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Antioxidative and Free Radical Scavenging Properties of Ethyl Acetate Fractions of *Persea americana* Seed and *Bryophyllum pinnatum* Leaf

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ABSTRACT

Persea americana and *Bryophyllum pinnatum* are important plants with age-long application in traditional medicine for the management of varieties of ailments. The seed of *P. americana* is a byproduct and the leaf of *B. pinnatum* constituting weed in some locality could be harnessed for marketable antioxidant potentials. The present study aims to assess the antioxidative and free radical scavenging potential of ethyl acetate fractions of *Persea americana* seed and *Bryophyllum pinnatum* leaf. This was determined using the effect of the plant fractions on hydroxyl, nitric oxide, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Total antioxidant capacity (TAC), reducing power assay and inhibition of H_2O_2-induced lipid peroxidation in rat stomach homogenates were assessed using standard methods. The extracts exhibited stronger DPPH radicals scavenging effect than tannic acid with threshold inhibitory concentration (IC_{50}) values of 53.10 ± 2.66, 65.59 ± 3.28, and 2039.49 ± 163.16 µg/mL for *P. americana*, *B. pinnatum*, and tannic acid respectively. The ethyl acetate fractions exhibited strong hydroxyl and nitric oxide radicals scavenging ability *in vitro*, with IC_{50} values of 370.37 ± 14.81, 107.45 ± 5.37, 623.96 ± 31.20 µg/mL and 377.26 ± 18.86, 691.48 ± 48.40,105.86 ± 6.35 µg/mL for *P. americana*, *B. pinnatum*, and catechin respectively. Furthermore, the *B. pinnatum* leaf fraction was found to be highly effective in the inhibition of H_2O_2-induced lipid peroxidation in rat stomach homogenates. The present study showed that the ethyl acetate fractions of *P. americana* seed and *B. pinnatum* leaf possess potent antioxidant properties evidenced in their radical scavenging ability.

***Keywords*:** Oxidative stress, Hydroxyl radical, Nitric oxide, *Persea americana*, *Bryophyllum pinnatum*

Introduction

Medicinal plants are known for their importance in the management of ailments and as sources of nutrients for growth and nutritional balance.[1] Plants' medicinal properties are attributable to their primary and secondary metabolites which exert their medicinal activity through antioxidative effects, free radicals scavenging, induction of enzymes, and activation of important biochemical pathways.[2] Free radicals are important in cellular phagocytosis, respiration, and mitochondrial energy generation.[3] Oxidative stress arises from an imbalance between stressors and stress-reducing factors. A shift of this delicate balance in favor of stressors such as the increase in reactive oxygen species, reduction in the concentration of antioxidants, and regenerating enzymes result in a distortion in important macromolecules such as lipids, carbohydrates, proteins, and nucleic acids.[4] Free radicals play a prominent role in the etiology of diseases such as diabetes mellitus, gastric ulcer, Alzheimer's disease, rheumatoid arthritis, respiratory diseases (asthma), cataract development e.t.c.[5,6] Recently, researchers have focused on harnessing plants and plant products in crude or purified forms as a remedy in oxidative stress management. *Persea americana* Mill. is an evergreen tree native to the Carribeans. It is commonly known as avocado, and locally in Igbo as *ube-beke*, *ube oyibo* or*ewépia* (Yoruba), and *ganyen piya* (Hausa). Documented and undocumented claims from traditional medicine practitioners show that the leaf, stem, seed, and bark of *P. americana* have been applied in ethnomedicine for a variety of purposes ranging from anti-inflammatory, antihypertensive, anti-ulcer, hypoglycaemic and hypercholesterolaemic, and parasitic skin diseases treatment.[7] The fruits of *P. americana* are rich in nutrients such as dietary fiber, carotenoids, tocopherol, folate, vitamins B and K, monounsaturated fatty acids, and important minerals such as potassium and magnesium.[8] Numerous studies on the medicinal properties of the plant have revealed significant dose-dependent antiulcer activity,[9] analgesic, and anti-inflammatory activity by the leaf extract.[10] Anticonvulsant,[11] hypoglycaemic and hypocholesterolemic,[12]free radical scavenging and anti-proliferative activities,[13] vasorelaxant and blood pressure reducing, activities in animal studies have also been reported.[7,11]

