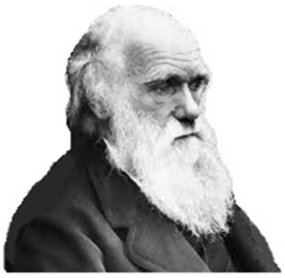
Available online at https://www.worldnewsnaturalsciences.com/



World News of Natural Sciences

An International Scientific Journal

WNOFNS 68 (2026) 88-107 EISSN 2543-5426

Comparative Phytochemical Characterization and Acute Toxicological Evaluation of *Chrysophyllum albidum* Fruit Pulp and Root Extract in Experimental Rodents

Ernest Emeka Ngwu[1], **Patrick Chinwe Ugwuezumba**[1], **Augustine Uchechukwu Obi**[2], **Eunice Ifenyinwa Agwah**[3], **Ebere Otuomasirichi Ibezim**[4], **Chigaemezu Remmy-Martin Akubuo**[5,*], and **Ezechukwu Gift Akandu**[6]

[1] Department of Human Physiology, Faculty of Basic Medical Sciences, Imo State University, Owerri, Imo State, Nigeria

[2] Department of Human Anatomy & Neurobiology, Faculty of Basic Medical Sciences, Imo State University, Owerri, Imo State, Nigeria

[3] Department of Dispensing Opticianry, School of Health & Related Technology, Federal Polytechnic Nekede, Owerri, Imo State, Nigeria

[4] Department of Surgery, Imo State University, Teaching Hospital Orlu, Imo State, Nigeria

[5] Department of Microbiology, Faculty of Biological Science, Imo State University, Owerri, Imo State, Nigeria

[6] Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, Imo State University, Owerri, Imo State, Nigeria

*E-mail address: martinakubuo@gmail.com

*ORCID: https://orcid.org/0009-0002-4939-5405

ABSTRACT

Chrysophyllum albidum is popularly eaten as a seasonal fruit and has been utilized for traditional medicine in some parts of West Africa. Comparative studies on the phytochemical profile, acute toxicity and body weight changes of the fruitpulp and root extract of the plant are limited. The phytochemical composition, acute oral toxicity and effect on body weight of fruit pulp and root extract of *C. albidum*

(Received 19 June 2026; Accepted 18 July 2026; Date of Publication 20 July 2026)

was evaluated on experimental rodents. Fresh ripe fruits and roots of *C. albidum* were harvested, identified and extracted in crude extracts. Phytochemical screening was conducted to find the presence and estimation of Saponins, Tannins, Alkaloids, Flavonoids, Phenolic compounds, Cardiac glycosides and Terpenes were conducted in both qualitative and quantitative manner. Acute oral toxicity was determined by giving single oral doses of 500, 1000, 2000, 3000, 4000 and 5000 mg/kg to mice, with observation for 7 days for symptoms of toxicity and death. Wistar rats were treated orally with 400 and 800 mg/kg of the fruit pulp and root extract for 30 days, respectively, for 30 days, to assess the effect of the extract on body weight. Data are shown as means ± SEM and were considered statistically significant at the 0.05 level. Saponins, tannins, alkaloids, flavonoids, cardiac glycosides and terpenes were present in both the extracts. Flavanoids and highly detected were present in fruit pulp while alkaloids were higher in the root extract. Flavonoids in the fruit pulp were significantly more (14.50 ±0.01 mg/100 g) than in the root extract (13.86 ±0.07 mg/100 g); and alkaloid content was significantly higher in the root extract than in the fruit pulp (15.57 ±0.01 mg/100 g vs 11.07 ±0.35 mg/100 g). There was no mortality in the mice when oral dosage of the extract up to 5000 mg/kg was taken. The oral LD_{50} is more than 5000 mg/kg in mice. The body weight gain was not significantly different between the 400 mg/kg of both extracts and the control in this 30 day study, but the 800 mg/kg of both fruit pulp extract and root extract caused a significant decrease in body weight gain. *C. albidum* fruit pulp and root extract exhibited low acute oral toxicity and several bioactive phytochemicals under the experimental conditions. The marked decrease in body weight gain at 800 mg/kg did indicate that chronic exposure to higher doses might have measurable effects. The results of this study clearly demonstrated the nutritional and ethnomedicinal significance of *C. albidum* and highlighted the necessity of dosage precautions and additional subacute, chronic, biochemical and histopathological safety tests.

Keywords**:** *Chrysophyllum albidum*, African star apple, fruit pulp, root extract, phytochemical characterization, acute oral toxicity, LD_{50}, body weight gain, experimental rodents, medicinal plants, bioactive compounds

1. INTRODUCTION

Medicinal plants continue to be valuable sources of bioactive compounds for food, traditional medicine and drug development. Plant-based preparations are commonly used in many communities across Africa due to cultural acceptance, availability and perceived safety. The World Health Organization (WHO, 2013) has stressed the need for more scientific evidence regarding quality, efficacy, safety and regulation of traditional medicinal products for a broader therapeutic use. The World Health Organization (WHO, 2013) has stressed the need for more scientific evidence regarding the quality, efficacy, safety and regulation of traditional medicinal products for a wider therapeutic use. This is an important factor because, the plant parts, extraction processes, doses, duration of exposure and route of administration could yield beneficial or harmful effects in plants (Jităreanu et al., 2022).

Secondary metabolites which are the phytochemicals are responsible for the pharmacological activities of medicinal plants. Alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, steroids, and glycosides are considered to be involved in the antioxidant and antimicrobial, anti-inflammatory, antidiabetic, and cytoprotective properties of the compounds. However, the fact that the plant contains active materials does not necessarily mean that it is safe, as some phytochemicals can be harmful if they are used too often or at a high concentration.

Thus, the phytochemical characterization needs to be associated with toxicological studies when evaluating medicinal plants for potential therapeutic use (Jităreanu et al., 2022; Mugale et al., 2024).

Chrysophyllum albidum G. Don., African star apple, is a member of the Sapotaceae family and is distributed throughout tropical and subtropical Africa. The ripe fruit is eaten in Nigeria and other west African nations and various parts of the plant have been traditionally used in the control of fever, diarrhoea, infections, malaria, inflammation, diabetes related disorders and other ailments. In the past, various bioactive constituents have been reported in *C. albidum*, along with its antioxidant, antimicrobial, antiplasmodial, anti-inflammatory, analgesic, and antidiabetic properties (Emudainohwo et al., 2015; Ihekwereme et al., 2017; Ajayi et al., 2021).

The fruit pulp of *C. albidum* has a special significance due to the fact that it is directly utilised as food, and it may also be a source of nutraceutical compounds. The nutritional and phytochemical analysis of edible parts of *C. albidum* fruit reveals that they contain alkaloids, tannins, saponins, flavonoids, terpenoids, carbohydrates, minerals and other constituents (Ewansiha et al., 2011). The antioxidant activity of the fruit parts of *C. albidum* and their inhibitory effect on carbohydrate-hydrolysing enzymes (α-amylase and α-glucosidase) have also been established through experimental studies, revealing potential application in metabolic health (Ajayi et al., 2020). Furthermore, the oral acute toxicity assessment of the fruit pulp (traditional food used) showed that there was no mortality at the concentrations tested in experimental animals, suggesting a relatively high safety margin when used as per the test conditions (Ihekwereme et al., 2017).

