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In vitro antiplasmodial activity of some medicinal plants from Nigeria

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Abstract
Evaluation of activity of some medicinal plants used as malarial remedies by the Ibibios of Niger Delta region of Nigeria against *Plasmodium falciparum* was carried out. Crude extracts as well as fractions from promising plants were investigated for antiplasmodial activity against erythrocytic stages of chloroquine (CQ)-sensitive 3D7 and CQ-resistant INDO strains of *Plasmodium falciparum* in culture using the fluorescence-based SYBR Green I assay. Cytotoxicity was determined against HeLa cells using MTT assay. GCMS of active fractions were carried out. Various plants extracts exhibited varying degrees of antiplasmodial activity. Promising antiplasmodial activity was found in *Setaria megaphylla* ethyl acetate leaf fraction [IC_{50} = 8.15 µg/mL (3D7) 8.05 µg/mL (INDO)] and good activities were found in *Hippocratea africana* [root ethyl acetate fraction IC_{50} = 25.95 µg/mL (3D7), 15.94 µg/ml (INDO)] and *Solenostemon monostachyus* (petroleum ether leaf fraction [IC_{50} = 25.99 µg/mL (3D7), 12.30 µg/ml (INDO)]. The leaf extract of *S. megaphylla* whose fractions exerted the highest activity against *Plasmodium falciparum* elicited moderate cytotoxicity (TC_{50}- 45µg/ml). GCMS results identified some pharmacologically active compounds. These results provide validation for the traditional usage of some medicinal plants against malaria by the Ibibios in Niger Delta region of Nigeria.

Keywords: Antiplasmodial, antimalarial, *Plasmodium falciparum*, ethnomedicine, Malaria

1. Introduction
The menace of malaria in the tropics especially in sub Saharan Africa cannot be over emphasized as the disease has continued to wreck havoc in terms of mortality and economic stress on the populace despite the availability of different control measures and treatments aimed at eradicating the disease. From vector control to combined therapies, none seems to provide a lasting effective solution to the eradication of the disease. There have been series of reports of treatment failure associated with the use of Artemisinin combined therapies which had proved to be effective in the treatment of resistant malaria in the past few years [1, 2] coupled to the already existing resistance to chloroquine, the cheapest and affordable antimalarial. Thus, the local poor populace for want of cheap remedy to their malarial infections still rely on the use of herbal products for the treatment of malaria. Different plants used from ancient time are still utilised traditionally in the fight against malaria. In Ibibio traditional medicine, a number of plants, some of which have been proven scientifically *in vivo* to possess antimalarial properties against rodent malaria models, are currently in use and sold on the street and markets to the general populace irrespective of status and education. Most people continue to patronise the herbal products even with their non standardisation and associated consequences though negligible.
However, most of these plants though proven scientifically using rodent malaria parasites models have not been tested on human malaria parasite, *Plasmodium falciparum*. This work was focussed on investigating the effect of the some plants used traditionally in Ibibio land of Niger Delta region of Nigeria on human malaria parasite both Chloroquine sensitive *Plasmodium falciparum* and chloroquine resistant *P. falciparum* strain to confirm their efficacy in the treatment of *Plasmodium falciparum* infections.

2. Materials and Methods
2.1 Selection of plants
The plants used in this study were selected based on documented ethnopharmacological information as antimalarial herbs used in Ibibio traditional medicine and reported *in vivo* antimalarial activity. All the plants selected satisfied the criteria mentioned above (Table 1), and are used by Ibibio tribe of Niger Delta region. The Ibibios are found in Akwa Ibom State in the Niger Delta region of Nigeria and has a population of about 2.2 million people. Akwa Ibom is a state in Nigeria.

It is located in the coastal southern part of the country, lying between latitudes 4°32′N and 5°33′N, and longitudes 7°25′E and 8°25′E. The state is bordered on the east by Cross River State, on the west by Rivers State and Abia State, and on the south by the Atlantic Ocean and the southernmost tip of Cross River State.

2.2 Drugs

Chloroquine diphosphate and artemisinin used in this study were from Sigma-Aldrich, Germany.

2.3 Microorganism (Parasite)

Plasmodium falciparum strains; Chloroquine sensitive, *Pf* 3D7 and chloroquine resistant, *Pf* INDO, were obtained from the International Center for Genetic Engineering and Biotechnology, New Delhi, India.

