

ANTIOXIDANT ACTIVITIES OF THE LEAVES OF *CHRYSOPHYLLUM ALBIDUM* G.

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ABSTRACT

Chrysophyllum albidum G. is a tropical plant and commonly found in Nigeria. It belongs to the sapotaceae family and used in folklore in the treatment of yellow fever, malaria, diarrhea, vaginal and dermatological infections. The study was aimed at investigating the antioxidant properties of this plant by employing the *in vitro* and *in vivo* experimental models. The effect of DPPH free radical scavenging activity on the fractions of petroleum ether, ethanol, butanol, ethylacetate, and water of *C. albidum* was determined. The ethyl acetate fraction was purified in column chromatography to obtain myricetin rhamnoside. Structure elucidation was done by NMR and mass spectroscopic techniques. Furthermore, ethanol extract was administered to five groups of eight rats per group. The animals in the normal group were administered with vehicle alone for 7 days. The positive control animals were given vehicle on the first four days, and with the vehicle and hepatotoxin (CCl₄) on the fifth, sixth and seventh day. The animals in the treatment category were respectively administered with 500, 1000 and 1500 mg/kg b.w. of extract & distilled water for the first four days, and with distilled water, extract and CCl₄ on the last three days. Animals were subsequently anaesthetized and blood samples were collected for catalase (CAT), malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) assays. The petroleum ether fraction showed the least antiradical activity (4057.5 ± 809.6 g/kg) while ethyl ether exhibited the highest activity (414.4 ± 92.0 g/kg). Myricetin rhamnoside also exhibited an excellent radical scavenging activity (314.1 ± 60.2) which was comparable to the positive control. Result from animal study showed that *C. albidum* exhibited significant (p < 0.05) differences on the activity of CAT, MDA and GSH. The plant could therefore be employed as sources of natural antioxidant boosters and for the treatment of some oxidative stress disorders in which free radicals are implicated.

Keywords: *Chrysophyllum albidum*, radical scavenging, antioxidant parameters, myricetin rhamnoside.

INTRODUCTION

The role of free radicals in disease initiation cannot be overemphasized. Most free radicals such as, hydroxyl radical (OH), the superoxide radical (O²⁻), lipid peroxide radicals and hydrogen peroxide (H₂O₂) are being implicated in some disease conditions. These include: cancer, gastrointestinal inflammation, asthma, cataracts, cardiovascular disease, diabetes mellitus, liver disorder, periodontal disease, and other inflammatory processes. These reactive oxygen species (ROS) are generated as a result of normal biochemical metabolism in the body which is due to high level of exposure to xenobiotics (Pourmorad *et al.*, 2006; Kumpulainen *et al.*, 1999; Cook and Samman, 1996). Pathological conditions result when the generation of ROS induced by stimuli in the organism exceeds the antioxidant capacity of the organism (Yang *et al.*, 2008). The harmful effect of these reactive species in normal metabolic processes which leads to disease condition is a consequence of their interaction with some biological compounds within and outside the cells.

Recently, many natural and synthetic free radical scavengers and antioxidants have been employed in protecting biomolecules against free radical mediated damages. Many medicinal plants from African origin are known to possess antioxidant properties. *Chrysophyllum albidum*, from the sapotaceae family, is commonly found in the Central, Eastern and Western Africa (Adebayo *et al.*, 2010a; Amusa *et al.*, 2003). They are distributed in Nigeria, Uganda, Niger, Cameroun and Cote d' Ivoire (Adebayo *et al.*, 2010a). It is often called the white star apple and distributed throughout the southern part of Nigeria (Idowu *et al.*, 2006). Across Nigeria, it is known by several local names and is generally regarded as a plant with diverse ethno-medicinal uses (Amusa *et al.*, 2003). In South-western Nigeria, the fruit is called "agbalumo" and known as "udara" in South-eastern Nigeria. The bark is used for the treatment of malaria and yellow fever, while the leaf is used as an emollient and for the treatment of skin eruption, stomachache and diarrhea (Idowu *et al.*, 2006). The cotyledons from the seeds of *C. albidum* are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria. The fruit pulp is rich in vitamin C and iron and an excellent

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source of raw material for industries (Akubugwo and Ugbo, 2007). Tannins, flavonoids, terpenoids, proteins, carbohydrates and resins are the phytochemicals that have been reported in *C. albidum* (Akaneme, 2008). Eleagnine, tetrahydro-2-methylharman and skatole have been isolated from this plant and eleagnine was the main compound responsible for its antimicrobial activity (Idowu *et al.*, 2003). The seed cotyledon has been reported to possess anti-hyperglycemic and hypolipidemic effects (Olorunnisola *et al.*, 2003). The search for potent antioxidant agents from medicinal plants cannot be over-emphasized; thus, the study investigated the *in vitro* and *in vivo* antioxidant activities of extracts and an isolated compound from *C. albidum*.

