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Full Length Research Paper

Polygalloyltannin isolated from the roots of *Acacia nilotica* Del. (Leguminoseae) is effective against *Plasmodium berghei* in mice

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Crude methanolic root extracts of *Acacia nilotica Del. (Leguminoseae)* demonstrated significant activity against chloroquine sensitive strain of *Plasmodium berghei* in mice. Purified extracts showed only a single fraction with significant antiplasmodial effects using bioguided essay techniques. The active *A. nilotica* isolate was highly polar dissolving readily in methanol, appeared as a single spot in different TLC conditions and was positive for tannins, melting with decomposition between 224 - 229°C. Its ^{1}H NMR spectra exhibited large signals at S 6.90 - 7.58 and 4.70 - 5.00. The Mass spectra (ES1 - Msn) of the isolate gave a large M - 1 signal of m/z 1395 consistent with the molecular formula $C_{62}H_{43}O_{38}$. Others at 1243, 1091, 939, 787, 635, 453 and 331 that differ by m/z 152 were accounted for by the progressive loss of a galloyl ($C_7H_4O_4$) moiety. A polygalloyltannin structure containing a central glucosyl moiety corresponding with 1, 3, 6 – digalloyl – 2, 4 monogalloyltannin was hence postulated.

Key words: *Acacia nilotica, Plasmodium berghei,* Bioguided assay ^{1}H NMR spectra, polygalloyltannin.

INTRODUCTION

The therapeutic potentials of *Acacia nilotica Del*. (Leguminoseae) extracts in herbal medicine have been widely reported. In Northern Nigeria, it is used in the treatment of malaria fever, gall bladder disease, indurations of the liver and spleen, hemorrhoids etc. (Jigam, 2008). It is a common Nigerian and West African plant reportedly native to Egypt but spread to the Arabian Peninsula, Indian subcontinent and most of Africa (Fagg, 1990). Malaria is by far the world's most important tropical parasitic disease, and kills more people than any other communicable disease except tuberculosis. The Disease remains a formidable medical challenge due to resistance by *Plasmodium* to commonly available drugs e.g. chloroquine. Morbidity and mortality to this infectious disease are quite alarming. Annually, about 500 million acute cases and two million deaths mostly among young children under 5 years, 90% of which are in sub-Saharan Africa are reported. This approximates to a death every 30 s (Idro et al., 2007).

There is at present an increased tempo globally in the search for new chemotherapeutic compounds against malaria. This is not only because the parasites and mosquitoes are increasingly resistant to available drugs and other control agents but also viable and relevant vaccines are not expected until many years into the future (Giles, 2005; Matuscheski, 2006; Apponte et al., 2007). Plants are the sources of diverse medicaments

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against a variety of diseases (Fletcher, 2007). It is estimated that 66-85% of the world's population depend directly on plants as medicines (Terry, 2000; Tagboto and Townson, 2001; Haidet, 2003).

An integral component of drug development include the selection of plants on the basis of traditional reputation for efficacy in the treatment of malaria or other diseases, biological and chemical analysis to produce pure compounds. Such plant based drugs include Digitalis (cardiac), Ephedrine (respiratory), Morphine (analgesic), Salicin (antipyretic), Vincristine and Vinblastin (cancer), Tubocurarine (muscle relaxant), Paclitaxel or Taxol (breast and ovarian cancer) etc (Tagboto and Townson, 2001).

Synthetic and semi-synthetic analogues of lead compounds with greater activity and preferably reduced toxicity are often developed. Quinine remains a first line medicine against malaria despite the development of semi synthetic derivatives such as chloroquine, amodiaquine, primaquine and mefloquine based on the quinoline ring structure (Foster, 1994).

Artemisinin is based on a complex tetracyclic 1, 2, 4-trioxane structure containing a peroxide moiety. A water soluble derivative Sodium artesunate, suitable for oral use and an oil-soluble derivative, artemether, suitable for intramuscular injection with improved antimalarial activity over artemisinin (Hien and White, 1993) have been developed. Over 1000 molecules called trioxanes and tetraoxanes similar to the active endoperoxide bridge have been synthesized. The mode of action of artemisinin and these synthetic compounds involve the cleavage of the oxo bridge by intraparasitic iron and haem, generating unstable free radicals that lead to the alkylation of specific malaria proteins (Meshnick et al., 1996).

