GVU JOURNAL OF SCIENCE, HEALTH AND TECHNOLOGY
Vol. 9(1), 2024; 62-69

p-ISSN: 2536-6866
e-ISSN: 2659-1529
ISAH et al. (2024)

Phytochemical screening and antioxidant investigation of *Persea americana*

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Abstract: This work investigated the phytochemical and antioxidant properties of aqueous and ethanol extract of the leaves, stem bark, seeds, and roots of *Persea americana*. The results of the qualitative phytochemical screening showed the presence of alkaloids, saponins, tannins, phenols, steroids, terpenoids, and flavonoids. Antioxidant activities were assayed using 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical scavenging capacity, reductive potentials, and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging. Results obtained from 15μg/ml of all extracts had the least percentage DPPH radicals, the values of DPPH for the leaves, stem bark, seeds, and root were 14.59±0.21, 6.24±0.04, 3.53±0.12, 9.75±0.17, and 1.66±0.09 respectively. The values of percentage DPPH radical scavenging increased with extract concentration. Just like DPPH radical scavenging, reducing power also increases with the concentration of extract. However, the leaves showed a higher percentage reductive power. The half maximal Inhibitory Concentration (IC50) and the maximal Effective Concentration (EC50) of extracts revealed that percentage values of ABTS were higher than those of DPPH in all samples. The EC50 of the reducing power showed variation in values, however, *P. americana* seeds sample had the highest value for reducing power (33.12 ± 0.01).

Keywords: Alkaloids, Antioxidant, inhibitory, macerated, medicinal plant, phytochemical

Introduction

Medicinal plants have continued to attract attention in the global search for effective antimicrobial agents that can combat resistant pathogens that have been rendering many conventional drugs obsolete in the treatment of infections. Many important drugs used in medicine today are directly or indirectly derived from plants. The most important of these bioactive constituents of plants are alkaloids, tannins, steroids, terpenoids and phenolic compounds.

Phytochemicals are biologically active, naturally occurring chemical compounds found in all the parts of plants. They protect plants from various diseases and contribute to plants' aroma, flavour and colour. In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as sources of medicinal agents. It is anticipated that phytochemicals with adequate antimicrobial efficacy will thus be used for the treatment of bacterial infections. Despite abundant literature on the antimicrobial properties of plant extracts, most of the plant-derived chemicals have not successfully been exploited for clinical use as antibiotics. A significant part of the chemical diversity produced by plants is thought to protect plants against microbial pathogens.

Persea americana is one of the 150 varieties of avocado pear. It is widely cultivated in tropical and subtropical regions of West Africa. It grows into a tree with a height of about 80 feet. It has leathery, evergreen leaves, and its flowers are rarely unisexual. The seed of *Persea americana* has diverse applications in ethnomedicine, ranging from treatment of diarrhea, dysentery, toothache, and intestinal parasites to skin treatment and beautification. The leaves have been reported to possess anti-inflammatory and analgesic activities. The seeds are rich in tannins, carotenoids, and tocopherols. The fruit has been shown to inhibit the in-vitro growth of prostate cancer cell lines, and "persin" from avocado leaves has been shown to have antifungal properties and to be toxic to silkworms. The effect of *P. americana* extract was evaluated on in-vitro rat lymphocyte proliferation. Antioxidant activity and phenolic content of seeds of avocado pear were found to be greater than 70

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Figure 1: MAP SHOWING THE LOCATION OF IKABIGBO COMMUNITY

In this study, the phytochemical and antioxidant activity of extracts of *P. americana* tree was carried out with a view to provide more scientific basis on the claim by traditional healers of its uses in traditional medicine in the context of the continued search for active therapeutic agents from plants.

Materials and Methods

Location

MAP SHOWING THE LOCATION OF IKABIGBO COMMUNITY

Sample Collection/Identification

Samples of the leaves, seed, stem, and root of *Persea americana* were obtained from a farm settlement in Ikabigbo, Uzairue, Etsako West Local Government Area of Edo state, Nigeria. The samples were authenticated in the herbarium laboratory of Botany Department, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The samples were rinsed with distilled water, air-dried in the laboratory for about a month, and then pulverized with a ceramic mortar and pestle.

