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Phytochemical Screening and Anti-plasmodial Activity of *Balsamodendron africanum* (A. Rich) (Burseraceae)

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Abstract: *Balsamodendron africanum* was popularly known to be present within tropical Africa and some Indian regions. The stem bark extract of the plant was investigated for phytochemical screening and *In vitro* anti-malarial bioassay using n-hexane, chloroform, ethylacetate and methanol solvent fractions. The phytochemical screening revealed the presence of flavonoids, alkaloids, terpenoids, tannins, saponins anthraquinones and carbohydrates. The bioassay results showed that, n-hexane fraction have the highest percentage of parasite elimination of 91.8% at 5000µg/ml lower than the standard anti-malarial drug (artemesinin combine treatment) with 92.8% at 5000µg/ml concentrations. Thus, it can be concluded that, further research on the plant may lead to the discovery of new potential anti-malarial drug in near future.

Keywords: *Balsamodendron africanum*, Anti-malaria, *in vitro* Bioassay, Phytochemical Screening

1. Introduction

The existence of malaria for over 35 years now has multiply in triple, spreading to over 100 countries with 300-500 million infections and 2.5 million deaths annually. Malaria parasite is primarily caused by *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale* being transmitted by over 70 species of anopheles mosquitoes with *P. falciparum* and *P. vivax* as predominant agents [1]. The most contagious specie within Africa is *P. falciparum* causes still birth during pregnancy and result to become severe in all age groups, killing approximately one million people annually, majority of which are children under the age of 5yrs covering about 86%. Among Asian's and American's, *P. vivax* was devastating with a far less mortality rate compared to *falciparum* [2].

Malaria treatment continues to become a problem in some areas of Asian continent, due to decline efficacy of the malarial drugs for resistivity reasons [3]. Drug resistance is the ability of a parasite to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than recommended, but within the tolerance of the subject [4]. Though, proof to the drug resistance were not identified yet, but inappropriate compliance with the recommended doses may attribute to the factor, also, malarial parasites with modifications in their gene can overpower the broader range of chemotherapeutic agents by over expression of a single gene [5]. Since 1934 4-aminoquinoline derivatives of Quinine and related compounds remain the most powerful and widely used antimalarial drug before resistance was established, and now, Artemisinin and its derivatives emerge the first-line drugs for the treatment of multi-drug-resistant *p. falciparum* infections as recommended [6].

Despite the lack of available licensed vaccine with fully protective acquired immunity, regulating the symptoms, occurrence and deaths by malaria could only be achieved using Insecticide-treated bed nets and chemotherapeutic means at the present situation [7].

Balsamodendron africanum (A. rich) (Burseraceae) is a plant found in tropical Africa, Transvaal and South West Africa and it is known as "Dashi" in Hausa [8, 9]. The plant serves as a good hedge in towns and farm lands in northern Nigeria [10]. In Batagarawa and Mani local governments of Katsina state the stem bark of the tree is used to treat malaria fever. It was reported that [9], all parts of the tree; were used by communities in east Africa to treat ailments such as malaria, colds, syphilis, typhoid, stomach troubles etc.

The plant doesn't grow more than 9 feet in height, normally, bearing knotted branches and branchlets ending in a spine. The leaves are trifoliate, scanty, small and very unequal, oval and entire. It is developed to have sturdy structure, though botanically, there is still lack of genuine origin and identity of the various species [11]. The stem back of the tree produces a gum-resin called *myrrh* for the *Balsamodendron myrrh* species while the *Balsamodendron mukul* and *Balsamodendron heudelotia* produce Bdellium as their gum resinous exudation. Both the myrrh and Bdellium are either reddish-yellow or reddish-brown in color, translucent, fractured and has feeble odor. The myrrh has strong pleasant odour, bitter and pungent while the Bdellium taste feebly bitter. However, the habitat known for the *Balsamodendron myrrh* is Somaliland, Arabia and Abyssinia, while *Balsamodendron Bdellium* is India and Africa [12, 13]

The hot Decoction prepared from the root *B. africanum* is used externally to treat stiff neck, while the stem bark in powdered form is mixed with porridge and eaten to cure malaria. The Fruit is used for typhoid treatment and stomach troubles. The gum-resin is applied to disinfect and seal up wounds [9] and also it could be used occasionally in plasters, as stimulant and tonic [12, 13].

