ORIGINAL RESEARCH

PHYTOCHEMICAL SCREENING AND *in vitro* ANTIOXIDANT PROPERTIES OF *persea americana* SEED GROWN IN NNEWI, SOUTH EASTERN NIGERIA

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ABSTRACT

Background: *Persea americana* (avocado) is a popular plant that has been used traditionally for its nutritional and medicinal values. The efficacy of a medicinal plant is contingent upon the bioactive compounds it possesses.

Aim: The study investigated the phytochemical and *in vitro* antioxidant properties of the ethanolic seed extract of *P. americana* obtained from rural part of Nnewi, Anambra State, Nigeria.

Methodology: The dried pulverized seeds of *P. americana* were extracted with ethanol by the maceration method. The extract obtained was subjected to phytochemical screening. In vitro antioxidant assays, (2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant properties (FRAP) were also carried out on the extract.

Results: The phytochemical screening of the extract revealed the presence of flavonoids, saponins, alkaloids, tannins, glycosides, steroids and oxalate. Kaempferol, a flavonoid was found to be the predominant phytochemical compound (50.890.002 µg/ml). The extract showed a higher DPPH scavenging activity than the ascorbic acid standard at lower concentrations. The DPPH

activity was highest (91.61±0.10 %) at concentration of 31.5 mg/ml. The extract also showed a concentration-dependent increase in FRAP activity, and was found to have a higher activity than the reference antioxidant, ascorbic acid.

Conclusion: This study revealed that the plant seed contains substantial amount of hydroxyl radical scavenging, and ferric reducing power activity, and could be a source of naturally occurring antioxidants that is beneficial to health.

Keywords: *Persea americana*, Flavonoids, Free radicals, Antioxidants, Medicinal plants

INTRODUCTION

Numerous bioactive secondary metabolites found in nature are utilized to treat and prevent a wide range of human illnesses. Additionally, these secondary metabolites serve as vital sources of inspiration for the creation of new medicinal agents[1]. Many of these natural remedies had long been available from the plant world, and it is believed that over 25% of our present medication arsenal comes from higher plants[2]. For the great majority of people worldwide, the traditional medical system, which mostly relies on herbal remedies, is crucial to their bodily and mental health[3]. Herbal medication is generally recognized due to its ease of accessibility, comparatively cheaper cost and relative safety profiles when compared to the synthetic alternatives. For example, almost 80% of the African population rely on herbal treatment[4], either independently or in conjunction with conventional medications[1], and the use of herbs is progressively receiving acknowledgment in western nations.

Free radicals are unavoidable components of everyday human existence. Free radicals are beneficial to health in moderation, but too many of them can lead to oxidative stress[5]. This condition speeds up the aging process and the onset of illness by causing oxidative damages at the level of cells, tissues, and organs[6]. Antioxidants are required to counteract and neutralize the effects of those free radicals. Medicinal plants include a diverse range of natural antioxidants, including flavonoids, coumarins, tocopherols, carotenoids, and phenolic acids[7]. Plant antioxidants can interact with pro-oxidant metals and behave as reducing agents, even though their primary role is to scavenge free radicals. Fruits and vegetables that we frequently eat, like avocados, can provide antioxidant compounds.

Persea americana, the avocado, as it is usually called, is a Central American edible fruit that may be readily adapted to tropical climates[8]. An underutilized resource, avocado seeds make up 16% of the weight of an avocado fruit[9]. Phytochemical studies on avocado seeds have identified various classes of natural compounds such as phytosterols, triterpenes, fatty acids, furanoic acids, abscisic acid, proanthocyanidins, flavonoids and polyphenols[10,11]. Given that the quantity of phenolic compounds and their phytochemical makeup in plants may be influenced by environmental conditions[12], the current study examined the phytochemical and *in vitro* antioxidant properties of *P. americana* seed obtained from rural part of Nnewi, Anambra State, Nigeria.

METHODS

Plant sample procurement and identification

Mature avocado fruits were obtained from Eke Amobi rural market in Otolo Nnewi

Anambra state, Eastern Nigeria. The seed was separated from the pulp, and was authenticated by a taxonomist from Botany Department of Nnamdi Azikiwe University, with Habarium number, NAUH-183[A].

