripe fruit of *H. umbellata* was washed and allowed to dry under normal room temperature. The fruits were opened and the seeds were extracted and the entire fruits and their components were sun-dried for two days to reduce the moisture content and also to prevent decomposition and later air-dried for 21 days. The dried fruits were then ground to powder using a mechanical grinder. Five hundred grams of the ground fruits were soaked in 100 mL of distilled water and allowed to stand for 72 hrs. After which, it was then filtered concentrated under pressure in a rotor vapour and air-dried to a constant weight in an oven set at 40°C for 48 hrs.

GC-MS analysis of the fruit extract of *H. umbellate*: Ten grams of the powdered sample were extracted with 20 mL of dichloromethane, concentrated to about 5 mL on a steam bath and purified to about 2 mL for gas chromatographic analysis by passing through a pasture pipette filled with silica gel and anhydrous sodium sulphate on a membrane. Prior to the analysis, the dichloromethane concentrate of the individual extracts was diluted with 98% hexane and 1 μ L^{-1} of each diluted sample was automatically fed into the Gas Chromatographic Model: 7890A (GC) interfaced with Mass Selective Detector Model: 5975C (MSD)[11].

Identification of bioactive constituents of the fruit extract of *H. umbellate*: Based on GC retention time on HP-5 column and spectrum matching with computer software using the Chem-software linked to the MS library, bioactive chemicals contained in the various extracts were identified. Utilizing the National Institute of Standards and Technology (NIST) database, detection of compounds present in each leaf sample was verified[11].

Experimental design for antimalarial study

Acute toxicity test (LD_{50}): Testing for acute toxicity has two main goals: To learn more about a chemical's biological activity and its mode of action. The information on acute toxicity produced by the test is utilized for hazard identification and risk management. The basis for the toxicologic classification of chemicals is the LD_{50} value, which is defined as the statistically calculated dose that, when administered in an acute toxicity test, is likely to cause death in 50% of the treated animals in a particular period[12]. Six groups of 5 rats each were orally administered 500, 1000, 1500, 2000, 2500 and 3000 mg kg^{-1} of the extract, respectively. They were closely monitored for 72 hrs for mortality and general abnormality.

Parasite inoculation: Mice infected with chloroquine-sensitive *Plasmodium berghei berghei* (NK65) were obtained from the National Institute for Medical Research in Lagos. After cutting the tails of the donor mice, thick blood films were taken on a microscope slide, allowed to dry and then stained with 10% Giemsa dye to detect their levels of parasitaemia. The slide was then treated with immersion oil and it was examined using the National Optical and Scientific Instruments Model 131-CLED (USA) Compound Microscope at a 40X magnification. For parasite inoculation, the technique described by Onyegeme-Okerenta *et al.*[13] was used.

Curative assessment: The curative effect of the fruit extract was evaluated using Rane's test. Groups 1-7 were assigned randomly and inoculated with *P. berghei*. Group 1 being the positive control received the chloroquine phosphate treatment of 5 mg kg^{-1} b.wt., group 2 received no treatment and they are referred to as the negative control group. Group 3-7 were treated with (500, 1000, 1500, 2000 and 2500 mg kg^{-1} b.wt.) of aqueous fruit extract of *H. umbellata*. Treatment was done for seven days, with the daily recording of parasitaemia levels.

Prophylactic assessment: The method described by Peters and Robinson[14] was adopted in the prophylactic assessment of the extract. The 7 groups of 5 mice each were pre-treated for four days using five graded doses (500, 1000, 1500, 2000 and 2500 mg kg^{-1} b.wt., day^{-1}) of aqueous fruit extract of *H. umbellata*. After 4 days, the mice in all the groups were inoculated intraperitoneally with an inoculums of *P. berghei* infected erythrocytes. After 72 hrs of post-inoculation, blood samples were collected for confirmation of parasitaemia level.

Chemo-suppressive assessment: Evaluation of the extracts' *in vivo* schizonticidal activity was done using the 4-day suppression test. Seven groups of 5 infected mice each were randomized from the infected animals. The treatment of the inoculated mice commenced 3 hrs from the time of inoculation with parasite and is recorded as day 0. The treatment continued for seven days (day zero inclusive). Groups 1 and 2 were given 5 mg kg^{-1} chloroquine and distilled water respectively. The remaining groups were treated using five graded doses (500, 1000, 1500, 2000 and 2500 mg kg^{-1} b.wt., day^{-1}) of aqueous fruit extract of *H. umbellata*. On the 7th day after treatment, blood samples were obtained to determine parasitaemia levels.

