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In Vitro and *In Vivo* Comparative Evaluation of Biological Activities in Freshly Homogenized and Oven-Dried *Chrysophyllum albidum* G. Don Fruits Extracts and Their Ethyl Acetate Fractions.

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Abstract

Chrysophyllum albidum (African star apple) is an edible fruit, found in tropical regions of the world. *C. albidum* fruits wastages have been reported at its peak season in Nigeria. Conventional oven drying has been reported to impart on the nutritional quality and heat-labile bioactive constituents in fruits. This study was designed to comparatively evaluate the *in vitro* and *in vivo* biological activities of extracts and fractions from freshly homogenized and oven-dried fruits of *C. albidum*. *C. albidum* fruits were obtained from a farm stead at Oyo town, Oyo state, Nigeria. The fruits were rinsed, deseeded and separated into two parts. One part was homogenized, the second part was oven-dried at 40^{o}C for one week. The homogenized and oven dried fruits were extracted in ethanol (70%) for 72-hours and the extracts obtained were sequentially partitioned in n-hexane and ethyl acetate. The phenolic contents were determined spectrophotometrically. The extracts and ethyl acetate fraction were tested for *in vitro* antioxidant activity using DPPH, NO, phosphomolybdate activity assays. *In-vitro* acetylcholinesterase inhibitory activity assay was also determined. Antidepressant-like effect of the extracts and ethyl acetate fraction were determined in male Swiss mice using the TST, FST and OFT. The oven-dried extract and its ethyl acetate fraction gave a higher extraction yield than the homogenized extract and its ethyl acetate fractions. Total phenolic content and *in vitro* antioxidant activity were significantly higher in homogenized extract and fraction than the corresponding oven-dried extract and fraction. The oven-dried ethyl acetate fraction had a higher total flavonoid content and flavonoid: phenolic ratio than the corresponding homogenized ethyl acetate fraction. The ethyl acetate fraction of oven-dried extract exhibited a significantly greater reduction in immobility time in the FST than the corresponding fraction of the homogenized extract. The results suggest that oven drying processing of fruits of *Chrysophyllum albidum* might affect the total phenolic content and *in vitro* antioxidant activity but not the *in vivo* biological activity.

Keyword: *Chrysophyllum albidum*, antioxidant, antidepressant, tail suspension test, forced swim test, open field test

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Introduction

The high prevalence of micronutrients deficiency in developing countries has been attributed to the insufficient knowledge of the nutritional value of fruits and vegetables, as well as their low consumption, despite their availability (Silva *et al*., 2017). The health-benefiting and health promoting properties of fruits are due to their richness in micronutrients and phytochemicals required for the growth, development, and optimal functioning of the human body (del Río-Celestino and Font, 2020). Fruits are vital components of diet that promote healthy living because they provide essential nutrients required for proper body functioning (Bvenura and Sivakumar, 2017; Wallace *et al*., 2020). Fruit-based diets have long been associated with increased life expectancy and a reduced risk of chronic and degenerative diseases (Hever and Cronise, 2017). Many juices, obtained from fruits or vegetables, are considered of interest as nutraceuticals for their high content in bioactive phytochemicals. Among phytochemicals, polyphenols, and anthocyanins in particular, attract a huge scientific interest (Muniyandi *et al*., 2019). Intake of polyphenols is associated with a lower incidence of several human diseases and lower mortality rates (Ferrari *et al*., 2011; Tresserra-Rimbau *et al*., 2014; Akhtar *et al*., 2015).

Chrysophyllum abidum (G. Don) fruit is a seasonal fruit with abundant health promoting phytochemicals. It is commonly known as the African star apple or white star apple in English. In Nigeria, it is locally called *Agbalumo*, *Udara,* and *Khada* by the Yoruba, Igbo, and Hausa tribes, respectively. This fruit is widely available from January through March in the South Western part of Nigeria. The fleshy pulp of the fruit is eaten especially as snack and enjoyed by both young and old (CENRAD, 1999). The fleshy and juicy fruits are the potential source of a soft drink (Okafor, 1981). The fruits are also useful for the production of fruit jams and jellies (Ureigho, et al, 2010). It is an excellent source of vitamins, irons, flavours to diets and raw materials to some manufacturing industries (Rafiu *et al.,* 2021*;* Gbesso, 2024).

The fruits have been reported to be rich in anti-inflammatory eleagnine, myricetin, rhamnoside, quercetin, linoleic acid and oleic acid (Ajayi *et al*., 2021). The health benefits of *C. albidum* are attributed to the presence of these phytochemical constituents, many of which have been proven to exert anti-inflammatory, anti-nociceptive and antioxidant activities (Idowu *et al*., 2006).

C. albidum fruits as many other indigenous fruits are largely wasted at the peak of its seasons (Bello and Henry, 2015). Despite its rich phytochemicals and commercial importance, poor post-harvest processing has negatively impacted the interest in cultivation and preservation of these fruits (Sunmonu, 2017; Erukainure *et al*., 2021). Shelf-life of freshly harvested *C. albdium* fruits is less than 5 days and can only be prolonged to 15 days when stored in the refrigerator (Adindu *et al*., 2003; Bello and Henry, 2015). Bio-deterioration of the fresh fruits began to be noticed by day 5 due to fungal infection, resulting in reduced micronutrient and its food value (Amusa *et al*., 2003). There are suggestions that post harvesting processing of fruits into dry materials through conventional oven drying might impart on the nutritional quality and heat-labile bioactive constituents in fruits (Mbondo *et al*., 2018).

Research relating to identifying phytoactive compounds, improving storage and demonstrating health effects are receiving great attention globally. There is scanty information on studies comparing the antioxidant activities of fresh and oven-dried extracts of *C. albidum* fruits. Hence, this present study aimed to comparatively evaluate *in vitro* and *in vivo* biological activities of extracts and fractions from freshly homogenized and oven-dried fruits of *C albidum*.

