

Evidence for Non-Toxicity of Aqueous Leaf Extract of *Chrysophyllum albidum*, a Commonly Used Nigerian Folk Medicine

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ABSTRACT

Chrysophyllum albidum is an important herb, used in treating or management of many diseases. Glycosides, flavonoids, saponins, alkaloids and tannins were detected in the extract with flavonoids (3.62 mg/QEq), glycosides (0.194 mg/100g), tannins (8.508mg/TAEq) and alkaloids (6.43%) were quantified. There was no sign of mortality or toxicity after the acute and chronic toxicity tests after 24 hr. Some hematological indices (red blood, white blood cells, packed cell volume, lymphocytes, neutrophil, mean cell volume) increased after the 28-day treatment regime. There was increase in the plasma concentration of high-density lipoprotein and triacylglycerides, with a decrease in the plasma concentration of low-density lipoprotein, with non-significant increase ($p>0.05$) in the activities of antioxidant enzymes. The extract did not show alteration in the liver function. Hence, prolonged consumption of the extract has not shown any sign of toxicity and may not predispose the animal to cardiovascular diseases.

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Authors' Contribution

KOO presented the concept. UDC and KOO arranged resources and supervised the project. JNO and OAL presented the methodology. JNO did data curation. OAL and KOO administered the project. EBO performed formal analysis and investigation. KOO, UDC and EBO wrote the manuscript.

Key words

Toxicological, Glycosides, flavonoids, saponins, alkaloids, tannins

INTRODUCTION

Microorganisms are important components of the ecosystem, where they are responsible for diversified activities. Most microbes are beneficial to humans, while some cause varying degrees of infections (Micheal *et al.*, 2014). These infections could be controlled using substance that possesses the capacity to inhibit their growth or kill them (Serwecinska, 2020). The resistance of microbes to known antibiotics is increasing with its attendant crises, leading to increase in global incidence of infectious diseases (Micheal *et al.*, 2014). The costs of these antibiotics are very high and not readily available making it difficult or impossible for people in under development parts of the world (especially Africa) to access and use

them adequately. This improper usage of antimicrobial agents by patients results in more serious infections that are very difficult to treat (Serwecinska, 2020). In Africa, herbs are employed in the treatment and management of different ailments such as malaria (Enechi *et al.*, 2016), increase in lipid concentration (Olorunnisola *et al.*, 2008), cholinesterase inhibition (Ezema *et al.*, 2021) and antibacterial infection (Goerge *et al.*, 2018).

Chrysophyllum albidum, a medicinal herb commonly used in Nigeria folk medicine belong to Sapotaceae family (Goerge *et al.*, 2018). According to Adebayo *et al.* (2010), it is also found in Cote d' Ivoire, Uganda, Cameroun and Niger. It has been employed as a therapeutic agent against many diseases including diseases caused by bacteria (Goerge *et al.*, 2018; Akubugwo and Ugbogu, 2007), diarrhea and vaginal infections (Dandare *et al.*, 2017), diarrhea (Idowu *et al.*, 2006), and as a hypolipidemic agent (Olorunnisola *et al.*, 2008).

However, there are several concerns about the toxicity or safety associated with the use of cold *Chrysophyllum albidum* leaf extract due to the ingestion of its constituents wholly as it is extracted using cold water. There is the need to scientifically evaluate the toxicity potential of *Chrysophyllum albidum* and not depend on safety information supply by the herbalist.

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MATERIALS AND METHODS

Ethical statement

The study was approved by the Ethics Committee of the Faculty of Biological Sciences, University of Nigeria, (approval number 013/11). Animals were handled in strict accordance with good animal practice as defined by the committee guideline.

Sample collection

Fresh leaf of *Chrysophyllum albidum* was plucked from its natural habitat at Edem-ani in Nsukka local government, south east Nigeria. The leaf was identified by Mr. Felix Nwafor of Pharmacognosy Department, University of Nigeria, Nsukka as *Chrysophyllum albidum* G. Don. (*Sapotaceae*) and deposited in their herbarium with voucher number PCG/UNN/0359.

Sample preparation

The leaf sample was dried at 25°C (room temperature) for 12 days, ground into fine powder using electric blender. 400 g of the powdered sample was soaked in 1000 mL of distilled water for 48 h in an airtight glass container. After maceration, the mixture was filtered, concentrated, and used for analysis.

