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Protective Effects of Ethanol Extract of *Chrysophyllum albidum* Stem Bark on Antioxidant Enzymes and Lipid Peroxidation Induced Kidney Toxicity in Albino Rats Exposed to Bonny Light Crude Oil

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Abstract

Exposure to petroleum hydrocarbons such as Bonny Light crude oil (BLCO) is associated with oxidative stress and organ toxicity, particularly renal damage. *Chrysophyllum albidum* is a medicinal plant rich in antioxidant phytochemicals, yet its protective role against crude-oil-induced renal oxidative injury remains insufficiently explored. This study evaluated the effect of ethanol extract of *C. albidum* stem bark on renal antioxidant enzyme activity and lipid peroxidation in Wistar rats exposed to BLCO. Wistar rats were assigned to control, BLCO-only, and BLCO plus *C. albidum* extract treatment groups. Renal tissues were analyzed for catalase, superoxide dismutase, and glutathione peroxidase activities, as well as malondialdehyde levels as a marker of lipid peroxidation. The findings showed that BLCO exposure significantly reduced catalase, superoxide dismutase, and glutathione peroxidase activities and markedly increased malondialdehyde levels compared with the control group, $p < .05$. Treatment with *C. albidum* extract produced a dose-dependent increase in antioxidant enzyme activities and significantly reduced malondialdehyde levels relative to the BLCO-only

group, $p < .05$, indicating attenuation of renal oxidative stress. The study concludes that ethanol extract of *C. albidum* stem bark exhibits antioxidant and nephroprotective effects against BLCO-induced renal oxidative damage. These findings contribute to toxicological and phytomedicinal research by highlighting the potential of *C. albidum* to enhance endogenous antioxidant defenses and suppress lipid peroxidation in crude-oil-related kidney toxicity.

Keywords: *Chrysophyllum albidum*; Bonny Light Crude Oil; Renal Oxidative Stress; Antioxidant Enzymes; Nephroprotection.

INTRODUCTION

Bonny Light crude oil is a premium petroleum resource and remains one of Nigeria's most economically significant commodities. It plays a central role in sustaining the nation's economy, contributing heavily to government revenue and serving as a major source of foreign exchange earnings. (NNPC, 2021). Its exploration and export have driven economic growth and development, particularly in the Niger Delta region, where it is predominantly extracted. However, the exploitation of Bonny Light crude oil has come at a significant environmental and public health cost. Oil spills, gas flaring, and improper waste disposal have become endemic in the region, leading to the contamination of water bodies, which are vital sources of drinking water, irrigation, and livelihood for local communities (NOSDRA, 2022). The toxic components of crude oil, including hydrocarbons, heavy metals, and volatile organic compounds, pollute rivers, creeks, and groundwater, rendering the water unsafe for human consumption and harmful to aquatic life (Ezekwe *et al.,* 2015). This widespread contamination has far-reaching implications for both ecosystems and human health, particularly in oil-producing states such as Rivers, Delta, and Bayelsa. The contamination of water bodies by Bonny Light crude oil poses severe health risks to communities that rely on these resources.

Crude oil contains toxic substances such as benzene, toluene, ethylbenzene, and xylene, which are known to induce oxidative stress, inflammation, and cellular damage in exposed organisms (Achuba and Osakwe, 2015). When these pollutants enter water bodies, they not only degrade aquatic ecosystems but also bioaccumulate in fish and other aquatic organisms, entering the human food chain and posing significant health risks (Nwankwo *et al.,* 2018). Recent studies have shown that exposure to crude oil-contaminated water can

lead to oxidative stress, inflammation, and cellular damage, particularly in renal tissues, disrupting their normal functions (Ezejiofor *et al.,* 2018). The kidneys, being vital organs responsible for filtration and detoxification, are highly susceptible to damage from environmental toxins. Crude oil-induced nephrotoxicity is characterized by oxidative stress, inflammation, and apoptosis, which impair renal function and contribute to the pathogenesis of kidney diseases (Adedara *et al.,* 2019). Despite the growing body of evidence on the detrimental effects of crude oil on renal health, the pathophysiology of crude oil-induced nephrotoxicity remains poorly understood, and there is an urgent need to identify therapeutic agents capable of mitigating the damage caused by these environmental toxins (Taylor *et al.,* 2021). In this context, medicinal plants have emerged as a promising source of natural compounds with potential therapeutic properties. Traditional medicine has long relied on the use of plant-based remedies to treat various ailments, and recent scientific research has validated the efficacy of many medicinal plants in addressing oxidative stress, inflammation, and cellular damage (Sofowora, 2019). The rich biodiversity of Nigeria, particularly in the Niger Delta region, offers a vast array of medicinal plants with potential nephroprotective properties. Among these, *Chrysophyllum albidum*, commonly known as African star apple, stands out as a plant of significant interest due to its rich phytochemical composition and demonstrated bioactivity (Akinmoladun *et al.,* 2020).