Materials and Methods

Drugs and Reagents

Folin-Ciocalteu reagents, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), acetylcholinesterase enzyme (electric eel, 1000 units/mg protein), Sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride were all products of Sigma Aldrich (USA). All other reagents and solvents are of analytical grade.

Experimental animals

Male Swiss mice (20-25 g) used in this study were purchased from the Central Animal House, University of Ibadan, Nigeria. The animals were kept in well-ventilated and hygienic compartments, maintained at standard environmental conditions and fed with standard rodent pellet and water ad libitum. The animals used in this study were handled in accordance with the United States National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH, 1985). The animal care and use experimental procedures followed the National Institute of Health guidelines in the "Principle of Laboratory Animal Care" (NIH Publication No. 85–23) and approved protocols by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/053-1222/12)

Extraction of Chrysophyllum albidum Fruit

Chrysophyllum albidum fruits was obtained directly from fruit farm in Oyo, Oyo State, Nigeria. Fruit identification and authentication was done by a botanist at the herbarium of Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria with voucher number FHI 110475. It was washed thoroughly to remove dirt and to reduce its microbial load. The fruit was then opened and deseeded and cut into smaller pieces to facilitate the process of blending and drying. The sliced sample was divided into two equal parts; a part was homogenized using a blender while the other part was dried in an oven at 40℃ for 72hrs. The dried sample was then

grinded into powder using a milling machine. Five hundred grams (500 g) of the homogenized fresh fruit and oven-dried powder were macerated in 1L of ethanol (70%) with intermittent stirring for 72 hr, the mixture was filtered using white muslin cloth and doubly filtered with filter paper. Ethanol was removed by evaporation under reduced pressure and temperature (40°C) using a rotary evaporator. The resulting extracts were freeze dried and percentage yield was calculated. The ethanol extracts were denoted as HEE for homogenized fresh fruits and OEE for oven-dried fruits of *C. albidum.*

Solvent-solvent extraction of extracts of homogenized and oven-dried fruits

Fifty grams (50 g) each of HEE and OEE were dissolved in 200 mL of water. The samples were first repeatedly partitioned with equal volume of n-hexane in a separating funnel. The partitioning was repeated until the n-hexane layer was found to be clear. Secondly, the aqueous layer was also repeatedly partitioned with ethyl acetate until the ethyl acetate layer became transparently clear. The fractions were evaporated to dryness and percentage yield calculated. Only the ethanol extracts and ethyl acetate fractions were used for further investigations. The extracts and fractions were labeled accordingly as follows; HEE: Homogenized ethanol extract, HEAF: Homogenized ethyl acetate fraction, HRAF: Homogenized Residual Aqueous fraction, OEE: Oven-dried ethanol extract, OEAF: Oven-dried ethyl acetate fraction, ORAF: Oven-dried Residual Aqueous fraction

Determination of Phenolic Composition of Extracts and Fractions

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of each extract/fraction was assessed in adherence to the colorimetric Folin–Ciocalteu method reported by Thaipong et al., (2006) with slight modification. Briefly, 100 µl sample or gallic acid solution dissolved in methanol at different concentration was mixed with 200 µl of 10% Folin–Ciocalteu reagent, then 800 µl Na_2CO_3 (700 mM) was added, and the mixture in assay tubes was incubated at 25°C for 2 hr. Finally, 200 µl mixture was transferred to a clean 96-well microplate, and the absorbance was quantified by a microplate reader (SpectraMax M2 Molecular Devices USA) at 765 nm. Gallic acid was used as reference standard by using seven-point analytical curves (measurements in duplicate), with concentrations ranging from 0–250µg/ml ($R2 > 0.999$). The results were expressed as mg Gallic acid equivalents (GAE)/mg sample.

Determination of total flavonoid content (TFC)

The TFC was ascertained using the classical colorimetric method as described by Sultana *et al.,* (2009). Briefly, 100 µl of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the sample extracts. Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 100 µl extract solution with 100 µl methanol without $AlCl_3$. The calibration curve was prepared using various concentrations of rutin (0–1000 µg/ml) dissolved in methanol solution. TFC results were shown as mg Rutin equivalent (RE)/mg sample.

In vitro antioxidant activity assays

DPPH free radical scavenging assay

The free radical scavenging activity of the flavonoid bands of each of the extract and fraction of the fruit was also estimated using the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) standard method as described by Aderogba *et al,* (2004) with minor modifications. Briefly, 100 µL of the extracts and fractions dissolved in methanol were added into 96-well plate at varying concentrations (62.50, 125, 250, 500, and 1000 µg/mL). Thereafter, 100 µL of freshly prepared DPPH (0.1M) in methanol was added in the dark, and the plates incubated in dark cup board for 30 min. Absorbance was measured in a microplate reader (LT4500, UK) at 540 nm, and the percentage of DPPH inhibition was calculated using the following equation:

$$\%\text{DPPH Inhibition} = \frac{A_{DPPH} - A_S \times 100}{A_{DPPH}}$$

where A_{DPPH} = absorbance of DPPH in the absence of the extract and A_S = absorbance of DPPH in the presence of either the extract or the standard. The DPPH scavenging activity was expressed as the concentration of the extract (IC_{50}). IC_{50} was graphically determined by plotting the percentage inhibition of DPPH radical against concentration of extract. The IC_{50} value was calculated from the transformed normalized response using the non-linear regression equation in GraphPad Prism® version 8.4.

Nitric Oxide free radical scavenging assay

Nitric oxide radical scavenging activity assay is based on the method described by Ajayi *et al.,* (2017) with slight modification. Briefly, Sodium nitroprusside (10 mM in 0.1M sodium phosphate buffer, pH 7.4), was incubated with extracts and fractions (62.50, 125, 250, 500, and 1000 µg/mL) and ascorbic acid (0-250 µg/mL) and incubated at room temperature for 150 min. The same reaction mixture without the extracts/fractions but with equivalent amount of water served as control. After incubation period, 100µL of Griess reagent (equal volume of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added to equal volume of samples in a 96-well plate. The absorbance was read at 540 nm in a microplate reader (LT4500, UK). Nitrite concentration was

extrapolated from sodium nitrite standard curve (6.25 -200 µM).

