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Phytochemical screening and antimalaria activity of extracts from the stem bark of *Adansonia digitata*

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ABSTRACT: Medicinal plants play an important role in curing of various kind of diseases. Particularly in Africa, where nature provides tremendous reservoir of these natural products. If properly harness this could help greatly in the primary health care delivery system in Africa. The bark of Adansonia digitata was extracted with ethanol and screened for bioactivity against *plasmodium falciperum*. The crude ethanol extracts was macerated with 60% aqueous methanol and partitioned into petroleum ether, chloroform, ethyl acetate and aqueous methanol extracts. Chloroform extract showed good activity on the test organism with 67% mortality. The results indicated that the extracts contained an active compound(s) which have anti-malaria property. Phytochemical screening of the extracts revealed the presence of Tannins, steroids, saponins, alkaloids, cardiac glycosides and flavonoids.

Keywords: *Adansonia digitata*, Antimalaria, *plasmodium falciperum*, phytochemical,

Introduction

Plant kingdom provides tremendous reservoir of various chemical substances with potential therapeutic properties. Plants that produce and accumulate constituents having medicinal values are generally regarded as medicinal plant (Sofowora, 1984). Malaria remains one of the world's most devastating diseases, killing millions of peoples yearly (Snow et al, 2005). It is the 2nd main killer disease after HIV/AIDS. It is caused by protozoan parasite of the genus (plasmodium Apicomplexa). In human, malaria is caused by P. falciparum, P. malaria, P. Ovalae and P. vivax. However P. Falciparum is the major protozoan that caused the malaria disease, responsible for 80% infections and 90% death (Snow et al, 2005).

The symptoms of malaria include, fever, shivering, vomiting, anemia caused by hemolysis, hemoglobinuria and convulsions. The parasites primary hosts and transmission vectors are female mosquitoes of anopheles genus. *Adansonia digitata*, the baobab, is the most widespread of the *Adansonia* species on the African continent, found in the hot, dry Savannahs of sub-Saharan Africa. *Adansonia digitata*, is a well known tree almost everywhere in tropical Africa. It has a mighty trunk which can reach a diameter of 2-6 (10) M, with a girth of more than 20M (Aliyu, 2006).

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Ethnomedicinal uses of the tree include: urinary tract disorders, anti- inflammatory and mild diarrhea (Iwu, 1993). anti-Malaria, insect bites, asthma, coughs, gastroenteritis and ulcers (Maydell, 1990). The fruit pulp is probably the most important food stuff. It is dry and mealy and it is used in cool and hot drinks. Pulp can be dissolved in water or milk and the liquid is used as a drink, as a sauce for food, as a fermenting agent in local brewing or as a substitute for cream of tartar in baking. The energy value of pulp is similar to that of baobab leaves (Becker, 1983).

Material and Methods

The bark of *Adansonia digitata* was collected from Yako village, Kiru Local Government Area, Kano State, Nigeria. The sample was identified by a Botanist in the Department of Biological Sciences, Bayero University Kano, and air-dried and ground into powdered material using a clean pestle and a mortar.

Extraction of Plant Materials

The ground plant material (200g) was soaked with 95% ethanol (750ml) at room temperature for two weeks. It was filtered after which the solvent evaporated using rotary evaporator at 40^0C. The crude ethanol extracts obtained was weighed and labeled AD1 for *Adansonia digitata*. Aqueous methanol (60%) was mixed with crude extracts (8g) and poured into separatory funnel and partitioned with 100ml x 3 of petroleum ether, chloroform, ethylacetate sequentially. The afforded fractions obtained were concentrated using rotary evaporator, weighed and labeled AD1-01, ADI-02, AD1-03 and AD1-04 respectively.

Phytochemical Screening

Phytochemical analysis for qualitative detection of secondary metabolites were carried out using procedures described by Harbone, 1975, Evans, 1995, Brain and Tunner, 1975, El-olemy et al,1994, sofowora , 1984 and ciulei, 1994.

Sample Preparation

The extract (0.2g) was dissolve in DMSO (2ml) to make a stock solution. Thereafter, 0.1, 0.2 and 0.5ml of stock solution were dissolved in 0.9, 0.8 ad 0.5ml of DMSO to make a 1000, 2000 and 5000µg/ml concentrations respectively.

Sampling of the Malaria Parasite

Blood samples positive for plasmodium *falciparum* were collected from Bayero university kano clinic in anticoagulant bottles (K_3-EDTA) in a thermo flask contained water maintained at 4^0C as demonstrated by Diacia (1968), and transported to the microbiology laboratory in Bayero university Kano.

Separation of the Erythrocytes from the Serum of the Blood Samples

Dextrose solution (4.5%) (0.03ml) was added to each blood samples (2ml) and defibrinated, and then centrifuged at 2500rpm for 15minutes in a (Spectramerlin centrifugation machine). The supernatant layers were discarded, and the sediments were diluted with normal saline (red blood cells diluting fluid) as demonstrated by Dacie (1968) and centrifuged at 2500rpm for 10minutes. Similarly, the supernatants were discarded, and samples with higher parasiteamia were diluted with fresh malaria parasite negative erythrocytes (Hann et al., 2002).

