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RESEARCH

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Effect of extraction technique, solvent polarity, and plant matrix on the antioxidant properties of *Chrysophyllum albidum* G. Don (African Star Apple)

Afolabi C. Akinmoladun, Olubusola Elizabeth Falaiye, Olubukola Benedicta Ojo*, Abimbola Adeoti, Zainab Abiola Amoo and Mary Tolulope Olaleye

Abstract

Background: *Chrysophyllum albidum* is a well-known medicinal plant in Africa and has many medicinal properties. This study investigated the effect of sonication, solvent polarity (acetone and ethanol), and plant matrix (bark and seeds) on the antioxidant property of *C. albidum*. The bark of *C. albidum* was subjected to sonication and soaking with acetone to evaluate the effect of sonication on the antioxidant property, and *C. albidum* bark and seeds were subjected to ultrasonic-assisted extraction of acetone and ethanol to evaluate the effect of solvents and plant matrix on the antioxidant property of *C. albidum*. The phytochemical composition, total flavonoid content, total antioxidant activity, total phenol content, lipid peroxidation (LPO) inhibition activity, nitric oxide, and 2, 2-diphenyl-1-picrylhydrazyl radicals scavenging activities were evaluated in all extracts.

Results: Sonication increased the percentage yield of extracts compared to maceration. Flavonoids and terpenoids were present, while saponins were absent in all extracts evaluated. Ultrasound-assisted extraction increased *C. albidum* antioxidant property compared to maceration. Ethanol was the most suitable solvent for *C. albidum* bark, while acetone was the most suitable solvent for *C. albidum* seeds. *C. albidum* bark extracts were most active as free radical scavengers, while the seed extracts were most active as inhibitors of lipid peroxidation.

Conclusions: Extraction technique, extraction solvent, and plant matrix significantly affect the antioxidant properties of *C. albidum*. This study indicates that the selection of an effective extraction process for medicinal plants depends on the phytochemical compound responsible for the biological activity of interest.

Keywords: Sonication, Ultrasound-assisted extraction, *Chrysophyllum albidum*, Antioxidant properties, Plant material, Extraction solvent

Background

Plants are known to possess biologically active compounds with significant folkloric health benefits. However, the identification, isolation, and characterization of these bioactive compounds from medicinal plants matrix require the careful selection of suitable extraction procedures and solvents (Azwanida 2015). Convenient, inexpensive, efficient, and highly applicable extraction technology is desirable in small- and medium-scale phytochemical exploration operations (de Morais Rodrigues et al. 2016; Sousa et al. 2014). Sonication is a highly reproducible and efficient modern extraction technique that accelerates bulk transfer and solvent diffusion through membranes to release cell contents (Toma et al.

*Correspondence: ojobenedicta@gmail.com
Department of Biochemistry, School of Life Sciences, The Federal University of Technology, Akure 340001, Nigeria

2001; Yang et al. 2009). Maceration is a convenient, less costly, and more applicable method of extraction (Dhanani et al. 2017; Vongsak et al. 2013). These extraction techniques require the use of solvents, and a suitable solvent increases phytochemical components of the extract which in turn influences the antioxidant properties (Dhanani et al. 2017; Vongsak et al. 2013). Plant parts have been reported to synthesize and store varieties of phytochemicals responsible for their antioxidant property and diverse biological activities (Iloki-Assanga et al. 2015; Rafat et al. 2010).

C. albidum, G Don-Holl (Sapotaceae) commonly known as African Star Apple, is a tree commonly found in Africa with diverse ethnomedicinal uses (Adebayo et al. 2010; Adebayo and Krettli 2011). *C. albidum* leaves, fruits, roots, bark, and seeds extracts have been reported to possess various pharmacological properties attributed to their phytochemical contents and antioxidant activities (Adebayo et al. 2010; Adebayo and Krettli 2011; Amusa et al. 2003; Abiodun 2014; Orijajogun et al. 2013). The plant is also known for its contraceptive (Onyeka et al. 2012), anti-plasmodial (Adewoye et al. 2010; Idowu et al. 2006), hypolipidemic, and anti-hyperglycemic (Olorunnisola et al. 2008) properties. Hence, it is important to investigate a suitable extraction technique, extraction solvent, and plant parts for the optimization of its antioxidant activity. This study investigated the effect of extraction technique, solvent polarity, and plant matrix on the antioxidant properties *of C. albidum*.

