

Vitamins Composition and Antioxidant Properties in Normal and Monosodium Glutamate-Compromised Rats' Serum of *Persea Americana* (Avocado Pear) Seed

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ABSTRACT

Avocado pear (*Persea americana*) seed flour (ASF) and the ethanolic extract (ASE) were respectively assessed for some vitamin contents and antioxidant properties (in vitro and in vivo), using standard protocols. The vitamins in ASF were in decreasing order thus: vitamin C > A > B1 > B2 > E > B3. Compared to the respective standards, ASE ferric reducing antioxidant power (FRAP), though lower at lower concentrations was higher at 1000 µg/ml and evidently concentration dependent while the exhibited initial 1,1-diphenyl-2-picryl hydrazyl radical scavenging activity (DPPH) at 250 µg/ml increased at 500 µg/ml though lower ($p < 0.05$). Monosodium glutamate (MSG) intoxication resulted to a higher ($p < 0.05$) glutathione concentration, superoxide dismutase (SOD) and catalase activity in the rats serum compared to control. Rats exposed to ASE alone had a reduced ($p < 0.05$) catalase activity compared to control and MSG-intoxicated rats whereas the glutathione concentration and the SOD activity was similar compared to the MSG-intoxicated rats. Simultaneous exposure to MSG together with increasing concentration of ASE showed a trend similar to, though less marked than, that on the respective parameters in ASE-exposed rats. Thus, ASF had appreciable vitamins, especially vitamin C and A contents while ASE exerted high antioxidant reducing, but not radical scavenging, power. MSG-intoxication of the determined antioxidant parameters was confirmed while ASE did not significantly improve or ameliorate respectively the determined antioxidant parameters in the normal and MSG-intoxicated rats' serum. Further studies are thus provoked and recommended, considering the ethno-medicinal use of avocado pear seeds.

Keywords: Vitamin C, Vitamin A, Catalase, Super oxide dismutase, Glutathione,

INTRODUCTION

Avocado plant (*Persea Americana*), family of *Lauraceae* and genus, *Persia*, bears avocado pear or alligator pear fruit that contains the avocado pear seed. The fruit is known as *ube oyibo* (loosely translated to 'foreign pear') in Ojoto and neighboring Igbo speaking communities south east Nigeria. Different parts of avocado pear are used in many ways including as an anti-microbial [1], anti - hypertensive, anti - hyperglycemic, antiviral, anti-hyper-cholesterol-emic, analgesic, anti-inflammatory and anti-ulcerative [2-4] agents. The fruit and leaf extracts are used in traditional medications [5].

Avocado pear seed is essentially discarded as solid waste although in Nigeria, owing to its ethno-medicinal use against hypertension, the seed is ground and incorporated into foods including soup and puddings and pap [3]. Reported uses of avocado pear seed include use in the management of hypertension, diabetes, cancer and inflammation [3-6]. Thus, avocado pear seeds could in particular affect monosodium glutamate-induced effects in the antioxidant capacity of animals.

Monosodium glutamate (MSG), a sodium salt of L-glutamic acid, serves as a food flavor enhancer [7]. It is common in packaged foods without appearing on the label [8]. The inadvertent use

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and possible abuse of MSG could elicit some adverse effects on especially MSG-sensitive individuals, and studies have either established or suggested toxic effects of MSG on some vital organs in experimental animals [9-11] but there was no regulation against the continued use of monosodium glutamate as flavor enhancer. These warranted continued search for natural products that could mitigate the adverse effects of MSG including on the antioxidant capacity of animals, necessitating this study aimed at determining some vitamin contents in avocado pear seed flour and the antioxidant properties (*in vitro* and *in vivo*) of the ethanolic extract of avocado pear seed. The objectives to achieving this aim was by determining in the sample flour some vitamins content including vitamin C, A, E, B₁, B₂ and B₃ as well as by determining in the antioxidant properties by standard protocols including the determination of FRAP and DHPP (for *in vitro*) as well as glutathione concentration, catalase and superoxide dismutase activity in normal and monosodium glutamate-intoxicated rats' serum.