Experimental animal

Fifteen adult albino rats purchased from Zoology Farm, University of Nigeria, Nsukka were used for the study. They were fed with commercial rat chow. The animals were grouped into five groups of three rats each. Aqueous extract of *Chrysophyllum albidum* was administered orally for 28 days as follows: Group I (5 mL of distilled water/kg body weight), Group II (50 mg/kg body weight), Group III (100 mg/kg body weight), Group IV (400 mg/kg body weight), Group V (500 mg/kg body weight).

Blood and other biological samples were drawn from the animals administered with the extract and the control respectively using chloroform as a sedative agent after day 28. The blood samples were taken to the laboratory and blood indices (haemocrit, white blood cells, haemoglobin concentration, red blood cells, platelet, mean cell volume, platelet concentration, and lymphocyte concentration) were evaluated by an Automated Hematology Sysmex Analyzer (Coulter Electronics, Bedfordshire, England). Body weights of animal were measured using Thermo-weighting balance. Fasting and random blood sugar of the normal rats were first determined followed by that of the experimental rats using Accu-chek glucometer by Roche Diagnostic according to the method of Marks and Dawson (1965).

Toxicity study

The toxicity evaluation was studied according to Lorke Method (1983). Mice orally received varying concentrations of the aqueous extract of *C. albidum* were monitored for any toxicity signs and symptoms for 24h. When no toxicity sign or death was recorded; the concentration of the administered aqueous extract was increased to the chronic toxicity phase.

Serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activity assays

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity was determined using method of Reitman and Frankel (1957). Color intensity was measured at 546nm. Alkaline phosphatase (ALP) activity was evaluated as described by Klein *et al.* (1960).

Assay of catalase and superoxide dismutase activity

Catalase (CAT) was measured using the method of Aebi (1984). This was carried out by monitoring the ultraviolet absorption of the sample at 240 nm as hydrogen peroxide is decomposed in the samples by catalase. Superoxide dismutase (SOD) activity was assayed as described by Martin *et al.* (1987).

Serum lipid profile

Serum high density lipoprotein (HDL) concentration was determined as described by Albers *et al.* (1978). Low density lipoprotein (LDL) concentration was evaluated according to Schriewer *et al.* (1984) method. Triacylglycerides concentration was evaluated according to the methods of Fossati and Prencipe (1982).

Statistical analysis

The statistical package used was the statistical package for social sciences (SPSS), version 1. One-way analysis of variance (ANOVA) was employed in analyzing the data. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical

Plants have served as remedy to the curing and management of different diseases affecting humans. *Chrysophyllum albidum* parts are employed in the treatment of many diseases in traditional medicine practice. According to Iwu (1982) the major challenge associated with traditional medicine, or the use of plant parts as medicinal remedies is non availability or inadequate information on their pharmacological potentials and toxicity. The aqueous

leaf extract yield was 23.77 %. The phytochemicals detected were flavonoids, glycosides, tannins, terpenoids, saponins and alkaloids as shown in Table I. Anthocyanins and steroids were not detected. The presence of many phytochemicals in the aqueous leaf extract of *C. albidum* corroborates the earlier reports of Okoli and Okere (2010), where phenols, tannins and alkaloids were detected. Also, Ibrahim *et al.* (2017) reported that tannins and alkaloids were detected in their work. Its anti-malarial properties could be attributed to the presence of alkaloids, which in addition to the presence of flavonoids could also confer antimicrobial, anti-allergic and anti-cancer benefits (Adisa, 2000), and hallucinogenic activity (Patel *et al.*, 2012). Quantitatively, the concentration of alkaloid was 6.43%, glycoside 0.194 mg/100g and tannins 8.505 mg/TAEq. High concentrations of bioactive compounds were obtained when compared to those reported by Ozioko *et al.* (2021) for *C. albidum*. The difference in the concentration of alkaloids and tannins may be because of the solvent used during extraction process, as the ethanol used may have destroyed some bioactive constituents of the leaf. One of the important antioxidant molecules is tannins. They have the ability to chelate free radicals (Deng *et al.*, 2019), thereby protecting the biological membranes that would have been attacked or degraded (Ashok and Upadhyaya, 2012). Also, some functions of tannins have been reported (Haslam, 1996; Khanbabae and van Ree, 2001). Similarly, glycosides are used in heart failure treatment (Braunwald *et al.*, 1961), hence, the aqueous extract of *C. albidum* could be used in the management of heart failure. Flavonoids, another important phytochemical present in the extract have been reported to possess antioxidant activity and also inhibit the progression of tumour (Kim *et al.*, 1994), and prevent platelet aggregation (Barakat *et al.*, 1993). Saponins were present in the extract. It has antibiotic activity, thus conferring more antimicrobial property to the extract (Sheikh *et al.*, 2013).

