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PHYTO CHEMICAL COMPOSITION AND *In vitro* ANTIPLASMODIAL POTENTIAL OF STEM BARK AND LEAF EXTRACTS OF *Ficus gnaphalocarpa* AND *Ipomea fistulos* USED IN YAURI AND ARGUNGU EMIRATES, KEBBI STATE, NIGERIA

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ABSTRACT

Malaria is the world's most parasitic disease endemic in about 100 developing countries. The search for new antimalarial drugs from natural products is necessitated by resistance of the parasites to known antimalarial agents. This study evaluated phytocmical composition and the *in vitro* antiplasmodial potentials of *Ficus gnaphalocarpa* stem bark and *Ipomea fistulos* leaf methanolic extracts obtained from two areas (one each from Argungu and Yauri emirates of Kebbi State, Nigeria) . The air dried samples of *Ficus gnaphalocarpa* (miq) C.C. Beng (stem bark) and *Ipomea fistulos L.* (leaves) were extracted with methanol for seventy two hours. The crude extracts were tested against chloroquine resistant strain of *Plasmodium berghei berghei* (NK-65) *in vitro* in 96 well microplates under anaerobic condition. The data obtained were analyzed using one way ANOVA and Duncan multiple comparison test with SPSS version 20.0. There was significant ($P<0.05$) reduction in the number of parasitized cells relative to control. *F. gnaphalocarpa* exhibited higher activity of 92.81% and IC_{50} (11.85 mg) while *I. fistulos* recorded an activity of 66.70% and IC_{50} (36mg). The plant extracts used in this study presented antiplasmodial activity. Further studies on *in vivo* assay, toxicity, histopathology, isolation and purification is recommended for possible development of their bio-constituents into antiplasmodial agents.

Keywords: Antiplasmodial activity, *Plasmodium berghei berghei, Ficus gnaphalocarpa, Ipomea fistulos,* malaria

INTRODUCTION

Malaria is an infectious disease caused by single-celled obligate parasite known as *Plasmodium* and it is transmitted to man through the vector Anopheles mosquito (Chinedu *et al*., 2014). Malaria in humans is caused by *P. Malariae, P. vivax, P. ovale* and *P. falciparum* (Shija *et al*., 2020). Malaria caused by *P. falciparum* and *P. vivax* represent the majority of malaria health burden worldwide with estimated incidences of 207 million and 8.5 million cases respectively in 2018 (WHO 2018, Muhammed *et al*., 2018).

P. falciparum is the most dominant and pathogenic species responsible for almost all mortality caused by malaria in tropical and sub-tropical countries (Ogundolie *et al*., 2017). *P. berghei* is a practical model organism in the laboratory for the study of human malaria aimed at developing a new management measure for the control and prevention of malaria (Muhammed *et al.*, 2018; Ogundolie *et al.*, 2017). The Parasites, *P. berghei*, is transmitted to rodents by the bites of an infected mosquito called *Anopheles dureni* (Muhammed *et al.*, 2018).

Malaria has contributed to major socioeconomic problems, consequence of which is global instability and poverty (CDC, 2016). Some complications due to malaria include respiratory distress resulting in metabolic acidosis, severe anemia and cerebral malaria which may lead to death (Chinedu *et al.*, 2014). Malaria remains the most prominent cause of death and illness in Africa particularly among pregnant women and children under age of 5 years (Chinedu *et al*., 2014; Schanzt – Dunn *et al*., 2009).

Various therapies have been developed for the treatment of malaria, some of which include, Chloroquine, Mefloquine, Quinine, Primaquine, Artemisinin and its derivatives like artesunate, artemeter and arteether (Adebayo and Kretlli, 2011). However, the current efforts to reduce the global burden of malaria are threatened by the rapid emergence and spread of *P. falciparum* resistance to artemisinin combination therapies, ACTs including artemisinin derivatives and their partner drugs (Muhammed *et al*., 2018; WHO, 2017). The possible and promising source of malaria treatment appears in the use of traditional herbal medicine which has been the most available, affordable and cheap sources of malaria treatment for most communities (Muhammed *et al*., 2018; Hosseinzadeh *et al*., 2015).

