Tropical Journal of Drug Research March 2026; 3 (2): 390 - 396
ISSN: 2476-8065 (Electronic)
Available online at http://www.tjdr.org
https://doi.org/10.26538/tjdr/v3i2.3

Original Research Article

In vitro Assessment of Antiplasmodial and Antioxidant Potentials of *Chrysophyllum albidum* Kernel Extracts

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Sent for review: 10 June 2025 Revised accepted: 04 April 2026

Abstract

Purpose*:* To evaluate the *In vitro* antiplasmodial, cytotoxic and antioxidant activities of *Chrysophyllum albidum* (African star apple) kernel extracts, with emphasis on their potential as dual-function antimalarial agents.
Methods*:* Ethanol, aqueous and essential oil extracts of *C. albidum* kernels were prepared using standard extraction procedures. Antiplasmodial activity was assessed against *Plasmodium falciparum* 3D7 (chloroquine-sensitive) and Dd2 (chloroquine-resistant) strains using a lactate dehydrogenase (LDH) fluorescence assay at concentrations of 1.56–25 µg/mL, with chloroquine and artemisinin as reference drugs and resistance index was dertermined. Cytotoxicity was evaluated on RAW 264.7 macrophage cells at 62.5–1000 µg/mL using a resazurin-based assay to determine CC_{50} and selectivity index (SI). Antioxidant activity was determined using DPPH (1–30 µg/mL), nitric oxide and hydrogen peroxide (10–100 µg/mL) scavenging assays. IC_{50} values were calculated by nonlinear regression analysis.
Results*:* Ethanol and essential oil extracts exhibited notable antiplasmodial activity (IC_{50} : 4.08–5.50 µg/mL) against both parasite strains, with slightly higher efficacy against the 3D7 strain compared to Dd2. The aqueous extract showed comparatively higher IC_{50} values, indicating lower potency. All extracts demonstrated low cytotoxicity (CC_{50} > 479 µg/mL) and high selectivity indices (SI > 10), suggesting preferential toxicity towards the parasite. Resistance indices (≤ 1.5) indicated minimal cross-resistance with standard drugs. The extracts also showed strong antioxidant activity across all assays, with IC_{50} values ranging from 33 to 46 µg/mL, and were most effective in DPPH radical scavenging, comparable to ascorbic acid.
Conclusion: These findings support the traditional use of *C. albidum* kernel extracts as potential sources of antiplasmodial and antioxidant agents, warranting further isolation of active compounds.

Keywords: *Chrysophyllum albidum*, kernel extract, antiplasmodial activity, *Plasmodium falciparum*, antioxidant activity, selectivity index

Tropical Journal of Drug Research is indexed by Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals
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INTRODUCTION

Malaria remains a major public health challenge, particularly in sub-Saharan Africa, necessitating the continuous search for new, effective and affordable antiplasmodial agents. Medicinal plants represent a valuable source of bioactive compounds with antiplasmodial and antioxidant properties.[1] The emergence of resistance to standard drugs like chloroquine and artemisinin derivatives necessitates the search for new therapeutic agents from natural sources.[2] Oxidative stress also plays a significant role in malaria pathogenesis, as plasmodium infection induce excessive production of reactive oxygen species during hemoglobin digestion and host immune responses.[3] While these reactive species contribute to parasite killing, their overproduction leads to oxidative damage of erythrocytes, resulting in hemolysis. Thus, antioxidant activity may help reduce malaria-associated complication while complementing antiplasmodial effects.[4]

Chrysophyllum albidum G. Don (Sapotaceae), commonly known as African star apple, is indigenous to Nigeria and other West African countries. Different parts of the plant, including the bark, leaves, fruit pulp. and seeds (kernel), have been used in traditional medicine for treating malaria infection and oxidative stress-related ailments. Previous studies have reported in vivo antiplasmodial activity of its fruit pulp and seeds, as well as antioxidant properties of its leaves and stem bark.[5] However, limited data exist on the kernel (seed cotyledon) extracts. This study investigates the *In vitro* antiplasmodial activity and antioxidant potential of various kernel extracts of *C. albidum*

MATERIALS AND METHODS

Plant Material and Extraction

The Kernel of *Chrysophyllum albidum* was gotten on 23rd of December 2025 from Abraka main market, Ethiope East Local Government Area of Delta State, Nigeria and was authenticated by a taxonomist at the Delta State University (DELSU) Herbarium Unit with voucher (DELSU-H/CA/1127).

