

Research Article

Nutritional, Phytochemical, and Biological Activities of *Chrysophyllum albidum* Fruit Extracts from Lagos

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This study investigated the nutritional, phytochemical, and biological activities of *Chrysophyllum albidum* aqueous (CAA) and ethanolic (CAE) fruit extracts from Lagos. Proximate, vitamin and mineral compositions, phytochemicals, and antioxidant and antibacterial properties of the extracts were analysed. There was no difference in the composition of moisture content, total ash, carbohydrate, crude protein, fat, fibre, and caloric values in CAA and CAE. Elemental analysis showed the presence of sodium, iron, zinc, magnesium, calcium, manganese, potassium, and copper, while vitamins B₁, B₂, B₃, B₆, B₁₂, C, D, and K were also present. The total tannin, flavonoid, and phenol contents of CAA were higher than those of CAE; however, CAE elicited stronger reducing power, antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl, and nitric oxide scavenging activities than CAA. The antibacterial activity of CAA was bactericidal on all tested bacterial clinical strains with the most promising activity against *Pseudomonas aeruginosa*. On the other hand, the antibacterial activity of CAE was bactericidal on all tested organisms except *Staphylococcus aureus* and *Staphylococcus faecalis* on which it was bacteriostatic. The most promising bactericidal activity was against *Staphylococcus epidermidis* and *P. aeruginosa*. These findings conclude the strong antioxidant effect of *C. albidum* fruit extracts in addition to a broad-spectrum antibacterial property.

1. Introduction

Chrysophyllum albidum G. Don, also called star apple, is a plant classified under the Sapotaceae family and Ericales order [1, 2]. It is commonly found in the West African tropics and native to countries like Nigeria, Benin, Cote d'Ivoire, and Cameroon [3]. The plant parts are of nutritional and medicinal value, with the fruits served as meals and desserts, prepared in various local methods into soups, jams, and salads. *C. albidum* fruits are commonly called "Agbalumo" in southwest Nigeria and "Udara" in the eastern and southern parts of Nigeria [4]. Fruits are consumed in large quantities as they contain several vitamins and minerals required for proper body buildup [5]. They are also a source of economic importance for farmers and marketers

involved in cultivation, logistics, and sales. Studies have shown that *C. albidum* fruit has numerous biological and health benefits. Seeds, fruit pulp, and fruit skin have been reported to contain sodium, potassium, magnesium, calcium, chlorine, and phosphorus [5]. It is also a rich source of antioxidants and promotes good health by truncating oxidative stress-related infectious and noninfectious diseases [2, 6]. The leaves are used ethnobotanically to tackle skin eruptions and dryness, remedy malaria and yellow fever, cure stomachache, and manage diarrhoea. All plant parts are rich in anacardic acid which is used in resin production and wood protection [3].

The antibacterial property of natural products is effective compared to synthesised agents coupled with their biodegradability, reduced toxicity, antioxidant, and other

infectious diseases abrogative properties [7]. Biologically active compounds found in these natural products have been reported to elicit antimicrobial and antioxidant capabilities and, thus, utilised as an alternative source of therapy that is cheap and easily accessible to locals [8]. They also serve as the basis for the synthesis of a quarter of prescribed medications in the world [9]. In recent times, there has been a recurrent challenge of antibiotic resistance worldwide [10]. This occurrence is a result of novel multidrug resistance patterns that have been developed by both Gram-positive and Gram-negative bacteria, making it difficult to properly treat curable infections or incurable in some cases using standard clinical medications [11]. The increase in clinically significant pathogens resistant to antibiotics has led to the emergence of new multiresistant strains [12]. Numerous mechanisms are utilised by these resistant organisms such as drug target site and/or drug modification, acquisition and emergence of antibiotic-resistant genes, biofilm production, mutation of efflux channels, and induced growth of persister cells, to impede and overcome the therapeutic effects of various classes of antimicrobials [13]. Infectious diseases caused by resistant microbes coupled with unavailable and expensive new-generation antibiotics with short half-lives are responsible for increased health costs and the spike in mortality and morbidity, particularly in low-income and lower-middle-income countries [12, 14]. The running cost of synthetic drug production is on the high side coupled with the negative side effects observed; therefore, the need to source effective antibacterial agents of natural and accessible origin or bioactives that could be potential leads for new antibacterial drugs is imperative [12]. This study attempts to investigate the nutritional, phytochemical, and biological activities of *Chrysophyllum albidum* G. Don crude extracts and their antibacterial effectiveness against clinically resistant bacterial strains.

2. Materials and Methods

2.1. Chemicals and Reagents. Vitamins A (palmitate) and D2 were obtained from Ranbaxy Pharmaceuticals Ltd. *n*-Hexane and methanol (HPLC grade) were obtained from Sigma-Aldrich GmbH. Mueller–Hinton and Sabouraud dextrose agar were bought from Lab M, United Kingdom, and HiMedia, India, respectively. Folin and Ciocalteu's phenol reagent, gallic acid, ascorbic acid, ferric chloride, Griess reagent, nitric acid, sodium nitrate, 2,2-diphenyl-1-picrylhydrazyl, aluminium chloride, hydrogen peroxide, and sodium carbonate were purchased from Solarbio, China. The remaining reagents and chemicals were of analytical grade.

