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Phytochemical investigation, *in vitro* antimalarial and cytotoxicity assessment of ethanol leaf extract of *Sabicea gigantistipula*

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Abstract

Phytochemical, *in vitro* antimalarial and cytotoxicity assessment of ethanol leaf extract of *Sabicea gigantistipula* (Schum) was investigated on the basis of the usage of the leaves of the plant in traditional medicine by Idoma tribe in Otukpo Benue State and other parts of Northern Nigeria to treat malaria disease. Crude ethanol leaf extract obtained from the plant was fractionated and screened for their antiplasmodial activity (*in vitro)*, existence of bioactive secondary metabolites and for activities in the *Artemia salina* Leah (Brine Shrimp) lethality assay for cytotoxicity profile. In the phytoconstituents screening, ethanol leaf extract shows the presence of saponins, tannins, flavonoids, alkaloids and steroids. At the maximum test dose of 5000 µg/ml, ethanol extract and its fractions revealed antiplasmodial suppression activity in the range of 85.25% - 91.80% while at the minimum dose of 500 µg/ml, the extract and fractions exhibited malaria parasites percentage elimination activity in the range of 64.75 % - 77.00% showing that the extract and fractions under investigation were less active when compared with the percentage parasitic inhibition of the standard drug at highest concentration of 5000 µg/ml (93.44%) and at the least dosage of 500 µg/ml (81.97%). Interestingly, extract and its fractions were non-cytotoxic as they all exhibited LC_{50} values > 1000 µg/ml since an extract or its fractions is considered non-cytotoxic if its LC_{50} value is greater than 1000 µg/ml. The findings of this research supports the continuous use of the extract from the leaves of *Sabicea gigantistipula* to cure malaria infection (as an antimalarial herb) as it has wide range of safety at relatively low/moderate dose and not at unregulated high dosage. Also, studies on the *in vivo* antiplasmodial and cytotoxicity of the extract/compounds from the leaf of the crude extract are worthy of further investigations.

Keywords: Phytochemicals; Cytotoxicity; Antiplasmodial; *Sabicea gigantistipula*

1. Introduction

Malaria remains one of the major pandemics and is a main public health problem especially in Africa [1]. Malaria is found in tropical and sub-tropical regions of the southern hemisphere. More than two billion people are at risk of contracting this disease worldwide. A microscopic parasite called *Plasmodium falciparum* is responsible for the most dangerous form of malaria. This parasite is transmitted by the female mosquito species belonging to the genus Anopheles [2]. Currently, despite the existing therapeutic arsenal, few drugs are available in the market and are not always accessible to the affected population. Additionally, the increased parasite's resistance to current treatments reinforces the urgent need to search for new antimalarial drugs. Due to the high cost of the prescribed drugs and the various activities displayed by medicinal plants against many diseases, 80% of the world population relies on medicinal plants for their basic and first healthcare in treating ill health. There are current antimalarial drugs which have been derived from medicinal plants and traditionally used in their countries of origin against fevers and malaria. This includes the bark of a tree native to the slopes of the Andean Cordillera (*Cinchona calisaya* and other species of Cinchona) and a

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native Chinese herb *Artemisia annua* [3]. These findings encourage the search for new antimalarial drugs in plant biodiversity. In Nigeria, a good number of plant species have been identified as antimalarial medicinal plants. Pure natural products compounds have been isolated from some of these plants and their antimalarial activities were comparable to or more active than chloroquine on sensitive and resistant strains of *Plasmodium falciparum* [4]. *Plasmodium falciparum* being one of the causative agents of malaria has high adaptability through mutation and is resistant to many types of anti-malarial drugs. This resistance is a serious setback to antimalarial programs since it precludes the use of cheap and previously effective drugs such as chloroquine. New classes of active compounds are needed, especially from natural sources so as to minimize the risk of resistance. An alternative solution in many endemic countries is the use of traditional medicinal plants since many of the available antimalarial drugs are from plants sources and the potential of plants to produce new antiplasmodial drugs are considerable. It is in line with this that researches have been intensified so as to find out more medicinal plants, the exact bioactive components present in them and their possible functions as therapeutics in traditional medicine. *Sabicea gigantistipula* (Schum) leaves have been known to be traditionally used for the treatment of malaria infection in Otukpo, Benue State and in other parts of Northern Nigeria. The ethnotherapeutical assertion of this plant species could possibly be harnessed if more investigations regarding its biological activity are investigated.