Preparation of Extracts

Pulverized leaves, seed, stem, and root were extracted using 80% ethanol and distilled water as solvents as follows: each of the pulverized samples was put in glass containers and the ethanol was added to each container; the same setup was repeated with distilled water as solvent for the aqueous extract. The setup was left for about 72 hours and agitated 6-hourly for the extraction of the bioactive components into the solvent. The solution was filtered with muslin cloth to remove the supernatant, and the leaves, seed, stem, and root shafts remaining as residues. The crude extract was obtained by evaporating the solvent using a rotary evaporator. The extracts obtained were then transferred into airtight glass containers for further analysis. Both the ethanolic and aqueous crude extracts were analyzed for phytochemical screening while only the aqueous extracts were investigated for antioxidant properties.

Phytochemical Screening

The preliminary phytochemical screening of the ethanolic and aqueous extracts of leaves, seed, stem, and root of *P. americana* were carried out in order to ascertain the presence of its phytoconstituents by utilizing standard conventional protocols. The extracts were screened for the presence of alkaloids, saponins, tannins, phenolics, steroids, terpenes, flavonoids, and cardiac glycosides as reported by Irabor et al.

Antioxidant Activity

Aqueous extracts of the plant parts were utilized for the antioxidants assay.

1,1-Diphenyl-2-PicrylHydrazyl (DPPH) Assay

The antioxidant activity by DPPH assay was carried out as described by Molyneux, using the stable free radical DPPH. To 1 ml of various concentrations of the extract, 1 ml of 0.1 mM DPPH was added to the test tube. Ascorbic acid was used as the standard for comparison. After incubation for 30 mins in the dark at room temperature, absorbance was read at 517 nm. The percent DPPH radical scavenging was calculated with the equation below and the experiment was carried out in triplicate.

Reductive Potential (Ferric cyanide (Fe^{3+}) Reducing Antioxidant Power Assay)

Reducing power of the extracts was measured by the direct reduction of $Fe^{3+}(CN)_6$ to $Fe^{2+}(CN)_6$ and was determined by absorbance measurement of the formation of the Perl's Prussian Blue complex following the addition of excess Fe^{3+}, as described previously. Different concentrations of extracts in 0.5 mL of distilled water were mixed with 1.25 mL of 0.2 M, pH 6.6 sodium phosphate buffer and 1.25 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50 °C for 20 min. After

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20 min incubation, the reaction mixture was acidified with 1.25 mL of trichloroacetic acid (10%). Finally, 0.5 mL of $FeCl_3$ (0.1%) was added to this solution and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates greater reducing capability.

2,2-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) Radical Scavenging Assay

The scavenging activity of extracts against ABTS radical was determined by following the method described by Roberta et al. with little modification. Briefly, the mixture of stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate ($K_2S_2O_8$) in equal volumes were allowed to stand in the dark for 12-16 h at room temperature. Prior to assay, ABTS solution was diluted in ethanol to give an absorbance of 0.700 ± 0.02 at 734 nm. 2800 l of the resulting solutions was allowed to react with 200 l of the plant extracts with different concentrations. The reaction mixture was incubated at room temperature for 30 min and absorbance was read at 734 nm. The same was done for the ascorbic acid standard of various concentrations (15 – 150 g/ml).

Statistical Analysis

All assays were carried out in triplicates and results were expressed as mean ± SEM. The data was subjected to one-way analysis of variance (ANOVA) and the differences between various concentrations were determined by Tukey's multiple comparisons test using GraphPad Prism 8.0 statistical. The P values of i 0.05 were considered significant. The IC_{50} and EC_{50} values were estimated by nonlinear curve-fitting and presented as their respective 95% confidence limits.

