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PHARMACOGNOSTIC STUDY AND ANTIMALARIAL ACTIVITY OF THE METHANOL EXTRACT AND FRACTIONS OF *BIXA ORELLANA* L., (FAMILY BIXACAEA) LEAVES (ANNATTO PLANT) ON ALBINO MICE

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ABSTRACT: The emergence of anti-malarial resistant parasites has posed a serious threat to the control and elimination of malaria. Therefore, there is need for alternative drug that is highly efficacious, affordable and safer for the treatment of malaria; hence, interest is now on the use of herbal medicine to treat malaria. As a matter of fact Ogidi community in Anambra State of Nigeria depends mainly on plants for treatment of malaria. *Bixa orellana* plant, belonging to the family Bixaceae, is a very popular medicinal plant in Nigeria where it is used to treat malaria, jaundice, diarrhea, dysentery, hypertension, as well as diabetes. The ethno-medicinal use against malaria prompted this investigation on the anti-plasmodial activity of the leaf of *Bixa orellana*. *Bixa orellana* leaves were obtained from Ogidi, Anambra, Nigeria, it was dried and pulverized to smaller particle size and extraction was by cold maceration with methanol which was fractionated into *n*-hexane and ethyl acetate fractions. The phytochemical analysis was carried out using standard procedures. The evaluation of antimalarial activity was done according to standard methods. Phytochemical analysis revealed the presence of saponins, tannins, terpenoids, proteins, alkaloids and flavonoids and absence of starch and phenols. Quantitatively, the percentage phytochemical content of the leaves of the plant are as follows; saponins (10.11%), tannins (11.11%), flavonoids (21.89%), glycosides (31.86%). *Bixa orellana* leaf has low moisture content and ash value of 6.13% and 4.58% respectively. The significant decrease in the parasitemia levels in mice was dose dependent for example in *n*-hexane extract 50 mg/kg dose gave a percentage decrease of 34.50% while the 100 mg/kg gave a percentage decrease of 80.15% after treatment which is the highest inhibition among all the other fractions. The positive control (Arthemeter and lumenfantrine) was more potent since it gave a 69.99% inhibition at a dose of 0.37 mg/kg. The treated groups were all very significant ($p<0.05$) except groups 1 and 3 ($p\geq0.05$). From the results obtained, *Bixa orellana* has antimalarial activity especially the *n*-hexane fraction of the leaf extract which confirms the claim of Ogidi community of Anambra State Nigeria.

INTRODUCTION: Malaria is a mosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the *Plasmodium* type.

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The disease is most commonly transmitted by an infected female Anopheles mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood. The parasites travel to the liver where they mature and reproduce [1]. Five species of Plasmodium can infect and spread by humans. Most deaths are caused by *P. falciparum* because *P. vivax*, *P. ovale* and *P. malariae* generally cause a milder form of malaria. The species *P. knowlesi* rarely causes disease in humans. Malaria is typically diagnosed by the microscopic examination of blood using blood

films, or with antigen-based rapid diagnostic tests. Methods that use the polymerase chain reaction to detect the parasite's DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity.

Because of resistance of malarial parasites to anti-malarial drugs, treatment of the infection has shifted to the use of herbal medicines especially in developing countries. Herbal medicine has proven to be effective in the treatment of some minor and critical illnesses, and has been used for ages in folk medicine. There has been a shift from the use of orthodox medicine to the use of herbs for therapy due to the search of more efficacious, safe and cost effective medicine, hence, this study was carried out to evaluate the anti-malarial effects of the plant *Bixa orellana* (Annatto plant) leaf extract.

***Bixa orellana*:** The Bixaceae family is one of the smallest plant families, consisting only of one genus, Bixa. There are only five species grouped under a single genus, and the most common species is *Bixa orellana*, an evergreen shrub grown not only because of its beautiful red flowers and ornamental red spiny fruits, but also for its economic value.