Phosphomolybdate Assay for total antioxidant capacity

Phosphomolybdate assay for determination of total antioxidant capacity using ascorbic acid as standard was performed according to method described by Khatoon et al., (2013). Briefly, the extracts and fractions at varying concentrations (62.50, 125, 250, 500, and 1000 µg/mL) were incubated with ammonium reducing solution containing ammonium molybdate (4 mM) dissolved in H_2SO_4 (0.2M) and acidified sodium phosphate buffer (28 mM). The reaction mixture was incubated in a water bath at 95°C for 30 min. Aliquot (200 µL) of the cooled mixture was added into 96-well plate and the absorbance was read at 695 nm in a micro plate reader (SpectraMax M2 Molecular Devices USA). Ascorbic acid standard calibration curve was used to determine the total antioxidant capacity equivalent in mg/g sample.

In vitro acetylcholinesterase inhibitory assay

Acetylcholinesterase Inhibitory activity of extracts and fractions was determined following the procedures described by Dzoyem and Eloff *et al*., (2015). Briefly, the extracts and fractions were dissolved in Tris-HCl buffer (50 mM, pH 8.0). Varying concentrations of the extracts/fractions (15.625, 31.25, 62.50, 125, 250 µg/mL) were incubated with acetylcholinesterase enzyme (0.01U/mL) and Dithiosbenzoic acid (1.5 mM) for 30 min. Thereafter, initial absorbance was taken at 405 nm in a microplate reader. Immediately acetylthiocholine (1.5 mM) was added and absorbance was read at 5 min for 20 min. Physostigmine was used as standard drug. Percentage inhibition of acetylcholinesterase enzyme activity was calculated by determining the reaction rates of samples and blank using the following formula:

$[(A_{control} - A_{blank}) - (A_{test} - A_{blank})/ [(A_{control} - A_{blank})] X 100$.

Where $A_{control}$, A_{blank} and A_{test} represent the absorbance of control well, blank well (without substrate) and extracts and fractions wells, respectively.

Experimental animals

Male Swiss mice (20-25 g) used in this study were purchased from the Central Animal House, University of Ibadan, Nigeria. The animals were kept in well-ventilated and hygienic compartments, maintained at standard environmental conditions and fed with standard rodent pellet and water ad libitum. The animals used in this study were handled in accordance with the United States National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH, 1985). The animal care and use experimental procedures followed the National Institute of Health guidelines in the "Principle of Laboratory Animal Care" (NIH Publication No. 85–23) and approved protocols by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/053-1222/12)

Evaluation of antidepressant-like activities of extracts and fractions

Male Swiss mice (40) were divided into 8 groups (n=5). Group 1 received normal saline, groups 2 received fluoxetine (20 mg/kg), groups 3-5 were treated with HEE, HEAF and HRAF, while groups 6-8 received OEE, OEAF and ORAF, respectively. The dose of the extracts and fraction (100 mg/kg) was chosen based on previous study (Ajayi et al., 2021) (Dulawa *et al*., 2004). The extracts/fractions were administered daily for 10 days and antidepressant-like activity was determined 24 hours after the last treatment using tail suspension test, open field test and forced swim test, respectively.

Tail Suspension Test (TST)

In the TST, the mouse was suspended by the tail and observed for the extent of immobility versus active movement (Steru *et al*., 1985). The mice were suspended by the tail from a bar, 50 cm above the floor using an adhesive tape placed 1-2cm from the proximal tail tip. The test duration was 6 min and the immobility was measured during the test period

Open-field test (OFT)

Locomotor behavior was monitored using the open field apparatus (Prut and Balzung, 2003). The apparatus consisted of a wooden box measuring 35 x 30 x 23 cm with visible lines drawn to divide the floor into 36 (20 cm × 20 cm) squares with a frontal glass wall and placed in a sound free room. The mice were placed in the rear left square and left to explore it. The number of lines crossed were counted for 5 min. After each mice session, the observation chamber was cleaned with 70% ethanol to remove residual odour.

Forced Swim Test (FST)

The forced swim test (FST) has been considered to be the most widely used pharmacological *in-vivo* model for assessing antidepressant activity (Porsolt *et al.,* 1978). The set-up consists of a clear plexi glass cylinder (26 x 12 cm diameter) filled with water to a 15cm depth. Water used during the experiment was kept at a temperature (34 ± 1°C). Mice were placed in a vertical glass cylinder (26 x 12 cm) with fresh water and allowed to swim. Time the animal stayed immobile or floating in an upright position within 6-minute duration was recorded (Soncini *et al*., 2012).

Statistical analysis

The analysis of data obtained was done by utilizing Graph pad prism software version 5.0, with the data expressed as mean ± SEM. Statistical analysis was done using one way

ANOVA followed by the Newman-keuls post hoc test. Thes statistically significant P values were those less than 0.05.

Results

Extraction Yield

Table 1 shows the smell, colour texture and yield of extracts and fractions. The oven-dried powder extract of *Chrysophyllum albidum* and its fractions have the highest yield after the extraction process. The OEE yield was almost 10 times higher than HEE.

Phenolic composition

The TPC of HEE and its fractions were significantly ($P < 0.05$) higher than the corresponding extract and fractions of the oven-dried powder of *C. albidum* (OEE). The TFC of HEE was not significantly higher than TFC of OEE, while OEAF showed a significantly ($P < 0.05$) higher concentration of flavonoid content and flavonoid and phenolic ratio to the corresponding HEAF.