2.4 Collection of plant materials

Plants' parts from 14 plants used in this study were collected fresh between July and August, 2015 from farmlands and compounds in Uyo, Ikono and Uruan Local Government Areas of Akwa Ibom State, Nigeria. The plants were identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo. Nigeria. Herbarium Specimens were deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

2.5 Preparation of extract and fractions

The plant parts (leaves, stems, stem barks and roots) were washed and air- dried on laboratory table for 2 weeks. The dried materials were pulverized using a pestle and mortar. The powdered material of each plant was macerated in 50% ethanol for 72 hours. The liquid ethanol extract obtained by filtration was evaporated to dryness in a rotary evaporator 40˚C. The crude ethanol extracts (10 g each) of active plants were further partitioned successively into each of petroleum ether, dichloromethane, ethyl acetate and butanol to give the corresponding fractions of these solvents. The extract/fractions were stored in a refrigerator at 4˚C until used for experiment reported in this study.

2.6 Evaluation of *in vitro* antiplasmodial activity

2.6.1 *In vitro* cultivation of *Plasmodium falciparum*

In vitro blood stage cultures of *Plasmodium falciparum* strains, CQ-sensitive strain 3D7 and CQ-resistant strain INDO, were used in this study to investigate the antiplasmodial potentials of the crude extract and fractions. The cultures were maintained at the Malaria Research Laboratory, International Centre for Genetic Engineering and Biotechnology, New Delhi, India. *Plasmodium falciparum* cultures were maintained according to the method of Trager and Jensen [3] with slight modifications. *Plasmodium falciparum* (3D7) cultures were maintained in fresh O+ve human erythrocytes suspended at 4% hematocrit in RPMI 1640 (Sigma) containing 0.2% sodium bicarbonate, 0.5% albumax, 45 µg/L hypoxanthine, and 50 µg/L gentamicin and incubated at 37 ◦C under a gas mixture of 5% O_2, 5% CO_2, and 90% N_2. Daily, infected erythrocytes were inoculated into fresh complete medium to propagate the culture. In the case of *Plasmodium falciparum* (INDO strain) in culture medium, 10% pooled human serum was used in place of albumax.

2.6.2. Drug dilutions

Dimethyl sulfoxide (DMSO) was used to prepare the stock solutions of each plant extract and fraction as well as artemisinin, while water (Milli-Q grade) was used in the case of CQ stock solution. Culture medium was used to dilute the stock solutions to their required concentrations exception of CQ. The final solution of each stock was constituted to contained nontoxic concentration of DMSO (0.4%), which was found to be harmless to the parasite. Drugs, test plant extracts and fractions were then placed in 96-well flat bottom tissue culture grade plates.

2.6.3. *In vitro* antiplasmodial assays

Evaluation of crude extracts and fractions of these plants for antiplasmodial potential against 3D7 and INDO strains of *Plasmodium falciparum* was carried out using SYBR green I-based fluorescence assay as described previously [4]. Sorbitol synchronized ringed stage parasites were incubated under standard culture conditions at 2% hematocrit and 1% parasitemia with or without varying concentrations of plant extract and fractions. CQ and artemisinin were used as positive controls, while negative control was 0.4% DMSO. The cultures were incubated for 48 h after which 100 µl of SYBR Green I solution (0.2 µl of 10,000× SYBR Green I (Invitrogen)/mL) in lysis buffer*{*Tris (20 mM; pH 7.5), EDTA (5 mM), saponin (0.008%, w/v), and Triton X-100 (0.08%, v/v)*}* was added to each well and mixed gently using multi-channel pipette and incubated in dark at 37 ◦C for 1 h. Fluorescence was measured with a Victor fluorescence multi-well plate reader (Perkin Elmer) with excitation and emission wavelength bands cantered at 485 and 530 nm, respectively. The fluorescence counts were plotted against the drug concentration and the 50% inhibitory concentration (IC_{50}) was determined by analysis of dose–response curves and IC_{50} estimator. Results were validated microscopically by examination of Giemsa stained smears of extract treated parasite cultures.

2.7 Cytotoxic activity on HeLa cells using MTT assay

Evaluation of cytotoxic effects of extract and fractions on host cells were carried out by functional assay as described previously [5] using HeLa cells cultured in RPMI containing 10% fetal bovine serum, 0.21% sodium bicarbonate (Sigma) and 50 µg/mL gentamycin (complete medium). Briefly, cells (10^4 cells/200 µl/well) were seeded into 96- well flat-bottom tissue culture plates in complete medium. 24 h after seeding, drug solutions were added and the plates further incubated for 48 h in a humidified atmosphere at 37 ◦C and 5% CO2. 10% DMSO was subsequently added as positive inhibitor. Twenty microliters of a stock solution of MTT (5 mg/mL in 1× phosphate buffered saline) was added to each well, gently mixed and incubated for another 4 h. The plate was centrifuged at 1500 rpm for 5 min, supernatant was removed and 100 µl of DMSO (stop agent) was added. Microtiter plate reader (Versa max tunable multi-well plate reader) was used to read the formation of formazon at 570 nm. The 50% cytotoxic concentration (TC_{50}) of drug was determined by analysis of dose–response curves and IC_{50} estimator.