2.2. Plant Collection and Preparation. Healthy ripe *C. albidum* fruits were procured in May 2012 from Ketu Market, Lagos, Nigeria (Figure 1). The fruit sample was authenticated and identified by comparing it with a previously deposited herbarium specimen at the Botany Department, University of Lagos, Nigeria, with the number 4999. The fruits were thoroughly cleaned with running tap water, and fleshy edible parts were separated from the seeds,



FIGURE 1: Ripe *Chrysophyllum albidum* fruits.

dried at room temperature for 18 days, and crushed in a Thomas–Willey milling machine. A respective weight of 140 and 150 g of the sample was soaked in 500 mL of distilled water and 98% ethanol for 2 days and filtered with a 125 mm filter paper (Whatman No. 42 m) to get an aqueous and ethanolic filtrate. The filtrates were then concentrated using a freeze dryer and a rotary evaporator to yield aqueous (CAA) and ethanolic (CAE) crude extracts used in the analysis.

2.3. Microorganisms. The slants of Gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus faecalis*) and Gram-negative (*Klebsiella pneumoniae* subsp. *ozaenae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Escherichia coli*) bacterial clinical strains were subcultured from stock at the Department of Pharmaceutical and Pharmaceutics Technology, University of Lagos, and maintained on Mueller–Hinton agar and Sabouraud dextrose broth.

2.4. Proximate, Mineral, and Vitamin Analysis. Proximate composition analysis of CAA and CAE for moisture, ash, fibre, crude protein, fat, and carbohydrate was assessed according to AOAC International standard methods of analysis [15] in triplicate. The energy values (kcal/g) for protein, fat, and carbohydrate were calculated using the dry sample.

2.4.1. Moisture Content. The oven-dried crucible was weighed empty (S1) and then weighed with 2 g of the sample (S2) and placed in an oven for 12 hours at 105°C. The sample was removed, cooled, and weighed (S3). The moisture content value was calculated using the following equation:

$$\text{moisture or total ash (\%)} = \frac{(S2 - S3)}{(S2 - S1)} \times 100. \quad (1)$$

2.4.2. Total Ash Content. The muffle furnace-dried crucible was weighed empty (S1) and then weighed with 1 g of the sample (S2) and placed in a muffle furnace for 4 hours at 550°C. It was removed, cooled in a desiccator, and weighed (S3). The total ash content value was calculated using equation (1).

2.4.3. Crude Protein. One gram of the sample was mixed thoroughly with digestate (10 mL H_2SO_4 and catalyst, i.e., K_2SO_4 : $CuSO_4$ in a ratio of 8:1) and heated to obtain a clear colour. The mixture was cooled, made up to 100 mL with distilled water before 10 mL, and aspirated in a distillation tube, and thereafter, 10 mL of 0.5 N NaOH was gently added to the tube. After 10 min of distillation, the ammonia produced was collected as ammonium hydroxide and mixed with boric acid (4%, 20 mL) and an indicator (methyl red) before titrating against 0.1 N HCl till pink appearance. Similarly, a blank sample underwent this outlined procedure, and the crude protein value was calculated using the following equation:

$$N(\%) = \frac{(S - B) \times N \times 0.14 \times D \times 100}{\text{weight of sample} \times V}, \quad (2)$$

$$\text{protein}(\%) = 6.25x \times \%N \quad (x = \text{correction factor}),$$

where S is the sample, B is the blank, N is the normality of HCl, D is the sample dilution after digestion, and V is the distillation volume.

2.4.4. Crude Fat. A well-wrapped moisture-free sample (1 g) was placed in an extraction tube fitted with a petroleum ether-filled receiving beaker, siphoned six times in which the ether was evaporated, and the beaker was detached before the last siphoning. The residue was poured onto a plate, placed in a water bath to evaporate residual ether, and oven-dried for 2 hours at $105^\circ C$. The crude fat value was calculated using the following equation:

$$\text{fat}(\%) = \frac{\text{weight of ether sample}}{\text{weight of sample}} \times 100. \quad (3)$$

2.4.5. Crude Fibre. A portion of the sample (150 mg, S1) was weighed in a porous crucible. Preheated sulphuric acid solution (150 mL) and 1-octanol (foam suppressor) were added to each column before boiling for half an hour. Thereafter, the acid was removed, and the column was rinsed thrice using distilled water. The procedure was repeated for potassium hydroxide before the sample was dried at $150^\circ C$ for 1 hour, cooled, and weighed (S2). The samples were later heated for 4 hours at $55^\circ C$ in a muffle furnace, cooled, and weighed again (S3). The crude fibre value was calculated as shown in the following equation:

$$\text{fibre}(\%) = \frac{S2 - S3}{S1} \times 100. \quad (4)$$

2.4.6. Carbohydrate. Carbohydrate was estimated by subtracting the sum values of other proximate compositions described above from 100.

The mineral content of *C. albidum* dried fruit blend was evaluated using an atomic absorption spectrometer (Perkin-Elmer model 2380) as a gram of the sample was placed in a Vycor dish, oven-dried at $150^\circ C$ for 1 hour in an air-forced oven, heated at $550^\circ C$ for 16 hours in a muffle furnace, and

cooled. Ash was dissolved in nitric acid and made up to 250 mL using distilled water. A flame emission photometer was used to ascertain the sample level of Na and K using their chloride salts as standards. Atomic absorption spectrometry was used to determine all other metals [16].