Table 1: Extraction yield of extracts and fractions

Extracts-Fraction	Smell	Color Texture	Yield (%)
HEE	Sweet smell	Dark-brown	3.67
HEAF	Sweet smell	Dark-brown	1.24
HRAF	Sweet smell	Dark-brown	12.24
OEE	Chocolate-like	Dark-brown	32.82
OEAF	Sweet smell	Dark-brown	30.10
ORAF	Sweet smell	Dark-brown	62.64

HEE: Homogenized ethanol extract, HEAF: Homogenized ethyl acetate fraction, HRAF: Homogenized Residual Aqueous fraction, OEE: Oven-dried ethanol extract, OEAF: Oven-dried ethyl acetate fraction, ORAF: Oven-dried Residual Aqueous fraction

Table 2: Phenolic contents in freshly homogenized and oven-dried powder extracts and fractions of *C. albidum* fruits

Samples	TPC (mgGAE/mg sample)	TFC (mgRE/mg sample)	Flavonoid/phenolic ratio
HEE	0.976 ± 0.021*	0.129 ± 0.009	0.132 ± 0.011
HEAF	1.518 ± 0.050*	0.158 ± 0.012	0.104 ± 0.008
HRAF	1.127 ± 0.019*	0.135 ± 0.012	0.120 ± 0.012
OEE	0.667 ± 0.010	0.059 ± 0.011	0.088 ± 0.016
-OEAF	0.736 ± 0.026	0.265 ± 0.05	0.368 ± 0.080
ORAF	0.469 ± 0.011	0.119 ± 0.026	0.250 ± 0.050

Each value is the Mean ± SEM of three replicates. Significance is indicated by a, b, and c ($P < 0.05$) for oven-dried samples in comparison to their fresh (homogenized) counterparts (HEE vs. OEE, HEAF vs. OEAF, etc.). TPC: Total phenolic content; TFC**:** Total flavonoid content

*In vitro Antioxidant Effect on DPPH free radical scavenging activity***.**

The inhibitory concentration (IC_{50}) of the homogenized extract (HEE) and its fractions was significantly lower [$F_{(8, 18)} = 6770$; $P < 0.0001$] than the corresponding oven-dried powder extract (OEE) and its fractions Figure 1B and C). The ethylacetate fractions of both freshly homogenized and oven-dried powder showed smaller IC_{50} values, respectively (Fig 1C). Therefore, HEE has higher potency than OEE in its ability to scavenge DPPH free radicals. Ascorbic acid has the lowest IC_{50} and this mean it is a potent antioxidant (Fig 1C).

Effect on Nitric Oxide free radical scavenging activity

HEE and OEE showed similar IC_{50} values on NO (Fig 2B). However, the IC_{50} of HEAF is significantly ($P < 0.0001$) lower compared with OEAF, while IC50 of ORAF was significantly ($P < 0.0001$) higher than HRAF (Fig 2B). Ascorbic acid showed a much higher free radical scavenging activity and much lower IC_{50} compared with the extracts and fractions (Fig 2A & 2B)

Total antioxidant capacity (TAC) of the extracts/fractions in the phosphomolydate Assay

The TAC results indicated that HEE was significantly higher than OEE. OEAF and ORAF showed a significantly

higher TAC than their corresponding HEAF and HRAF, respectively (Fig 3A & 3B)

Acetylcholinesterase inhibitory activity of extracts and fractions

The acetylcholinesterase inhibitory activity as demonstrated by extracts and fractions is shown in Fig 4A & B. The IC_{50} of OEE and its fractions were significantly ($p < 0.05$) smaller compared with HFEE and its fractions (Fig 4B). Physostigmine showed a more potent inhibition of the enzyme than the extracts and fractions.

Antidepressant-like activity of extract/fractions in Tail Suspension Test

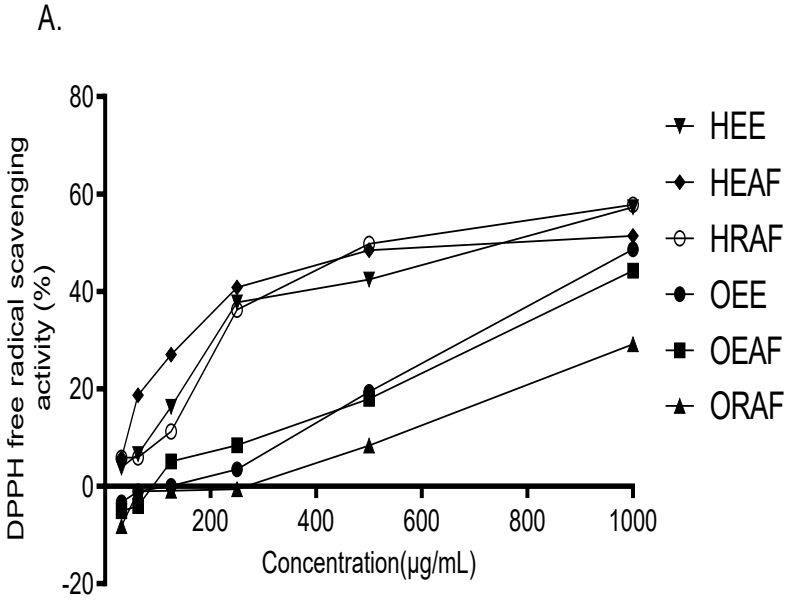
Repeated administration of extracts and fractions in mice did not show significant reduction in immobility time in TST as shown in Figure 5. Although the immobility time in OEE and its fractions were significantly ($P < 0.05$) lower than HEE and its fractions, Standard positive drug, fluoxetine (20 mg/kg) administration demonstrated significant reduction in immobility time in the TST.

