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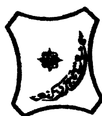

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PHYTOCHEMICAL AND *IN VITRO* ANTIPLASMODIUM ACTIVITIESOF LEAF EXTRACTS OF *Cassia nigricans* VAHL. (CAESALPINACEAE)

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ABSTRACT
The study was aimed at evaluating the phytochemicals, acute toxicity and in vitro antiplasmodium activity of aqueous and chloroform leaf extracts of Cassia nigricans using the basic phytochemical screening, Lorke's and Candle Jar methods. Steroid glycosides, reducing sugars and alkaloids were detected in both extracts, while saponins and flavonoids were only detected in the aqueous extract. The median lethal dose (LD_{50}) of the aqueous leaf extractwas found to be 471.17mg/kg. The in vitro antiplasmodium screening showed that both extracts have antiplasmodium activity; with chloroform extract having the highest activity at the concentration of 2, 3 and 4 mg/ml which was statistically significant($P<0.05$) in comparison with the positive control. The chloroform extract had the most potent half maximal inhibitory concentration (IC_{50})value of 0.60 mg/ml. It can be concluded that C. Nigricans possess strong antiplasmodium activity and this may accounts for its use in traditional medicine in the management of malaria.
Keywords: Acute toxicity, antiplasmodium, malaria, phytochemicals and traditional medicine

INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoa belonging to the genus *Plasmodium* (WHO, 2014), a widespread group of protozoa that parasitizes the human liver and red blood cells. It is transmitted by the bite of an infected female anopheles mosquito. It is characterized by cycles of chills, fever, pain and sweating (Heyneman, 2016).
Four species of *Plasmodium* infects human cells, and this includes; *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax*and *Plasmodium ovale*. *P. falciparum* is the most important cause of the disease, responsible for about 80% of all malaria cases and about 90% of deaths (Mendis *et al.*, 2001).
Consequences of severe malaria include coma and death if untreated. It is thought to have been the greatest selective pressure on the human genome in recent history, and this is due to the high levels of mortality and morbidity caused by malaria (Kwiatkowski, 2005).
The World Health Organization (WHO) reported that there were 198 million cases of malaria worldwide in 2013, and this resulted in an estimated 584,000 to 855,000 deaths, 90% of which occurred in Africa (WHO, 2014). There is an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria(WHO, 2014). Globally, malaria is the third leading cause of death for children under five years (i.e after pneumonia and diarrheal disease) (WHO, 2012).
Traditional methods of malaria treatment could be a promising source of new antimalaria drugs; this is because efforts are being made to screen medicinal plants to obtain new classes of compounds which can be used as antimalarials. In Africa, more than 80% of people use traditional medicine and most families have recourse to this medicine based on plants extract for the curative treatment of malaria (Wright and Phillipson, 1990). Medicinal plants have become popular over the past decade and are used for treatment all over malaria-infected countries of the world (WHO, 2003).
*Cassia nigricans*belongs to the family Caesalpinaceae. It is locally known as "Madacin-kasa", "Jiwotsamiya" or "Shuwakangargari" by the Hausa people of Northern Nigeria. The plant has many uses in both human and veterinary medicine. Throughout West and East Africa, an infusion of the leaves or aerial parts is taken against fever, malaria, stomachache, diarrhea and worms. Methanol extract of this plant has shown analgesic and anti-inflammatory, antiulcers effects (Chidume *et al.,* 2001).
The present study was aimed to evaluate the phytochemical, acute toxicity and antiplasmodium properties of aqueous and chloroform leaf extractsof *Cassia nigricans* used traditionally in the treatment of malaria in many parts of Northern Nigeria.

MATERIALS AND METHODS

Malaria Parasites

Malaria parasite positive and negative blood samples were obtained from Murtala Muhammad Specialist Hospital, Kano State, Nigeria. Consent of the Hospital's management allowed the collection of 15 ml and 10 ml each of positive and negative samples respectively (5 ml from each patient). The blood samples were immediately transferred into a K_3-EDTA bottle and preserved at 4^oC as demonstrated by Diacie and Lewis (1968).

Animals

Swiss albino mice of both sexes, weighing 18-25 g were obtained from the Animal House of the Department of Pharmacology, Bayero University, Kano, Nigeria. They were housed under standard conditions of temperature (25 ± 2°C), 12/12 hour light/dark cycle, fed with standard diet and given water*ad libitum*.

Plant Collection, Identification and Preparation

The plant was collected from Usmanu Danfodiyo University, Sokoto State, Nigeria. It was identified and authenticated by a Taxonomist at the Ethnobotany Unit of Bioresources Development Centre, Kano. A reference sample number, BDCKN/EB/1795 has been deposited in the Herbarium. The leaves were dried and powdered using pestle and mortar.