Determination of parasitaemia level: The thick blood films were prepared by cutting the tails of the mice and the blood was collected onto cleaned microscope slides and allowed to air-dry for 45 min. After 5 min of rinsing with buffered distilled water, the slides were stained with 10% Geimsa stain and left to dry. A drop of immersion oil was placed on the slides and examined using a light microscope. The amount of parasitized red blood cells from a total of 500 red blood cells was then counted manually to determine the parasitaemia levels[15].

Percentage parasitaemia was calculated using the formula[15]:

$$PP = \frac{\text{Total number of PRBC}}{\text{Number of RBC}} \times 100$$

Where,
PP = Percentage parasitaemia
PRBC = Parasitized red blood cells
RBC = Red blood cells

Percentage suppression (PS) was calculated using the formula[15]:

$$PS\ (\%) = \frac{\text{Parasitaemia in negative control} - \text{Parasitaemia in study group}}{\text{Parasitaemia in negative control}} \times 100$$

Statistical analysis: The Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 21.0) was used to conduct the study's statistical analysis. The results of the One-Way Analysis of Variance (ANOVA) were presented as mean standard deviation after comparing the means at a 95% confidence level.

RESULTS

Acute toxicity test: During the 72 hrs observation period, mice exposed to the aqueous fruit extract of *H. umbellata* showed no evidence of toxicity, indicating that a dose of 2500 mg kg^{-1} of the leaf extract was safe.

Proximate analysis: The result obtained from the proximate analysis showed the following composition: Moisture content (7.13±0.19), protein (15.21±0.14), ash (2.42±0.21), carbohydrate (46.71±0.46), fibre (5.70±0.53) and lipid (22.84±0.19). The fruit of *H. umbellata* is a rich source of carbohydrate.

Phytochemical screening and bioactive compounds present in the fruit extract of *H. umbellate*: Preliminary phytochemical screening of the plant extract indicated the presence of alkaloids, cardiac glycosides, flavonoids, saponins, phenols tannins, phlobatannins and sterols. However, Quinoline,2-methyl 13.27%, Hyoscyamine 9.42%, Benzyl Benzoate 7.62%, Beta-Caryophyllene 6.75% and Coumaric acid 6.48% were the predominant among the 30 bioactive components identified in the dichloromethane extract of the fruit of *H. umbellata* (Table 1).

Prophylactic effects of aqueous fruit extract of *H. umbellata* on albino mice infected with chloroquine-sensitive *P. berghei*: The effects of the prophylactic test showed a dose dependent mode of parasitaemia levels (Table 2). When compared to the positive and negative control groups, an increase in the extract doses from 500 to 2500 mg kg^{-1} (groups 3-7) elicited a corresponding significant ($p<0.05$) decrease in the parasitaemia count from 50.00±4.00 to 32.00±5.00 in day 1 and from 41.00±3.00 to 20.00±2.00 in day 7, respectively with a significant ($p<0.05$) dose dependant increase in percentage suppression (Fig. 1).

Chemo-suppression properties of aqueous fruit extract of *H. umbellata* on albino mice infected with chloroquine-sensitive *P. berghei*: Chemo-suppression assessment revealed an overall dose-dependent pattern. The aqueous fruit extract of *H. umbrellata* at 2000 and 2500 mg kg^{-1}, respectively, significantly ($p<0.05$) decreased percentage parasitaemia with a corresponding increase in percentage suppression. The percentage parasitaemia in the negative group on Day 7 after infection with the parasite was 62.00±5.00 and the positive control has 14.00±0.00 (Table 3) while the percentage suppression was 0.00 and 71.43%, respective (Fig. 2).

