

Research Article

Toxicity Study, Phytochemical Characterization and Anti-parasitic Efficacy of Aqueous and Ethanolic Extracts of *Sclerocarya birrea* against *Plasmodium berghei* and *Salmonella typhi*

Gabi Baba, A.A.J. Adewumi and Saudatu Ahmed Jere

Department of Applied Science, College of Science and Technology Kaduna Polytechnic
Kaduna, Nigeria

Abstract: In screening drugs, determination of its toxicity is usually part of the initial step in their assessment and evaluation, phytochemical constituents and their isolation can subsequently be determined. *Sclerocarya birrea* is a plant employed ethnically in the treatment of different parasitic infections such as malaria. This study examined the anti-malarial and antibacterial activity of plant leaf extracts against *Plasmodium berghei* using Swiss albino mice *in-vivo* and *Salmonella typhi* isolates. Also acute toxicity (LD₅₀ as the index), phytochemical screening and FTIR characterization of the aqueous and ethanolic leaf extracts of the plant were conducted. The LD₅₀ was based on Lorke's method using mice, while phytochemical screening and FTIR characterization based on the routine procedures. The results indicated that LD₅₀ of the aqueous and ethanolic extracts were 566 and 800 mg/kg body weight, (intra-peritoneal), respectively. The phytochemical screening revealed the presence of alkaloids, Anthraquinones in the Free State, Carbohydrate (Reducing sugar) and Flavonoids. Also present include Phlobatanins, saponins, tannins and terpenoids all of which are detected in both extracts. However, Cardiac Glycoside and steroids are only detected in the aqueous extract. FTIR spectroscopy indicated the presence of functional groups such as C-H, C=O and O-H, indicating the peaks for alkanes, alcohols, aromatics and Carboxylic acid, while C-O-C, C-C(O)-C, C-N, C-I, N-H indicated the peak for ether, ester, Amine, amide, aldehyde and (alkyl halide). P=O (Phosphine oxide) and C=C (conjugate) (Alkene) also indicated in the extracts. Ethanolic extract is characterized with certain peculiar functional groups such as C-C(O)-C, C-O-C, C-H bend and C-H stretch relating to ethers, esters, aromatics and aldehyde/ketones. The mice were grouped according to their weights and the extracts administered curatively against *Plasmodium berghei* based on standard procedures. The research finding shows that both aqueous and ethanolic leaf extracts of *Sclerocarya birrea* were 100% effective against *Plasmodium berghei*. Double dilution method was used to determine the antibacterial activity of the identified *S. birrea in vitro*. The aqueous and ethanolic extract of *Sclerocarya birrea* showed an MIC of 3×10^{-4} mg/mL and 4×10^{-4} mg/mL, respectively; while both extracts were found to have the same MBC of 4×10^{-4} mg/mL. The values of their MIC and MBC prove promising in further antibacterial research breakthrough.

Keywords: FTIR, *in-vivo*, *in-vitro*, LD₅₀, *Plasmodium berghei*, *Salmonella typhi*, *Sclerocarya birrea*

INTRODUCTION

Sclerocarya birrea (A. Rich.) Hochst. is a common species throughout the semi-arid savannas of sub-Saharan Africa. It is commonly known as marula (English), Danyaa (Hausa) Jinjere goyi (Nupe), the family Anacardiaceae which encompasses 73 genera and 352,600 species (Van Wyk *et al.*, 1997), locally known as marula. It is widespread in Africa from Ethiopia in the north to KwaZulu-Natal in the south. In South Africa it is more dominant in the Baphalaborwa area in the Limpopo province. It occurs naturally in various types of woodland, on sandy soil or occasionally on sandy loam (MacGaw *et al.*, 2007). It has multiple uses, including the fruits that are eaten

fresh and sucking the juice or chewing the mucilaginous flesh after removal of the skin. The ripe fruit has an average vitamin C content of 168 mg/100 g which is approximately three times that of oranges and comparable to the amounts present in guavas (Wilson, 1980; Christensen and Kharazmi, 2001). The kernels are eaten and cooking oil can be extracted from them. The leaves are browsed by livestock and have medicinal uses (Muok *et al.*, 2009) as does the bark. Several researchers have reported biological activities of *S. birrea* extracts, but no comprehensive anti-Plasmodia activities of the plant have been reported, although it is widely used by traditional healers. (Eloff, 2001) reported antibacterial activity of acetone extracts of *S. birrea* against *Staphylococcus aureus*,

Corresponding Author: Gabi Baba, Department of Applied Science, College of Science and Technology Kaduna Polytechnic Kaduna, Nigeria

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

Pseudomonas aeruginosa, *Enterococcus faecalis* and *Escherichia coli*. (MacGaw *et al.*, 2007) have also reported its antibacterial, antihelmintic and cytotoxic effects. Anticonvulsant effect of *S. birrea* stem-bark aqueous extract in mice was reported by Ojewole and Adewole (2007) and Runyoro *et al.* (2006) have investigated 34 medicinal plants used by Tanzanian traditional healers in the management of *Candida* infections and they reported that the ethanolic extract of dried stem bark of *S. birrea* showed antifungal activity against *C. albicans*.

Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases; this practice is employed as an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (Sofowora, 1995; Zhu *et al.*, 2002). Unfortunately, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. More so, there is limited scientific evidence regarding the anti-malaria and anti-typhoid efficacy to back up its continued therapeutic application. The rationale for plant utilization has rested largely on long-term clinical experience (Zhu *et al.*, 2002). However, with the upsurge in the use of herbal medicines, a thorough scientific investigation of these plants will go a long way in validating their folkloric usage (Sofowora, 1993). This study is therefore designed to investigate the anti-malarial and anti typhoid activity of the aqueous and ethanolic extracts of *S. birrea* and to further assess the acute toxicity using LD₅₀ as the index to detect the phytochemicals and carried out the FTIR characterization of the aqueous and ethanolic leaf extracts of the plant.

MATERIALS AND METHODS

Plant materials: Fresh leaves of *Sclerocarya birrea* plant were collected from Zaria road and Maguzawa Rigasa, Kaduna between March and April. The sampled leaf was duly authenticated at applied science department, Kaduna Polytechnic, Kaduna. The leaves were collected and washed thoroughly with water and air dried in a shady area at room temperature (25°C) for about two weeks. The dried leaves were pulverized using pestle and mortar. The processed leaves were then stored in a brown bottles away from sunlight until required.

Extraction: 100 g of the plant sample was weighed on a watch glass and transferred into a porous thimble which was placed in soxhlet extractor. The plant extract was extracted with 500 mL of ethanol. While the aqueous extract was obtained by maceration sequentially using the dried residue of the leaf of *S. birrea* obtained from the ethanol extraction. 100 g powdered of the leaf was macerated in 500 mL of distilled water for 48 h with an occasional shaking. It

was then filtered by the use of cheese cloth, after which it was properly suction filtered and concentrated by the use of water bath at 60°C (Sofowora, 1995). The extracts were collected in a flask with air tight cover and kept for further tests. The percentage yield of oil was calculated as follows:

$$\text{Percentage yield} = \frac{\text{weight of n-hexane extract obtained}}{\text{Total Weight of Sample}} \times 100$$

Acute toxicity test:

Acute toxicity studies: The LD₅₀ was carried out by adopting the method outlined by Lorke (1983) with a little adjustment. A total 26 Swiss albino mice (*Mus musculus*) were used for the study of the two extracts. In the initial phase, nine mice were grouped into three groups of three mice each and they were treated with extract at doses of 10, 100 and 1000 mg/kg body weight intra-peritoneally and observed for 24 h. In the second phase which is deduced from the first phase, four mice were grouped into four groups of one mouse each and they were treated with doses of 400, 800, 1600 and 3200 mg/kg body weight intra-peritoneally. They were also observed for 24 h as in the first phase and final LD₅₀ value was determined (Lorke, 1983).

Phytochemical screening: All extracts obtained were screened for phytochemical constituents which include Alkaloids, Anthraquinones, Carbohydrate (Reducing sugar), Cardiac Glycoside, Flavonoids, Phlobatanins, Saponins, Steroids and Tannins using standard quantitative procedures (Harbone, 1973; Trease and Evans, 1989; Sofowora, 1993).

Characterization procedure: The routine procedure for the infrared analysis by Egwaikhide and Gimba (2007) was adapted, with little modification, using Fourier Transformed Infrared (FTIR) spectrophotometer model 8400s. The extracts were scanned in accordance with ASTM 1252-98. A drop of each extract was applied on a sodium crystal disc using a special mould and a hydraulic press to obtain a thin layer. The cell was mounted on the FT IR and scanned through the IR region.

In-vivo test:

Parasite inoculation: The *Plasmodium berghei* was obtained from National Pharmaceutical Research Institute Abuja through the Department of Biochemistry, Federal University of Technology, Minna and maintained by blood transfer in albino Swiss mice of body weight (21-24 g) of the same sex.

The *Plasmodium berghei* was prepared and inoculated as described by Okonkon *et al.* (2008). The blood was prepared by determining the percentage parasitemia and the erythrocytes count of the donor mouse and further diluting the blood with normal saline in proportion such that 0.2 cm³ of the blood contains

$1.0 \times 10^7 P. berghei$ parasitized erythrocytes. 0.2 cm^3 of the infected blood was inoculated intra-peritoneally to each mouse on day 0.

Animal grouping: Albino Swiss mice of weight 21-24 g of the same sex were obtained from Animal house of Ahmadu Bello University Zaria. They were maintained on standard animal pellets and enough water. The animals were grouped into four groups of 3 mice each identified as group A, B, C and D.

Curative schizontal activity of the extract: The evaluation of anti-plasmodia activity of the extracts was carried out using the curative method described by Ryley and Peters (1970) with the following modification. Each mouse was inoculated on the first day (0) intra-peritoneal with 0.2 cm^3 of infected blood containing about $1.0 \times 10^7 P. berghei$ parasitized RBCs. Seventy two hours later Group A animals were administered Chloroquine orally at 10 mg/kg/day . Equivalent volume of distilled water (Negative control) was given to Group B animals. Group C and D were orally administered with aqueous and ethanolic extracts respectively using concentrations of extracts 40 mg/kg/day . The drug and the extracts were given once daily for 5 days. The parasitemia level was monitored using a prepared thin film and Giemsa stained obtained from the mice tail daily for 5 days. The mean survival time for each group was determine by arithmetic means (Average survival time/day) of the mice after inoculation in each group over a period of 15 days (day 0 to day 14) as described by Okonkon *et al.* (2008).