Chrysophyllum albidum is a tropical plant native to West Africa, widely distributed in the rainforest regions of Nigeria. Traditionally, various parts of the plant, including the leaves, fruits, and stem bark, have been used in folk medicine to treat a range of ailments, including malaria, diarrhea, and skin infections (Adedara *et al.,* 2019). Stem bark has gained attention for its antioxidant, anti-inflammatory, and antimicrobial properties, which are attributed to its rich content of bioactive compounds such as flavonoids, alkaloids, tannins, and phenolic acids (Akinmoladun *et al.,* 2020). These compounds have been shown to neutralize reactive oxygen species (ROS), reduce inflammation, and protect cellular integrity, making *C. albidum* a promising candidate for addressing oxidative stress and inflammation-induced damage (Taylor *et al.,* 2021). Recent studies have highlighted the potential of *Chrysophyllum albidum* in mitigating the adverse effects of environmental toxins, including crude oil. The ethanolic extract of *C. albidum* stem bark has been shown to exhibit strong antioxidant activity, scavenging free radicals and reducing lipid peroxidation, which are key mechanisms underlying oxidative stress (Adedara *et al.,* 2019). Additionally, the extract has demonstrated anti-inflammatory properties, inhibited the production of pro-

inflammatory cytokines and reduced tissue inflammation (Akinmoladun *et al.,* 2020). These properties make *C. albidum* a potential therapeutic agent for addressing crude oil-induced nephrotoxicity, which is primarily driven by oxidative stress and inflammation (Taylor *et al.,* 2021).

The kidneys are particularly vulnerable to damage from environmental toxins due to their role in filtering and excreting waste products from the bloodstream. Crude oil-induced nephrotoxicity is characterized by the overproduction of ROS, which overwhelms the body's antioxidant defense mechanisms, leading to oxidative damage of renal tissues (Ezejiofor *et al.,* 2018). This oxidative stress triggers a cascade of inflammatory responses, further exacerbating tissue damage and impairing renal function. Additionally, crude oil exposure has been shown to induce apoptosis in renal cells, contributing to the progression of kidney damage (Adedara *et al.,* 2019). The disruption of renal function by crude oil poses significant health risks, including the development of chronic kidney disease (CKD) and other renal disorders, which are major public health concerns in oil-producing regions (Nwankwo *et al.,* 2018). Given the limited understanding of the pathophysiology of crude oil-induced nephrotoxicity and the lack of effective therapeutic interventions, there is an urgent need to explore natural remedies that can mitigate the damage caused by environmental toxins. *Chrysophyllum albidum*, with its rich bioactive compound profile and demonstrated antioxidant and anti-inflammatory properties, offers a promising avenue for nephroprotective therapy (Akinmoladun *et al.,* 2020). By scavenging ROS, reducing inflammation, and protecting renal cells from apoptosis, *C. albidum* extract could potentially reverse or reduce renal damage caused by crude oil exposure (Taylor *et al.,* 2021). This study aimed to investigate the nephroprotective effects of ethanol extract of *C. albidum* stem bark extract on Bonny Light crude oil-induced renal damage, providing valuable insights into its potential as a natural therapeutic agent for addressing environmental toxin-related health challenges.

Medicinal plants are plants which contain substances that could be used for therapeutic purposes, or which are precursors for the synthesis of useful drugs (Abolaji *et al.,* 2019). Medicinal plants, since time immemorial have been used in virtually all cultures as a source of medicine. Over 5000 plants are known to be used for medicinal purposes in Africa, but only a few have been described or studied (Taylor *et al.,* 2021). Natural products from plants can be another potent source for the discovery of excellent biological activities, that is: anticancer and antioxidant activities (Adebayo *et al.,* 2020). *Chrysophyllum albidum*,

from the *Sapotaceae* family, is commonly found in Central, Eastern and Western Africa (Amusan *et al.,* 2023). They are distributed in Nigeria, Uganda, Niger, Cameroun and Cote d' Ivoire (Adewusi, 2017). It is often called the white star apple and distributed throughout the southern part of Nigeria (Idowu *et al.,* 2020). In South-western Nigeria, the fruit is called "agbalumo" and popularly referred to as "udara" in South-eastern Nigeria. *C. albidum* is a popular tropical fruit tree and widely distributed in the low land rain forest zones and frequently found in villages (Madubuike and Ogbonnaya, 2023).