Toxicity of *C. albidum*

Toxicity protocol is used in the determination of the harmful potential of an active species given to an organism under a specified period (Krishnaraju *et al.*, 2005) with respect to mortality, changes in body weight, behavior, and other general well-being of the mice (Kharchoufa *et al.*, 2020). The aqueous extract could be adjudged to be practically non-toxic according to the classification of Berezovskaya (2003), since at $LD_{50} = 5000$ mg there was no mortality or other toxicity signs.

Table I. Phytochemical analysis of *C. albidum* aqueous extract.

Phytochemicals	Concentration
Flavonoid	3.62 mg/QEq
Glycoside	0.194mg/100g
Tannin	8.508 mg/TAEq
Alkaloid	6.43%
Anthocyanins	Below detectable limits
Saponin	Below detectable limits
Steroid	Below detectable limits

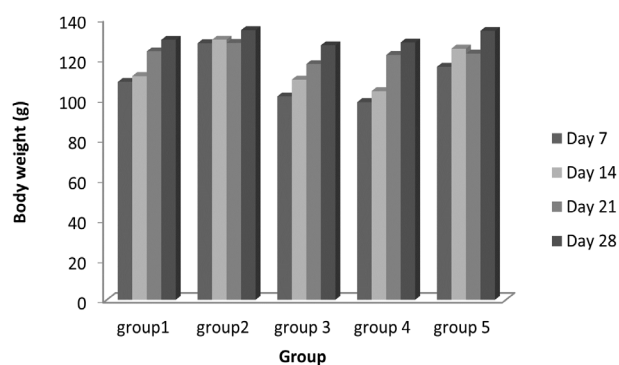


Fig. 1. Effect of aqueous extract of *C. albidum* on body weight.

Effect of body weight

Figure 1 shows the aqueous extract activity on body weight of albino rat. There was a nonsignificant ($p < 0.05$) increase in the body weight of the animals when compared with the control group. Change in body weight is an important indicator of adverse effect caused by an active compound; it shows whether the animal has been exposed to the active species or otherwise (Teo *et al.*, 2002). There was no significant change in the body weight among the groups. This indicates that the aqueous leaf extract of *C. albidum* does not contain any phytochemical that interferes or adversely affect the animal's appetite or growth. The result may be caused by the presence of diverse phytochemicals in the extract that could be working synergistically, thereby altering the metabolic processes not to produce excess biological material that would result to storage in the body or serve as precursors for the biosynthesis of other products. A reduction in the body weight of animals after oral administration of plant extract was reported by (Riva *et al.*, 2013), which they attributed to decrease in water and food intake, or possession of antilipidemic activity by the extract (Ijioma *et al.*, 2014). Also, Chibuogwu *et al.* (2021) reported a loss in body weight of wistar rat in their methanol extract of *J.*

tanjorensis leaf. The differences in the results could be the effect of the solvent used in the extraction since water was used in this study.

Effect on hematological indices

The result of the aqueous leaf extract on hematological indices of albino rat as shown in Table II, indicates that there an increase ($p < 0.05$) in white blood cells (WBC), red blood cells (RBC), packed cell volume (PCV) and mean corpuscular volume (MCV) in groups IV (400mg/kg body wt.) and V (500mg/kg body wt.). Similarly, LYM (lymphocytes) and NEU (neutrophils) concentration increased significantly ($p < 0.05$) when compared to the control. Although, a significant decrease ($p > 0.05$) as shown in Table II.

The roles of some hematological indices in transporting of molecules in the body have made them essential indicators for toxicity evaluation, thus could serve as an indicator for evaluating the presence of toxic molecules in biological tissues. The increase of RBC and WBC concentration ($P > 0.05$) observed across the groups is an indication of a nontoxic molecule and indicates safety on the two important blood indices. Also, the increase in the concentration of PCV suggests the extract could be used in the management of anaemia. The decrease in platelet concentration could be due to low concentration of polyphenols in the extract responsible for the protection of the platelet (Barakat *et al.*, 1993), hence suggesting the extract could initiate or induce thrombocytopenia (Kharchoufa *et al.*, 2020).