Generally, vitamins are important in animal physiology including as co-enzymes hence each has its minimum recommended daily allowance (RDA) for adult and children [12,13]. DPPH free radical scavenging activity tests preliminary radical scavenging activity of a compound or a plant extract and determines the anti-radical power of an antioxidant by measuring the absorbance of DPPH at 517 nm [14]. Ferric reducing antioxidant power measures antioxidant capacity based on the capability of antioxidant to reduce ferric tripyridyltriazine (TPTZ Fe³⁺) complex to ferrous form (Fe²⁺) and forming an intense blue color complex with an absorption maximum at 540 nm [15]. Superoxide dismutase catalyzes the dis-mutation of superoxide to hydrogen peroxide while catalase catalyses the conversions of hydrogen peroxide to water [16].

MATERIALS AND METHODS

Collection, Identification, Preparation and Extraction of Plant Materials

Monosodium brand (99 % purity) used in this study was procured from *Ubani* market, a daily food condiments market in Umuahia, south east Nigeria. Chemicals and solvents used in this study were products of reputable companies procured from reputable chemical dealers and were used without further purification.

Matured avocado pear fruits were bought in a local market in Umuahia close to Michael

Okpara University of Agriculture Umudike, during the fruiting season of June, 2015 and identified as *Persea Americana* mill (*Lauraceae*) in the Plant Science Department of Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Following deseeding, the seeds were washed with clean tap water, crushed into smaller pieces using manual grater and sun-dried for three days. The dried seeds were milled into powder using a laboratory miller (ED-5, U.S.A) and stored in an air tight container until used for the determination of vitamins as in the study design.

The avocado seed flour was extracted with ethanol by cold maceration method. The extraction method involved weighing 700 g of the avocado pear seed flour into a volumetric flask, soaking in 1400 ml of 90 % ethanol with intermittent shaking and stirring for three days and thereafter filtering with No 1 What mann filter paper. The filtrate was concentrated using water bath at 60 °C and was further dried in an oven at 50 °C. The extract was packed into a sample bottle and stored in a refrigerator until used as in the animal study design to assess the *in vitro* antioxidant properties and the effect on normal and monosodium glutamate-intoxicated rats' serum antioxidant capacity.

Animal Experimentation

The MSG-intoxicating dose for the rats was 8000 mg/kg body weight for 14 days according to Mariyamma *et al.* [9] as supported by other studies [17-20]. The ethanolic extract of avocado pear seed (1g) was dissolved in 10 ml of distilled water as the stock solution and three graded doses were selected as follows: low, medium and high doses (100 mg/kg body weight, 300 mg/kg body weight and 500 mg/kg body weight) respectively.

Twenty-four albino rats (*Rattus norvegicus*) of either sex (mean body weight, 96.00±10.00 g) used in this study were obtained from the animal breeding unit of the College of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatized for 1 week and then randomized (based on weight) to six experimentation groups with sample size of four rats as described below.

Rats in the normal control group were sham-dosed with distilled water (without either the extract or MSG) while rats in the MSG group (negative control) were fed intoxicating dose (8000 mg/kg body weight) of MSG according to Mariyamma *et al.* [9]. Rats in the extract group

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(Extract control group) were fed ethanolic extract of avocado pear seed flour at 300 mg/kg body weight while rats in the MSG + low extract group were concomitantly exposed to ethanolic extract of avocado pear seed flour (100 mg/kg body weight) and intoxicating dose of MSG (8000 mg/kg body weight) whereas rats in the MSG + medium extract group were co-administered 300 mg/kg body weight of ethanolic extract of avocado pear seed flour and intoxicating dose of MSG (8000 mg/kg body weight). Rats in the MSG + high extract group were concomitantly exposed to ethanolic extract of avocado pear seed flour (500 mg/kg bw) and intoxicating dose of MSG (8000 mg/kg body weight). The exposure was *per oral* using orogastric tube and daily for 2 weeks (14 days).

Ethical Consideration

The animals were placed in rat cages kept in a well ventilated room and allowed free access to standard feed and clean tap water throughout the experimentation period. Animals were exposed to natural room temperature with a 12 hour day/night cycle.