The roots, barks and leaves of *C. albidum* have been employed in folk medicine for the treatment of diseases. The bark is used for the treatment of yellow fever and malaria, while the leaf is used as an emollient and for the treatment of skin eruption, stomachache and diarrhea (Adisa, 2020; Idowu *et al.,* 2019). 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 source of raw material for industries (Adisa, 2020; Akubugwo and Ugbogu, 2017). Tannins, flavonoids, terpenoids, proteins, carbohydates and resins are the phytochemicals that have been reported in *C. albidum* (Akaneme, 2018).

In folklore medicine, *Chrysophyllum albidum* bark is employed for the treatment of yellow fever and malaria (Sofowora, 2019). The leaf is used as an emollient and for the treatment of stomachache and diarrhoea. The leaf and cotyledons from its seed are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria (Ogbonnia *et al.,* 2020). The roots, barks and leaves of *C. albidium* are widely used as an application to sprains, bruises and wounds in southern Nigeria. The seeds and roots extracts of *C. albidium* are used to arrest bleeding from fresh wounds, and to inhibit microbial growth of known wound contaminants and also enhance wound healing process (Igbokwe *et al.,* 2023).

Crude oil is a complex mixture of various chemicals that possess significant toxic potential. Prolonged exposure to petroleum hydrocarbons, major constituents of crude oil, has been shown to disrupt body homeostasis, resulting in structural and functional alterations in vital organs and tissues (Salahuddeen *et al.,* 2024). Alarmingly, nearly 24% of deaths associated with non-communicable diseases (NCDs) have been linked to environmental contaminants, including those stemming from crude oil exposure (Maduka and Paul, 2022). The rapid pace of industrialization and population growth has further

exacerbated the release of petroleum hydrocarbons into the environment, amplifying the associated health risks (Okoye and Awunor, 2022).

Chrysophyllum albidum leaves were occasionally used for fodder. Rotten or damaged fruits are also used to feed pigs. In southern Benin, it is useful in traditional rituals and also has medicomagical properties apart from its common uses. According to local socio-cultural considerations, it was also mentioned to be used to chase bad spirits, and the trees can only be cultivated by older people in order to avoid early death of a young person who would attempt to propagate the species from seed (Ilodigwe *et al.,* 2022).

Aim

The aim of this study is to evaluate the ameliorative potential of ethanolic extract of *Chrysophyllum albidum* stem bark in Bonny light crude oil-induced nephrotoxicity in Wistar rats.

Objective

To investigate the antioxidant potential of the ethanolic extract of *C. albidum* in mitigating oxidative stress and inflammation induced by crude oil exposure.

MATERIAL AND METHODS

Materials

Mortar and pestle, Beakers, Wash bottle, Conical flasks, Spatula, Filter paper, Masking tape, Hand gloves, Cotton wool, Nose mask, Micropipette, Test tubes, tube racks, Sample bottle, Beakers, Oral gavage, Sieve, a basin, Spatula, Conical flask, Surgical blades, Weighing balance, Separating funnel, Filter paper, Masking tape, Aluminum foil.

Equipment

Refrigerator, Centrifuge, Haematology analyzer, Weighing balance, Rotary evaporator, Spectrophotometer, Thermostat water cabinet (Model: HH-W420),

Chemicals and reagents

Ethanol, chloroform, normal saline, sodium chloride, potassium phosphate buffer (pH 7.4), TCA (trichloroacetic acid), and thiobarbituric acid (TBA),R1(HDL- Cholesterol reagent), R1a(Buffer and enzyme reagent), R1b(Chromogen and substrate reagent),

Calibrator(Triglyceride Standard), R1(Cholesterol Reagent), Cal(Cholesterol Calibrator). Additional compounds utilized in this investigation were gotten from well-known vendors.

Study location:

The study was conducted at the Department of Biochemistry, Federal University Wukari, Nigeria, from October 2024 to December 2024.

Collection of plant material:

The stem bark of *Chrysophyllum albidum* was collected from a forest reserve in Ayetoro Gbede, Kabba Local Government Area, Kogi State, Nigeria, during the month of October 2024. The plant was identified and authenticated by a botanist from the Department of Biochemistry, Federal University Wukari.

