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A new indole alkaloid and anti-malarial activity of the stem bark extract of *Chrysophyllum albidum*

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Abstract

Background to the study: *Chrysophyllum albidum* stem bark extract had been shown to be antimalarial active in various studies but the compound responsible have not been identified.

Methods: Its stem bark ethanol extract was successively partitioned to obtain n-hexane, ethyl acetate, butanol fractions and the aqueous phase with the aim of isolating the antimalarial constituent(s). The fractions were all tested for chemosuppressive activities at doses 0-80 mg/kg using Peter's four-day test in Plasmodium berghei berghei-infected mouse model with chloroquine (10 mg/kg) and normal saline as positive and negative control respectively

Result: The partitioned fractions: n hexane, butanol and aqueous with ED_{50} 44.71±4.88, 54.84±4.25 and 54.42±3.84 and 56.6±4.64 respectively were comparable ($p>0.05$) in activity to each other. The ethyl acetate fraction with strong reactions to Dragendorff's reagent and similarly has good weight, was further purified by repeated column chromatography leading to the isolation of a new indole alkaloid identified and characterized as 4, 5, 7, 8-tetrahydro-1H-1, 4-epoxyazepino [1′, 2′: 1, 2] pyrido [3, 4-b] indole-2, 3, 3(2H, 13H, 13bH)-triol (1) named as albidumine with a molecular formula C16H18N2O4. In addition, eleaginine (2), previously characterised from the seed was also identified in this stem-bark through LC-MS. To the best of our knowledge, compound 1 is a new alkaloidal constituent in *Chrysophyllum sp*. The structure was elucidated using the data from mass spectra, 1D (1H, 13C NMR and DEPT) and 2D-NMR (HMQC and HMBC) spectra.

Conclusion: A new indole alkaloid isolated from ethyl acetate fraction of the ethanol extract of the stem-bark of *Chrysophyllum albidum* and characterised as albidumine may likely be an antimalarial constituent of the plant

Keywords: *Chrysophyllum albidum*; Stem-bark; Indole alkaloids; Albidumine; Antimalarial

1. Introduction

One disease which has become a foremost public health issue in the world, is malaria. It has been widely reported to cause about 0.7-1 million deaths per year and half of the world's populace is at risk from malaria; with about 78% occurrence in Africa, 15% in Southeast Asia and 5% in Eastern Mediterranean regions [1].

As a result of the growing resistance of the parasite to currently available drugs, there is the need for concerted effort at searching for novel antimalarial agents with focus on plant-derived active principles, from some medicinal plants that have been used locally for treatment. According to WHO (2010) [2], more than 80% of world's population are dependent

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on traditional medicine, which is principally plant-based, for their primary health care needs. In literature, over 160 families of plants, both lower (especially ferns) and higher with over 1200 species including *Chrysophyllum albidum* have been documented to be used traditionally for the treatment of malaria and some of these have been scientifically validated *in vitro* and/or *in vivo* for their claimed activity against the infection [3, 4, 5, 6]. This has inspired many researchers especially in Africa to further intensify the search for antimalarial agents from plant sources.

The plant, *Chrysophyllum albidum*, frequently called African star Apple, belongs to the family *Sapotaceae* and is used in folkloric medicine for the management of several ailments. The plant is prevalent in tropical and subtropical regions of the world. In traditional medicine, it is used as an anthelmintic, antiseptic, mosquito repellent, for stomach ailments, headache, colds, coughs tonic, antiscorbutic, astringent, diuretic, arthritis, digestive and appetite stimulant, an anti-oxidant and for ~~s~~ore throats [1].

In the last two decades, various researchers have subjected the plant, *Chrysophyllum albidum* to phytochemical, pharmacological and clinical studies and it has been reported to have several biological activities. [7, 8, 9, 10, 1, 11, 12, 13, 14, 15].

In particular, the stem-bark explored in this study has been reported in literature as a remedy for malaria and yellow fever [16, 9, 11, 12, 15]. In our laboratory, earlier investigations of the antimicrobial constituents of *Chrysophllum albidum* seed cotyledons led to the isolation and characterization of three alkaloids viz: eleaginine, tetrahydro-2-methylharman and skatole [7] and from the stem-bark, stigmasterol, epicatechin, epigallocatechin and procyanidin B5 [17]. In addition, [15] established that the ethanolic extract of the stem bark has *in vivo* prophylactic, chemosuppressive and curative activities against *Plasmodium berghei berghei* in mice.

This study was thus designed to validate the antimalarial activity of different solvent fractions of *Chrysophyllum albidum* bark and to isolate and characterize its chemical constituents.