High-performance liquid chromatography (Agilent 1100 Series Isocratic Pump HPLC) was used to evaluate the fat- and water-soluble vitamins using ZORBAX SB-C8 columns 75×4.6 mm, $3.5 \mu m$, and 4.6×150 mm, $5 \mu m$, at a flow rate of 1 mL/min.

2.4.7. Vitamin B Complex. Two grams of sample was mixed with 25 mL of 100 mN H_2SO_4 solution, heated for 30 min at $121^\circ C$, brought to room temperature, and adjusted to pH 4.5 using sodium acetate (2.5 M). Fifty milligrams of diastase was added to the mixture, stored overnight at $35^\circ C$, and filtered using Whatman No. 4 to obtain a filtrate that was made up to 50 mL with distilled water. A $0.45 \mu m$ filter was used to further clarify the solution before $20 \mu L$ was injected for HPLC estimation [17].

2.4.8. Vitamin C. An extraction solution (1.4 M acetic acid and 0.3 M metaphosphoric acid) was homogenized with the sample (10 g) and agitated for 15 min at 10,000 rpm. The standard was prepared by dissolving 100 mg of L-ascorbic acid in the extraction solution to a final concentration of 0.1 mg/mL and thereafter filtered with a membrane ($0.45 \mu m$) for HPLC assessment. A linear range of the measured concentration levels was developed by converting the calibration line [17].

2.4.9. Vitamins A, D, and K. A solution made up of the sample (10 g), pyrogallol (1 g), ethanol (70 mL), and KOH (30 mL, 50%) was refluxed in a water bath ($50^\circ C$, 40 min). Extracts were harvested using varying concentrations of ether (20–50 mL) before neutralization and dehydration using distilled water and Na_2SO_4 , respectively. After concentration to approximately 5 mL by using a water bath at $50^\circ C$, methanol was used to dilute the extract to 10 mL and thereafter filtered using a membrane ($0.45 \mu m$) for HPLC evaluation. Serial dilutions of vitamins A, D3, and K were injected in HPLC, and peak areas were determined to generate standard curves [17].

2.5. Phytochemical Assessment. Chemical tests to identify the presence of anthraquinones, flavonoids, terpenoids, cardiac glycosides, alkaloids, saponins, phlobatannins, tannins, steroids, and phenols were carried out on CAA and CAE using standard procedures [18–20]. Total tannin, flavonoid, and phenol contents of CAA and CAE were also estimated [21].

2.6. In Vitro Antioxidant Assay

2.6.1. Nitric Oxide Radical Scavenging Assay. Various concentrations (25–100 $\mu g/mL$) of CAA and CAE were placed in a tube of sodium nitroprusside (10 mM), made up

to 3 mL final volume, and incubated for 150 min at 25°C. Control experiments containing an equivalent amount of buffer in place of CAA, CAE, and standard were prepared similarly in phosphate buffer saline (0.025 M, pH 7.4). Thereafter, 500 µL of the incubated solution was diluted with an equal volume of the Griess reagent and allowed to incubate for 30 min before reading absorbance at 546 nm. The inhibition activity was calculated using a positive control [22].

2.6.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Assay. The radical scavenging activity of CAA and CAE was examined in the presence of DPPH radicals. In brief, 1 mL of the graded concentrations of CAA and CAE (25–100 µg/mL) was made up to 4 mL with methanol before DPPH (2 mL) was added and placed in the dark for 30 minutes. Absorbance was read at 514 nm. Ascorbic acid served as the standard, while control experiment void of CAA, CAE, and standard served as a negative control [18, 21].

2.6.3. Reducing Power. One millilitre of the graded concentrations of CAA and CAE (25–100 µg/mL) was pipetted into a tube containing phosphate buffer (0.025 M, pH 7.4, 2.5 mL). Thereafter, a volume of the mixture (2.5 mL) was incubated with freshly prepared ferric chloride (0.5 mL, 0.1%) with absorbance measured at 700 nm and compared to the gallic acid standard [18, 21].

2.6.4. Total Antioxidant Capacity. Graded concentrations of CAA and CAE (25–100 µg/mL; 0.3 mL) were mixed with TAC buffer (3 mL), boiled for 1.5 h, and cooled. Sample absorbance was recorded at 695 nm against a methanol blank and compared to the ascorbic acid standard curve [18, 21].

2.7. Antibacterial Assessment. The *in vivo* antibacterial potential was evaluated by the well diffusion technique. The antibacterial diffusion test was carried out using a cell suspension of about 3.0×10^8 cfu/mL obtained from a 0.5 McFarland turbidity standard [23]. The antimicrobial activities of CAA and CAE were evaluated by measuring the zone of inhibition diameter around the extracts at 200, 400, and 800 mg/mL. Minimum inhibitory concentration (MIC) was assessed using the microtitre broth dilution method of graded working concentrations (0.25–128 mg/mL) of CAA and CAE. Concentrations with potent inhibitory potential were further assessed for their minimum bactericidal concentration (MBC) and MIC/MBC ratio [23, 24].

2.8. Statistical Analysis. The results were recorded as the mean ± SEM of the experiment(s) in three replicates. Values of the proximate analysis, quantitative phytochemical evaluation, and antibacterial activity were analysed using an unpaired *T*-test, while those of the antioxidant assessment were subjected to the one-way analysis of variance (ANOVA) with mean values separated at $p < 0.05$ using Dunnett's multiple comparison test on IBM SPSS v23 (USA).