Table 1: Bioactive compounds present in the fruit extract of *H. umbellata*

Compound	Retention time (min)	Molecular weight (g mol^{-1})	Peak area (%)	Development time (min)
Cynarin	8.674	516.451	1.48	28
Demecolcine	9.193	371.433	0.81	43
Penta-triacontene	9.852	236	2.69	19
Hyoscyamine	10.438	289.375	9.42	72
Kheltin	10.613	260.24	3.17	48
Acetyldigoxin	12.948	300.2	1.53	17
Quinoline,2-methyl	13.531	143	13.27	52
Coumaric acid	14.721	164	6.48	19
Copaene	15.698	204.36	1.71	73
Benzyl Benzoate	16.213	212.25	7.62	42
Caryophyllene	16.784	204.36	2.55	50
Danthron	18.362	240.211	2.78	48
Lupenone	18.759	424.713	4.46	72
Beta-Amyrene	19.427	410	1.18	13
Ephedrine	19.693	165.24	3.15	52
24-Methylene ergosta-5-en-3β-ol	19.845	398	5.70	93
Galanthamine	20.268	287.354	1.64	47
Kawain	20.611	230.2592	2.18	25
Agrimophol	21.253	478.7	2.42	37
Eugenol	21.647	164.2	1.07	51
Anabasine	22.401	162.3	0.94	72
Benzylisoquinoline	22.698	219.28	3.53	68
Carpaine	22.953	478.7	1.20	24
Phytol	23.374	296.53	2.19	34
Phytic acid	23.642	660.04	1.40	18
Myrcene	23.908	136.23	0.16	27
Loboline	24.527	337.455	3.13	69
Beta-caryophyllene	24.859	204.36	6.75	28
Sparteine	26.214	234.285	3.92	10
Emetine	26.796	480.639	1.47	52

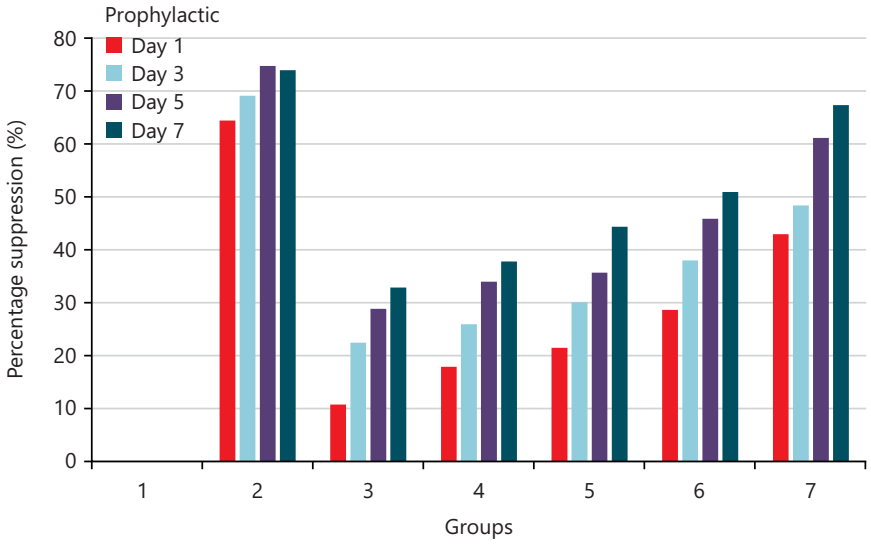

Fig. 1: Prophylactic assessment of percentage suppression level of albino mice treated with aqueous extract of *H. umbellata*

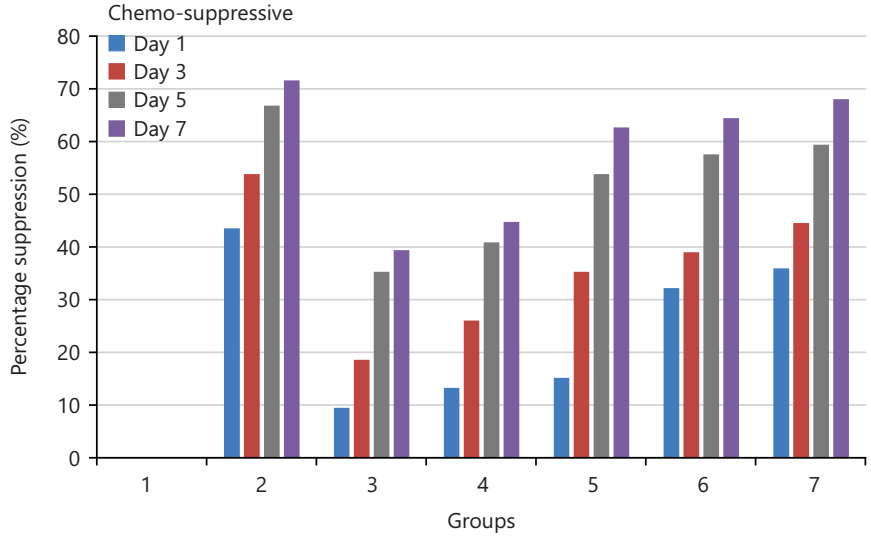

Fig. 2: Chemo-suppressive assessment of percentage suppression level of albino mice treated with aqueous extract of *H. umbellata*