Percentage parasitaemia: Percentage parasitaemia was calculated before and after treatment from:

$$\% \text{ parasitaemia} = \frac{\text{Number of infected RBCs}}{\text{Total number of RBCs}} \times 100$$

From the thin films made from the tail blood of each mouse, parasitaemia level was determined by counting the number of parasitized RBCs out of 200 erythrocytes in random fields of the microscopes. Average percentages (%) were then calculated.

Physical parameters such as body temperature, heartbeat rate and body weight of the animals were examined, before and after treatments.

Bacterial isolates and bioassays:

Source of bacterial isolates: The bacterial isolated used was *Salmonella typhi* which was clinical isolated from Yusuf Dantsoho Memorial Hospital Microbiology Laboratory. The bacterial isolates were then gram-stained, after which this biochemical tests (which include the Lysine decarboxylase reaction) was carried out.

Biochemical test to identify *Salmonella typhi*: The Kligler Iron Agar (KIA) and Rosco Enzyme tests (based on Lysine decarboxylase and KIA) are used to identify *Salmonella typhi* (Cheesbrough, 2005). The gram-stained growth was emulsified with a small amount of KIA in a loop full of physiological saline on a slide. It was then mixed by tilting the slide backwards for about 30 sec. It was then examined for agglutinations against a dark background. There was no agglutination observed. One loop full of test anti-serum was then added and mixed. It was then re-examined for agglutination. The presence of a story clear agglutination within one minute confirms that the strain in *Salmonella typhi*.

Anti-microbial activity of the extract: The anti-microbial activity of the extracts was done by the Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) as adopted by Dewanjee *et al.* (2007) with some modification.

MIC was determined by tube dilution method for the test organism in triplicates (Doughari *et al.*, 2007). To 2 mL of varying concentrations of the extracts (range of $10\text{-}50 \mu\text{g/mL}$ for both aqueous and ethanol extracts), 2 mL of nutrient broth was added and then a loopful of the overnight test organism previously diluted to 0.5 McFarland turbidity standard (Cheesbrough, 1999), was introduced to the tubes. The procedures were repeated on the test organisms using standard antibiotics Ampicillin. A tube containing nutrient broth only seeded with the test organism served as control. Tubes containing bacterial cultures were then incubated at 37°C for 24 h. After incubation the tubes were examined for microbial growth by observing the turbidity.

To determine the MBC for the set of test tubes in the MIC determination, a loop full of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar by streaking. Plates inoculated are then incubated at 37°C for 24 h. After incubation the concentration at which no visible growth was seen was noted as MBC.

RESULTS

Percentage yield: The ethanolic extract of *Sclerocarya birrea* has higher percentage yield with 13.336% while aqueous has 12.86% (Table 1).

(LD₅₀ for aqueous extracts is given by the square root the two values):

$$\begin{aligned} \text{LD}_{50}(\text{aqueous}) &= \sqrt[2]{400 \times 800} \\ &= \sqrt{320000} \\ &= 566 \text{ mg/kg (i.p)} \end{aligned}$$

Table 1: The percentage yield of the extracts

Extracts	%Yield of extracts	Colour of extracts
Ethanollic	13.33	Bluish-green
Aqueous	12.86	Pale brown

Table 2: Acute toxicity tests of the aqueous and ethanollic extracts on the leaves of *Sclerocarya birrea*

Doses (mg/kg)	Survival rate (phase I)		
	Aqueous	Ethanol	
10	0/3	0/3	
100	1/3	0/3	
1000	1/3	1/3	
Doses (mg/kg)	Survival rate (phase II)		
	400	0/1	0/1
	800	0/1	0/1
	1600	1/1	0/1
	3200	1/1	1/1

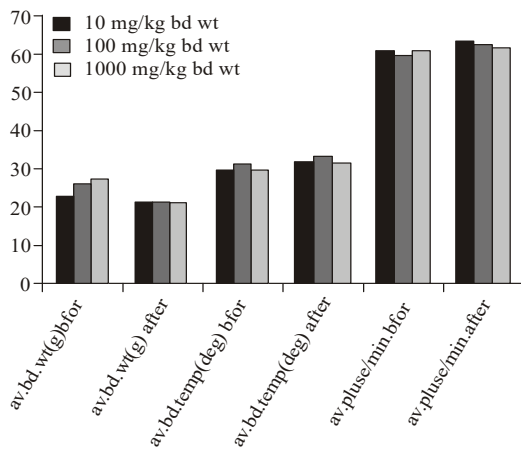


Fig. 1: Changes in some physiological parameters for the mice treated with aqueous extracts: Phase I

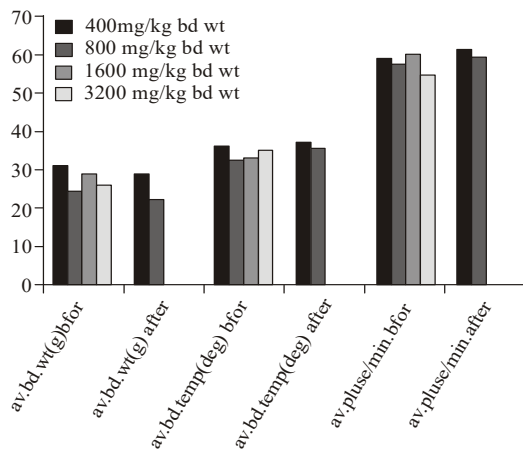


Fig. 2: Changes in some physiological parameters for the mice treated with aqueous extracts: Phase 2

