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Assessment of *in-vitro* antioxidant and diuretic potential of an ethanol seed extract of *Persia americana* in wistar albino rats

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Abstract *Persea americana* commonly known as avocado is classified in the flowering plant family 'Lauraceae'. It is a medicinal plant that serves as both a preventive measure and a cure to several diseases. Ethanol seed extract of *Persea americana* was studied for in-vitro antioxidant activity and diuretic effect. Different models such as DPPH radical scavenging activity, Nitric oxide scavenging activity and Anti-lipid peroxidation activity were used to determine antioxidant activity. Acute toxicity testing was performed to determine therapeutic dose of the extract using the Litchfeild and Wilcoxon (1949) method using swiss albino mice weighing between 20-25 g. Healthy adult wistar albino rats of both sex weighing 120-180g was used to determine diuretic effect of the ethanol seed extract, volume of urine output was determined using the rat metabolic cages, Na^+, K^+, Cl^- and HCO_3^- were determined using flame photometric method. Comparisons were made between the control and the different concentration of the extract which includes 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.50 mg/ml, 31.25 mg/ml and they were represented using a one way analysis of variance (ANOVA). The results were expressed as mean± standard deviation using bar charts at $P< 0.05$ level of significance. Results revealed that ethanol seed extract of *Persea americana* exhibited its antioxidant activity in a concentration dependent manner, it scavenged DPPH radical by 87.55± 0.63, Nitric oxide radical by 91.44± 0.15, and lipid peroxidation by 75.51± 0.014 all at the dosage of 500mg/ml which is the maximum concentration. Volume of urine and electrolyte Na^+, Cl^- and K^+ was increased significantly when compared to the normal control, HCO_3^- did not show any significant increase. The results clearly indicate that ethanol seed extract of *Persea americana* exhibit diuresis and has potential to scavenge free radicals.

Keywords Medicinal, antioxidant, acute toxicity, *Persea americana*, diuretic and therapeutic dose

Introduction

Plants are the back bone of all the life on earth and an essential resource for human well-being. The origin of plants is dated as far back as the origin of man. It is conceivable that early man through such instincts or divine revelation as claimed by many herbalist discovered this plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions [1]. Plants are directly used as medicines by a majority of cultures around the world.

Ever since ancient times, in search for rescue for their diseases, the people looked for drugs in nature. The beginnings of the medicinal plants use were instinctive as is the case with animals [2]. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illness or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific

medicinal plants for treatment of certain diseases were being discovered; thus, the medical plants usage gradually abandoned the empiric framework and became founded on explicatory facts [3]. Medicinal plants have been identified and used throughout human history it has played and essential role in the development of human culture for example religions and different ceremonies. Cultivation and preservation of medicinal plants protect biological diversity e.g. metabolic engineering of plants. Edible plants and herbal medicine mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs, most conventional drugs are derivatives from plants. Herbal medicines do not differ greatly from conventional drugs in terms of how they work and have some potential to cause harmful side effects [4]. According to the World health organization, approximately 25% of modern drugs used in the United States have been derived from plants [5]. At least 7000 medical compounds in modern pharmacopoeia are derived from plants [6]. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived [7].

Oxidation reactions are crucial for life but they can also be damaging; Oxidative stress seems to play a significant role in many human diseases. It is damage to cell structure and function by overly reactive oxygen containing molecules and chronic excessive inflammation. The use of antioxidant in pharmacology is intensively studied particularly as a treatment for diseases resulting from oxidative stress [8].

In conditions such as congestive heart failure, liver cirrhosis, hypertension, water poising, pregnancy toxemia and certain Kidney diseases, diuretic substance can help in the management and treatment of such conditions by increasing the excretion of water from the body. Drugs that increases the rate of urine flow are called diuretics, drug induced diuresis is beneficial in many life threatening disease. There are several categories of diuretics, each class perform its action in a distinct way [9]. Sodium excretion is used to adjust the volume and composition of body fluids in variety of clinical situations.

