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In-vitro Anti-plasmodia activity of leaf extracts of *Ximenia americana* (Wild Plum)

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Abstract
Ximenia americana was investigated in an attempt to evaluate its medicinal potential. The phytochemical constituents of ethanol extract reveals, the presence of Carbohydrates, Reducing sugar, Alkaloids, Saponins, Tannins, Steroids, Flavonoids and Terpenes. More so, the percentage yield of the extract reveals that ethanol yielded 25.1%. The antimalaria result showed that the activity of the extract is cytotoxic for *Plasmodium falciparum,* at 5000mg/ml and 2000mg/ml with a percentage (%) elimination of 86.4% and 74.5% respectively, when compared with the Standard with percentage elimination of 88.6% and 84.2%. The higher percentage elimination of the extract justify it antimalaria activity. While that of 1000mg/ml and 500mg/ml may also show growth elimination. The result of the phytochemicals study validates the use of ethanol extract of this species in ethno medicine and could provide a lead in the isolation of novel drugs candidate from ethanol extract of the plant.

Keywords: phytochemicals, antimalarials, secondary metabolites, plasmodium falciparum

Introduction
Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities. The local people have a long history of traditional plant usage for medicinal purposes. The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 4000 - 5000 B.C. and Chinese used first the natural herbal preparations as medicines [1].
Medicinal plants are frequently used as raw materials for the extraction of active ingredients which used in the synthesis of different drugs. Medicinal plants are an integral component of research developments in the pharmaceutical industry. Such research focuses on the isolation and direct use of active medicinal constituents or on the development of semi-synthetic drugs, or still again on the active screening of natural products to yield synthetic pharmacologically-active compounds [2].

Malaria
Malaria is caused by infection with a single-cell parasite, *Plasmodium*. Four *Plasmodium* spp. cause malaria in human beings which are: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*and *Plasmodium malariae. P. falciparum* is the most important because it accounts for the majority of infections and causes the most severe symptoms. Malaria remains one of the leading causes of morbidity and mortality in the tropics.
According to the [3, 4], there were 106 malaria endemic countries in 2010. There were 216 million cases of malaria in 2010; 81% of these were in the World Health Organization (WHO) African region. An estimated 3.3 billion people are at risk of malaria. Among the 655,000 persons died of malaria in 2010, 86% of these victims were children under the age of five.

Statement of Problem
Malaria is a major global health problem that threatens the lives of 40% of the world's population – over 2200 million people. Malaria is estimated to kill more than 1 million people annually, majorly young children. The most prevalent and dangerous type of malaria is caused by *Plasmodium falciparum. Plasmodium vivax* is a common cause of malaria in Latin America, Asia, and Oceania, but not Africa.

General botanical description of *Olacaceae* family
The genus *Ximenia* belongs to the family Olacaceae and comprises about 8 species: *X. roiigi*, *X. aegyptiaca*, *X. parviflora*, *X. coriaceae*, *X. aculeata*, *X. caffra*, *X. Americana* and *X. aegyptiaca*. *Ximenia americana* Linn. Is the most common. It is a plant of diverse habitats in semi-arid bushland, dry woodland, sandy open woodland and coastal bushlands. It is frequently found on coastal dunes, along water courses and on stony slopes, and occurs at altitudes up to 2000m above sea level and where rainfall exceeds 500mm per year and temperatures of 14 -30ºC [5]. The plant is widely distributed in northeast Brazil [6]. Its fruit contains one large endospermic seed [7]. The plant is widely used in folk medicine of different countries to treat several human ailments [8].

Material and Methods
Collection and preparation of plant materials
The fresh leaves of *Ximenia americana* L. was collected on 3rd December, 2016 at Chaza Suleja, Niger State. The plant was identified and authenticated with a Voucher number NPRD/H/7042 at the Herbarium Unit department of Medicinal Plant Research and Traditional Medicine at the National Institute of Pharmaceutical Research and Development (NIPRD) Abuja. After collection, it was air dried in a room for a period of 2 weeks. It was then crushed and reduced into small sizes using pestle and mortal. The ground powder was store for latter research work.

Extraction of plant material
The air dried sample of the plant 100g was macerated in 90% ethanol (250cm^3) in an enclosed container, at room

temperature for two weeks. It was then decanted and filtered using no. 1 Whatman filter paper. The filtrate was collected into a weighed beaker and allowed to evaporate at room temperature.

Phytochemical screening of extract

Ethanolic crude of the plant sample was tested for the presence of secondary metabolite according to the standard method [9, 11].

Malaria parasite assay

Sourcing of malaria parasite for assay

Malaria parasite of infected blood samples containing a parastemia of *Plasmodium falciparum* were collected from the Department of Haematology, Bayero University Hospital, Kano. The samples were received in K3-EDTA coated disposable plastic sample bottles with tightly fitted plastic corks, and transported to the Microbiology Laboratory of Bayero University Kano.

Preparation of *Plasmodium falciparum* Culture Medium

Venous blood (2cm^3) from the main vein of white healthy rabbit pinnae was withdrawn using a disposable 5cm^3 syringe (BD 205 WG). This was defibrinated by allowing it to settle for at least one hour. The defibrinated blood was centrifuged at 1500rpm using spectre merlin centrifuged for 10minutes and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500rpm for 5minutes, and the supernatant layer was added to the first test tube. The sediment were discarded and the serum collected was supplemented with the salt of RPMI 1640 salt medium and sterilized 50µg/ml gentamac in sulphate [12].