(By determining square root of the two values, (Lorke, 1983)):

$$\begin{aligned}
 LD_{50}(\text{Ethanollic extract}) &= \sqrt[3]{1600 \times 800 \times 400} \\
 &= \sqrt[3]{512,000,000} \\
 &= 800 \text{ mg/kg (i.p)}
 \end{aligned}$$

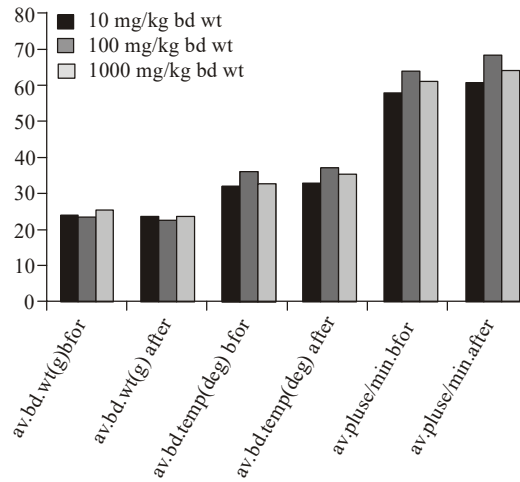


Fig. 3: Changes in some physiological Parameters for the mice treated with ethanollic extracts: Phase I

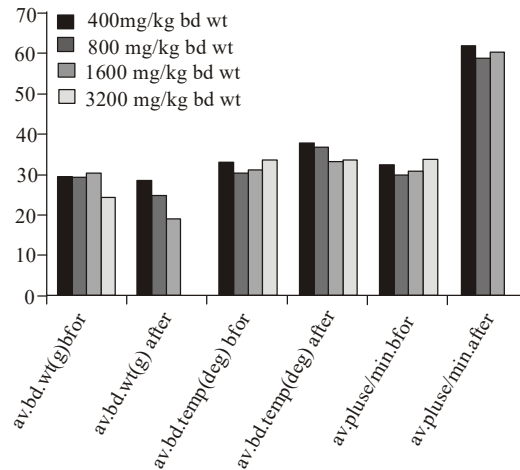


Fig. 4: Changes in some physiological Parameters for the mice treated with ethanollic extracts: Phase II

(By determining cube root of the three values, (Lorke, 1983))

The LD₅₀ of the aqueous and ethanol extracts of *S. birrea* were found to be 566 and 800 mg per kg body weight, (intra-peritoneal) respectively (Table 2).

The animals after 24 h of the administration of the extracts of *S. birrea* show weight loss, increased body temperature and heartbeat and a slight decrease in food consumption (Fig. 1 and 2).

The animals after 24 h period, after the administration of the extracts of *S. birrea* show weight loss, increased body temperature and heartbeat and a slight decrease in food consumption (Fig. 1 and 2).

The average increase in body temperature and heartbeat was comparatively high (Fig. 2 to 4) and some mice in the phase II died as a result, which cannot be unconnected to the increased concentration of the extracts.

Table 3: Phytochemicals of the aqueous and ethanolic extracts of *Sclerocarya birrea* leaf

Phytochemicals	Aqueous	Ethanollic
Alkaloids	+	+
Anthraquinones (free state)	+	+
Carbohydrate (Reducing sugar)	+	+
Cardiac Glycoside	-	+
Flavonoids	+	+
Phlobatanins	+	+
Saponins	+	+
Steroids	-	+
Tannins	+	+
Terpenoids	+	+

+ = present; - = Negative

Phytochemical screening: The phytochemical screening revealed the presence of alkaloids, Anthraquinones in the free state, Carbohydrate (Reducing sugar) and Flavonoids. Also present include Phlobatanins, saponins, tannins and terpenoids all of which are detected in both extracts of *Sclerocarya birrea*. However, Cardiac Glycoside and stereroids are only detected in the aqueous extract (Table 2).

FTIR characterization: FTIR spectroscopy as shown in Table 3 indicted the presence of functional groups such as C-H, C = O and O-H, indicating the peaks for alkanes, alcohols, aromatics and Carboxylic acid, While C-O-C, C-C(O)-C, C-N, C-I, N-H indicated the peak for ether, ester, Amine, amide, aldehyde and (alkyl halide). P = O (Phosphine oxide) and C = C (conjugate) (Alkene) also indicated in the extracts. Ethanolic extract

is characterize with certain peculiar functional groups such as C-C(O)-C, C-O-C, C-H bend and C-H stretch relating to ethers, esters, aromatics and aldehyde/ketones. Aqueous extract on the other hand is peculiar with C = O stretch relating to amides (Table 4).

