



Bioactive Composition and Acute Oral Toxicity Studies on *Persea Americana* Seed Ethyl Acetate Fraction

E. S. Asiwe^{1*}, C. U. Igwe¹, K. M. E. Iheanacho¹, I. O. Onyeocha²
and V. A. Onwuliri¹

¹Department of Biochemistry, Federal University of Technology Owerri, Imo State, Nigeria.

²Department of Biotechnology, Federal University of Technology Owerri, Imo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors CUI, ESA, VAO, KMEI and OIO designed the study and wrote the protocol, while authors CUI, ESA, OIO and KMEI performed the statistical analysis and interpretation of study data. Authors ESA and JNI did the literature searches, while ESA wrote the first draft of the manuscript and incorporated all corrections from co-authors. Authors CUI, VAO and KMEI critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Persea americana* (*P. americana*) dubbed 'green gold' is a highly sought after fruit today, with insatiable export market. Different parts of avocados have been consumed both for nutritional and health benefits across regions of the world. Therefore, this study investigates the bioactive composition of *P. americana* seed ethyl acetate fraction and acute toxicological effects.

Place and duration of study: Department of Biochemistry, Federal University of Technology Owerri, Imo State, Nigeria; between May 2019 and October, 2019.

Methodology: Quantitative phytochemical composition was assessed using gas chromatography fitted with flame ionization detector (GC-FID) and acute toxicity determined using standard method.

Results: Result of quantitative phytochemical composition of *P. americana* seed fraction shows a rich presence of phytochemicals such as epicatechin, kaempferol, proanthocyanin, rutin, resveratrol,

*Corresponding author: E-mail: nnaemeka.asiwe@yahoo.com;

ribalinidine, naringin, spartein, quinine, flavan-3-ol, anthocyanin, lunamarin, sapogenin, flavonones, flavones. The quantitative phytochemical composition of *P. americana* seed shows that among other phytochemicals, the seed is relatively rich in anthocyanin, quinine, epicatechin, tannin and proanthocyanin with concentrations of $69.39 \pm 8.33 \mu\text{g/g}$, $22.16 \pm 1.77 \mu\text{g/g}$, $21.88 \pm 2.53 \mu\text{g/g}$, $19.86 \pm 1.19 \mu\text{g/g}$ and $10.98 \pm 0.55 \mu\text{g/g}$ respectively. The acute toxicity studies on the seed reveal that the ethyl acetate fraction of *P. americana* seed did not elicit any lethal signs of morbidity and mortality at doses up to 5000mg/Kgb.wt. and are therefore considered generally safe.

Conclusion: *P. americana* seed ethyl acetate fraction contains essential phytochemicals with useful phyto-medicinal and nutraceutical benefits. The implications of these findings are further discussed.

Keywords: *Persea americana*; seed; acute toxicity; phytochemicals; GC-FID.

1. INTRODUCTION

Plants have been identified as important source of phyto-active compounds with verifiable medicinal properties and nutraceutical benefits [1,2] Medicinal plants are applied in herbal-based medication for therapeutic, healing or alleviation of diseases in humans [3]. It is estimated that 80% of Asians and Africans living in rural communities depend on herbal medicine for their primary healthcare [4]. The increased acceptance and consumption of herbal remedies calls for a close assessment of toxicological effect and safety in short term or long term. Indiscriminate consumption of herbal remedies and/or associated chemical compounds may present risk to vital organs such as liver, Kidney or heart.

Persea americana Mill. is commonly known as avocado. It is locally known as Ube-beke, Ube oyibo (Igbo) or Ewé pia (Yoruba) and Ganyen piya (Hausa). The avocado is an evergreen tree native to the Carribeans, geographically distributed and adaptable in tropical regions including Nigeria [5]. There are at least 500 named cultivars, Fuerte and Mexican-Guatemalan hybrid with smooth-skinned, shiny green, pear-shaped fruits are the most popular cultivars. Avocados are loaded with nutrients such as dietary fiber, vitamin E, potassium, magnesium and folate [6]. They are also cholesterol and sodium free. It is an excellent source of monounsaturated fatty acids, vitamins B and K [7]. Different parts of the plant have been documented to possess pharmacological potentials such as analgesic and anti-inflammatory [8], anticonvulsant [9], hypoglycaemic and hypocholesterolaemic [10], vasorelaxant and blood pressure reducing [9,11] activities in animal studies. The aqueous bark extract of the tree is used by traditional medicine practitioners in Nigeria for the treatment of parasitic skin diseases [11]. The avocado seed