Bryophyllum pinnatum is a member of the Crassulaceae family. They are routinely known across different regions by the names"air plant", "love plant", "miracle leaves", "life plant", all attributable to their identified characteristics.[14]The plant is indigenous to Madagascar, but largely distributed with dominance in the rain forest belt and tropical countries. *B. pinnatum* occurs in Southern Nigeria, known by the Igbos of South-Eastern Nigerian '*odaa opue*', and '*ewe abamoda*' or '*odundun*' by the Yorubas of South-Western Nigeria.[16] Many types of research have focused on Crassulaceae family especially *B. pinnatum* due to its xeromorphic characteristics and medicinal properties.[17] Its application in folk medicine cuts across the tropics of Africa, Asia, America, and Australia.[17] The leaf and stem of *B. pinnatum* are hyperacidic with a characteristic bitter taste, painkilling, and

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antispasmodic properties. They are ethnopharmacologically applied in the management of many maladies including runs, vomiting, earache, burns, abscesses, gastric ulcers, insect stings, lithiasis, coughs, bronchial infections, blood dysentery, jaundice, and gout[18,19]. It is also applied for homeostasis, tissue regeneration, and facilitation of the dropping of the placenta of a newly born baby[20]. Hepatoprotective activity, increased vascular integrity, [21] anti-inflammatory effects,[22] anti-ulcer activity,[23] free radical scavenging activities have also been reported.[24] The chemical, proximate, mineral, lipids compositions, as well as prostaglandins in the stem of *B. pinnatum*, have been reported.[25-28]

The present study seeks to examine the antioxidative and free radical scavenging properties of the phenolic fraction of *P. americana* seeds and *B. pinnatu*m leaf. It will validate claims on the medicinal properties of the plants and provide further justification for its wide application in ethnomedicine. In addition, provide empirical evidence to back similar studies which were chiefly carried out with crude extracts; and will also use non-linear mathematical models to describe inhibition patterns of the extracts on studied free radicals.

Materials and Methods

Plant materials:
A fresh sample of *B. pinnatu*m leaf was collected from a garden at Umunam village, Ngor Okpala L.G.A., Imo State, while fresh fruits of *P. Americana* were harvested from a farm in Ugiri-Ike Autonomous community, Ikeduru L.G.A. Imo State in May 2019. The plant materials were identified by Prof. F. N. Mbagwu a plant taxonomist at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State, Nigeria. The samples were deposited in the institution's herbarium with voucher numbers IMSUH 0225 and IMSUH 0226 respectively.

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. The fresh leaves of *B. pinnatum* and *P. americana* seeds were shed-dried at room temperature (28 ± 2^{o}C) and crushed to a fine powder using a Kenwood blender (BL357). The powder (800 g) was extracted with 80% ethanol (2.0 L) using a Soxhlet extractor. The ethanol extract was partitioned between ethyl acetate and water to recover the ethyl acetate soluble component of the extract. Recovery of the extract was carried out by distillation in a rotary evaporator at 49^{o}C. The slurry was further concentrated to paste in a desiccator and stored in a refrigerator (1.7-4.0°C) until used.

Free radicals scavenging potentials
The antioxidative property of the extracts was assessed using their effect on nitric oxide radicals, hydroxyl radicals, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals, as well as reducing power assay, *in vitro* inhibition of lipid peroxidation, and total antioxidant capacity.

Determination of nitric oxide radical scavenging effect
Nitric oxide radical scavenging effect of fractions was based on the method of Marocci *et al.*,[29] as described by Alisi and Onyeze.[30] The assay was carried out in a 4 mL volume consisting of varying concentrations of extract (0 - 2000 µg/mL) and quercetin stock solution (0-1000 µg/mL) in phosphate buffer (PB) (pH 7.2). To the tubes, 1mL of 5 mM sodium nitroprusside solution prepared in PB (pH 7.2) was added and incubated in a water bath at 29°C for 2 hours. Nitrite formed was measured by reacting 2 mL of the earlier solution with 1mL Griess reagent (equal volume of 1% sulphanilic acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (NED). The absorbance was measured in a spectrophotometer (UV-VIS 752N) at 550 nm. Inhibition of nitrite formation by extracts or quercetin was calculated relative to the negative control which was void of extract or quercetin.

$$\%\ Inhibition = \frac{Absorbance\ control - Absorbance\ test}{Absorbance\ control} \times 100$$