The myrrh contains volatile oil 2.5-7%, resin (myrrhin $C_{33}H_{48}O_{10}$) 20-30%; Ash 5-10%, gum 50-60%, the gum is soluble in water and forms a precipitate with acetate of lead, the resin is soluble in chloroform and alcohol but partially soluble in alkalis and bisulphide of carbon while the insoluble part of the resin from bisulphide dissolve in ether. The volatile oil (myrrhol $C_{10}H_{14}O$) has variable specific gravity (S.G) of 0.98-1.018; Tincture of myrrh becomes purple on addition of $HNO_3$and ethereal solution gives a similar reaction with bromine vapor.

The bdelliums contain volatile oil 7%, specific gravity (S.G) -0.8836, have a terpene, bisabolene and oxygenated portion as $C_{56}H_{96}O$ [12, 13].

The Biological activities of *Balsamodendron* include; anti-inflammatory, hypolipidemic activity, reduced blood cholesterol level and cardioprotective properties [14].

2. Materials and Methods

2.1. Plant Material

The stem back of *Balsamodendron Africanum* was collected by Hajia Ade Sulaiman and Sagiru Sulaiman on 18th June 2014 from Nassarawar Charambi, Mani local government Area of Katsina State. The sample was authenticated at Biological Science Department Herbarium of Ahmadu Bello University Zaria by Mallam Musa Muhammad and voucher number 900367 was given. They were air-dried and ground into powder.

2.2. Extraction and Fractionation

The air-dried sample (200g) was percolated using ethanol 800ml for a period of 14 days. The extract obtained from the stem bark of *B. africanum* was concentrated at 40°C on a rotary evaporator, weighed and recorded.

The crude ethanol extracts were macerated using chloroform, n-hexane, methanol, and ethyl acetate. The macerated fractions were dried by exposing to air at room temperature.

2.3. Phytochemical Screening

A stock solution was prepared by dissolving 1g in 100ml of each macerated fraction using n-hexane, chloroform, ethyl acetate and methanol respectively as adopted by [15]. The presence of secondary Metabolites was identified and the results were recorded.

2.3.1. Test for Alkaloids

The extract (2ml) was put into a test tube; 0.2ml of dilute HCl was added, followed by 1ml of Dragendroff's reagent. Orange yellow color indicates the presence of alkaloids.

2.3.2. Test for Flavonoids

A portion of plant extract was heated with 10ml ethylacetate over a steam bath for 3mins. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. Yellow coloration indicates the presence of flavonoids.

2.3.3. Test for Saponins

1ml of the extract was taken in a test tube and added 20ml of Distilled water. It was shaken with hand for 15mins. A foam layer was obtained on the top of the test tube. This foam layer indicates the presence of saponins.

2.3.4. Test for Tannins

The stock solution (3ml) was taken in a test tube and diluted with chloroform and added acetic anhydride (1ml), finally, sulphuric Acid (1ml) was added carefully by the side of the test tube to the solution. A green color was formed which shows the presence of tannins.

2.3.5. Test for Steroids

The crude plant extract (1mg) was taken in a test tube and dissolved with 10ml chloroform, then added equal volume of concentrated sulphuric Acid to the test tube by sides, the upper layer in the test tube was turn into red and the acid layer showed yellow with green fluorescence. The steroids are present.

2.3.6. Test for Anthraquinones

The crude extract (0.5g) was taken into dry test tube and 5ml chloroform was added and shaken for 5mins and filtered,

the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red color in the lower layer indicates the presence of anthraquinones.