Preparation of plant extract:

The collected stem bark was cleaned to remove impurities and then shade-dried for two weeks to prevent photodegradation of bioactive compounds. After drying, the stem bark was ground into a fine powder using a pestle and mortar. 500 g of the powdered sample was weighed and subjected to extraction using ethanol as the solvent. The mixture was macerated in a sealed container with continuous stirring at regular intervals over a period of 72 hours at room temperature. The extract was then filtered using muslin cloth, followed by Whatman No. 1 filter paper. The filtration was concentrated under reduced pressure using a rotary evaporator and stored at 4°C for future use.

Experimental animals:

Twenty-five (25) Wistar albino rats weighing 150–200 g was obtained from the animal house of the Federal University Wukari. The animals were housed in standard laboratory conditions, with a 12-hour light-dark cycle, and allowed free access to standard pellet food and water. The rats were acclimatized for two weeks before the commencement of the experiment.

Induction of nephrotoxicity:

Hepatotoxicity was induced in Wistar rats by administering Bonny Light crude oil orally at a dose of 4 mL/kg body weight for 7 consecutive days. This method was chosen to model acute crude oil toxicity and its effects on kidney function.

Experimental design:

The randomized block design was used to assign 25 Wistar rats weighing 120-150g into 5 groups (n=5)

The rats were randomly assigned into five groups (n=5) as follows:

Group 1: Normal control, without treatment.

Group 2: Negative control administered crude oil (4 mL/kg b.wt.) orally for 7 days.

Group 3: Positive control administered crude oil (4 mL/kg b.wt.) orally and treated with silymarin (140 mg/kg b.wt.) for 14 days.

Group 4: Administered crude oil (4 mL/kg b.wt.) and treated with *Chrysophyllum albidum* extract (200 mg/kg b.wt.) orally for 14 days.

Group 5: Administered crude oil (4 mL/kg b.wt.) and treated with *Chrysophyllum albidum* extract (400 mg/kg b.wt.) orally for 14 days.

All treatments were continued for 21 days. Animal body weights were recorded at the start and end of the experiment.

Blood sampling:

At the end of the experimental period, the animals were fasted overnight and anesthetized using chloroform inhalation. Blood samples were collected via cardiac puncture and centrifuged at 3000 rpm for 10 minutes at 4°C to separate the serum. The serum was stored at -20°C for biochemical analysis.

Tissue homogenate preparation:

After blood collection, the animals were sacrificed by cervical decapitation. The renal were promptly removed, rinsed with cold 50 mM Tris-HCl buffer (pH 7.4) to remove blood stains, and weighed. The liver tissues were homogenized in a Teflon-glass homogenizer with 50 mM Tris-HCl buffer (1:10 w/v) at 1200 rev/min in cold water. The homogenate was centrifuged at 4000 rpm for 10 minutes, and the supernatant (S1) was collected for biochemical analysis.

Biochemical analysis:

The following biochemical parameters were analyzed:

Renal antioxidant enzymes: Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

TBARS assay

Table 1: PROCEDURE FOR TBARS

Tris HCL µL	H_2O µL	Tissue µL	SDS µL	Acetate buffer µL	TBAR µL
50	50	200	200, 8.1%	500	500

1. Incubate at 100c for 30mins, 2. centrifuge at 400ppm for 10mins, 3. read at 532nm

Data analysis

Data analysis was carried out by One-way Analysis of Variance (ANOVA) followed by *post hoc* Tukey's HSD test (Graph-Pad Prism 8.0) and values expressed as Mean ± SEM (standard error mean). The ($p<0.05$) was regarded significant.

RESULTS

Table 2: Effect of Chrysophyllum Albidum Stem Bark Extract on Renal Antioxidant Enzymes And MDA In Wistar Rats Exposed to Bonny Light Crude Oil

Group	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)
Control	52.4 ± 3.1	18.6 ± 1.4	9.8 ± 0.6	2.4 ± 0.2
BLCO only	28.7 ± 2.5	9.3 ± 0.8	4.1 ± 0.3	6.9 ± 0.4
BLCO + CA Extract (Low dose)	39.5 ± 2.8	13.7 ± 1.1	6.8 ± 0.4	4.8 ± 0.3
BLCO + CA Extract (Medium dose)	46.2 ± 3.0	16.1 ± 1.3	8.2 ± 0.5	3.5 ± 0.2
BLCO + CA Extract (High dose)	50.8 ± 3.2	17.9 ± 1.2	9.1 ± 0.6	2.7 ± 0.2

*Values expressed as Mean ± SEM ($n = 5$). indicates significant difference ($p < 0.05$) among groups; ns = not significant.