Ethanol, aqueous (decoction), and essential oil extracts of *Chrysophyllum albidum* kernel were prepared using standard methods describe by Joseue (2014).[6] The ethanol extract was obtained by macerating 50 g of kernel powder in 1 L of ethanol for 72 h, followed by filtration and oven-drying at 40 °C. The aqueous extract was prepared by steeping 50 g of powder in 1 L of hot distilled water, filtering after cooling. and drying at 50 °C. The essential oil was extracted using ethanol as a solvent and stored at 2 ℃ in brown bottles until use.[7]

Antiplasmodial Activity of *C. albidum* kernel extracts

Plasmodium falciparum strains (chloroquine-sensitive 3D7 and multidrug-resistant Dd2) were cultured in human O+ red blood cells using the Trager and Jensen method in supplemented RPMI 1640 medium under controlled gaseous conditions (92% N_2, 5% CO_2, and 3% O_2) at 37 °C.[8] Cultures were synchronized to the ring stage using D-sorbitol before assays. *In vitro* antiplasmodial activity was evaluated using an LDH fluorescence assay, where synchronized parasites were exposed to different concentrations of kernel extracts (1.56, 3.12, 6.25, 12.5 and 25 µg/mL), with artemisinin and chloroquine as positive controls with concentrations ranging from 25 to 1.56 µg/mL and 1% DMSO as a negative control, followed by fluorescence measurement to determine IC_{50} and resistance index.[9]

Resistance index (RI)

$$(RI) = \frac{IC_{50}\ Dd2}{IC_{50}\ 3D7}$$

Cytotoxicity of C. albidum kernel extracts against RAW Cell line

Cytotoxicity of the extracts was assessed on RAW 264.7 macrophage cells using a resazurin-based assay. Cells were seeded into a 96-well plates, treated with varying extract concentrations (62.5, 125, 250, 500 and 1000 µg/mL). and incubated for 48 h, followed by resazurin solution (0.15 µg/mL) addition. Absorbance was measured at 540 nm to determine the CC_{50} values. and the selectivity index (SI) was subsequently calculated.[10]

Selective index (SI)

$$(SI) = \frac{CC_{50}}{IC_{50}}$$

Antioxidant Activity

The antioxidant activity of the extracts was evaluated using DPPH radical scavenging, hydrogen peroxide scavenging. and nitric oxide inhibition assays. DPPH scavenging activity was determined by measuring percentage inhibition across different extract concentrations (30. 10, 3, and 1 µg/mL) using ascorbic acid as a reference standard with the same concentration range.[11] Hydrogen peroxide scavenging capacity was assessed spectrophotometrically at 230 nm at various concentrations (100. 50, 25, and 10 µg/mL), while nitric oxide inhibition was measured based on chromophore formation at 530 nm with various concentrations (100. 50, 25, and 10 µg/mL), and

antioxidant potential expressed as percentage inhibition in all assays.[12]

STATISTICAL ANALYSIS

All experiments for the *In vitro* antiplasmodial and antioxidant assays were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation (SD). Antiplasmodial activity was calculated as percentage parasite inhibition relative to untreated controls, while antioxidant activities were expressed as percentage radical scavenging for DPPH assay, Nitric oxide scavenging assay, and Hydrogen peroxide scavenging assay. The half-maximal inhibitory concentration (IC_{50}) values for both antiplasmodial and antioxidant assays were determined by nonlinear regression analysis of log-transformed concentrations against percentage inhibition using a sigmoidal dose–response (variable slope) model. The goodness of fit was assessed using the coefficient of determination (R^2), and IC_{50} values were reported with 95% confidence intervals (95% CI). Statistical comparisons among different extracts and standard compounds were carried out using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. Pearson correlation analysis was used to evaluate the relationship between antiplasmodial activity and antioxidant capacities. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. All analyses were performed using GraphPad Prism (version X.X).[13]

RESULTS AND DISCUSSIONS

The present study evaluated the antiplasmodial activity of different extracts against Pf3D7 and PfDd2 using the standard *In vitro* culture technique described by Trager and Jensen method[14].