Hydroxyl radical scavenging assay
The hydroxyl radical scavenging effect of fractions was determined by assessing 2-deoxyribose degradation by Fe^{3+} / ascorbate / EDTA / H_2O_2system-generated hydroxyl radicals as described by Hillwell.[31] The reaction was carried out using an extract concentration range of 0 – 3000 µg/mL in 1.0 mL final volume. The assay contained2.8mM deoxyribose,0.1mM $FeCl_3$, 0.1mM EDTA(0.1mM), 1mM H_2O_2, 0.1 mM ascorbic acid, 20mM KH_2PO_4/K_2HPO_4–KOH buffer (pH 7.4); incubated at 37^{o}C for 1 hr.The level of deoxyribose degradation was measured by determining thiobarbituric acid reactive substances (TBARS) formation by the method of Ohkawa*et al.*,[32], as modified by Liu *et al.* [33] The test setup was further treated with 1.5 mL of 20% acetic acid and 0.8% thiobarbituric acid (TBA) respectively, followed by 0.2 mL of 8.1% sodium dodecyl sulfate (SDS) and the mixture heated at 100^{o}C for 1 hr. This was cooled to room temperature and proteins precipitated by the addition of trichloroacetic acid (TCA) (2mL), vigorously shaken, and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant obtained was measured in a spectrophotometer (UV-VIS 752N) at 532nm wavelength. Hydroxyl radical scavenging was calculated as follows:

$$\%\ Inhibition = \frac{Absorbance\ control - Absorbance\ test}{Absorbance\ control} \times 100$$

Determination of DPPH radical scavenging
The effect of fractions on DPPH radicals was determined using the method of Blois [34]as described by Velazquez *et al*.[35] Aliquot of 1mL of graded concentrations (0 – 500 mg/mL) of extract prepared in methanol was incubated with 2 mL of 0.02 mg/mL DPPH (Fluka Chemie, Switzerland) in methanol at room temperature for 15 Minutes. The absorbance of the set-up was measured in a spectrophotometer (UV-VIS 752N) at 517 nm. Tannic acid (0 – 500 mg/mL) was used as standard. The DPPH radical-scavenging effect was calculated with the formula:

$$\%\ DPPH\ radical\ scavenging = \frac{Blank\ absorbance\ - Absorbance\ test}{Blank\ absorbance} \times 100$$

The scavenging data were fitted into mathematical equations with the highest correlation coefficient and used to evaluate IC_{50}.

Determination of reducing power
The reducing power of the fraction was assessed using $Fe^{3+}/Fe^{2+}$by the test compound as described by Oyaizu[36]and Hsu[37]. The assay was carried out in 2.5 mL graded concentration of extract (0 – 1000 µg/mL) in 200 mM PB (pH 6.6), to which 2.5 mL of 1% $K_3Fe(CN)_6$ was added, thoroughly vortexed, and incubated at 50^{0}C for 20 minutes. Thereafter, 2.5 mL of 1% TCA was pipetted into the test set-up and centrifuged at 3000 rpm for 5 minutes. Furthermore, 1:1 dilution of the supernatant with distilled water was treated with 0.5mL of 0.1% $FeCl_3$. The absorbance of the assay was measured in a spectrophotometer (UV-VIS 752N) at 700nm. Reducing power (RP 0.5_{AU}) was calculated from the plot of optical density with the concentration of fraction. This is equivalent to the concentration of extract that produced a 0.5 absorbance reading.

Determination of Inhibition of H_2O_2-induced lipid peroxidation in rat stomach homogenate
The inhibition of lipid peroxidation in rat stomach was determined by incubating rat stomach homogenate treated with H_2O_2 (10 µM) and graded concentrations of the fraction (0 – 1000 µg/mL). A mixture of 200 µL rat stomach homogenate (4% w/v), extract (0 – 1000 µg/mL) and 200 µL H_2O_2(10µM) was incubated for 1 hr at room temperature. TBARS formed was quantified according to the method of Liu *et al.*,[33]. The set-up contained 0.75mL of 20% acetic acid (pH 3.5), 0.75 mL of TBA (1.0%) and 0.2 mL of SDS (8.1%). This was placed in a water bath at 100ºC for 1 hr; after which it was allowed to cool at room temperature and 2mL of 10% TCA was added to the tubes. It was centrifuged at 3000 rpm for 10 minutes and the absorbance of the resulting supernatant was measured in a spectrophotometer (UV-VIS