The root of *C. albidum* is less frequently used for food, while the root bark has been studied for medicinal uses; however, the root's uses also have not been widely reported. The root has been reported to exhibit hypoglycaemic, antioxidant and hepatoprotective effects on alloxan induced diabetic rats, suggesting that the plant possesses biologically active compounds which may be used in the treatment (Onyeka et al., 2013). The concentrations, pharmacological activities, and toxicological risks of phytochemicals may be different, however, between the underground parts and the edible fruit pulp, as the roots tend to be readily loaded with defensive secondary metabolites. This makes it imperative to compare the fruit pulp with root extract either directly or indirectly, particularly if both plant parts are used or looked upon for treatment purposes (Onyeka et al., 2013; Mugale et al., 2024).

The assessment of potential toxicity is an essential first step in the elucidation of the safety profile of plant extracts, which is known as acute toxicological evaluation. It offers initial data on effects that may occur after brief exposures such as death, behavioural changes, changes in body weight, neurological effects, gross pathological effects, etc. To minimize the number of animals used for toxicity testing, the Organisation for Economic Co-operation and Development has recommended standardised procedures for assessing acute oral toxicity, such as the fixed-dose procedure and the acute toxic class method. This assessment is especially important for medicinal plants, since traditional usage does not necessarily prove the plants are safe for use (Jităreanu et al., 2022).

Previous studies mainly evaluated the nutritional, phytochemical, antidiabetic, antioxidant and toxicological aspects of some parts of *C. albidum*, but there is little evidence to compare the phytochemical and acute toxicity profile of the edible fruit pulp and root extract under similar experimental conditions. This is because the pulp is frequently consumed and is considered safe while the root could have higher concentrations of bioactive compounds that

may have different safety profile. Hence, the present study is justified due to the need to compare the phytochemical constituents and the acute toxicological effects of the root extract and fruit pulp of *C. albidum* in experimental rodents which is needed to ensure safer ethnomedicinal usage and further pharmaceutical development (Ihekwereme et al., 2017; Onyeka et al., 2013).

2. METHODOLOGY

2. 1. Study Design

The study was an experimental laboratory study aimed at comparing the Phytochemical composition, Acute oral toxicity profile and effect on the body weight of *Chrysophyllum albidum* Fruit pulp and root extract in experimental rodents. The study comprised plant material collection and authentication, preparation of fruit pulp and root extract, qualitative and quantitative phytochemical screening, acute oral toxicity of the plants in mice and 30 days treatment assessment in Wistar rats. The animal study was performed in compliance with the accepted principles of the care and use of laboratory animals and reported following the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

2. 2. Collection and Authentication of Plant Materials

The fresh ripe fruits of *Chrysophyllum albidum* were bought from Orie-Udeora market, Umuna, Orlu Local Government Area of Imo state, Nigeria while the roots were collected from a local farm settlement in Amaifeke, Orlu Local Government Area, Imo State, Nigeria. The plant materials were then brought to the Department of Forestry College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria for botanical identification in clean labelled containers. Authenticating the specimens as fruits and roots of *Chrysophyllum albidum* G. Don. A voucher number, CVM/VPP/17/014 was assigned and representative specimens were deposited in the herbarium of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike for future reference.

2. 3. Preparation of *Chrysophyllum albidum* Fruit Pulp Extract

The fresh ripe fruits were washed with clean water to remove dirt and external contaminants. The fruits were hand opened and the pulp removed from the epicarp and seeds. The collected pulp was homogenized in a clean manual blender and twice filtered through the clean muslin cloth to get the pulp filtrate. The filtrate was concentrated in a hot-air oven at 60 °C to get crude fruit pulp extract of dark brown colour. The weight of the crude fruit pulp extract was 480 g out of the total weight of the 2000 g of fruit, or 24.0% yield. All extracts were placed in sterile labeled containers and then refrigerated until needed for phytochemical and experimental analysis.

The percentage yield was given by:

$$\text{Percentage yield} = \frac{weight\ of\ extract\ obtained}{Initial\ weight\ of\ plant\ materials} \times 100$$

$$\text{Percentage yield} = \frac{480}{2000} \times 100 = 24\%$$

2. 4. Preparation of *Chrysophyllum albidum* Root Extract

Newly grown roots were rinsed with plenty of water to loosen soil particles and other impurities. The roots were air-dried at room temperature for 21 days then cut up into smaller pieces with a clean cutlass. The root portions dried were ground to a coarse powder in a clean mechanical blender. The powdered root material was placed inside the extraction chamber of a Soxhlet extractor and the extraction was then performed with ethanol. The extraction temperature was kept at 48 hours at 60 °C.

The crude root extract was a dark brown product obtained by low-temperature evaporation of the ethanol at the end of the extraction in a hot-air oven. The percentage yield was calculated to be 6.40% as the result was 3.20 g of crude root extract from 50 g of the powdered root material. The extract was kept in sterile, labelled containers, in a refrigerated condition, until needed.

The percentage yield was determined using the equation:

$$\text{Percentage yield} = \frac{weight\ of\ extract\ obtained}{Initial\ weight\ of\ plant\ materials} \times 100$$

$$\text{Percentage yield} = \frac{3.20}{50} \times 100 = 6.4\%$$

2. 5. Qualitative Phytochemical Screening

The fruit pulp and root extracts of *C. albidum* were qualitatively screened for phytochemical constituents using standard phytochemical technique for medicinal plant analysis. Qualitative screening of extracts was conducted for the presence of saponins, tannins, alkaloids, flavonoids, phenols, cardiac glycosides and terpenes. These phytochemical groups were identified by applying the standard colour change tests, frothing tests, emulsion tests, precipitation tests and layer reaction tests. The observed reaction intensity gave each constituent detected a relative intensity of high (+++), moderate (++), or low (+). It was carried out using the methods of Trease and Evans, (1989), Harborne, (1973) and Sofowora, (1993) and also used by Deka and Kalita, (2012).

Test for Saponins

2 mls of each extract were diluted with 5 mls of distilled water and shaken for 2 minutes. A few drops of olive oil were added and the mixture was shaken again. Saponins were present when a stable emulsion was formed.

Test for Tannins

Each of the extracts was diluted with 4 mL distilled water and a few drops of ferric chloride solution were added to each. The mixture was monitored for a colour change. The presence of tannins was indicated by the development of blue-black, blue-green or greenish colour.

Test for Alkaloids

The extracts were each treated with a few drops of Dragendorff's reagent and two millilitres were used. The formation of an orange or reddish-brown precipitate was a sign of the presence of alkaloids.

Test for Flavonoids

5 ml of 5% sodium hydroxide solution were added to each extract. Yellow coloration was detected during development and was an indication of flavonoids.

Test for Phenolic Compounds

A drop of each extract was added to distilled water in a test-tube and warmed gently. Add 2 mL of $FeCl_3$ solution. Green, blue or bluish black colouration formed was due to the presence of phenolic compounds.