2. Materials and methods

2.1. Chemical Reagents, Chemicals and Apparatuses/Equipments

Analytically graded solvents, chemicals and chemical reagents used in this research work include; Ethanol (Aldrich), Petroleum ether (Aldrich), n-hexane (Aldrich), Chloroform (Aldrich), Ethyl acetate (Aldrich), Methanol (BDH), Potassium iodide, Silica gel powder, DMSO, Sodium hydroxide, Lead Ethanoate, Aqueous Ammonia , Ferric chloride, gentamycin injection, Gemsta stain, candle wax, Hydrochloric acid, Basic Bismuth Nitrate, Glacial acetic acid, Potassium hydroxide and antimalarial drug (Lumefantrine and Arthemether) was procured from a reputable pharmaceutical and drug store in Kano, Nigeria which was used as a positive control, standard or reference drug.

Apparatuses used include round bottom flasks, separation funnel, conical flasks, beakers, test tubes, measuring cylinder, weighing balance, chromatographic column, spatula, media, glass slides, cover slides , anaerobic bell jar glass, microscope EDTA bottles, insulin syringes, pasteur pipette, autoclave, centrifuging machine and labeling sticker.

2.2. Collection and Authentication of Plant Materials

Matured fresh leaves of *Sabicea gigantistipula* (Schum) were collected from Utukpo in Benue State by Idris Aliyu on 10th of June, 2019. The leaves of the plant were authenticated with voucher No. 0280 at the herbarium of the Federal University of Agriculture, Makurdi, Benue state, Nigeria. The voucher specimen was dumped in the herbarium for future reference. The mature and fresh leaves collected were air dried at room temperature for three (3) weeks and ground to powder form and subjected to further analysis.

2.3. Preparation, Extraction of Plant Materials and Fractionation Procedure

The matured fresh leaves of *Sabicea gigantistipula* were collected and air/shade dried at room temperature for three weeks and the dried leaves were ground into fine powder using pestle and mortar. Two hundred grams (200 g) of pulverized form of leaves sample was percolated in 1.2 litres of ethanol (96%) in a sample bottle repeatedly at room temperature for (2 weeks). The ethanol liquid crude extract was then decanted into a flat bottom flask and further filtered using No. 1 Whatman filter paper in order to remove the suspended and fine residue present in the liquid extract. The ethanol liquid extract was concentrated using rotatory evaporator (R110) at 40°C and later evaporated to dryness by exposure at room temperature so as to obtain dried crude ethanol extract. The ethanol crude liquid extract was allowed to dry properly, weighed and labeled as SAG-1. The ethanol crude extract (SAG-1) was fractionated with n-hexane, chloroform, ethyl acetate and methanol according to the standard method [5]. The order of fractionation was as follows: n-hexane < Chloroform < Ethyl acetate < Methanol. Each of the dried fractions of ethanol crude extract (n-hexane, chloroform, ethylacetate and methanol fraction) was coded as SAG-1-01, SAG-1-02, SAG-1-03 and SAG-1-04 respectively, weighed and stored until investigated for their phytochemicals, *in vitro* antiplasmodial and *in vitro* cytotoxicty activities.

2.4. Phytochemical Screening

2.4.1. Preparation of Test Solutions/Samples for Phytochemical Screening

The test solutions/samples were subjected to phytochemical screening for the presence or absence of secondary metabolites using standard procedures [6][7][8]. The secondary metabolites investigated include alkaloids, saponins, tannins, flavonoids, steroids and terpeniods.

2.5. Brine Shrimp Lethality Test of Ethanol Crude Extract and Its Fractions

Eggs of Brine shrimp (*Artemia salina*-Leach) were hatched in a hatching chamber which contained salt water obtained from the ocean or sea. The chamber used for hatching was positioned under a natural light for forty eight (48) hours to enable the eggs to rapidly hatch into the larvae.