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752N) at 532 nm. Quercetin was used as a standard antioxidant; the percentage inhibition of lipid peroxidation by fraction or quercetin was calculated relative to the negative control.

$$\%\ Inhibition = \frac{Absorbance\ control - Absorbance\ test}{Absorbance\ control} \times 100$$

Determination of Total antioxidant capacity

The TACof the fraction was estimated according to the method described by Prieto *et al.*[38] The test consisted of about 0.4 mL of extract (1000 µg/mL) dissolved in methanol, and 4 mL phosphomolybdenum reagents consisting of 4 mM ammonium molybdate and 28 mM sodium phosphate prepared in 0.6 M H_2SO_4. The set-up was heated at 95ºC for 90 minutes in a water bath. This was then cooled at room temperature (28±2ºC) and the absorbance measured at 695 nm in a spectrophotometer (UV-VIS 752N). The TAC of the sample was calculated from a standard calibration curve prepared for ascorbic acid; and expressed in concentration unit of mg ascorbic acid equivalents (AAE)/g of extract.

Statistical Analysis

The dose-response data from the assessment of free radical scavenging potentials of the plant fractions were fitted into non-linear models using sigmaplot statistical software (version 10) to obtain the respective threshold inhibitory concentration (IC_{50}). This is defined as the concentrations of the plant fraction that scavenged free radicals by 50%.

$$\%\ Inhibition\ = \frac{100}{1\ + \left(\frac{x}{IC_{50}}\right)^{b}} (\mathbf{1})$$

Where *x* represents the concentration of the fraction, IC_{50} expresses the concentration that caused 50% inhibition; *b* is the relative slope of the curve at IC_{50}.

The percentage inhibition data (y) generated was fitted into non-linear mathematical equations; the models with the highest R^2 value and low fit standard error were chosen as best fit. The data largely fitted into logistic dose-response model (LDR (a,b,c,d) (equation 2), and sigmoid a,b,c (equation 3).

$$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^{d}} \quad (2)$$

Where x represents the concentration of the fraction, a and b are the minimum and maximum response of negative control respectively, c represents the IC_{50}, d is a constant that determines the slope at IC_{50}.

$$y = \frac{a}{1\ + exp\left(-\left(\frac{x-b}{c}\right)\right)} \quad (3)$$

Where x represents the concentration of the extract, *a* is the maximum response (of negative control), *b* is the curve transition center (IC_{50}), c is constraints: c≠0.

Results and Discussion

Nitric oxide radical scavenging

Figure 1.0 (A) presents the nitric oxide radical scavenging properties of ethyl acetate fractions of *P. americana* seed, *B. pinnatum* leaf, and catechin standard. Results presented in Figure 1.0 (A) showed that the extracts of *P. Americana* and *B. pinnatum* effectively scavenged nitric oxide radicals. The scavenging properties of *P. Americana* and catechin followed a logistic dose-dependent model (LDR a,b,c,d) with R^2 values of 0.993 and 0.983 respectively. However, the scavenging pattern of *B. pinnatum* extracts fitted into a sigmoidal model (sigmoid a,b,c), R^2=0.936. Evaluated IC_{50} values were 377.26 ± 18.86 µg/mL, 691.48 ± 48.40 µg/mL, and 105.86 ± 6.35 µg/mL for *P. americana*, *B. pinnatum*, and catechin standard respectively (Table 1.0). Comparison of evaluated IC_{50} values indicated that nitric oxide radical scavenging was in the order catechin>*P. americana*> *B. pinnatum.*

Hydroxyl radical scavenging

Figure 1.0 (B) shows the hydroxyl radicals scavenging properties of ethyl acetate fractions of *P. Americana* seed, *B. pinnatum* leaf, and catechin standard. Results presented in figure 1.0 (B) showed that the extracts of *P. americana*, *B. pinnatum*, and catechin's hydroxyl radical scavenging effect were logistic dose-dependent. Evaluated IC_{50} values of the extracts were 370.37 ± 14.81 µg/mL, 107.45 ± 5.37 µg/mL, and 623.96 ± 31.20 µg/mL for *P. americana*, *B. pinnatum*, and catechin standard respectively (Table 1.0). The R^2 value ranged from 0.981, 0.989, and 0.998 respectively, indicating a highly significant ($p<0.05$) correlation of the inhibitory response and concentration with the equation LDR a,b,c,d. Comparison of evaluated IC_{50} values showed that hydroxyl radical scavenging was in the order *B. pinnatum*>*P. americana*>catechin.