The avocado is classified in the flowering plant family "lauraceae" [10]. It is a fruit botanically a large berry that contains a single seed. They have olive green-skinned fleshy body that maybe pear-shaped, egg-shaped or spherical. The fruit is normally used for human consumption. Most plants have various therapeutic uses, avocado is not an exception. The fruit is sometimes called an avocado pear or alligator pear due to its shape and the rough green skin of some cultivars [11]. Avocado is known as "butter fruit" in parts if India, Vietnamese and eastern China. In Taiwan, it is known as "cheese pear". In Portuguese it is "abacate", South American Spanish- speaking countries use a Quechua derived word "Palta". French uses the word "Avocatier" meaning lawyer and advocate forms of the word appear in several Germanic language such as the (now obsolete) German "Advogato- Birne", the Old Danish "Advokat- paere" and the Dutch "advocaatpeer".Avocado pear has different traditional names in Nigeria. Among the Igbos, it is known as "Ubebeke" or "Ubeoyibo", "Igba" or "apoka" in Yourba etc. Avocado is sometimes considered as a vegetable. The avocado is popular in vegetarian cuisine as a substitute for meats in sandwiches and salads. In the Philippines, Brazil, Indonesia, Vietnam, Southern India, Morocco, Ethiopia and Mexico, avocados are frequently used for milkshakes, ice-creams and other desserts. In African countries such as Kenya, Ghana and Nigeria, avocado is often eaten as a fruit eaten alone or mixed with other fruits in a fruit salad or eaten with bread [12].

Many studies have suggested that increasing consumption of plant food like avocado decreases the risk of obesity, diabetes and heart diseases by lowering bad cholesterol, maintaining blood sugar and increasing good ones, carotenoids, Vitamin K and fibre present in avocados reduces the risk of vision impairment, osteoporosis and improves digestion respectively, and significant amount of folic acid is essential to fight cancer, reduce depression and produce healthy babies that are strong. According to the Linus Pauling institute at Oregon state university and university of Maryland medical center, Vitamin C and E in avocados helps keep skin nourished and glowing and avocado creams may be useful in treating Psoriasis. Avocado is essential for overall mortality.

Persea americana seeds have been used in traditional medicine as a treatment for several diseases. In this work, the ethanol seed extract of *Persea americana* was evaluated with respect to its *s* antioxidant properties in terms of its DPPH radical scavenging activity, Nitric oxide inhibition and its anti- lipid peroxidation activity and also diuretic

properties which includes parameters such as sodium, potassium, chloride and bicarbonate levels in albino rats were evaluated.

Materials and Methods

Sample Collection

Samples of ripe avocado pears (*Persea Americana*) were purchased at Urbani market, Umuahia North local government, Abia state. The plant material was identified and authenticated by Dr. Garuba Omosun of the department of plant science and biotechnology, Michael Okpara university of Agriculture, Umudike, Abia state, Nigeria. The succulent part of the fruit was removed to obtain the seeds. The seeds were minced by means of grater and dried to a constant weight in an oven at 45 ^{o}C for 24 hours before being ground to powder and passed through a sieve to obtain a uniform sized powder and stored in a plastic.

Preparation of Extract

About 100 g of dried ground seed material was weighed using a weighing balance and soaked in 500ml of ethanol which was measured out by a measuring cylinder and stirred using a sterilized glass rod. The solution was left for 72 hours to ensure proper extraction. Then the solution was passed through a filter paper for filtration and the filtrate obtained was left to evaporate. A semisolid mass of extract was obtained after evaporation and stored at room temperature for further use.

Dose Preparation

2 g of the ethanol extract of seeds of *Persea americana* was dissolved in 1ml of distilled water to yield 2000 mg/ml concentration of the extract, a doubling dilution was made to obtain various concentration which includes 1000 mg/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml, 62.50 mg/ml, 31.25 mg/ml.

Experimental Animals

Swiss albino mice weighing 20-25 g and Wistar albino rats weighing 120-180 g of both sex were obtained from a local farm and were used for acute toxicity study and evaluation of pharmacologic activity (diuretic effect) respectively. Animals were housed in standard husbandry conditions for acclimatization period of 20 days and fed with commercial pellet diet (Vital growers mash by Grand Cereal and Oil mills, Nigeria), before the experiment was performed.

Reagents and Apparatus

Distilled water, Test tubes, Ethanol seed extracts, Metallic cages, 2 ml and 5 ml syringe, Ethanol, DPPH (1,1-diphenyl-2-picryhydrazyl), Ascorbic acid used as standard, Sodium nitroprusside, Phosphate buffer, Distilled water, Spectrophotometer, Potassium dichromate, Silver nitrate solution (N/50), Flame photometer, Metabolic cages, Standard drug furosemide.