represents 13–18% of the fruit, and it is a byproduct that may be of interest to industry as a source of bioactive compounds [12]. The seed have also been widely applied in ethnomedicine, for benefits ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites, skin treatment and beautification etc. [11,13,14]. An active extract of avocado containing carotenoids and tocopherols was shown to inhibit the growth of both androgen dependent (LNCap) and androgen independent (PC-3) prostate cancer cell lines in vitro [15]. cardiotoxic effects of the leaves have been reported in mammals and birds [16-18]. The present study seeks to address the safety concerns of consumption and uses of the seed in alternative medicine, as well elucidate the bioactive composition of the plant seed fraction.

2. MATERIAL AND METHODS

2.1 Plant Materials

Fresh *P. americana* fruits were harvested from a farm in Ugiri-ike Autonomous community, Ikeduru L.G.A. Imo State. The plant materials were authenticated by Prof. F. N. Mbagwu a plant taxonomist at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State. Plant specimens were deposited in the institution herbarium with voucher number IMSUH 0226.

2.2 Preparation of Extract

The fresh seeds were collected by carefully cutting the fruits open; the seeds were peeled and cut into cubes for easy drying at 30°C; this was reduced to a coarse powder in a mill (Kenwood BL357). The powder (800g) was extracted with 2 L of 80% ethanol using a Soxhlet extractor; and partitioned between ethyl acetate and water to recover ethyl acetate

soluble component of the extract. The extracts were recovered by distillation under reduced pressure at 49°C in a Buchi rotavapour (Switzerland), then dried to solid forms in vacuum desiccators, and stored in a freezer ($\leq 4^{\circ}\text{C}$) until used.

2.3 Quantitative Phytochemical Content Determination

One gram (1g) of processed sample was weighed into a test tube and 15ml ethanol and 10ml of 50% m/v potassium hydroxide were added. The mixture in the test tube was allowed to react in a water bath at 60°C for 60mins. Later, the reaction product was transferred to a separation funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which were all transferred into the funnel. The combined extracts were washed three times with 10ml of 10% v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent evaporated. The extract was solubilized in 1000 μl of pyridine of which 200 μl was transferred to a vial for analysis.

The quantitation was according to the method of Kelly and Nelson, (2014). The analysis of free steroids was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250 μm x 0.15 μm) was used. The injector temperature was 280°C with split less injection of 2 μl of sample and a linear velocity of 30 $\text{cm}\cdot\text{s}^{-1}$, Helium 5.0 $\text{pa}\cdot\text{s}$ was the carrier gas with a flow rate of 40 $\text{ml}\cdot\text{min}^{-1}$. The oven operated initially at 200°C, was heated to 330°C at a rate of 3°C min^{-1} and was kept at this temperature for 5min. The detector operated at a temperature of 320°C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentrations of the different phytochemicals were expressed in $\mu\text{g}/\text{g}$.

2.4 Acute Toxicity Study

A total of twenty two (22) Wistar albino rat weighing 80-100g were used for the study. Animals were randomly grouped for the study according to body weight. The animals were fasted overnight, but with access to portable drinking water *ad libitum* and then treated orally with the extract. Acute toxicity study was determined according to the method of [19]. The acute toxicity study was carried out in two

phases. The first phase consisted of twelve rats randomly divided into four groups of three rats each; administered extract doses of 10mg/kg.b.wt., 100mg/kg.b.wt., and 1000mg/kg.b.wt. and control treated with normal saline to possibly establish the range of doses producing any toxic effect. The second phase was carried out using another fresh set of ten rats randomly divided into five groups of two rats each, and was administered extract doses of 1200, 1600, 2900 and 5000 mg/kg body weight and control treated with normal saline respectively. The doses were chosen based on deduction from the first phase of the study. The animals were observed for 24 hours for paw licking, salivation, stretching of the entire body, weakness, respiratory distress, coma and possible death in the first 4 hrs and subsequently observed daily for 14 days in both phases of the study. Thereafter, the LD₅₀ value was calculated as the square root of the product of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