Methods

Chemicals

Naphthalene ethylenediamine dihydrochloride, trichloroacetic acid (TCA), ammonium molybdate, thiobarbituric acid (TBA), orthophosphoric acid, sodium carbonate, sodium acetate, chloroform, concentrated sulfuric acid, trisodium chloride, hexane, acetic acid, sodium phosphate monobasic, potassium phosphate dibasic salt, sodium orthophosphate, sodium nitroprusside, sulfanilamide, sodium dodecyl sulfate, DPPH (1,1 diphenyl-2-picrylhydrazyl), ferrous sulfate. All chemicals and reagents used for this study are of analytical grade and are purchased from Merck Millipore and Sigma-Aldrich Inc. St Louis, USA.

Plant material

The bark and fruits of *C. albidum* were harvested from Owena-Ijesha community farm, Osun State, Nigeria. They were then identified and assigned specimen voucher number FHI 110105 and deposited at the Forest Research Institute Nigeria (FRIN). The bark and seeds of *C. albidum* were air-dried for 14 days at room temperature and pulverized.

Preparation of *Chrysophyllum albidum* extracts

The extraction of *C. albidum* bark and seeds was done with two solvents (99.8% acetone and 99.8% ethanol) using two methods: maceration and sonication. The extracts were prepared as follows: powdered bark (5 g) was macerated in 100 ml (99.8%) of acetone for 30 min with constant shaking, and the solvent was evaporated to dryness to yield the dried extract labeled CABAM. Another set of powdered bark (10 g) was soaked in 200 ml acetone (99.8%) and subjected to sonication (Model: Sonicator (XUB18UK, Grant) for 30 min to yield the extract labeled CABAS. The powdered seeds (10 g) were soaked in 200 ml acetone (99.8%) and subjected to sonication (Model: Sonicator (XUB18UK, Grant) for 30-min labeled CASAS 30. A separate set of powdered bark (10 g) and powdered seeds (10 g) was soaked in ethanol (200 ml) and subjected to sonication (Model: Sonicator (XUB18UK, Grant) for 30 min, respectively, and the extracts were labeled CABES and CASES, respectively.

Phytochemical screening of the extracts

The extracts were tested for the presence of saponins, steroids, and terpenoids as described by Deepti et al. (2012), Edeoga et al. (2005), and Ghagane et al. (Ghagane et al. 2017); tannins, phlobatannins, and cardiac glycosides (Salkowski's test) according to the method described by Deepti et al. (2012), Ghagane et al. (2017), and Evans and Trease (2002); and flavonoids as described by, Deepti et al. (2012), Ghagane et al. (2017), and Sofowora (1993).

Evaluation of antioxidant property of extracts

Determination of the total phenolic content of extracts

The total phenolic present in each extract was evaluated by a method previously described by Ghagane et al. (Ghagane et al. 2017), Muhammad et al. (2021), and Singleton et al. (Singleton et al. 1999). In brief, 1 mg/ml of crude extract (200 µL) was mixed with Folin-Ciocalteu reagent (0.5 mL), followed by 20% (w/v) sodium carbonate (2 mL) after 3 min. The mixture was kept in the dark for 1 h and the absorbance was read at 650 nm. A calibration curve was prepared for the estimation of the total phenolic content in each sample using gallic acid. The results were expressed as milligram of gallic acid equivalent per gram dry weight of the extract.

Determination of the total flavonoid content of extracts

The aluminum chloride method previously described by Ghagane et al. (2017), Muhammad et al. (2021), and Chang et al. (2002) was used to determine the total flavonoid contents of the extracts. Briefly, 50 µL of crude extract (1 mg/mL) was mixed with methanol to make 1 ml of the mixture. The mixture was further mixed with

4 mL of distilled water and incubated for 5 min. Then, 0.3 mL of $AlCl_3$ (10%) and 0.3 mL of $NaNO_2$ (5%) were added to the mixture and allowed to settle. After 6 min, 1 M NaOH (2 mL) was added to the mixture and incubated for 15 min. The absorbance of each mixture was taken at 510 nm and the total flavonoid content was estimated from a quercetin calibration curve. The result was expressed as milligram quercetin equivalent per gram dry weight of the extract.

Determination of the total antioxidant capacity of the extracts

The total antioxidant activity of each extract was calculated by the method described by Ghagane et al. (2017), Muhammad et al. (2021), and Prieto et al. (1999). 0.1 mL of sample (100 µg) solution was combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). It was then incubated at 95 °C in a water bath for 90 min. The absorbance was read at 695 nm against blank in a spectrophotometer, after cooling to room temperature. An equal volume of solvent used to dissolve the sample was incubated with 1 mL of reagent solution under the same conditions as the samples, which served the blank. The antioxidant capacity of each extract was expressed as equivalents of ascorbic acid.