Variation in the physical parameters: Table 5 showed that mice in all the groups showed variations in the average rate of heart beat, body temperature and the body weight before and after treatment. A general increase in the body temperature in all the groups between 32°C and 35°C is observed, which can be considered as possible indication of manifestation of *Plasmodium species*. That was followed by subsequent fall in the temperature between 30 to 31°C of the treated groups. The temperature of the control group increases progressively from 35 to 43°C (Table 5). There was progressive increase in the average heartbeat rate between 60 and 64 pulses per minute after infection, before treatment. However, during and after the treatment a remarkable decrease was observed the heartbeat rate was between 62 and 50 pulses per minute, however the control group remain progressively high up to 67 pulses per minute, which could be an indication of progress in malaria ailment (Table 5). There was an average weight loss within 2 of the treated groups (0.8 to 2.8% loss) and control groups (the weight loss is about 8.1%). The effect of which cannot be unconnected to the infection, based on the

Table 4: The FT infrared characterization of *Sclerocarya birrea* aqueous and ethanolic leaf extract

Molecular motion	Peak cm ⁻¹		Functional group
	Aqueous extract	Ethanollic extract	
C-I Strech	467.75		Alkylhalids
C-H Bend (ortho)		713.69	Aromatics
C-H bend (meta)		882.46	Aromatic
C-N Strech (Alkyl)	1076.32	1, 041.60	Amines
C-O-C Stretch		1, 117.58	Ethers
P = O stretch	1159.26	1159.26	Phosphine oxide
C-C(O)-C stretch (Diaryl)		1240.27	Ester
CH ₃ bend		1376.26	Alkane
OH bend	1, 424.48		Carboxylic acid
CH ₂ bend		1459.20	Alkanes
N-H bend (primary 1 ^o)		1560.46	Amines
C = C stretch (conjugate)		1617.37	Alkenes
C = O stretch	1654.01		Amides
		1488.13	Aromatic
	2356.13		Aromatics Acetic Acid
C-H Stretch		2852.81	Aldehyde/Ketone
C-H Stretch	2917.43	2923.22	Alkanes Carboxylic acid
O-H Stretch	3420.87	3406.40	

Table 5: Variations in some physiological parameter of the mice before and after treatment with chloroquine and extracts of *Sclerocarya birrea*

Chloroquine /Extracts	Groups	Before treatment		After treatment		Before treatment	After treatment
		*Ave Temp °C	Ave. Temp °C	Ave. Pulse/min	Ave. Pulse/min	Ave. Body weight (g)	Ave. Body Weight (g)
Chloroquine	A	34	30.5	60	50	22.2	23
Distilled water	B	35	43	62	67	22.2	20.4
Aqueous extract	C	33	30	61	55	23.0	22.8
Ethanolic extracts	D	32	31	64	62	20.8	20.2

*Ave = average

Table 6: Means percentage parasitaemia per day (%)

Groups	Days								% Clearance after treatment (%)
	0	1	2	3	4	5	6	7	
A	0±0.00	03.5±0.3	06.0±0.6	11.0±0.6	6.8±0.9	3.2±0.2	0±0.0	0±0.0	100
B	0±0.00	03.3±0.2	06.3±0.3	10.7±0.7	19.0±0.6	27.7±1.6	34.2±0.8	37.3±1.2	00
C	0±0.00	02.8±0.2	05.5±0.3	08.7±0.3	06.5±0.3	03.7±0.3	02.3±0.3	00±0.0	100
D	0±0.00	03.8±0.4	04.5±0.3	09.0±0.9	06.3±0.7	03.5±0.3	01.0±0.5	00±0.0	100

Data showed the means and standard Error of mean of parasitaemia samples taken in Triplicate

Table 7: Mean survival time of mice after 14 days of infection

Group	Initial number	Number of death	Percentage survival (%)
A	3	0	100
B	3	3	0
C	3	0	100
D	3	0	100

Table 8: The minimum inhibitory concentration and the minimum bactericidal concentration of the extracts

MIC X10 µg/mL	MBC X10 µg/mL	Extract concentration (x10 µg/mL)	Extract concentration (x10 µg/mL)					
			0	1	2	3	4	5
3	4	Aqueous	+	+	+	-	-	-
4	4	Ethanol	+	+	+	+	-	-
1	1	Ampicillin	+	-	-	-	-	-

fact that the proportional growth rate within the period retarded and the growth of the animals was maintained within the same range as obtained before and after treatment.

In vivo anti-malarial activity: The group of mice treated with Chloroquine phosphate showed almost total clearance of about 100% at day 6 (Table 6) and showed 100% protection from the parasites after treatment (Table 7). The group of mice that were left as control and treated with distilled water group B showed daily progressive increase in the average parasitaemia from 3% (day 2) to 19% (day 4) to 34% (day 6) and a total mortality at the end of the experiment. The group C mice treated with aqueous extract also showed daily increase from 3% (day 1) to 8% (day 3) and suddenly reduced to 6.5% (day 4) down to 0% (day 7) and the extract showed 100% protection (Table 7).

In group D mice treated with ethanolic showed daily percentage increase from 4% (day 1) to 9% (day 3) then reduced to 6% (day 4) to 0% (day 7). The extract showed 100% protection at the end of the experiment (Table 7).

Identification of *S. typhi*: The strains of the isolates were motile and showed a positive test to lysine decarboxylase by the presence of agglutination. This confirms that the isolate is *S. typhi*.

McFarland turbidity standards: The concentration of the bacterial cells was found to be 6×10^8 /mL, this is because test tube 2 corresponds to the turbidity of the standard inoculant and hence the cell density, which is equivalent to 6×10^8 /mL.

The MIC of the aqueous and ethanol extracts of *S. birrea* were found to be 30 µg/mL and 40 µg/mL, respectively and their MBC were found to be 40

µg/mL; while the MIC and MBC of the standard antibiotic (Ampicillin) was found, both to be 10 µg/mL (Table 8).