2. Material and methods

Column chromatography (CC) was performed using the Accelerated Gradient Chromatography (AGC) with silica gel 60 (230-400 mesh ASTM, 0.040-0.063mm, E. Merck, Darmstadt, Germany). Thin Layer Chromatography (TLC) was performed on pre-coated silica gel (Kieselgel 60) GF_{254} plates (0.25 mm, Merck). In the TLC, hex-CH_2Cl_2 (3: 7) and CH_2Cl_2-MeOH (9: 1.5) was used to develop the plates except where stated otherwise and visualization was by viewing under UV light and spraying with Dragendorff's reagent and sulphuric acid. Proton Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded at 300 and ^{13}C-NMR at 75 MHz, on a Bruker Avance DPX 300 spectrometer at the University of Botswana, Botswana. Chemical shifts are expressed in ppm in δ values relative to TMS. 2D-Heteronuclear shift correlation spectra (HMQC and HMBC) were recorded in the inverse mode at 300. EIMS was done on Finnigan MAT SSQ 7000 Single Quadruple Instrument at 70 eV and the ESI were done on Finnigan LQC Deca. L

2.1. Plant collection, authentication and extraction

The stem-bark of *Chrysophyllum albidum* was collected at Ede Road Ile-Ife in February 2018. It was identified by Mr. I. I. Ogunlowo of the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife. A voucher specimen, UHI 16181, was deposited at the University Herbarium, Ile-Ife (UHI), Nigeria. The sample was air dried for 7 days at room temperature, subsequently in an oven at 40 ^{o}C and finally powdered using Christy Norris grinding machine. The powdered stem-bark (1.1 kg) was extracted with 80% ethanol (3 x 5 L) at room temperature for 72 hours and the pooled extract was concentrated to dryness *in vacuo*.

2.2. Solvent partitioning of the active extract

The crude ethanolic extract (CASB, 110 g) was suspended in distilled water and partitioned with *n*-hexane (6 x 300 mL), ethyl acetate (6 x 300 mL) and *n*-butanol (3 x 300 mL) successively which were in turn concentrated to dryness *in vacuo* to obtain the *n*-hexane (CASBHF, 1.45 g), ethyl acetate (CASBEAF, 12.27 g), *n*-butanol (CASBBF, 7.15 g) fractions and the aqueous fraction (CASAF, 36.6 g) respectively.

Chemosuppressive antimalarial screening of the partition fractions.

2.3. Mice

Thirty (30) Swiss albino mice weighing between 18 to 24 g (male and female, not pregnant) were purchased from the Animal House, College of Health Sciences, Obafemi Awolowo University, Ile –Ife. They were housed in aluminium cages

exposed to 12 hours day/night cycle and fed with grower's marsh and clean water with acclimatisation for at least 10 days before use. They were randomly divided into 6 groups (**I-VI**) containing 5 mice per group.

2.4. Parasite

A donor mouse infected with *Plasmodium berghei berghei* NK 65, maintained by serial intraperitoneal passaging in mice [18], was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan. The mouse was euthanized after attaining at least 30 % parasitaemia; the blood obtained was diluted so that 0.2 ml will contain 1×10^7 infected erythrocytes. Each of the acclimatized and grouped mice was inoculated each with 0.2 ml of the diluted blood.

2.5. Preparation of the Partition fractions and standard drug

Each of the partitioned fractions of the stem bark extract was weighed in a proportion of 6.0 mg, 12.0 mg, 24.0 mg, 48. 0 mg and solubilized each into 6.0 mL of distilled water with the aid of Tween 80.to obtain final doses of 10, 20, 40 and 80 mg kg^{-1} respectively. In addition, 8.33 mg of chloroquine phosphate was dissolved in small quantity of distilled water and made up to 6.0 mL. with distilled water to give a dose of 10 mg kg^{-1} for 5 animals for the duration of the experiment

2.6. Antimalarial activities of the partition fractions

The partitioned fractions *n*-hexane (CASBHF), ethyl acetate (CASBEAF), *n*-butanol (CASBBF) and aqueous phase (CASBAQF) fractions were screened for antimalarial activities at doses 10, 20, 40, and 80 mg kg^{-1} prepared above. In each case, the solutions were injected to the animals based on the weight such that a 20.0 g of mice will take 0.2 mL of the solutions prepared according to the weight.

Briefly, an inoculum of 0.2 mL of diluted blood containing 1 x 10^7 *P. berghei* parasitized red blood cells was given to each of thirty (30) test mice intraperitoneally and were randomly divided into six groups (**I-VI**) (n=5). Two hours later (D_0), mice in Groups **I** and **VI** were orally administered with distilled water (0.2 mL) and *p.o.* chloroquine (10 mg/kg) respectively while Groups **II-V** received 10, 20, 40 and 80 mg/kg of the *n*-hexane partition fractions (CASBHF) respectively after recording their rectal temperatures using a digital thermometer. The procedure above was repeated for three consecutive days (D_1-D_3) afterwards. The same procedure was repeated for ethyl acetate (CASBEAF) and *n*-butanol (CASBBF) and aqueous phase (CASBAQF) fractions. The blood smear for each mouse was taken from the blood withdrawn from the tail on the fifth day (D_4) and the level of parasitaemia was determined as percentage parasitaemia per dose, after fixing the smear with methanol and staining with Giemsa followed by cell counting using the microscope The mice were further observed for a period of 28 days for mortality from the day of drug administration in each case and recorded [19].