Table II. Effect of aqueous extract of leaves of *Chrysophyllu albidum* on haematological profile, liver function profile, Lipid profile, antioxidant enzymes and fasting blood sugar of albino rats.

Haematological parameter	Dosage of leaf extract (mg/kg body weight)				
	Control	50mg	100mg	400mg	500mg
WBC ($\times 10^9/L$)	8.17 \pm 0.51	8.17 \pm 0.51	7.63 \pm 0.03	9.58 \pm 0.48	9.11 \pm 0.55
NEU (%)	24.11 \pm 0.63	26.10 \pm 0.84	28.77 \pm 0.32	25.30 \pm 1.02	25.33 \pm 0.87
RBC ($\times 10^{12}/L$)	8.05 \pm 1.37	11.14 \pm 0.93	10.12 \pm 2.32	9.88 \pm 1.92	10.20 \pm 0.75
Hb (g/dl)	10.07 \pm 3.35	9.86 \pm 1.41	10.01 \pm 0.09	8.97 \pm 0.05	9.26 \pm 0.23
PCV (fl)	31.93 \pm 0.04	32.71 \pm 0.06	35.94 \pm 0.55	33.70 \pm 0.49	37.80 \pm 0.03
MCV (pg)	16.33 \pm 0.60	18.11 \pm 0.02	16.99 \pm 0.18	17.15 \pm 0.93	17.28 \pm 0.22
LYM (%)	65.08 \pm 0.04	65.16 \pm 0.29	68.93 \pm 0.17	66.47 \pm 0.93	66.85 \pm 1.39
PLT ($\times 10^9/L$)	259.82 \pm 0.90	236.66 \pm 1.44	227.13 \pm 0.03	243.18 \pm 2.14	238.04 \pm 1.00
Total protein (mg/dL)	7.13 \pm 0.22	8.16 \pm 1.10	8.39 \pm 0.82	7.47 \pm 2.08	6.98 \pm 0.54
AST (IU/L)	154.16 \pm 0.32	141.39 \pm 1.45	136.91 \pm 0.14	148.04 \pm 2.31	155.09 \pm 0.10
ALT (IU/L)	29.31 \pm 0.11	33.19 \pm 0.63	31.83 \pm 0.92	30.65 \pm 2.91	31.22 \pm 0.66
ALP (IU/L)	86.33 \pm 0.40	84.57 \pm 1.62	83.63 \pm 0.72	79.49 \pm 3.85	82.74 \pm 0.90
HDL (mmol/L)	0.77 \pm 0.04	0.96 \pm 0.17	0.85 \pm 2.73	0.83 \pm 0.64	0.83 \pm 1.59
LDL (mmol/L)	0.23 \pm 0.39	0.04 \pm 0.64	0.15 \pm 1.00	0.17 \pm 2.95	0.17 \pm 0.15
Triacylglyceride (mmol/L)	0.74 \pm 1.72	0.90 \pm 0.40	0.83 \pm 3.03	1.18 \pm 0.59	0.84 \pm 2.17
CAT (IU/L)	153.62 \pm 0.06	155.73 \pm 3.00	151.93 \pm 0.97	154.01 \pm 1.54	155.39 \pm 0.57
SOD (IU/L)	182.38 \pm 4.07	178.51 \pm 0.79	181.37 \pm 0.42	185.49 \pm 3.65	180.17 \pm 1.35
Fasting blood sugar (mg/dL)	115.29 \pm 3.94	95.68 \pm 0.71	138.09 \pm 0.52	105.68 \pm 0.33	118.42 \pm 2.08

WBC, white blood cell; NEU, neutrophils; RBC, red blood cells; Hb, haemoglobin; PVC, packed cell volume; MCV, mean corpuscular volume; LYM, lymphocytes; PLT, platelet count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; HDL, high density lipoprotein; LDL, low density lipoprotein; CAT, catalase; SOD, superoxide dismutase.