2.5.1. Preparation of Test Solutions/Samples for Brine Shrimp Lethality Test

Each of the dried crude leaf extract and its fractions (20 mg) was dissolved separately in 2 ml of methanol which constituted the stock solutions. Also, 20 mg of a positive control, potassium heptaoxodichromate (VI) was dissolved in 2 ml of distilled water. 500 µl, 50 µl and 5 µl were measured using micropipette from the stock solution of each test extract/fraction including the positive control which are equivalent to 1000, 100 and 10 µg/ml and were transferred into the vials respectively. A negative control contains sea/ocean salty water without any of the test samples. Each of the doses was tested in triplicates. In specific terms, each of the test solutions were measured out from stock solutions of the crude extract and each of its fractions were put in 9 vials (9 vials per test solution) and 9 vials for positive control were air dried or evaporated to dryness under a shade in about 48-hours (forty eight hours) around 25°C. Subsequently, the dried test samples were tested for their activity against Brine Shrimp Larvae: *Artemia salina*. Also, 500 µl, 50 µl and 5 µl of negative control (salty sea water) were measured by using micropipette and transferred into 9 vials (3 vials for each dose in triplicates) [9].

2.5.2. Procedure for Brine Shrimp Lethality Test

To each dried test sample in a vial, 1-2 drops of Dimethyl sulphur (IV) oxide (DMSO) solvent was introduced in order to rapidly dissolve each of the test samples in the vials before transferring 10 (Ten) *Artemia salina larvae* and salty sea water. A pasteur pipette was used to transfer ten larvae of the brine shrimps into each of the vials containing the test solution/sample, after which natural salty sea water was added into the vials until the volume of salt water rose to 5 ml in each of the vials. Immediately after twenty four hours later, the surviving brine shrimp larvae were counted at each dosage and recorded. *Artemia salina* Leach larvae were considered or regarded as dead if they were lying immobile at the base of the vials. The total number of mortality and then percentage of mortality for each dose in triplicate for all test samples including positive and negative control were determined. Finally, LC_{50} values (µg/ml) at 95% confidence interval for each concentration for all test samples plus positive and negative control were calculated by employing a computer software application [10].

$$\% \text{ Mortality} = \frac{\text{Total No. of Dead Artemia salina Larvae per Dosage}}{\text{Total Initial No. of Live Artemia salina Larva per Dosage}} \times 100$$

2.6. Malaria Parasite Bioassay of the Extract and Fractions

2.6.1. Preparation of Test Solutions

A stock solution of 10,000 µg/ ml was prepared by dissolving 20 mg in 2 ml of dimethyl sulphoxide (DMSO). Solution of 500 µg/ml, 1000 µg/ ml, 2000 µg/ml, and 5000 µg/ml were prepared from the stock solution by serial dilution.

2.6.2. Source of Malaria Parasite for Assay

Infected human blood samples containing parasitaemia of *Plasmodium falciparum* were collected from Bayero University Clinic, Murtala Mohammed Specialist Hospital, Kano and Aminu Kano Teaching Hospital, Kano in K3-EDTA coated disposable plastic sample bottles tightly fitted with plastic corks [2]. Venous blood from patients recommended for malaria parasites test (MP Test) using 5 cm^3 disposable plastic syringes and needles (BD and 20 SWG) was collected and the samples were immediately transferred into K3-EDTA disposable plastic sample bottles with tightly fitted plastic corks and mixed thoroughly and then transported to the Microbiology laboratory at Bayero University in a thermo flask containing water maintained at 40°C [11]..

2.6.3. 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 2 mm from one end. A cover slip was placed at angle 45°C in front of each drop and drawn backward to make contact with each drop. The drop was run along the full length of the edge of the cover slip. Smears were formed by moving the cover slip forward on glass slide. The thin smears were immersed in 30 ml of methanol contained in a Petri dish for 15 minutes. Geimsa's stain was dropped on each smear and allowed for about 10 minutes [12].. Excess stain was washed with clean tap water. The smears were air dried by hanging the glass slides inverted in a rack. Each dried smear was microscopically observed under a high power objective (x 100) using oil immersion after which an average parasitaemia was determined using the reading of 3 microscopic fields [12].