Curative potentials of aqueous fruit extract of *H. umbellata* on albino mice infected with chloroquine-sensitive *P. berghei*: The *in vivo* curative antiplasmodial effect of the aqueous extract of *H. umbellata* showed significant effect ($p<0.05$) with different dose of 500, 1000, 1500, 2000 and 2500 mg kg^{-1} bodyweight, when compared to the negative (untreated) group of the albino mice. On the 7th day of treatment, the percentage parasitaemia for groups treated with 500 and 2500 mg kg^{-1} b.wt., showed a decrease from 39.00±7.00 to 22.00±3.00, respectively when compared to the negative control (62.00±5.00) (Table 4). There was a significant decrease ($p<0.05$) in the percentage parasitaemia with a corresponding significant increase ($p<0.05$) in percentage suppression (Fig. 3). Parasitaemia suppression is proportional to the dosage of extract given to individual groups, the higher the dosage, the higher the level of suppression.

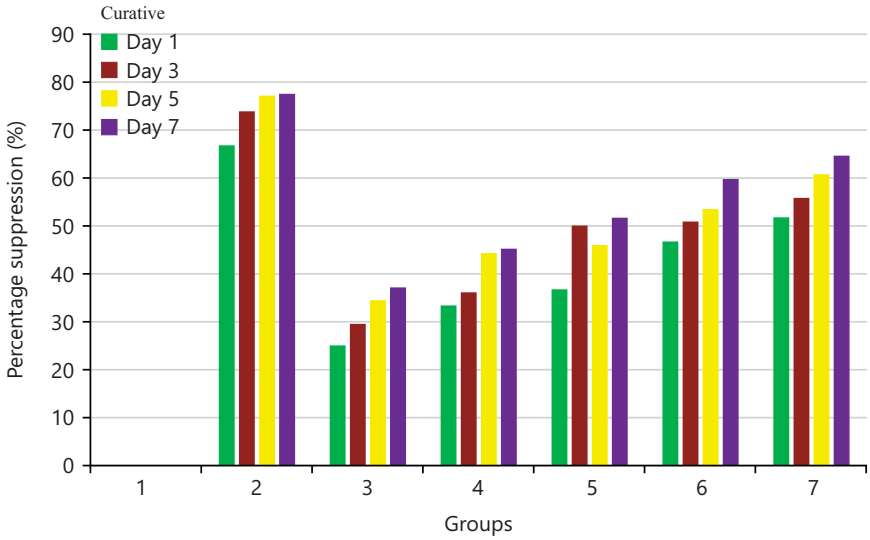

Fig. 3: Curative assessment of percentage suppression level of albino mice treated with aqueous extract of *H. umbellata*

Table 2: Prophylactic assessment of percentage parasitaemia (%) level of albino mice treated with aqueous fruit extract of *H. umbellata*

Group	Day 1	Day 3	Day 5	Day 7
Group 1	56.00±3.00	58.00±7.00	59.00±2.00	61.00±2.00
Group 2	20.00±3.00	18.00±1.00	15.00±3.00	16.00±4.00
Group 3	50.00±4.00	45.00±3.00	42.00±2.00	41.00±3.00
Group 4	46.00±6.00	43.00±1.00	39.00±4.00	38.00±1.00
Group 5	44.00±3.00	40.00±1.00	38.00±6.00	34.00±3.00
Group 6	40.00±3.00	36.00±3.00	32.00±3.00	30.00±1.00
Group 7	32.00±5.00	30.00±2.00	23.00±4.00	20.00±2.00

Percentage parasitaemia was reported as mean±SD and n = 5

Table 3: Chemo-suppressive assessment of percentage parasitaemia (%) level of albino mice treated with aqueous fruit extract of *H. umbellata*