1 Results and Discussion

The phytochemical screening of *P. americana* revealed the presence of some of the phytochemicals screened and are shown in Table 1. The phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, terpenes and steroids, phenolics and tannins. Saponins were conspicuously absent in the aqueous extract of *P. americana* seed. Terpenoids and steroids are absent in ethanol extracts of *P. americana* stem; this is in agreement with Doughari (2012). Also, the absence of alkaloids was recorded in the aqueous extract of *P. americana* leaves.

These phytochemicals are known to support bioactive activities in medicinal plants and may therefore be responsible for the antioxidant properties of the leaves, seed, stem, and root. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [**?**]. Tannins are generally known to be useful in the treatment of inflamed and ulcerated tissues and have remarkable activity in cancer prevention [**?**]. Thus, the presence of these phytochemical constituents in *P. americana* may justify the plant's inclusion in folk preparation of the popular antimalarial potion, "Agbo" in the west and central African region, particularly in Nigeria.

The antioxidant properties analyzed were assessed with regard to their antioxidant activity as typified by their reducing power and free radical scavenging ability. Data obtained showed that *P. americana* possessed strong antioxidant activities, although the ranking of antioxidant activity of samples may vary with the analytical methods because of different mechanisms for each analysis. Hence the choice of DPPH and ABTS radical scavenging activity in this research work is due to the fact that they are the most widely used and reliable methods for screening the antioxidant activity of plant extracts.

The DPPH radical scavenging of the studied plant leaves, seed, stem, and root extracts showed a good scavenging activity as shown in Table 2. Figure 1 compares the DPPH radical scavenging activities of *P. americana* root with standard ascorbic acid. *P. americana* exhibited high radical scavenging activities at 30 to 150 μg/ml and it was revealed that extracts were dose-dependent. Figure 2 shows DPPH radical scavenging activity of ascorbic acid and extracts of *P. americana* stem at varying concentrations. Figure 3 compares the DPPH radical scavenging activities of *P. americana* root with standard ascorbic acid. Figure 4 compares the DPPH radical scavenging activities of *P. americana* leaves with ascorbic acid.

There was a statistically significant difference between extracts and ascorbic acid used as standard (pi0.05). The scavenging effects of all extracts at a concentration of 150 μg/ml were 34.84 – 39.90%. The scavenging activity was not as good for the *P. americana* (17.43 ± 0.56% for the seed, 25.27 ± 0.39% for the leaves, 38.03 ± 0.36% for the stem, and 40.86% for the root) as compared to ascorbic acid. DPPH radical scavenging activities of the extracts were dose-dependent; the radical scavenging activities increased with concentrations of extracts.

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Table 1: Table 1: Phytochemicals in aqueous and ethanolic extracts of *Persea americana*

Phytochemicals	P. americana aqueous extract				P. americana ethanolic extract			
	Seed	leaves	stem	Root	Seed	leaves	stem	Root
Alkaloids	+	-	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Saponins	-	+	+	+	+	+	+	+
Terpene/steroids	+	+	+	+	+	+	-	+
Phenolics	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+

KEY: + = present, – = absent

Table 2: **Table 2:** DPPH radical scavenging (%) of ascorbic acid and extracts of leaves, seed, stem, and root of *P. americana* at varying concentrations

Extracts (μg/ml)	Ascorbic acid	Leaves	Seed	Stem	Root
15	14.59 ± 0.62	8.65 ± 0.07	7.12 ± 0.33	2.21 ± 0.29	5.46 ± 0.21
30	31.26 ± 0.33	8.96 ± 0.07	15.21 ± 0.36	10.71 ± 0.13	13.58 ± 0.26
60	41.26 ± 2.85	27.38 ± 0.49	19.09 ± 0.09	13.33 ± 0.21	19.43 ± 0.19
90	62.92 ± 1.46	32.11 ± 0.45	24.19 ± 0.31	20.90 ± 0.15	20.36 ± 0.60
120	77.09 ± 0.24	34.64 ± 0.04	26.42 ± 0.12	27.30 ± 0.11	24.35 ± 0.21
150	90.91 ± 0.15	39.90 ± 0.39	40.21 ± 0.55	36.85 ± 0.15	34.84 ± 0.12

(All values are expressed as mean ± standard error of three replicates. Values in the same range along the same row are not significantly different (pi0.05).)