2.7. Average percentage parasitaemia and the median effective doses

Each of the Giemsa-stained blood smears was viewed under the microscope using oil immersion (x 100) objective and ten fields with uniform distribution of red cells were counted. For each of the fields selected, the numbers of parasitised (PRBC), as well as unparasitised red blood cells (UPRBC) were counted. The percentage parasitaemia for each field was calculated from the number of parasitised red blood cells divided by the total number of parasitised and unparasitised red blood cells, multiplied by 100. The average value obtained for each mouse and subsequently for each group of the five mice was calculated and recorded as the mean percentage parasitaemia for each group [19].

Percentage chemosuppression per dose was also determined from the average percentage parasitaemia using the formula:

$$\%\ chemosupression\ =\ 100\left(\frac{\%\ \text{parasitaemia}\ negative\ control\ -\ \%\ \text{parasitaemia}\ Test\ dose}{\%\ \text{parasitaemia}\ negative\ control}\right)$$

The median effective dose, ED_{50} and the dose that will cause 90% efficacy, ED_{90} as measurements of the antimalarial activities of each of the partition fractions/ standard drugs were estimated from the graph of percentage chemosuppression against doses [19], using the Microsoft Office Excel 2010.

2.8. Survival times and percentage survivors

The treated mice were observed for mortality for 28 days from the day of drug administration in order to determine the survival times and percentage survivors elicited by the extracts in the mice. The survival time for each mouse was recorded, in days and the average for each group determined as days ± SEM. The percentage survivor or survival rate

for each group was estimated from the average survival time as the percentage number of mice eliciting survival time that falls within the average for the whole group [6].

2.9. Statistical analysis

Values of percentage parasitaemia, percentage chemosuppression and the effective doses for the various extracts and the partitioned fractions were expressed as mean±SEM and analyzed statistically using One-way Analysis of Variance (ANOVA) followed by Student Newman Keul's post-hoc test for comparisons to determine the source of significant difference for all values. Values of $p < 0.05$ were considered to be of statistical significance.

2.10. Column chromatography of the ethyl acetate fraction (CASBEAF)

The ethyl acetate fraction (7.0 g) was chromatographed on silica using a gradient of *n*-hexane, dichloromethane, and methanol. The fractions collected were analysed by TLC in hex-CH_2Cl_2 (3: 7) and CH_2Cl_2-MeOH (9: 1.5) and visible spots were detected using Dragendorff's spray reagents. Fractions having similar TLC profiles were pooled together resulting in six different fractions coded F1 (1-9, 0.32 g), F2 (10-26, 0.16 g), F3 (27-55, 0.32 g), F4 (56-64, 0.55 g), F5 (65-76, 1.6 g) and F6 (77-100, 3.10 g). Only F4 and F5 gave strong reaction to Dragendorff's with 3 and 2 spots respectively. F4 (500 mg) was further separated on silica gel column using isocratic elution with dichloromethane - methanol (95:5). Fractions collected were analyzed by TLC in CH_2Cl_2 - MeOH (9 : 1.5) and fractions having the similar TLC profiles were bulked together resulting in four different fractions coded F4A (1-26, 0.163 g)) , F4B (27-31, 0.029 g, **compound 1**), F4C (32-50, 0.105 g), and F4D (51-60, 0.19 g).