DPPH radical scavenging

Figure 1.0 (C) shows the DPPH radical scavenging properties of ethyl acetate fractions of *P. Americana* seed, *B. pinnatu*m leaf, and tannic acid. Results presented in Figure 1.0 (C) and Table 2.0 showed that the ethyl acetate fraction of *P. americana*, *B. pinnatum*, and tannic acid demonstrated an effective inhibitory effect against DPPH radicals. Mathematical modeling of the results (Table 2.0) showed that the scavenging data fitted into logistic dose-dependent model LDR (a,b,c,d) with R^2 values of 0.983, 0.991,and 0.979 respectively. This indicates a highly significant ($p<0.05$) correlation of the inhibitory response and concentration to a logistic pattern model (LDR a,b,c,d) with the equation shown in Table 2.0. Evaluated IC_{50} values of the extracts were 53.10 ± 2.66 µg/mL, 65.59 ± 3.28 µg/mL, and 2039.49 ± 163.16 µg/mL for *P. Americana* seed, *B. pinnatum* leaf, and tannic acid standard respectively. Furthermore, a comparison of evaluated IC_{50} values showed that DPPH radical scavenging potency was in the order *P. americana*>*B. pinnatum*> tannic acid.

H_2O_2 induced lipid peroxidation

Results presented in Figure 1.0 (D) show the *in vitro* inhibition of H_2O_2 induced lipid peroxidation of rat stomach homogenates by ethyl acetate fraction of *P. americana* seed extract, *B. pinnatum* leaf extract, and tannic acid standard. Results obtained for the inhibitory effect of the plant fractions on H_2O_2 induced lipid peroxidation in rat stomach homogenate showed that the extracts of *B. pinnatum* were more potent than *P. Americana* extract and the standard. The threshold inhibitory concentration (IC_{50}) obtained were 2045.05 ± 25.10 µg/mL, 191.61 ± 12.33 µg/mL, and 409.82 ± 18.55 µg/mL for *P. americana, B. pinnatum*, and tannic acid respectively. Furthermore, mathematical modeling of the results showed that inhibition of lipid peroxidation were largely logistic (LDR a,b,c) and LDR a,b,c,d for *P. americana* and *B. pinnatum* fractions respectively; while the effect of tannic acid was sigmoidal (sigmoid a,b,c). The R^2 values obtained were 0.981, 0.978 and 0.849 respectively (Table 2.0).

Reducing power determination

Figure 2.0 shows the reducing power activity of ethyl acetate fractions of *P. americana* seed, *B. pinnatum* leaf, and ascorbic acid standard. Results obtained from the study showed that ascorbic acid was a more potent reducing agent with $RP_{0.5}$ = 21.05 ± 0.84 µg/mL, while *P. americana* and *B. pinnatum* ethyl acetate fractions had reducing power of 460.37 ± 23.02 µg/mL and 337.22 ± 26.98 µg/mL respectively (Table 3.0). The results presented in Figure 2.0 demonstrated a sigmoidal reducing response (Sigmoid a,b,c) of extracts and ascorbic acid on ferric ions.

Total antioxidant capacity (TAC) of ethyl acetate fractions of P. americana seed and B. pinnatum leaf

The TAC of *P. americana* seed ethyl acetate fraction was 72.45 ± 6.51 mg AAE/g extract while that of *B. pinnatum* leaf ethyl acetate fraction was 49.38 ± 7.47 mg AAE/g extract.