Ficus gnaphalocarpa is one of the most important general in the plant family *Moraceae* (Babandi *et al*., 2019). It has reported biological activities such as anti-pyretic, gastroprotective property, antioxidant, antimicrobial activity (Babandi *et al*., 2019, Mbosso *et al*., 2016b; Phan *et al*., 2012). The traditional medicine practitioners in Yauri Emirate claimed to be using the plant to treat malaria.

Ipomea fistulos is a shrub like species of morning glory belonging to plant family, *convolvulaceae* (Chand and Rohatgi, 2005). It has sedative and anticonvulsant property (Chand and Rohatgi, 2005). The gylcosidicsaponin purified from *I. carnea* possess anticarcinogenic and oxytoxic properties (Chand and Rohatgi, 2005). The traditional medicine practitioners in the study area claimed it's being used to treat malaria. Therefore, the study was aimed at evaluating the phytochemicals and antiplasmodial efficacy of methanolic crude extract of *Ficus gnaphalocarpa* (Stem bark) and *Ipomea fistulos* (leaf).

MATERIALS AND METHODS

Collection of Plants' Parts, Identification and Processing

Ethnobotanical Survey

The ethnobotanical survey was carried out by administration of Questionnaires to fourty respondents including traditional medicine practitioners and herbal sellers in each Local Government of Argungu and Yauri Emirates of Kebbi State.

This was to ascertain the plants which are traditionally used for treatment of malaria in the study area.

Selection Criteria
One plant with highest frequency and whose little or no research has been conducted was selected from each Emirate.

Collection and Identification of the selected Plants.
Stem bark of *Ficus gnaphalocarpa* and *Ipomea fistulos* leaves were collected from Tondi near Yauri and Argungu towns of Kebbi, State in January, 2021. They were subsequently identified and authenticated through their leaves by a botanist in Plant Science and Biotechnology Department, Kebbi State University of Science and Technology, Aliero, Nigeria where voucher specimen numbers 511 and 34 respectively, were given to the plants. The fresh plant materials were washed with clean water and shade dried. The stem bark was cut into pieces before drying. The dried pieces were subsequently made to powder using laboratory grinding machine.

Extraction of Plant's Materials.
Extraction of the powdered samples was done using the method described by Olasehinde *et al.* (2016). Two hundred grammes (200g) of the dried powdered form of each plant material were soaked in 1000ml methanol for 72 hours with constant stirring at regular interval. Then, filtered using sterile muslin cloth and whatman filter paper no.1 and evaporated to dryness using rotary evaporator at 40ºC. The extracts were then stored in air- tight containers and kept in a refrigerator at 4ºC in Biochemistry Laboratory, Kebbi State University of Science and Technology, Aliero until needed for use.

Source of Malaria Parasite Stock
The chloroquine resistance strain of *Plasmodium berghei berghei* (NK-65) was obtained from the National Institute for Pharmaceutical Research and Development, NIPRD, Abuja, Nigeria in April, 2021. The infected mice were kept in Animal house of Kebbi State University of Science and Technology, Aliero until needed for use. The parasite was kept alive by continuous re-infestation (IP) with the blood from infected mice in albino rats every four days.

Phytochemical Screening of the Crude Extracts.
Crude extracts of *F. gnaphalocarpa* and *I, fistulos* were screened for phyto-constutients such as alkaloids, saponins, tannins, anthraquinones, glycosides, cardiac glycosides, terpenoids, steroids and flavonoids using standard methods of Harborne (1984), Trease and Evans, (1989) and Sofowora (1993).