Results obtained from DPPH radicals scavenging suggest that DPPH radical was significantly bound by the extracts of leaves of *P. americana* (with IC50 value of 44.25 ± 1.59 μg/ml). These values of extract scavenging abilities are comparable to that of ascorbic acid (with IC50 value of 37.57 ± 0.22 μg/ml) as shown in Table 5, showing no significant difference (Pi0.05).

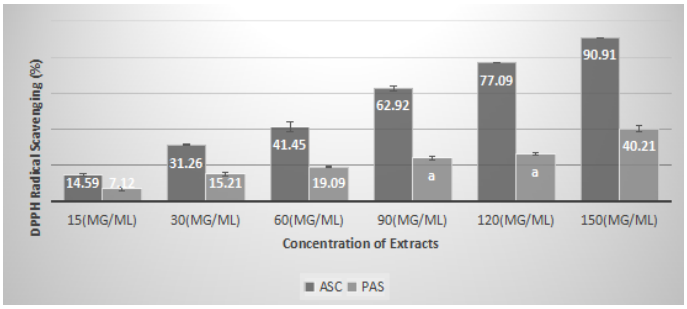

Figure 2: DPPH radical scavenging activity of ascorbic acid and extracts of *P. americana* seed at different concentrations. ASC, ascorbic acid; PAS, *P. americana* seed. (The bars represent mean ± SEM (n = 3), bars with the same alphabet along the same group are not statistically different pi0.05).

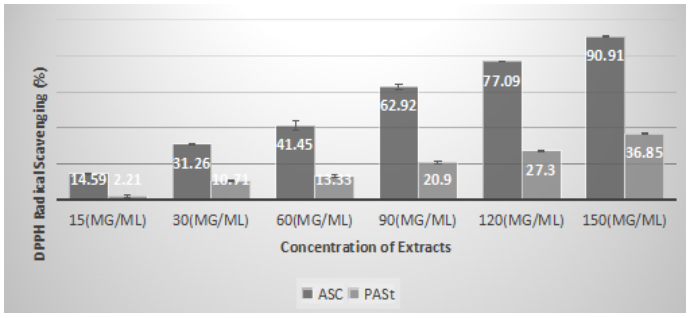

Figure 3: DPPH radical scavenging activity of ascorbic acid and extracts of *P. americana* stem at varying concentrations. ASC, ascorbic acid; PASt, *P. americana* stem. (The bars represent mean ± SEM (n = 3), bars along the same group are significantly different p<0.05).

Results for the ABTS assay are presented in Table 4, and the inhibiting power was found to be dosage-dependent. Figure 5 compares the ABTS radical scavenging activities of *P. americana* leaves and ascorbic acid. Figure 6 compares the ABTS radical scavenging activities of *P. americana* seed and ascorbic acid. Figure 7 shows ABTS radical scavenging activity of extracts of *P. americana* stem at

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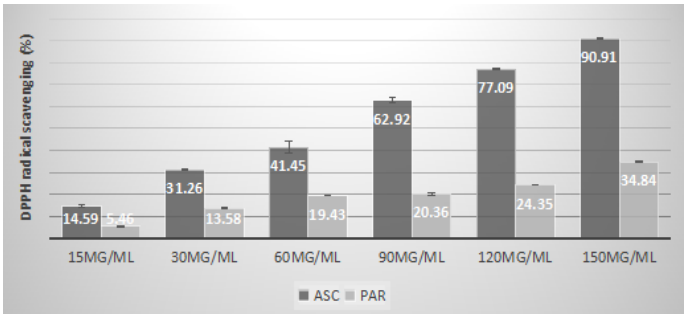

Figure 4: DPPH radical scavenging activity of ascorbic acid and extracts of *P. americana* root at different concentrations. ASC, ascorbic acid; PAR, *P. americana* root. (The bars represent mean ± SEM (n = 3), bars with the same alphabet along the same group are not significantly different p<0.05).