3. Results and Discussion

3.1. Proximate, Mineral, and Vitamin Composition. There was no difference ($p < 0.05$) in the composition of moisture content, total ash, carbohydrate, crude protein, crude fat, and crude fibre in CAA (47.02 ± 0.92 , 4.68 ± 0.04 , 25.17 ± 0.11 , 2.68 ± 0.03 , 10.79 ± 0.06 , and $11.05 \pm 0.44\%$) and CAE (48.38 ± 0.08 , 4.18 ± 0.03 , 24.26 ± 0.05 , 2.75 ± 0.05 , 10.94 ± 0.40 , and $10.48 \pm 0.03\%$) as shown in Table 1. Caloric values of CAE and CAA were 206.5 and 208.53 kcal/g, respectively (Table 1). The moisture content and ash compositions of CAA and CAE were higher than those of a previous study [25], while crude carbohydrate was comparable to the finding of Oyebade et al. [26]. The high carbohydrate content and low fat content suggest rich energy value. These results support a previous report suggesting the use of *C. albidum* fruits in managing diabetic and obese patients [2, 6, 27]. Consumption of *C. albidum* fruits may reduce the incidence of diseases like coronary heart disease, colon cancer, diabetes, obesity, high blood pressure, and other digestive disorders due to the high crude fibre content [28, 29]. Crude fibre consumption increases faecal bulk and the intestinal transit rate and induces intestinal cholesterol mop-up and prebiotic effects. In Figure 2(a), it can be seen that *C. albidum* dried fruit blend had sodium, iron, zinc, magnesium, calcium, manganese, potassium, and copper elemental compositions of 123.05, 42.45, 34.45, 34.05, 24.55, 4.1, 2.05, and 0.95 mg/100 g of dried blend, respectively. The presence of sodium, magnesium, calcium, and potassium suggests that *C. albidum* fruit can be utilised as a nutritional supplement of these elements. Thus, it may play a role in the absorption of glucose and some amino acids in the small intestine, maintaining acid-base balance, osmotic balance between cells and interstitial fluid, muscle contraction, and nerve function due to its sodium and potassium content. The presence of calcium and magnesium indicates that ingesting *C. albidum* fruits may aid in the stabilisation of ATP, DNA, and RNA synthesis, bone formation, and mineralisation. Interestingly, *C. albidum* fruit consumption may supply the required daily allowance (RDA) of iron, zinc, manganese, and copper in adults as their elemental composition surpasses the RDA [30]. Concentrations of lead and cadmium were not detected; thus, there was no heavy metal contamination, making the fruit safe for consumption. Despite vitamin A being absent, *C. albidum* dried fruit blend contained phyloquinone and vitamins B₁, B₆, C, B₁₂, B₃, B₂, and D at 0.354, 0.187, 0.033, 0.031, 0.020, 0.015, 0.005, and 0.001 mg/100 g, respectively (Figure 2(b)). These values show that *C. albidum* fruit is a rich water- and fat-soluble vitamin source. Hence, their consumption can help build the immune system, modulate various metabolic pathways that require these vitamins, and prevent a host of nutritional-associated diseases.

3.2. Phytochemical Assessment. Table 2 shows the presence of terpenoids, tannins, cardiac glycosides, flavonoids, phlobatannins, and phenols in both CAA and CAE, while anthraquinones were absent in both. Despite alkaloids, saponins, and steroids being present in CAA, they were absent

TABLE 1: Proximate analyses of *C. albidum* fruit ethanolic and aqueous extracts.

Proximate content	CAE	CAA	<i>p</i> value
Moisture content (%)	48.38 ± 0.08	47.02 ± 0.92	0.48
Total ash content (%)	4.18 ± 0.03	4.68 ± 0.04	0.11
Crude protein (%)	2.75 ± 0.05	2.68 ± 0.03	0.15
Crude fat (%)	10.94 ± 0.40	10.79 ± 0.06	0.57
Carbohydrate (%)	24.26 ± 0.05	25.17 ± 0.11	0.29
Crude fibre (%)	10.48 ± 0.03	11.05 ± 0.44	0.43
Caloric value (kcal/g)	206.50 ± 0.92	208.53 ± 0.05	0.62

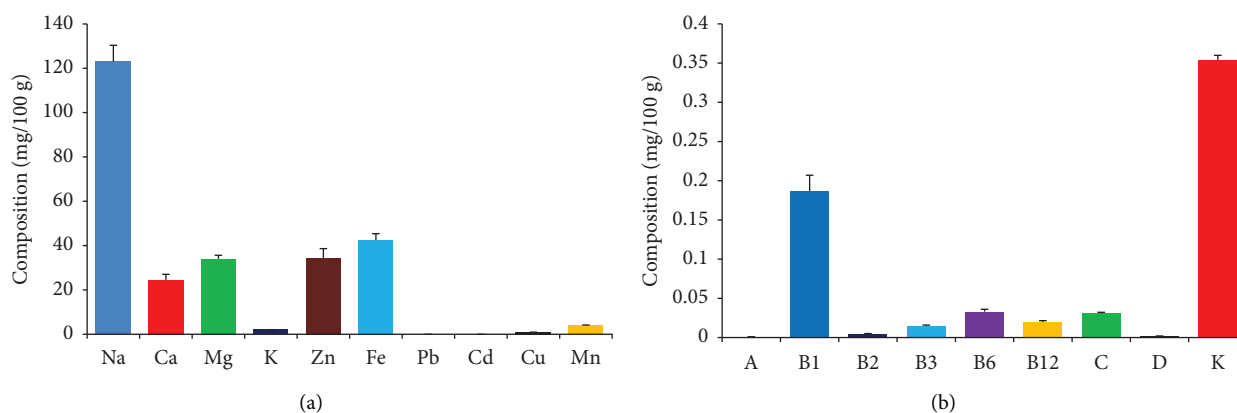
FIGURE 2: (a) Mineral and (b) vitamin compositions of *C. albidum* dried fruit blend.