Preparation of plant material

The avocado seed was prepared according to the method postulated by Egbuonu *et al*.[13]. The avocado seed was sun-dried and milled, and the powder extracted with cold maceration using 90% ethanol in ratio 1:2 solid to solvent. Afterwards, the extract was filtered using a linen. The filtrate was concentrated at 60℃ using a water bath and dried at 50℃ with an oven.

Phytochemical screening

This was done using standard method as described by Sharma *et al*.[14]. One gram of the extract was weighed and transferred into a test tube. This was followed by the addition of 15 ml ethanol and 10 ml of potassium hydroxide to the test tube. The test tube was warmed in a water bath for 60 min at 100 ^{0}C. After this, the solution was transferred to a separating funnel. The test tube was washed with 20 ml of ethanol, 10 ml of cool water, 10 ml of hot water and 3 ml of hexane respectively. The extract was washed three times with 10 ml of 10 %w/v ethanol:aqueous solution, and dried with anhydrous sodium sulphate and the solvent evaporated. The sample obtained was solubilized in 1000ul of pyridine of which 200 ul was transferred to a vial for analysis. The analysis of each of the phytochemicals in the extract was performed using gas chromatography equipped with flame ionization detector.

***In vitro* antioxidant assay**

The *in vitro* antioxidant assays, 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant properties (FRAP*)* assays were determined according to the method described by Shwetha and Sudha[15].

2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Serial dilution of the sample (31.25, 62.50, 125, 250, 500 mg/ml) was filtered using Whatman 125 mm filter paper and 4 ml of each was mixed with 1 ml of DPPH radical reagent. Exact serial concentrations of ascorbic acid were used as standard. The mixtures were shaken and incubated for 30 mins at room temperature. The absorbance was measured at 517 nm using UV-VIS spectrophotometer (Model 752, China)

Calculations:

$$\text{Percentage inhibition} = \frac{Ao - A1}{Ao} \times 100$$

Where A_0 = Absorbance of Blank

A1 = Absorbance of test sample. Ascorbic acid was used as standard.

Ferric reducing antioxidant properties (FRAP) assay

Nine hundred microlitre (900µL) of FRAP reagent was mixed with 90 ml of distilled water and 30 ml of various concentrations of the extract (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg/ml). The reaction mixture was incubated at 37^{o}C for 30 mins using incubator (model DNP-9052A, China) and the absorbance was measured at 593 nm using UV-VIS spectrometer (model 752, China).

Calculation:

The percentage inhibition of ferrous-TPTZ complex formed was calculated using:

$$\frac{Ao - A1}{Ao} \times 100\ (\%)$$

Where A_0 = Absorbance of blank

A_1 = Absorbance of test sample

Data analysis

Descriptive statistics was carried out on the data generated by analysis of variance (ANOVA). Results were expressed as mean±SEM of triplicate determinations.

RESULTS

The results of quantitative phytochemical analysis of ethanolic seed extract of *Persea americana* revealed that kaempferol, a type of flavonoid was the predominant phytochemical compound (50.89±0.002 µg/ml) present in the seed's extract. Aphyllidine and dihydrocysisine, are alkaloid compounds found in moderately high quantity, 10.68±0.002µg and 12.87±0.001µg respectively. Tannin, oxalate, cardiac glycoside, steroid, sapogenin (saponin) were also detected in moderate quantities (Table 1). Table 2 showed the *in vitro* DPPH scavenging activity of 31.25 mg/ml, 62.5 mg/ml, 125 mg/ml, 250 mg/ml and 500 mg/ml of the *P. americana* seed extract using ascorbic acid of corresponding concentrations as a reference standard. There was a concentration dependent decline in the scavenging activity of the extract. The activity was lowest for 500 mg/ml (61.21±0.37%) and highest for 31.25 mg/ml (91.61±0.10%). The extract showed a higher DPPH scavenging activity than ascorbic acid. Table 3 showed the *in vitro* ferric reducing antioxidant power of 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1.0 mg/ml and 1.2 mg/ml of the *P. americana* seed extract using corresponding concentrations of ascorbic acid as reference standard. There was a concentration-dependent increase in the FRAP of the extract from 0.2 to 1.2 mg/ml in relation to the standard. The activity was highest for 1.2 mg/ml (67.65±0.04%) and lowest for 0.2 mg/ml (18.63±0.01%). The extract showed a higher FRAP reducing power than the standard.