Preparation of Culture Media
Culture medium was prepared by mixing 15ml stock solution of RPMI 1640 medium (pH7.3) with equal volume of plasmodium free screened red blood cells (O+) obtained from Federal Medical Centre Birnin Kebbi, kebbi State, Nigeria. Parasitized erythrocytes were obtained from a donor-infected rat by cardiac puncture into heparin bottle and made up to 20 ml with normal saline.

Anti-plasmodial Assay of the crude Extracts
The *in vitro* antiplasmodial assay was carried out in triplicate in 96 wells microlitre plate according to method described by Ngemenya *et al*. (2006). Unto each well was measured 100µl of each extract at concentrations of 0.6mg, 1mg and 2mg followed by 100µl of culture medium and 100µl parasite (isolates), *Plasmodium berghei berghei* (NK65) respectively. This was incubated at 37^0C for 48hrs under anaerobic conditions using Thelco High Performance Laboratory Incubator (Model No. 3501).
Thin smear of the harvested cells from the wells was done and stained with leisman stain for 3minutes, parasite growth was counted in 10 fields using x100 objective microscope and the mean was calculated (Ngemenya *et al*., 2006).
Artesunate (20mg/ml) was used as drug control (DC) medium plus parasite free blood as normal control (NC) and only parasitized cells as induced control (IDC).
Percentage (%) inhibition was calculated as;

$$(\%)\ \text{inhibition} = \frac{\text{Parasitemia in induced control well} - \text{Parasitemia test wells}}{\text{Pasasitemia in induced control well}} \times 100$$

Concentration required to inhibit the parasite growth by 50% (IC_{50}) was determined by linear interpolation from the parasite growth inhibition curves (concentration versus percentage inhibition) ge nerated from parasite – extract interaction (Mustofa, *et al*., 2007).

Data Analysis
Results of the study was analyzed with one-way analysis of variance (ANOVA) using SPSS version 20.0 and the results expressed as mean ± standard error of the mean (M ± SEM). Treatments were separated using Duncan's multiple range test (Zar, 1984). The result was considered statistically significant at 95% confidence level and p-value less than 0.05 ($p<0.05$).

RESULTS
Phytochemical Analysis of the Crude Methanolic Extracts
The results obtained showed the presence of alkaloids, saponins, tannins, anthraquinones, glycosides, steroids and flavonoids in both plants while cardiac glycosides were absent in *I. fistulos* and terpenoids absent in *F. gnaphalocarpa* (Table1).

Table1: Phytochemical Profile of *F. gnaphalocarpa* (stem bark) and *I. fistulos* (leaf) Extracts.

Phytochemical	*F. gnaphalocarpa*	*I. fistulos*
Saponin	+	+
Tannins	+	+
Anthraquinones	-	-
Cardiac glycoside	+	-
Steroids	+	+
Terpenoids	-	+
Flavonoids	+	+
Glycosides	+	+
Alkaloids	+	+

Detected = +, Not detected = -

Parasitemia Reduction Performances by the Crude Extracts

The results in Table 2 showed that parasitemia load decreased with increase in concentration of the crude extract. For instance, the results in Figure 1 showed that the parasitemia reduction performance by *F. gnaphalocarpa* was highest (13) at 2.0mg/ml and lowest (128) at 0.6mg/ml respectively. Also, the parasitemia reduction performance by *I. fistulos* was highest (72) at concentration of 2mg/ml and lowest (224) at 0.6mg/ml (Figure 2).