Test for Cardiac Glycosides

About 0.5 mL of each extract was mixed with 1 mL of glacial acetic acid containing traces of ferric chloride. A concentrated sulphuric acid was slowly poured down the side of the test tube, 1 ml of it. A reddish-brown ring at the intersection of the two layers, and the bluish-green color of the upper layer, were signs of cardiac glycoside.

Test for Terpenes

Two mls of each extract was then added to chloroform and concentrated sulphuric acid was carefully added to the test tubes alongside to form a layer. A reddish-brown interface formed, which showed the presence of terpenes.

2. 6. Quantitative Phytochemical Analysis

The fruit pulp and root extracts have been subjected to quantitative phytochemical analysis to determine the amounts of selected secondary metabolites. The phytochemicals quantified were saponins, tannins, alkaloids, flavonoids, phenolic compounds, cardiac glycosides and terpenes. Appropriate spectrophotometric and gravimetric methods were used and results were reported as mg/100 g of extract. The quantitative analysis was conducted and direct comparison was made between the phytochemical composition of the fruit pulp and root extract. It was carried out using the methods of Morsy, (2014).

Determination of Saponin Content

Each of the powdered samples was heated in 100 mL of 20% aqueous ethanol at approximately 55 °C for 4 hours with constant stirring. The mixture was filtered and re-extracted with 200 mL of 20% ethanol. The combined extracts were concentrated down to 40 mL over a water bath at ~ 90 °C and extracted using diethyl ether. The water phase was collected and re-extracted using n-butanol. Water phase was recovered and re-extracted with n-butyl alcohol. The extracts were extracted as a combined n-Butanol extract and washed twice with 5% of sodium chloride solution, evaporated, dried to constant weight and used to measure the content of saponins.

Determination of Tannin Content

The measured amount of each extract was added to distilled water and boiled for 30 minutes. The material was filtered and distilled water added to make up the volume of the solution. Kjeldahl aliquots were treated with potassium ferricyanide and ferric chloride solutions and distilled water was added to bring the volume to 100.0 mL. The absorbance was measured at 5 minutes after the addition using a spectrophotometer at 720 nm. A standard calibration curve was used to calculate the concentration of the tannin.

Determination of Alkaloid Content

Five grams of each extract was placed in a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added to each beaker. After 4 hours the mixture was filtered. The filtrate was concentrated on water bath to 1/4 of its original volume. Concentrated ammonium hydroxide was then added dropwise until it was all precipitated. The precipitate was allowed to settle, collected, washed with dilute Ammonium Hydroxide, filtered, dried and weighed.

Determination of the content of flavonoids

The methanolic extract solution of each plant part was mixed with 120 mM potassium acetate (0.5 mL) and Aluminium chloride (0.5 mL) to make a total volume of 2 mL. The mixture was left at room temperature for 30 minutes. The absorbance was determined at 415 nm by a spectrophotometer and concentration of flavonoids was determined from the standard curve.

Determination of Total Phenolic Content

Each extract was diluted to 1 mL and mixed with 0.1 mL of Folin–Ciocalteu reagent and left to stand for 15 minutes. Five mL of saturated sodium carbonate solution was poured on it and stood for 30 minutes at room temperature. A spectrophotometer was used to measure absorbance at 760 nm. The phenolic content was quantified using gallic acid as reference.

Determination of Cardiac Glycoside Content

Each plant sample was placed in 10 mL of 70% alcohol and soaked for 2 hours then filtered. Lead acetate and disodium hydrogen phosphate solutions were used for purification of the filtrate. Baljet's reagent was freshly prepared and added to the samples and absorbance of the colour produced was taken at 495 nm on the spectrophotometer. The absorbance values were used to estimate the amount of cardiac glycoside in the preparation.

Determination of Terpene Content

A standard spectrophotometric method was used to determine the amount of terpene. Each extract was added to a measured amount of chloroform and concentrated sulphuric acid and the coloured layer produced was measured spectrophotometrically. The concentration of terpenes was determined by reading the absorbance values and then expressed in mg/100 g of extract.

2. 7. Experimental Animals

The acute oral toxicity study was conducted on healthy adult mice and 30-day body weight study was conducted on adult Wistar rats. The animals used were purchased from the

Animal Production Unit of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, College of Veterinary Medicine. Standard feed and clean water ad libitum were provided under standard laboratory conditions in cages which were clean and well-ventililated. Animals were acclimatized prior to treatment, and treated humanely during the experiment.

2. 8. Ethical Approval and Animal Welfare

Prior to the study, ethical approval was granted. All animal procedures were conducted according to accepted guidelines of laboratory animal care and use. Precautions were taken to reduce discomfort, stress and undue suffering. Animal handling, reporting were carried out according to the Guide for the Care and Use of Laboratory Animals and ARRIVE (Percie du Sert et al., 2020) recommendations for experimental animal studies.

2. 9. Acute Oral Toxicity Evaluation

The acute oral toxicity assessment was performed on mice separately for fruit pulp and root extracts. Thirty mice were used for each extract in 6 groups of 5 mice each. The groups received single oral doses of 500, 1000, 2000, 3000, 4000, and 5000 mg/kg body weight of the respective extract. Oral gavages were used to provide extract. Mice were then monitored for toxicity and death.

The animals were carefully watched in the first 24 hours after the administration and then daily for 7 days. The clinical signs evaluated for toxicity were locomotor activity change, posture change, grooming, feeding behaviour, salivation, piloerection, tremor, convulsion, diarrhoea, respiratory distress, weakness, sleepiness, coma and death. The median lethal dose was estimated by counting the number of deaths at each dose level. The principles of general acute oral toxicity testing and classification of toxicity (Lorke, 1983) were used in guiding the acute toxicity assessment.

2. 10. Determination of Median Lethal Dose

The median lethal dose was estimated from the mortality pattern that was observed following the administration of graded doses of each extract. The toxic dose for 50% of the animals (LD_{50}) has been determined using the relationship proposed by Lorke:

$$LD_{50} = \sqrt{A \times B}$$

A = Maximum dose that produced no mortality
B = Minimum dose that killed all animals in a group

In the present study, no mortality was recorded at the highest administered dose of 5000 mg/kg body weight.

Therefore, the oral LD_{50} was not directly determined but was considered to be:

$$LD50 > 5000 \text{ mg/kg}$$

2. 11. Thirty-Day Treatment and Body Weight Assessment in Wistar Rats

The 30 day treatment phase was carried out on 60 adult Wistar rats. The rats were randomly allocated to six groups of 10 rats in each cage, which were all of clean aluminium

material. The control group was group A, which was given the vehicle. The dose of *C. albidum* fruit pulp extract was given to group B1 (400 mg/kg body weight). The group B2 was given 400 mg/kg body weight of *C. albidum* root extract. C1 group was treated with 800 mg/kg body weight of *C. albidum* fruit pulp extract. All the C2 group was administered with *C. albidum* root extract at the dose of 800 mg/kg body weight. The Proviron was administered to group D at 50 mg/kg body weight. The oral treatments were given by gavage for 30 days/day.