Antidepressant-like activity of extract/fractions in Forced Swim Test

There was a significant ($P < 0.05$) decrease in immobility time in the FST in all groups pre-treated with HEE and its fractions and OEE and its fractions. Immobility time in OEE and OEAF treated mice were significantly ($P < 0.05$) reduced when compared with corresponding HEE-and HEAF treated mice. HEAF showed the highest reduction (73%) in immobility time among all treatment. Standard positive drug, fluoxetine (20 mg/kg) administration also demonstrated significant reduction in immobility time in the FST (Figure 6).

Effect of extracts and fraction on locomotor activity in the open field

There was no significant ($P < 0.05$) alteration of the locomotor activity in mice pretreated with HEE, HEAF, OEE, OEAF, ORAF. However, HRAF showed significant ($P < 0.05$) increase in number of line crossing. Fluoxetine (20 mg/kg) administration did not significantly affect locomotor activity in the open field test (Figure 7).



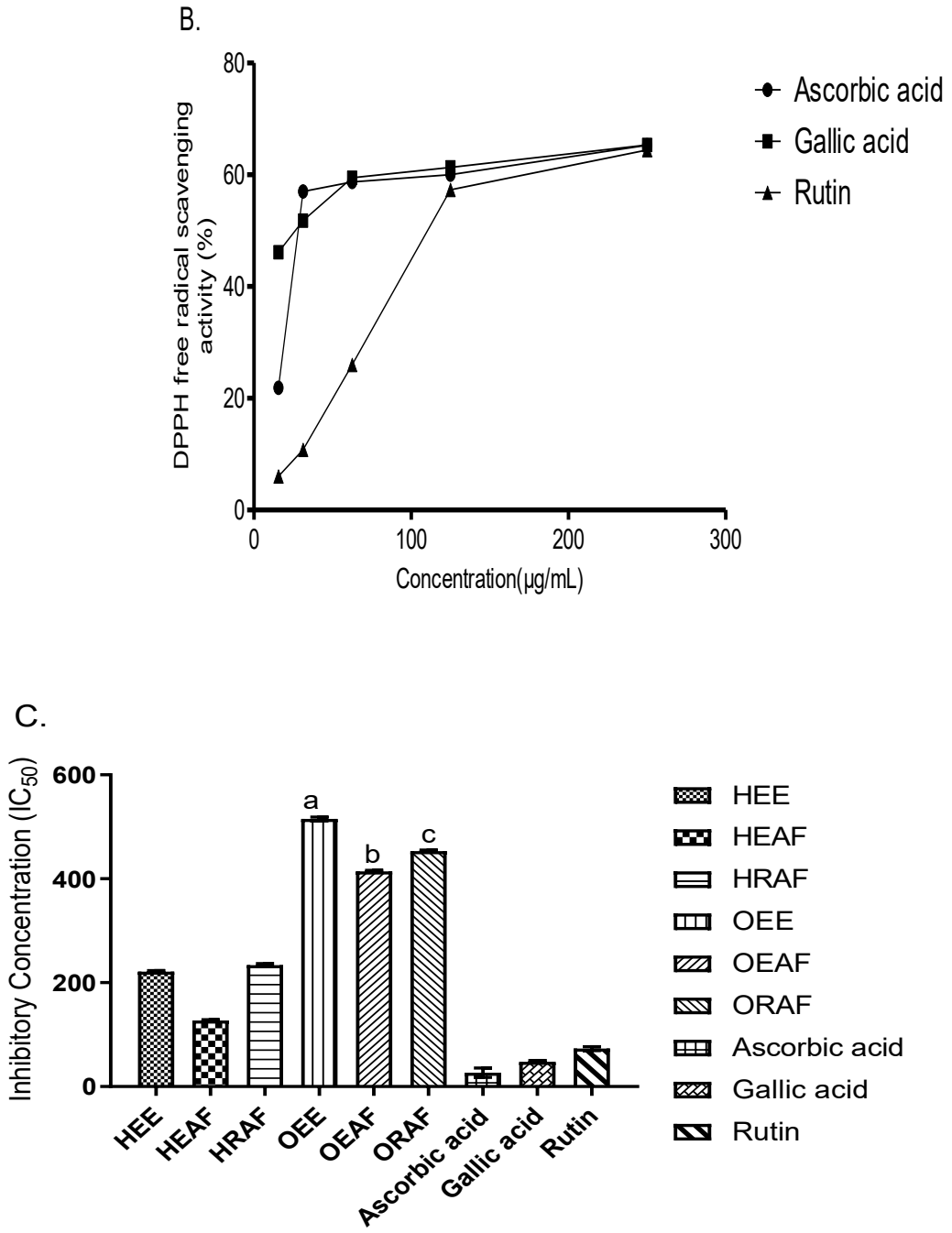


Figure 1: DPPH free radical scavenging activity of (A) freshly homogenized and oven-dried powder extracts and fractions of *C. albidum* fruits, (B) compounds, and (C) inhibitory concentration (IC_{50}). Letters indicate significant difference in when compared with corresponding extract using one-way ANOVA followed by pairwise comparison using Tukey's post hoc test.