Group	Day 1	Day 3	Day 5	Day 7
Group 1	53±2.0	54±1.12	54±1.21	56±2.56
Group 2	30±1.8	25±1.55	18±1.21	16±1.28
Group 3	48±0.50	44±3.42	35±3.54	34±1.28
Group 4	46±1.11	40±3.12	32±1.28	31±1.36
Group 5	45±1.21	35±2.10	25±6.14	21±3.14
Group 6	36±0.13	33±0.10	23±5.00	20±3.44
Group 7	34±6.40	30±1.03	22±4.16	18±2.24

Percentage parasitaemia was reported as mean±SD and n = 5

Table 4: Curative assessment of percentage parasitaemia (%) level of albino mice treated with aqueous extract of *H. umbellata*

Group	Day 1	Day 3	Day 5	Day 7
Group 1	60.00±3.00	61.00±6.00	61.00±2.00	62.00±5.00
Group 2	20.00 ±5.00	16.00 ±4.00	14.00±3.00	14.00±0.00
Group 3	45.00±8.00	43.00±4.00	40.00±3.00	39.00 ±7.00
Group 4	40.00±2.00	39.00±4.00	34.00± 5.00	34.00±3.00
Group 5	38.00±7.00	36.00±3.00	33.00±2.00	30.00±5.00
Group 6	32.00±4.00	30.00±3.00	26.00±5.00	25.00±5.00
Group 7	29.00±4.00	27.00±4.00	24.00±4.00	22.00±3.00

Percentage parasitaemia was reported as mean±SD and n = 5

DISCUSSION

Crude fibre is very essential for the digestion of food materials in the food canal of animals. The total fat content in *H. umbellata* if further analysed may contain fatty acids as well as vitamins as it contains moderate percentage of fat. Also from the results Ash content of 2.42% dry matter (DM) was obtained. Ash in food contributes for residue remaining after all the moisture has been removed as well as organic material (fat, protein, carbohydrates, vitamins, organic acids etc.) have been incinerated at a temperature of about 500°C. Ash content is generally taken to be a measure of mineral content of original food. The percentage of moisture content obtained in the plant is 7.13%. The moderate moisture content provides for an activity of water soluble enzyme and coenzyme needed for metabolic activities of the plant[16].

The LD_{50} could not be ascertained as no death was recorded after 24 hrs of administration of the various doses of extract (500, 1000, 1500, 2000, 2500 and 3000 mg kg^{-1} b.wt.). The LD_{50} gives a measure of the immediate or acute toxicity of a test substance[17]. The result of this study revealed that the lethal toxicity (LD_{50}) test of *H. umbellata* did not show any toxicity on mice at the concentration below 3000 mg kg^{-1} b.wt. This suggests that *H. umbellata* had no apparent toxic and lethal effects on the animals which probably indicate that the extract has a high safety index. Ihekwereme *et al.*[18], reported that LD_{50} values of test substances above 5000 mg kg^{-1} are considered safe. Toxicity studies are usually undertaken to define the toxicity and effect of extract, access the susceptible species, identify target organs, provide data for risk assessment in case of acute exposure to the chemical or drug, provide information for the design and selection of dose levels for prolonged studies[19,20].

Secondary plant metabolites are organic compounds produced by plants during secondary metabolism[21]. They are generally the products of primary metabolites and are produced from biosynthetic modifications, including methylation, glycosylation and hydroxylation. These compounds are often involved in plants protection against biotic or abiotic stresses and a good number of them are used as chemicals such as drugs, flavours, fragrances, insecticides and dyes and thus have a great economic value[22].

Quinoline,3-methyl is the most abundant bioactive compound present in the fruit extract of *H. umbellata*, it constitutes about 13.27% of the peak area in the chromatogram. The peak area is directly proportional to the concentration of the bioactive compound present in the extracted sampled. Quinoline,3-methyl, is an organic chemical compound and a tertiary hexanol used in the synthesis of the tranquilizer emylcamate and has similar sedative and anticonvulsant actions itself[23,24]. Quinoline compounds have been found to have antibacterial, antifungal and antiviral properties and has been studied for its potential use as an antimicrobial agent. It has also been found to have cytotoxic and apoptotic effects on cancer cells and to have antioxidant and anti-inflammatory properties. Additionally, 3-methylquinoline has been found to have an effect on the expression of certain genes, including those involved in cell cycle regulation, apoptosis and inflammation[25-29]. Its presence in *H. umbellata* may be responsible for the antiplasmodial activity observed in this study.