2.8 Gas chromatography-Mass spectrometry analysis

GC-MS analyses of the active fractions were carried out to generate qualitative data. The fraction was injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/ splitless injector. The column used was an DB-5 (Optima-5), 30 m, 0.25 mm i.d., 0.25 µm df, coated with 5 % diphenyl-95 % polydimethylsiloxane, operational oven temperature programme was: 50 ˚C, held for 1 min, rising at 3 ˚C/min to 250 ˚C, held for 5 min, rising at 2 ˚C/min to 280 °C, held for 3 min; injection temperature and

volume, 250 ˚C and 1.0 µl, respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8 KPa; detector temperature, 280 ˚C; hydrogen, flow rate, 50 ml/min; air flow rate, 400 ml/min; make-up (H_2/air), flow rate, 50 ml/min; sampling rate, 40 ms. Data were acquired by means of GC solution software (Shimadzu). Agilent 6890N GC was interfaced with a VG Analytical 70-250s double -focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250 ◦C. The GC was fitted with a 30 m x 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of GC analysis described above.

2.8.1 Identification of the compounds

The components of the active fractions of the plant extracts were identified based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley and Nist Libraries [6, 7].

2.9 Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using ANOVA (One-way) followed by a post test (Turkey-Kramer multiple comparison test). Differences between means was considered significant at 1% and 5% level of significance, that is $P \leq 0.01$and 0.05.

3. Results

3.1 *In vitro* antiplasmodial and cytotoxic activities

A total of 14 plants were screened for *in vitro* activity against chloroquine sensitive *Plasmodium falciparum* strains (Pf 3D7) and Chloroquine resistant *P. falciparum* strain (Pf INDO). Five (35.7%) out of the 14 plants had considerably moderate activity against Pf 3D7 with IC_{50} < 50 µg/ml. Their potency order were *S. monostachyus* > *H. africana*> *M. africana*> *B. nivosa* >*S. megaphylla* (Table 2). Other plants had IC_{50} >50 µg/ml with *H. letestui* and *P. maximum* having IC_{50} values of 56.33±3.02µg/ml and 57.85±3.78 µg/ml respectively. Only one (7.14%) plant (*H. africana*) with IC_{50} value of 32.90±1.25 µg/ml had IC_{50} value < 50 µg/ml against Pf INDO strain. Other plant extracts had IC_{50} values >50 µg/ml against Pf INDO (Table 2). Three plant extracts (*H. africana, S. monostachyus* and *S. megaphylla*) were selected based on prominent activity against either Pf 3D7 or Pf INDO, fractionated and screened against the 2 strains, Pf 3D7 and Pf INDO. Ethyl acetate fraction of *H. africana* exerted the highest activity than other fractions from the same plant with IC50 values of 25.95±1.82 µg/ml and 15.94±2.10 µg/ml against Pf3D7 and Pf INDO respectively (Table 3). The potency order was ethyl acetate> chloroform>n-butanol. Petroleum ether fraction of *S. monostachyus* was observed to exert a better activity than other fractions with IC_{50} values of 25.99±2.28 µg/ml and 12.30±1.25 µg/ml against Pf 3D7 and Pf INDO respectively. Next in potency was ethyl acetate fraction followed by chloroform and n-butanol fractions. Ethyl acetate fraction of *S. megaphylla* exhibited the highest activity against both Pf 3D7 and Pf INDO. The potency order was ethyl acetate>chloroform>petroleum ether>n-butanol (Table 3). Seven plant extracts were cytotoxic against HeLa cells with TC_{50} values <50 µg/ml. They include; *A. comosus, S. monostachyus, T. occidentalis, B. nivosa, S. anomalum, E. indica* and *S. megaphylla* (Table 2). Other extracts had TC_{50} values>100 µg/ml.