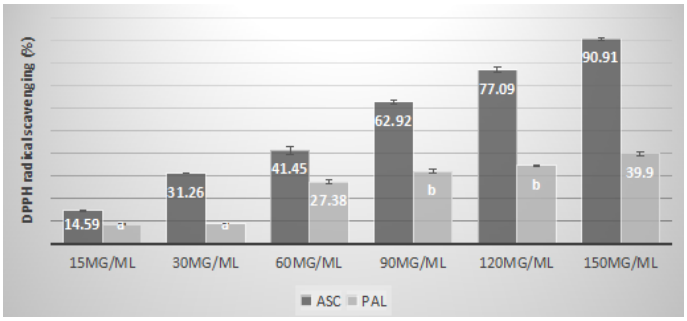

Figure 5: DPPH radical scavenging activity of ascorbic acid and extracts of leaves of *P. americana* at different concentrations. ASC, ascorbic acid; PAL, *P. americana* leaves. (The bars represent mean ± SEM (n = 3), bars with the same alphabet along the same group are not significantly different p<0.05).

varying concentrations. Figure 8 compares the ABTS radical scavenging activities of *P. americana* root with "standard" ascorbic acid.

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and chain-breaking antioxidants. The extracts of *P. americana* effectively scavenged ABTS radicals generated by the reaction between 2,2-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) ammonium persulphate. Table 4 shows the activity of extracts of the plant parts, and the ascorbic acid standard was found to increase in a dose-dependent manner from 2.68 to 52.27% (*P. americana* leaves), 6.69 to 17.43% (*P. americana* seed), 6.39 to 38.03% (*P. americana* stem), 2.14 to 40.86% (*P. americana* root), and 17.33 to 98.58% (ascorbic acid standard) at different concentrations from 15 to 150μg/ml. The extracts of *P. americana* seed, *P. americana* leaves, and the standard ascorbic acid exhibited comparative IC50 values of 55.68μg/ml, 78.68μg/ml, and 55.42μg/ml respectively. Therefore, the recorded IC50 values for the extracts of the plant indicated their ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.

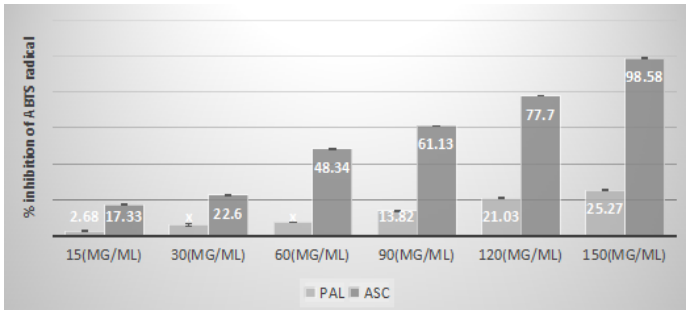

Figure 6: ABTS radical scavenging activity of ascorbic acid and extracts of leaves of *P. americana* at different concentrations. ASC, ascorbic acid; PAL, *P. americana* leaves. (The bars represent mean ± SEM (n = 3), bars with the same alphabet along the same group are not significantly different, pi0.05).

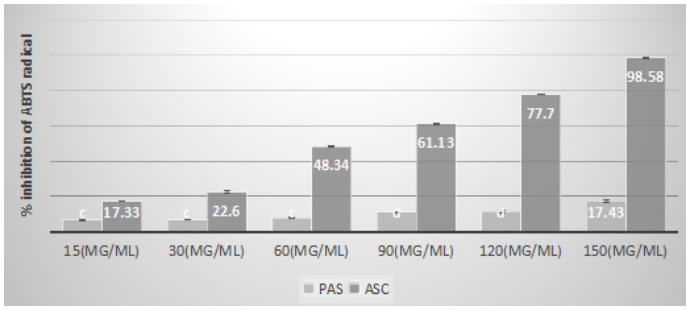

Figure 7: ABTS radical scavenging activity of ascorbic acid and extracts of *P. americana* seed at different concentrations. ASC, ascorbic acid; PAS, *P. americana* seed. (The bars represent mean ± SEM (n = 3), bars with the same alphabet along the same group are not significantly different, pi0.05).