***In-vitro* Assay of the Activity of the Extract on *Plasmodium falciparum* Culture**

A test solution (0.1ml) and the culture medium (0.2ml) were added into a test tube containing 5% parasitaemia erythrocytes are and mixed thoroughly. The sensitivity of the parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to each tested fractions at 500, 1000, 2000, and 5000µg/ml was determined microscopically at 37^{0}C after 24 and 48 hours of incubation. The incubation was carried out under a bell jar system with a lighted candle that ensured the condition being atmospherically inert (about 5% O_2, 2% and 93% nitrogen gas) as demonstrated by [13].

Determination of the Activity

At the end of the incubation periods usually between 24 to 48 hours, a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscope slides and stained by Giemsa's staining techniques. The mean number of erythrocytes appearing as blue discloid cells containing life rings of the parasites (that appeared red pink) was estimated and the average percentage elimination by samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasite after incubation periods of 24 and 48 hours, using the formula below;

$$\% = \frac{N}{Nx} \times 100$$

Where

% = Percentage activity of the extract

N = Total number of cleared Red Blood Cells (RBC)

N_x= Total number of parasitized Red Blood Cells [13].

Results and Discussion

The powered material (100g) was extracted with ethanol yield 25.1g with a percentage (%) yield of 25.1%.

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development [14]. The phytochemicals screening in this research has revealed the presence of Carbohydrates, Reducing sugar, Alkaloids, Saponins, Tannins, Steroids, Flavonoids and Terpenes which is shown in Table 1 above. However, previous studies on the phytochemical analysis of the leaves of *Ximenia americana* showed the presence of Tannins, Steroids, Flavonoids and Terpenes [15]. Also, Saponins, Tannins and Flavonoids were reported present in the leaves [16]. Additionally [17], reported the presence of Carbohydrates, Tannins, Steroids, Flavonoids and Terpenes. These are in agreement with the result of this research.

Table1: Result of Phytochemicals screening of the ethanol extract

Phytochemicals	Results
Carbohydrate	+ve
Reducing sugar	+ve
Alkaloids	+ve
Saponins	+ve
Tannins	+ve
Steroids	+ve
Flavonoids	+ve
Terpenes	+ve

Note: +ve = Present-ve = absent

Antimalaria screening of *Ximenia americana* leaves

The results of anti-malaria activity of the extract were shown in Table 2. The microscopic examination of Geimsa's stained slides for the fractions at 5000µg/ml showed a lesser number of parasites after 24 and 48 hours and has the most interesting anti-plasmodial activity. These observations suggest that the antimalaria activity of the extract is cytotoxic for *Plasmodium falciparum*, at 5000mg/ml and 2000mg/ml with a percentage (%) elimination of 86.4% and 74.5% respectively, when compared with the Standard with percentage elimination of 88.6% and 84.2%. The higher percentage elimination of the extract justify it antimalaria activity. While that of 1000mg/ml and 500mg/ml may also show growth elimination. Currently, no data is available from literature regarding the antimalarial activity carried out *Ximenia americana* plant Total counts of infected and non-infected Red Blood Cell = 218 Initial parasitemia counts of infected Red Blood Cell/ fields before use = 184

Control: Artemether (20mg) Lumefantrine (120mg)

Table 2: Antimalaria result of *Ximenia americana* leaves

Concentration of extract used (µg/ml)	Final parasitemia counts of infected Red Blood Cell	Final parasitemia counts of control	Percentage (%) elimination at the end of incubation	Percentage (%) elimination of control
5000	25	21	86.4	88.6
2000	47	29	74.5	84.2
1000	58	-	68.5	-
500	72	-	60.9	-

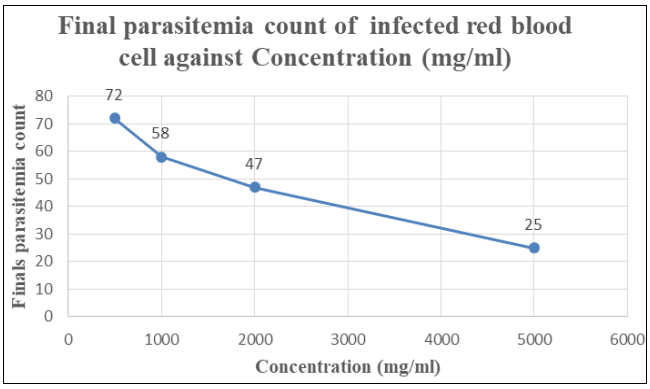

Fig 1: A plot of Final parasitemia count of infected red blood cell after incubation against Concentration of extract (mg/ml)

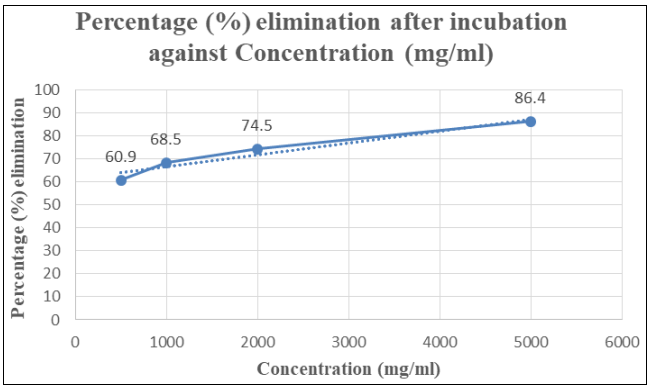

Fig 2: A plot of Percentage (%) elimination at the end of incubation against Concentration of extract (mg/ml)

Conclusion

The qualitative phytochemicals analysis of ethanol extract reveal the presence of Carbohydrate, Reducing sugar, Alkaloids, Saponins, Tannins, Steroids, Flavonoids and Terpenes. Most of these phytochemicals are responsible for the antimalaria property of the plant and thus, their presence has justified the use of the leaves of the plant in traditional medicine. The antimalarial result also shows that the leaves of *Ximenia americana* can be used as an alternative source of antimalaria.

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