3. Results

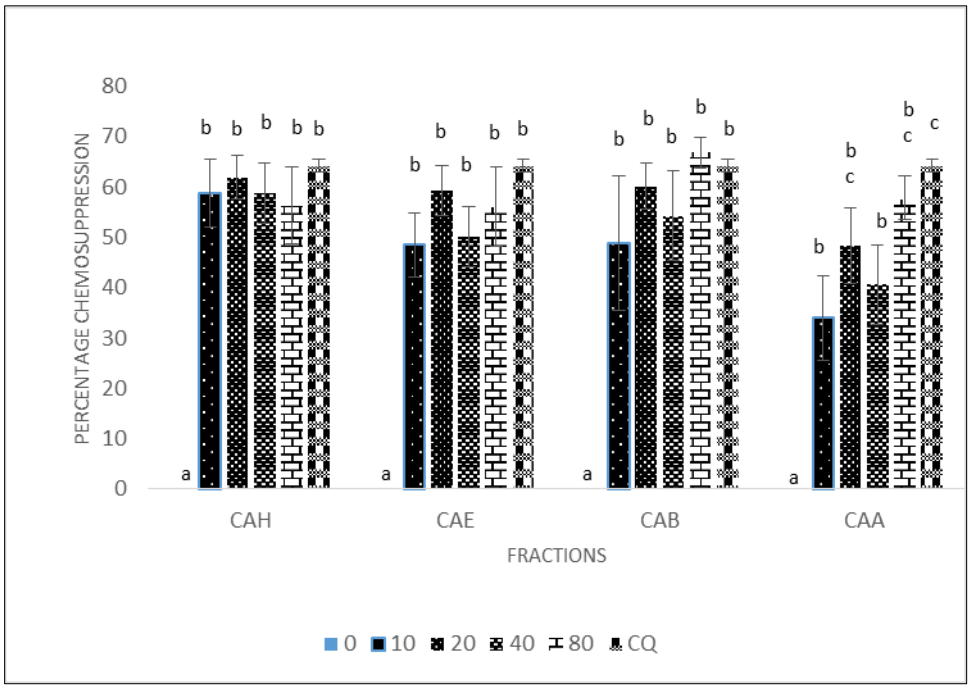

Figure 1 Percentage chemosuppression elicited by the partition fractions of the stem bark of *C. albidum*. Keys: Data show the mean ± SEM, n = 5.: NC (negative control): Tween 80 in normal saline; PC: CQ = Chloroquine (10 mg/kg) as positive control. CAH: n-hexane fraction; CAE: ethyl acetate fraction; AB: butanol fraction; CAA: aqueous fraction. Values with different superscripts, a,b or c within columns on the bar are significantly different ($p < 0.05$), one-way analysis of variance followed by the Student–Newman–Keuls' test. Superscripts a, b or c are statistical notations to depict significantly different activities in the values within columns

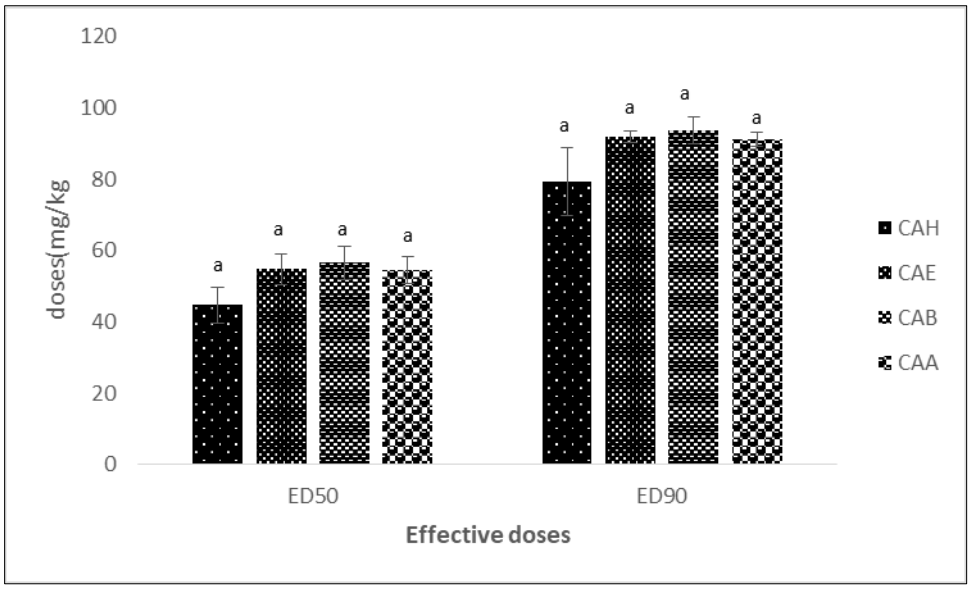

Figure 2 The effective doses (ED_{50} and ED_{90}) elicited by the partition fractions of the stem bark of *C. albidum*. Keys: Data show the mean ± SEM, n = 5. ED_{50}, ED_{50}: Doses that produced 50 and 90 % activities; Values with similar superscripts, a within columns on the bars shows activities are not significantly different (p >0.05), one-way analysis of variance followed by the Student–Newman–Keuls' test

Table 1 Survival times as percentages relative to the negative control and percentage survivor (in parenthesis) elicited by the partition fractions of the stem bark of *C. albidum*

Doses (mg/kg)	FRACTIONS			
	Hexane	**Ethyl acetate**	**butanol**	**aqueous**
0	100.00±0.00 a (40)	100.00±0.00 [a] (40)	100.00±0.00 [a] (40)	100.00±0.00 [a] (40)
10	159.06±38.0 [a] (60)	128.81±37.0 [a] (60)	125.40±37.8 [a] (60)	141.18±34.12 [a] (80)
20	191.80±43.57 [a] (80)	86.08±20.62 [a] (60)	103.90±36.8 [a] (80)	143.82±16.01 [a] (80)
40	160.71±55.34 [a] (60)	100.16±36.29 [a] (40)	143.77±45.17 [a] (60)	105.18±30.56 [a] (60)
80	163.27±38.71 [a] (60)	136.07±14.32 [a] (80)	155.91± 55.47 [a] (40)	134.59±32.26 [a] (60)
CQ (10)	192.53±60.64 [a] (60)	192.53±60.64 [a] (60)	173.92±23.16 [a] (60)	173.92±23.16[a] (60)