Other pure compounds with antiplasmodial activity from herbal origins are bitter pentacyclic terpenoids (quassinoids) from species of Simaroubaceae, corialstonine and Corialstonidine (indole alkaloids) isolated from

Astonia coriaceae; cryptolepine from *cryptolepis sanguinolenta*, *ancistrocladine,* dioncopeltine A and dioncopeltine C from species of Ancistrocladaceae *and* Dioncophyllaceae, a bisbenzylisoquinoline alkaloid from *Triclisia patens* etc (Tagboto and Townson, 2001). *Azadirachta indica* (neem) reportedly contains limonoids such as gedunin; nimbolides and rutin with antiplasmodial action (Jigam, 2008).

MATERIALS AND METHODS

Plant materials

Fresh roots of *A. nilotica* were collected between June - September in Minna, Northern Nigeria and identified at the Department of Biological Sciences, Federal University of Technology Minna, Nigeria.

Preparation of crude extracts

40 g of air dried roots were micronised and extracted exhaustively (48 h) in the cold with 1.5 L methanol. The extract was filtered with muslin cloth and solvent removed under reduced pressure in a rotary evaporator. A brown coloured paste was obtained and weighed prior to further analysis.

Animals

Healthy Swiss albino mice of either sex, about 4 - 6 weeks old weighing between 20 - 30 g each were obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Abuja and used for the experiments. The rodents were conveniently housed under standard environmental conditions: temperature; 27±2°C; 70% relative humidity; free access to commercial food pellets and water and natural 12 h daylight/night cycles. Experiments were conducted in strict compliance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC, 1997).

Parasites

Plasmodium berghei berghei NK65 chloroquine sensitive strain was obtained from NIPRD Abuja, Nigeria and maintained in our laboratory by serial passage in mice.

Phytochemical analysis

Standard screening tests were used to detect secondary plant metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides and volatile oils etc, in the crude extract (Odebiyi and Sofowora, 1978; Trease and Evans, 1989).

Safe dose and acute toxicity (LD_{50})

Five groups of four mice each were used and the animals were given extracts intraperitoneally (i.p.) at doses of 200, 400, 600, 800, 1200 mg/kg body weight (bw) respectively. Extracts were dissolved in dimethylsulphoxide (DMSO) (Sigma chemicals; St Louis, MO, USA).

A control group was given normal saline (0.9% w/v NaCl) at 20 ml/kg bw. Mice were observed over 72 h, clinical signs and mortality were recorded. LD_{50} was obtained as the intercept of % mortality (y - axis) and dosages (x - axis).

Preliminary antiplasmodial test

Extract purification

Air-dried *A. nilotica* roots (80 g) were micronised and exhaustively (48 h) extracted in the cold sequentially with 1.5 L × 2 each of hexane, ethyl acetate and methanol. The marc was dried after extraction with a single solvent. The extracts were filtered using muslin cloth and the solvents recovered under reduced pressure with a rotary evaporator. The crude extracts were each weighed and employed in mice challenged with *P. berghei*. The most promising methanolic extract was partitioned in chloroform and water (1:1) before subjecting the active chloroform fraction to column chromatography (3.0 cm i.d. × 30 cm) over silica gel (G60, 70 - 230 mesh; Merck). A mixture of hexane/ethyl acetate/methanol

Table 1. Summary of results of phytochemical analysis of root extracts of *A. nilotica.*

Active principle	Extract
Alkaloids	+
Morphine alkaloids	+
Glycosides	+
Cardiac glycosides	-
Steroids	+
Terpenoids	+
Tannins	+
Flavonoids	+
Saponins	-
Caffeine	-

+ = Present; - = Absent.

Table 2. Results of antiplasmodial effects of crude *A. nilotica* extracts in mice.