DISCUSSION

The results of this investigation showed that ethanolic extract of *Sclerocarya birrea* have high percentage yield 13.33% yield, while aqueous extract yield 12.86%. This might probably explain why ethanolic extract gives higher number of characteristic functional groups, even though the differences in proportion is small (Table 1).

The acute toxicity study of the extracts revealed that LD₅₀ values of aqueous and ethanolic extracts are 566 and 800 mg/kg (i.p.) which are greater than 500 mg/kg body weight. Therefore, the *sclerocarya birrea* can be categorized as relatively non-toxic based on the scale proposed by Lorke (1983). This study shows that the ethanolic extract could be safer for oral consumption. However, in higher concentration the animals showed slight loss of appetite and reduction in fluid consumption. The animals within 24 h period after the administration of the extracts of *S. birrea* showed loss of weight, increased body temperature and heartbeat and a slight decrease in food consumption. Colour of the eyes was darker than those of the control. However, the mice that survived regained full health within 48 h after the study. This is because they show an increased consumption of food, even greater than that of the control and a more steady heartbeat, body weight and body temperature was recorded. There was no much effect on the group of mice in phase I, due to the lower level of concentration of the extracts administered (Fig. 1 and 2). Some mice in the phase II died, which can possibly be associated to increased concentration of the extracts (Table 2). Before their death, they showed some physiological changes such as weakness of the body (sluggishness), dark eye coloration and reluctance to feeding within the first 24 h after administration. In a related development The LD₅₀ value of 400 mg/kg body weight in mice *i.p.* signifies the extract to be slightly toxic to the experimental model as stated by Matsumura (1975) who classified the chemical based on their LD₅₀ values and pointed out that LD₅₀ of 500-5000 mg/kg as slightly toxic to the experimental model. The increased body temperature and heartbeat and a decrease in food consumption effects of the aqueous extract can reduce the fluid intake in rats and may be responsible for the

loss in body weight of the animals (Fig. 3 and 4). Such effect might also be attributed to tannin and saponin which are anti-nutritional factors (Umaru *et al.*, 2007). Tannins have the ability to precipitate certain proteins and can therefore, combine with digestive enzymes thereby making them unavailable for digestion (Abara, 2003; Binita and Khetapaul, 1997). The changes in the physiological parameters such as increase in body weights are peculiar to diseased organisms (Sofowora, 1995). The administration of the extracts of *S. birrea* at high concentrations above the LD₅₀ might trigger such effects. The pale coloration of some internal organs of the mice killed by a high dose of extracts may be as a result of some physiological changes of the internal organs caused by the administration of the extracts (Barlow and Goh, 2002). This however requires a further study to determine the actual cause of death.

This research indicated that phytochemicals identified from *Sclerocarya birrea* leaf extracts include alkaloids, Anthraquinones in the free state, Carbonhydrate (Reducing sugar), Cardiac Glycoside and Flavonoids. Also present include Phlobatanins, saponins, stereroid, stannins and terpenoids (Table 4). The spectral analysis of the extracts shows the presence of functional groups C-H, C=O and O-H, indicating the peaks for alkanes, alcohols, aromatics and Carboxylic acid, While C-O-C, C-C(O)-C, C-N, C-I, N-H indicated the peak for ether, ester, Amine, amide, aldehyde and (alkyl halide). P = O (Phosphine oxide) and C = C (conjugate) (Alkene) also indicated in the extracts (Table 4). The presence of phenols, amine, Carboxylic acid, amide, aldehydes/ketones and alcohols which are the principal functional groups of the identify phytochemicals correlated with the findings from the spectral analysis of the extracts. This support the report by Ndhlala *et al.* (2007) that the pulp of *S. birrea* possesses phenolics flavanoids and condensed tannins; although he reported was based on the pulp of the plant, the relationship strongly be correlated.

The finding from this study showed that both aqueous and ethanolic extracts of the leaf of *Sclerocarya birrea* contains anti-*Plasmodia* substances with properties that showed curative effects on the established malaria parasites infection. Both aqueous and ethanolic extract showed high potential that is compatible to the standard drug, Chloroquine as demonstrated by the mean survival time of the mice after treatment (Table 6). The doses of the crude extracts administered (40 mg/kg) and that of the chloroquine was 10 mg/kg body. it therefore implies that if the crude extract can be further purify for the lead compound to be isolated higher potential might speculated. The parasite clearance capacity demonstrated by the plant from the result could be describe as time dependent. This is based on the fact that initially, there was increase in parasitamea levels in all groups progressively before the commencement of

the treatment. On the initiation of treatment there was drastic reduction in the parasite level as recorded (Table 8). The identified phytochemicals are directly link to the medicinal activity of the herbs. More so, the constituent compounds identify may act singly or synergistically with one or more phytochemicals to achieve the desire activities as suggested by Kirby *et al.* (1989).