2.3.7. Test for Triterpenoids

The dry crude plant extract 5mg was dissolved in chloroform (2ml) and the acetic anhydride (1ml) was added to it. Concentrated sulphuric acid (1ml) was added to the solution. Formation of reddish violet color shows the presence of triterpenoids.

2.3.8. Test for Carbohydrates

Extracts (1g) were dissolved separately in 5ml distilled water and filtered; the filtrates were treated with Benedict's reagent and heated gently. An orange red precipitate indicates the presence of Carbohydrates.

2.4. Malaria parasite Assay

2.4.1. Preparation of Test Solution

A stock solution (10,000µg/ml) was, prepared by dissolving the extracts (20mg) obtain from *B. africanum* in Dimethyl sulphoxide, DMSO, (2ml). Serial dilution of 500µg/ml, 1000µg/ml, 2000µg/ml and 5000µg/ml concentration using serial dilution formula

$$C_1V_1 = C_2V_2 \quad (1)$$

Where:

C_1= Initial ConcentrationC_2= Final Concentration

V_1= Initial VolumeV_2= Final Volume

2.4.2. Sourcing of Malaria Parasite for Assay

Malaria parasites of infected blood samples containing a parasiteamia of *Plasmodium falciparum* was collected from the Microbiology laboratory of General Hospital Katsina, in a K3-EDTA coated plastic bottle and refrigerated.

2.4.3. Determination of Plasmodium Falciparum (Positive Blood Samples) Using Thin Smear Method

Using a clean capillary tube, a small drop of each blood sample was placed at the centre of a clean glass slide at least 2mm from one end. A cover slip was placed at angle of 45^0 in front of each drop. The drop was run along the full length of the edge of the cover slip. The cover slip was moved forward on each glass slide for a smear to be formed. The thin smear was immersed in methanol under control in a Petri dish for about 15 minutes. Geimsa's stain was dropped on each smear and allowed to stay for about 10 minutes. Excess stain was washed with clean water. The smear was dried in air by hanging the glass sides' upside-down on a rack. Each dried smear was observed under a high power objective lens microscope [16]

2.4.4. Separation of the Erythrocytes from the Serum of the Blood Samples

Dextrose solution (50%, 0.5ml) was added to each 5ml blood samples and the centrifuge for 15 minutes. The supernatant layer was diluted with normal saline and centrifuge for 10 minutes after being separated from the sediments. The resulting supernatant was discarded and sample with higher parasiteamia was diluted with fresh malaria parasite negative erythrocytes [17]

2.4.5. Preparation of Plasmodium Falciparum Culture Medium

Blood sample (2ml) was withdrawn from the main vein of a healthy rabbit using 5ml disposable syringe and defibrinated after allowing it to settle for about 45 minutes. The defibrinated blood sample was further centrifuge for 10 minutes. The supernatant layer was collected and sterilized. The sediment was separated and centrifuge further for about 5 minutes. The supernatant layer was added to the first one in a test tube. The sediment was discarded and the serum was supplemented with RPMI 1640 salt medium and sterilized 50µg/ml gentamycin sulphate. The description of the composition for the RPMI salt is as follows; KCl 5.37mM, NaCl 10.27mM, $MgSO_4$ 2.56mM, $NaHPO_4$ 17.73mM, $Ca(NO3)_2$ 0.42mM, $NaHCO_3$ 2.5mM and glucose 11.0mM demonstrated by [18]

2.4.6. In vitro Assay of the Activity of the Extracts on Plasmodium Falciparum Culture

A test Solution (0.1ml) and the culture medium (0.2ml) were added into a test tube containing 5% parasiteamia erythrocytes and mixed. The sensitivity of the parasites to each test fractions was determined under microscope at 37°C after 24 and 48 hours of incubation with the serial concentrations of 500, 1000, 2000 and 5000µg/ml.