Hyoscyamine is a naturally occurring tropane alkaloid and plant toxin. It is a levorotary isomer of atropine and is sometimes known as levo-atropine[30]. Hyoscyamine has also been used to treat some heart issues, some Parkinson's disease symptoms, abnormal respiratory symptoms and hypermucus secretions in patients with lung disease. It is an anticholinergic agent used in the treatment bladder spasms[31,32].

Benzyl benzoate is a benzoic acid ester, a colourless, faintly aromatic liquid, used chiefly as a fixative and solvent in the manufacture of flavourings and perfume. It is available as a generic medication for the treatment of scabies and lice[33] and as an insect repellent[34]. It is on the WHO's list of essential medicines[35]. The fruit of *H. umbellata* contains benzyl benzoate as one of its bioactive compounds. This can be explored for the treatment of scabies and mice as well as an additive for perfumes.

Coumaric acid is among the bioactive compounds present is the fruit extract of *H. umbellata*. According to literatures, it is a phenolic compound that can be found in a wide variety of edible plants and mushrooms. *In vitro* and *in vivo* studies have demonstrated that p-CA possesses multiple bioactivities of pharmacological qualities that support its ability to have impacts on blood sugar, hyperlipidaemia, neurodegeneration, analgesia, arthritis, anxiolysis, cancer, ulcers, oxidation, anti-platelet aggregation and inflammation[36-38]. Coumaric acid have also been report to prevent liver damage induced by CCl_4 or BDL (necrosis and cholestasis) and exhibits amoebostatic activity against *Entamoeba histolytica*[39].

One of the ample phytosterols identified in the fruit extract of *H. umbellata* is 24-methylene-ergosta-5-en-3-ol. It was demonstrated to exhibit potent anti-leukaemic cell proliferation properties along with lupenone and other sterols (IC_{50} = 2.80 and 32.89 g mL^{-1})[40]. Beta-caryophyllene was among the bioactive constituents of fruit extract of *H. umbellata*. It is a bicyclic sesquiterpene compound widely found in the plant kingdom. Beta-caryophyllene has been demonstrated to have the ability to improve chronic pathologies marked by inflammation and oxidative stress, especially neurological and metabolic diseases by decreasing pro-inflammatory mediators like tumour necrosis factor-alfa (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-B). Obesity, non-alcoholic steatohepatitis and fatty liver disease, diabetes, cardiovascular disease, pain and other nervous system problems are all positively impacted by beta-caryophyllene. It is also beneficial in treating seizures and osteoporosis and has antimicrobial, antibacterial and antioxidant properties[41].

Lupenone a triterpenoid found in *H. umbellata*. Many plant families and vegetarian diets contain lupenone. The variety of pharmacological actions exhibited by lupenone include anti-cancer, antiviral, anti-inflammatory, anti-diabetes and improving Chagas disease with insignificant toxicity. It is known to promote the inhibition of protein tyrosine phosphatase 1B (PTP1B)[40,42].

Sparteine is an alkaloid that belongs to a family of antiarrhythmic drugs. It is a sodium channel blocker with a weak affinity for the dopamine and serotonin transporters and is thought to bind the bivalent cations calcium and magnesium[43]. Several plants including *H. umbellata* contain the alkaloid ephedrine, which has a phenethylamine skeleton. It is a CNS stimulant that is frequently used to guard against low blood pressure during anaesthesia. It is on the WHO's list of essential medicines and acts by boosting the activation of the and adrenergic receptors. Ephedrine is a bronchodilator, improves blood lipid profiles (enhances HDL cholesterol and decreases LDL cholesterol and triglycerides) and may alleviate motion sickness. It also increases mild short-term weight loss[2,44-47].

Similarly, danthron was among the bioactive compounds listed in the fruit extract of *H. umbellata*. It an anthraquinone, a natural product that has been widely administrated as a laxative since the 1900s. Its use is also carefully limited to the elderly and the terminally sick of all ages. It only plays a little part in the management of constipation. Dantron can be given orally or rectally as an enema, either alone or in conjunction with other laxatives[48]. Phytol was also detected in the fruit extract of *H. umbellata*. It was discovered to be cytotoxic against breast cancer cell lines (MCF7) and to have substantial antioxidant and antinociceptive activity, as well as antimalarial, antibacterial, antifungal, anti-inflammatory and diuretic property. It is a precursor of synthetic vitamin E and vitamin K1[49-51].