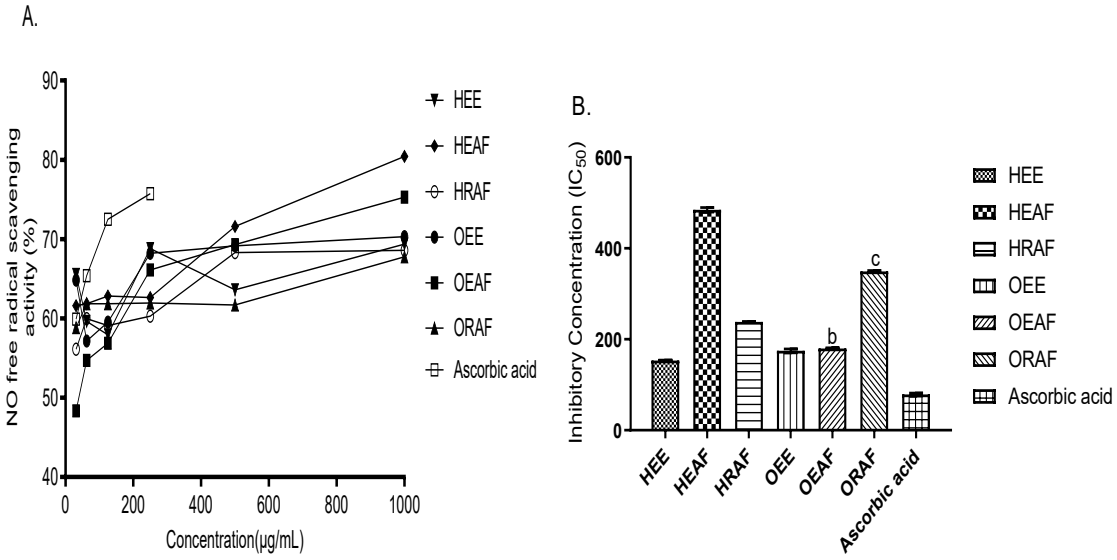


Figure 2: NO free radical scavenging activity of (A) freshly homogenized and oven-dried powder extracts and fractions of *C. albidum* fruits, (B) inhibitory concentration (IC_{50}). Letters indicate significant difference in when compared with corresponding extract/fraction using one-way ANOVA followed by pairwise comparison using Tukey's post hoc test at 95% confidence interval of difference

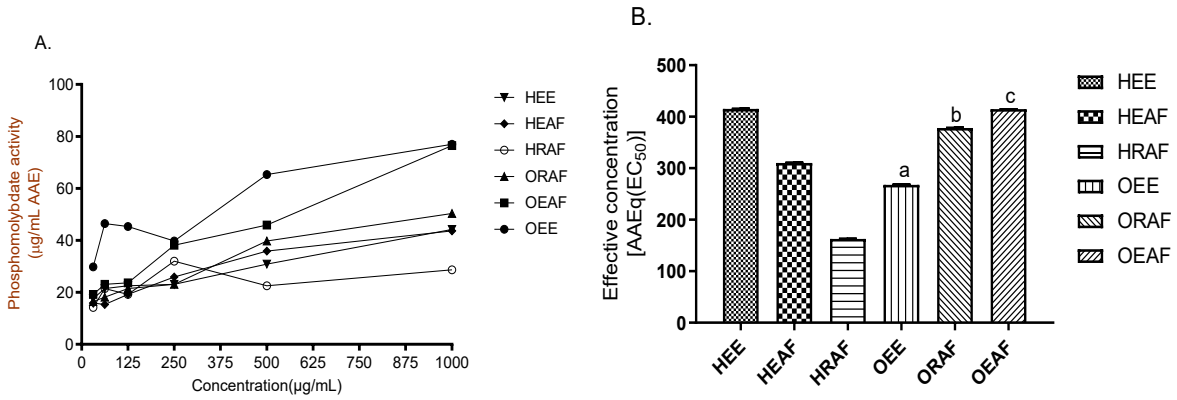


Figure 3: Total antioxidant capacity in phosphomolybdate assay (A) freshly homogenized and oven-dried powder extracts and fractions of *C. albidum* fruits, and (B) inhibitory concentration (IC_{50}). Different letters indicate statistically significant differences between extracts, as determined by one-way ANOVA followed by Tukey's post hoc test. AAE**:** Ascorbic Acid Equivalent

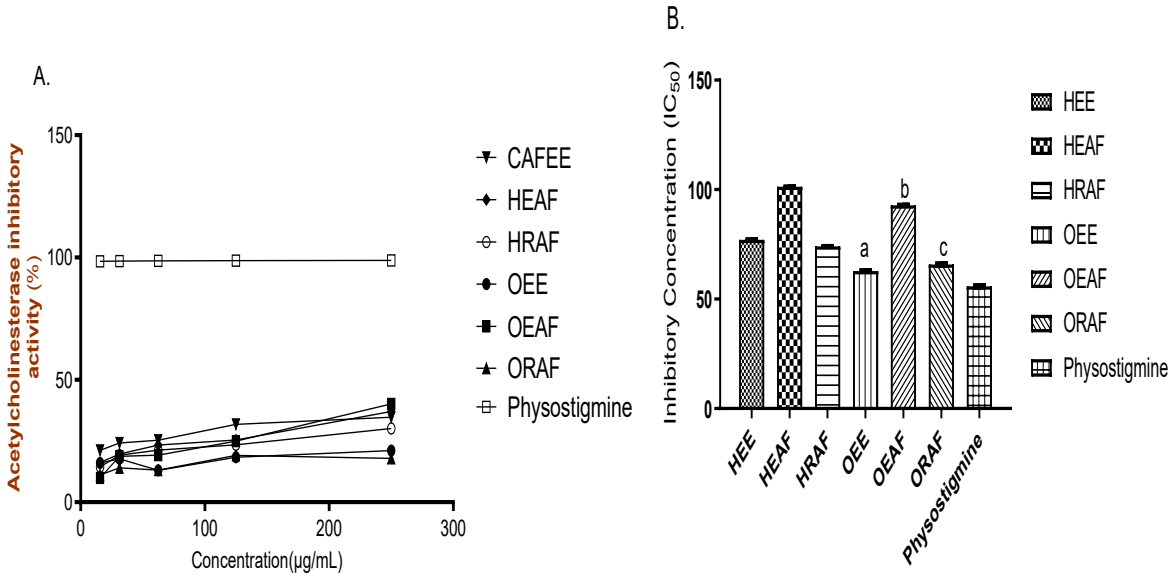


Figure 4: Acetylcholinesterase inhibitory activity (A) freshly homogenized and oven-dried powder extracts and fractions of *C. albidum* fruits, and (B) inhibitory concentration (IC_{50}). Letters indicate significant difference when compared with corresponding extract using one-way ANOVA followed by pairwise comparison using Tukey's post hoc test. AAE- ascorbic acid equivalent