Evaluation of DPPH radical scavenging activity of the extracts

In this experiment, the DPPH radical scavenging activity was determined according to the method of Ghagane et al. (2017), Muhammad et al. (2021), and Manzocco et al. (1998). 0.0158 g of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was dissolved in 400 ml of methanol. 1 ml of it was added to 1 ml of extracts of various concentrations ranging from 5 µg/ml to 100 µg/ml and allowed to react at room temperature for 30 min. The reaction mixture was vortexed thoroughly and incubated at room temperature in the dark for 30 min. The absorbance of the mixture was read at 517 nm. DPPH radical scavenging activity of extracts and standard was expressed as % scavenging activity. It was calculated using the given formula: % Scavenging activity $= ((Ao - A)/Ao) \times 100$ where Ao is the absorbance of the control and A is the absorbance of the extract. The percentage of DPPH radical scavenging activity was used to acquire the IC_{50} value of each extract, defined as the concentration of extract necessary to cause 50% inhibition. The smaller the IC_{50} value, the higher is the antioxidant activity.

Evaluation of nitric oxide scavenging activity of the extracts

In this experiment, the nitric oxide radical scavenging activity was determined according to the method of Marcocci et al. (1994). 2 mL of 10 mM sodium nitroprusside dissolved in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations (5 µg/ml to 100 µg/ml). The mixture was then incubated at 25 °C. After 150 min of incubation, 0.5 ml of the incubated solution was withdrawn and mixed with 0.5 mL of Griess reagent (2% sulfanilamide in 5% phosphoric acid, and 0.2% naphthyl ethylenediamine dihydrochloride (NEDD)). The mixture was then incubated at room temperature for 30 min and its absorbance was measured at 546 nm. Nitric oxide radical scavenging activity of extracts and standard (ascorbic acid) was expressed as % scavenging activity. It was calculated using the given formula: % Scavenging activity $= ((Ao - A)/Ao) \times 100$ where Ao is the absorbance of the control and A is the absorbance of the extract. The percentage of nitric oxide radical scavenging activity was used to acquire the IC_{50} value of each extract, defined as the concentration of extract necessary to cause 50% inhibition. The smaller the IC_{50} value, the higher is the antioxidant activity.

Assay of lipid peroxidation inhibition activity of the extracts

Yolk sample (50 g) was dissolved in 150 ml of chloroform and 1% sulfuric acid in methanol (75 ml). The mixture was mixed in a stoppered tube for 2 h. 50 ml of 5% NaCl solution was added and the required esters were extracted with hexane (3 × 30 ml) using Pasteur pipette, and the layer was separated. The hexane aliquot was washed with 2% $KHCO_3$ (40 ml) and dried over anhydrous sodium sulfate. The solution was then filtered to remove the drying agent (Kaźmierska et al. 2005). Lipid peroxidation inhibitory activity of each extract was determined according to the method of Ohkawa et al. (Ohkawa et al. 1979). The tissue (egg yolk lipid extract) was homogenized in 0.1 M buffer pH 7.4 with a Teflon-glass homogenizer. Lipid peroxidation (LPO) in this homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced primarily. Extracts at varying concentrations (5 µg/ml to 100 µg/ml) were added to 100 µl of (15 mM) ferrous sulfate followed by the addition of 3 ml of homogenate. After incubation for 30 min, 0.1 ml of this reaction mixture was mixed with 1.5 ml of 10% TCA and incubated for 10 min. 1.5 ml of 0.67% TBA (in 50% acetic acid) was added and placed in a boiling water bath for 30 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its OD (optical density) was measured at 532 nm against an appropriate blank without the sample. The lipid peroxidation inhibitory activity of the extracts and

standard (ascorbic acid) was expressed as % inhibition. It was calculated using the following formula: % Inhibition = $((Ao - A)/Ao) \times 100$ where Ao is the absorbance of the control and A is the absorbance of the extract. The percentage of nitric oxide radical scavenging activity was used to acquire the IC_{50} value of each extract, defined as the concentration of extract necessary to cause 50% inhibition. The smaller the IC_{50} value, the higher is the antioxidant activity.

Statistical analysis

All the analyses were run in triplicates. Results were then computed using Microsoft Excel software (Microsoft Corporation, Redmond, WA) and analyzed using appropriate analysis of variance (ANOVA) followed by Duncan's new multiple range test (DNMRT) and Pearson's correlation analysis using GraphPad Prism 6.01 (GraphPad Software Inc., CA, USA). The criterion for statistical significance level was set at $p < 0.05$.