		Suppressive		Curative	
Treatment	**Dose (mg/kgbw)**	**Parasitamia***	**Decrease (%)**	**Parasitamia***	**Decrease (%)**
A. nilotica	150	50.67±2.81	62.59	80.67± 5.80	29.83
Chloroquine	5	15.17± 3.35	86.59	16.50±1.54	85.22
Normal saline	20 ml	125.50±5.85	0.00	115.00±7.38	0.00

n = 6, *Mean= ±SEM.

(2:7:1) was used to elute the column. 5 bands were visible and collected as separate fractions. Each fraction was evaluated for antiplasmodial activity in mice. The most active fraction was rechromatographed by TLC on silical gel using methanol/water (8:2). This was further tested for activity in parasitized mice.

Melting point determination

The melting point of the isolate was determined using the Fisher-Johns apparatus. Fischer Scientific (Vogel, 1964) Pittsburg Pa.

Spectral analysis

The active *A. nilotica* isolate was analyzed by nuclear magnetic resonance (NMR) and mass spectroscopy (MS). NMR spectra were recorded with an Oxford YH200 spectrometer (200 MHZ for ^{1}H; solvent, CD_3OD). 2D 'H – H' COSY and NOESY spectra were obtained using the same instrument. Mass spectra were determined on CEC (Agilent Scribs 103C); coupled to a mass (Finningan LCQ) spectrometer using the positive ion (Na^{+}) and negative (HCOOH) electron spray modes.

RESULTS

The phytochemicals detected in the crude extracts of *A. nilotica* roots are indicated in Table 1 below. Crude methanolic extracts of *A. nilotica* were generally safe with apparently no adverse clinical symptoms at doses below 1000 mg/kg with LD_{50} of 3000 mg/kg body weight of mice. The preliminary antiplasmodial screening of the crude methanolic extracts (Table 2) showed appreciable suppressive effect which was comparable to the standard chloroquine treatment. The purified extracts however demostrated greater curative activity (Table 3).

The purified, active *A. nilotica* isolate started browning at 140°C and melted with decomposition between 224 - 229°C. Preliminary phytochemical tests of the isolate were positive for tannins.

Proton assignments in the Nuclear Magnetic Resonance data (Figure 1) of *Acacia* isolate were facilitated by COSY NMR and NOESY NMR spectra. H_5, H_4, H_3 and H_2 were observed as multiplets at resonances of *S*0.03, *S*3.96, *S*4.98 and *S*5.52, respectively. H_2, and H_3 were Cosy to each other but H_4 and H_2 were Noesy.

H_1 and H_6 were however observed as doublets at *S*6.12 and *S*6.23 respectively. The large signals between *S*4.70 - *S*5.00 and *S*6.90 - *S*7.58 are characteristic of aromatic 0H and aromatic protons respectively.

In the mass spectrum (ESI – MSn) represented in Figure 2, a large M -1 signal at M/z 1395 was observed. Subsequent signals down to 331 differed by M/z 152. This value is consistent with a galloyl moiety ($C_7H_40_4$) while m/z 331 is for a glucosylgallate unit.

Based on the spectral data and thermodynamic considerations, the compound isolated from *A. nilotica*

Table 3. Results of antiplasmodial effects of purified *A. nilotica* extracts.

		Suppression		Curative	
Treatment	**Dos (mg/kgbw)**	**Parasitamia***	**Decrease (%)**	**Parasitamia***	**Decrease (%)**
A. nilotica	150	164+4.72	12.02	73.67+7.10	52.57
Chloroquine	5	35.33+ 2.01	77.26	40.52+2.38	74.19
Normal saline	20ml	155.43+4.82	0.00	155.33+7.38	0.00

n = 6; *Mean = ±SEM.CQ eq of isolate: Curative = 44.09; Suppressive = 192.83.