3.2 GCMS analysis

The GCMS analyses of the ethyl acetate fractions of *A. laxiflora* leaf, root extract of *H. africana* and petroleum ether fraction of *S. monostachyus* leaf revealed the presence of bioactive compounds with major and minor ones as presented in Tables 4, 5 and 6.

4. Discussion

Malaria continues to be one of the greatest health challenges worldwide threatening about 3.2 billion people, about half of the world's population [8]. According to the World Health Organisation's (WHO) world malaria report of 2015, 78% of people who die from malaria are children under 5, mostly in sub-Sahara Africa. About 214 million cases**,** and 438 000 deaths from malaria were recorded worldwide in 2015 [8]. Even though reduced mortality rate was recorded, Democratic Republic of Congo and Nigeria accounted for 35% of the global total malaria deaths in 2015 [8]. This implies that Nigeria among other sub Saharan African countries still bear a serious scourge from malaria necessitating serious effort into researches aim at discovering readily cheap and available antimalarials probably from natural products and medicinal plants.

This present study was aim at investigating antimalarial plants used by the Ibibios of Niger Delta region of Nigeria for activity against *Plasmodium falciparum,* the major causative agent for malaria in this endemic region.

A number of medicinal plants are used traditionally as malaria remedies and/or febrifuge by Ibibio tribe, but only a few of them have been screened scientifically to ascertain their activities and these were done using rodent malaria model only (Table 1). This investigation is the first report of activity of these plants understudy against human malaria parasite (*P. falciparum*) exception of *Setaria megaphylla*.

The 14 plants investigated had varying degree of activity with 3 plant extracts and their fractions exhibiting prominent activities. They are *Hippocratea africana* root, *Setaria megaphylla* leaf and aerial parts of *Solenostemon monostachyus*

Setaria megaphylla (Steud) Dur & Schinz (Poaceae) also called broad leafed brittle grass is a tall, robust, tufted, perennial grass which occurs in tropical and subtropical areas of Africa, America and India where there is high rainfall [9, 10]. The plant is used mainly as pasture grass and traditionally by the Ibibios in Akwa Ibom State, Nigeria in the treatment of various ailments such as malaria, inflammation and diabetes. Antiplasmodial activity; *in vitro* [11] and *in vivo* [12], hypoglycaemic and antidiabetic activities [13, 14], antiiflammatory and antinociceptive [15] activities have been reported on the leaf extract. Antileishmanial, anticancer and immunomodulatory activities have also been reported on the leaf extract whose fractions were found to contain various polyunsaturated fatty acids, mono and sesquiterpenes [16], which have variously been implicated in antiplasmodial activities of plants. The present study showed that the plant extract and ethyl acetate fraction exhibited promising antiplasmodial activity (IC_{50} - 8.15±1.10 µg/ml (3D7) and 8.05 ± 0.12 µg/ml (INDO). Clarkson *et al*., [11] had reported IC_{50} of 4.5 µg/ml from MeoH:DCM whole plant extract against D10 strain. Our results in this study corroborate their findings and further confirm the antiplasmodial potential of the plant. *Setaria megaphylla* (100–300 mg/ kg/day) was found to exhibit significant ($p < 0.05$) blood schizonticidal activity in 4-day early infection and in established infection with a significant ($p < 0.05$) mean survival time comparable to that of the standard drug, chloroquine, 5 mg/kg/day

according to report by Okokon *et al*., [12]. GCMS analysis of ethyl acetate fraction revealed the presence of (E)-β-ocimene, P-metha-1(7),8-diene, D:A-friedooleannan-3-ol,(3a)-,Stigmastone-3,6-dione,(5a), Bicyclo[2.2.1]heptan-2-ol,4,7,7-trimethyl, P-cymene. Some of these compounds have reports of antimalarial activity [16]. Earlier study by Okokon *et al*., [17] had shown the presence of ᵞ-Elemene, Urs-12-ene, Bicyclogermacrene, α-muurolene, Germacrene- A, Guaiol in the extract which are mono and sesquiterpene compounds implicated in antiplasmodial activity of many plants [18].