Extraction of the Powdered Plant Material

The powdered plant material (10 g) was percolated in 200 ml of distilled water/chloroform and allowed to stand for 24 hours. The mixture was then shaken and filtered using a filter paper. The filtrate obtained was evaporated to dryness at 40 ^{0}C using a rotary evaporator and a water bath.

Preliminary Phytochemical Screening

The Preliminary Phytochemical screening of aqueous and chloroform extracts of *Cassia nigricans* was conducted using the standard laboratory procedures (Evans, 1996, Ciulel, 1994, Brain and Turner, 1975).

Acute Toxicity Studies

The intraperitoneal median lethal dose (LD_{50}) of aqueous leaf extract of *C. nigricans* was conducted using the method of Lorke (1983).

Determination of Malaria Positive Blood Samples (Using Thin Smear Method)

This was conducted as described by Stanley (1983) and Kanai (1988). After thorough mixing, a small drop of each blood sample was placed at the centre of a clean glass slide at least 2 mm from one end using a capillary tube. A clean cover slip was placed in front of each drop at an angle of 45°, and then drawn backward to be in contact with blood, the drop was then allowed to run along the full length of the edge of the cover slip. With a fast and smooth movement, the cover slip was pushed forward to form even thin smear on each glass slide. The smears were immersed in a petri dish containing absolute methanol for 15 minutes, and then covered with several drops of Giemsa's stain for 10 minutes. Excess stain was washed with distilled water and dried on a rack with glass slide kept upside down. The air-dried smears were observed under a microscope using a higher objective (x 100) under an oil immersion. The smears were traversed thoroughly for malaria parasite infected erythrocytes. An average parasitemia was obtained from the reading of 3 microscopic fields. Blood samples with 5 % parasiteimia were used for the study (Hanne *et al.,* 2002).

Separation of Erythrocytes (with 5 % Parastemia) from the Serum of the Blood Samples

A 0.05 ml of 50 % dextrose solution was added to 5 ml of the blood samples, defibrinated and centrifuged at 2500 rpm for 15 minutes in a spectra merlin centrifuging machine. The supernatants were discarded and the sediments were diluted with normal saline as described by Diacie and Lewis (1968). The sediment was recentrifuged at 2500 rpm for 10 minutes. Also, the supernatants were discarded and the sediment was diluted with fresh negative erythrocytes of malaria parasite (Hanne *et al.,* 2002).

Preparation of Malaria Parasite Culture Medium

The method of Trager (1982) and Devo *et al.* (1985) were adopted. The samples were subjected to centrifugation at 1500 rpm for 10 minutes. The supernatant layers were collected in a clean and sterilized test tube. The sediments were centrifuged at 1500 rpm for 5 minutes and the supernatant layers were collected into the first tube. The sediments were discarded and serum collected was supplemented with hypoxanthine and the salts of RPMI 1640 medium. The prepared medium was sterilized by addition of 40 ug/ml gentamicin sulphate.

***In Vitro* Antiplasmodium Activity of the Extracts**

Aliquot of 0.2 ml of the culture medium was distributed into a set of clean and sterilized vials with tightly fixed plastic corks, 0.05 ml of the diluted extracts at the concentration of 0.5, 1, 2, 3 and 4 mg/ml were transferred into each vial containing the culture medium. One of the vials was kept extract free (negative control). With a 1 ml disposable syringe, 0.1 ml of the 5 % parastemia erythrocytes were added into each vial and mixed thoroughly. The vials were then transferred into a glass bell jar containing a lighted candle supplying about 93 % nitrogen, 5 % carbon dioxide and 2 % oxygen as demonstrated by Trager (1982); Symith (1994); Hanne *et al.,* (2002).

Statistical Analysis

The level of significance was tested using One-way ANOVA followed by Duncan Multiple Range Test (DMRT). Results were regarded as significant when $P<0.05$.

RESULTS

Preliminary Phytochemical Screening

The preliminary phytochemical screeningof aqueous and chloroform extracts revealed the presence of steroid glycosides, reducing sugars and alkaloids. In addition, saponins and flavonoids were detected in the aqueous extract (Table 1).

