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RESEARCH ARTICLE

Physicochemical Composition and Antioxidant Role of *Chrysophyllum albidum* Seed Endosperm in Monosodium Glutamate-Intoxicated Rats

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ABSTRACT

Background and Objectives: *Chrysophyllum albidum* seed is usually discarded as food waste and this constitutes an environmental challenge. Finding its medicinal use could lighten this burden and proffer natural solution to peroxidation which has been implicated as an underlying cause of many disease conditions. The objectives of this study are thus, to determine phytochemical compositions and antioxidant parameters of *Chrysophyllum albidum* seed. **Materials and Methods:** Physicochemical composition of *Chrysophyllum albidum* seed endosperm flour and the antioxidant role of its ethanol extract in monosodium glutamate-intoxicated rats were evaluated using standard protocols. SPSS version 22 was used for the one-way ANOVA analysis. **Results:** Proximate evaluation of the seed endosperm flour revealed the presence of dry matter > carbohydrates > moisture content > crude lipids > crude proteins > ash > fiber, while its Vitamin estimation was in the order vitamin A > riboflavin B₂ > thiamine B₁ > niacin B₃. However, the anti-nutrients composition included oxalates > alkaloids > tannins > flavonoids > saponins > hydrocyanide while its mineral composition was in the order; sodium > potassium > calcium > magnesium. For In-vitro antioxidant activity, the extract showed higher DPPH radical inhibition at 250 µg mL⁻¹ (60.73%) compared to that of ascorbic acid standard (58.83%). The *in-vivo* antioxidant result for Malondialdehyde (MDA) a marker of lipid peroxidation was significantly (p<0.05) increased in MSG (8000 mg kg⁻¹) treated rats but showed slightly reduced peroxidation across the cotreated groups. **Conclusion:** In conclusion, *C. albidum* seed endosperm can be a rich source of minerals and vitamins and can be made a nutritional option for feed incorporation in the diets of man.

Key words: Antioxidant, *Chrysophyllum albidum*, Monosodium glutamate, physicochemical, toxicity, mineral, vitamins

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INTRODUCTION

Plant-derived substances are known for their antioxidant activity and their consumption is associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with aging¹. In recent years, several natural antioxidants have been isolated from plants such as berry crops, teas, herbs, oilseeds, beans, fruits such as *Chrysophyllum albidum* and vegetables¹.

Chrysophyllum albidum (African star apple), family Sapotaceae is an indigenous tropical plant with edible fruits. In Nigeria, the plant is known by various tribal names such as Agbalumo (Yoruba), Udara (Ibo, Efik and Ibibio), Ehya (Igala) and Agwaluma (Hausa) and is also used for several ethnomedicinal purposes². The plant is a genus of about 70-80 species of trees native to tropical regions of the world with the greatest number of species found in Northern South America and central, eastern and western Africa^{3,4}. In Africa, it is widely distributed in countries such as Nigeria, Uganda, Niger, Cameroun and Cote d'Ivoire³. The bark is used for the treatment of malaria and yellow fever, while the leaf is used as an emollient and for the treatment of skin eruption, stomach ache and diarrhea while the endosperms from the seeds are used as ointments for the treatment of vaginal and dermatological infections⁵. The fruit pulp is rich in vitamin C and iron⁶ and anti-nutrient factors⁷ and show antioxidant effects via mechanisms including free radical scavenging, decreased lipid peroxidation and increased endogenous blood antioxidant enzymes levels⁸. Eleagnine, tetrahydro-2-methylharman and skatole have been isolated from the seed endosperm of this plant with eleagnine identified as the main compound responsible for its antimicrobial activity⁹.

Monosodium glutamate (MSG), one of the most abundant naturally occurring amino acids, is frequently added to food to enhance flavor. Many deleterious effects have been attributed to the consumption of MSG including neuro-toxicity¹⁰, obesity¹¹, induced oxidative cardiac tissues¹² and implicated in hepatic assault¹³. Increased oxidative stress due to MSG consumption may cause changes in the membrane lipids and proteins, which could be responsible for the initiation of metabolic disorders. Oxidative stress is a biochemical disequilibrium occurring due to excessive production of free radicals and reactive oxygen species, which aggravates oxidative damage to biomolecules that cannot be counteracted by anti-oxidative defense systems¹⁴. Antioxidants are capable of stabilizing, or deactivating, free radicals before they damage cells. The body's antioxidant defense system comprises endogenous antioxidant enzymes, viz. superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, etc., that catalyze free radical quenching reactions. In addition, dietary antioxidants, like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids and other low molecular weight compounds, such as glutathione also aid in stabilizing free radicals.