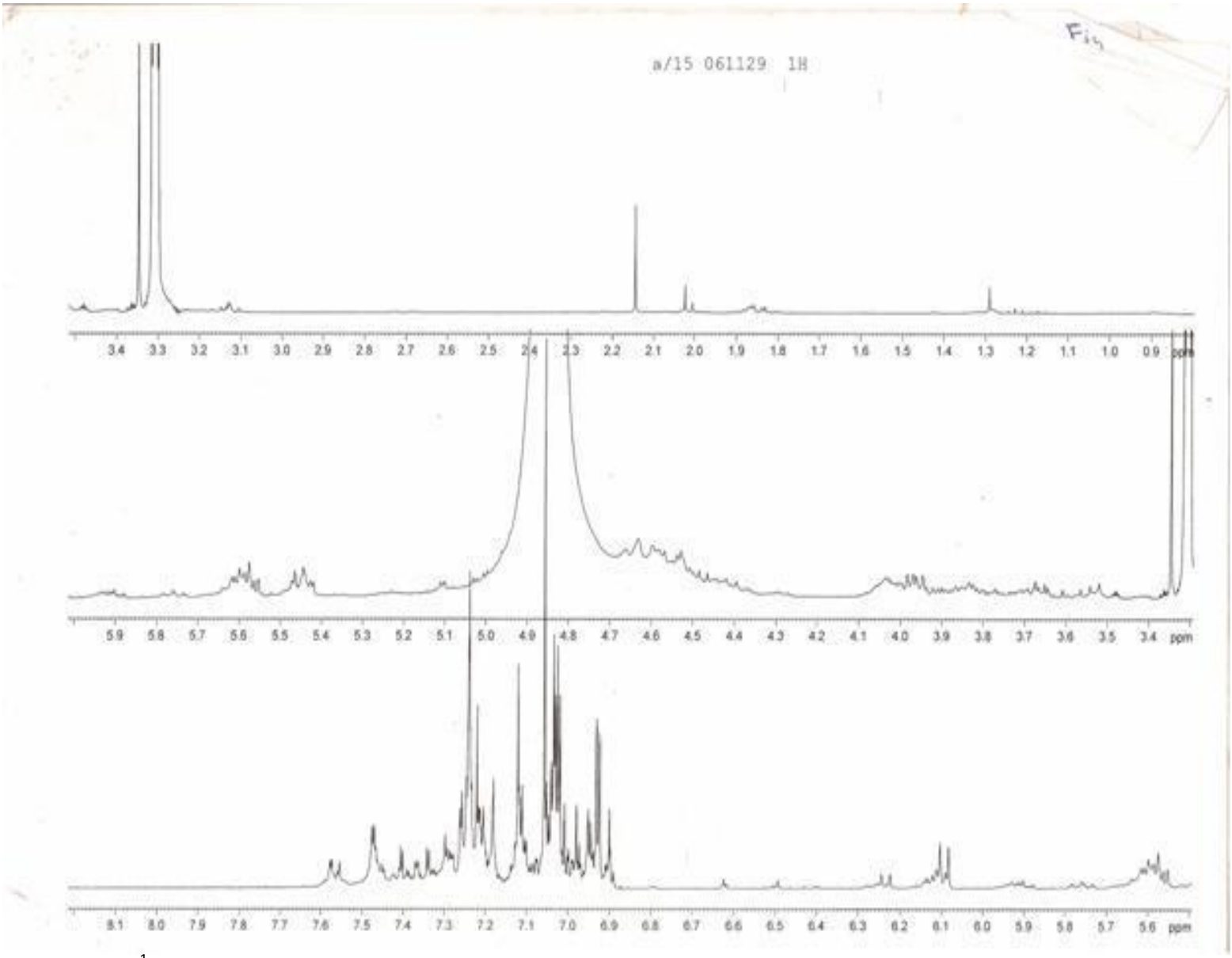

Figure 1. ^{1}H of purified *A. nilotica* extract.

roots was represented as shown in Figure 3. This corresponds with 1, 3, 6 – dilgalloyl – 2, 4 – monogalloyltannin.