On Day 1, prior to treatment, and Day 30 after treatment, the body weights were recorded. Weight gain was determined by subtracting the weight at the beginning from the weight at the end. Body weight percentage increase was determined by the following formula:

Body weight gain = Final body weight – Initial body weight

Percentage rise in body weight was calculated using the formula:

$$\text{Percentage rise in body weight} = \frac{Final\ body\ weight - Initial\ body\ weight}{Initial\ body\ weight} \times 100$$

Initial body weight = body weight recorded on Day 1 before treatment

Final body weight = body weight recorded on Day 30 after treatment

To assess the effect of repeated oral administration of the fruit pulp and root extracts on the growth pattern or the general physiological status of the treated rats, a body weight assessment was included.

2. 12. Outcome Measures

The main phytochemical results were the qualitative intensity and quantitative concentration of the extracts obtained from the fruit pulp and root of the plant, namely the saponins, tannins, alkaloids, flavonoids, phenolic compounds, cardiac glycosides and terpenes. The main acute toxicity effects were clinical signs of toxicity, mortality, percentage mortality and estimated LD_{50} values. Day 1 body weight, day 30 body weight, absolute weight gain, and percentage increase in body weight were the body weight outcomes measured.

2. 13. Statistical Analysis

IBM SPSS Statistics software was used for analysing data. The quantitative phytochemical value and the body weight parameters were represented in terms of mean ± SEM. The differences in phytochemical composition between the fruit pulp and root extract were analysed by independent samples t-test. One-way analysis of variance with post-hoc comparison (when necessary) was used for the body weight parameters of the treatment groups. The frequencies and percentage of mortality and clinical signs of toxicity were summarized. The level of statistical significance was p-value < 0.05.

2. 14. Quality Control

Phytochemical analysis was done using analytical grade all the reagents. To avoid contamination, glassware and laboratory equipment were cleaned and dried before they were

used. Extracts were well labelled and kept in the refrigerator when needed. Where applicable, spectrophotometric assays were done using reagent blanks and standards.

The grouping of animals, administration of extracts and monitoring of toxicity was performed in standardized laboratory conditions, increasing the reliability and reproducibility of the experiment.

3. RESULTS

3. 1. Qualitative phytochemical composition of *Chrysophyllum albidum* fruit pulp and root extract

Qualitative screening indicated that both fruitpulp of *C. albidum* and root extract are rich in saponins, tannins, alkaloids, flavonoids, phenolic compounds, cardiac glycosides and terpenes. The content of saponins was highly detected in both extracts, and that of alkaloids was highly detected in the root extract whereas that of flavonoids was highly detected in the fruit pulp (Table 1).

Table 1. Qualitative phytochemical composition of *Chrysophyllum albidum* fruit pulp and root extract.

Phytochemical constituent	Fruit pulp	Root extract
Saponins	+++	+++
Tannins	++	++
Alkaloids	++	+++
Flavonoids	+++	++
Phenolic compounds	+	+
Cardiac glycosides	+	+
Terpenes	+	+

Key: +++ = high intensity; ++ = moderate intensity; + = low intensity.

3. 2. Quantitative phytochemical composition of *Chrysophyllum albidum* fruit pulp and root extract

The quantitative phytochemical analysis of fruit pulp and root extract of *C. albidum* is presented in Table 2 and Figure 1. The flavonoids and alkaloids were significantly higher in the fruit pulp and root extract respectively. The results of this study showed no significant difference between both extracts in terms of saponins, tannins, phenolics, cardiac glycosides and terpenes.

Table 2. Quantitative phytochemical composition of *Chrysophyllum albidum* fruit pulp and root extract.

Phytochemical constituent	Fruit pulp (mg/100 g)	Root extract (mg/100 g)
Saponins	16.69 ± 0.02	17.73 ± 0.05
Tannins	8.85 ± 0.50	9.73 ± 0.02
Alkaloids	11.07 ± 0.35*	15.57 ± 0.01
Flavonoids	14.50 ± 0.01*	13.86 ± 0.07
Phenolic compounds	3.54 ± 0.02	4.71 ± 0.08
Cardiac glycosides	0.93 ± 0.60	1.28 ± 0.01
Terpenes	2.88 ± 0.01	2.65 ± 0.02

Values are presented as mean ± SEM. *Indicates a significant difference between fruit pulp and root extract within the same row at $p < 0.05$.

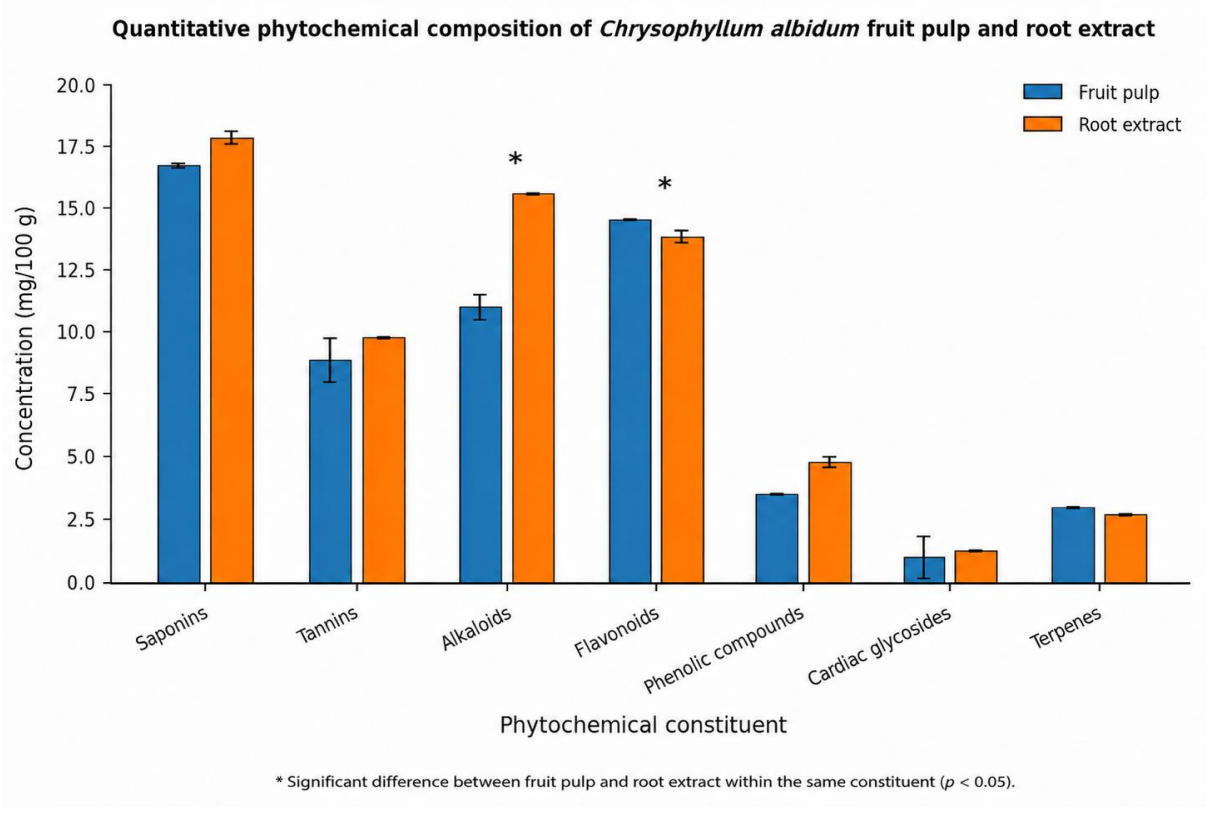


Figure 1. Quantitative phytochemical composition of *Chrysophyllum albidum* fruit pulp and root extract.