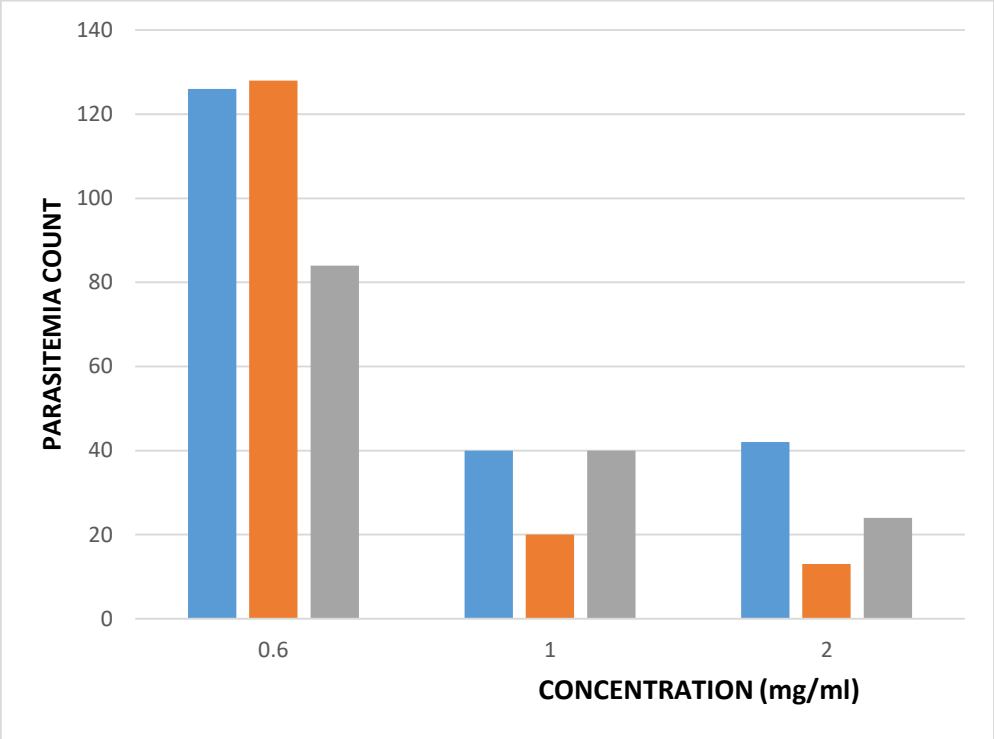

Figure 1: Parasitemia Reduction Performance by *F. gnaphalocarpa* Stem Bark Extract