DISCUSSION

The secondary plant metabolites including alkaloids,

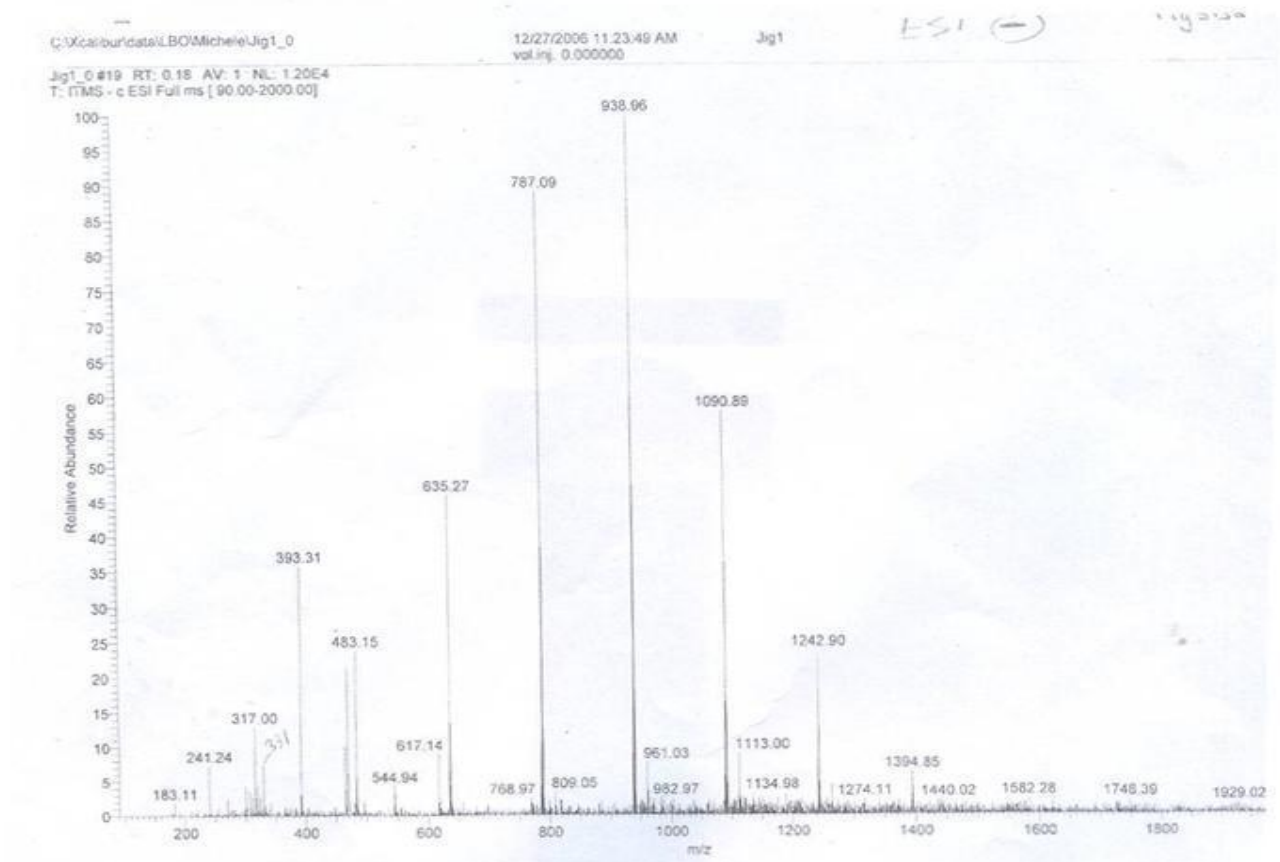

Fig. 3.52 Mass Spectrum (MS) of JIG1 Fraction (A.nilotica)

M/Z	mol. Formula
1395	C62H43O38
1243	C55H39O34
1091	C48H35O30
939	C41H31O26
787	C34H27O22
635	$C_{27}H_{23}O_{18}$
483	$C_{20}H_{19}O_{14}$
331	$C_{13}H_{15}O_{10}$