The anti-*Plasmodial* activity of the two extracts could be associated with the presence of Alkaloids (Harborne and Williams, 2001; Heinrich *et al.*, 2004), terpenoids (Bruneton, 1999; Heinrich *et al.*, 2004). Although the mechanism of their action is yet to be elucidated, literature had shown that alkaloids such as cryptolepine interact with DNA of the parasite; it appeared that two nitrogen atoms N and N-CH₃ of cryptolepine interact with adenine-thymine base pair. More so the possible formation of π - π charge transfer complex between purine-pyrimidine bases is speculated (Kirby *et al.*, 1989). Sesquiterpene on the other hand is believed to interfere with the protein synthetic pathway of the parasite. Moreover one of the mechanisms of action is due to its inhibition of cytochrome oxidase, which occurs at the plasma, the nuclear and the food vacuole-limiting membranes as well as in the mitochondria of the trophozoites of *P. berghei* (Zhao *et al.*, 1986). Some other plant extracts phytochemicals are said to exhibit their anti-plasmodia activity by causing the oxidation of red blood cells (Etkin, 1997) depending on the phytochemical constituents. However, the constituent compounds identify may act singly or synergistically with one or more phytochemicals to achieve the desire activities as suggested by (Kirby *et al.*, 1989).

The antibacterial activity of the extracts of *S. birrea* showed that the MBC and MIC of both extracts inhibited and consequently kill the bacteria *S. typhi* at an appreciable concentration with the aqueous extract exhibiting higher sensitivity (MIC 30 μ g/mL and MBC 40 μ g/mL) (Table 8) while ethanolic extract (MIC 40 μ g/mL MBC 40 μ g/mL) (Table 8). However both are considered highly sensitive when compare with study of Dewanjee *et al.* (2007). The antibacterial activity of the standard antibiotics was found to be higher (MIC and MBC 10 μ g/mL), probably because the lead compound is isolated. However, further characterization, identification and isolation of the biologically active component of *S. birrea* may be useful for the further elucidation, since the mass of the crude extract which contains different components was equal to the mass of the powdered antibiotic, but the concentration of the active components differs. This research work confirm the report of Eloff (2001) who reported antibacterial activity of *S. birrea* leaves and bark extracts against Gram-positive and G-negative bacteria such as *S. aureus*, *P.aeruginosa*, *E.coli* and *E. faecalis* with MIC values ranging from 0.15 to 3

mg/mL and further reported that based on minimum inhibitory concentration values, inner bark extracts tended to be the most potent followed by the outer bark and leaf extracts (Masoko *et al.*, 2008). The sensitivity of the extracts might be attributed to the presence of secondary metabolites such as flavonoids, phenolic groups and steroids as suggested by previous reports (Kosalec *et al.*, 2005; Pereira *et al.*, 2007; Caceres *et al.*, 1993). Thus our findings revealed the medicinal potential value of the extract of *Sclerocarya birrea* and the lead compound need to be isolated.

CONCLUSION

The result of the present study showed that *Sclerocarya birrea* leaf extracts are active against both *Plasmodium berghei* and *Salmonella typhi* parasites, respectively. This therefore justifies the traditional usage of this plant as malaria and typhoid remedy. *Sclerocarya birrea* leaves are therefore a potential source of drug for the effective treatment of malaria and typhoid.

REFERENCES

- Abara, A.E., 2003. Tannin content of *Dioscorea bulbifera*. J. Chem. Soc. Niger., 28: 55-56.
- Barlow, P.J. and L.M. Goh, 2002. Antioxidant capacity in *Ginkgo biloba*. Food Res. Int., 35: 815-820.
- Binita, R. and N. Khetarpaul, 1997. Probiotic fermentation: Effect on antinutrients and digestibility of starch and protein of indigenously developed food mixture. Nutr. Health, 11(3): 139-147.
- Bruneton, J., 1999. Pharmacognosy, Phytochemistry and Medicinal Plants. Intercept. Ltd., England, U.K.
- Caceres, A., L. Fletes, L. Aguilar, O. Ramirez, L. Figueroa, A.M. Taracena and B. Samayoa, 1993. Plants used in Guatemala for the treatment of gastrointestinal disorders. 3. Confirmation of activity against enterobacteria of 16 plants. J. Ethnopharmacol., 38(1): 31-38.
- Cheesbrough, M., 1999. District Laboratory Practice in Tropical Countries. Cambridge University Press, New York, pp: 240-258.
- Cheesbrough, M., 2005. District Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, New York, pp: 240-258.
- Christensen, S.B. and A. Kharazmi, 2001. Antimalarial Natural Products. Isolation, Characterization and Biological Properties. In: Trigalic, C. (Ed.), Bioactive Compounds, from Natural Sources Isolation, Characterization and Biological Properties. Taylor & Francis, London, UK, pp: 379-432.
- Dewanjee, S., M. Kundu, A. Maiti, R. Majumdar, A. Majumdar and S.C. Mandal, 2007. *In Vitro* Evaluation of antimicrobial activity of crude extract from plants *Diospyros peregrina*, *Coccinia grandis* and *Swietenia macrophylla*. Trop. J. Pharm. Res., 6(3): 773-778.
- Doughari, J.H., A.M. Elmahmood and S. Manzara, 2007. Studies on the antibacterial activity of root extracts of *Carica papaya* L. Afr. J. Microbiol. Res., 1(3): 037-041.
- Egwaikhide, P.A. and C.E. Gimba, 2007. Analysis of the phytochemical content and anti-microbial activity of *Plectranthus glandulosus* whole plant. Middle-East J. Sci. Res., 2(3-4): 135-138.
- Eloff, J.N., 2001. Antibacterial activity of Marula (*Sclerocarya birrea* (A. rich.) Hochst. subsp. *Caffra* (Sond.) Kokwaro) (Anacardiaceae) bark and leaves. J. Ethnopharmacol., 76(3): 305-308.
- Etkin, N.L., 1997. Antimalarial plants used by Hausa in northern Nigeria. Trop. Doct., 27: 12-16.
- Harbone, J.B., 1973. Phytochemical Methods: A Guide to Modern Techniques of Plant analysis Chapman and Hall, London, pp: 279.
- Harborne, J.B. and C.A. Williams, 2001. Anthocyanins and other flavonoids. Nat. Prod. Rep., 18: 310-333.
- Heinrich, M., J. Barnes, S. Gibbons and E.M. Williamson, 2004. Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Elsevier Science Ltd., UK.
- Kirby, G.C.O., J.D. Philipson and D.C. Warhurt, 1989. *In vitro* studies on the mode of action quassionoids with activity against chloroquine-resistant *Plasmodium falciparum*. Biochem. Pharmacol., 38: 4367-4374.
- Kosalec, I., S. Pepeljnjak and D. Kustrak, 2005. Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., Apiaceae). Acta Pharmaceut., 55(4): 377-385.
- Lorke, D.A., 1983. New approach to acute toxicity testing. Arch. Toxicol., 54: 275-287.
- MacGaw, L.J., D. Van der Merwe and J.N. Eloff, 2007. *In vitro* anthelmintic, antibacterial and cytotoxic effects of extracts from plants used in South African ethno veterinary medicine. Vet. J., 173(2): 366-372.
- Masoko, P., T.J. Mmushi, M.M. Mogashoa, M.P. Mokgotho, L.J. Mampuru and R.L. Howard, 2008. *In vitro* evaluation of the antifungal activity of *Sclerocarya birrea* extracts against pathogenic yeasts. Afr. J. Biotechnol., 7(20): 3521-3526.
- Matsumura, F., 1975. Toxicology of Insecticides. Plenum Press, New York and London, pp: 2-24.
- Muok, B.O., A. Matsumura, I. Takaaki and W.O. David, 2009. The effect of intercropping *Sclerocarya birrea* (A. Rich.) Hochst., millet and corn in the presence of arbuscular mycorrhizal fungi. Afr. J. Biotechnol., 8(5): 807-812.