Preparation of the *Plasmodium falciparum* Culture Medium

Blood samples (2ml) withdrawn from two healthy Rabbits (black and white) were transferred into sterilized test tubes and defibrinated as demonstrated by Dacie (1968). The defibrinated blood samples were subjected to centrifugation at 1500rp, in a (Spectra merlin centrifugation machine) for 10 minutes the supernatant layers were collected in a clean and sterilized test tube. The sediments were centrifuged

at 1500rpm for 5 minutes, and the supernatant layers were collected into the test tube. The sediments were discarded. The serum collected was supplemented with the salt of the RPMI 1640 medium as demonstrated by Devo et al (1985). The prepared medium was sterilized by addition of 40µg/ml gentamacin sulphate (Trager, 1982).

Bioassay Procedure

Aliquot of the culture medium (0.2ml) was distributed into a set of clean and sterilized test tubes with tightly fitted plastic corks. Then 0.05ml of the British pharmacopoeia Reagent substance (BPCRS) and the fractions was transferred into the test tubes containing the culture medium and one of the test tubes was kept anti-malaria free. The test tubes were labeled to indicates the each concentration, site of sampling and samples number while the test tube containing the culture medium without the anti-malaria was marked as control (C) with a 1ml disposable syringe and needle (OMN_1FIX-F) the parasiteamia erythrocytes (0.1ml) was added into each test tubes containing the tested organism were transferred into a glass bell jar containing a lighted candle supplying about 93% nitrogen, 5% carbon dioxide ad 2% oxygen as demonstrated by Muktar et al, (2006). Immediately the flame of the candle went off, the whole set up was transferred into an incubator maintained at 37^0C for 48hours.

Activity of the Extracts against *Plasmodium falciparum* after 48 Hours

This was done by removing a small drop of the content of each test tube after thoroughly mixing to the center (2cm from one end) of clean glass slide using a capillary tube. With the aid of a clean cover slip placed at angle of 45^0C in front of each drop and drawn backward to be in contact with each drop, thin smears were made on each glass tube slide by pushing the cover slips forward with smooth and fast movements. The smears formed were air dried and fixed in absolute methanol for 15minutes. These were then covered with several drops of a Giemsa's stain for 10 minutes. The excess stain was washed with a clean tap water. The glass slides were hanged on a rack with the smear sides facing downward and allowed to air dry. The air dried smears were observed under a microscope (CETI Belgium, BUK/BIO/99/01) using a high power objective (x100) under an oil immersion. The number of infected erythrocytes and the parasite density in each infected erythrocytes were noted, and average from reading of 3 microscopic fields were calculated and converted into percentage using the formular below:

$$\% = \frac{N}{Nx} \text{ X } 100$$

Where, % = Percentage activity of the extracts
N = Total number of cleared erythrocytes Nx = Total number of infected erythrocytes (Muktar et al., 2006.)

Results and Discussion

The results of phytochemical screening of the plant's extracts are shown in table1. And that of antimalaria activity of the extracts are shown in table 2

Table1: Phytochemical Screening Result of the Plant Extracts

FRACTIONS	ALKALOIDS	SAPONINS	TANNINS	FLAVANOID	STEROID
AD1	+	+	+	+	-
AD1-01	-	+	-	+	-
AD1-02	+	+	-	+	-
AD1-03	-	+	+	+	-
AD1-04	+	+	+	-	-

Keys: AD = Adansonia digitata, , AD1 = ethanol extract, AD1-01 = petroleum ether extract, AD1-02 = chloroform extract, AD1-03 = ethyl acetate extract, AD1-04 = methanol extract

Table 2: Antimalaria Activity of *Adansonia digitata* Extracts

Plant extracts	% Mortality per extracts concentration (μg/ml)		
	1000	2000	5000
AD1	31	48	62
AD1-01	26	31	52
AD1-02	36	50	67
AD1-03	24	40	50
AD1-04	0	38	50

Keys: AD = Adansonia digitata, , AD1 = ethanol extract, AD1-01 = petroleum ether extract, AD1-02 = chloroform extract, AD1-03 = ethyl acetate extract, AD1-04 = methanol extract

Discussion

Result of the phytochemical analysis (table1) revealed the presence of the following secondary metabolites in the plant extracts; Alkaloids, Saponins, Tannins, and Flavonoids. But steroids is absent in all the fraction of the plant extracts while saponins were found present in all the fractions. Anti-malarial activity test was determined at three different concentrations as shown in table 2. It is observed that activity of the extract increases with increase of concentration. From table 2; chloroform extract (AD1-02) showed highest total mortality. However, no activity is seen in methanol extract (AD-04) at 1000 μg/ml. Moderate anti-malarial activity was found in n-hexane extract (AD1-01) and methanol extract at 5000 μg/ml.

Conclusion

The present research work shows that, the extracts of the bark of *Adansonia digitata* have good activity against plasmodium parasite particularly the chloroform fraction (AD1-02). Thus, the results obtained, in this work establishes the efficacy of the plant extracts used in traditional medicine for treatment of malaria.

Recommendations

We recommend further work be carried out on the other parts of the plant such as leaves, fruits as well as their toxicity studies to ascertain safety for human consumption.

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