Seed Endosperm of *Chrysophyllum Albidum* is usually discarded as waste thus, identifying its nutritional composition and possible pharmacological importance could help put it to better use hence this study designed to investigate the physicochemical composition of *Chrysophyllum albidum* seed endosperm flour and the antioxidant role of its ethanol extract in monosodium glutamate-intoxicated rats.

MATERIALS AND METHODS

Study area: This study was carried out between February-June 2018 at the laboratory unit of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Collection of samples and preparation of extract: Fresh fruits of *C. albidum* were collected from a local farm at the Afoegbe market, Mbaise Local Government Area of Imo State, Nigeria. The fruits were identified by Professor M.C. Dike of the College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The endosperms of the fruits were removed from the seed epicarp after crushing the seeds in a local Nigerian made mortar with pestle. The removed endosperms were subsequently cleaned, oven-dried at 40°C and ground into powder using Arthur Thomas Laboratory Mill (Crypto model, USA). Part of the flour was used for the physicochemical analysis while five hundred grams (500 g) of the powdered material was first soaked in 900 mL of 70% ethanol for 5 days with intermittent shaking to facilitate the extraction process, after which it was filtered using a Whatman filter paper. The filtrate was then concentrated at 40°C in a Laboratory oven to obtain a dried extract that was preserved in a refrigerator and hereafter referred to as *Chrysophyllum albidum* seed endosperm extract (CASEE).

Animals: Sixty adult male albino rats (120-160 g) obtained from the animal house unit of the Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria were used. After acclimatization for 2 weeks, the animals were randomly divided into six treatment groups of 10 rats each and with each group-housed in an aluminum cage measuring 20"×15". All animals had access to food and water *ad libitum* and were maintained under standard laboratory conditions with light and dark cycles of 12 hrs each and room temperature of 25°C. All guidelines involving the use and care of laboratory animals were duly observed

Proximate analysis of CASEE

The proximate Moisture content determination: The determination of moisture content of *Chrysophyllum albidum* seed was achieved using the method as described by AOAC.¹⁵

Ash content determination: The ash content of *Chrysophyllum albidum* seed was determined using the method proposed by AOAC.¹⁵

Crude protein determination: Crude protein of the sample (*Chrysophyllum albidum* seed) was determined using Micro-Kjeldahl¹⁵.

Crude fat determination: Crude fat of the sample was determined and calculated using the as proposed by AOAC.¹⁵

Crude fiber determination: The crude fiber of *Chrysophyllum albidum* seed sample was determined using the method proposed by AOAC.¹⁵

Carbohydrate content determination: The carbohydrate content was determined by using the following formula¹⁶:

$$\text{Carbohydrate (\%)} = 100 - (\text{Crude fat (\%)} + \text{Crude protein (\%)} + \text{Ash (\%)} + \text{Moisture content (\%)})$$

Estimation of calorific Value (Energy): The calorific value of the samples was calculated by the Atwater factor method as described by Osborne and Voogt¹⁷. The value of the protein, carbohydrate and fat were multiplied by 4, 4 and 9 Kcal, respectively and their sum was taken as the total energy value.

Determination of mineral elements content: The method as described by AOAC.¹⁸ was employed for the determination of mineral content of *Chrysophyllum albidum* seed.

Determination of Anti-nutrients: Hydrocyanide and Oxalate contents were determined following the method described by AOAC.¹⁵ Tannin content was established by the method proposed by Sofowara¹⁹. Alkaloid content was ascertained by the method proposed by Harbone²⁰. Flavonoid content was determined by the method by Bohm²¹ while vitamin content was determined by the method proposed by Onwuka²².

IN-VITRO ANTIOXIDANT ACTIVITY OF CASEE

Ferric reducing antioxidant power (FRAP) assay: FRAP assay was carried out following the method by Benzie and Strain²³.

DPPH free radical scavenging assay: DPPH scavenging assay was carried out based on the method described by Brand-Williams *et al.*²⁴

Induction of toxicity: Toxicity was induced to the animals using 8000 mg kg⁻¹ b.wt. of the monosodium glutamate via oral administration daily for 14 days as described by Mariyamma²⁵.