2.6.4. Separation of the Erythrocytes (5% parasitaemia) from the Serum of the

Blood Samples

A 50% Dextrose solution (0.5 ml) was added to each of the 5 m1 defibrinated blood samples and then centrifuged at 2500 rpm for 15 minutes in a spectral merlin centrifugation machine. Supernatant layers were separated from the sediments. The later was diluted with 2-3 drops of normal saline solution [11] and further centrifuged at 2500 rpm for 10 minutes. The resulting supernatants were discarded. Samples with higher parasitaemia (above 5%) were diluted with fresh malaria parasite negative erythrocytes [12].

2.6.5. Preparation of Plasmodium falciparum Culture Medium

Venous blood (2 ml) from the main vein of white healthy rabbit's pinnae was withdrawn using a disposable 5 ml syringe (BD 205 WG). This was defibrinated by allowing it to settle for at least one hour [11]. The defibrinated blood was centrifuged at 1500 rpm using spectre merlin centrifuge for 10 minutes and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500 rpm for five minutes, and the supernatant layer was added to the first test tube. The sediments were discarded and the serum collected was supplemented with the salt of RPMI 1600 medium (KCl 5.37 mM, NaCl 10.27 mM, $MgSO_4$ 0.4 mM, $NaHPO_4$ 17.73 mM, Ca $(NO_3)_2$ 0.42 mM, $NaHCO_3$2.5 mM, and glucose 11.0 mM.(BDH Ltd, UK) [13]. The medium was sterilized by 40 µg/ml gentamicin sulphate [14].

2.6.6. In Vitro Bioassay of the Activity of Ethanol Extract and its Fractions on Plasmodium falciparum Culture

A 0.1 ml of test solution and 0.2 ml of the culture medium were added into a tube containing 0.1 ml of 5% parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to the test fraction/samples was determined microscopically after incubation for 24, 48 and 72 hours at 32°C. The incubation was undertaken in a glass bell jar containing lighted candle to ensure the supply of required quantity of CO_2, about 5% O_2 gas, 2% and 93% of nitrogen gas [15].

2.6.7. Determination of the Activity

At the end of the incubation period of 72 hours (3 days), a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and stained by Giemsa's staining techniques. The mean number of erythrocytes appearing as blue discoid cells containing life rings of the parasite (that appeared red pink) was estimated and the percentage elimination by the samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasites after incubation period of 72 hours, using the formula below:

% = N/Nx x 100

Where,

%=Percentage Activity of the Extracts/Fractions
N = Total number of cleared RBC after 72 hrs
Nx = Total number of parasitized RBC
RBC= Red Blood Cells [15].

3. Results and discussion

The screening results of phytochemicals indicated the presence of some secondary metabolites in the crude extract and its fractions. In Table 1, the ethanol crude extract revealed the presence of tannins, saponins, alkaloids, flavonoids, steroids and absence of terpenoids. N-hexane fraction showed the presence of saponins, flavonoids, steroids, tannins and absence of steroids and terpenoids. Chloroform fraction indicated only the presence of saponins, tannins, flavonoids , alkaloids and absence of steroids and terpenoids. The presence of saponins, tannins, steroids and alkaloids were detected in ethyl acetate fraction with exception of terpenoids. Methanol fraction showed the presence of saponins, tannins and flavonoids and absence of steroids, terpenoids and alkaloids (Table 1). The phytochemical investigation of this study revealed the presence of some secondary metabolites (alkaloids, tannins, saponins, steroids and flavonoids) in the leaf extract of the plants (Table 1).

Table 1 Screening Results of Phytochemical Investigation of Ethanol Crude Extract and its Fractions

Phytochemical Constituents	SAG-1	SAG-1-01	SAG-1-02	SAG-1-03	SAG-104
Tannins	+	+	+	+	+
Saponins	+	+	+	+	+
Alkaloids	+	+	-	+	-
Flavonoids	+	-	-	+	+
Steroids	+	-	-	+	-
Terpenoids	-	-	-	-	-

Key: SAG-1: Ethanol Crude Extract SAG-1-01: Chloroform Soluble fraction SAG-1-02:Chloroform soluble fraction SAG-1-03:Ethyl acetate soluble fraction SAG-1-04:Methanol soluble fraction. **+** indicates present and – indicates absent.