TABLE 2: Phytochemical assessment of CAA and CAE.

Phytochemicals	Qualitative		Quantitative	
	CAE	CAA	CAE	CAA
Saponins	-	+	ND	ND
Tannins	+	+	62.94 ± 0.22 mg/g TAE	84.10 ± 0.20 mg/g TAE*
Phenols	+	+	45.85 ± 0.06 mg/g GAE	83.36 ± 0.11 mg/g GAE*
Flavonoids	+	+	18.69 ± 0.51 mg/g RE	24.96 ± 0.21 mg/g RE*
Cardiac glycosides	+	+	ND	ND
Steroids	-	+	ND	ND
Terpenoids	+	+	ND	ND
Phlobatannins	+	+	ND	ND
Alkaloids	-	+	ND	ND
Anthraquinones	-	-	ND	ND

+: present, -: absent, CAE: *C. albidum* fruit ethanolic extract, CAA: *C. albidum* fruit aqueous extract, and ND: not determined. Bars = mean ± SEM, *n* = 3; *significantly different at *p* < 0.05.

in CAE. The presence of saponins justifies the use of the fruit in managing metabolic conditions [2, 31, 32]. Phenolic, terpenoids, alkaloids, and cardiac glycosides are compounds that possess health-promoting effects and medicinal properties [33, 34]. The total tannin, flavonoid, and phenol contents of CAA (84.10 ± 0.20 mg/g TAE, 24.96 ± 0.21 mg/g RE, and 83.36 ± 0.11 mg/g GAE) were higher than those of CAE (62.94 ± 0.22 mg/g TAE, 18.69 ± 0.51 mg/g RE, and 45.85 ± 0.06 mg/g GAE) (Table 2). Phenolics are the most diverse phytochemical group and have been identified as the bioactive responsible for the antioxidant activity of numerous plants and their crude extracts [35]. The presence of tannins and flavonoids indicates possible anti-inflammatory effects of the fruit in managing a myriad of diseases linked to oxidative stress. Flavonoids are potent water-soluble

antioxidants and radical scavengers that have strong anti-cancer activity, prevent oxidative cell damage, and inhibit tumour growth [36]. The tannin content of CAA and CAE was higher than what was previously reported for the stem; however, the flavonoid content was lower than the reported values of the seed [37].

3.3. In Vitro Antioxidant Analyses. Figure 3 depicts the dose-dependent scavenging activity of DPPH by gallic acid CAE and CAA. Gallic acid (IC₅₀: 2.62 ± 0.98 µg/mL) exhibited better DPPH scavenging activity (*p* < 0.05) at all concentrations than both CAE (IC₅₀: 53.98 ± 0.06 µg/mL) and CAA (IC₅₀: 66.07 ± 0.83 µg/mL) (Table 3). However, DPPH scavenging activity of CAE was higher than that of CAA.

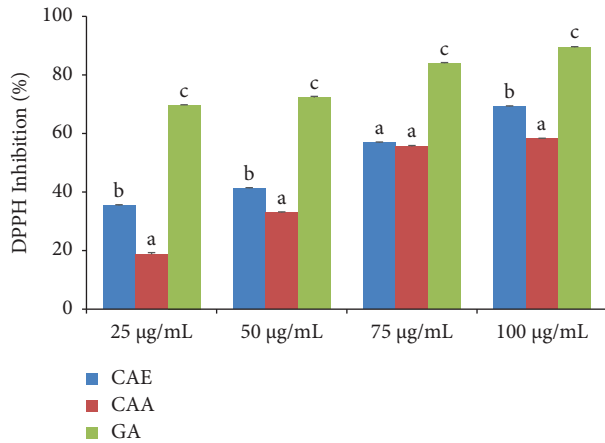


FIGURE 3: DPPH scavenging activity of *C. albidum* fruit ethanolic and aqueous extracts. Bars = mean \pm SEM, $n = 3$. Different superscripts on each bar = $p < 0.05$. Similar superscripts on each bar = $p > 0.05$. CAE: *C. albidum* fruit ethanolic extract, CAA: *C. albidum* fruit aqueous extract, and GA: gallic acid.

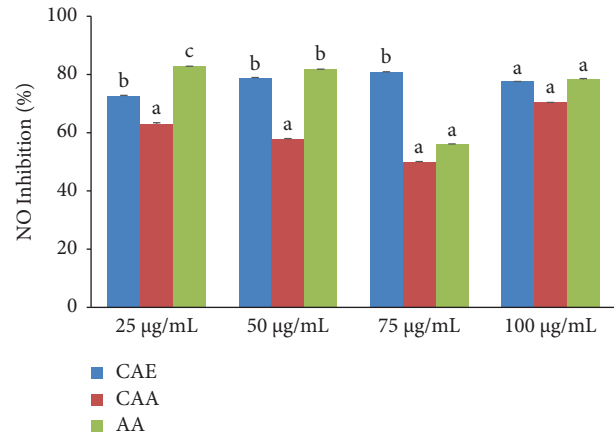


FIGURE 4: Nitric oxide scavenging activity of *C. albidum* fruit ethanolic and aqueous extracts. Bars = mean \pm SEM, $n = 3$. Different superscripts on each bar = $p < 0.05$. Similar superscripts on each bar = $p > 0.05$. CAE: *C. albidum* fruit ethanolic extract, CAA: *C. albidum* fruit aqueous extract, and AA: ascorbic acid.