Methods

Acute Toxicity

Determination of Lethal Dose 50 (LD_{50}) test was carried out by earlier reported method [13-14]. Swiss albino mice of both sex weighing between 25-30g were divided into different groups comprising of 4 animals each and labelled A, B, C, D and E. The first group received 2000 mg/kg B.W of seed extract, the second group received 1000 mg/kg B.W of seed extract, the third group received 500 mg/kg B.W, the fourth group received 250 mg/kg B.W, the fifth group received 125 mg/kg B.W. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioural changes. Thereafter, they were then kept under observation up to 72 hours to look out for mortality if any. The method of Litchfield and Wilcoxon, 1989 was used for the determination of LD_{50} [15].

Investigation of Diuretic Effect

The method of Lipschitz was employed for the assessment of diuretic activity. In this method, albino rats of both sex weighing between 120-180 g deprived of food and water 18 hours prior to the experiment, were divided into 7 group at 2 rats each. The first group of animal serving as negative control received normal saline, the second group serving as positive control received 20 mg/kg furosemide [16] other groups received doses of extract (500, 250, 125, 62.50 and 31.25 mg/kg B.W). Immediately after administration, animals were placed in metabolic cages (1 per cage) and kept at 37 ± 0.5°C. the volume of urine collected was measured at the end of 24 hours. During this period food and water were not made available to the animals. The parameters taken were total urine volume and concentration of Na^+, K^+, $Cl^-$and HCO_3^-. Sodium and Potassium ion concentration were determined by flame spectrophotometer. Chloride and bicarbonate concentration were estimated by titration method [17] with silver nitrate solution (N/ 150) using 3 drops of 5% potassium dichromate as indicator [17].

Evaluation of Antioxidant Activity

DPPH Radical Scavenging Activity

Evaluation of radical scavenging activities by antioxidants in the plant extract was carried out using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radicals [18]. Different volumes of 0.2 mg/ml of the plant extract were added to 200 µl of (0.36 mg/ml concentration) DPPH solution in methanol. A series of concentration ranging from 2 to 15 µg of dried extract were tested. The mixtures were vigorously shaken and incubated in the dark for 30minutes after which the reduction of DPPH absorption was measured at 517 nm. Percent inhibition by sample treatment was determined by comparing it with the methanol- treated control group. The ability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging activity (%) = $(A_0 - A_1)/ (A_0 \times 100$ where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The IC_{50} values denote the concentration of each sample required to give 50 % of the optical density shown by the control, using a non-linear regression analysis. All test analysis were run in duplicates and both values were reported. Ascorbic acid (300 mg/ml) was used as the positive control.

Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity was conducted based on the Greiss illosvosy reaction [19]. 2.0 mL of 10 Mm sodium nitroprusside and 5.0 mL of phosphate buffer were mixed with 0.5 mL of different concentrations of the seed extract and incubated at 25 °C for 150 minutes. The sample was run and the blank was replaced with water. After incubation period, 2 mL of the above incubated solution was added to 2 mL of Greiss reagent and incubated at room temperature for a period of 30 minutes. Scavengers of Nitric oxide compete with oxygen, leading to reduced production of nitrite ion, a pink coloured chromophore is formed. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Percentage inhibition was calculated as

NO scavenging activity (%) = $(A_0-A_1)/ A_0\times100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Anti-lipid Peroxidation Activity

The effect of extract on lipid peroxidation inhibition was determined by the ammonium thiocyanate method [20]. The principle is based on the measure of the absorbance of red colour at 500 nm which decreases in the presence of antioxidants. Different concentration (0.2-6 mg/ml) of the extract (0.5 ml) were mixed with 0.2 ml of diluted linoleic acid (25 mg/ml in 99 % ethanol) and 0.4 ml of 50 mm phosphate buffer (pH 7.4). After 15 minutes of incubation at 40 °C, an aliquot (0.1 ml) from the reaction mixture was mixed with reaction solution containing 3 ml of 70 % ethanol, 0.1 mL of ammonium thiocyanate (30 mg/ml in distilled water) and 50 µL of ferrous chloride (2.45 mg/ml in 3.5 % hydrochloric acid). The final reaction solution was mixed and incubated at room temperature for 3 minutes. The absorbance was then measured at 500 nm. Linoleic acid emulsion without extract served as control and vitamin C (0.2- 1 mg/ml) was used as standard control. Inhibition of linoleic acid oxidation was calculated by using the following formula:

Anti-lipid peroxidation activity (%) = $(A_0-A_1)/ A_0\times100$, Where A_0 is the absorbance of control and A_1 absorbance of the sample extract. IC_{50} values denote the concentration of sample which is required to inhibit 50% of Linoleic acid oxidation.