Hippocratea africana (Willd.) Loes. ex Engl. (Celastraceae) syn. *Loeseneriella africana* (Willd.) N.Hallé is a green forest perennial climber widely distributed in tropical Africa [19]. It is commonly known as African paddle-pod and 'Eba enang enang' by the Ibibios of Nigeria. The Ibibios of the Niger Delta region of Nigeria used the root of the plant traditionally in the treatment of various ailments such as fever, convulsion, malaria, body pains, diabetes and diarrhea [20]. Decoction of the plant's root is also use as an antidote or antipoison to treat liver diseases such as jaundice and hepatitis [21-23]. The plant (root) has been reported by Okokon *et al*., [20] to possess *in vivo* antiplasmodial activity, tested at oral doses of 200–600 mg/kg/day, demonstrated promising blood schizontocidal activity both in early and established infections. Other biological activities include; antiinflammatory and analgesic [24], antidiarrheal, antiulcer [25], antidiabetic, and hypolipidemic activities [26], hepatoprotective activity [27], cytotoxicity against HeLa cells, cellular antioxidant and antileishmanial activities [28]. In the present study, the ethyl acetate was found to exhibit promising antimalarial activity with IC_{50} values of 25.95±1.82µg/ml and 15.94±2.10µg/ml against 3D7 and INDO strains respectively. The phytochemical components of this fraction was revealed by GCMS to include; Spirohexane-1-carboxylic acid, ethyl ester, 3-methoxy-2-methylphenol, 2,3-Benzofurandione,6-hydroxy-4-(p-hydroxybenzyl), ᵟ-3-Carene and α-terpineol. Okokon *et al*., [27] had reported the presence of monoterpenes (thujene, limonene, linalool, α-phellandrene, α-terpineol and sabinene) and sesquiterpenes (Dehydromevalonic lactone), in the n-hexane fraction of the root extract. These compounds which are potent antimalarial molecules are likely to be responsible for the antiplasmodial activity observed in this study.

Solenostemon monostachyus P. Beauv (family Lamiaceae) is an annual weed in anthropogenic habitats and rocky savannahs that is widespread in West and Central Africa. It is slightly succulent, aromatic and grows up to 100 cm tall [29]. The aerial parts of the plant are used in various decoctions traditionally by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, fever/malaria [23, 30], hemorrhoid and other inflammatory diseases. The plant is also used as an hypertensive as well as a diuretic [31]. *Solenostemon monostachyus* leaves contain phytoconstituents such as diterpenoids [32], flavonoids, coumarin, polyphenol [33, 34]. The leaf essential oil of *S. monostachyus* contain; β-pinene, oct-1-en-3-ol, β-caryophyllene, octan-3-ol and (*E*,*E*)-α-farnesene [35]. Biological activities of the plant include; antioxidant [33, 34, 36], antihypertensive [37] and antimicrobial [38]. We had reported the *in vivo* antimalarial activities of the leaf extract and fractions (chloroform and aqueous) in mouse model, which showed prominent activity against *P. berghei* infections in suppressive, repository and established infection [39]. In this study, the leaf extract was found to exhibit moderate activity (IC_{50}-30.07±2.84µg/ml against 3D7 and 51.76±1.02µg/ml against INDO), while the petroleum ether fraction exerted prominent activity during the *in vitro* study (IC_{50}-25.99±2.28µg/ml against 3D7 and 12.30±1.25µg/ml against INDO). GCMS analysis revealed the presence of phenolics, flavonoids, terpenoids and polyunsaturated fatty acids (Table 6), which have been variously implicated in the antiplasmodial activity of plants. These compounds maybe responsible for the observed *in vitro* antiplasmodial activity of this plant.

Of interest are the vegetables; *T. occidentalis* (Hook F.) Vahl (Family Cucurbitaceae), *Heinsia crinata* (Afzel.) G. Taylor (Rubiaceae) and *Lasianthera africana*. P.Beav (Stemonuraceae), which are basic vegetables employed in the preparation of soups by the Ibibios. Apart from their nutritional values, these vegetables are also employed in Ibibio traditional medicine in the treatment of a number of diseases such as malaria, diabetes, inflammation, pains, ulcer among others. Reports of their ethnomedical uses as malarial remedies are published in literatures [40-43]. *in vivo* antimalarial activities of these plants have been published previously as they had shown significant activity against *P. berghei* infections in mice [40-43]. Although, their activities were prominent in the *in vivo* studies, their *in vitro* activities were weak against 3D7 and INDO. This finding supports earlier report by Okokon *et al*., [42], that these vegetables may as well be immunostimulatory in activity against malarial infections and probably may be acting to alleviate the symptoms of malaria. The analgesic and antipyretic activities of these vegetables have earlier been reported [44-46], giving credence to their ability to alleviate symptoms of malaria. The immunostimulatory activities of *L. africana* and *T. occidentalis* have also been reported [47, 48], confirming immunostimulation to be one mode of action of these plants.