TABLE 3: Total antioxidant capacity, DPPH, and nitric oxide IC_{50} values.

	DPPH IC_{50} ($\mu\text{g/mL}$)	NO IC_{50} ($\mu\text{g/mL}$)	TAC (mg/AAE)
CAE	53.98 ± 0.06^b	11.88 ± 0.23	$191.81 \pm 0.23^*$
CAA	66.07 ± 0.83^c	14.03 ± 0.51	121.05 ± 0.20
GA	2.62 ± 0.98^a	—	—
AA	—	8.67 ± 0.10	—

Bars = mean \pm SEM, $n = 3$. Different superscripts on each value = $p < 0.05$. Similar superscripts on each value = $p > 0.05$. *Significantly different at $p < 0.05$. CAE: *C. albidum* fruit ethanolic extract, CAA: *C. albidum* fruit aqueous extract, GA: gallic acid, AA: ascorbic acid, and TAC: total antioxidant capacity.

Generally, antioxidants suppress reactive oxygen species formation by augmenting the antioxidant enzyme activities, inhibiting enzymes, or chelating elements responsible for generating free radicals and reactive species [38, 39]. CAA and CAE scavenged the DPPH radical by donating hydrogen ions to inhibit its activity [40]. In Figure 4, it can be seen that ascorbic acid, CAE, and CAA nitric oxide scavenging activities were not dose-dependent. The scavenging property of CAE was most efficient at all concentrations compared to that of CAA and ascorbic acid; nonetheless, there was no difference in their IC_{50} values (Table 3). Peroxynitrite (ONOO⁻), nitric oxide (NO⁻), and nitrogen dioxide (NO₂) are reactive nitrogen intermediates that are also involved in the inflammatory processes [41] and disease pathogenesis [42, 43]. Thus, scavenging these radicals might truncate the steps involved in the pathogenesis of numerous diseases. Ascorbic acid exhibited better reducing power ($p < 0.05$) at all concentrations than both CAE and CAA. However, CAE's reducing power was better than CAA's (Figure 5). Likewise in Table 3, the total antioxidant capacity was higher in CAE (191.81 ± 0.23 mg/AAE) than in CAA (121.05 ± 0.20 mg/AAE). These results further show the antioxidant potential of CAE and CAA which may be ascribed to their phytochemical constituents.

3.4. Antibacterial Effect. In Figure 6, it can be seen that all examined concentrations of CAE and CAA were able to prevent the growth of *S. faecalis*, *P. aeruginosa*, *S. epidermidis*, *S. typhi*, *K. pneumoniae*, *B. subtilis*, *S. aureus*, and *E. coli* evident by zones of inhibition diameter ranging from 13.00 ± 0.00 to 21.33 ± 0.33 and 13.33 ± 0.88 to 28.17 ± 0.17 mm, respectively. *S. faecalis* and *S. typhi* were the most susceptible microbes, while *B. subtilis* and *E. coli* were the least susceptible microbes to CAA at all concentrations. In the case of CAE, 400 mg/mL was the concentration that elicited the highest zones of inhibition against all tested microbes, with *B. subtilis* and *S. faecalis* being the least and most susceptible, respectively. Plant crude extracts, tinctures, infusions, and their purified actives have been reported to elicit antibacterial effects in numerous studies [34, 44, 45]. Natural products with known antibacterial properties can be utilised in alternative therapy. The antimicrobial activities of CAE and CAA against the tested microorganisms indicate a significant medicinal potential against a broad spectrum of infectious pathogens. CAA and CAE possess active principles that could serve as cheap and effective alternative medicines against common microbial infections. These findings are in consonance with a previous study that reported the antimicrobial properties of *C. albidum* exudates against *E. coli*, *S. aureus*, *S. pyogenes*, *K. pneumoniae*, and *C. albicans* [46].

The MIC of CAA on *P. aeruginosa* and *S. faecalis* was 8 mg/mL after 24 and 72 hours. Similarly, the growth of *E. coli*, *S. aureus*, and *B. subtilis* was repressed by CAA at 8 mg/mL after 24 hours; however, further incubation for 72 hours increased the respective MICs to 16, 64, and 16 mg/mL. Inoculum incubation of *K. pneumoniae*, *S. typhi*, and *S. epidermidis* showed that CAA elicited an MIC of 32 mg/mL after 24 h which increased to 64 mg/mL for *K. pneumoniae* and *S. typhi* and 128 mg/mL for *S. epidermidis* after 72 hours of incubation (Table 4). From Table 5, it can be seen that the MIC of CAE on