Study design for *in-vivo* antioxidant activity of CASEE: The 6 groups of experimental animals were treated according to the protocol below:

- Group 1** : Feed and water only and served as the normal control group
- Group 2** : 200 mg kg⁻¹ b.wt. of CASEE only
- Group 3** : 8000 mg kg⁻¹ b.wt. of monosodium glutamate (MSG) only and served as the negative control
- Group 4** : 8000 mg kg⁻¹ b.wt. of MSG and 200 mg kg⁻¹ of CASEE
- Group 5** : 8000 mg kg⁻¹ b.wt. of MSG and 400 mg kg⁻¹ of CASEE
- Group 6** : 8000 mg kg⁻¹ b.wt. of MSG and 600 mg kg⁻¹ of CASEE

At the end of 14 days of treatment, the animals were sacrificed and blood was collected by cardiac puncture into plain bottles for determination of liver enzymes. Superoxide dismutase (SOD) activities were assayed using Randox commercial kits and following standard methods prescribed by the producer, Randox Laboratories, UK.

Determination of Malondialdehyde (MDA) Concentration was achieved according to the method as described by Varshey and Kale²⁶. The Catalase activity was assayed using the method described by Aebi²⁷. Glutathione concentration was determined according to the method as described by Habig *et al.*²⁸.

Statistical analysis: Descriptive statistics and tests for significance in mean were carried out on the data generated by one-way analysis of variance (ANOVA) with the statistical package for social sciences (SPSS) version 22. The turkey *post hoc* test was used to identify the means that differ significantly at $p < 0.05$. Results were expressed as mean \pm standard error of mean SEM.

RESULTS

The result of Table 1 showed that the highest proximate composition of *Chrysophyllum albidum* flour was Dry matter 69.7 \pm 0.03 followed by Carbohydrate

Table 1: Proximate composition and functional properties of seed endosperm of *Chrysophyllum albidum*

Component	Composition (%)
Carbohydrate	61.13±0.07
Lipid	5.35±0.04
Protein	2.10±0.01
Fiber	0.91±0.01
Ash	1.15±0.01
Dry matter	69.7±0.03
Moisture	30.1±1.03

Table 2: Anti-nutritional composition of ethanolic extract of seed endosperm of *Chrysophyllum albidum*

Antinutrient	Composition (mg/100 g)
Oxalates	12.25±0.03
Alkaloids	1.88±0.01
Flavonoids	1.67±0.01
Saponins	1.00±0.01
Tannins	0.76±0.01
Hydrocyanide	0.32±0.01

Table 3: Vitamin composition of seed endosperm of *Chrysophyllum albidum*

Vitamin	Composition
Vitamin A	11.03±0.51 I.U
Riboflavin	0.08±0.00 mg/100 g
Thiamin	0.06±0.00 mg/100 g
Niacin	0.02±0.00 mg/100 g

Table 4: Mineral composition of seed endosperm of *Chrysophyllum albidum*

Minerals	Composition (mg/100 g)
Sodium	26.41±0.00
Potassium	22.15±0.02
Calcium	20.39±0.00
Magnesium	13.35±1.15

61.13±0.07. It also indicated a very low ash content 1.15±0.01 and fiber 0.91±0.01. The result of Table 2 shows that the highest anti-nutrient factors found present in *Chrysophyllum albidum* flour were Oxalates (12.25±0.03 mg/100 g) while the least factor found was, hydrocyanide (0.32±0.01 mg/100 g). This could be indicative that *Chrysophyllum albidum* relatively has a low anti-nutrient composition.

The result of Table 3 showed the highest vitamin composition of *Chrysophyllum albidum* flour to be Vitamin A (11.03±0.51 I.U) while the lowest vitamin composition was niacin (0.02±0.00 mg/100 g). This could also imply that *Chrysophyllum albidum* flour has very low vitamin composition.

The result of Table 4 indicated that *Chrysophyllum albidum* has relatively high vitamin composition. The highest mineral composition of seed endosperm of *Chrysophyllum albidum* was Sodium (26.41±0.00 mg/100 g) while the lowest in composition was Magnesium (13.35±1.15 mg/100 g).

The result of Table 5 shows an increase in DPPH inhibition of CASEE with an increase in concentration. However, a reduction in scavenging activity was observed at concentrations above 250 meanwhile, these values are all relatively lower compared to the standard at the tested concentrations.

The result as observed in Table 6 indicated an increase in the ferric reducing power of CASEE with an increase in concentration. However, reduction in reducing power was observed at concentrations above 250 and these values are all comparatively lower than the standard at the tested concentrations.