Scientific Classification:

Kingdom : Plantae
Subkingdom : Tracheoblonta
Superdivision : Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Dillenildae
Order : Violales
Family : Bixaceae
Genus : Bixa L.
Species : *B. orellana* L.
Lipstick plant

Geography and Distribution: *Bixa orellana*, also known as "annatto", is native to tropical America [2], but widely cultivated and naturalized throughout the tropics, including Malaysia. *Bixa orellana* is a plant native to Brazil but grows in other regions of South and Central America. It is grown in tropical countries such as Peru, Mexico, Ecuador, Indonesia, India, Kenya and East Africa. Despite the existence of several species, the most common in our country is *Bixa orellana* L., named after Francisco Orellana, who was the first European to navigate the Amazon. 'Bixa' is derived from a local South American name.

Local Names: Arabic (galuga); Bengali (latkan); Creole (chiót, woukou); English (lipstick tree, arnato tree, annatto tree); Filipino (sotis, echuete); French (chiote, annatto, roucou, rocouyer); Hindi (latkan); Indonesian (kunyit jawa, kesumba, jarak belanda), Portuguese (urucum); Spanish (bija, anato, achiote); Swahili (mzingefuri).

FIG. 1: A PICTORIAL REPRESENTATION *OF BIXA ORELLANA*

Botanical Description: *Bixa orellana* is an evergreen shrub or small tree, 2 - 8 m high; trunk up to 10 cm in diameter; bark light to dark brown, tough, smooth, sometimes fissured, lenticellate; inner bark pinkish towards the outside with orange sap, slightly bitter; twigs green with minute, rusty, reddish-brown scales, becoming dark brown. Leaves spirally arranged, simple, stipulate, ovate, 7.5-24 × 4-16 m, shallowly cordate to truncate at base, longly acuminate at apex, green or dark green above, grey or brownish-green beneath; scaly when young, glabrous; petiole terete, thickened at both ends, 2.5 - 12 cm long.

Flowers in terminal branched panicles, 8 - 50 flowered, fragrant, 4 - 6 cm across; pedicel scaly, thickened at the apex, bearing 5 - 6 large glands; sepals 4 - 5, free, obovate, 1 - 1.2 cm long, caducous, covered with reddish brown scales; petals 4 - 7, obovate, 2-3 × 1-2 cm, pinkish, whitish or purplish tinged; stalks scaly; stamens numerous, 1.6 cm long; anthers violet; pistil 1.6 cm long, composed of bristly 1-celled, superior ovary; style thickened upwards, 12-15 mm long; a short, 2-lobed stigma.

Fruit a spherical or broadly elongated ovoid capsule, 2-4 × 2-3.5 cm, flattened, 2 valved, more or less densely cloaked with long bristles, green, greenish-brown or red when mature; seeds numerous, obovoid and angular, 4.5 mm long, with bright orange-red fleshy coats.

Ethnomedicinal Uses: *Bixa orellana* is well known for its coloring agent and medicinal value. The seeds are sources of food coloring and a dye called annatto. Besides that, they are also used as treatment for illnesses like gonorrhea and asthma and have been traditionally used as gargle for sore throats. The bark and root can be used to treat fever; the leaves are used as a cure for snakebites, jaundice, diabetes and hypertension, especially in Trinidad and Tobago, while also being used as a postpartum medicine in Malaysia. The leaves of *Bixa orellana* have been reported to have antimicrobial, antifungal, antileishmanial [3], anticonvulsant, analgesic [4] and anti-inflammatory activities [5]. Leaves are applied to the head andto sprains to relieve aches; a decoction is gargled as a cure for mouth and throat infections.

Leaves may also be used in baths to relieve colic or to get rid of worms in children. A macerated seed decoction is taken orally for relief of fever, and the pulp surrounding the seed is made into an astringent drink used to treat dysentery and kidney infection. Oliguria and jaundice are treated using root teas; infusions of root in water and rum are used to treat venereal diseases. The dye is used as an antidote for prussic acid poisoning caused by poorly treated *Manihot esculenta.* Seeds are used as expectorant.

Aims: To evaluate the antimalarial property of the leaves of *Bixa orellana* L. plant (Annatto plant).