Antiplasmodial Activity

In vitro antiplasmodial activity of *C. albidum* kernel extracts against Pf3D7 and PfDd2 expressed as IC_{50} values are displayed in figure 1. The essential oil and ethanol extracts exhibited strong antiplasmodial activity against both parasite strains, with IC_{50} values comparable to standard antimalarial drugs. The aqueous extract showed relatively higher IC_{50} values, indicating lower potency. Chloroquine and artemisinin demonstrated the lowest IC_{50} values, confirming their high efficacy. Overall, the extracts were more active against the 3D7 strain than the Dd2 strain.

The results demonstrated that all tested extracts exhibited measurable inhibitory activity against

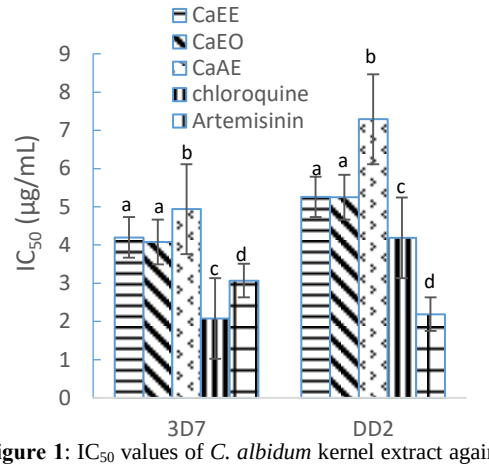

Figure 1: IC_{50} values of *C. albidum* kernel extract against *P. falciparum* 3D7 and Dd2 strains.
CaEE – *C. albidum* Ethanol extract
CaEO – *C. albidum* Essential oil extract
CaAE – *C. albidum* Aqueous extract
Values that bear different alphabets for each strain differ significantly.

both parasite strains, although with varying degrees of potency. Among the extracts, the essential oil and ethanol extracts showed relatively higher antiplasmodial activity compared to the aqueous extract, with lower IC_{50} values observed against both 3D7 and Dd2 strains. This suggests that the bioactive constituents responsible for the antiplasmodial effect are more efficiently extracted in organic solvents than in water. The comparatively lower activity of the aqueous extract may be attributed to the limited solubility of certain active phytochemicals in polar solvents. Similar observations have been reported in previous studies, where organic extracts demonstrated enhanced antiplasmodial efficacy due to higher concentrations of lipophilic bioactive compounds.[15] The observed IC_{50} values of the extracts (ranging approximately from 4.08 to 7.29 µg/mL) indicate moderate antiplasmodial activity. According to established criteria for plant extracts, IC_{50} values below 10 µg/mL are considered indicative of promising antiplasmodial potential[16]. Therefore, the findings suggest that the plant extracts possess bioactive compounds that may serve as leads for the development of novel antimalarial agents. The standard drugs, Chloroquine and Artemisinin, also demonstrated inhibitory activity against the parasite strains. However, their IC_{50} values were observed to be higher than the nanomolar range commonly reported in the literature. This deviation may be attributed to several factors, including differences in experimental conditions such as parasite density, hematocrit levels, and incubation duration. In addition, the use of microscopy-based assessment methods, which are generally less sensitive than

fluorescence or radioisotopic assays, may contribute to relatively elevated IC_{50} values.[17]

Resistance Index (RI)

The resistance index (RI) of the kernel extracts and standard drugs, which indicates the level of resistance of the Dd2 strain relative to the 3D7 strain as shown in figure 2. All extracts exhibited low resistance indices (R I $\leq$ 1.5), indicating minimal cross-resistance with standard antimalarial drugs to which the Plasmodium falciparum Dd2 strain is resistant, particularly chloroquine. This suggests that the extracts may possess distinct mechanisms of action and retain efficacy against chloroquine-resistant parasites. The aqueous extract showed a slightly higher RI compared to the essential oil and ethanol extracts, although still within the range indicating low cross-resistance.[18]