Quantitative Estimation of Na^+, K^+, Cl^- and HCO_3^-

Sodium Assay

Step 1- precipitation of Na^+ and protein: 1ml of precipitating reagent (for sodium) was pipetted into clean, dry centrifuge tubes; one for the standard reagent labeled as S and the other for 8 test tubes labeled as T, one each for all the experimental groups namely, T_1(Control), T_2 (15.5mg/kg), T_3 (31.25mg/kg), T_4 (62.50 mg/kg), T_5 (125 mg/kg), T_6 (250mg/kg), T_7 (500mg/kg), T_8(Standard drug- furosemide). Then 10 µl of standard reagent was added into the test tubes and 10 µl of urine to all T series test tubes. The solution was shake vigorously and incubated at room temperature for 5 min, then centrifuged at 2000-3000 r.p.m for 2 minutes to obtain a clear supernantant.

Step 2- Colour development: 1Ml of colour reagent (for sodium) was pipette into each of the clean, dry test tubes labelled B for blank, S for standard and 8 test tubes labelled T_1 to T_8 as above. 20 µl of the appropriate supernatant from step 1 was then added into the corresponding test tube S and tubes of all series T into test tube B. 20 µl of the precipitating reagent (for sodium) was added. Pipetting of sodium precipitate reagent (in step 1) and quick transfer of supernatant (in step 2) was followed. The solution was mixed well and allowed to stand at room temperature for 5minutes, then the absorbance of Blank (Abs B), Standard (Abs S), and test series (Abs T_1, Abs T_2, Abs T_3, Abs T_4, Abs T_5, Abs T_6, Abs T_7 and Abs T_8 against distilled water on a photo colorimeter with 530 nm within 10 minutes was measured. Na^+ in mMol/L= [(Abs of B- Abs of T) / Abs of B- Abs of S)] × 150. This procedure is linear up to 200 m Mol/L for Na^+

Potassium Assay

1ml of boron reagent for potassium was pipetted into dry clear centrifuge tube labeled standard (S) and test (T) T_1, T_2, T_3, T_4, T_5, T_6, T_7 and T_8 as described for step 1 in Na^+ assay. 50 µl of standard reagent was added into the test tube S, and the same amount of corresponding urine sample was added to the T series of test tubes. The solution was mixed well , incubated at room temperature for 5 minutes, then the absorbance of standard (Abs S) and that of test series (Abs T_1, Abs T_2 …… Abs T_8) against distilled water on a photo-colorimeter with 620 nm within 10 minutes was measured. The urine K^+ for each group/ test series was then measured by the formula: K^+ in mMol/ L= (Abs of T/ Abs of S) ×5. This procedure is linear up to 7 m Mol/L for K^+. If values exceed these limits, urine samples were diluted with deionized water and assay repeated, and the final volume was calculated using the dilution factor.

Chloride Assay

Urine specimen was diluted 1+1 with distilled water 1ml of colour reagent was pipette into test tubes labeled Blank(B), Standard (S) and Test (T)- T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8. 10 µl of the appropriate urine sample was added to the corresponding T series test tubes. The solution was mixed well and the absorbance of the standard (Abs S) and test (Abs T) against blank (Abs B) were read at 530 nm. The Cl^- content was calculated using the given formula : Cl^- in Mmol/L= [(Abs of B- Abs of T) / Abs of B- Abs of S)] × 150. The final value was calculated by multiplying the dilution factor 2.

Bicarbonate Assay

Urine specimen was diluted 1+1 with distilled water 1ml of colour reagent was pipette into test tubes labeled Blank(B), Standard (S) and Test (T)- T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8. 10µl of the appropriate urine sample was added to the corresponding T series test tubes. The solution was mixed well and the absorbance of the standard (Abs S) and test (Abs T) against blank (Abs B) were read at 530nm. The HCO^-_3content was calculated using the given formula: HCO_3^- in Mmol/L= [(Abs of B- Abs of T) / Abs of B- Abs of S)] × 150. The final value was calculated by multiplying the dilution factor 2.