MATERIALS AND METHODS

Plant material

The leaves of *C. albidum* plant were obtained from Covenant University, Canaan land, Ota, Ogun State, Nigeria in November, 2009. Voucher specimen (PCGH 435) was deposited in the Herbarium of Pharmacognosy Department, University of Lagos, Lagos, Nigeria.

Preparation of extracts

The procedure described by Adebayo *et al.* (2010b) was adopted. The leaves of *C. albidum* were obtained and air-dried in the laboratory for 14 days. They were subsequently mashed into fine powder. Four hundred (400) grams of the plant leaves were extracted exhaustively with 95% ethanol. Evaporation of the extract in a rotary evaporator at 40°C yielded 98 g. The residue (98 g) was dissolved in 300 mL deionized water and successively extracted with petroleum ether (0.5 L × 5), ethylacetate (0.5 L × 5), and n-butanol (0.5 L × 5) solvents. The petroleum ether, ethylacetate, n-butanol, and water fractions yielded 27.0, 12.7, 14.1 and 10.5 g, respectively. The fractions were evaporated to dryness under reduced pressure and subsequently screened for their antiradical activity.

Purification and structure elucidation of isolated compound

Ethyl acetate fraction (12.7 g) was fractionated by column chromatography (CC) (130 g silica gel, mesh 100-200; CHCl₃: MeOH 9:1, 7:3, 1:1, 3:7 and 1:4). Fraction 2 was purified by CC (1. Sephadex LH-20 CHCl₃: MeOH 2:1; 2. Silica gel CHCl₃: MeOH 4:1, 3:1) and afforded myricetin 3-rhamnoside (41.8 mg). 5,7-dihydroxy-3-((2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yloxy)-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one, myricetin 3-rhamnoside, yellow powder, C₂₁H₁₈O₁₂, ¹H-NMR (500 MHz, C₅P₅N), δ_H: 6.94 (s, H-2', 6'), 6.51 (d, *J* = 2.9, H-1"), 6.35 (d, *J* = 2.0, H-6), 6.19 (d, *J* = 2.0, H-8), 5.89 (dd, *J* = 1.7, 6.4 Hz, H-4"), 0.95 (d, *J* = 11.2 Hz, CH₃-6"). ¹³C-NMR (100 MHz, CD₃OD), δ_C: 159.4 (s, C-2), 136.3 (s, C-3), 179.7 (s, C-4),

105.8 (s, C-4a), 163.2 (s, C-5), 99.8 (d, C-6), 165.8 (s, C-7), 94.7 (s, C-8), 105.8 (s, 8a), 121.9 (s, C-1'), 109.5 (d, C-2'), 147.0 (s, C-3'), 137.9 (s, C-4'), 146.8 (s, C-5'), 109.5 (d, C-6'), 103.6 (d, C-1"), 71.9 (d, C-2"), 72.0 (d, C-3"), 73.3 (d, C-4"), 72.1 (d, C-5"), 17.7 (q, C-6"). FAB-MS *m/z* (%): 463 [M-H]⁻ (10).

Carbon-13 (¹³C) Nuclear Magnetic Resonance (NMR) and Proton (¹H)-NMR spectra were measured on Bruker AM-400 and DRX-500 spectrometers (Germany) with tetramethylsilane (TMS) as internal standard. FAB-MS was measured on a VG Auto Spec-3000 spectrometer (Britain). Column chromatography was carried out over silica gel (200-300 mesh, Qingdao Marine Chemical Inc., China) and sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co., Ltd., Sweden), respectively.

DPPH antiradical assay

The effects of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potentials of the fractions were determined according to the methods described by Brand-Williams *et al.* (1995) with slight modifications. Aliquots of 5 μL of dimethyl sulphoxide (DMSO) solutions each containing extract or compound of four replicates were added to 195 μL of pure methanol solution of DPPH (0.025 g/L). The absorbance at 515 nm against a methanol blank was read after 30 minutes in a microplate spectrophotometer (SPECTRA MAX 340, USA). Six serially diluted concentrations of samples were prepared. Absorbance of each sample concentration against a methanol blank was measured. The EC₅₀ value, defined as the concentration of the test sample required to decrease the initial DPPH concentration by 50%, was calculated from the non linear regression curve of percentage activity against concentration for each sample.