MATERIALS AND METHODS:

Reagents and Solvents: Dragendoff's reagent (solution of potassium bismuth iodide), Molisch reagent, Million's reagent, Wagner's reagent (iodine and potassium iodide), Hager's reagent (saturated solution of picric acid), 0.1% ferric chloride, chloroform, glacial acetic acid, ethanol, coal tar, normal saline solution, giemsa stain, blood samples, sodium chloride (JHD), acetic anhydride, ethyl acetate (by JHD), *n*-hexane(JHD), distilled water, concentrated sulphuric acid (JHD).

Apparatus and Equipments: Water bath, beakers, evaporating dish, test tubes, test tubes rack, hand gloves, microscope, watch glass, spatula, glass slides, conical flasks, measuring cylinder, chromatograph, oven, desicator, refrigerator (thermocool), weighing balance, Soxhlet extractor (Ahaus Poland), crucible and funnel.

Drugs: Coartem (Arthemeter and Lumenfantrine combination). Ethanol, ethyl acetate and *n*-hexane fractions of *Bixa orellana* leaves.

Animals and Housing: Mice within the weight range of 20 to 30 kg were used for this study. The animals were obtained from the animal house of the Department of Pharmacology and toxicology, Faculty of Pharmaceutical Scinces, Nnamdi Azikiwe University, Awka, Agulu Campus. The mice were properly housed in a well ventilated and neat cage with easy access to food and water. This is in accordance with the animal experimental protocols which were approved by our institutions animal Ethics committee and were in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/ EEC).

Malaria Parasite Strain: A chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the animal house of the University of Nigeria, Nsukka in Enugu state, Nigeria. The parasite was maintained by weekly blood passage in mice.

METHODS:

Sample Collection and Preparation: The leaves of *Bixa orellana* plant were obtained from its plant in Ogidi, Anambra state, Nigeria on the 19th of April, 2017. It was identified by a Taxonomist Mr Ozioko. Plant authenticated voucher specimen number is PCG/474/A/054. The leaves were properly selected and dried for three (3) weeks, it was then pulverized using a generator powered grinding mill to obtain smaller sizes for a large surface area. The grounded leaves were carefully packed in a bag and avoided spillage and water contact.

Method of Extraction: A 400g of the grounded *B. orellana* leaves were macerated in 1000ml of methanol and left for 2 days, then was filtered and

concentrated using rotary evaporator as well as water bath at 50 ºC, the extract was placed in a labeled container**.**

Fractionation of the Crude Extract: Fractionation of the crude extract into *n*-hexane and ethyl acetate fractions was done using liquid-liquid method of fractionation. The crude extract was dissolved in 250 ml of methanol. The mixture was transferred into a separating funnel, after which 250 ml of *n*-hexane was poured into the mixture. The separating funnel was shaken vigorously, releasing pressure at intervals. The mixture was allowed to stand undisturbed for 2 h to ensure proper separation and the *n*-hexane fraction which is less dense was collected in a fresh beaker. This was repeated with fresh *n*-hexane of the same volume until almost clear fraction was obtained.

Furthermore, 250 ml of ethyl acetate was later poured into the separating funnel and shaken vigorously the mixture was allowed to stand for 2 h for proper separation after which the less dense fraction was collected in a clean beaker. This was repeated with a fresh ethyl acetate until almost clear fraction was obtained. The collected fractions were concentrated respectively using water bath, and afterwards stored in the refrigerator (18 ºC - 25ºC) for later use.

Microscopical Evaluation of *B. orellana* Leaves: About 0.5g of the powdered leaves were placed on a clean slide, then chloral hydrate which acted as a clearing agent was added and a smear was made on the slide. The smear was now passed over the Bunsen burner several times to heat fix the smear made on the slide. Then a drop of glycerol was added to the smear, it was then covered with a cover slip and viewed under the microscope at x10 magnification. The observed cellular component arrangement was snapped.

Phytochemical Tests on Crude *Bixa orellana* Extract: The phytochemical analysis of the grounded *Bixa orellana* leaves were carried out using the standard procedures to identify the constituents as described by Trease and Evans (1989) and Harborne (1973) [6].

Qualitative Phytochemical Analysis:

Test for Alkaloids: To test for the presence of alkaloids in the plant material, five grams of the grounded leaves of *Bixa orellana* was placed in at test tube and 20 ml of methanol was poured into the test tube. The mixture was allowed to boil for 2 min in a water bath, cooled and filtered. The filtrate was then used for the following:

Two drops of Dragendoff's reagent (solution of potassium bismuth iodide) was added to 2 ml of the filtrate and the colour change was noted.