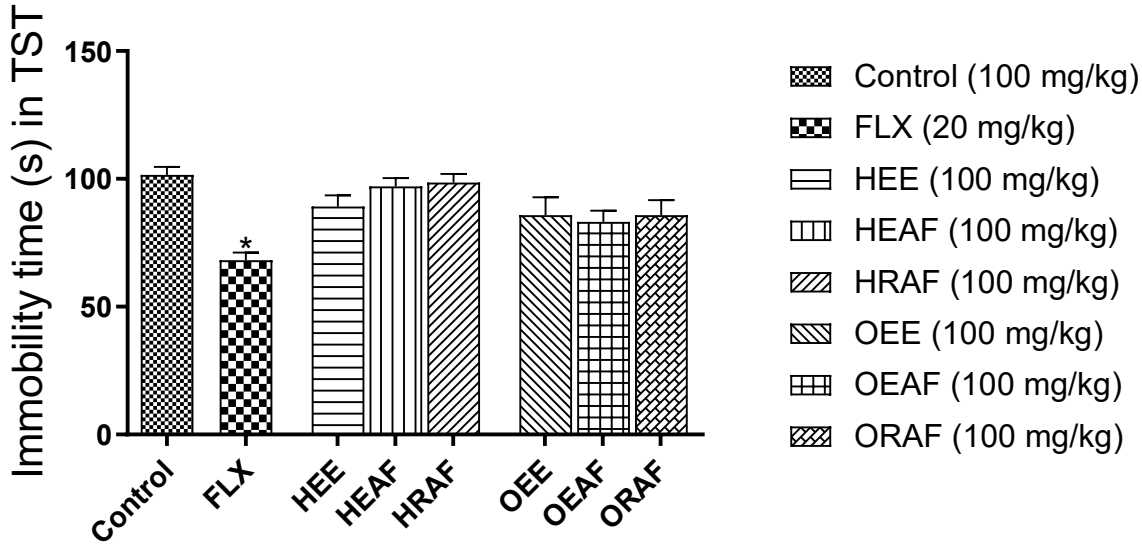


Figure 5: Antidepressant activity of homogenized and oven-dried powder extracts and fractions of *C. albidum* fruits in TST. Bar represents mean ±SEM (n=6). * $P < 0.05$ vs control using one-way ANOVA followed by pairwise comparison using Tukey's post hoc test.

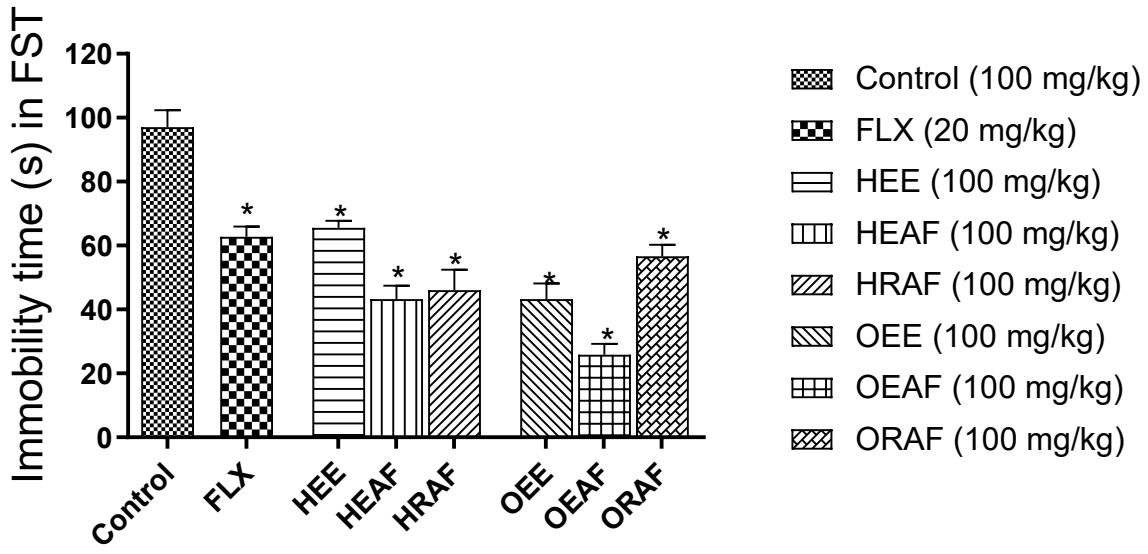


Figure 6: Antidepressant activity of homogenized and oven-dried powder extracts and fractions of *C. albidum* fruits in FST. Bar represents mean ±SEM (n=6). * $P < 0.05$ vs control, a $P < 0.05$ vs corresponding extract using one-way ANOVA followed by pairwise comparison using Tukey's post hoc test.