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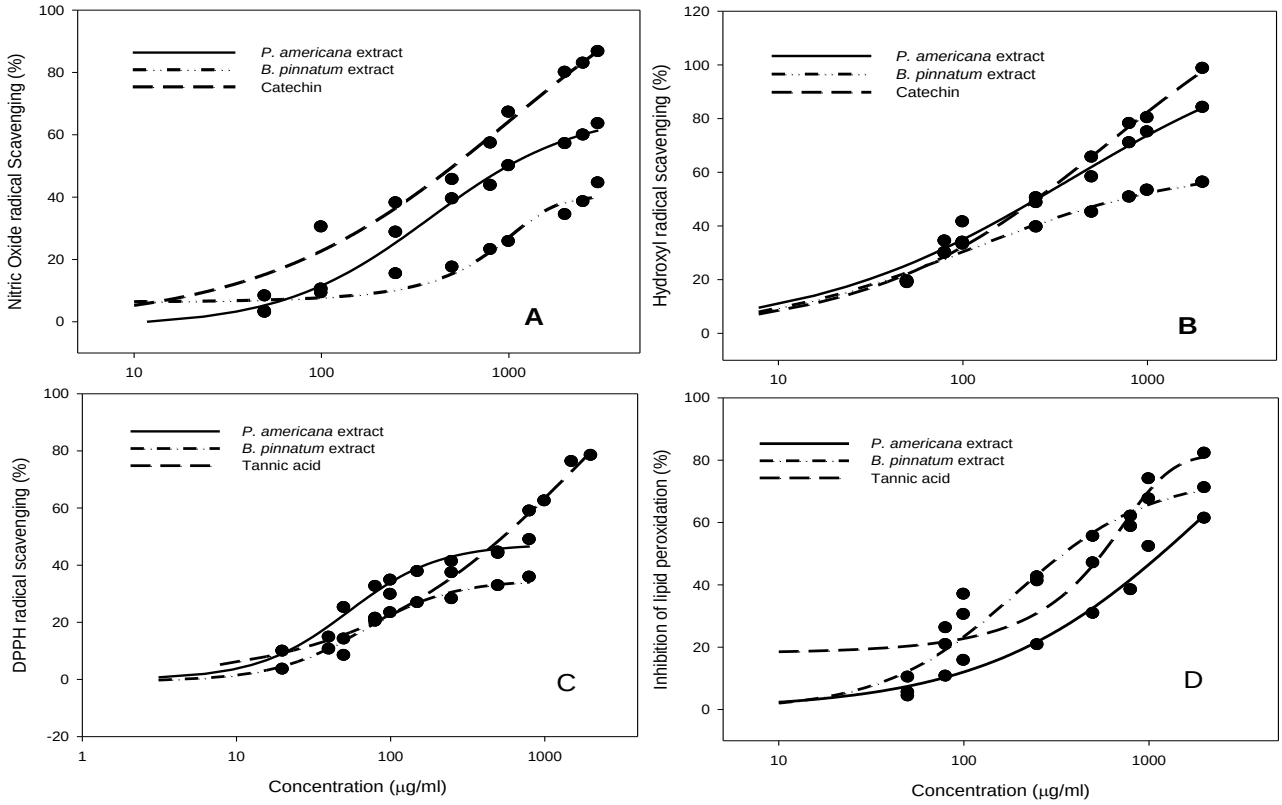

Figure 1: Free radicals scavenging properties of ethyl acetate fraction of *P. americana* seed, *B. pinnatum* leaf, and standards (catechin and tannic acid). Nitric oxide radical (A), Hydroxyl radical (B), DPPH radical (C), and inhibition of H_2O_2 induced lipid peroxidation of rat stomach homogenates (D).

Table 1: Threshold Inhibitory concentration (IC_{50}) of ethyl acetate fraction of *P. Americana* seed, *B. pinnatum* leaf extract, and catechin standard on nitric oxide and hydroxyl radicals

	Nitric oxide radical scavenging			Hydroxyl radicals		
Extracts	**(IC_{50})**	**Mathematical model**	**R^2**	**(IC_{50})**	**Mathematical model**	**R^2**
P. americana extract	377.26 ± 18.86	Logistic a,b,c,d	0.993	370.37 ± 14.81	Logistic a,b,c,d	0.981
B. pinnatum extract	691.48 ± 48.40	Sigmoid a,b,c	0.936	107.45 ± 5.37	Logistic a,b,c,d	0.989
Catechin	105.86 ± 6.35	Logistic a,b,c,d	0.983	623.96 ± 31.20	Logistic a,b,c,d	0.998

Results are mean ± Standard deviation of 3 determinations.

Table 2: Threshold Inhibitory concentration (IC_{50}) of ethyl acetate fraction of *P. americana* seed, *B. pinnatum* leaf and tannic acid standard on DPPH radical and *in vitro* H_2O_2 induced lipid peroxidation of rat stomach homogenates.