Table 5: DPPH radical scavenging activity of the ethanol extract of *Chrysophyllum albidum* seed endosperm

Concentration ($\mu\text{g mL}^{-1}$)	Inhibition (%)	
	<i>C. albidum</i> extract	Ascorbic acid
31.5	47.93±0.13	73.92±1.21
62.5	57.63±1.02	82.27±1.01
125.0	59.33±1.14	78.87±1.44
250.0	60.73±1.47	77.42±1.32
500.0	54.90±1.44	75.36±0.58
1000.0	48.20±1.13	74.20±1.44

Table 6: Ferric Reducing Anti-oxidant Power of the ethanol extract of *Chrysophyllum albidum* seed endosperm

Concentration ($\mu\text{g mL}^{-1}$)	Absorbance	
	<i>C. albidum</i> extract	Gallic acid (Standard)
31.5	0.07±0.00	0.34±0.01
62.5	0.08±0.04	0.39±0.01
125.0	0.09±0.01	0.42±0.01
250.0	0.09±0.01	0.54±0.01
500.0	0.08±0.02	0.64±0.01
1000.0	0.07±0.02	0.68±0.01

Table 7: Effects of CASEE on hepatic reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels in monosodium glutamate (MSG) treated rats

Groups	GSH ($\mu\text{mol mg}^{-1}$)	SOD (IU L ⁻¹)	CAT (IU L ⁻¹)
Normal saline	24.03±1.60	87.36±0.70	1.8±0.03
<i>C. Albidum</i> 200 mg	21.06±0.43**	82.09±1.12	1.63±0.10
MSG 8000 mg kg ⁻¹	21.07±0.40*	79.5±1.30*	1.46±0.10*
CASEE 200 mg + MSG 8000 mg kg ⁻¹	20.49±0.40	79.2±1.30	1.41±0.10
CASEE 400 mg + MSG 8000 mg kg ⁻¹	20.61±0.26	81.03±1.47	1.52±0.10
CASEE 600 mg + MSG 8000 mg kg ⁻¹	20.15±0.15	80.18±0.51	1.44±0.04

Results are expressed as Mean±SEM (n = 4), *: Represents significance at p<0.05 while **: Represents significance at p<0.05 for CASEE 200 mg when compared to normal saline group

Table 8: Effects of CASEE on MDA concentrations in monosodium glutamate (MSG) treated rats

Treatment groups	MDA concentration (nmol mg ⁻¹)
Normal control	1.17±0.16
CASEE 200 mg kg ⁻¹	2.12±0.10
MSG 8000 mg kg ⁻¹	2.69±0.10*
CASEE 200 mg kg ⁻¹ + MSG 8000 mg kg ⁻¹	1.82±0.37
CASEE 400 mg kg ⁻¹ + MSG 8000 mg kg ⁻¹	2.31±0.47
CASEE 600 mg kg ⁻¹ + MSG 8000 mg kg ⁻¹	2.00±0.50

Results are expressed as Mean±SEM (n = 4), *: Represents significance between MSG group and positive control

The results as shown in Table 7 indicated that the MSG group produced a significant (p<0.05) change in GSH, SOD and CAT levels in treated rats when compared to the normal control groups. However, the results of the co-treated groups showed a dose-dependent increase across the stated parameters when compared to MSG.

The result of Table 8 indicated that the MSG group produced a significant (p<0.05) increase in MDA levels when compared to the normal control. Further look indicated a significant (p<0.05) decrease in the MDA concentration of the co-treated groups when compared to the MSG group.

DISCUSSION

In this study, the physicochemical composition and antioxidant property of *Chrysophyllum albidum* seed endosperms in monosodium glutamate intoxicated rats have been evaluated. The physicochemical analysis of the flour of *C. albidum* seed endosperms as shown in Table 1 indicated a high moisture content (30.3%) and was higher than those reported in other similar works 28.5²⁹, 20.10³⁰ and 26.26%³¹. The slight variation may be attributed to handling processes during seed storage³². The high moisture content of the seed endosperm flour of *C. albidum* may reduce the durability or storage stability of the sample flour. The ash content (1.12%) suggested that the flour will be a rich source of inorganic matter and oxides since the ash content of any material is a measure of the total amount of minerals present within the food. The presence of lipid in the flour of seed endosperm of *C. albidum* (5.35%) is suggestive of its nutritional value. Lipids provide an excellent source of energy that enhances the transport of fat-soluble vitamins and protects internal organs. The high energy value of the flour of the seed endosperm of *C. albidum* (301.10 kcal) also suggested that the flour could facilitate protein utilization and possibly avert protein-energy malnutrition which is very common in some parts of Africa due to high cost of dietary proteins³³. The Vitamin contents compare favorably with that of common fruits like *Mangifera indica* and *Citrullus lanatus* reported in other works^{34,35} and points at the value of *C. albidum* seed endosperm in the maintenance of body processes.