Table 1:Phytochemical Constituents of Aqueous and Chloroform Leaf Extracts of C. *nigricans*

Phytochemicals	Extracts	
	Chloroform	Aqueous
Tannins	-	-
Resins	+	-
Steroid glycosides	+	+
Saponins	-	+
Reducing sugars	+	+
Flavonoids	-	+
Alkaloids	+	+

Key:
+ = Present

- =Absent

Acute Toxicity Study

The median lethal dose (LD_{50}) of the aqueous leaf extract of *C. nigricans* was found to be 471.17mg/kg. Deaths were recorded in the third group (1000 mg/kg body weight) and fourth group (600 mg/kg body weight) of first and second phase respectively (Table 2).

Table 2: Acute Toxicity Study of Aqueous Leaf Extract of *C. nigricans*

First Phase		Second Phase	
Dose (mg/kg)	**Mortality**	**Dose (mg/kg)**	**Mortality**
10	0/3	140	0/1
100	0/3	225	0/1
1000	3/3	370	0/1
		600	1/1

***In Vitro* Antiplasmodium Activity**

The *in vitro* antiplasmodium screening of aqueous and chloroform leaf extracts of *C. nigricans* showed that both extracts have antiplasmodium activity. The highest activity was observed in chloroform extract which afforded a dose dependent antiplasmodium activity at the tested concentrations which is statistically significant ($P<0.05$)when compared to the positive control(Table 3). Also, the half maximal inhibitory concentration (IC_{50})values of chloroform and aqueous extracts were 0.60 and 1.27 mg/ml respectively.

Table 3:*In vitro* Antiplasmodium Activity of Aqueous and Chloroform Leaf Extracts of *C. nigricans*

Concentration (mg/ml)	% Inhibition	
	Artesunate Aqueous Extract	**Chloroform Extract**
0.5	$87^{b}27^{d}$	47^{c}
1.0	$100^{a}45^{c}$	40^{c}
2.0	$100^{a}9^{d}$	100^{a}
3.0	$100^{a}82^{b}$	100^{a}
4.0	$100^{a}100^{a}$	100^{a}

Values in the same column with different superscript differs significantly ($P<0.05$)

DISCUSSION

The phytochemical analysis of aqueous and chloroform leaf extracts of *C. nigricans* showed the presence of different classes of phytochemicals. Steroid glycosides, reducing sugars and alkaloids were detected in both extract, while saponins and flavonoids were only detected in the aqueous extract. These variations could be attributed to the differences in the polarity index of the two solvents used in this study (Imam *et al.,* 2016). The presence of saponins and flavonoids in the aqueous extract supported the finding of Ayo and Amupitan (2007).

The antiplasmodium activity of*C. nigricans* may be due to the alkaloids detected in both extracts. Previous studies showed that alkaloids are commonly implicated in the antiplasmodium activity of many medicinal plants (Okokon*et al.,* 2006). Also, flavonoids were also reported to have significant antiparasitic activity against different parasites strain of malaria; however, the exact mechanism of antiplasmodium activity of flavonoids is not fully established (Al-Ahdroey *et al.,* 2011; Imam *et al.,* 2016).

The acute toxicity test gives an idea on the range of doses that could be toxic to the animals (Abubakar *et al.,* 2017). The aqueous leaf extract of *C. nigricans* has an LD_{50}than can be considered as slightly or moderately toxic (Matsumura, 1975).

The present study showed that both aqueous and chloroform extracts of *C. nigricans* have antiplasmodium activity. The chloroform extract had the highest activity; it gave 100 % inhibition at the concentration of 2, 3 and 4 mg/ml of the extract which is statistically significant ($P<0.05$)when compared to the group treated with a standard drug, artesunate. This finding is in agreement with Obiageri (2005) and Obodozie *et al.,* (2004) who reported the antiplasmodium activity of *C. nigricans* against chloroquine resistant strains of *P. falciparum.* The development of new therapeutic approach to malaria is very much needed since resistance of parasites to different antimalarial drugs is fast developing (Wright and Phillipson, 1990). Due to the important roles that plant derived compounds have played in drug discovery and development for the management of many diseases, the isolation of new bioactive compounds from medicinal plants based on traditional uses appears to be a very good approach (Newman, 2008).

CONCLUSION

The findings in this study showed that the leaf extracts of *C. nigricans* possess strong antiplasmodium property; this may accounts for its use in traditional medicine in the management malaria. A study is currently going on to isolate, characterize and identify the bioactive compounds responsible for the antiplasmodium property of this important plant.

Contribution of Authors

Fatima, A. performed the laboratory work, literature search and results interpretation, while Abubakar, U. S. wrote the first draft of the manuscript and also participated in the results interpretation. Abdullahi, M. S. and Yusuf, K. M. designed and co-supervised the work. Finally, Abdu, G. T.made extensive corrections to themanuscript.

Conflict of Interest

The authors declare no conflict of interest.

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