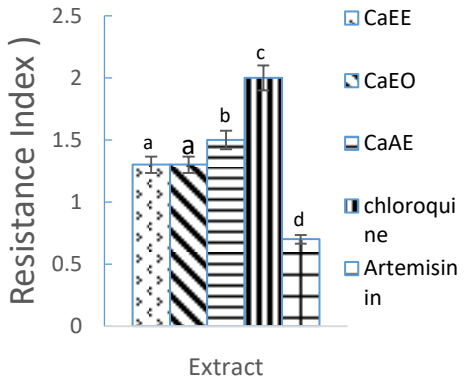

Figure 2: Resistance index values of C. albidum kernel extracts calculated from IC50 value against Plasmodium falciparum 3D7 and Dd2 strains. RI>1 indicate resistance.
CaEE – C. albidum Ethanol extract
CaEO – C. albidum Essential oil extract
CaAE – C. albidum Aqueous extract
Values that bear different alphabet differ significantly.

Cytotoxicity (CC_{50})

The cytotoxic effects of *C. albidum* kernel extracts on mammalian cells are presented in figure 3, expressed as CC_{50} values. All extracts demonstrated high CC_{50} values, indicating low cytotoxicity. The aqueous extract showed the highest CC_{50} value, followed by the essential oil and ethanol extracts. These results suggest that the extracts are relatively safe at concentrations effective against *Plasmodium falciparum*. Similar antiplasmodial effects have been reported for methanolic bark extracts of *C. albidum*, which reduced parasitaemia in vivo with minimal toxicity, indicating the presence of bioactive compounds across plant parts.[19] Recent isolation of a novel indole alkaloid (albidumine)

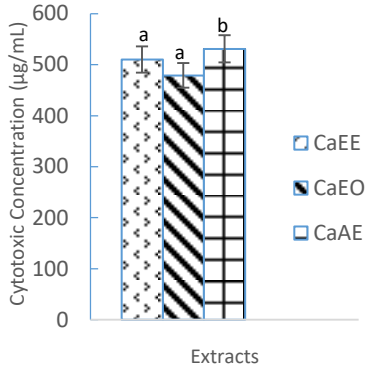

Figure 3: Cytotoxicity profile of C. albidum kernel extracts.
CaEE – C. albidum Ethanol extract
CaEO – C. albidum Eseential oil extract
CaAE – C. albidum Aqueous extract
Values that bear different alphabet differ significantly

from stem bark fractions further supports the antimalarial potential of *C. albidum*, with chemosuppressive activity in *Plasmodium berghei* models.[20]

Selectivity Index (SI)

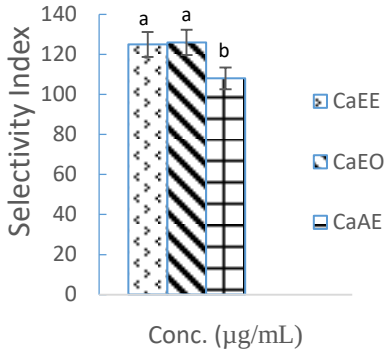

Figure 4: Selective index of C. albidum kernel extract against *P. falciparum.*
CaEE – *C. albidum* Ethanol extract
CaEO – *C. albidum* Eseential oil extract
CaAE – *C. albidum* Aqueous extract
Values that bear different alphabet differ significantly

Figure 4, presents the selectivity index (SI) of the extracts, which reflects their safety and specificity toward the malaria parasite compared to mammalian cells. All extracts showed high selectivity index, with the ethanol and essential oil extracts exhibiting the highest SI values. This indicates that the extracts are more toxic to the parasite than to host cells, supporting their potential as safe antimalarial agents. High selectivity index (>100) and CC_{50} values (>479 µg/mL) indicate

preferential activity against parasites over host cells. The aqueous extract showed the highest CC_{50} value, followed by the essential oil and ethanol extracts, supporting a favorable safety profile consistent with traditional uses.

Antioxidant Activity

Assessment of antioxidant activities of the extracts was done using nitric oxide, hydrogen peroxide, and DPPH radical scavenging assays as shown in figure 5, 6 and 7 below, the essential oil and ethanol extracts demonstrated strong antioxidant activities across all assays, with IC_{50} values comparable to ascorbic acid. The aqueous extract showed slightly reduced antioxidant potency. Among the assays, the extracts were most effective against DPPH radicals, indicating strong free radical scavenging ability.