Croton zambesicus Muell. Arg. (Euphorbiaceae) (syn *Croton amabilis* Muell. Arg. *Croton gratissimus* Burch), an ornamental tree, *Mammea africana* Sabine (Guttiferae) (syn. *Ochrocarpus africana* Oliv.), a large forest tree, *Homalium letestui* Pellegr (Flacourtiaceae), forest tree, and *Breynia nivosa* (W. Bull) Small (syn. *Phyllanthus nivosus* W. G. Sm., *Breynia disticha* J.R. Forst. & G.Forst.) (Euphorbiaceae), a rounded ornamental shrub, are extensively used in Ibibio traditional medicine in the treatment of malaria and reports of their *in vivo* antimalarial activities have been published [49-53]. These plants have been found in this study to possess moderate activities against the two strains (Pf 3D7 and INDO) of *P. falciparum* tested. Considering their *in vivo* activities and their reputation in Ibibio traditional medicine in the treatment of malaria, their *in vitro* activities seem to be affected by the crude nature of the various extracts. However, the activities of these plants suggest the involvement of immune system as these plants may also act by stimulating the immune system. Reports of immunostimulatory activities of *H. letestui*, *M. africana*, and *C. zambesicus* have been published [54-56], supporting their ability to stimulate the immune system. This is the first time these plants are tested against *P. falciparum.*

Panicum maximum. Jacq (poacace), a perennial, tuft grass and *Eleuscine indica* (L.) Gaertn.(Poaceae), an annual or short-lived perenial tufted grass, are two grasses extensively used in Ibibio traditional medicine in treatment of various ailments including malaria. We have published the antimalarial activity of both plants [57-59] showing their significant *in vivo* activity against *P. berghei* infections. These plants showed moderate activities against 3D7 and INDO strains *of P. falciparum* in this study probably due to their crude nature. However, these plants are known strong immunostimulators [60, 61], which suggests that their *in vivo* antimalarial activities may be potentiated by their immunostimulatory effect. Moreover, the plants have been reported to posses antipyretic and analgesic

properties [59, 62] which is an added advantage to their antimalarial actions. Thus, these results confirmed the utilization of these plants as malarial remedies in Ibibio traditional medicine.

Solanum anomalum Thonn. ex Schumach.(*Solanaceae*) and *Ananas comosus* (L.) Merr. (Bromeliaceae) are two tropical plants with edible fruits whose leaves and fruits are used in Ibibio traditional medicine to treat various ailments including malaria. However, their activities in this study were very weak as their IC_{50} values were >100µg/ml. Report of *in vivo* antimalarial activities of these plants against *P. berghei* infection in mice corroborates this weak antimalarial activity [63, 64]. It further suggests that these plants may either be immune stimulants or they may act to alleviate the symptoms of malaria such as pains and fever among others. As it has been documented that some plants claimed to be malarial remedies traditionally may only be active in alleviating the symptoms associated with malaria without any significant effect on the parasites as is the case in this study [65].

Moreso, plant compounds that merely slow down or temporarily arrest the growth of the parasite (plasmodistatic) as well as those that act as an immune stimulant or help to alleviate symptoms and reverse some pathological results of malaria infection are reported to potentiate malaria resistance and antiplasmodial activity in immune individuals living in endemic areas [65]. So these plants maybe contributing to boast immune system to develop resistance to malaria and in a way are malaria remedies.

Table 1: Ethnopharmacological and *in vivo* antimalarial activity information

Plant no.	Plants' name	Parts	Local name	English name	Antimalarial activity Information
1	*Setaria megaphylla* (Steud) Dur & Schinz	leaf	Nkwongo	broad leafed brittle grass	11,12
2	*Croton zambesicus* Muell. Arg.	Leaf, root	Eto aduma	Thunder plant	49,52
3	*Homalium letestui* Pellegr	stem	Otong idim	African homalium	50
4	*Lasianthera africana* P. Beav	leaf	Editan	-	40
5	*Mammea africana* Sabine	stembark	Edeng	African mammy apple	51
6	*Panicum maximum* Jacq	Leaf	Nyayaha	Elephant grass	51
7	*Telfairia occidentalis* (Hook F.) Vahl	Leaf	Ikong ubong	Fluted pumkin	43
8	*Solanum anomalum* Thonn. ex Schumach	leaf	Mkpa erong	Children's tomato	64
9	*Eleusine indica* (L.) Gaertn	leaf	Nkim enang	Stubborn grass	54,66
10	*Hippocratea africana* (Willd.) Loes. ex Engl.	root	Mba enang enang	African paddle-pod	20
11	*Heinsia crinata* (Afzel.) G. Taylor	leaf	Atama	Bush apple	42
12	*Solenostemon monostachyus* P. Beauv	Aerial parts	Ntoro ikwot	-	39
13	*Ananas comosus* (L.) Merr.	leaf	pineapple	Pineapple	63
14	*Breynia nivosa* (W. Bull) Small	leaf	Uyai ikong	Snow bush	53