Statistical Analysis

The data are expressed as mean ±standard deviation using bar charts. Comparisons made between the control, standard and the five groups with different concentrations and represented using the one way Analysis of variance (ANOVA) followed by post HOC LSD test. The result were analysed with mean standard deviations of sample using the student T test and P< 0.05 *i.e.* 95 % level of significance or confidence limit. The statistical significance of difference of the means was evaluated by Dunnet's test.

Results

Table 1: Result of acute toxicity testing

Group	Conc. of extract (mg/ml)	Log. of concentration	No. of animal per group	Number of Mortality	% Mortality
A	2000	3.301	4	4	100
B	1000	3.000	4	2	50
C	500	2.699	4	1	25
D	250	2.398	4	0	0
E	125	2.097	4	0	0

LD_{50}= [highest concentration with no mortality × least concentration with mortality] $^{1/2}$; LD_{50} = [2000×250] ^{½}; LD_{50}= 707mg/ml; LD_{50} is a standard measurement of acute toxicity, LD_{50} represents the individual dose required to kill 50% of a population of test animals i.e. Rats.

DPPH Radical Scavenging Activity

Table 2: The percentage inhibition of DPPH radical scavenging activity

Concentration (mg/ml)	% Inhibition
500 mg/ml	87.65 ± 0.63
250 mg/ml	82.27± 0.95
125 mg/ml	77.49± 0.41
62.50 mg/ml	74.90± 0.42
31.25 mg/ml	70.92± 1.53
Vitamin C (300 mg/ml)	96.01±0.59

Percentage inhibition values are represented as Mean value ± standard deviation of two replicates. Increase in concentration shows a relative increase in percentage inhibition. Values of percentage inhibition shows significant difference when compared to the standard. The IC_{50} value is given as 20.91.

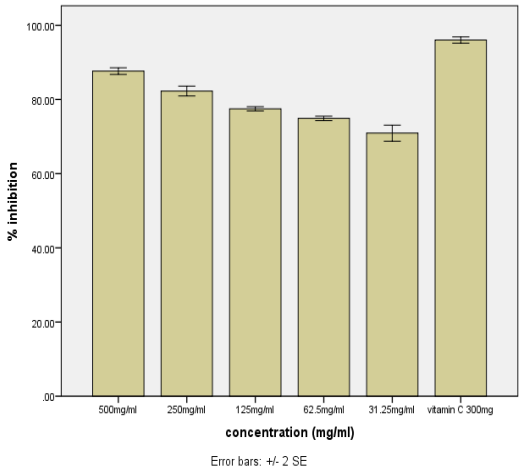

Figure 1: A bar chart showing the DPPH radical scavenging activity

Result for Nitric Oxide Inhibition

Table 3: The percentage inhibition for Nitric oxide scavenging activity

Concentration (mg/ml)	% Inhibition
500mg/ml	91.44± 0.15
250mg/ml	82.88± 0.29
125 mg/ml	77.73± 0.38
62.50 mg/ml	67.76± 2.22
31.25 mg/ml	59.53± 2.54
Vitamin C (300mg/ml)	98.29± 0.42

Percentage inhibition values are represented as Mean value ± standard deviation of two replicates. Increase in concentration shows a relative increase in percentage inhibition. Values of percentage inhibition shows significant difference when compared to the standard. The IC_{50} value is given as 32.42.