3. 3. Acute oral toxicity of *Chrysophyllum albidum* fruit pulp and root extract

The acute oral toxicity of fruit pulp and root extract of *Chrysophyllum albidum* was evaluated. The fruit pulp extract and root extract of *Chrysophyllum albidum* were investigated for their acute oral toxicity. The fruit pulp and root extract of *C. albidum* did not cause any mortality in the mice when they were treated with a single dose of 500, 5000 mg/kg for 7 days (Tables 3 and 4). Thus, the oral LD_{50} of both extracts was estimated to be above 5000 mg/kg under the experimental conditions.

Table 3. Acute oral toxicity profile of *Chrysophyllum albidum* fruit pulp extract in mice.

Group	Dose administered (mg/kg)	Number of deaths	Mortality (%)
I	500	0	0.00
II	1000	0	0.00
III	2000	0	0.00
IV	3000	0	0.00
V	4000	0	0.00
VI	5000	0	0.00

Table 4. Acute oral toxicity profile of *Chrysophyllum albidum* root extract in mice.

Group	Dose administered (mg/kg)	Number of deaths	Mortality (%)
I	500	0	0.00
II	1000	0	0.00
III	2000	0	0.00
IV	3000	0	0.00
V	4000	0	0.00
VI	5000	0	0.00

3. 4. Effects of *Chrysophyllum albidum* fruit pulp and root extract on body weight changes in Wistar rats

The body weight changes of Wistar rats after 30 days treatment are given in Table 5. There was no significant effect of the fruit pulp extract and root extract (400 mg/kg) on body

weight gain compared to control. The 800 mg/kg doses of both extracts, however, caused significant decreases in body weight gain and percentage weight gain when compared to control.

Table 5. Effects of *Chrysophyllum albidum* fruit pulp and root extract on body weight changes in Wistar rats.

Group	Treatment/dose	Body weight Day 1 (g)	Body weight Day 30 (g)	Weight gain (g)	Rise in weight (%)
A	Control	161.12 ±2.64	195.85 ±2.94	34.73 ±3.85	21.82 ±2.51
B1	Fruit pulp, 400 mg/kg	152.74 ±4.51	186.54 ±6.97	33.80 ±5.43	22.29 ±4.06
B2	Root extract, 400 mg/kg	150.42 ±3.46	182.89 ±7.23	32.47 ±7.98	21.56 ±5.86
C1	Fruit pulp, 800 mg/kg	156.96 ±6.11	171.34 ±3.83*	14.38 ±5.05*	9.99 ±2.88*
C2	Root extract, 800 mg/kg	164.32 ±4.81	183.60 ±4.93	19.28 ±2.39*	11.88 ±1.57*
D	Proviron, 50 mg/kg	168.40 ±5.59	194.94 ±5.27	26.54 ±1.56	16.00 ±1.17

Values are presented as mean ± SEM. *Indicates a significant difference compared with the control group at $p < 0.05$.

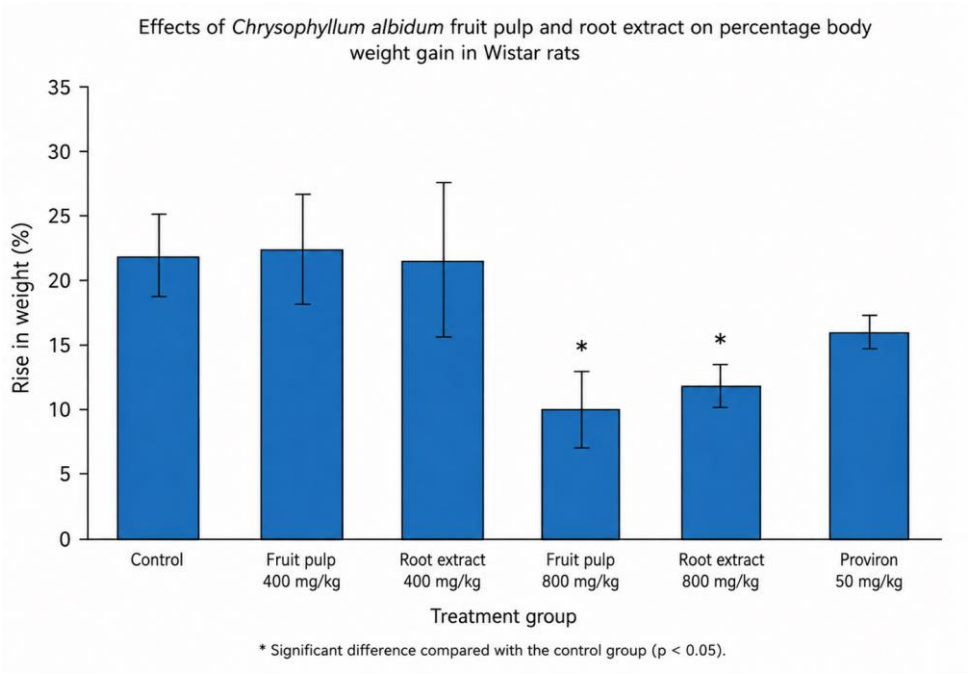


Figure 2. Effects of *Chrysophyllum albidum* fruit pulp and root extract on percentage body weight gain in Wistar rats.

4. DISCUSSION

In this study, the phytochemical composition, the acute oral toxicity and effect on body weight of the extract of the fruit pulp and root of *Chrysophyllum albidum* were compared between experimental rats. The results indicated the presence of major secondary metabolites such as saponins, tannins, alkaloids, flavonoids, phenolic compounds, cardiac glycosides and terpenes in both the extracts. But, their relative concentrations varied with the part of the plant, the fruit pulp had significantly higher concentration of flavonoids and the root extract had significantly higher concentration of alkaloids. No mortality was observed with either of the extracts at dosages up to 5000 mg/kg, indicating that they were non-lethal under the present experimental conditions. In Wistar rats, however, repeated doses at 800 mg/kg caused a significant decrease in body weight gain, showing that lack of acute mortality does not rule out dose-dependent physiological changes. According to toxicological guidelines and recent reviews on medicinal plant safety (Mugale et al., 2024; Maru et al., 2025), weight loss, clinical signs, biochemical data, organ weights, and repeated dose responses are also critical indicators of systemic tolerance besides mortality.

The qualitative phytochemical profile revealed a considerable similarity between the fruit pulp and root extract, with the difference in intensity of some of the phytochemicals. The presence of saponins was detected highly while the presence of flavonoids was detected more intense in fruit pulp and in the root extract the presence of alkaloids was detected more intense. This finding is consistent with the biological fact that the phytochemical classes may occur in similar proportions in the different parts of a plant due to variation in tissue function, biosynthetic activity, storage function and ecological defence function. Fruits tend to store antioxidants and pigments to protect and disperse seeds, while roots store higher amounts of defensive metabolites against soil pathogens and environmental stressors (Panche et al., 2016; Hassanpour et al., 2023; Lemanowicz et al., 2026). Ewansiha et al. (2011), Ihekwereme et al. (2017), Akinpelu et al. (2016) and Nartey et al. (2021) reported the presence of related secondary metabolites in the fruit pulp, edible fruit parts, stem bark, leaves, fruits, bark and roots of *C. albidum* respectively.