	DPPH radicals scavenging			Inhibition of lipid peroxidation		
Extracts	**(IC_{50})**	**Mathematical model**	**R^2**	**(IC_{50})**	**Mathematical model**	**R^2**
*P. americana*extract	53.10 ± 2.66	LDR a,b,c,d	0.983	2045.05 ± 25.10	Logistic a,b,c	0.981
B. pinnatum extract	65.59 ± 3.28	LDR a,b,c,d	0.991	191.61 ± 12.33	Logistic a,b,c,d	0.978
Tannic acid	2039.49 ± 163.16	LDR a,b,c,d	0.979	409.82 ± 18.55	Sigmoid a,b,c	0.849

Results are mean ± standard deviation of 3 determinations.

Free radical scavenging properties of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fractions were assessed using their effects on nitric oxide radical, hydroxyl radical, DPPH radical, inhibition of H_2O_2-induced lipid peroxidation, ferric reducing antioxidant power as well as their total antioxidant capacity. Results of our nitric oxide radical scavenging studies showed that the extracts compared favorably with catechin standard.

The nitric oxide scavenging properties of *P. americana* and catechin were describable using logistic dose-dependent model (Logistic a,b,c,d), and sigmoidal model (sigmoid a,b,c) for *B. pinnatum* extract. The threshold inhibitory concentration (IC_{50}) of the extracts indicated that nitric oxide radical scavenging potential of *P. americana* fraction was greater than that of *B. pinnatum*, however, the standard catechin exhibited a greater scavenging potential. Nitric oxide radicals are

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generated *in vivo* as cell-to-cell messengers,[39] responsible for such effects as decreasing blood pressure and neutrophils phagocytic response.[40]Nitrosative stress plays a role in the development and pathogenesis of many disease conditions like heart disease, congestive heart failure,[41,42] hypertension, cerebrovascular accidents, and diabetic complications.[43] The deleterious effect of NO radicals lies in their ability to react with superoxide anion to produce highly reactive hydroxyl radical ($^{\cdot}OH$) implicated in the initiation of lipid peroxidation process and DNA fragmentation.[44] Nitric oxide radical scavenging ability has been associated with phenolic compounds content.[30,45] The nitric oxide scavenging activities of flavonoid and phenolic compounds have been extensively reported.[46,47] Also, the ability of the extract to inhibit the Fenton-type generation of hydroxyl radical showed that our plant fractions were appreciably more effective at scavenging hydroxyl radical than catechin. The hydroxyl radical scavenging effect of the extracts was tightly logistic dose-dependent (Logistic a,b,c,d), with R^2 values of 0.981, 0.0.989, and 0.998 for *P. americana*, *B. pinnatum*, and catechin respectively, indicating a highly significant ($p<0.05$) correlation of the inhibitory response and concentration. Studies on the effect of the fractions on DPPH radical demonstrated similar trends observed against hydroxyl radicals; scavenging effects were tightly logistic dose-dependent (Logistic a,b,c,d), with R^2 values of 0.983, 0.991, and 0.979 for *P. americana*, *B. pinnatum*, and tannic acid respectively. The fractions' DPPH radical scavenging effect was in the order *P. americana*>*B. pinnatum*> tannic acid. Tannic acid effect on the radicals could not match the effectiveness of ethyl acetate fractions of *P. americana* and *B. pinnatum.* Comparative analysis of evaluated IC_{50} values indicates that *P. americana* was 97.39% more effective, while *B. pinnatum* was 96.78% more effective than tannic acid. DPPH radical scavenging is an index of free radical scavenging ability of compounds.[48,45]

However, the inhibition of the extracts on *in vitro* H_2O_2-induced lipid peroxidation of rat stomach homogenates indicated a strong inhibitory effect of *B. pinnatum* ethyl acetate fraction on lipid peroxidation than tannic acid and *P. americana* fraction. This result corroborates with the earlier findings on the hydroxyl radical scavenging effect of *B. pinnatum*. The fractions of *P. americana* and *B. pinnatum* inhibitory effects were largely logistic (LDR a,b,c) and LDR a,b,c,d respectively; while the effect of tannic acid was sigmoidal (sigmoid a,b,c).