Table 1 Percentage yield and phytochemical constituents of *C. albidum* extracts

Extracts	CABAM	CABAS	CABES	CASAS	CASES
% Yield	2	4	12	5	13
Saponins	−	−	−	−	−
Steroids	+	+	+	−	+
Tannins	+	+	+	−	+
Terpenoids	+	+	+	+	+
Phlobatannins	+	+	+	−	−
Flavonoids	+	+	+	+	+
Cardiac glycosides	−	+	+	−	+

+ denotes presence, − denotes absence

CABAM C. albidum bark acetone extract obtained by maceration for 30 min, *CABAS C. albidum* bark acetone extract obtained by sonication for 30 min, *CABES C. albidum* bark ethanol extract obtained by sonication for 30 min, *CASAS C. albidum* seeds acetone extract obtained by sonication for 30 min, *CASES C. albidum* seeds ethanol extract obtained by sonication for 30 min

Results

The percentage yield and phytochemical constituents of *C. albidum* extracts

The percentage yield and phytochemical screening results of *C. albidum* crude extracts are presented in Table 1. CASES had the highest yield of 13%, while CABAM had the lowest yield of 2%. Sonication (4%) increased the percentage yield compared to maceration (2%). Ethanol gave a higher percentage yield (bark: 12% and seeds: 13%) compared to acetone (bark: 4% and seeds: 5%). The percentage yield of the seeds was high compared to the bark. Terpenoids and flavonoids were present in all extracts, while saponins were absent in all extracts. Bark acetone extract obtained by sonication had cardiac glycosides which were absent in the extract obtained by maceration. Ethanol extracts had more phytochemical constituents compared to acetone extracts, especially the ethanol seeds extract which had steroids, tannins, phlobatannins, and cardiac glycosides that were absent in acetone seed extract. Phlobatannins were present in the bark extracts but absent in seeds extracts.

Total phenolic content, total flavonoid content, and total antioxidant capacity of *C. albidum* extracts

The total phenolic content and flavonoids content of *C. albidum* extracts are shown in Table 2. The total phenolic content of extract obtained by sonication (74.40 ± 10.15 mg GAE/100 g) was not significantly different ($p < 0.05$) from the extract obtained by maceration (55.58 ± 3.49 mg GAE/100 g). Acetone seeds extract (84.65 ± 7.57 mg GAE/100 g) had significantly higher total phenolic content ($p < 0.05$) compared to ethanol seeds extract (63.72 ± 6.93 mg GAE/100 g). Ethanol and acetone bark extracts was not significantly different from each other. The total phenolic content of bark acetone and ethanol extracts were not significantly different from seeds acetone and ethanol extracts, respectively.

Table 2 Total phenolic content, total flavonoid content, and total antioxidant content of *C. albidum* extracts

Extracts	Total phenolic content (mg GAE/100 g)	Total flavonoids content (mg QE/100 g)	Total antioxidant capacity (mg AAE/100 g)
CABAM	55.58 ± 3.49	74.69 ± 1.59	914.60 ± 23.37
CABAS	74.40 ± 10.15	$35.68 \pm 0.09^{*}$	$1431.82 \pm 96.42^{*}$
CABES	77.91 ± 6.07	$69.84 \pm 4.22^{\#}$	$2625.34 \pm 6.31^{\#}$
CASAS	84.65 ± 7.57	50.10 ± 2.49^{o}	975.20 ± 87.65^{o}
CASES	$63.72 \pm 6.93^{\#}$	$27.34 \pm 4.76^{\#o}$	$2014.46 \pm 14.61^{\#o}$

Values represent Mean ± Standard Deviation ($n = 3$). $^{*}p < 0.05$ versus maceration; $^{\#}p < 0.05$ versus acetone; $^{o}p < 0.05$ versus Bark

GAE gallic acid equivalent, *QE* quercetin equivalent, *AAE* ascorbic acid equivalent, *CABAM C. albidum* bark acetone extract obtained by maceration for 30 min, *CABAS C. albidum* bark acetone extract obtained by sonication for 30 min, *CABES C. albidum* bark ethanol extract obtained by sonication for 30 min, *CASAS C. albidum* seeds acetone extract obtained by sonication for 30 min, *CASES C. albidum* seeds ethanol extract obtained by sonication for 30 min

CABAM had the highest total flavonoid content (74.69 ± 1.59 mg QE/100 g), while CASES had the lowest total flavonoid content (27.34 ± 4.76 mg QE/100 g). The total flavonoid content of extract obtained by maceration (74.69 ± 1.59 mg QE/100 g) was significantly ($p < 0.05$) higher compared to extract obtained by sonication (35.68 ± 0.09 mg QE/100 g). Ethanol bark extract (69.84 ± 4.22 mg QE/100 g) had significantly ($p < 0.05$) higher total flavonoid content than acetone bark extract (35.68 ± 0.09 mg QE/100 g), while acetone seed extract (50.10 ± 2.49 mg QE/100 g) had significantly ($p < 0.05$) higher total flavonoid content than ethanol seed extract (27.34 ± 4.76 mg QE/100 g). The total flavonoid content of bark ethanol extract was significantly ($p < 0.05$) higher compared to seed ethanol extract, while seed acetone extract was significantly ($p < 0.05$) higher compared to bark acetone extract.