Table 2: Cytotoxicity and antiplasmodial screening of crude extracts on *Pf* 3D7 and *Pf* INDO

Sample no.	Plants' name	IC_{50}, *Pf3D7, (µg/ml)*	IC_{50},*Pf INDO (µg/ml)*	Cytotoxicity TC_{50}(µg/ml) HeLa cells
1	*S. megaphylla*	49.50±1.24	79.96±3.29	45
2	*C. zambesicus*	73.48±2.04	58.67±2.35	>100
3	*H. letestui*	56.33±3.02	57.43±2.47	>100
4	*L. africana*	84.08±2.13	>100	>100
5	*M. africana*	45.77±3.12	58.87±2.47	>100
6	*P. maximum*	57.85±3.78	67.83±2.87	>100
7	*T. occidentalis*	91.70±4.18	>100	28.06
8	*S. anomalum*	>100	>100	35.35
9	*E. indica*	85.60±43.23	>100	15.23
10	*H. africana*	39.86±2.44	32.90±1.25	>100
11	*H. crinata*	74.29±1.28	>100	>100
12	*S. monostachyus*	30.07±2.84	51.76±1.02	32.98
13	*A. comosus*	>100	>100	21.76
14	*B. nivosa*	45.85±3.46	87.05±2.66	26.48
15.	Chloroquine	0.021	0.258	>200
16.	Artemisinin	0.0045	0.0045	>200

Table 3: Antiplasmodial activity of fractions of some medicinal plants on *pf3D7* and *pf INDO*

S/ no.	Plants' name	Fraction	IC50, *Pf3D7 (µg/ml) assay*	IC50, *PfINDO (µg/ml) assay*	CYTOTOXIITY TC50 ON HeLa cells
1	*Hippocratea africana* root	Pet. ether	>100	40.17±1.46	>100
		Chloroform	37.99±2.10	25.44±1.22	>100
		Ethyl acetate	25.95±1.82	15.94±2.10	44.75
		n-butanol	74.32±3.19	53.33±2.45	>100
		Aqueous	>100	73.48±1.67	>100
2.	*Solenostemon monostachyus* leaf	Pet. ether	25.99±2.28	12.30±1.25	NT
		Chloroform	40.39±3.19	21.54±2.19	NT
		Ethyl acetate	27.99±1.56	28.23±1.08	NT

		n-butanol	72.97±2.88	63.55±1.23	NT
		Aqueous	85.72±2.19	73.29±1.212	NT
3.	*Setaria megaphylla Leaf*	Pet. ether	10.34±1.16	10.20±0.84	NT
		Chloroform	11.43±2.18	8.05±0.12	NT
		Ethyl acetate	8.15±1.10	8.94±1.26	NT
		n-butanol	46.75±0.89	56.71±1.27	NT
		Aqueous	>100	>100	NT

Table 4: GC –MS analysis of ethyl acetate fraction of *Setaria megaphylla*

S/No.	NAME OF COMPOUND	RI	MOL.WT	CHEMICAL FORMULA
1.	2-propenoic acid, 3-(2 hydroxy-phenyl,(E)-	101	164	$C_9H_8O_3$
2.	Benzaldehyde, 3-methyl	200	120	C_8H_8O
3.	Phenol,2,6-dimethoxy	280	154	$C_8H_{10}O_3$
4.	Desulphosinigrin	528	279	$C_{10}H_{17}N0_6S$
5.	D-mannose	367	180	$C_6H_{12}O_6$
6.	(E)-β-ocimene	810	136	$C_{10}H_{16}$
7.	P-metha-1(7),8-diene	999	136	$C_{10}H_{16}$
8.	D:A-friedooleannan-3-ol,(3a)-	1011	428	$C_{10}H_{16}$
9.	Stigmastone-3,6-dione,(5a)-	1012	428	$C_{29}H_{48}O_2$
10.	Bicyclo[2.2.1]heptan-2-ol,4,7,7-trimethyl	282	154	$C_{10}H_{18}O$
11.	P-cymene	1012	134	C_10H_{14}