The oral median lethal dose was calculated using the formula:

$$\text{LD}_{50} = \sqrt{\text{Maximum tolerated dose} \times \text{Minimum toxic dose}}$$

3. RESULTS AND DISCUSSION

3.1 Quantitative Phytochemical Content of *P. americana* Seed Ethyl Acetate Fraction

GCFID analysis revealed that *P. americana* seed ethyl acetate fraction contains proanthocyanin, rutin, quinine, flavan-3-ol, anthocyanin, lunamarin, sapogenin, phenol, flavonones, steroids, epicatechin, kaempferol, phytate, oxalate, resveratrol, flavones, tannin, ribalinidine, naringin, spartein (Table 1). The results obtained (Table 1) shows that among other phytochemicals, *P. americana* seed is relatively rich in anthocyanin ($69.39 \pm 8.33 \mu\text{g}/\text{g}$), quinine ($22.16 \pm 1.77 \mu\text{g}/\text{g}$), epicatechin ($21.88 \pm 2.53 \mu\text{g}/\text{g}$), tannin ($19.86 \pm 1.19 \mu\text{g}/\text{g}$) and proanthocyanin ($10.98 \pm 0.55 \mu\text{g}/\text{g}$).

3.2 Acute Toxicity Studies on *P. americana* Seed Ethyl Acetate Fraction

Result of acute toxicity studies on *P. americana* seed ethyl acetate fraction showed that the fraction caused no observable toxicological signs such as depression, writhing, diarrhoea,

hypermotility and aggression compared to the control, and no death was recorded at the test dose range of 10-5000 mg/kg b.wt. (Table 2).

Table 1. Quantitative phytochemical content of *P. americana* seed ethyl acetate fraction

Phytochemical components	<i>P. americana</i> seed ($\mu\text{g/g}$)
Proanthocyanin	10.98 \pm 0.55
Rutin	1.69 \pm 0.10
Quinine	22.16 \pm 1.77
Flavan-3-ol	11.43 \pm 0.57
Anthocyanin	69.39 \pm 8.33
Lunamarin	0.43 \pm 0.02
Sapogenin	18.98 \pm 1.14
Phenol	14.43 \pm 0.72
Flavonones	5.47 \pm 0.22
Steroids	18.7 \pm 1.12
Epicatechin	21.88 \pm 2.53
Kaempferol	1.15 \pm 0.05
Resveratol	8.03 \pm 0.48
Flavones	3.57 \pm 0.25
Ribalinidine	3.83 \pm 0.19
Naringin	4.48 \pm 0.13
Sparteine	6.59 \pm 0.26
Phytate	3.89 \pm 0.23
Oxalate	8.48 \pm 0.51
Tannin	19.86 \pm 1.19

Results are Mean \pm SD of 3 determinations

Flavan-3-ols (catechins, epicatechin gallate and epigallocatechin gallate) are reportedly responsible for the protective effect of red wine against atherosclerotic cardiovascular disease [20-22]. Epicatechin have shown protective effect in streptozotocin-induced pancreatic islets changes [23,24]. Other phytotherapeutic constituents detected through GCFID include rutin, ribalinidine, kaempferol, resveratrol, Naringin and Sparteine. Rutin exhibit anti-inflammatory properties through inhibition of PLA2, COX and LOX in the AA pathway [25,26].