CABES had the highest total antioxidant capacity (2625.34 ± 6.31 mg AAE/100 g), while CABAM had the lowest total antioxidant capacity (914.60 ± 23.37 mg AAE/100 g). Sonication significantly ($p < 0.05$) increased the total antioxidant capacity (1431.82 ± 96.42 mg AAE/100 g) of bark extract compared to maceration (914.60 ± 23.37 mg AAE/100 g). Ethanol extracts (bark: 2625.34 ± 6.31 mg AAE/100 g; seed: 2014.46 ± 14.61 mg AAE/100 g) had significantly ($p < 0.05$) higher total antioxidant capacity compared to acetone extracts (bark: 1431.82 ± 96.42 mg AAE/100 g; seed: 975.20 ± 87.65 mg AAE/100 g). The total antioxidant capacity of the bark extracts was significantly ($p < 0.05$) higher compared to the seed extracts.

Inhibition and free radical scavenging activity of *C. albidum* extracts

Figure 1 shows the DPPH scavenging activity of *C. albidum* extracts. All extracts evaluated scavenged DPPH radical in a concentration-dependent manner. The nitric oxide scavenging activity of *C. albidum* extracts is shown in Fig. 2. Nitric oxide scavenging activity of the studied extracts increased with increasing concentration. Figure 3 shows lipid peroxidation inhibitory activity of *C. albidum* extracts. All extracts were able to inhibit lipid peroxidation in a concentration-dependent manner. The IC_{50} values for DPPH scavenging activity, nitric oxide scavenging activity, and lipid peroxidation inhibition activity of *C. albidum* extracts are presented in Table 3. CASAS had the highest DPPH scavenging activity (IC_{50} value = 5.34 ± 0.85), while CABAM had the lowest DPPH scavenging activity (IC_{50} value = 41.52 ± 5.46). There was no significant difference in the DPPH scavenging activity of bark extract obtained by maceration compared to bark extract obtained by sonication. Ethanol bark extract (IC_{50} value = 18.66 ± 1.42) had significantly ($p < 0.05$) higher DPPH scavenging activity compared to acetone bark extract (IC_{50} value = 39.44 ± 4.62), while acetone seed extract (IC_{50} value = 5.34 ± 0.85) had significantly ($p < 0.05$) higher DPPH scavenging activity compared to ethanol seed extract (IC_{50} value = 20.04 ± 1.62). The DPPH scavenging activity of bark ethanol extract was not significantly different from seed ethanol extract, while seed acetone extract had significantly ($p < 0.05$) higher DPPH scavenging activity compared to bark acetone ext ract.

CASAS had the highest nitric oxide activity (IC_{50} value = 0.02 ± 0.01), while CABAM had the lowest nitric

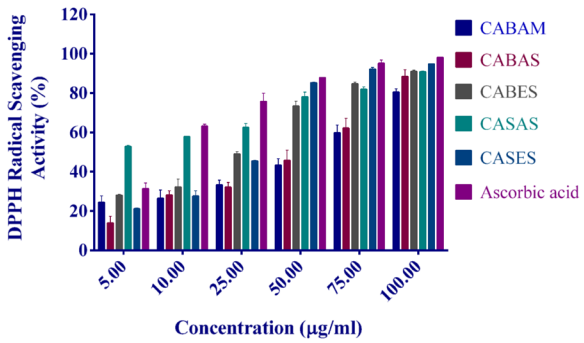

Fig. 1 DPPH radical scavenging activity of *C. albidum* extracts. Values represent mean ± standard deviation ($n = 3$). *CABAM C. albidum* bark acetone extract obtained by maceration for 30 min, *CABAS C. albidum* bark acetone extract obtained by sonication for 30 min, *CABES C. albidum* bark ethanol extract obtained by sonication for 30 min, *CASAS C. albidum* seeds acetone extract obtained by sonication for 30 min, *CASES C. albidum* seeds ethanol extract obtained by sonication for 30 min, *Asc. Acid* ascorbic acid