The results of the reducing power assay of *P. americana* extracts are summarized in Table 4. Figure 9 compares the reducing power of leaves of *P. americana* with ascorbic acid used as a standard. Increase in absorbance indicates an increase in reducing power. Figure 10 compares the reducing power of seed of *P. americana* with ascorbic acid used

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Table 3: ABTS radical scavenging (%) of extracts of leaves, seed, stem, and root of *Persea americana*

Extracts (μg/ml)	Ascorbic acid	Leaves	Seed	Stem	Root
15	17.33 ± 0.25	2.68 ± 0.19	6.69 ± 0.31	6.39 ± 0.24	2.14 ± 0.27
30	22.60 ± 0.54	6.09 ± 0.47	6.68 ± 0.10	9.23 ± 0.53	4.32 ± 0.36
60	48.34 ± 0.40	7.62 ± 0.10	7.86 ± 0.38	10.15 ± 0.34	7.79 ± 0.27
90	61.13 ± 0.17	13.82 ± 0.56	11.28 ± 0.28	21.31 ± 1.37	14.04 ± 0.08
120	77.70 ± 0.18	21.03 ± 0.39	11.44 ± 0.54	26.83 ± 0.68	29.31 ± 0.27
150	98.58 ± 0.24	25.27 ± 0.39	17.43 ± 0.56	38.03 ± 0.36	40.86 ± 0.51

(All values are expressed as mean ± standard error of three replicates. Values in the same range along the same row are not significantly different (p<0.05).)

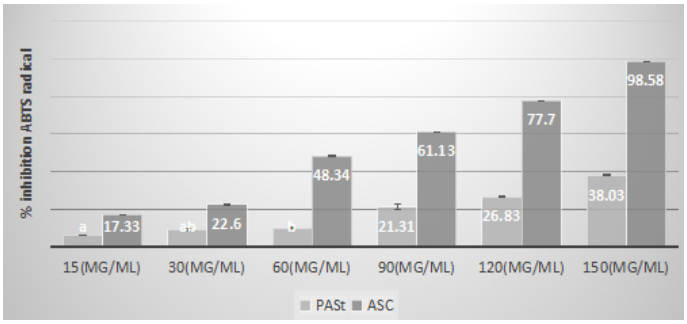

Figure 8: ABTS radical scavenging activity of ascorbic acid and extracts of *P. americana* stem at different concentrations. ASC, ascorbic acid; PASt, *P. americana* stem. (The bars represent mean ± SEM (n = 3), bars with the same alphabet along the same group are not significantly different, pi0.05).

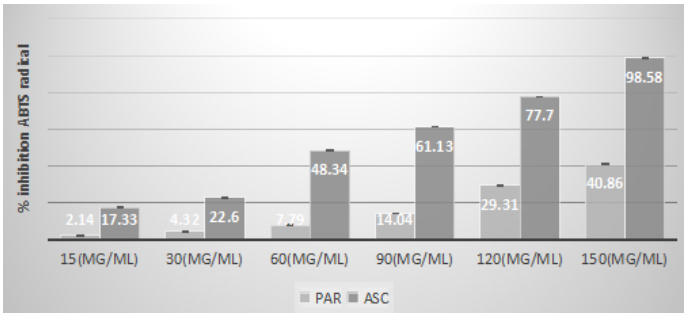

Figure 9: ABTS radical scavenging activity of ascorbic acid and extracts of *P. americana* root at different concentrations. ASC, ascorbic acid; PAR, *P. americana* root. (The bars represent mean ± SEM (n = 3), bars with the same alphabet along the same group are not significantly different, pi0.05).

as a standard. Increase in absorbance indicates an increase in reducing power. Extracts and the standard are dose-dependent. Figure 11 shows the reducing power of root of *P. americana* and ascorbic acid used as a standard. Increase in absorbance indicates an increase in reducing power. Figure 12 shows the reducing power of stem of *P. americana* and ascorbic acid used as a standard. Increase in absorbance indicates an increase in reducing power.