Table 1: Quantitative phytochemical analysis of ethanolic seed extract of *Persea americana*

Compound	Concentration (µg/ml)
Flavonoids	
Kaempferol	50.89±0.002
Proanthocyanidin	25.24±0.001
Anthocyanin	27.70±0.001
Catechin	0.02±0.000
Flavonones	8.75±0.001
Narigenin	0.02±0.00
Flavone	0.01±0.00
Alkaloids	
Aphyllidine	10.68±0.002
Dihydrocysisine	12.87±0.001
Ribalinidine	7.27±0.001
Ammodendrine	2.91±0.001
Epihedrine	9.37±0.002
Tannin	8.16±0.002
Cardiac glycoside	9.16±0.001
Oxalate	1.84±0.001
Steroid	17.89±0.001
Sapogenin	2.78±0.001

Each value represents Mean±SEM of triplicate determinations.

Table 2: DPPH free radical scavenging activity of the *P. americana* extract

	DPPH inhibition (%)	
Conc. (mg/ml)	Ascorbic acid	Extract
31.25	10.77±0.09	91.61±0.10
62.5	17.62±0.05	84.87±0.22
125	21.04±0.04	82.26±0.17
250	25.02±0.03	76.49±0.26
500	28.42±0.02	61.21±0.37

Each value represents Mean±SEM of triplicate determinations.

Table 3: FRAP activity of the *P. americana* extract

	FRAP activity (%)	
Conc. (mg/ml)	**Ascorbic acid**	**Extract**
0.2	10.51±0.00	18.63±0.01
0.4	15.30±0.01	48.17±0.06
0.6	17.29±0.01	49.26±0.02
0.8	23.72±0.01	57.59±0.02
1.0	59.15±0.01	62.84±0.22
1.2	65.45±0.11	67.65±0.04

Each value represents Mean±SEM of triplicate determinations

DISCUSSION

Nigeria has wide variety of medicinal herbs with therapeutic benefits. Alkaloids, polyphenols, terpenes, glycosides, and other compounds with potential medicinal uses have been found in Nigerian plants, according to studies on them[16]. The phytochemical screening of the extract revealed the presence of flavonoids, saponins, alkaloids, tannins, glycosides, steroids, oxalate in the ethanolic seed extracts of *P. americana.* Flavonoids were the predominant class of compounds detected, with kaempferol having the highest value (50.89±0.002 µg/ml). This result is in agreement with the previous analysis of *P. americana* seed extracts obtained by Okoye *et al*.[17], except for the absence of steroids in their extract. Egbuonu *et al*.[13] reported similar finding, except for the absence of oxalate. Phenols detected in *P. americana* seeds examined in this study may suggest that these seeds have anti-inflammatory, anticoagulant, antioxidant, immune-boosting, anticarcinogenic, and antiaging properties. Flavonoids are powerful water-soluble super antioxidants that scavenge free radicals[18]. They protect against all phases of carcinogenesis, have potent anticancer properties, and mitigate oxidative cell damage. They are the most prevalent and extensively distributed class of plant phenolic chemicals, and they reduce the risk of inflammation and heart disease[19]. Alkaloids are important secondary metabolites found in plants that have medicinal use. Because of their bactericidal and analgesic properties, alkaloids in their isolated, pure form as well as their synthesized derivatives are employed as fundamental therapeutic agents. The seed extract's application in ethnomedicine may be justified by the presence of alkaloids. The free astringency and bitter taste of *P. americana* seeds may be due to the tannin content[20]. Saponins are a broad collection of chemicals that are stable in aqueous solutions and create soap-like foams. According to Henry *et al*. [21], saponins have the ability to lower blood pressure and heart rate because they bind to cholesterol and create insoluble complexes. The amount of

saponin in the seed sample was moderate and thus, would not have a negative impact on the animals' growth. The quantity of oxalate in the seed sample was less than the (3-5 g) lethal dosage of soluble oxalate intake [22]. The phytochemical findings of this study are in line with the report of Omorovbiye *et al*.[18]. These phytochemical substances may be the reason why the plant's seed extract used in this investigation is applied in folklore medicine, and could be explored more for pharmaceutical applications.