- Ndhlala, A.R., A. Kasiyamhuru, C. Mupure, K. Chitindingu, M.A. Benhura and M. Muchuweti, 2007. Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. Food Chem., 103(1): 82-87.
- Ojewole, J.A.O. and S.O. Adewole, 2007. Hypoglycaemic and hypotensive effects of *Globimetula cupulata* (DC) Van Tieghem (Loranthaceae) aqueous leaf extract in rats. Cardiovasc. J. South Afr., 18(1): 9-15.
- Okonkon, J.E., E. Etebong and A. Bassey, 2008. In vivo antimalarial activity of ethanolic leaf extract of *Stachytarpheta cayennensis*. Indian J. Pharmacol., 40(3): 111-113.
- Pereira, A.P., I.C.F.R. Ferreria, F. Marcelino, P. Valentao, P.B. Andrade and R. Seabra, 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. cv. *cobrancosa*) leaves. Molecules, 12: 1153-1162.
- Runyoro, D.K.B., O.D. Ngassapa, M.I.N. Matee, C.C. Joseph and M.J. Moshi, 2006. Medicinal plants used by Tanzanian traditional healers in the management of *Candida* infections. J. Ethnopharmacol., 106(2): 158-165.
- Ryley, J.F. and W. Peters, 1970. The antimalarial activity of some quinolone esters. Ann. Trop. Med. Parasit., 64(2): 209-222.
- Sofowora, A., 1993. Medicinal Plants and Traditional Medicines in Africa. 2nd Edn., Spectrum Books Ltd., Ibadan, Nigeria, pp: 1-153.
- Sofowora, A., 1995. Medicinal Plants and Traditional Medicines in Africa. Chichester Sohn, Willey and Sons, New York, pp: 256.
- Trease, G.E. and W.C. Evans, 1989. Pharmacognicy. 11th Edn., Braillia Trindall Can Ltd., Macmillan Publisher, London, pp: 45-50.
- Umaru, H.A., R. Adamu, D. Dahiru and M.S. Nadro, 2007. Levels of antinutritional factors in some wild edible fruits of Northern Nigeria. Afr. J. Biotechnol., 6(16): 1935-1938.
- Van Wyk, B.E., B. Van Oudtshoorn and N. Gericke, 1997. Medicinal plants of South Africa. Briza Publications, Pretoria, South Africa.
- Wilson, C.W., 1980. Guava. In: Nagy, S. and P.E. Shaw (Eds.), Tropical and Subtropical Fruits: Composition, Properties and Uses. Avi Publications, Westport, Connecticut, USA, pp: 279-299.
- Zhao, Y., W.K. Hanton and K.H. Lee, 1986. Antimalarial agents: Artesunate, an inhibitor of cytochrome oxidase activity in *Plasmodium berghei*. J. Nat. Prod., 49: 139-142.
- Zhu, M., K.T. Low and P. Loung, 2002. Protective effects of Plant formula on ethanolic-induced gastric lesions in rats. Phytother. Res., 16: 276-280.