High absorbance depicts high reducing power. The data obtained in this study showed that all the extracts are dose dependent; that is, absorbance increased with concentration. Among the tested extracts, *P. americana* leaves showed the highest reducing power (EC50 = 19.58 ± 0.10 μg/ml). This value was even higher than that of ascorbic acid (EC50 = 21.99 ± 0.12 μg/ml). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing properties are generally associated with the antioxidant action of reductones. Gordon (1990) reported that the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen ion. The reduction of ferrous iron (Fe^{3+}) to ferric iron (Fe^{2+}) is measured by the intensity of the resultant blue-green solution which absorbs at 700 nm. The results linked the marked ferric reducing power activity of the extract to the presence of polyphenols, which may act similarly to reductones by donating electrons and reacting with free radicals to convert them into more stable products and terminate free radical chain reactions (Muanya, 2007).

Conclusion

In the present study, the aqueous extracts of leaves, seed, stem, and root of *P. americana* were found to contain phytochemicals, as revealed in both aqueous and ethanolic extracts. Antioxidant and radical scavenging activities, as assessed through various models including DPPH, ABTS, and reducing power assays, demonstrated that the plant parts analyzed

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Table 4: **Reducing power of extracts of leaves, seed, stem, and root of *P. americana* at varying concentrations**

Extracts (μg/ml)	Ascorbic acid	Leaves	Seed	Stem	Root
15	0.075 ± 0.002	0.018 ± 0.002	0.006 ± 0.001	0.024 ± 0.001	0.013 ± 0.001
30	0.157 ± 0.004	0.054 ± 0.001	0.016 ± 0.004	0.036 ± 0.005	0.017 ± 0.003
60	0.352 ± 0.001	0.117 ± 0.018	0.024 ± 0.003	0.094 ± 0.004	0.050 ± 0.002
90	0.483 ± 0.006	0.172 ± 0.015	0.067 ± 0.005	0.142 ± 0.003	0.090 ± 0.011
120	0.647 ± 0.004	0.174 ± 0.016	0.095 ± 0.003	0.174 ± 0.002	0.114 ± 0.009
150	0.912 ± 0.007	0.269 ± 0.001	0.290 ± 0.008	0.241 ± 0.010	0.137 ± 0.004

(All values are expressed as mean ± standard error of three replicates. Values in the same range along the same row are not significantly different (p<0.05).)

Table 5: IC50 and EC50 of leaves, seed, stem, and root extracts of *P. americana*.

Samples	**DPPH (IC50 (μg/ml))**	**ABTS (IC50 (μg/ml))**	**Reducing Power (EC50 (μg/ml))**
AAC	37.57 ± 0.22	55.42 ± 1.06	21.99 ± 0.12
PAL	44.25 ± 1.59	78.68 ± 2.29	19.58 ± 0.10
PAS	62.34 ± 5.73	55.68 ± 7.42	33.12 ± 0.01
PASt	66.98 ± 0.64	88.16 ± 7.01	21.63 ± 0.45
PAR	50.08 ± 4.53	97.58 ± 0.55	20.65 ± 0.86

(Values are expressed as mean ± standard error of three replicates. Values were tested by one-way ANOVA followed by Tukey's multiple comparisons test. Values with the same subset in a group are not significantly different (pi0.05). Key: PAL (P. americana leaves), PAS (P. americana seed), PASt (P. americana stem), and PAR (P. americana root).)