Experimental animals

Male albino Wistar rats (40) obtained from the University of Agriculture, Abeokuta, Ogun State, Nigeria were used for the experiment. The animals weighed between 200-230 g and they were maintained in 12-h light; 12-h dark at a controlled temperature (25 ± 3°C), humidity (60 ± 5%) and housed in the Department of Biological Sciences, Covenant University, Ogun State, Nigeria. The animals were allowed to acclimatize for six weeks. Feed and water were given *ad libitum*. All animals were treated in accordance with the recommendations of National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH, 1985).

Experimental design

The procedures described by Chakraborti and Handa (1989) were adopted with some modifications. The rats were divided into five groups of eight rats per group. The animals of group A served as normal control group and were administered only vehicle (distilled water, 1 ml/kg b.w.) for 7 days. Animals in group B which served as positive control group were administered with vehicle on

the first four days, and with the vehicle and CCl_4 (2 ml/kg b.w. of 50% solution of CCl_4 in liquid paraffin) on the fifth, sixth and seventh day. The animals of groups C, D and E were respectively administered with 500, 1000 and 1500 mg/kg b.w. of leaf extract & distilled water for the first four days, and with distilled water, leaf extract and CCl_4 on the last three days. Animals were subsequently anaesthetized and blood samples were collected for catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) assays.

Blood collection and preparation of sample

At the end of the treatment period, the rats were anaesthetized in diethylether prior to dissection. The blood was then collected by cardiac puncture into lithium heparinized bottles. Blood was centrifuged at 10,000 rpm for 15 mins into clean bottles and stored at -20°C until required for biochemical assays (Adebayo *et al.*, 2006).

Analysis of biochemical parameters

Commercial test kits obtained from Randox Laboratories, United Kingdom were used for all biochemical parameters measured. Standard methods were used to estimate GSH (Moron *et al.*, 1979), TBARS was analysed and expressed as the amount of MDA formed (Niehaus and Samuelson, 1968). Superoxide dismutase was assayed utilizing the technique of Misra and Fridovich (1972) while catalase activity was performed by the methods of Sinha (1972).

STATISTICAL ANALYSIS

Values were expressed as mean \pm standard error and Tukey's post hoc test was carried to analyze significance of difference between different groups using the statistical analysis software package SPSS (Version 13). Values with $p < 0.05$ were regarded as significant.

RESULTS

Characterization of isolated compound

The compound (fig. 1) obtained was yellow powder and its molecular weight as analyzed by negative FAB-MS was 462. From the ^{13}C -NMR, ^1H -NMR, and DEPT spectra, the molecular formula, $\text{C}_{21}\text{H}_{18}\text{O}_{12}$ was derived. From the spectra data, it was found that the structure of the compound belongs to flavonol glycoside and when the structure was compared with established reference data, it was identified as myricetin 3-rhamnoside (Arot, 1996).

DPPH antiradical assay

The EC_{50} values on the effect of extracts of *C. albidum* on DPPH scavenging activity showed that petroleum ether (4057.5 \pm 809.6 g/kg) had the least antiradical activity while ethyl ether (414.4 \pm 92.0 g/kg) had the highest activity (fig. 2). Similarly, the EC_{50} values of myricetin 3-rhamnoside (TCA-3) and vitamin C (standard control) were 314.1 \pm 60.2 and 127.1 \pm 8.6 g/kg respectively (fig. 2). The order of scavenging activity is such that myricetin 3-rhamnoside > ethylacetate extract > n-butanol extract > ethanol extract > petroleum extract.

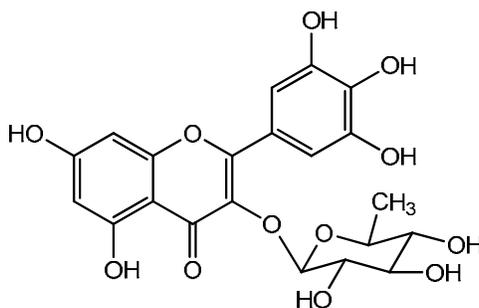
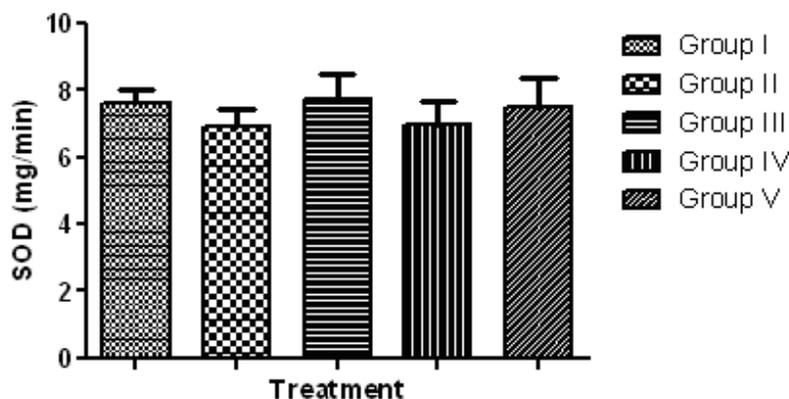


Fig. 1: Structure of myricetin 3-rhamnoside.