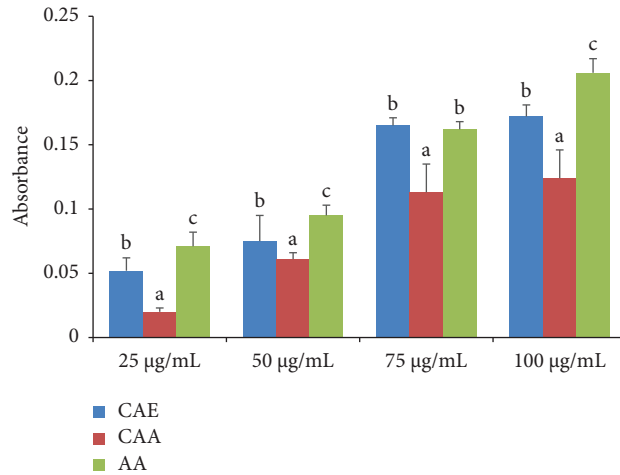


FIGURE 5: Reducing power of *C. albidum* fruit ethanolic and aqueous extracts. Bars = mean ± SEM, n = 3, Different superscripts on each bar = p < 0.05. Similar superscripts on each bar = p > 0.05. CAE: *C. albidum* fruit ethanolic extract, CAA: *C. albidum* fruit aqueous extract, and AA: ascorbic acid.

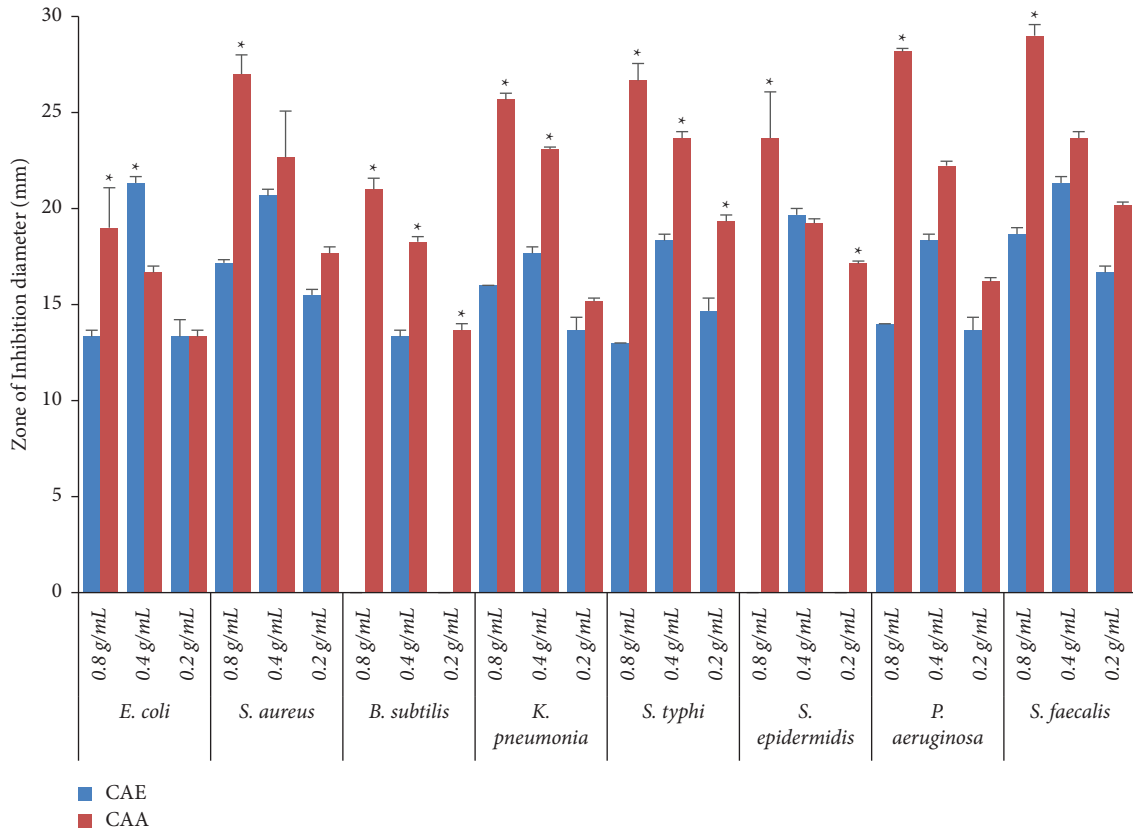


FIGURE 6: Antibacterial activity of *C. albidum* fruit ethanolic and aqueous extracts. *Significantly different at p < 0.05 for each concentration.

P. aeruginosa, *B. subtilis*, and *K. pneumoniae* was 16, 32, and 32 mg/mL after 24 and 72 hours. Similarly, the growth of *S. aureus* (8 mg/mL), *S. epidermidis* (16 mg/mL), *E. coli* (16 mg/mL), *S. typhi* (32 mg/mL), and *S. faecalis* (2 mg/mL) was inhibited by CAE after 24 hours; however, further incubation for 72 hours increased the respective MICs to

32, 32, 32, 64, and 4 mg/mL (Table 5). The MBC of CAA ranged from 8 to 32 mg/mL, with *P. aeruginosa* being the most susceptible, while CAE's MBC ranged from 32 to 64 mg/mL, with *P. aeruginosa*, *S. epidermidis*, and *S. faecalis* being the most susceptible organisms (Table 6). The antimicrobial activities of CAA and CAE were effective on

TABLE 4: Minimum inhibitory concentration of *C. albidum* fruit aqueous extract at 24 and 72 hours.