The *in vitro* cytotoxicity test results obtained by using *Artemia salina* Leah larvae (Brine shrimp Test) whose values were expressed in LC_{50} µg/ml at 95% confidence interval indicates that high toxicity level for Potassium heptaoxodichromate (VI) (4 µg/ml) serving as a positive control was significantly less than the values obtained for toxicity levels of ethanol crude leaf extract (>1000), n-hexane fraction (>1000), chloroform fraction (>1000), ethyl acetate fraction (>1000) and methanol fraction having LC_{50} µg/ml of >1000 µg/ml (Table 2). From these test results of the samples, it was discovered that the ethanol crude extract and all it fractions exhibitng LC_{50} µg/ml values which are >1000 are non-toxic due to the fact that their LC_{50} µg/ml values were higher than 100 (Table 2). The consideration of LC_{50} µg/ml values less than 1000 suggest toxicity while LC_{50}µg/ml values which are greater than 1000 signify non toxicity [9].

Table 2 *In vitro* Cytotoxicity Test (BSLT) Results of Ethanol Leaf Extract, n-hexane, Chloroform, Ethylacetate, Methanol soluble fraction, Positive and Negative Control.

Extract/Fraction	Conc. (µg/Ml)	Number of Nauplii used	Total No. of Nauplii Dead after 24 Hrs	Percentage (%) Mortaliity	LC_{50} (µg/mL)
SAG – 01	1000	10	2	0.07	> 1000
	100	10	0	0	
	10	10	0	0	
SAG – 01- 1	1000	10	2	0.07	> 1000
	100	10	2	0.07	
	10	10	0	0	
SAG – 01 – 2	1000	10	5	17	> 1000
	100	10	2	0.07	
	10	10	0	0	
SAG – 01- 3	1000	10	3	10	

	100	10	3	10	> 1000
	10	10	0	0	
SAG – 01 – 4	1000	10	4	13.3	> 1000
	100	10	2	0.07	
	10	10	0	0	
Positive control ($K_2Cr_2O_7$)	1000	10	30	100	> 1000
	100	10	30	100	
	10	10	11	37	
Sea Water (-ve control)	----------	10	10	0	0

Man utilizes herbs due to the presence of secondary metabolites in plants which determines some biological activities in animals and man [16]. The existence of these phytochemicals in the plant has led to the justification of orthodox uses of *Sabicea gigantistipula* leaves in traditional medicine practices in treating malaria infection which has been reported on the plant. The ethanol crude leaf extract and each of its fractions showed various degrees of antiplasmodial activity (Table 3). At concentration of 5000 µg/ml, the ethanol leaf crude extract disclosed highest parasitic elimination of 91.80%, followed by chloroform fraction (90.98%), methanol fraction (89.34%), n-hexane fraction 88.52% and ethyl acetate fraction (85.25%).

Table 3 Results of *In-Vitro* Antiplasmodial Evaluation of SAG-1 to SAG-1-04

Extract/Fraction	**Parasitemia Initial Count Per Dose**	**Conc. (µg/ml)**	**Parasitemia Final Count Per Dose**	**No of Parasitemia Mortality After 72 hrs Per Dose**	**% Elimination of Parasites at the end of 72 hrs of Incubation Per Dose/Conc.**
Artemether and Lumenfantrine (+ control)	122	5000	8	114	93.44
	122	2000	12	110	90.16
	122	1000	18	104	85.25
	122	500	22	100	81.97
SAG-1	122	5000	10	112	91.80
	122	2000	22	100	81.97
	122	1000	27	95	77.87
	122	500	41	77	66.39
SAG-1-01	122	5000	14	108	88.52
	122	2000	12	110	83.61
	122	1000	16	106	74.59
	122	500	21	101	64.75
SAG-1-02	122	5000	12	110	90.98
	122	2000	16	106	81.15
	122	1000	22	100	72.95

	122	500	24	98	77.00
SAG-1-03	122	5000	9	113	85.25
	122	2000	10	112	78.69
	122	1000	13	109	75.41
	122	500	14	108	65.57
SAG-1-04	122	5000	9	113	89.34
	122	2000	10	112	84.43
	122	1000	11	111	78.69
	122	5000	15	107	75.41

Key: SAG-1: Ethanol Crude Extract SAG-1-01: Chloroform Soluble fraction SAG-1-02:Chloroform soluble fraction SAG-1-03:Ethyl acetate soluble fraction SAG-1-04:Methanol soluble fraction **+** indicates present and – indicates absent.