2.4.7. Determination of the Activity

After 24 and 48 hours of Incubation, an aliquot of the culture medium was dropped on a microscopic slide, stained and viewed under oil immersion. The average percentage elimination of the erythrocytes that appeared as blue discoid's cells was determined using the formula as follows:

$$\% = \frac{N}{Nx} \quad (2)$$

% = percentage activity of the extracts,

N = Total number of cleared red blood cells (RBC),

Nx = Total number of parasite (RBC) [19]

3. Results and Discussions

The phytochemical screening of *B. africanum* indicate the presence of the following secondary metabolites in all the extract fractions of n-hexane, chloroform, ethylacetate and methanol; alkaloids, saponins, steroids, triterpenoids. However, flavonoids and tannins found to be absent in methanol fraction but, present in n-hexane, chloroform and ethylacetate. Likewise, Anthraquinone and carbohydrate were absent both in n-hexane and ethylacetate while present in chloroform and methanol fractions.

Numerous researchers have investigated the phytochemistry of myrrh, reporting a number of different chemical constituents within the resin, gum and oil [20]. The phytochemical Screenings of *B. africanum* indicate the

presence of steroids, triterpenoids, and tannins (Table 1). Though, tannin is absent in methanol fraction. But, OMARIA the antimalarial drug said to be effective against *P. falciparum* and *P. vivax* uses ellagic acids and tannins. Despite that, alkaloids may have deleterious effects on the placental parenchyma and ellagi-tannins seem to up-regulate healthy conditions and post partum [3]. However, they may be responsible for the antimalarial activity shown.

Table 1. *Phytochemical screening results of B. africanum.*

Metabolites	n-Hexane	Chloroform	ethylacetate	Methanol
Alkaloids	+	+	+	+
flavonoids	+	+	+	-
Saponins	+	+	+	+
Tannins	+	+	+	-
Steroids	+	+	+	+
anthraquinones	-	+	-	+
triterpenoids	+	+	+	+
carbohydrates	-	+	-	+

The antiplasmodial bioassay was conveyed on all the solvent fractions obtained from the stem bark extract of *B. africanum* and demonstrated remarkable activities at all concentrations; n-hexane fraction showed highest activity (Table 2) with percentage elimination of 91.8% at 5000µg/ml, 88.7% at 2000µg/ml 85.6% at 1000µg/ml and 84.3% at 500µg/ml, compared to *Commiphora kerstingii* 96.4% [21] at higher concentration of 5000ug/ml.

Table 2. *Malarial Parasite percentage elimination of B. africanum bark extract.*

Fractions	Concentration (µg/ml)	Average parasite before incubation	Average parasite after incubation	% elimination
Control	0	32	32	0%
Petroleum ether	500	32	6.3	80.3
	1000	32	5.3	83.4
	2000	32	4.3	86.5
	5000	32	3.3	89.6
n-Hexane	500	32	5	84.3
	1000	32	4.6	85.6
	2000	32	3.6	88.7
	5000	32	2.6	91.8
Chloroform	500	32	4.6	85.6
	1000	32	4.3	86.5
	2000	32	3.3	89.6
	5000	32	3	90.6
ethylacetate	500	32	6	81.2
	1000	32	5	84.3
	2000	32	4	87.5
	5000	32	3	90.6
Methanol	500	32	6	81.2
	1000	32	4	87.5
	2000	32	3	88.3
	5000	32	3	90.6
Artemesinin	500	32	5.3	83.4
Combine	1000	32	5.3	83.4
Treatment	2000	32	5	84.3
	5000	32	2.3	92.8

4. Conclusion

Plants in Nigeria can provide biologically active natural Products that will lead to the drug Development with enhanced activity in curing different ailment. terpenoids and alkaloids happen to be the most active phytochemical constituents responsible for anti-malarial activity shown by so many African plants [22]. However, *B. africanum* showed similar trend of the existing results from the anti malarial research and therefore, is biologically significance.

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