Organisms	Concentration (mg/mL)																			
	0.25		0.50		1		2		4		8		16		32		64		128	
	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-
<i>Staphylococcus epidermidis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-
<i>Escherichia coli</i>	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	-	-	-
<i>Bacillus subtilis</i>	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-
<i>Salmonella typhi</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-
<i>Streptococcus faecalis</i>	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-

T[#]: 24 hours, T^{*}: 72 hours, -: inhibition of organism growth, and +: growth of the organism.

TABLE 5: Minimum inhibitory concentration of *C. albidum* fruit ethanolic extract at 24 and 72 hours.

Organisms	Concentration (mg/mL)																			
	0.25		0.50		1		2		4		8		16		32		64		128	
	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]	T [#]	T [*]
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
<i>Staphylococcus epidermidis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
<i>Escherichia coli</i>	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-
<i>Bacillus subtilis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
<i>Salmonella typhi</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-
<i>Streptococcus faecalis</i>	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-

T[#]: 24 hours, T^{*}: 72 hours, -: inhibition of organism growth, and +: growth of the organism.

TABLE 6: Minimum bactericidal concentration of *C. albidum* fruit ethanolic and aqueous extracts.

Organisms	Concentration (mg/mL)									
	8		16		32		64		128	
	CAE	CAA	CAE	CAA	CAE	CAA	CAE	CAA	CAE	CAA
<i>Pseudomonas aeruginosa</i>	+	-	+	-	-	-	-	-	-	-
<i>Escherichia coli</i>	+	+	+	-	+	-	-	-	-	-
<i>Staphylococcus aureus</i>	+	+	+	+	+	-	-	-	-	-
<i>Streptococcus faecalis</i>	+	+	+	+	+	-	-	-	-	-
<i>Salmonella typhi</i>	+	+	+	+	+	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	+	+	+	+	+	-	-	-	-	-
<i>Bacillus subtilis</i>	+	+	+	+	+	-	-	-	-	-
<i>Staphylococcus epidermidis</i>	+	+	+	+	-	-	-	-	-	-

CAE: *C. albidum* fruit ethanolic extract, CAA: *C. albidum* fruit aqueous extract, -: bactericidal, and +: growth of the organism.

Gram-negative and Gram-positive organisms, which is in line with the findings from a past study [47]. Gram-negative organisms differ from Gram-positive bacteria as they possess an outer phospholipidic membrane linked by lipoproteins to a thinner sheet of peptidoglycan which houses porins and lipopolysaccharide structural components. Hence, they are impervious to lipophilic solutes and high molecular mass hydrophilic solutes [48]. These cellular features would have enabled CAA and CAE to permeate through the porins of the cell wall to enact their antimicrobial activity on the Gram-negative organisms [49]. The MBC/MIC ratio is important in identifying the antibacterial activity (bactericidal or bacteriostatic) of

natural compounds and other substances. If the ratio is ≤ 4 , activity is considered bactericidal, while if the ratio is > 4 , it is considered bacteriostatic [24]. The MBC/MIC ratio presented in Table 7 shows that CAA antimicrobial activity was bactericidal on all tested organisms with the most promising bactericidal activity against Gram-negative *P. aeruginosa*. On the other hand, CAE antimicrobial activity was bactericidal on all tested organisms except *S. aureus* and *S. faecalis* on which it was bacteriostatic. The most promising bactericidal activity was against *S. epidermidis* and *P. aeruginosa*. The bactericidal activity of CAA on these bacterial species suggests its possible broad-spectrum use.

TABLE 7: MBC/MIC ratio of *C. albidum* fruit ethanolic and aqueous extracts.

Organisms	CAE	CAA
<i>Pseudomonas aeruginosa</i>	2(+)	1(+)
<i>Escherichia coli</i>	2(+)	4(+)
<i>Staphylococcus aureus</i>	8(-)	4(+)
<i>Streptococcus faecalis</i>	8(-)	4(+)
<i>Salmonella typhi</i>	2(+)	1(+)
<i>Klebsiella pneumoniae</i>	2(+)	1(+)
<i>Bacillus subtilis</i>	4(+)	2(+)
<i>Staphylococcus epidermidis</i>	2(+)	1(+)

CAE: *C. albidum* fruit ethanolic extract, CAA: *C. albidum* fruit aqueous extract, -: bacteriostatic, and +: bactericidal.

4. Conclusion

Chrysophyllum albidum fruit consists of various nutrients and is a rich source of Na, Ca, Mg, Zn, Fe, and vitamins B₁, B₆, C, and K that are sufficient to meet the required daily allowance when consumed. The fruit's low caloric value and high crude fibre may help reduce the incidence of metabolic diseases. A high concentration of tannins and phenolics in CAA suggests that the sap of the fruit may help to alleviate metabolism-induced oxidative stress and combat pro-inflammatory microbes. *C. albidum* fruit extracts exhibited good antibacterial activity with low inhibition zones, MICs, MBCs, and MBC/MIC ratios; thus, they may be useful in managing broad-spectrum infections and diseases associated with clinical bacterial strains (Gram-negative and Gram-positive). These findings corroborate the ethnobotanical use of *Chrysophyllum albidum* as a remedy for the management of infectious bacteria. However, further studies on *in vivo* assessment of the precise target and the molecular mechanisms are imperative since studies have reported no correlation with *in vitro* activity.

Data Availability

The data that support the findings of this study are available from the corresponding author upon request.

Disclosure

Ngozi Awa Imaga and Franklyn Nonso Iheagwam are the co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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