Keys: Data show the mean ± SEM, *n* = 5. *: **NC** (negative control): Tween 80 in normal saline; **PC**: CQ = Chloroquine (10 mg/kg) as positive control Values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test

3.1. The physical and spectroscopic data and characterization of Compound 1

The physical and spectroscopic data on the isolated compounds are as recorded below:

Brown amorphous solid, ESI-MS m/z (rel. int. %) 303 $[M+H]^+$ (100), 285 $[M+H-18]^+$ (37), 325 $[M+Na]^+$ (13); ^{1}H- and ^{13}C-NMR (CD_3OD). **^{1}HNMR** (300 MHz, CD_3OD) δ ppm: 4.54 (1H, *d*, *J* = 1.2 Hz, H-1), 4.26 (1H, *d*, *J* = 2.4 Hz, H-2), 3.81 (1H, t, H-4), 2.82 (2H, *dd* , *J* = 10.5 Hz, H-5), 3.19 (2H, t, H-7), 2.52 (2H, t, H-8), 7.40 (1H, *d*, *J* = 7.5Hz, H-9), 6.97 (1H, *dd*, *J* = 7.35, 1.1 Hz, H-10), 7.05 (1H, *dd*, *J* = 7.5, 1.4 Hz, H-11), 7.30 (1H, *d*, *J* = 7.8 Hz, H-12), 4.07, (1H, *d* , J = 1.8 Hz, H-13b). **^{13}CNMR** (75 MHz, CD_3OD) δ ppm: δ 80.40 (C-1), 80.47 (C-2), 103.19 (C-3), 84.14 (C-4), 48.87 (C-5), 49.03 (C-7), 15.86 (C-8), 107.23 (C-8a), 117.12 (C-9), 127.25 (C-9a), 118.23 (C-10), 120.65 (C-11), 110.54 (C-12), 136.51 (C-12a), 130.90 (C-13a), 55.43 (C-13b).

Table 2 ^{1}H- and ^{13}C-NMR Spectral Data for compound I (F4B) in CD_3OD at 300 MHz

No.	^{1}H	^{13}C	Observed connectivity in HMBC spectrum	Observed connectivity in COSY spectrum
C-1	4.54 (1H, *d*, *J* = 1.2 Hz)	80.40	80.47, 84.14, 103.19, 130.90	H-2, H-13b, H-4
C-2	4.26 (1H, *d*, *J* = 2.4 Hz)	80.47	55.43, 80.40, 84.14	H-4, H-13b, H-1
C-3	-	103.19	-	
C-4	3.81 (1H, t)	84.14	48.87, 80.47, 80.14, 103.19	H-5, H-2, H-1
C-5	2.87; 2.77 (2H, dd, J = 10.5 Hz)	48.87	49.03, 55.43, 84.14, 103.19	H-13b, H-4
N-6	-	-	-	
C-7	3.19 (2H, t)	49.03	48.87, 55.43, 107.23, 15.86	H-8, H-13b, H-5
C-8	2.52 (2H, t)	15.86	49.03, 107.23	H-7, H-13b
C-8a	-	107.23		
C-9	7.40 (1H, *d*, J = 7.5Hz)	117.12	107.23, 127.25, 118.23, 120.65, 136.51	H-10, H-11
C-9a	-	127.25		
C-10	6.97 (1H, *dd*, *J* = 7.35, 1.1 Hz)	118.23	110.54, 117.12, 120.65, 127.25	H-9, H-11, H-12
C-11	7.05 (1H, *dd*, *J* = 7.5, 1.4 Hz)	120.65	118.23, 117.12, 110.54, 136.51	H-10, H-9, H-12
C-12	7.30 (1H, *d*, *J* = 7.8 Hz)	110.54	120.65, 118.23, 127.25	H-11, H-10
C-12a	-	136.51		
N-13	-	-	-	
C-13a	-	130.90	-	
C-13b	4.07, (1H, *d* , J = 1.8 Hz)	55.43	48.87, 49.03, 107.23, 80.47, 130.90	H-8, H-5, H-7

Coupling patterns and coupling constants (J) in Hz are given in parentheses

4. Discussion

The search for antimalarial molecules from natural sources especially plants is an active area of research and *Chrysophyllum albidum* has received some attention. Although, antimalarial or antiplasmodial activities have been reported in literature, the constituents responsible for the observed activities have not been isolated. The present work was therefore aimed at obtaining from the stem bark of this plant, the constituent(s) that may be responsible for its antimalarial activities.