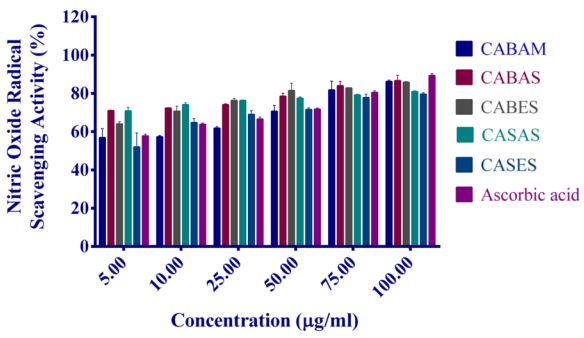

Fig. 2 Nitric oxide radical scavenging activity of *C. albidum* extracts. Values represent mean ± standard deviation ($n = 3$). *CABAM C. albidum* bark acetone extract obtained by maceration for 30 min, *CABAS C. albidum* bark acetone extract obtained by sonication for 30 min, *CABES C. albidum* bark ethanol extract obtained by sonication for 30 min, *CASAS C. albidum* seeds acetone extract obtained by sonication for 30 min, *CASES C. albidum* seeds ethanol extract obtained by sonication for 30 min, *Asc. Acid* ascorbic acid

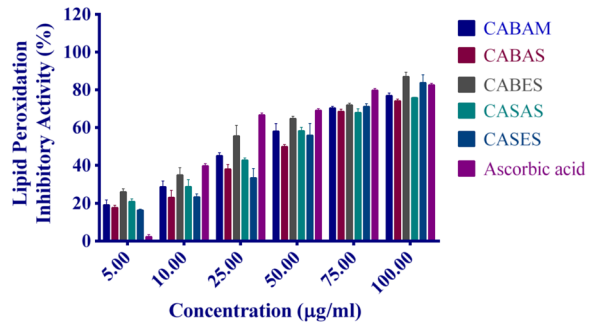

Fig. 3 Lipid peroxidation inhibitory activity of *C. albidum* extracts. Values represent mean ± standard deviation ($n = 3$). *CABAM C. albidum* bark acetone extract obtained by maceration for 30 min, *CABAS C. albidum* bark acetone extract obtained by sonication for 30 min, *CABES C. albidum* bark ethanol extract obtained by sonication for 30 min, *CASAS C. albidum* seeds acetone extract obtained by sonication for 30 min, *CASES C. albidum* seeds ethanol extract obtained by sonication for 30 min, *Asc. Acid* ascorbic acid

Table 3 Radical scavenging activity and lipid peroxidation inhibitory activity of *C. albidum* extracts

Extracts	DPPH radical scavenging activities	Nitric oxide scavenging activities	Lipid peroxidation inhibitory activities
	IC_{50} Values (µg/ml)		
CABAM	**41.52 ± 5.46**	**4.16 ± 1.11**	**29.47 ± 1.17**
CABAS	39.44 ± 4.62	0.28 ± 0.16*	38.22 ± 2.11*
CABES	18.66 ± 1.42#	1.10 ± 0.19	19.71 ± 1.34#
CASAS	5.34 ± 0.85σ	0.02 ± s0.01	30.63 ± 1.23σ
CASES	20.04 ± 1.62#	3.15 ± 0.68#σ	34.97 ± 2.52σ
Asc. acid	8.12 ± 0.48	2.89 ± 0.76	19.50 ± 2.05

Values represent Mean ± Standard Deviation ($n = 3$). *$p < 0.05$ versus maceration; #$p < 0.05$ versus acetone; σ$p < 0.05$ versus Bark

CABAM C. albidum bark acetone extract obtained by maceration for 30 min, *CABAS C. albidum* bark acetone extract obtained by sonication for 30 min, *CABES C. albidum* bark ethanol extract obtained by sonication for 30 min, *CASAS C. albidum* seeds acetone extract obtained by sonication for 30 min, *CASES C. albidum* seeds ethanol extract obtained by sonication for 30 min, *Asc. Acid* ascorbic acid

oxide scavenging activity (IC_{50} value = 4.16 ± 1.11). Sonication (IC_{50} value = 0.28 ± 0.16) significantly ($p < 0.05$) increased the nitric oxide scavenging activity of bark acetone extract compared to maceration (IC_{50} value = 4.16 ± 1.11). The nitric oxide scavenging activity of ethanol bark extract was not significantly different from acetone bark extract, while acetone seed extract (IC_{50} value = 0.02 ± 0.01) had significantly ($p < 0.05$) higher nitric oxide scavenging activity compared to ethanol seed extract (IC_{50} value = 3.15 ± 0.68). Bark ethanol extract (IC_{50} value = 1.10 ± 0.19) had significantly ($p < 0.05$) higher nitric oxide scavenging activity than seed ethanol extract (IC_{50} value = 3.15 ± 0.68), while the nitric oxide scavenging activity of bark acetone extract was not significantly different from seed acetone extract.