This study considered and adhered to the standard ethical use of experimental animals. Throughout out the experimentation (acclimatization and exposure periods), all rats were housed at 25°C in stainless steel cages under normal daylight/dark cycle and humid tropical conditions. The rats were allowed free access to rat feed (Vital feed, Jos Nigeria) and tap water, and generally received humane care in accordance with the guidelines of the National institute of Health, USA for ethical treatment of laboratory animals as approved by the various (departmental and college) ethical committees of Michael Okpara University of Agriculture Umudike, Nigeria.

Sacrifice and Blood Sample Collection

After 2 weeks (14 days) exposure, the rats were sacrificed the next day after overnight fast by cervical dislocation and the blood sample of the respective rats was collected individually from the heart using a syringe into a clean non-anti-coagulated polystyrene tube, allowed to clot, centrifuged at 3000 rpm for 5 minutes and the serum collected and stored in a refrigerator until used.

Determination of Studied Parameters

Vitamins A, B₁, B₂, B₃, C and E contents of the sample flour were determined using spectro

photometric method as described in AOAC [21]. The ferric reducing antioxidant power (FRAP) of ASE was determined as described by Benzie and Strain [22] using Gallic acid as standard while the DPPH free radical scavenging activity was determined as described by Mensor *et al.* [23] using ascorbic acid as a reference standard.

Catalase activity in the serum was determined by the method based on the principle that catalase splits hydrogen peroxide to water and molecular oxygen hence the activity could be measured by the amount of hydrogen peroxide consumed. The superoxide dismutase was determined by the method of Marklund and Marklund [24] based on the principle that pyrogallol auto oxides rapidly in alkaline solution generating superoxide ions. However, SOD inhibits the auto oxidation of pyrogallol by dis-mutating the superoxide ions to hydrogen peroxide and molecular oxygen. And, the activity of 50 % inhibition by SOD could be measured at 450nm. The serum glutathione concentration was determined by the method of Ellman [25]. This involved the development of a yellow color using the Ellmans reagent 5,5' dithio-bis-2-nitrobenzoic acid, DTNB, to compounds containing sulphhydryl groups and reading the absorbance at 412 nm.

Statistical Analysis

Descriptive statistics and test for significance difference in mean were carried out on the data generated by analysis of variance (ANOVA) with the statistical package for social sciences (SPSS) for Windows version 16. The turkey HSD 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 AND DISCUSSION

Table 1. Some vitamins composition of avocado pear (*Persea americana*) seed flour

Vitamins	Concentration
A	10.11 \pm 0.01 (IU)
B ₁	0.33 \pm 0.00 (mg/100g)
B ₂	0.29 \pm 0.00 (mg/100g)
B ₃	0.06 \pm 0.00 (mg/100g)
C	97.8 \pm 0.00 (mg/100g)
E	0.12 \pm 0.01 (mg/100g)

Values are mean \pm SEM for $n = 2$ (duplicate determinations). Difference considered statistically significant at $p < 0.05$

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The determined vitamins content (unit as indicated on Table 1) was in the order vitamin C > A > B₁ > B₂ > E > B₃.

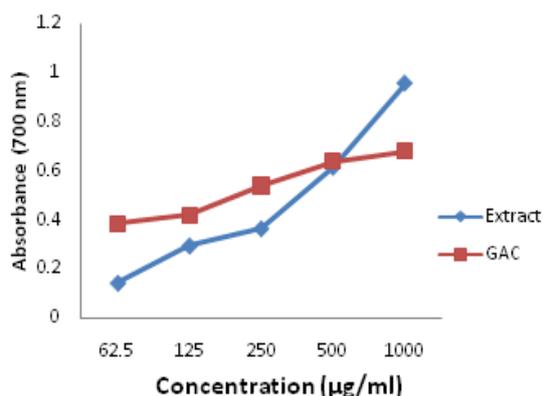


Figure 1. Ferric reducing antioxidant power of ethanolic extract of *P. Americana* and Gallic acid

The ferric reducing antioxidant power of the sample extract as compared with the standard, Gallic acid, was lower at the tested lower concentrations (62.5, 125 and 250 µg/ml), similar at 500 µg/ml, higher at 1000 µg/ml and evidently concentration dependent (Figure 1).