Saponins, tannins, alkaloids, flavonoids, phenols, cardiac glycosides, and terpenes are detected in both extracts, thus indicating the phytochemical richness of *C. albidum*, which justifies its reported ethnomedicinal and functional value. The phytochemical constituents of stem bark of *C. albidum* and those of the leaves, fruits, bark and roots of *C. odoratissimus* include flavonoids, tannins, saponins, alkaloids, steroids, reducing sugars and terpenoids (Akinpelu et al., 2016; Nartey et al., 2021). Lately, Adadi et al. (2026) summarized the nutritional, phytochemical and functional attributes of African star apple and its by-products, underlining the significance of its use as a source of bioactive compounds for food and health applications. The current finding of several classes of phytochemicals with overlapping but quantitatively varying classes of compounds (Akinpelu et al., 2016; Nartey et al., 2021; Adadi et al., 2026) is supported by the aforementioned reports.

The root extract had significantly greater flavonoids than fruit pulp, as determined quantitatively. This is biologically relevant as flavonoids are polyphenols and are of major importance in plants, which have antioxidant, anti-inflammatory, metabolic and cytoprotective activities. They are thought to have anti-oxidant, anti-inflammatory, and anti-microbial properties, and have been shown to interact with cell-signalling pathways, oxidative enzymes, and inflammatory mediators, as well as chelate metals, though the extent to which these

activities are useful depends on the specific context (Panche et al., 2016; Safe et al., 2021; Hassanpour et al., 2023; Lemanowicz et al., 2025). This result could be one of the reasons for the functional value assigned to the fruit of African star apple. The fruit parts of *C. albidum* exhibited antioxidant activity and inhibitory activity against α-amylase and α-glucosidase (2020, Ajayi et al.) and the fruit pulp powder of *C. albidum* modulated genes associated with antioxidant, inflammatory and glucose metabolism in diabetic rats (2021, Oyetayo et al.). The observations agree with the current results showing a relatively higher concentration of flavonoids in the edible fruit pulp which could be attributed to the nutraceutical properties of the plant (Ajayi et al., 2020; Oyetayo et al., 2021; Yang, 2025).

The alkaloids content of the root extract was on the other hand significantly higher than that of the fruit pulp. Depending on their chemical structure and concentration, the alkaloids are pharmacologically active secondary products that could be responsible for antimicrobial, anti-diabetic, analgesic, anti-inflammatory and neuroactive activity. But at high doses or with long-term use, some alkaloids can cause toxicity, highlighting the toxicological importance of the higher concentration of these compounds in the root extract (Anywar et al., 2021; Mugale et al., 2024). This could be responsible for the systemic pharmacological actions of root and root bark preparations of *C. albidum* as reported in earlier studies. Onyeka et al. (2013) reported that ethanolic root bark extract of *C. albidum* exhibited hypoglycaemic, antioxidant and hepatoprotective effect in alloxan induced diabetic rats suggesting that the root bark contains significant bioactive compounds. In conclusion, the extract of the root contains a significant amount of alkaloids, which suggests potential therapeutic benefits, but careful dosing and additional safety testing are recommended.

The moderate tannin content and high saponin detection in both extracts could also add to the biological activities of *C. albidum*. Tannins possess antioxidant and antimicrobial properties, and, when present in large amounts, may also interact with the digestive enzymes and dietary proteins, affecting nutrient bioavailability. A wide range of antimicrobial, anti-inflammatory, cholesterol-lowering, and membrane-active properties have been reported for saponins, but in some experiments, overdosage could impact nutrient absorption, gastrointestinal tolerance, and growth performance (Freeland et al., 1985; Salim et al., 2023; Singh et al., 2023). The safety in the acute phase might be due to this dual biological nature, and the decrease in weight gain at repeated high exposure was probably due to this effect. Also latest review on antinutrient and medicinal plant safety has also focused on the importance of phytochemicals at moderate doses while the high doses may have adverse physiological effects (Salim et al., 2023; Singh et al., 2023; Mugale et al., 2024).

Phenolic compounds, cardiac glycosides and terpenes were present in small amounts, but they are still biologically significant. Phenolic compounds and terpenes can have antioxidant, antimicrobial, anti-inflammatory and cytoprotective properties, which can be additive or synergistic effects in combination with flavonoids, alkaloids, saponins and tannins (Hassanpour et al., 2023; Nartey et al., 2021; Yang, 2025). The presence of cardiac glycosides also corroborates Ihekwereme et al. (2017) who detected cardiac glycosides in preparations of *C. albidum* pulp. However, cardiac glycosides have been found to be biologically active, even at low concentrations, suggesting that they need to be assessed for toxic effects in addition to traditional use as a proof of safety (Ihekwereme et al., 2017; Mugale et al., 2024; Philips et al., 2024).

The acute oral toxicity results revealed no mortality among mice, when given fruit pulp or root extract at 500 – 5000 mg/kg. The LD_{50} of the extracts was, therefore, estimated to be

more than 5000 mg/kg under the experimental conditions. This result is in good agreement with Ihekwereme et al. (2017) who reported that the oral acute toxic dose of *C. albidum* pulp extract was more than 5000 mg/kg as all animals survived at that dose. It also correlates with the OECD 2002 interpretation of acute toxicity (absence of death at high doses suggests low acute oral lethality under the given test conditions). Acute toxicity studies, however, give only preliminary information regarding toxicity and do not entirely rule out delayed, subacute, chronic, reproductive, biochemical, genotoxic and histopathological toxicity (Mugale et al., 2024; Maru et al., 2025).

The lack of death in the root extract group is especially significant because roots may have more levels of secondary metabolites which are dangerous for the organism than edible fruits. In the present study the root extract showed significantly higher alkaloid content, but showed no mortality up to 5000 mg/kg. This indicates that the root extract is not highly acute orally toxic even though it contains higher level of alkaloids. The same has been observed in studies on the safety of medicinal plants, where extracts with high levels of secondary metabolites might be non-lethal at acute doses but become toxic at repeated doses (Bemidinezhad et al., 2023; Mugale et al., 2024; Mafogang et al., 2025). Therefore, the present results do not confirm the long-term safety of the extract, particularly the root part which is not consumed as food.

The results of the body weight determinations were a useful supplement to the acute toxicity data. The fruit pulp and root extract at concentration of 400 mg/kg did not significantly affect the body weight gain which indicated that the lower dose was well tolerated during the study period of 30 days. The body weight gain and percentage body weight gain were significantly decreased by both extracts at 800 mg/kg. Weight gain is a particularly sensitive overall indicator in repeated dose toxicology as decreased weight gain can be associated with decreased food intake, impaired nutrient uptake, altered metabolism, systemic toxicity, organ damage, or intolerance of treatment (Balkrishna et al., 2023; Mugale et al., 2024). Acute and subacute toxicity studies have also been conducted in recent years, and body weight, organ weight, clinical observations, haematology and biochemical markers are still considered as core endpoints to define safety and tolerability of herbal products (Maru et al., 2025; Mafogang et al., 2025).