Two drops of Wagner's reagent (solution of iodide and potassium iodide) was added to 5 ml portion of the filtrate and the colour change was noted.

Two drops of Hager's reagent (saturated solution of picric acid) was added to 5ml portion of the filtrate and the color change was noted.

Test for Flavonoids: To test for the presence of flavonoids, 10 ml of ethyl acetate was added to 0.2 g of the grounded leaves and heated on a water bath for 3 min, filtered and the filtrate was used for the following tests:

1. Ammonium Test: 4 ml volume of the filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammonium layer indicates the presence of flavonoids.

2. Aluminium Chloride Test: Another 4 ml portion of the filtrate was shaken with 1ml of 1% aluminium chloride solution. The layers were allowed to separate. A yellow color in the aluminium chloride layer indicates the presence of flavonoids.

Test for Cardiac Glycosides: Five ml of extract was treated with 2 ml of glacial acetic acid containing a drop of 0.1% ferric chloride solution. This was under laid with concentrated sulphuric acid. A reddish brown ring at the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring appears below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Test for Tannins: One gram of the grounded leaves was boiled with 50 ml of water, filtered and used for the following tests:

1. Ferric Chloride Test: A few drops of 0.1% ferric chloride were added to 3ml of the filtrate and

observed for brownish green or a blue black or a greenish-black coloration.

2. Lead Sub-acetate Test: Few drops of lead sub-acetate were added to 3 ml of filtrate and the color of the precipitate formed was noted. A cream precipitate appearing indicates the presence of tannins.

Test for Saponins: 20 ml of water was added to about 0.25g of the grounded leaves in a 100 ml beaker and boiled gently on a water bath for 2 min. The mixture was filtered hot and allowed to cool and filtrate was used for the following tests:

1. Frothing Test: 5 ml of the filtrate was diluted with 20 ml of water and vigorously shaken and a stable froth (foam) upon standing indicates the presence of saponins.

2. Emulsions Test: 2 drops of olive oil was added to the frothing solution and the content shaken thoroughly. The formation of an emulsion indicates the presence of saponins.

Determination of the Haemolytic Effects of Saponins on Red Blood Cells: Into each of two test tubes, 5 ml of 5% suspension of red blood cells was placed in normal saline solution. To one test tube, 5 ml of normal saline solution was added and to the other test tube, 5 ml of *Bixa orellana* extract into which 0.045 g sodium chloride has been previously dissolved to render it isotonic with blood was added.

Both test tubes were shaken gently and were allowed to stand for some time. The liquid in the first tube will remain opaque *i.e*. will contain a suspension of the red blood cells, whereas a clear liquid will be formed in the second tube, indicating that hemolysis of the cells has taken place.

Test for Proteins:

1. Millions Test: To a little portion of the filtrate of the powdered seeds in a test tube, 2 drops of millons reagent were added. A white precipitate indicates the presence of protein.

2. Picric Acid Test: A few drops of picric acid were added to a little portion of the filtrate. A yellow precipitate indicates the presence of protein.

Test for Starch: General tests which include;

1. Molisch Test: The powdered leaf (0.1 g) was boiled with 2 ml of water and filtered. To the filtrate, a few drops of naphtha solution in ethanol (Molisch reagent) were added. Concentrated sulphuric acid was then gently poured down the test tube to form a lower layer. A purple interfacial ring indicates the presence of carbohydrates.

2. Iodine Test: A drop of iodine solution was added to 0.1g of the powdered leaf. A blue black colour indicates the presence of starch.

Test for Steroids: Two ml of acetic anhydride was added to ethanol extract of each sample with two ml concentrated sulphuric acid. A colour change from violet to blue or green in the sample indicates the presence of steroids.

Test for Terpenoids (Salkowski Test): Five ml of each extract was mixed in 2 ml of chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration at the interface indicates the presence of terpenoids.