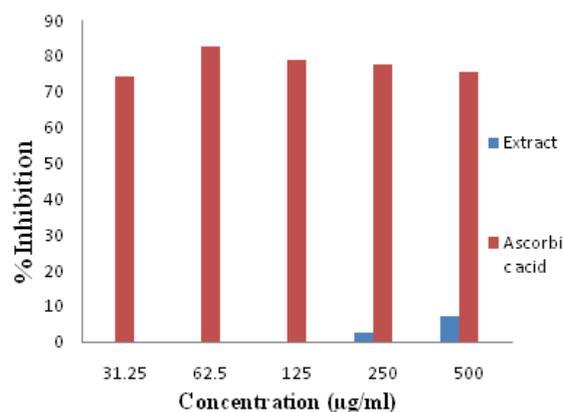


Figure 2. DPPH radical scavenging activity of the ethanolic extract of *P. Americana*

As depicted on Figure 2, the ethanolic extract of the sample did not show any DPPH radical scavenging activity at the lower tested concentrations (31.25, 62.5 and 125 µg/ml) but exhibited initial DPPH radical scavenging activity at 250 µg/ml (which increased at 500 µg/ml). However, the observed DPPH radical scavenging ability exhibited by the ethanolic extract of the sample was significantly ($p < 0.05$) lower compared with that of the standard, ascorbic acid.

Table 2. Effect of ethanolic extract of avocado pear seed (ASE) on some antioxidant parameters of normal and monosodium glutamate-intoxicated rats' serum

Groups	Glutathione (mg/dl)	Catalase (µ/l)	SOD (µ/l)
Normal control (feed + water only)	2.36±0.50	2.31±0.15	11.34±0.05
MSG / Negative control (8000 mg/kg bw MSG)	3.39±0.22	4.93±0.23	11.45±0.01
Extract group (300 mg/kg bw extract)	3.14±0.95	1.58±0.19	11.44±0.01
Low extract co-treated group (MSG, 8000 mg/kg bw +100mg/kg bw extract)	3.10±0.57	1.52±0.13	11.44±0.01
Medium extract co-treated group (MSG, 8000 mg/kg bw +300 mg/kg bw extract)	3.52±0.79	1.78±0.22	11.41±0.06
High extract co-treated group (MSG, 8000 mg/kg bw +500mg/kg bw extract)	3.86±0.68	1.98±0.41	11.36±0.06

Values are mean ± SEM for n= 4. Difference considered statistically significant at $p < 0.05$

As shown in Table 2, MSG intoxication resulted to a significantly ($p < 0.05$) higher glutathione concentration, higher superoxide dismutase and catalase activity in the rats serum compared to control rats. Rats exposed to the ethanolic extract of the sample had a significant ($p < 0.05$) reduction in the catalase activity compared to the control and MSG-intoxicated rats whereas the glutathione concentration and the SOD activity was similar compared to the MSG-intoxicated rats, hence higher ($p < 0.05$) compared to that of the control. Exposing rats to intoxicating dose of MSG together with increasing concentration of the

ethanolic extract of avocado pear seed showed a trend similar to, though less marked than, the observation on the respective parameters in rats exposed to the ethanolic extract of the sample alone.

Avocado pear (*Persea Americana*) seed flour (ASF) and the ethanolic extract (ASE) were respectively assessed for some vitamin contents, *in vitro* antioxidant properties as well as effect on some antioxidant capacity parameters of normal and monosodium glutamate-intoxicated rats serum. The determined vitamins content in

decreasing order was vitamin C > A > B₁ > B₂ > E > B₃. Vitamins, though required minute concentration ensure proper functioning of cells, including in metabolic regulations and modification of enzymes activities [26] hence the composition of these vitamins in ASF may be nutritionally important and deserves follow up.