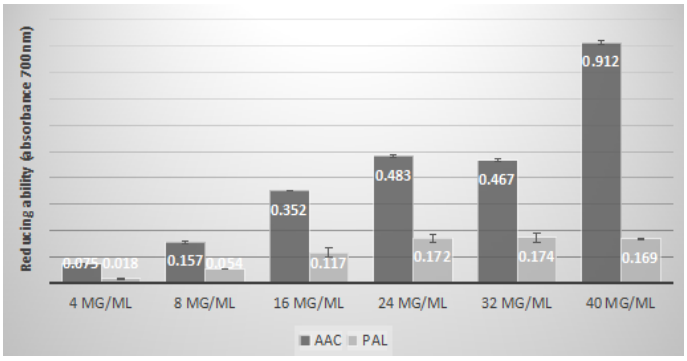

Figure 10: Reducing power of extracts of leaves of ascorbic acid and *P. americana* at different concentrations. AAC, ascorbic acid; PAL, *P. americana* leaves. (The bars represent mean ± standard deviation of three replicate values)

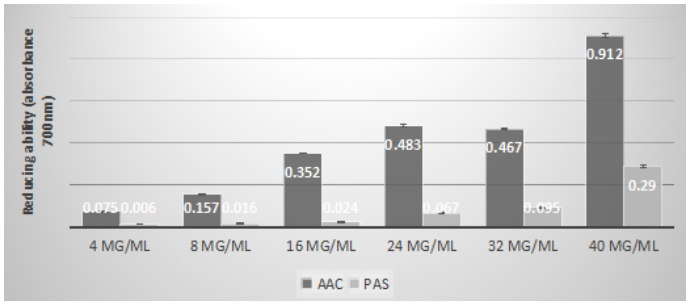

Figure 11: Reducing power of extracts of seed of ascorbic acid and *P. americana* at different concentrations. AAC, ascorbic acid; PAS, *P. americana* seed. (The bars represent mean ± standard deviation of three replicate values)

have significant potential in radical scavenging compared to ascorbic acid, a standard antioxidant. This supports the use of these plant parts in modern traditional medicine. The plant may also enhance the nutritional value of various foods.

All extracts exhibited concentration-dependent antioxidant and radical scavenging activities. Notably, the extracts of leaves and seeds showed the highest ability to quench DPPH radicals and scavenge ABTS radicals. In general, the leaves, seed, and stem of *P. americana* exhibit promising antioxidant effects that warrant further investigation for potential therapeutic applications. Adeyemi, O. O., Okpo, S. O., Ogunti, O. O. (2002). Analgesic and anti-inflammatory effect of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). *Fitoterapia, 73*, 375-380.

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e-ISSN: 2659-1529
ISAH et al. (2024)

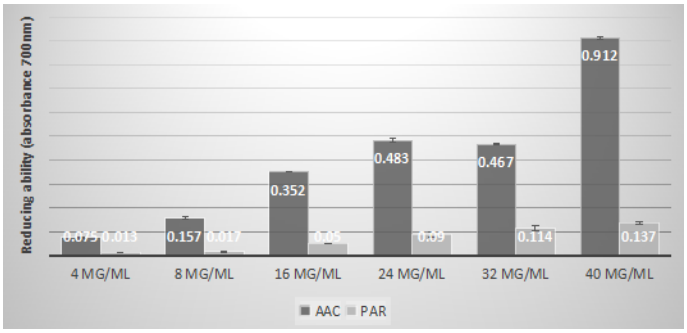

Figure 12: Reducing power of extracts of root of ascorbic acid and *P. americana* at different concentrations. AAC, ascorbic acid; PAR, *P. americana* root. (The bars represent mean ± standard deviation of three replicate values)

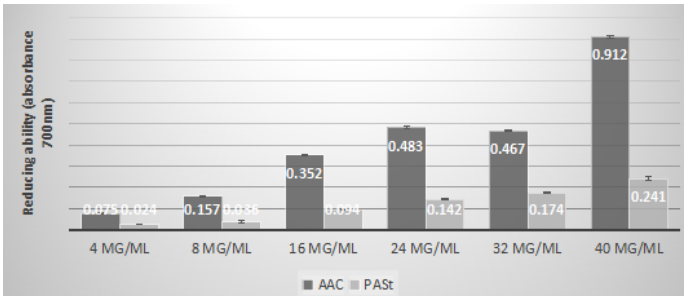

Figure 13: Reducing power of extracts of stem of ascorbic acid and *P. americana* at different concentrations. AAC, ascorbic acid; PASt, *P. americana* stem. (The bars represent mean ± standard deviation of three replicate values)

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