Quantitative Phytochemical Analysis:

Test for Alkaloid: A 5 g of powdered *Bixa orenella* leaf sample was added into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. It was covered and allowed to stand for 2h. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle, the precipitate formed was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried, weighed and expressed as Percentage of the alkaloids in the leaves.

Test for Saponin: To 20 g of powdered *Bixa orenella* leaf sample was placed in a conical flask, 100 ml of 20% ethanol added. This was heated over in a water bath for 4h with continuous stirring at 55ºC. The mixture was filtered and the residue was extracted with 200 ml of 20% ethanol. The extracts were combined and then concentrated to 40 ml over a water bath at 90 ºC. The concentrate was transferred into a 250 ml separating funnel, extracted twice with 20 ml diethyl ether and shaken vigorously. The ether layer was discarded while the aqueous layer was retained.

To the aqueous layer, 60 ml of *n*-butanol was added. Then the *n*-butanol extract was washed twice with 10ml of 5% aqueous sodium chloride and the remaining solution was heated on a water bath. After evaporation, it was dried in the oven (40 ºC) to a constant weight. Finally, the saponins content was calculated as percentage of the initial weight of the sample taken.

Determination of Tannins: Folin-Denis Spectro-photometric method described by Pearson (1976) was used.

Procedure: A measured weight of each sample (1.0) was dispersed in 10 ml distilled water and agitated. This was left to stand for 30 min at room temperature being shaken every 5 min. At the end of the 30 min, it was centrifuged and the extract gotten. 2.5 ml of supernatant (extract) was dispersed into a 50 volumetric flask. Similarly, 2.5 ml of standard tannic acid solution was dispersed into a separate 50 ml flask.

A Folin-Denis reagent was measured into each flask, followed by 2.5 ml of saturated Na_2CO_3 solutions. The mixture was diluted to mark in the flask, (50 ml) and incubated for 90 min at room temperature. The absorbance was measured at 250nm in a Genway Model 6000 electronic spectrophptpmeter. Readings were taken with the reagent blank to zero; the tannin conyent was given as follows:

$$\% \text{ Tannin} = An/As \times c \times 100/w \times Vf/Va$$

Where, An = Absorbance of the test sample, As = Absorbance of the standard solution, C = Concentration of the standard solution, W = Weight of sample used, V = Total volume of extract, Va = Volume of extract analyzed

Determination of Flavonoids: 1g of powdered *Bixa orellana* leaf sample was extracted with methanol, 20 ml of acetone, 2 ml of 25% HCl and 1ml of 0.5 % hexamethylenetetramine was added to 25 ml of the extract and refluxed at 56 ºC for 30 min. The extract was filtered and was re-extracted twice with 20 ml of acetone for 10 min. After cooling and filtration, the extract was made up to 100 ml with acetone (basic sample solution, BSS), 20 ml of BSS was mixed with 20 ml of water and then extracted with ethyl acetate (first with 15 ml and then three times with 10 ml).

The ethyl acetate extracts were rinsed twice with water then filtered and made up to 50 ml with ethyl acetate (SI). To 10 ml of SI, 0.5 ml of 0.5% solution of sodium citrate and 2 ml of $AlCl_3$ (prepared by dissolving 2g of $AlCl_3$ in 100 ml of 5% acetic acid in methanol) was added and was made up to 25 ml with 5% methanol solution of acetic acid (sample solution, SS). The same procedure was performed with blank sample solution but without $AlCl_3$. After 45 min, the yellow solutions were filtered and the absorbance reading at 425nm evaluated. Then the yield was calculated as quercetin percent using the following expression:

$$g\ \% = A \times 0.772/b$$

Where, A is absorbance and b represents the mass of dry herbal material in grams.

Determination of Ash Values:

Total Ash: About 2g of accurately weighed *Bixa orellana* grounded leaves was placed into a nickel crucible that has been heated, cooled and stored in a desiccator (the grounded leaves were 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 (450 ºC) 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. The ash value was calculated with relevance to air dried drug. Experiment was carried out in triplicate.

Acid Insoluble Ash: The ash obtained from method 1 above was boiled in a crucible with 25 m of dilute hydrochloric acid (2M) for 5 min; 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 paper 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 30 min and then weighed.