Some vitamins content as in this study compared with, while others were either higher or lower than, the corresponding values reported earlier [27,28], for various plants and parts. In particular, Vitamin C (97.8%) content of ASF in this study was higher compared with the values reported in various studies [28,29], and the RDA (60mg/day) hence the consumption of ASF may be beneficial in vitamin C-deficiency-related cases and in boosting antioxidant capacity [13]. In animals, including humans, vitamin C is an effective anti-oxidant involved in the alleviation of oxidative stress [30]. Vitamin A content in ASF was also higher than most of the other determined vitamins hence, the consumption of ASF may boost the antioxidant capacity while alleviating vitamin A-deficiency-related symptoms in animal [31]. Thus, consumption of avocado pear seed may exert high antioxidant capacity in animals. The vitamin E content reported in this study (Table 1) is lower than (4.14 %) reported by Achikanu *et al.* [32] for *Mucuna pruriens*. Thus, avocado pear seed flour may not be a good source of vitamin E. Thiamine (B₁) acts as a co-enzyme for the decarboxylation of α -keto acids and the content in ASF is higher than the minimum RDA (1.0 mg for adults and 0.4-1.3mg for children) hence consumption of avocado pear seed flour may prevent the onset of beri-beri. In particular, riboflavin (B₂) and niacin (B₃) contents in ASF were lower than the corresponding RDA hence consumption of ASF may not be useful in vitamin B₂-deficiency-related ailments [12] or vitamin B₃-deficiency-related dysfunctions.

DPPH [14] and FRAP [15,33] measured antioxidant capacity *in vitro*. The ferric reducing antioxidant power of the sample extract as compared with the standard, gallic acid, was lower at the tested lower concentrations (62.5, 125 and 250 μ g/ml), similar at 500 μ g/ml, higher at 1000 μ g/ml and evidently concentration dependent suggesting apparent better antioxidant capacity (via free radical reduction) of ASF than gallic acid notably at higher concentrations. The ASE did not show any DPPH radical scavenging

activity at the lower tested concentrations (31.25, 62.5 and 125 μ g/ml) but exhibited initial DPPH radical scavenging activity at 250 μ g/ml which increased at 500 μ g/ml but significantly ($p < 0.05$) lower compared with that of the standard, ascorbic acid. This suggested lower antioxidant capacity (via free radical scavenging or quenching mechanisms) of ASE compared to the ascorbic acid. This however contradicted the high antioxidant vitamins (vitamins C and A) contents and attendant suggestion in this study. This could be attributed to possible structural and functional derangement following the extraction method as used in this study that required concentrating the filtrate using water bath at 60 °C and further oven-drying 50 °C. This may be so since vitamins are generally heat labile and the antioxidant vitamins (vitamins C and A) along with others may have been destroyed by the extracting temperature conditions.

Glutathione as an antioxidant protects cells against reactive oxygen species and other toxic substances [34]. Catalase catalyzes the two stage conversion of hydrogen peroxide to water and oxygen. Superoxide dismutase catalyzes the dismutation of superoxide to hydrogen peroxide. The hydrogen peroxide is then removed by catalase or glutathione peroxidases. Monosodium glutamate intoxication resulted to a significantly ($p < 0.05$) higher glutathione concentration, higher superoxide dismutase and catalase activity in the rats serum compared to control rats. This agreed with the reports of Soliman [34]. Rats exposed to the ethanolic extract of the sample had a significant ($p < 0.05$) reduction in the catalase activity compared to the control and MSG-intoxicated rats whereas the glutathione concentration and the SOD activity was similar compared to the MSG-intoxicated rats, hence higher ($p < 0.05$) compared to that of the control. This could be a pointer to weak antioxidant potential of ASE in the rats. Exposing rats to intoxicating dose of MSG together with increasing concentration of the ethanolic extract of avocado pear seed showed a trend similar to, though less marked than, the observation on the respective parameters in rats exposed to ASE alone seemingly confirming the weak antioxidant potential of ASE and further suggesting that ASE could not ameliorate MSG-induced adverse influence on the antioxidant capacity of the rats. The findings in this study negated the ethno-medicinal use of avocado pear seed but it is reasonable that the possibly damaged

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antioxidant vitamin contents of ASE as indicated above may have downplayed on the antioxidant capacity of the sample as noted in this study warranting further studies to perhaps could control the noted shortcomings of this study.

CONCLUSION

Thus, ASF had appreciable vitamins, especially vitamin C and A contents while ASE exerted high antioxidant reducing, but not radical scavenging, power. MSG-intoxication of the determined antioxidant parameters was confirmed while ASE did not significantly improve or ameliorate respectively the determined antioxidant parameters in the normal and MSG-intoxicated rats' serum. Further studies are thus provoked and recommended, considering the ethno-medicinal use of avocado pear seeds.

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