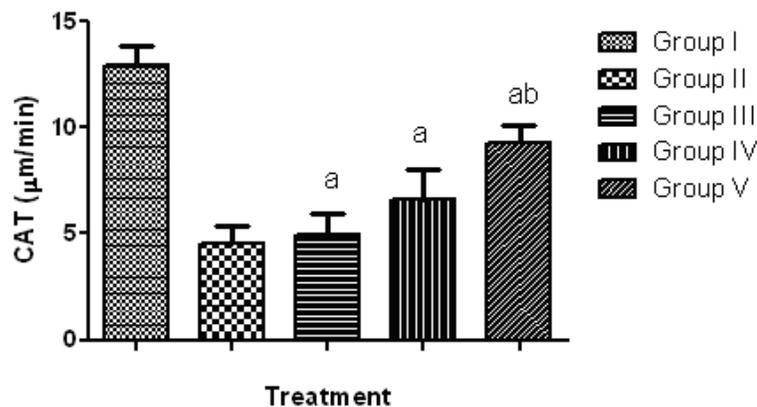


Fig. 2: DPPH scavenging activity of extracts and compound from *Chrysophyllum albidum*.



Values represent mean \pm S.E. of 8 replicates; $p > 0.05$

Fig. 3: Effect of *C. albidum* on superoxide dismutase (SOD) activity in carbon tetrachloride induced liver damage of Wistar rats.



Values represent mean \pm S.E. of 8 replicates; ^a $p < 0.05$, significantly different from negative control (Group I); ^b $p < 0.05$, significantly different from positive control (Group II).

Fig. 4: Effect of *C. albidum* on catalase (CAT) activity of carbon tetrachloride induced liver damage of Wistar rats.

In vivo antioxidant activity

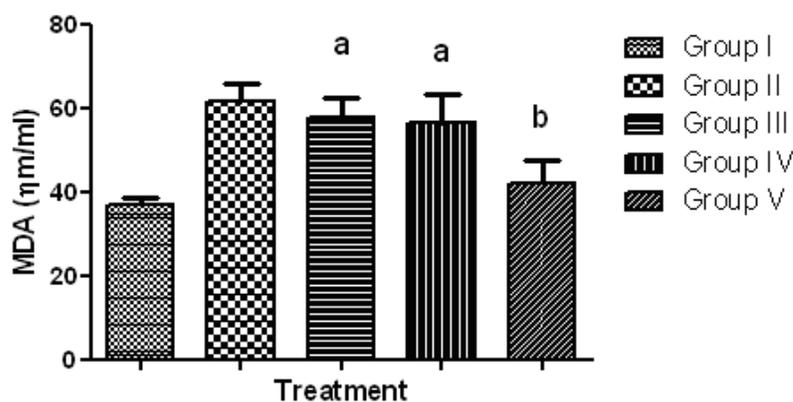
The effect of the ethanolic fraction of *C. albidum* on some biochemical parameters was examined. There was no significant difference in the activity of SOD in all the treated groups (fig. 3). All animals treated with the extract showed a significant ($p < 0.05$) dose-dependent decrease in the activity of CAT when compared with the negative control; however, the group treated with 1500 mg/kg bw had significantly ($p < 0.05$) high CAT activity when compared with the positive control group (fig. 4). Similarly, animals treated with 500 and 1000 mg/kg bw of the extract exhibited a significantly ($p < 0.05$) elevated MDA activity when compared with the negative control group. Also, the group treated with 1500 mg/kg bw had significantly ($p < 0.05$) reduced level of MDA when compared with the positive control group (fig. 5). All the animals in treated groups showed a significantly ($p < 0.05$) lowered level of GSH when compared with both the negative and positive control groups (fig. 6).