One commonly used method for assessing the antioxidant properties of plant extracts is the scavenging of DPPH free radicals[23]. "DPPH is a stable free radical that can be used to test the ability of the sample's polyphenols to scavenge free radicals" [24]. The result of the DPPH activity revealed that the *P. americana* extract has DPPH radical scavenging property. The extract showed a higher scavenging activity than the ascorbic acid standard which may indicate increased ability of the components of the extracts to donate hydrogen ions, reducing the radical to corresponding hydrazine. This further suggest that the extract could be an effective therapeutic agent for the treatment of pathological damages caused by free radicals. However, there was a dose-dependent decline in radical scavenging activity of the extract, Olasunkanmi and Ogunyemi[25] reported similar pattern of result. The decline in activity with dose could be as a result of high concentrations of antioxidants and the presence of colour compounds in the extract. Antioxidants lose their beneficial effects when present in excess[19,26]. Phenolic compounds in excess can transit from antioxidants to prooxidants[27]. Colour pigments present in plant-food samples can interfere with the elaborate absorbance readings of the DPPH radicals because the absorb in the same wavelength range[28]. Previous studies have reported DPPH radical scavenging activity of *Persea americana* seeds extract[29,23,24,30]. Also, Onyedikachi *et al*[30], reported DPPH radical scavenging property of *P. americana* seed oil. The radical scavenging activity of the *P. americana* extract could be attributed to polyphenols such as flavonoids present in the extract. Flavonoids are powerful hydrophilic antioxidants and scavengers of free radicals, that mitigate oxidative cell damage and prevent growth of cancers[31]. The plant's seed high antioxidant activity is indicative of its significance in natural product development.

"The principle of FRAP assay was to reduce the colourless Fe^{3+}–TPTZ complex to produce blue-coloured Fe^{2+}–TPTZ complex under low pH condition by antioxidants present in the sample extract"[23]. The findings of this study revealed that *P. americana* seed extract demonstrated ferric reducing antioxidant property. The FRAP activity of the extract showed a dose-dependent increase, and was found to have a higher activity than the standard ascorbic acid. In general, substances that break the chain of free radicals by donating a hydrogen atom are associated with reducing characteristics[32]. Due to the development of the Fe^{2+}-TPTZ complex with increasing concentration, which was also observed in the reference antioxidant, the extract's ferric reducing property increased noticeably. This could indicate that the plant seeds can provide electrons to free radicals that are stable in the food and biological systems, thus, preventing radical-related damages and deteriorations Previous studies have reported FRAP activity of *Persea americana* seed extract [33,29,24]. Also, Onyedikachi *et al*.[30], demonstrated FRAP activity of *P. americana* seed oil. The FRAP activity of the extract could be attributed to phenolic compounds present in the extract. Due to their strong

redox reactivity, phenolic compounds have effective antioxidant potential, reducing free radicals and averting harmful cascade events[24]. The *in vitro* antioxidant properties of the ethanolic seed extract of *P. americana* could be an indication of its *in vivo* antioxidant potentials.

CONCLUSION

The findings of this study have shown that the ethanolic seed extract of *P. americana* contains good amounts of bioactive compounds, with alkaloids, flavonoids, and tannins abundantly present, which possess high antioxidant and free radicals scavenging activities. The *in vitro* antioxidant activities of the extract indicated that the plant seed possesses a substantial amount of hydroxyl radicals scavenging and ferric reducing power activity in comparison to the reference antioxidant ascorbic acid, and could serve as a source of naturally occurring antioxidants for health benefits.

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Conflict of interest: The authors declare that they have no known competing interest

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