Effect on blood sugar

Table II shows the effect of aqueous extract of leaves of *C. albidum* on blood sugar, liver function and lipid profiles and antioxidant enzymes of rats. There was no observable increase in the fasting blood sugar among the groups (Group

II, IV and V). Also, a significant increase ($p > 0.05$) in the fasting blood sugar of animals in Group III (138.09 \pm 0.52) when compared to the control. The observation that the aqueous extract did not cause alterations to the glucose concentration of rat at the studied concentration indicates

that the extract contains some bioactive compounds that are responsible for the proper regulation of carbohydrate metabolism and secretion of insulin. Thus, its consumption may not predispose the consumer to high blood glucose concentration. This activity may be due to the presence of flavonoids in the extract. The presences of flavonoids and polyphenols in plant material have been asserted to be responsible for the proper metabolism of glucose in animals (Ong *et al.*, 2011).

Effect on liver function

The plasma total protein, AST, ALT and ALP were used to determine the health status of the liver. The activity of the leaf extract of *C. albidum* to the experimental animal produced significant increase ($p>0.05$) in the plasma concentration of protein (Groups II and III; dosages of 50 and 100mg/kg body weigh), a nonsignificant increase in group IV and a significant decrease ($p<0.05$) was observed in group V administered with 400 and 500mg/ kg body weight respectively (Table II). This suggests that the oral administration of the extract at high concentration (500 mg) could impair the liver ability to perform its protein biosynthetic function since the liver is the main site of plasma protein. Adeyemi *et al.* (2010) had stated that the decreased in the concentration of plasma proteins suggests an impaired function of the liver. The activity of the liver and kidney is important to ascertain the toxicity study, due to their functions in metabolism and excretion. There was a nonsignificant ($p>0.05$) increase in all the liver enzyme assayed (Table II). This signifies that the function of liver was not overwhelmed by the varying concentrations of the extracts administered. Chibuogwu *et al.* (2021) stated that increase in the liver enzyme activity is an indication of liver damage, thus, there was no damage to the liver of the rats' since there was no increase in the activities of the enzymes assayed (Table II) and could be considered safe for consumption.

Effect on antioxidant enzymes

Similarly, antioxidant enzymes were studied to ascertain their status after the consumption of the extract. There was a nonsignificant increase of CAT and SOD among the groups when compared to the control. SOD is one of the important enzymes that scavenge free radicals in living organisms (Stephanine *et al.*, 2020). The result obtained suggests that the oral administration of the extract did not generate ubiquitous amount of free radicals or that the extract contains high concentration of external antioxidant that would have augmented the scavenging of the excess free radicals produced. The result of the study agrees with the report of Ezema *et al.* (2021). Also, the nonsignificant ($p>0.05$) increase in CAT

activity among all groups (Table II) when compared with the control, is another indication that the aqueous extract did not initiate or generate additional free radicals from the membrane of the rat. CAT plays essential role in the protection or scavenging of free radical in the body of an organism (Ighodaro and Akinloye, 2017). Though, high concentration of polyphenols and vitamins in an extract serve as remedy in mobbing of free radicals in the body of an organism.

Effect on lipid profile

The effect of aqueous leaf extract of *C. albidum* on lipid profile of albino rat is shown in Table II. There was a significant increase ($p>0.05$) in the plasma concentration of HDL and triacylglyceride (TAG) in the test groups after 28 days of orally receiving varying concentrations of the leaf extract. In contrast, a dose dependent decrease ($p<0.05$) in plasma concentration of LDL ($p<0.05$) in all the groups was observed when compared to the control. Previous researchers have reported that increase in HDL concentrations aids in management of cardiovascular diseases (Olukanni *et al.*, 2013). The corresponding increase in HDL concentration and reduction in LDL concentration indicates that prolonged consumption may not predispose one to heart related diseases. This result is corroborated by the report of Adeyemi and Orekoya (2014). The decrease in LDL and increase in TAG also show that extract is safe and may not cause athero-related diseases after exposing animals to it.

CONCLUSION

The cheap and readily available antibiotic aqueous leaf extract of *Chrysophyllum albidum* toxicity potential was evaluated in rat. No mortality recorded at 5000mg/kg body weight. The extract is rich in diverse phytochemicals such as flavonoids, tannins, alkaloids, which would have led to the increase in blood indices such as WBC, RBC, PCV, LYM, NEU and MCV at high concentration of the extract. The extract did not produce excess free radicals in the body and may not predispose the animal to cardiovascular diseases as suggested from the results as the animals did not show any sign of toxicity within the concentrations studied.

Statement of conflict of interest

The authors have declared no conflict of interest.

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