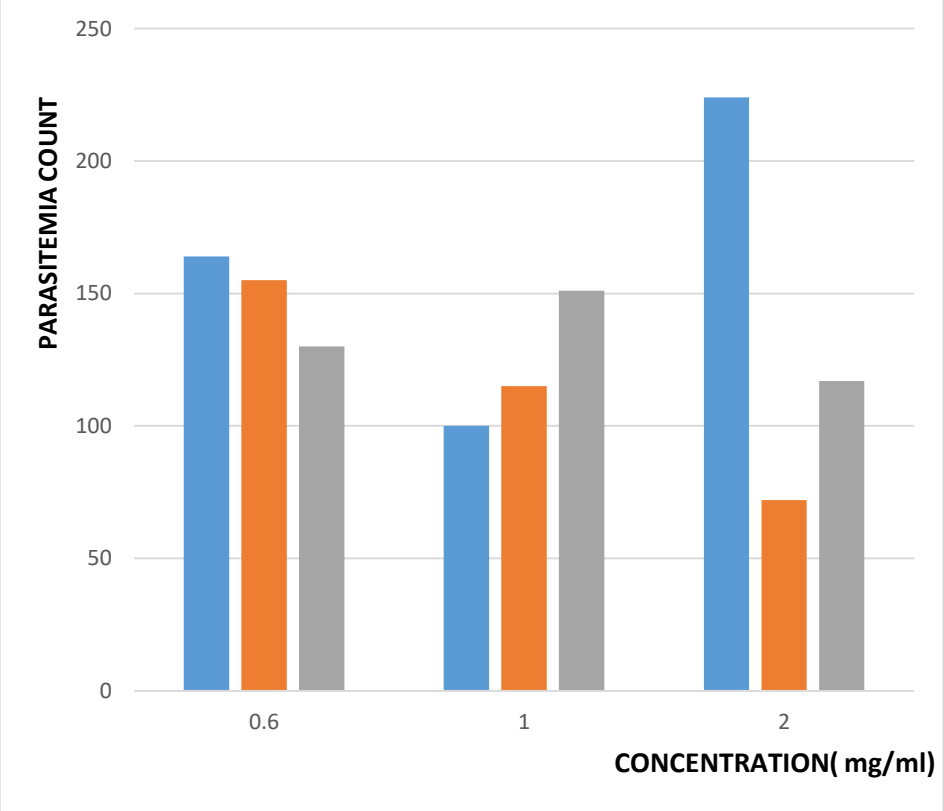

Figure 2: Parasitemia Reduction Performance by *I. fistulos* Leaf Extract

Antiplamodial Activities of *F. gnaphalocarpa* (stem bark) and *I. fistulos* (leaf) extracts.

The results in Table 2 indicated that mean parasitemia count for *F. gnaphalocarpa* was lowest (26.33±8.45) at 2.0mg/ml and the lowest mean parasitemia count for *I. fistulos was* 122.00±15.13 at 2.0mg/ml. Table 2 results also showed that the highest activity exhibited by *F. gnaphalocarpa* and *I. fistulos* extracts were 92.81% and 66.7% while concentrations required to eliminate the parasite by 50% (IC_{50}) were 11.85mg/ml for *F. gnaphalocarpa* and 36.0mg/ml for *I. fistulos* respectively. There was no significant difference in the antiplasmodial activity of 1mg and 2mg of *F. gnaphalocarpa* extract and also among all the various concentrations (0.6mg, 1.0mg and 2mg) of *I. fistulos* compared to that of normal control. However, there was significant difference between the antiplasmodial activity of 1mg, 2mg and 0.6mg of *F. gnaphalocarpa* at P≤ 0.05.

Table 2: *In vitro* antiplasmodial activity of *F. gnaphalocarpa* (stem bark) and *I. fistulos* (leaves) Extract

Treatments	Concentration (mg/ml)	Activity (SEM)	Percentage (%)IC_{50}	Inhibition
Controls DC	20	10.666±2.609[a]	97.27	
IDC		366.33±32.692[e]	0.00	
NC		0.00±0.00[a]	100.00	
F. gnaphalocarpa	0.6	112.67±14.34 [cd]	69.24	11.85
	1.0	33.33±6.67 [ab]	90.90	
	2.0	26.33±8.45[ab]	92.81	
I. fistulos	0.6	137.67±45.67 [cd]	62.42	36.00
	1.0	149.67±10.17 [cd]	59.14	
	2.0	122.00±15.13 [cd]	66.70	

NC= Normal control; IDC = Induced control, DC= Drug control and SEM= Standard error of mean

The results with the same superscript showed that there was no significant (P≤ 0.05) difference in the parasitemia reduction by the extracts.

DISCUSSION

The abundance of phytochemicals detected in the selected plants' extracts of this study support their medicinal values. The presence of these bioactive compounds in the plants' extracts is in agreement with results of previous researches. For instance, a comparative study on phytochemical screening conducted by Babandi *et al.* (2019) and Bello *et al*. (2013) using *Ficus syncomorus* stem bark with different solvents reported the presence of tannins, alkaloids, saponnins and flavonoids. Report of a similar study on *Ficus syncomorus* leaves conducted by Alsiddig and Sufyan (2015) also revealed the presence of secondary metabolites such as tannins, alkaloids, terpenens, phenols, saponins and flavonoids using n-hexane, chloroform, ethylacetate, butanol and aqueous solvents. There is little variance in the phytochemicals of *F. Syncomorus* between Babandi *et al*. (2019) report and the present study. Saponins was absent in the former's report but present in this study. This may be due to difference in geographical distribution with different soil type and climatic conditions and also different plant parts possess varied phytochemical constituents. In the present study, stem bark was used as against fruit used by Babandi *et al*. (2019).