The GC-MS analysis of fruit extract of *H. umbellata* showed the presence of emetine compound. Emetine, belongs to the class of organic compounds known as emetine alkaloids, characterized by the presence of both an isoquinoline and a benzoquinolizidine nuclei. It has been used in phytomedicine to induce vomiting and to treat cough and severe amoebiasis. According to Möller *et al.*[52], emetine may induce apoptosis in leukaemia cells. It makes leukaemia cells more susceptible to the apoptosis caused by cisplatin and was also discovered to severely impair cell viability as well as cause apoptosis and caspase

activation. Additionally, it amplified the apoptosis that cisplatin causes and exhibited an additive effect. Another major alkaloid present in the leaf extracts of *H. umbellata* is Carpaine. Carpaine has been reported to affect the myocardium due to its macrocyclic dilactone structure which is a cation chelating structure[53].

The *in vivo* antimalarial activities of *H. umbellata* used in most African traditional medicine against *P. berghei* infection in mice in a seven-day suppressive test model are reported. The result of the prophylactic, chemo-suppressive and curative studies showed that the reference drug, chloroquine was effective in the treatment of the malaria infection. However, the aqueous fruit extract of *H. umbellata* significantly reduced the parasitaemia level and increased the percentage suppression of *P. berghei* in the infected mice in a dose-dependent manner. Parasitaemia refers to the level of infection by a particular parasite. A decline in parasitaemia level is essential for recovery from symptomatic malaria. The lowest parasitaemia load was observed in group 7 that has a dosage value of 2500 mg kg^{-1}. White[54], reported similar findings, where it was documented that higher doses correlated with higher effectiveness of the drug most especially at the first administration until the development of tolerance for the drug or plant extract. Also, Mekuria *et al*.[55], reported the reduction of the parasitaemia level in chloroquine-sensitive *P. berghei berghei* (NK65) infected-mice using seed extracts of *Schinus molle* respectively. The chemo-suppression exhibited by *H. umbellata* is in line with other medicinal plants such as *Annona senegalensis*[56], *Adhatoda schimperiana*[57] and *Artemisia annua*[58], which showed suppression values of 91.1, 53.1 and 92.00%, respectively.

The curative result is in line with the research done by Onyegeme-Okerenta *et al*.[13]. The result indicated that there was a significant decrease ($p<0.05$) in the percentage parastaemia with an increase in dosage concentrations of the extract. It is also worthy to note that each of the extract administration (beginning from day one) had a significant effect ($p<0.05$) and percentage parasitemia level decreased progressively which indicates that the aqueous fruit extract of *H. umbellata* has antiplasmodial therapeutic properties. According to Muñoz *et al*.[59], compounds that can elicit about 30% or more of parasitemia inhibition are considered to possess antimalarial activities. Aqueous fruit extract of *H. umbellata* used in this study, was able to exhibit much higher suppression, which indicates the antiplasmodial potential of the plant.

This study investigated the phytochemical composition and antiplasmodial potential of fruit extract of *H. umbellata* on chloroquine-sensitive *Plasmodium berghei berghei* (NK65)-infected albino. However, further investigations should be carried out to isolate the active compound eliciting the antimalarial response and assess the toxicological effect of the fruit extract on vital body organs like the kidney, liver, heart and even the reproductive gonads. Also, the pharmacological properties of the leaves of *H. umbellata* should be investigated.

CONCLUSION

This research contributes to a plethora of studies investigating bioactive compounds present in plants as their uses as an alternative medication for the treatment of infections including malaria. It also contributes to a pool of investigative study on the pharmacological properties of *H. umbellata* and gives credit to the information provided by the locals and justify the use of the plant extract in the treatment of malaria in Nigeria folk medicine.

SIGNIFICANCE STATEMENT

Nigeria has the highest percentage of malaria infections (26.8%) and deaths (31.9%) worldwide and treatment for people with malaria is becoming increasingly challenging due to the growing issue of drug resistance. This has led to the use of plant-based combinations that can reduce the activities of the *Plasmodium* spp. These herbs have been traditionally used due to their widespread availability, low cost and putative efficacy. The presence of bioactive compounds was screened in the fruit extract of *Hunteria umbellata* and its proximate composition and antiplasmodial potentials were evaluated in this study. The results concluded that unripe fruit extract of *H. umbellata* is high in crude fibre, carbohydrates and the bioactive compounds have significant antiplasmodial potentials.

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