Furthermore, polyphenolic compounds, flavonoids inclusive identified in our study, are

known antioxidants that prevent the oxidative stress resulting from inflammatory processes [27-30]. They are also beneficial antimicrobial, antiulcer, antiviral agents [31], in addition, their tumor suppressing and hepatoprotective effect have been reported [1,32]. The scavenging activity of flavonoids have been reported to be in the order: myricetin > quercetin > rhamnetin > morin > diosmetin > naringenin > apigenin > catechin > 5,7-dihydroxy-3',4',5'-trimethoxyflavone > robinin > kaempferol > flavone [33]. Plant alkaloids have been isolated, explored and reported to possess strong anti-cancer potential [34,35]. Their anti-inflammatory effects [36], antimalarial activity [37], neuroprotective activities [38] have also been reported. They are also selective inhibitor of acetylcholinesterase (AChE) [39,40] with promising potential as therapeutic option for Alzheimer's disease treatment [41]. Our findings are consistent with the report of Shafika et al., [42]; they identified major polyphenols in avocado seeds to be catechin and pyrogallol, hesperidin, naringin and rutin. Anti-nutrient identified includes phytate, tannin and oxalate. Oxalate binds with calcium to form calcium-oxalate crystals associated with blockage of renal tubules [43]. Tannins are water soluble phenolic compounds which occur in all vascular plants, known to precipitate proteins from aqueous solution. Tannins are documented to be responsible for reduced feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals [44,45]. Their breakdown products constitute a large amount of compounds, which can be toxic. Phytic acid chelates certain essential minerals like iron, zinc, magnesium and calcium impeding their absorption [46]. This produces highly insoluble phytate salts that are poorly absorbed by the gastrointestinal tract resulting to lower bioavailability of minerals. Phytates also inhibit digestive enzymes like pepsin, trypsin and amylase [47].

Table 2. Acute toxicity studies of *Persea americana* seed ethyl acetate fraction

Group of rats	Dose of <i>P. americana</i> (mg/kg b.wt.)	No. of death recorded
Phase 1		
GP 1	10	0/3
GP 2	100	0/3
GP 3	1000	0/3
Phase 2		
GP 1	1200	0/3
GP 2	1600	0/3
GP 3	2900	0/3
GP 4	5000	0/3

Result of acute toxicity studies on *P. americana* seed ethyl acetate fraction in rats showed that the extracts in the two phases of the acute toxicity studies at the test dose of 10-5000 mg/kg b.wt. caused no observable toxicological signs such as depression, writhing, diarrhoea, hypermotility and aggression compared to the control, and no death was recorded (Table 2). An acute dose study provides a guideline for selecting doses for sub-acute dose study [48]. These results revealed that the LD₅₀ value of *P. americana* seed was estimated to be greater than 5000 mg/kg. This places the seed fraction in class 5 status (LD₅₀ > 5000 mg/kg), i.e., in the lowest toxicity class [49] according to OECD labelling and classification of acute systemic toxicity. The ethyl acetate fractions of these plants are considered generally safe for animal consumption up to 5,000 mg/kg b.wt. Similar results were reported by Ozolua *et al.* (50), they reported no noticeable toxicity of *P. americana* seed hydroalcoholic extract in doses up to up to 10000mg/kgb.wt. Also, studies on the alcoholic and hydro-alcoholic extract [51], aqueous extract and mucilage [52] of *P. americana* seed in Wistar rats showed that the lethal dose (LD₅₀) was higher than 2500 mg/kg body weight as no observable toxicity was recorded in animals exposed to 2000mg/Kgb.wt of the extracts. However, Eduardo *et al.*, [12] reported the LD₅₀ for avocado seed extract to be 1200.75mg/kgb.wt and 250mg/Kg was for genotoxic effect. The observed disparity in findings on toxicity profile may be attributable to difference in extraction solvents, altered chemical composition of attributable to cultivar differences, season or botanical source changes [53,54].

4. CONCLUSION

The present study has demonstrated that the seed of *P. americana* seed contains notable number of bioactive compounds, with beneficial properties such as neuroprotective, antioxidant and antitumor activities. Also, oral acute toxicity studies showed that *P. americana* seed did not elicit any lethal signs of morbidity and mortality at doses up to 5000mg/Kgb.wt. and are therefore considered generally safe. They is need for further studies on sub-acute toxicity, genotoxic activity using different cell lines to further strengthen its capacity for use in cosmetic or pharmaceutical products.

DISCLAIMER

The products used for this research are commonly and predominantly used products in

our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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