The lower increase in body weight seen at 800 mg/kg may be due to several factors. Higher exposure to tannins and saponins could affect nutrient utilization. Tannins are proteins and digestive enzyme chelators that can interact with proteins, thereby decreasing digestibility and energy availability, and saponins interact with membranes, gastrointestinal tolerance and nutrient absorption, depending on the dose and biological context (Freeland et al., 1985; Salim et al., 2023; Singh et al., 2023). Secondly, elevated concentrations of phytochemicals could lead to increased hepatic biotransformation and renal elimination needs which may lead to greater energy expenditure for detoxification and elimination. Third, the increased amount of alkaloids present in the root extract may be responsible for metabolic or apetite related effects, but the fruit pulp also exhibited a weight loss at 800 mg/kg, indicating that the effect was more likely to be attributable to the combined phytochemical burden of the root extract and fruit pulp (Anywar et al., 2021; Mugale et al., 2024).

Both the 800 mg/kg fruit pulp and 800 mg/kg root extract resulted in a decrease in weight gain; indicating a dose threshold effect. The phytochemical load may not have exceeded a physiologically tolerated range at 400 mg/kg, whereas at 800 mg/kg, the effect of the phytochemicals (saponins, tannins, alkaloids, flavonoids, phenolics, cardiac glycosides and

terpenes) combined may have influenced normal growth or weight gains. This interpretation is supported by repeated dose toxicity studies, where low acute lethality does not necessarily indicate that there will be no physiological effects after longer exposure (Bemidinezhad et al., 2023; Maru et al., 2025). Thus, the present results suggest additional subacute and chronic toxicity studies be conducted to establish no-observed-adverse-effect levels and to evaluate organ specific safety endpoints.

Body weight gain values of Proviron-treated group were not significantly different from control group, while those of 800 mg/kg fruit pulp and root extract group showed significant difference. This indicates that the lower weight gain seen in the plants exposed to plant extract was more strongly linked to high doses of plant extract than the comparator treatment. But the mechanism is not simply the weight or mass of the body. To assess whether the decrease in body weight gain was due to decreased feed consumption, changes in metabolism, systemic toxicity or due to a specific pharmacological effect more data on feed consumption, organ weight, haematological indexes, liver enzymes, renal markers, lipid profile, hormonal profile and histological architecture would be required. OECD repeated-dose toxicity guidance also includes the interpretation of body weight along with the clinical signs, food intake, clinical chemistry, haematology, organ weights and histopathology (Balkrishna et al., 2023).

The present results are consistent with previous studies, which indicated that the species, *C. albidum*, possesses high phytochemicals and biological activities. The presence of flavonoids, alkaloids, tannins, saponins, cardiac glycosides and terpenes corroborates the reports of other researchers, such as Ewansiha et al. (2012), Akinpelu et al. (2016), Ihekwereme et al. (2017), and Nartey et al. (2021), who detected the same phytochemical classes in various parts of *C. albidum*. The increased concentration of flavonoids in the fruit pulp is in line with the previous studies on the African star apple fruit and its antioxidant and metabolic benefits (Ajayi et al., 2020; Ajayi et al., 2021; Oyetayo et al., 2021). The increased level of alkaloids in the root extract is also similar to the pharmacologic activity of *C. albidum* root bark extract in diabetic rats (Onyeka et al., 2013). African star apple is also recommended as a nutritious, phytochemical, functional and bioactive fruit according to the latest reviews (Adadi et al., 2026).

The discrepancies with previous studies could be attributed to variations in plant part, extraction solvent, geographic origin, plant maturity, storage conditions and analytical technique. The present study was a direct comparison between fruit pulp and root extract, while many previous studies were focused on either stem bark, seed, leaf, fruit pulp, essential oils or root bark (Akinpelu et al., 2016; Ihekwereme et al., 2017; Nartey et al., 2021; Onyeka et al., 2013). This distinction is significant as the concentration of secondary metabolites can be affected by biosynthesis, environmental stress, season of harvest and extraction efficiency depending on the tissue. Hence, the pharmacological and safety results obtained from one part of the plant does not automatically imply to the another part and in particular, from the edible fruit pulp to that of the non-edible medicinal root preparations (Mugale et al., 2024; Philips et al., 2024; Adadi et al., 2026).

The study overall indicates that *C. albidum* fruit pulp and root extract is composed of various bioactive phytochemical classes with low acute oral lethality up to 5000 mg/kg. The fruit pulp seems to have more nutraceutical potential related to flavonoids, whereas the root extract has higher content of alkaloids, which could have better pharmacological potential and needs more attention in regard to safety. Importantly, a reduction in body weight gain at 800 mg/kg suggests that chronic exposure to higher doses may be physiologically measurable in the

absence of acute death. These findings strongly suggest the ethnomedicinal value of *C. albidum*, and highlight the importance of the four key factors: part, dose, route and duration of exposure in determining safety and biological response, as stipulated in the OECD guidelines (Mugale et al., 2024; Mafogang et al., 2025; Adadi et al., 2026).

5. CONCLUSION

The findings of this study showed that the fruit pulp and the root extract of *Chrysophyllum albidum* have important bioactive phytochemicals such as saponins, tannins, alkaloids, flavonoids, phenolic compounds, cardiac glycosides and terpenes. Both extracts had similar phytochemical classes but the relative proportions varied with the fruit pulp having significantly higher levels of flavonoids and root extract having significantly higher levels of alkaloids. According to this, the fruit pulp might have more nutraceutical and antioxidant benefits, and the root extract more pharmacologically active properties that should be evaluated with caution. Both extracts have low acute oral toxicity, having LD_{50} values of 5000 mg/kg or higher, based on their lack of mortality in mice after single oral doses up to 5000 mg/kg. Despite the fact that 800 mg/kg did not appear to be lethal, there was a significant decrease in body weight gain at this dose group following repeated administration, suggesting that higher doses may have measurable physiological effects. The overall results indicate the possible nutritional and ethnomedicinal importance of *C. albidum*, and highlight the role of the plant parts, dosage and period of exposure as safety factors. The fruit pulp and root extract are recommended for further subacute and chronic studies in order to establish their long term safety profile, biochemical studies, haematological, and histopathological studies.