At 2000 µg/ml methanol fraction exhibited highest bioactivity of 84.43% followed by n-hexane fraction (83.61%), ethanol fraction (81.97%), chloroform fraction (81.15%) and ethylacetate fraction (78.69%). At 1000 µg/ml, the sequence of antiplasmodial activity of the extract and its fraction is, methanol fraction.(78.69%) > ethanol extract (77.87%) > ethylacetate fraction (75.41 %) > n-hexane fraction (74.59 %) > chloroform fraction (72.95 %), which suggest that the extract and the fractions showed a remarkable antiplasmodial activity. At the lowest dose of 500 µg/ml employed in this study, chloroform fraction (77.00%) revealed the maximum antimalarial activity, followed by methanol fraction (75.41%), ethanol fraction (66.39%), ethylacetate fraction (65.57 %) and n–hexane fraction (64.75 %) while positive control (artemether and lumenfantrine) exhibited percentage suppression activity of 93.44% at dosage 5000 µg/ml, 90.16 % at a dose of 2000 µg/ml, 85.25 % at concentration of 100µg/ml and 81.97 % at a dose of 500 µg/ml. When the antiplasmodial activities of the standard drug/positive control were compared to the tested ethanol extracts and its fractions, it was observed that there is no significant difference in the activity of positive control at the highest tested dose of 5000 µg/ml at (93.44%) with the ethanol crude extract and fractions with the range of their percentage elimination of malaria parasite from 85.25 % - 91.80% while at the least tested concentration of 500 µg/ml, it was also noticed that no significant difference exist in the activity of positive control (81.97 %) and chloroform fraction (77.00 %) and methanol fraction (75.41%). However, at the lowest tested dose of 500 µg/ml, a relative significant difference was observed between the antiplasmodial activity of reference standard drug (81.97 %) and ethanol extract (66.39 %), n-hexane fraction (64.75%) and ethyl acetate fraction (65.57 %). The inhibitory activity of the crude extract and its fraction against multidrug resistant *Plasmodium falciparum* strain K_1 (*in vitro*) was as a result of the synergetic effects of the phytochemicals which were present in the chemical mixture of extract and its fraction. It should be noted that the positive control is a standard antimalarial drug which contains two chemical compounds namely, artemether and lumenfantrine (ACT) which are physically combined together. The tested extract, fraction or bioactive compound which could be regarded as exhibiting best activity is the one that suppressed or killed all or most of the parasites at the lowest tested dose, dosage or concentration in any *in vitro* or *in vivo* investigation. Hence, chloroform fraction of ethanol crude extract indicated best antiplasmodial activity (77%) against multidrug resistant *Plasmodium falciparum* strain K_1 (*in vitro*) at the lowest dose of 500µg/ml even though in this present study, it was noticed that the inhibitory activity of the extract and fractions were concentration/dose dependent implying that the higher the dosage, the higher the antiplasmodial activity (or % suppression activity) and vice versa or tested concentration of the extract, fractions and reference drug is directly proportional to antiplasmodial (or parasitic elimination) activity. The antiplasmodial activities of ethanol extract and its fractions could be due to the existence of bioactive secondary metabolites such as tannins, steroids, flavonoids and alkaloids in the leaves of the plant since flavonoids, alkaloids, terpenoids, tannins and steroids which has been reported to possess antiplasmodial activity [17]. This finding actually confirms that *Sabicea gigantistipula* leaves have been effectively and efficiently employed in folkloric medicine for the treatment of malaria.

4. Conclusion

The current study indicates that ethanol extract of *Sabicea gigantistipula* (schum) leaves possessed plant metabolites such as saponins, tannins, flavonoids, steroids and alkaloids. The results obtained in the *in vitro* antiplasmodial and cytotoxicity suggests that ethanol leaf extract of *Sabicea gigantistipula* and fractions indicated appreciable antiplasmodial activity and apparent *in vitro* non-toxicity in the Artemia salina (Brine shrimp) bioassay. Hence, the continuous use of the leaves of the plant to treat malaria infection in traditional medicine is scientifically confirmed and encouraged because of its wide range of safety especially at relatively low or moderate dosage. Also, studies on the *in*

vivo antiplasmodial and cytotoxicity of the extract/compounds from the leaf of the crude extract are worthy of further investigations.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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