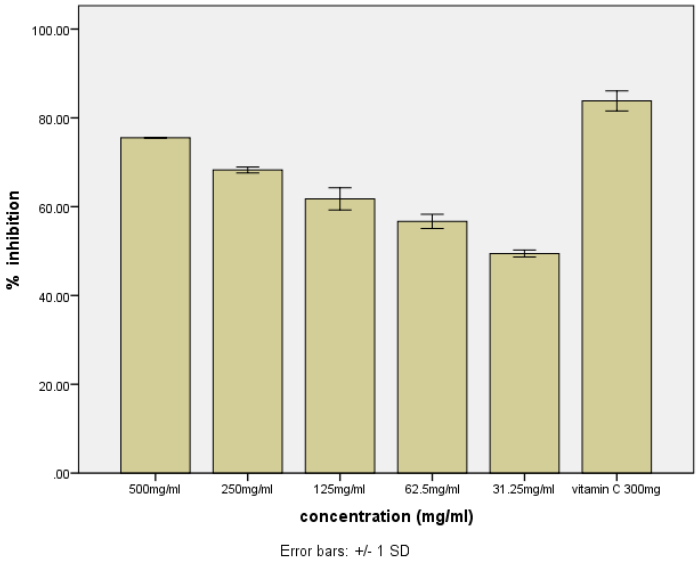

Figure 2: A bar chart showing Nitric oxide inhibition

Anti-lipid Peroxidation Activity

Table 4: The percentage inhibition of anti-lipid peroxidation activity

Concentration (mg/mg)	% Inhibition
500 mg/ml	75.51± 0.014
250 mg/ml	68.27± 0.69
125 mg/ml	61.77± 2.50
62.50 mg/ml	56.69± 1.59
31.25 mg/ml	49.44± 0.78
Vitamin C (300mg/ml)	83.82± 2.26

Percentage inhibition values are represented as Mean value ± standard deviation of two replicates. . Increase in concentration shows a relative increase in percentage inhibition. Values of percentage inhibition shows significant difference when compared to the standard. The IC_{50} value is given as 12.88.

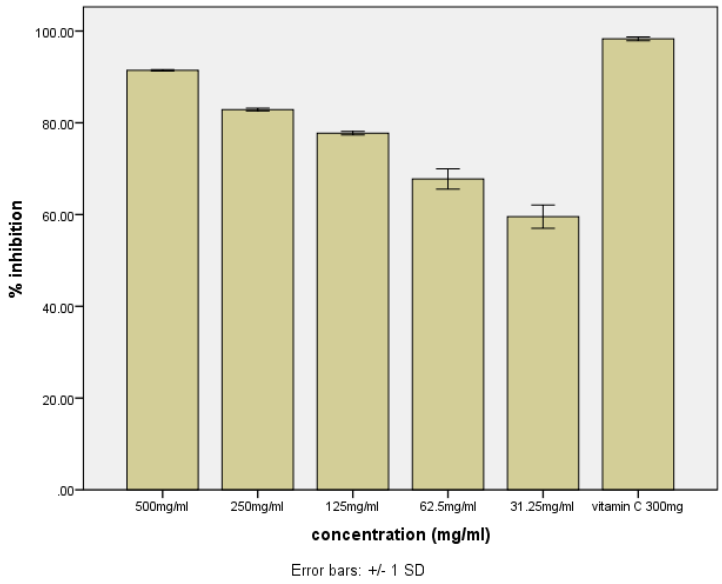

Figure 3: A bar chart showing Anti-lipid peroxidation activity

Diuretic Activity of Ethanol Extract of *Persea americana* and Frusemide

Table 5: The volume of urine and sodium, potassium, chloride and bicarbonate ion excretion of furosemide and different concentration of the extract

Treatment	Volume of urine/ 24hrs	Sodium mEq/24hrs	Potassium mEq/ 24hrs	Chloride mEq/24hrs	Bicarbonate mEq/ 24hrs
Normal saline	0.65 ± 0.71	112.5± 3.54	8.25± 0.35	97.50± 0.71	36.50± 0.70
Furosemide 20mg/kg B.W	9.15± 0.49	190.00± 2.83	31.25± 1.77	143.60± 1.97	39.35± 1.06
500 mg/kg B.W	5.25± 0.35	104.65± 1.20	25.50± 0.98	97.25± 1.06	37.95± 3.18
250 mg/kg B.W	3.60± 0.57	93.90± 1.84	19.50± 0.98	81.30± 0.98	35.05± 4.17
125 mg/kg B.W	2.65± 0.21	79.30± 0.99	14.60± 0.85	61.10± 1.27	36.90± 1.27
62.25 mg/kg B.W	1.65± 0.21	34.00± 0.28	8.25± 0.212	31.35± 1.06	35.50± 0.70
31.25 mg/ kg B.W	1.15± 0.70	32.55± 0.78	6.40± 0.57	26.75± 0.49	34.00± 0.00
15.50 mg/kg B.W	0.85± 0.70	25.15± 1.48	3.90± 0.14	21.50± 0.99	33.40± 0.57

Values are represented as Mean value ± standard deviation of two replicates and show significant difference when compared to the standard.