The acid -insoluble ash was calculated in mg/kg with reference to the air dried drug. Experiment was carried out in triplicates

Water Insoluble Ash: To the crucible containing total ash, 25 ml of distilled water was added and boiled for 5 min. The insoluble matter was collected on an ashless filter paper. Then it was washed with hot water and ignited in a crucible for 15 min at a temperature not exceeding 450 ºC. 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.

Determination of Moisture Content:
Loss on Drying: To an evaporating dish which has been heated to constant weight and stored in a desicator, 3g of *Bixa orellana* grounded leaves was accurately weighed into the dish. Then it was placed in an oven at 100 - 105 ºC for 5 h and the sample was weighed. The drying and weighing was continued at 1 h 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

Curative Study: In this model of study, a total of 27 mice were used. They were grouped into nine (9) groups of three (3) mice per group. Blood was collected from donor mouse infected with the parasite (*P. berghei*) by occula-puncture and was diluted with normal saline such that 0.2 ml contains approximately 1×10^6 injected with *Plasmodium berghei* by single intraperitoneal administration of 0.2ml of the diluted blood and was left for 72 h for the infection to be established. Then the animals started receiving treatment as follows:

Group 1: received 5 mg per kg of distilled water
Group 2: received 0.37 mg per kg of ACT
Group 3: received 50 mg per kg of crude extract
Group 4: received 100 mg per kg of crude extract
Group 5: received 200 mg per kg of crude extract
Group 6: received 100 mg per kg of ethyl acetate extract
Group 7: received 200 mg per kg of ethyl acetate extract
Group 8: received 100 mg per kg of *n*-hexane extract
Group 9: received 200 mg per kg of *n*-hexane extract.

A thin blood film was made from the tail, blood stained with Giemsa stain and was examined for parasitemia on day 0 and day 5 of treatment respectively. The percentage curative was calculated using the formula:

$$= D0 – DA / D0 \times 100$$

Where, D0 = Day 0 mean parasitemia, DA = Treated day mean parasitemia.

Statistical Analysis: The data was analyzed using one way analysis of variance (ANOVA). Data were tabulated as mean ± SEM (Standard error of mean); p value < 0.05 was considered significant.

RESULTS: An extractive value of the methanol fraction (crude extract) the *Bixa orellana* leaves is 14.05%.

Phytochemical Analysis:

TABLE 1: RESULTS OF PHYTOCHEMICAL ANALYSIS OF *BIXA ORELLANA*

S. no.	Analysis	Results
1.	Alkaloids	++
2.	Saponins	++
3.	Tanins	++
4.	Flavonoids	+
5.	Glycosides	+
6.	Resins	+
7.	Starch	-
8.	Terpenoids	+
9.	Phenols	-

- = absent, + = trace/mildly present, ++ = moderately present, +++ = abundantly present.

TABLE 2: PERCENTAGE PHYTOCHEMICAL CONTENT OF *B. ORELLANA*

Parameters	% Phytochemical content
Glycoside	31.86
Flavanoids	21.89
Saponins	10.11
Tanins	11.11
Phenols	121.89

TABLE 3: PROXIMATE COMPOSITION (%) OF *B. ORELLANA*

Parameters	% Composiion
Moisture	6.13
Ash	4.58
Water soluble ash	1.97
Acid soluble ash	0.14

Anti-malarial Test Result - Effect of the Methanol (Crude) Leaf Extract and Other Fractions on the Malaria Infected Mice:

TABLE 4: RESULTS OF MEAN PARASITEMIA

Groups		Mean parasitemia (pretreatment)	Mean parasitemia (post treatment)
1	5mg per kg distilled water	19.08	19.12
2	0.37mg/ kg ACT	15.83	4.75
3	50mg/kg extract	19.92	9.08
4	100mg/ kg extract	19.92	5.0
5	200mg/ kg extract	16.17	4.25
6	100mg/ kg ethyl acetate fraction	29.0	7.13
7	200mg/ kg ethyl acetate fraction	29.0	11.08
8	100mg/ kg *n*-hexane fraction	31.25	20.47
9	200mg/kg *n*-hexane fraction	34.0	6.75