Furthermore, reducing power studies indicate a strong reducing effect of our extracts on ferric ions, reducing Fe^{2+} to Fe^{3+}. Although ascorbic acid was most effective, our plant fractions were appreciably effective. A strong reducing power is a significant indicator of a good primary or secondary antioxidative property, mediated via electron donation and reduction of oxidized reactive intermediates in free radical pathways.[49] Also, the total antioxidant capacity assay shows that the *P. americana* seed fraction possesses a higher antioxidant capacity than the *B. pinnatum* leaf fraction. Total antioxidant capacity is also a strong indication of the electron-donating ability of the extracts. These suggest that the extracts may be able to transform free radical species into stable products and protect cells from oxidative damage.

Plants' secondary metabolites like flavonoids, phenolic acids, tannin, and saponins are rich in complex aromatic ring(s) containing valence electrons and oxidizable hydrogen atoms responsible for their antioxidant properties.[45] Phenolics form a large portion of exogenous antioxidants and show stronger antiradical activity than some vitamins and carotenoids.[50] Other studies have also shown that different solvent fractions of plants are rich in flavonoids such as catechin, rutin, quercetin, kaempferol, and isorhemnetin.[51] The observed radical scavenging potentials of our plants' fractions may be accounted for by a rich presence of a battery of phytochemicals or other groups in the plant fractions which may be acting in synergy to achieve a good scavenging response. The antioxidants effect of phenolic compounds is mediated through inhibition of lipid/protein oxidation reaction or by binding to the proteins.[52] They have also been associated with free radical scavenging effects.[53] The antiradical mechanism may be attributed to the ability of phenolic compounds to bind to proteins or form complexes that may act as radical scavengers, and their ability to act as proton sinks.[54,55]Some transition metals ions($Cu^{+}$and Fe^{2+}) act as pro-oxidants; and flavonoids may exert antioxidant activity by chelating these metal ions involved in initiating free radical formation.[56,57,6] Flavonoids also directly attenuate radical species by disrupting radicals chain reactions;[53] others are involved in the regeneration of tocopheryl radicals, oxidized glutathione, and ascorbyl radical to α-tocopherol, reduced glutathione and ascorbate monoanion.[54,55] The metal chelating ability of phenolic compounds are important in preventing unwanted free radical-mediated damage.[58]

Table 3: Reducing power activity of ethyl acetate fraction of *P. americana* seed, *B. pinnatum* leaf extract, and ascorbic acid standard.

Extracts	Reducing Power ($RP_{0.5}$) (μg/mL)	Mathematical model	R^2
P. Americana extract	460.37 ± 23.02	Sigmoid a,b,c	0.985
B. pinnatum extract	337.22 ± 26.98	Sigmoid a,b,c	0.943
Ascorbic acid	21.05 ± 0.84	Sigmoid a,b,c	0.965

Values are mean ± standard deviation of three determinations

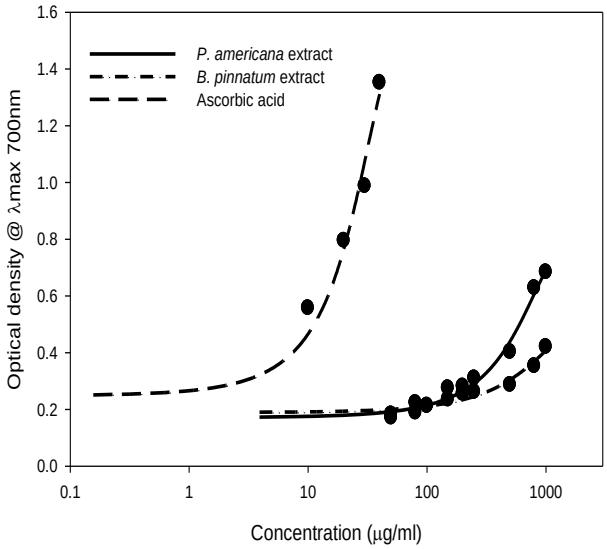

Figure 2: Reducing power activity of ethyl acetate fraction of *P. americana* seed ethanol extract, *B. pinnatum* leaf ethanol extract, and ascorbic acid standard

Conclusion

The present study has assessed the antioxidative and free radical scavenging properties of ethyl acetate fractions of *P. americana* seed and *B. pinnatum* leaf. The findings of this study reveal that *P. americana* seed and *B. pinnatum* leaf ethyl acetate fractions were appreciably effective at scavenging nitric oxide, hydroxyl, and DPPH radicals. They also showed good reducing power and antioxidative properties which were comparable and in some cases more potent than the standards used in the study. This potent radical scavenging effect may be responsible for their wide application in ethnomedical practice.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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