Discussion

Natural antioxidants that are present in plants generally are responsible for inhibiting or preventing the deleterious consequences of oxidative stress that is being suggested as the root cause of aging and various human diseases such as atherosclerosis, stroke, diabetes, cancer and neurodegenerative. The ability of a molecule to donate a hydrogen atom to a radical determines its antioxidant potentials. DPPH accepts hydrogen atom from an antioxidant and becomes a stable diamagnetic molecule. DPPH is a stable free radical and can be reduced in the presence of an antioxidant molecule, it usage has been widely applied for evaluating antioxidant activity in a number of studies [18]. The reactivity of ethanol extract of seeds of *Persea Americana* was analyzed with DPPH, a stable free radical scavenger, the absorption decreases with increase in concentration and the resulting discolouration is stechiometrically related to the number of electron gained. The DPPH radical scavenging activity is shown in Table 1, extract of seeds of *Persea americana* exerted an inhibition of 87.65% at 500mg/ml compared to vitamin C at 96.02%.

The nitric oxide is a potent pleiotropic inhibitor of physiological processes such smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messengers, vasodilation and antimicrobial and antitumor activities. Suppression of released nitric oxide may be partially attributed to direct nitric oxide scavenging as the extract of seeds of *Persea americana* decreased the amount of nitrite generated from the decomposition of SNP *in vitro*. The scavenging activity of the extract was increased in dose dependent manner. At the maximum concentration of 500mg/ml ethanol extract of *Persea americana* scavenged the radical by 91.43% compared to Vitamin C which is 98.25%.

Peroxidation of lipid is a natural phenomenon and occurs on its exposure to oxygen. Recently, free radicals- induced lipid peroxidation has gained much importance because of its involvement in several pathological conditions. The result of this investigation showed that the extract inhibited lipid peroxidation in a dose dependent manner. The ethanol extract inhibited lipid peroxidation maximally by 75.26% compared to Vitamin C which is 83.82%.

Diuretics are drugs capable of increasing level of urine, so they are useful in the treatment of diseases that are related with retention of fluids. Many herbal diuretics exert their action by directly affecting electrolytes. The present study revealed that ethanol seed extract of *Persea americana* significantly increased the urine volume as well as urinary electrolyte concentration. This effect is dose dependent, this agreed with the finding of [21]. Determination of urinary hydrogen bicarbonate concentration revealed that hydrogen bicarbonate concentration was not significantly increased. This shows that diuretic activity of ethanol extract of seeds of *Persea americana* does not make use of carbonic anhydrase inhibition as its mechanism of action [17]. In the present study, ethanol seed extract of *Persea americana* showed a minimal level of potassium in the urine which may increase the risk of hyperkalemia which could indicate that it may not have potassium- sparing capabilities. The increase in the ratio of concentration of excreted sodium and potassium ion indicates that the extract increased sodium ion excretion to a greater extent than potassium which is a very essential quality of a good diuretic with less hyperkalemia. The regulation of sodium and potassium is intimately related to renal control of acid-base balance [22]. The chloride ion excretion was elevated significantly indicating that the Natiuretic effect found was high in urine. The data was found statistically significant when compared with normal control. This also indicates that the component of the seeds of *Persea americana* responsible for diuresis has shorter half-life and quick onset of action. The result obtained in this study provides a qualitative basis to explain the characterization of *Persea americana* as a medicinal plant.

Conclusion

From the result obtained, it can be concluded that the ethanol extract of the seeds of *Persia americana* possess significant diuretic activity by increasing the total urine output and increased excretion of sodium, potassium and chloride ions levels in the urine of the albino rats and it can also be concluded that the extract of the seeds of *Persea americana* can attenuate oxidative stress via its antioxidant properties.

The activity of the extract was not comparable in terms of qualitative activity elicited by the control (furosemide and Vitamin C), this could be due to the use of crude extracts. Hence, isolation of the active principle will be advantageous to produce novel bioactive constituent from these extract which may possess more significant activity. Further phamacodynamic investigation are required to investigate its *in-vivo* antioxidant activity and the different antioxidant mechanism, and the active constituent and mechanism of diuretic effect exhibited by ethanol extract of *Persea americana.*

Consent

It is not applicable.

Ethical Issues

All animal experiments were in compliance with the National Institute of Health Guide for care and use of laboratory animals (Pub. No. : 85-23, revised 1985). An approval for the use of animals' experimental protocols was secured from the university committee of ethical and appropriate use of animals.

Competing Interests

Authors have declared that no competing interests exist.

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