TABLE 5: PERCENTAGE INHIBITION OF THE MALARIA PARASITE

Groups	Treatments	% Inhibition
1	5mg/kg distilled water	0.20%
2	0.37mg/kg ACT	69.99%
3	50mg/ kg crude extract	43.85%
4	100mg/ kg crude extract	74.90%
5	200mg/ kg crude extract	78.66%
6	100mg/ kg ethyl acetate extract	75.41%
7	200mg/ kg ethyl acetate fraction	47.04%
8	100mg/ kg *n*-hexane fraction	34.50%
9	200mg /kg *n*-hexane extract	80.15%

DISCUSSION: The results obtained shows that the leaves of the plant is a good source of phytochemicals as it contains many secondary metabolites with saponins being the most abundant. The presence of such metabolites suggests that the leaves might be of great importance in phytomedicine. The presence of these secondary metabolites in *Bixa orellana* leaves may be responsible for its anti-malarial activity. For example, flavonoids are reported to chelate with nucleic acid base pairing of the parasite [7] and terpenoids are potent inhibitors [5].

These compounds (flavonoids and terpenoids) present in this plant extracts may in part have contributed to the anti-malarial activity of this extract and therefore explained the mechanism of anti-malarial effect of the leaf extract. Saponin, flavonoids and tannins have been suggested to act as primary antioxidant or free radicals scavengers that can counteract the oxidative damage induced by malaria parasite. These compounds could be acting singly or in synergy with one another to exert the anti-plasmodial activity observed in this study [8, 9]. *Plasnodium berghei* has been used in studying the activity of potential anti-malarials in mice [10] and in rats [11].

It produces diseases similar to those of human plasmodium infection [12, 13]. It is also the parasite of choice because other species of the plasmodium parasites cannot survive in mice. From the anti-malarial test, the positive control (Arthemeter-Lumenfantrine combination) gave a significant parasitemia reduction from 15.83 to 4.75 this means that the positive control has anti-malarial effect far more than the negetive control. For the crude extract, it is observed to be dose dependent because the mean parasitemia reduction increases as the dose of the extract increases with 200 mg/kg having the highest effect, it can be concluded that the crude extract (methanol fraction) has anti-malarial effect [14].

For the fractions, the 100 mg/kg dose ethyl acetate gave a significant parasitemia reduction from 29.0 to 7.13 unlike the 200 mg/kg dose which gave a reduction of 20.92 to 11.08 while for the n-hexane fraction, the100mg/kg dose gave a reduction 31.25 to 20.47 and the 200mg/kg dose gave a significant reduction of 34.0 to 6.75, it can be agreed that both the ethyl acetate and the *n*-hexane fractions have anti-malarial properties.

It is observed that the different fractions of the leaf extract inhibited the parasitemia more when compared with the results of the standard treatment (Coartem - arthemeter and Lumenfantrine combination) at the dose of 0.37 mg /kg giving an inhibition of 69.99% while the dose of 200 mg/kg of *n*-hexane fraction gave the highest inhibition followed by 200 mg per kg dose of the crude extract and then the 100 mg per kg dose of ethyl acetate fraction with 100 mg per kg dose of *n*-hexane fraction having the least inhibition.

The low percentage chemosuppression observed in the positive control, 50mg/kg crude extract, 100mg/kg and 200mg/kg of *n*-hexane may be due to the fact that treatments at the dose administered had not accumulated sufficiently to bring about considerable chemosuppression. However, the prolonged administration of the treatments may lead to the total clearance of the parasites.

CONCLUSION: *Bixa orellana* has proven to have some anti-malarial properties that could be used in the formulation of cheap alternative drug for treatment of malaria with its qualitative and quantitative phytochemical constituents determined. Hence, the results of this work proved the claim of the local community for the use of this plant as an anti-malarial drug.

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RECOMMENDATION: The isolation and characterization of the biological active compounds of the plant should be encouraged for the purpose of finding new lead compounds for fighting malaria resistant parasites. Further studies on the effect of the leaf extract on vital organs like liver, kidney, heart *etc*. should be encouraged. The long term and short term stability studies of the leaf extract to determine the suitable storage conditions for the product should also be encouraged.

CONFLICT OF INTEREST: Nil

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