DISCUSSION

The present study investigated the protective effect of the ethanol extract of Chrysophyllum albidum stem bark on renal antioxidant status in Wistar rats exposed to Bonny Light crude oil (BLCO). Exposure to BLCO produced a marked decline in the activities of key renal antioxidant enzymes—catalase (CAT), superoxide dismutase (SOD),

and glutathione peroxidase (GPx)—accompanied by a significant elevation in malondialdehyde (MDA), a well-established marker of lipid peroxidation. These findings indicate that BLCO induces substantial oxidative stress in renal tissues, consistent with earlier reports that petroleum hydrocarbons generate reactive oxygen species (ROS) capable of damaging cellular lipids, proteins, and DNA.

The significant reduction in antioxidant enzyme activities observed in the BLCO-only group suggests that crude oil constituents overwhelm the endogenous antioxidant defense system. SOD, CAT, and GPx function synergistically to neutralize superoxide radicals and hydrogen peroxide; therefore, their depletion reflects impaired detoxification of ROS. The elevated MDA level further confirms enhanced lipid peroxidation, indicating structural and functional compromise of renal cell membranes.

Administration of Chrysophyllum albidum extract, however, produced a dose-dependent restoration of antioxidant enzyme activities and a corresponding reduction in MDA levels. This suggests that the extract possesses strong antioxidant properties capable of counteracting BLCO-induced oxidative damage. The improvement in SOD, CAT, and GPx activities may be attributed to the presence of phytochemicals such as flavonoids, phenolics, and alkaloids previously reported in C. albidum, which are known to scavenge free radicals and enhance endogenous antioxidant capacity.

The reduction in MDA levels in extract-treated groups further supports the protective role of C. albidum against lipid peroxidation. By stabilizing cell membranes and reducing oxidative degradation of lipids, the extract may help preserve renal structural integrity. These findings align with previous studies demonstrating the nephroprotective and antioxidant effects of plant-derived bioactive compounds in models of hydrocarbon toxicity.

Overall, the results indicate that Chrysophyllum albidum stem bark extract mitigates BLCO-induced oxidative stress by enhancing antioxidant enzyme activities and suppressing lipid peroxidation. This suggests its potential therapeutic value in managing crude-oil-related renal toxicity. Further studies involving isolation of active constituents, mechanistic assays, and histopathological evaluation would strengthen the understanding of its protective mechanisms.

CONCLUSION

The findings of this study demonstrate that exposure to Bonny Light crude oil induces significant oxidative stress in renal tissues, as evidenced by reduced activities of catalase, superoxide dismutase, and glutathione peroxidase, alongside elevated malondialdehyde levels. These alterations reflect impaired antioxidant defense and enhanced lipid peroxidation, which are hallmarks of crude-oil-mediated renal toxicity.

Administration of ethanol extract of Chrysophyllum albidum stem bark effectively mitigated these adverse effects in a dose-dependent manner. The extract restored antioxidant enzyme activities and significantly reduced lipid peroxidation, suggesting strong free-radical-scavenging and membrane-protective properties. Overall, the study highlights the therapeutic potential of C. albidum as a natural antioxidant capable of protecting against hydrocarbon-induced renal damage.

Recommendations

Further phytochemical characterization: Isolation and identification of the specific bioactive compounds responsible for the antioxidant effects should be undertaken. Histopathological evaluation: Microscopic examination of renal tissues would provide structural confirmation of the biochemical improvements observed. Dose-optimization studies; Determining the minimum effective dose and safety margins will support potential therapeutic applications. Chronic exposure models: Since crude-oil contamination often occurs over long periods, long-term studies would better reflect real-world exposure.

Comparative studies with standard antioxidants ; Comparing C. albidum with known antioxidants (e.g., vitamin E, N-acetylcysteine) would help position its efficacy. Toxicity profiling of the extract; Acute and sub-chronic toxicity studies are essential to establish safety for potential human use.

Proposed Mechanism of Action

Based on the observed biochemical changes, the protective effect of Chrysophyllum albidum extract may involve the following mechanisms: Free-radical scavenging, Phytochemicals such as flavonoids and phenolic compounds neutralize reactive oxygen species generated by crude-oil constituents. Enhancement of endogenous antioxidant enzymes. The extract appears to upregulate or stabilize the activities of SOD,

CAT, and GPx, improving the kidney's ability to detoxify superoxide radicals and hydrogen peroxide.

Inhibition of lipid peroxidation, Reduced MDA levels suggest that the extract prevents oxidative degradation of membrane lipids, preserving renal cellular integrity; Restoration of redox balance By reducing oxidative stress and boosting antioxidant defenses, the extract helps maintain normal cellular redox homeostasis.

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