Table 5: GC –MS analysis of ethyl acetate fraction of *Hippocratea africana*

S/No.	NAME OF COMPOUND	RI	MOL.WT	CHEMICAL FORMULA
1.	Spirohexane-1-carboxylic acid, ethyl ester	365	154	$C_9H_{14}O2$
2.	3-methoxy-2-methylphenol	238	138	$C_8H_{10}O2$
3.	2,3-Benzofurandione,6-hydroxy-4-(p-hydroxybenzyl)	901	270	$C_{15}H_{10}O_5$
4.	$^{\delta}$-3-Carene	1008	136	$C_{10}H_{16}$
5.	α-terpineol	1186	154	$C_{10}H_{18}O$

Table 6: GC –MS analysis of petroleum ether fraction of *Solenostemon monostachyus*

S/No.	NAME OF COMPOUND	RT	MOL.WT	CHEMICAL FORMULA
1.	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	8.750	180	$C_{11}H_{16}O_2$
2.	Ethyl 4-hydroxybenzoate	19.213	166	$C_9H_{10}O_3$
3.	1,2-benzenedicarboxylic acid, diethyl ester	20.103	222	$C_{12}H_{14}O_4$
4.	9-(3,3-dimethyl-2-oxiranyl)-2,7-dimethyl-2,6-nonadien-1-o	21.113	238	$C_{15}H_{26}O_2$
5.	9,12,15-octadecatrien-1-ol	21.757	264	$C_{18}H_{32}O$
6.	2,6,10-trimethyl,14-ethylene-14- pentadecne	25.283	278	$C_{20}H_{38}$
7.	2-pentadecanone, 6,10,14-trimethyl-	25.440	268	$C_{18}H_{36}O$
8.	p-Hydroxycinnamic acid, ethyl ester	25.817	192	$C_{11}H_{12}O_3$
9.	Hexadecanoic acid, methyl ester	27.140	270	$C_{17}H_{34}O_2$
10.	Hexadecanoic acid	28.713	256	$C_{16}H_{32}O_2$
11.	Hexadecanoic acid, ethyl ester	28.863	284	$C_{18}H_{36}O_2$
12.	(Z,Z)-6,9-cis-3,4-epoxy-nonadecadiene	32.127	278	$C_{19}H_{34}O$
13.	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]-	32.560	296	$C_{20}H_{40}O$
14.	Ethyl (9Z,12Z)-9,12-octadecadienoate	33.543	308	$C_{20}H_{36}O_2$
15.	Ethyl (9Z,12Z)-9,12-octadecadienoate	33.723	308	$C_{20}H_{36}O_2$
16.	(Z,Z)-6,9-cis-3,4-epoxy-nonadecadiene	33.863	278	$C_{19}H_{34}O$
17.	Octadecanoic acid, ethyl ester	34.163	312	$C_{20}H_{40}O_2$
18.	4,8,12,16-Tetramethyl heptadecan-4-olide	36.667	324	$C_{21}H_{40}O_2$
19.	1,1,4,7-tetramethyldecahydro-1H-cyclopropa[e]azulen-	37.160	222	$C_{15}H_{26}O$
20.	2-methyloctacosane	38.193	408	$C_{29}H_{60}$
21.	Bis(2-ethylhexyl) phthalate	38.783	390	$C_{24}H_{38}O_4$
22.	Heptadecanoic acid, ethyl ester	39.160	298	$C_{19}H_{38}O_2$
23.	Tetracontane	40.170	562	$C_{40}H_{82}$
24.	Docosanoic acid, ethyl ester	41.280	368	$C_{24}H_{48}O_2$
25.	Squalene	41.727	410	$C_{30}H_{50}$
26.	Tetracontane	42.600	562	$C_{40}H_{82}$
27.	Stigmast-5-en-3-ol, (3.beta.)-	46.590	414	$C_{29}H_{50}O$
28.	Octadecanal	49.727	268	$C_{18}H_{36}O$
29.	-)-Beta-sitosterol	52.677	414	$C_{29}H_{50}O$
30.	Olean-12-en-3-one	57.420	424	$C_{30}H_{48}O$

Conclusion

The findings of this study shows that most of the plants used as malaria remedies by the Ibibios actually possess antimalarial activity as could be seen in their pronounced effects on the parasites strains studied. Some of the plants were very active against chloroquine resistant strain and could be useful in the treatment of chloroquine resistant malaria. Some plants were found to possess weak activity against the

parasites suggesting the involvement of the immune system in their activity and may also work to alleviate the symptoms of the disease.

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