CABES had the highest lipid peroxidation inhibition activity (IC_{50} value = 19.71 ± 1.34), while CABAS had the lowest lipid peroxidation inhibition activity (IC_{50} value = 38.22 ± 2.11). The lipid peroxidation inhibition activity of extract obtained by maceration (IC_{50} value = 29.47 ± 1.17) was significantly higher compared to extract obtained by sonication (IC_{50} value = 38.22 ± 2.11). Ethanol bark extract (IC_{50} value = 19.71 ± 1.34) had significantly higher lipid peroxidation inhibition activity compared to acetone bark extract (IC_{50} value = 38.22 ± 2.11), while the lipid peroxidation inhibition activity of ethanol seed extract was not significantly different from acetone seed extract. Bark ethanol extract (IC_{50} value = 19.71 ± 1.34) had significantly ($p < 0.05$) higher lipid peroxidation inhibition activity than seed ethanol extract (IC_{50} value = 34.97 ± 2.52), while seed acetone extract (IC_{50} value = 30.63 ± 1.23) had significantly ($p < 0.05$) higher lipid peroxidation inhibition activity compared to bark acetone extract (IC_{50} value = 38.22 ± 2.11).

Correlation of phytochemical content and antioxidant activity of *C. albidum* extracts

The Pearson correlation coefficients of *C. albidum* extracts are shown in Table 4. Total phenol content had a very strong positive relationship with nitric oxide scavenging activity ($r = 0.861$) and DPPH scavenging activity ($r = 0.717$), while total flavonoid content ($r = 0.745$) and total antioxidant capacity ($r = 0.552$) had a strong positive relationship with lipid peroxidation inhibition.

Discussion

The bioactive phytochemicals are present in accurate concentrations. Hence, the selection of a suitable extraction method and corresponding extraction parameters

Table 4 Pearson's correlation coefficients of *C. albidum* extracts antioxidant parameters

r	Total phenols content	Total flavonoids content	Total antioxidant capacity
DPPH scavenging activity	0.717	− 0.098	− 0.058
Nitric oxide scavenging activity	0.861*	− 0.183	− 0.350
Lipid peroxidation inhibition	0.223	0.745	0.552

* Significant at $p < 0.05$. *r*: correlation coefficient; DPPH: 1, 1-diphenyl-2-picrylhydrazyl. The value $r = 1$ means a perfect positive correlation, the value $r = 0$ means no correlation, and the value $r = -1$ means a perfect negative correlation

is very important in the plant-based drug discovery process. Different parts of *C. albidum* had been reported to possess arrays of phytochemicals with medicinal and physiological benefits. However, this study aimed to investigate the effect of extraction parameters on the antioxidant activity of *C. albidum* in in vitro models.

In this study, saponins were absent, while terpenoids were present in all extracts examined. Steroids, tannins, terpenoids, flavonoids, cardiac glycosides, and phlobatannins were present in most of the *C. albidum* extracts evaluated in this present study. The absence of saponins might be attributed to the type of solvents used, and saponins are extracted majorly with solvents of higher polarity like water and methanol (Tiwari et al. 2011). Terpenoids are known to have anti-diarrheal properties. These classes of phytochemical compounds are known to show medicinal activity against several diseases, and it is not surprising that *C. albidum* extracts are used traditionally by herbalists to cure oxidative stress-related ill-health (Njoku and Obi 2009).

In this present study, ultrasound-assisted extraction (UAE, Sonication) increased the percentage yield of *C. albidum* extracts compared to maceration. *C. albidum* extracts obtained by UAE possessed cardiac glycosides which were absent in extracts obtained by maceration. Sonication significantly increased the total antioxidant content and nitric oxide scavenging property of *C. albidum* bark extract compared to maceration. Maceration significantly increased the total flavonoid content and lipid peroxidation inhibition capacity of *C. albidum* bark extracts compared to sonication. The extraction time (30 min) used in this study may be responsible for the inability of ultrasound-assisted extraction to significantly optimize the total flavonoids content of *C. albidum* extracts compared to maceration. Prolonged ultrasound-assisted extraction duration has been reported to lead to decomposition of some bioactive phytochemicals and formation of free radicals once equilibrium of inner and outer concentration is attained (Kaufmann and Christen 2002; Thoo et al. 2010). Ferreira et al. (Ferreira et al. 2019) suggested 60 min to be the best time for ultrasound-assisted extraction of flavonoids.