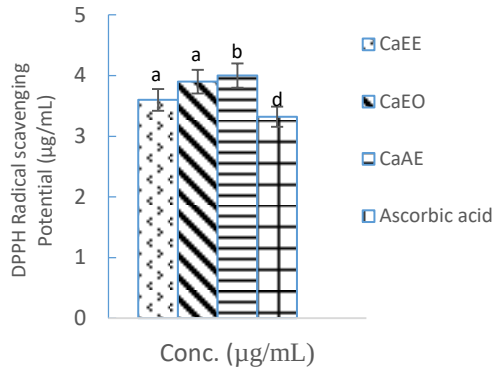

Figure 5: DPPH Radical scavenging activity of C. albidum kernel extracts.

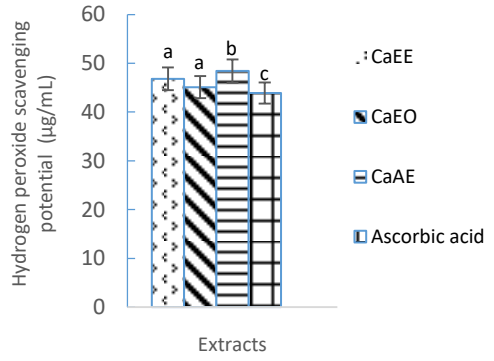

Figure 6: Hydrogen peroxide scavenging IC50 value of C. albidum kernel extracts

Antioxidant results consistent potency across nitric oxide, H_2O_2. and DPPH assays, with essential oil marginally superior and all extracts comparable to ascorbic acid. These findings align with previous reports on *C. albidum* leaves, which exhibited strong DPPH scavenging and in vivo modulation of oxidative markers like catalase and glutathione.[21]

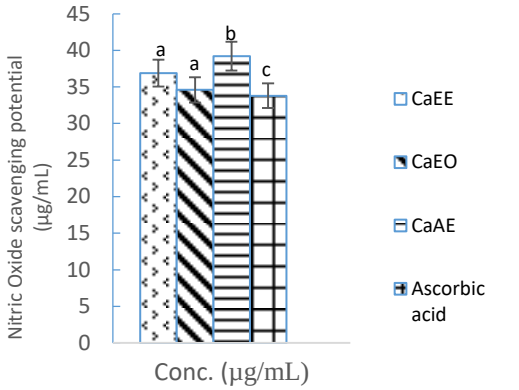

Figure 7: Nitric Oxide scavenging activity of C. albidum kernel extracts
CaEE – C. albidum Ethanol extract
CaEO – C. albidum Essential oil extract
CaAE – C. albidum Aqueous extract
Values that bear different alphabet differ significantly.

Fruit-supplemented diets have also demonstrated antioxidant effects in brain tissues, reducing oxidative stress and proinflammatory cytokines in lipopolysaccharide-induced models.[22] The dual antiplasmodial and antioxidant properties observed may enhance therapeutic efficacy in malaria management by mitigating parasite-induced oxidative damage, a mechanism supported by the plant's phytonutrient content.[23]

Descriptive comparisons highlight solvent-dependent extraction efficiency, with non-polar (essential oil) and semi-polar (ethanol) solvents yielding more active extracts than aqueous ones. This solvent polarity effect is consistent with studies showing that extraction techniques influence the yield of bioactive compounds in *C. albidum*.[22] These results validate the traditional ethnomedicinal applications of *C. albidum* for malaria treatment, where leaves and bark are used in southern Benin and Nigeria.[24] Fruit pulp consumption during pregnancy in south-eastern Nigeria may serve as intermittent preventive therapy against malaria, with both pulp and seed extracts showing suppressive and curative effects in rodent models.[25] While prior research focuses on bark, leaves and fruit, this study extends evidence to kernel extracts of ethanol as most potent followed by essential oil and aqueous in order of poteny[26], positioning *C. albidum* as a promising source for novel antimalarials and antioxidants warranting compound isolation and clinical evaluation

CONCLUSION

Kernel extracts of *C. albidum* possess significant *In vitro* antiplasmodial and antioxidant activities with

favorable safety profiles. Further studies on bioactive compound isolation and in vivo efficacy are recommended.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

ACKNOWLEDGEMENT

Authors are very thankful to the Technologists of the Department of Medical Biochemistry, Delta State University for their inputs in the course of the laboratory work.

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