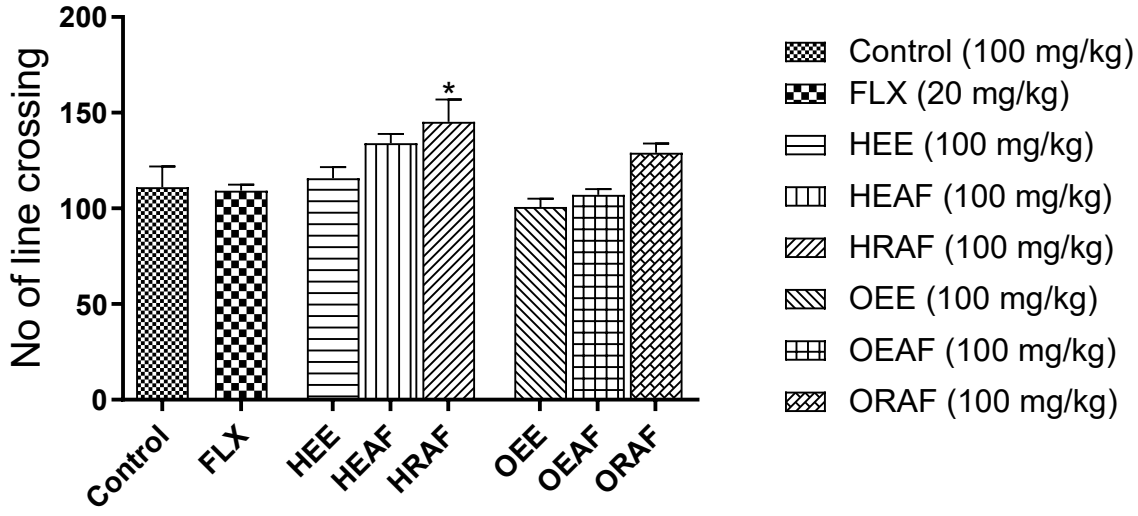


Figure 7: Effect of homogenized and oven-dried powder extracts and fractions of *C. albidum* fruits on locomotor activity in mice. Bar represents mean ±SEM (n=6). * $P < 0.05$ vs control using one-way ANOVA followed by pairwise comparison using Tukey's post hoc test.

Discussion

The study has provided information on health benefits that may accrue from consumption of fresh and oven dried fruits of *C. albidum*. Children and adolescents are nutritionally vulnerable age groups either categorized as undernourished or over-nourished (Tzioumis and Adair, 2014). Although prevalence data on fruits intake in Nigeria is scarce, a study reported fruits consumption prevalence of 12% among in-school adolescents in Ibadan, South West Nigeria (Ilesanmi *et al*., 2014). This is far below the World Health organization recommendation (WHO, 2023). It is important to note that socio-cultural acceptability of indigenous fruits can be greatly influenced by the knowledge of the health promoting potentials in such fruits. This study further provides information on the nutritional and health benefits of *C. albidum*.

In this comparative study, extraction yield was higher in the oven-dried fruits than in the freshly homogenized extract. It has been shown previously that conventional oven drying of fruits reduced moisture content and increases extraction yield, and hence its phytochemical constituents (Stephenus *et al*., 2023). Oven drying also increases shelf –life of the fruits powder, hence could be considered as a method of preserving and storage of tropical fruits at the peak season. This will enable the fruits products to be available as functional food ingredients and nutraceuticals.

Phenols and flavonoids are the most common phytoconstituents of different fruits, vegetables, and medicinal and aromatic plants, which account for their antioxidant activities. The TPC and TFC of freshly homogenized fruits of *C. albidum* were significantly higher than the oven-dried powder extract. These results correlate well with report on some fruits such as banana, mango and pineapple that showed higher polyphenolic content in fresh fruits compared with dried fruits extracts (Mongi et al., 2015). Drying has been shown to affect other minerals and vitamin C contents of *C. albidum* fruits (Bolarinwa and Ajetunmobi, 2020). However, the flavonoid and phenolic ratio of OEE extract and fractions are higher than that of HEE and fractions. The *in vitro* antioxidant assessment of *C. albidum* extracts and fractions showed variation in their antioxidant activity.

In the *in vitro* antioxidant assessment, DPPH radical scavenging assay is among the most frequently used methods and offers the first approach for evaluating antioxidant activity. In this study the *C. albidum* extracts showed DPPH radical scavenging activity with the Inhibitory concentration (IC_{50}) of the homogenized extract (HEE) and fractions lower than the oven-dried powder extract (OEE) and fractions. Hence, HEE and its fractions are more potent than OEE and its fractions in their DPPH free radicals scavenging activity.

Nitric oxide (NO) is a pro-inflammatory mediator involved in various physiological events and its production is essential in body defense mechanism. However, its overproduction can cause tissue damage and activation of pro-inflammatory mediators associated with acute and chronic inflammation (Dzoyem *et al*., 2016). The homogenized extract (HEE) has the lowest IC_{50} and this implies that it is more potent than the oven-dried powder extract (OEE) in scavenging NO free radical.

Antidepressants are a class of drugs that reduce symptoms of depressive disorders by correcting chemical imbalances of neurotransmitters in the brain. The behavioural despair test is centered on a rodent's response to the threat of drowning, whose result has been interpreted as measuring susceptibility to negative mood (Yankelevitch-Yahav *et al*., 2015). The TST and FST are mouse behavioral tests useful in measuring the potential antidepressant-like effects of drugs, and assessing of other manipulations that are expected to affect depression related behaviours. These tests have good predictive validity and allow for rapid screening of agents with potential antidepressant-activity (Castagné *et al*., 2010). In this study, both HEE and OEE and their fractions showed significant reduction in immobility time in the FST but not in the TST. Surprisingly, the oven-dried extract and fractions showed a more significant antidepressant-like activity when compared with their corresponding homogenized fresh fruit extract. In addition, ethyl acetate fraction of OEE was shown to produce a more profound reduction in immobility time in the FST. This may not be unconnected with a high flavonoid: phenolic ratio present in the fraction.

The open field test is a widely used to evaluate locomotor function when mice are placed in an open field arena. There was no significant alteration in number of lines crossed in animal treated with OEE and its fractions. A similar pattern was observed for HEE and its fractions, with the exception of the HRAF treated animals which had significant alteration in number of lines crossed. Overall, this means that the antidepressant-like activity observed in the FST is not due to stimulatory effect of the extracts. What account for these differences in the fresh fruit extract and the oven-dried extract remains to be investigated?

Conclusion

This study reveals that oven-drying of fruits of *Chrysophyllum albidum* reduces the total phenolic content, *in vitro* antioxidant activity but increased extraction yield and antidepressant activity. Therefore, it is more advantageous to take the fresh fruit than the dried, and oven drying might serve to preserve the fruit for nutraceutical purpose.

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