Both plants screened for antiplasmodial activity exhibited antimalarial potentials. This agreed with the report of similar studies. Babandi *et al*. (2019) reported that *F. Syncomorus* leaves possessed antiplasmodial activities. There is yet to be established scientific proof of the antiplasmodial activity of *I. fistulos* probably due to the fact that *Ipomea* species had been reported as poisonous in animals. *I. asarifolia* as reported by Salles *et al*. (2011) possess a toxic principle "lecithin" which causes natural intoxication of livestock. However, the traditional medicine practitioners in the study area asserted the addition of potash (potassium carbonate) during the preparation of its decoction in order to reduce or eliminate any trace of poison in the plant materials.

The dose-dependent antiplasmodial activity observed was because the percentage parasite elimination revealed that higher concentrations exhibited higher activity. The mean parasite elimination was least at 0.6mg (112.67) and highest at 2mg (26.33) for *Ficus gnaphalocarpa* while least (149.67±10.17) parasite elimination at 1.0mg and highest elimination (122.00±15.13) was recorded for *I. fistulos*.

The low IC_{50} (11.8 and 36.0 mg/ml) recorded by the plants indicates that the plants can be considered as having antimalarial property. Gessler *et al.,* (1994) reported thresholds for *in vitro* antimalarial activity of water and ethanol extracts of *A. occidentale* with $IC_{50}$of 16.0 µg/ml and 11.7µg/ml respectively, are considered as moderate antimaliarial activity. Though IC_{50} recorded in this study are higher than the reported values, this could be due to the different plants and solvents used.

The plants' demonstration of antiplasmodial activities may have been due to their possession of bioactive compounds such as flavonoids, alkaloids, tannins and saponins whose presence was also confirmed in this study. Alkaloids exhibit toxicity against foreign cells which are widely used in cancer therapy (Oyi *et al.,* 2007) and antiplasmodial activity (Babandi *et al.,* 2019; Deguchi *et al.,*2012). Babandi *et al.* (2019) also asserted that alkaloids act as defense compound in plants and effective against pathogens and predators due to their toxicity. It disrupts protein function after ingestion and altars the central nervous system. This was supported by Anigboro *et al*. (2020) and Rathnapala *et al*. (2017) report that some plants exhibit antiplasmodial activity by thwarting protein synthesis perhaps as a result of the presence of certain phytochemical constituents.

Flavonoids were previously reported by Monbrison *et al.* (2006) to exert antiplasmodial activities against different strains of malaria parasites. The mechanism of antiplasmodial action of flavonoids is yet to be established (Babandi *et al.,* 2019) but some have been reported by Elford (1986) to inhibit the influx of L-gluitamine and myoinositol into *P. falciparum* infected red blood cells while flavones, glycoside from *Phlomis brunneogaleata* and iridoid from *Scrophularia lepidota* have been shown to inhibit Fab I enzymes of *P. falciparum* (Tasdemir *et al.,* 2005; Kirmizibe-Kmez *et al*., 2004). Flavonoids may also act as primary antioxidant or free

radical scavengers that fight oxidative damage induced by the malaria parasite.

More so, tanins were reported to exhibit antiplasmodial activity in *Punica granatum* L. fruit rind (Adia *et al*., 2016). However, it can be suggested that any of the classes of bioactive compounds, which may have acted as individual, additive, antagonistic or synergy may be responsible for the observed antiplasmodial potentials of the extracts. Therefore, *F. gnaphalocarpa* and *I. fistulos* extracts containing appreciable amounts of these phytochemicals might show antiplasmodial efficacy via any of these mechanisms.

CONCLUSION

The two plants exhibited antiplasmodial efficacy but *F. gnaphalocarpa* exhibited a relatively higher activity than *I. fistulos*. These findings validates the claim of the Traditional Medical Practitioners in malaria therapy. Hence, further studies should be carried out on *in vivo* assay, cytotoxity, characterization and purification of active ingredients of these plants for the purpose of developing new Antimalarial agents.

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