The result of the chemosuppressive antiplasmodial tests as recorded in Figure 1 showed the percentage chemosuppression elicited by the partitioned fractions. The median effective doses, depicting the doses that will reduce parasitaemia in mice by 50 and 90%, were also determined and recorded in Figure 2.

From the results, the percentage chemosuppression for all the partitioned fractions were significantly different ($p<0.05$) from that of the negative control, showing intrinsic antiplasmodial activities in the fractions, and confirming the earlier reports on the stem bark extract [9,10,11,13,14, 15].

However, a comparison of the percentage chemosuppression of the fractions at each of the tested doses showed that they were all of comparable ($p>0.05$) activities (Fig.1). Also, comparison along the same doses for each of the individual fractions gave the same comparable activities. The effective doses (ED_{50} and ED_{90}) obtained also confirm the comparable activities of these partitioned fractions (Fig 2). The mean survival times for each of the partitioned fractions at each of the doses tested were not significantly different ($p>0.05$) from that elicited by the negative control (100%) (Table 1). Also, those elicited by each of the fractions along the doses were not significantly different from each other neither from that of the original ethanol extract (91.0%). A value of 200% survival time for a curative activity test was taken as an activity against the parasite [20]. The above observation implies that none of the partition fractions could prolong the life of the mice beyond the day of drug administration (Table 3, where is Table 3?).

Considering the percentage survivor of 40% elicited by the negative control mice, the highest value of 80% at the lowest dose of 10mg/kg by the aqueous, at 20mg/kg, by the hexane and the butanol and at 80mg/kg, by the ethyl acetate fraction, the order of activity of the partitioned fractions may be taken as: CAA>CAH=CAB>CAE. We may then infer from the percentage chemosuppression and survival times that the stem bark extract of the plant did not benefit from the partitioning exercise aimed at purifying it for improved antiplasmodial activities but the percentage of mice that survived (percentage survivor), gave a hint of the possible relative effects of the partitioned fractions on the parasite. It now means that any of the fractions could be further purified to obtain the active constituents of the plant. The ethyl acetate fraction with a good yield was therefore chosen for further purification which led to the isolation and characterization of a new indole alkaloid (Compound 1) identified as albidumine.

4.1. Structural elucidation of the alkaloidal compound

The compound **1** isolated from the ethyl acetate fraction gave the characteristics of an indole and was new. The ESI-MS recorded in the positive mode exhibited a $[M+H]^+$ at m/z 303.1 which is compatible with the molecular formula $C_{16}H_{18}N_2O_4$ and with ^1H and ^{13}C NMR spectra data obtained.

Its NMR spectrum was obtained in deuterated methanol (CD_3OD). The ^{13}C NMR spectrum showed 16 signals characterized as three methylenes (sp^3) resonating at δc 15.86, 48.87 and 49.03, one non-aromatic methine (sp^2) resonating at δc 55.43, three non-aromatic oxygenated methine (sp^2) peaks resonating at δc 80.40, 80.47 and 84.14; four aromatic methines (sp^2) peaks resonating at δc 117.12, 118.23, 120.65 and 110.54, five quaternary carbon peaks (four sp^2 and one sp^3) resonating at δc 127.25, 136.51, 130.90, 107.23 and 103.19 by carbon-13 NMR and DEPT experiments. The $^1H - ^1H$ – COSY, HMQC and HMBC experiment established germinal and vicinal hydrogen interactions as well as direct ($^1J_{CH}$) and two and three bonds' correlations between carbons and hydrogen in the structure (Table 2). The presence of the indole nucleus was clearly indicated by the ^1H and ^{13}C aromatic signals (Table 2). The geminal proton signals at δ_H 2.87 (d) and δ_H 2.77 (d) were located on a single carbon (δc 48.87, C-5), those at δ_H 3.19 (m) and δ_H 3.07(m) on C-7 (δc 49.03) and those at δ_H 2.55 (t) and δ_H 2.50 (t) on C-8 (δc 15.86). The aromatic methines at positions 9, 10, 11, and 12 appeared at δc 117.12, δc 118.23, δc 120.65 and δc 110.54 respectively.

The observed ^1H - ^1H COSY indicated the correlations of H-13b to H-1 (cross peak H-7 and H-5); H-7 to H-8 (cross peak H-13b and H-5); H-8 to H-7; H-9 to H-10 (cross peak H-11); H-10 to H-9 and H-11, (cross peak H-12), H-11 to H-10 and H-12 (cross peak H-9); H-12 to H-11(cross peak H-10); and H-1 to H-13b (cross peak H-2); H-2 to H-1 (cross peak H-4); H-4 to H-5 (cross peak H-2); H-5 to H-4 (cross peak H-13b).