DISCUSSION

Free radicals have very important roles in various pathogenesis, inflammatory diseases and can result in necrosis of the liver (Kehrer, 1993; Gressner, 1991). It has been recognized that flavonoids exhibit antioxidant potential and their effects on human health and nutrition are considerable. The mechanisms of action of flavonoid are through chelating or scavenging processes (Cook and Samman, 1996; Kessler *et al.*, 2003). From our *in vitro* study, all the extracts (except petroleum ether extract) from *C. albidum* were rapidly scavenged by the radical, indicating the ability of the extract to donate enough hydrogen atoms quickly. The mechanism of antioxidant effect of DPPH radical is considered to be due to their proton donating potential (Yu *et al.*, 2002). A glucoside flavonoid, myricetin 3-rhamnoside was isolated from the *C. albidum* and found to rapidly scavenge DPPH radical, its effect was comparable to that of vitamin C which

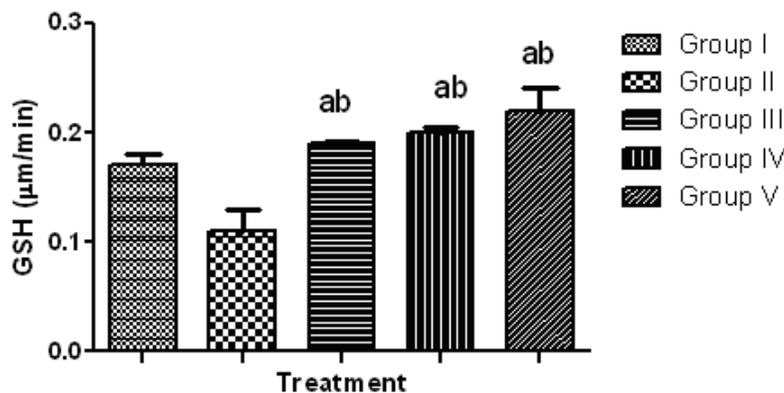
served as the control. Chemically, the remarkable antioxidant properties of flavanoids are due to: i) the hydrogen donating substituents (hydroxyl groups), which is attached to the aromatic ring structures of flavonoids, which enable the flavonoids to undergo a redox reaction and thus helping them to rapidly scavenge free radicals easily; ii) a stable delocalization system, which consist of heterocyclic and aromatic rings as well as multiple unsaturated bonds, and helps to delocalize the resulting free radicals, and iii) the presence of certain structural moieties which are capable of forming transition metal-chelating complexes that can regulate the production of reactive oxygen species such as O_2^{2-} and OH^\cdot (Peng *et al.*, 2003; Rice-Evans *et al.*, 1997). It could be deduced that myricetin isolated from *C. albidum* is a free radical inhibitor or scavenger and thus responsible for conferring the antioxidant property of the plant. Animal study was also conducted to validate the antiradical property of the

plant by examining some antioxidant parameters. Glutathione (GSH) is a tripeptide found in most cells and reacts with the free radicals to protect cells against hydroxyl radical, singlet oxygen and superoxide radical (Schulz *et al.*, 2000). The activity of GSH reduced in CCl_4 control is an indication of decreased liver functions (Ahmad *et al.*, 2002; Hentze *et al.*, 2000). Its level increased significantly in the treated groups to a near normal value; this shows the ability of the leaf extract in increasing the competency of liver in detoxification of xenobiotics, as GSH is a major detoxifier in the liver. This shows that the leaf extract possesses antioxidant properties that helps to stabilize the integrity of cell membrane and also prevent hepatic insult mediated free radicals. There was no significant difference in the levels of SOD across the treated groups, which is the only enzyme that employs the superoxide anions as a substrate and produces the hydrogen peroxide as a metabolite, this



Values represent mean \pm S.E. of 8 replicates; ^a $p < 0.05$, significantly different from negative control (Group I); ^b $p < 0.05$, significantly different from positive control (Group II).

Fig. 5: Effect of *C. albidum* on malondialdehyde (MDA) activity of carbon tetrachloride induced liver damage of Wistar rats.



Values represent mean \pm S.E. of 8 replicates; ^a $p < 0.05$, significantly different from negative control (Group I); ^b $p < 0.05$, significantly different from positive control (Group II).

Fig. 6: Effect of *C. albidum* on reduced glutathione (GSH) activity of carbon tetrachloride induced liver damage of Wistar rats

is more toxic than O₂ radical and has to be disposed by catalase (Harman, 1991; Carrillo *et al.*, 1992). The group treated with 1500 mg/kg of the extract showed a decreased level of MDA. MDA is the end product of lipid peroxidation and measures free radical generation. Thus, validating the scavenging property of the extract at high concentration against free radicals generated. The extract also showed a significant increase in levels of catalase in all the treated groups, indicating the aqueous extract scavenges the hydrogen peroxide, which is generated by SOD.

CONCLUSION

The present investigation showed that the *C. albidum* has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes levels.

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