Figure 2. Mass Spectrum (MS) of purified *A. nilotica.*

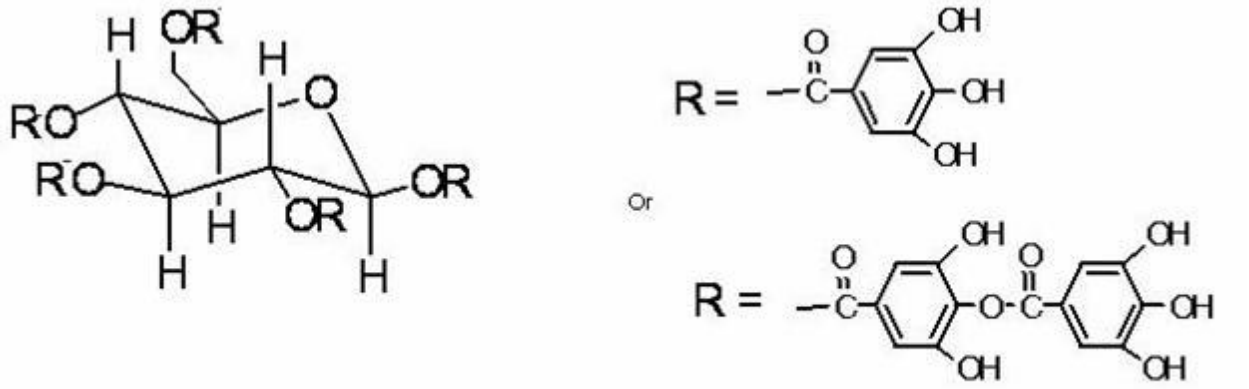

Figure 3. Postulated structure of purified *A. nilotica.*

glycosides, steroids, tannins and flavonoids, identified in the crude extract of *A. nilotica* roots is in agreement with earlier reports (El-Sayyad and Ross, 1983; Gupta and Bokadia, 1975; Banso, 2009) hence useful as index to the final isolation of the bioactive principle with antiplasmodial effect. It also signifies the medicinal potentials of the plant (Dafallah and Al-Mustapha, 1996; Sotohy et al., 1997; Banso, 2009). The phytochemicals were analysed primarily because of their established pharmacologic activity, for example alkaloids are tranquilizers e.g. reserpine; anti-malarials e.g. quinine; analgesics e.g. morphine etc (Jigam et al., 2004). The higher parasite suppression by crude extracts issignificant in the sense that most reports about the anti malarial effects of *Acacia* are not specific as to whether it is anti plasmodial or effective by some other mechanism. Such reports were mostly based on *in vitro* studies (Etkin, 1997; Jigam et al., 2009). Crude plant extracts have generally been suggested to be more plasmodistatic than plasmodicidal probably because unpurified bioactive principles may require initial conversions which time lag allows for parasite proliferation (Noedl et al., 2003). The observed potency of crude *A. nilotica* extracts in mice is a confirmation of the rationale for its use in malaria treatment among indigenous Nigerians (Togboto and Townson, 2001; Gathirwa, et al., 2007; Fletcher, 2007).

The purified bioactive principle was more effective as a curative than suppressive antiplasmodial agent hence could be useful as radical cure for malaria. This is because tissue forms of *Plasmodium* are responsible for the recrudescence and therefore relapse in the disease (Hien and White, 1993; White and Olliero, 1996; Bloland, 2001).

The isolation and characterization of an active principle is noteworthy. Pharmacological and medicinal effects of gallotannins include anti-diarrhoel, haemostatic and anti-haemorrhoidal properties (Watterman and Mole, 1994; Pauuponen-Pimia et al., 2004).

Orisidape et al. (2004) isolated and characterized a 1, 2, 3, 4, 6-Pentagalloylglucose which was effective against *Mycobacteria* from the leaves of *Entandrophagma angolense* further confirming the antimicrobial and medicinal potentials of gallotannins. Tetracycline and clindamycin are useful as antimalarials thus establishing a link between some antimicrobials and *Plasmodium* (Bloland, 2001).

Since the isolation of quinine an alkaloid from *Cinchona ledgeriana* in 1820, a variety of potent phytochemicals against malaria have been identified. These include *Artemisinin* a sesquiterpene lactone from *Artemisia annua* (Tiagboto and Townson, 2001).

Literature is however scarce in respect of the efficacy of gallotannins as antiplasmodial agents. The present finding may thus represent such an early association. The mechanism of action of the isolate could be determined and synthetic analogues with improved efficacy and other pharmacological parameters synthesized.

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