Careful analysis of the HMBC spectrum further assisted proper assignment of both aromatic and non-aromatic protons. Particularly diagnostic were the correlations from aromatic methines at position 9 (cross peaks H-9/ C-9a, C-8a, C-10, C-11, and C-12a); position 10 (cross peaks H-10/ C-9, C-9a, C-11, and C-12); position 11 (cross peaks H-11/ C-10, C-9, C-12, and C12a); position 12 (cross peaks H-12/ C-12a, C-9a, C-11, and C-10) and these are diagnostic features of indole nucleus 6].

The non-aromatic signals at δ_H (H-13b) showed long range correlations in the HMBC spectrum to C-48.87 (C-5), 49.03 (C-7), 107.23 (C-8a), 80.40 (C-1), 80.47 (C-2), 130.90 (C-13a); δ_H (H-7) showed linkage with 48.87 (C-5), 55.43 (C-13b),

107.23 (C-8a), 15.86 (C-8); δ_H (H-8) revealed linkage with 49.03 (C-7), 107.23 (C-8a); δ_H (H-1) showed an HMBC correlation with C-13a (130.90) of the indole nucleus, C-2 (δc 80.47), C-3 (103.19) and C-4 (84.14). There is also a cross-peak between δ_H 4.26 (H-2) with C-13b (55.43) of the indole ring, C-1 (δc 80.40) and C-4 (δc 84.14). The HMBC in addition showed cross-peak between δ_H 2.82 (H-5) and C-13b (55.43) and C-7 (δc 49.03) of the indole ring.

Thus, the complete analysis of this compound with the diagnostic tools of ^{1}HNMR, ^{13}CNMR, DEPT, COSY, HMQC and HMBC, enabled the characterization and identification of the new and remarkable indole alkaloid as 4, 5, 7, 8-tetrahydro-1*H*-1, 4-epoxyazepino [1′, 2′: 1, 2] pyrido [3, 4-*b*] indole-2, 3, 3(2*H*, 13*H*, 13b*H*)-triol named as **albidumine,** which is reported for the first time from *Chrysophyllum albidum, Chrysophyllum* genus and from natural sources.

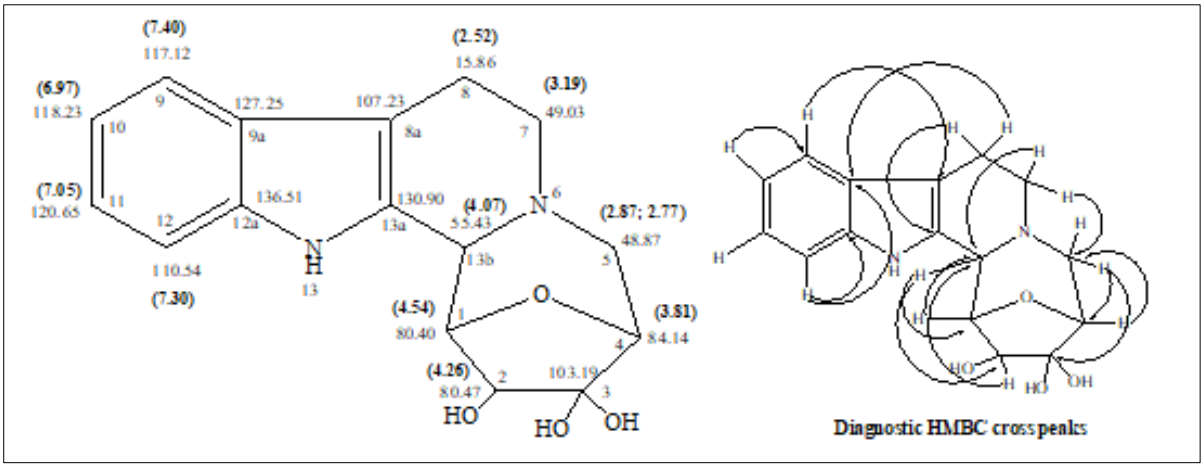

Figure 3 Diagnostic Heteronuclear Multiple Bond Coupling cross peaks of Albumidine

5. Conclusion

To the best of our knowledge, the identified compound has neither been reported in *Chrysophyllum albidum* nor in *Chrysophyllum* genus before. Though alkaloids with indole nucleus had been reported isolated from the seed cotyledons. However, this is the first report of this compound in the plant and in nature.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Umar MB, Ogbadoyi EO, Falarin AA, Adamu Z, Abubakar AN. Evaluation of the Antimalarial and Liver Function Potentials of Methanol Extract of *Chrysophyllum albidum* Stem Bark in Plasmodium berghei - Infected Mice. Journal of Biomedical and Pharmaceutical Sciences, 2018; 1: 106.

[2] World Health Organization Guideline for the treatment of malaria. WHO, Geneva. 2010

[3] Chen W, Van Wyk B-E, Vermaak I, and Viljoen, AM, (). Cape aloes—A review of the Phytochemistry, Pharmacology and Commercialization of Aloe ferox. Phytochemistry Letters, 2012; 5(1): 1-12.