References

[1] Ajayi, A. M., Adedapo, A. D., Badaki, V. B., Oyagbemi, A. A., & Adedapo, A. A. (2021). Chrysophyllum albidum fruit ethanol extract ameliorates hyperglycaemia and elevated blood pressure in streptozotocin-induced diabetic rats through modulation of oxidative stress, NF-κB and PPAR-γ. *Biomedicine & Pharmacotherapy*, 141, 111879. https://doi.org/10.1016/j.biopha.2021.111879

[2] Ajayi, O. B., Oyetayo, F. L., & Akomolafe, S. F. (2020). Starch composition, glycemic indices, antioxidant properties and carbohydrate hydrolyzing enzymes activities of African star apple fruit parts. *BMC Complementary Medicine and Therapies*, 20(1), 260

[3] Akinpelu, D. A., Odewade, J. O., Aiyegoro, O. A., Ashafa, A. O., Akinpelu, O. F., & Agunbiade, M. O. (2016). Biocidal effects of stem bark extract of Chrysophyllum albidium G. Don on vancomycin-resistant Staphylococcus aureus. *BMC Complementary and Alternative Medicine*, 16(1), 105

[4] Anywar, G., Kakudidi, E., Byamukama, R., Mukonzo, J., Schubert, A., Oryem-Origa, H., & Jassoy, C. (2021). A review of the toxicity and phytochemistry of medicinal plant species used by herbalists in treating people living with HIV/AIDS in Uganda. *Frontiers in Pharmacology*, 12, 615147

[5] Balkrishna, A., Haldar, S., & Varshney, A. (2023). OECD-407 Driven 28-day-repeated-dose non-clinical safety evaluation of Tinospora cordifolia (Giloy) stem aqueous extract in Sprague-Dawley rats under GLP compliance. *Frontiers in Pharmacology*, 14, 1095083

[6] Bemidinezhad, A., Zojaji, S. A., Jamshidi, S. T., Mohammadi, M., Alavi, M. S., & Ghorbani, A. (2023). Evaluation of acute, subacute, and subchronic toxicity of a hepatoprotective herbal formulation. *Toxicology Reports*, 11, 452-459

[7] Emudainohwo, J. O. T., Erhirhie, E. O., Moke, E. G., & Edje, K. E. (2015). A comprehensive review on ethno-medicine, phytochemistry and ethnopharmacology of Chrysophyllum albidum. *Journal of Advances in Medical and Pharmaceutical Sciences*, 3(4), 147-154

[8] Ewansiha J. U., Garba S. A., Mawak J. D., Oyewole O. A. (2012). Antimicrobial Activity of Cymbopogon Citratus (Lemon Grass) and It's Phytochemical Properties. *Frontiers in Science*, 2(6), 214-220. doi: 10.5923/j.fs.20120206.14

[9] Freeland, W. J., Calcott, P. H., & Anderson, L. R. (1985). Tannins and saponin: interaction in herbivore diets. *Biochemical Systematics and Ecology*, 13(2), 189-193. https://doi.org/10.1016/0305-1978(85)90078-X

[10] Hassanpour, S. H., & Doroudi, A. (2023). Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. *Avicenna Journal of Phytomedicine*, 13(4), 354

[11] Hoenders, R., et al., (2024). A review of the WHO strategy on traditional, complementary, and integrative medicine from the perspective of academic consortia for integrative medicine and health. *Frontiers in Medicine*, 11, 1395698

[12] Ihekwereme, C. P., Okoye, F. K., Agu, S. C., & Oli, A. N. (2017). Traditional consumption of the fruit pulp of Chrysophyllum albidum (Sapotaceae) in pregnancy may be serving as an intermittent preventive therapy against malaria infection. *Ancient Science of Life*, 36(4), 191-195

[13] Jităreanu, A., Trifan, A., Vieriu, M., Caba, I. C., Mârțu, I., & Agoroaei, L. (2022). Current trends in toxicity assessment of herbal medicines: a narrative review. *Processes*, 11(1), 83

[14] Ko, K., Onajobi, F. D., Osilesi, O., Ho, I., & Oo, A. (2017). Nutrients compositions and phytochemical contents of edible parts of Chrysophyllum albidum fruit. *Journal of Nutrition & Food Sciences*, 7(02)

[15] Lemanowicz, J., Gawlińska, K., Jaskulska, I., Jaskulski, D., & Sar, M. (2026). Flavonoids in Plants and Human Health: From Biosynthesis to Neurodevelopmental and Neurodegenerative Disorders. *Molecules*, 31(1), 66. https://doi.org/10.3390/molecules31010066

[16] Mafogang, B., Ponka, R., Mukam, J. N., & Fokou, E. (2025). Nutrients and phytochemicals characterisations, acute and sub-acute oral toxicity studies of BobyGuard C, a polyherbal nutraceutical with anti-breast cancer properties. *Frontiers in Toxicology*, 7, 1598185.

[17] Maru, S., & Belemkar, S. (2025). Acute and subacute oral toxicity study of a herbal formulation containing asparagus racemosus, tinospora cordifolia, and trigonella foenum-graceum in mice. *Journal of Toxicology*, 2025, 8221552. https://doi.org/10.1155/jt/8221552

[18] Mugale, M. N., et al., (2024). A comprehensive review on preclinical safety and toxicity of medicinal plants. *Clinical Complementary Medicine and Pharmacology*, 4(1), 100129. https://doi.org/10.1016/j.ccmp.2024.100129

[19] Nartey, D., Gyesi, J. N., & Borquaye, L. S. (2021). Chemical composition and biological activities of the essential oils of Chrysophyllum albidum G. Don (African star apple). *Biochemistry Research International*, 2021, 9911713

[20] Onyeka, C. A., Nwakanma, A. A., Bakare, A. A., Okoko, I. I., Ofoego, U. C., Wali, C. C., & Abengowe, F. C. (2013). Hypoglycemic, Antioxidant and Hepatoprotective Activities of Ethanolic Root Bark Extract of Chrysophyllum albidum in Alloxan-Induced Diabetic Rats. *Bangladesh Journal of Medical Science*, 12(3), 298-304

[21] Oyetayo, F. L., Akomolafe, S. F., Jegede, F. O., Elekofehinti, O. O., Akinjiyan, M. O., & Odeniyi, I. A. (2021). Effect of Chrysophyllum albidum fruit pulp powder on antioxidant and proinflammatory genes in non-diabetic and type 2 diabetic rats. *Journal of Diabetes & Metabolic Disorders*, 20(2), 1663-1674

[22] Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, 5, e47

[23] Philips, C. A., & Theruvath, A. H. (2024). A comprehensive review on the hepatotoxicity of herbs used in the Indian (Ayush) systems of alternative medicine. *Medicine*, 103(16), e37903

[24] Safe, S., Jayaraman, A., Chapkin, R. S., Howard, M., Mohankumar, K., & Shrestha, R. (2021). Flavonoids: structure–function and mechanisms of action and opportunities for drug development. *Toxicological Research*, 37(2), 147-162

[25] Salim, R., Nehvi, I. B., Mir, R. A., Tyagi, A., Ali, S., & Bhat, O. M. (2023). A review on anti-nutritional factors: Unraveling the natural gateways to human health. *Frontiers in Nutrition*, 10, 1215873

[26] Singh, P., Pandey, V. K., Sultan, Z., Singh, R., & Dar, A. H. (2023). Classification, benefits, and applications of various anti-nutritional factors present in edible crops. *Journal of Agriculture and Food Research*, 14, 100902 https://doi.org/10.1016/j.jafr.2023.100902

[27] Yang, Y., & Ling, W. (2025). Health benefits and future research of phytochemicals: a literature review. *The Journal of nutrition*, 155(1), 87-101. https://doi.org/10.1016/j.tjnut.2024.11.007