The presence of tannins, saponins, alkaloids, flavonoids and other chemical substances in *C. albidum* seed endosperm intolerable amounts is indicative of its medicinal potentials and maybe the reasons why the plant has been used over the years for the treatment of various ailments. The saponins (1.00 mg/100 g), tannins (0.76 mg/100 g) and alkaloids (1.88 mg/100 g) of *Chrysophyllum albidum* in are all comparably higher than that of *Persea americana* 0.14±0.01, 0.68±0.06 and 0.51±0.21, respectively³⁶. Alkaloids are reported to contain a large group of nitrogenous compounds that are widely used as cancer chemotherapeutic agents³⁷. Many tannin components have been reported to be anti-carcinogenic and protect the body from cellular oxidative damage due to the antioxidant activities. Tannins also inhibit the generation of superoxide radicals³⁸. The ability of tannins to react with protein to provide a typical tannin effect which is important for the treatment of inflammatory or ulcerated tissues has also been reported³⁹. Saponins have also been found to be useful in the management of increased cholesterol levels in humans as it binds to cholesterol in the body to inhibit the reabsorption of the later thereby facilitating its excretion from the body. The antioxidant activities of flavonoids and alkaloids via the free radical scavenging mechanism have been reported^{40,41}. However, the low flavonoid composition of *Chrysophyllum albidum* in (1.67 mg/100 g), as compared to *Persea Americana* 8.11±0.14 could be indicative of low antioxidant activity.

The presence of various mineral elements including sodium, potassium, calcium and magnesium further attests to the nutritional value of *C. albidum* seed endosperm and suggests a possible role in the maintenance of muscle tone and body electrolytes. The role of these mineral elements in health maintenance has been highlighted⁴². The sodium content of *Chrysophyllum albidum* (26.41 mg/100 g) is comparatively higher than 9.57 for *P. foetida* fruit⁴³ while the potassium and calcium content of *Chrysophyllum albidum* (22.15 mg/100 g and 20.39 mg/100 g) were all comparatively higher than 17 mg/100 g and 12 mg/100 g respectively for *F. flavicarpa*⁴⁴.

The *In-vitro* antioxidant study of *C. albidum* seed cotyledon showed significant scavenging of DPPH free radical ion when compared to ascorbic acid standard while the FRAP assay showed its ability to reduce TPRZ-Fe (III) complex to TPRZ-Fe (II) with values which were significantly lower when compared to that obtained for Gallic acid standard. Oxidative stress has indeed been implicated in various disease conditions in humans⁴⁵. The MSG induces toxicity by increasing oxidative stress in treated animals with characteristic lipid peroxidation, neuronal necrosis in the hypothalamic arcuate and possible damage to the liver and kidneys and other organs⁴⁶. In the current study, the serum MDA concentration, a marker of lipid peroxidation increased significantly ($p < 0.05$) in all rats following 14 days treatment with MSG thus agrees with previous study¹³. Interestingly, the co-treated groups showed little ameliorative effects suggesting a poor role in beefing up antioxidant systems of the human body. This relatively poor antioxidant activity could be attributed to its low flavonoid composition.

CONCLUSION

In conclusion, the results of this work have shown that the flour of *C. albidum* seed endosperm can be a rich source of minerals and vitamins and can be made a nutritional option for feed incorporation in the diets of man. This pharmacological importance of *C. albidum* seed endosperm could increase its usage as a nutritional supplement and could change its fate as food waste which would be of environmental benefits.

SIGNIFICANCE STATEMENT

This study discovered the rich vitamin and vitamin composition of *C. albidum* seed endosperm that can be beneficial since of *C. albidum* seed endosperm is discarded as waste, this finding could help divert this waste into being incorporated diets hence lighten the environmental burden created by excessive discarding of the said part. This study will help the researchers to uncover the critical areas of food waste that many researchers were not able to explore. Thus a new theory on the use of food waste as a nutritional supplement for human wellbeing may be arrived at.

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