[4] Sangsopha W, Lekphrom R, Kanokmedhakul S, Kanokmedhakul K. Cytotoxic and antimalarial constituents from aerial parts of Sphaeranthus indicus. Phytochemistry Letters, 2016; 17: 278-281.

[5] Tuenter E, Zarev Y, Matheeussen A, Elgorash E, Pieters L, Foubert K. Antiplasmodial prenylated flavonoids from stem bark of Erythrina latissima. Phytochemistry Letters. 2019; 30: 169-172.

[6] Odediran SA, Akosile OR. Bamigboye RA. In Vivo Chemosuppressive Activities of Combinations of Four Nigerian Ethnomedicinal Antimalarial Ferns. GSC Biological and Pharmaceutical Sciences. 2022; 20(01):145–158.

[7] Idowu TO, Onawunmi GO, Ogundaini AO, Adesanya SA. (). Antimicrobial constituents of Chrysophllum albidum seed cotyledons. Nigerian Journal of Natural Products and Medicine 2003; 7:33 – 36.

[8] Idowu TO, Iwalewa EO, Aderogba MA, Akinpelu BA, Ogundaini AO () Antinociceptive, Anti-inflammatory and Antioxidant activities of Eleaginine: An alkaloid isolated from seed cotyledon of *Chrysophyllum albidum*. Journal of Biological Science 2006; 6: 1029–1034.

[9] Adewoye EO, Salami AT, Taiwo VO. Anti-plasmodial and toxicological effects of methanolic bark extract of *Chrysophyllum albidum* in albino mice. Journal of Physiology and Pathophysiology 2010; 1 (1):1 – 9.

[10] Adewoye EO, Salami AT, Jagun AT. Attenuation of Plasmodium berghei berghei -induced Bone Marrow Suppression by Bark Extract and fractions of *Chrysophyllum albidum* on bone marrow response to haemolytic conditions in male albino mice. Afr. J. Biomed. Res. 2013;16 : 47 – 53

[11] Omodamiro OD, Jimoh MA, Eze OS. Anti-Plasmodial and Toxicological Effects of Methanolic Bark Extract of *Chrysophyllum albidium* in Albino Mice. International Journal of Pharmacy & Pharmaceutical Research, 2018; 12 (1): 385-403.

[12] Ademoye M.A, Lajide L, Owolabi BJ, Onubogu CC. GC-MS Analysis of Raw and Roasted Seeds of *Chrysophyllum albidum*, a Medicinal Plant Used for the Treatment of Tuberculosis. International Journal of Scientific Research and Management 2018; 06 (08): 1-11.

[13] Ihekwereme CP, Melidem CO, Maduka IC, Okoyeh, JN. In Vivo Antiplasmodial and Toxicological Effects of Extracts of Fruit Pulp of *Chrysophyllum albidum* G. Don (Sapotaceae). Tropical Journal of Natural Product Research. 2018; 2(3):126-131.

[14] Salami AT, Emikpe BO. Effect of Methanol Extract and Fractions of *Chrysophyllum albidum* Bark on Gut Morphology in bled and Plasmodium-Infected Mice, Archives of Basic and Applied Medicine 2019; 7: 85 – 94.

[15] Odediran SA, Awosode KE, Adegoke TA, Odebunmi KA, Oladunjoye BB, Obasanya AA, Omilani IM, Soyinka KT, Ugbo JU, Ogunleke AJ, Adesida SA Adebajo AC. Combinations of *Chrysophyllum albidum* and Citrus aurantifolia as Antimalarial Agents and their Effects on Orthodox Antimalarial Drugs in Mice. Annals of Complementary and Alternative Medicine. 2020; 2(1): 1007.

[16] Adisa SA, Garba SA, Ijagbone IF, Iyagba AO, Iyamu AO, Kiliani AS, Ufaruna N. Protein and Mineral content of African Apple (*Chrysophyllum albidum*) Proceedings of the 18th Annual Conference of NIST Vitamin C. 2000; 141–146.

[17] Idowu TO, Ogundaini AO, Adesanya SA, Onawunmi GO, Osungunna MO, Obuotor EM, Abegaz BM. Isolation and characterization of chemical constituents from *Chrysophyllum albidum* G. Don-Holl stem-bark extracts and their antioxidant and antibacterial properties. African Journal of Traditional Complementary and Alternative Medicine. 2016; 13 (5):182-189.

[18] Iyiola O. A, Tijani AY, Lateef KM. Antimalarial activity of ethanolic stem bark extract of Alstonia boonei in mice. Asian Journal of Biological Sciences, 4: 235-243.

[19] Peters W. Drug resistance in Plasmodium berghei Vincke and Lips, 1948 I. In Chloroquine resistance. Experimental Parasitology. 1965; 17(1): 80-89.

[20] Mukherjee, P. K. Quality control of herbal drugs: An approach to evaluation of botanicals, (1st ed); 2019; 784.