In this study, ethanol extracts had a higher percentage yield, extracted more phytochemical components, and possessed more total antioxidant content than acetone extracts. Ethanol extract of *C. albidum* bark possessed significantly higher total flavonoid content, DPPH radical scavenging activities, and lipid peroxidation inhibitory properties compared to an acetone extract of *C. albidum* bark. Conversely, total phenolic content, total flavonoid content, DPPH radical, and nitric oxide scavenging activities of acetone extracts of *C. albidum* seed were significantly high compared to ethanol extract of *C. albidum* seed. The result suggests that ethanol (relative polarity = 0.654; eluent strength = 0.88; dielectric constant = 24.3) is a more suitable solvent for UAE of bark, while acetone (relative polarity = 0.355; eluent strength = 0.56; dielectric constant = 24.3) is a more suitable solvent for UAE of seeds (Reichardt and Welton 2011). The extraction of bioactive compounds from plant material is strongly affected by the physical and chemical properties of the solvent system. The difference in the observed phytochemical composition and antioxidant activities of ethanol and acetone extracts may be due to the variation of their solvent strength and polarity which alters the solubility of bioactive compounds in the plant matrix (Do et al. 2014; Ngo et al. 2017). Moreover, ethanol is alcohol with hydrogen bonding like water, while acetone is a ketone with a keto group which may be responsible for the variation in the dissolution of polyphenolic compounds from *C. albidum* bark and seeds (Do et al. 2014).

The results of this study show that the percentage yield of *C. albidum* seed extracts was higher than the bark extracts. *C. albidum* bark extracts had more phytochemicals compared to *C. albidum* seed extracts especially phlobatannins which were present in all bark extracts and absent in all seed extracts. The seeds had significantly higher total flavonoid content compared to the bark extracts. The bark extracts had significantly higher total antioxidant capacity compared to the seed extracts. The scavenging properties of the bark and seed extracts were highly dependent on the extraction solvent. Ethanol was the best solvent for bark extraction, while acetone was the best solvent for seeds extraction. The antioxidant properties of *C. albidum* bark and seed extracts confirm their efficacy in the treatment of infections and inflammatory reactions (Idowu et al. 2006; Adisa 2000). The difference in antioxidant capacities of *C. albidum* bark and seeds observed in this study confirms that the anatomical distribution of bioactive compounds in a specific plant part dictates its solubility in a specific solvent (Do et al. 2014).

The statistical relationship between each antioxidant parameter of *C. albidum* extracts was measured by Pearson's correlation coefficient. Pearson's value is between + 1 and − 1, where 1 means perfect positive correlation, 0 means no correlation, and − 1 means perfect negative correlation. The very strong positive correlation between the total phenolic content and radical scavenging activity of *C. albidum* extracts observed in this study shows that the phenolic compounds in the extracts are responsible for their free radical scavenging properties which are supported by previous studies (Thoo et al. 2010). The strong positive correlation between the total flavonoid content and lipid peroxidation inhibition

activity of *C. albidum* extracts observed in this study shows that the flavonoid compounds in the extracts are responsible for their lipid peroxidation inhibitory properties. Previous reports suggest that flavonoid compounds possess lipophilic character and are more thermally stable than phenolic compounds (Njoku and Obi 2009). This suggests that some phytochemical compounds in the extracts apart from phenolic compounds also contribute to their free radical scavenging properties.

Conclusions

This study provides useful insight into the optimum extraction conditions (technique, solvent, and plant matrix) for the antioxidant property *of C. albidum*. Extraction technique, extraction solvent, and plant matrix significantly influence the *C. albidum* antioxidant properties. However, the ultrasound-assisted extraction of bioactive compounds from *C. albidum* can further be optimized using a response surface methodology (RSM) for industrial application. This study indicates that the selection of an effective extraction process for medicinal plants depends on the phytochemical compound responsible for the biological activity of interest.

Abbreviations

UAE: Ultrasound-assisted extraction; AAE: Ascorbic acid equivalent; QE: Quercetin equivalent; GAE: Gallic acid equivalent; CABAM: *Chrysophyllum albidum* Bark acetone extract obtained by maceration for 30 min; CABAS: *Chrysophyllum albidum* Bark acetone extract obtained by sonication for 30 min; CABES: *Chrysophyllum albidum* Bark ethanol extract obtained by sonication for 30 min; CASAS: *Chrysophyllum albidum* Seeds acetone extract obtained by sonication for 30 min; CASES: *Chrysophyllum albidum* Seeds ethanol extract obtained by sonication for 30 min; Asc. Acid: Ascorbic acid.

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Authors' contributions

ACA MTO conceived and designed the experiments. OEF, AA performed the experiments. ACA, OBO, ZAA, MTO supervised the experiment. OBO ZAA analyzed the data. OBO ACA wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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