Trop J Nat Prod Res, November 2021; 5(11):2037-2042 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phenolic Profile and *In Vitro* Antioxidant Potential of Four Medicinal Plants

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ARTICLE INFO

Article history:
Received 18 September 2021
Revised 22 October 2021
Accepted 04 November 2021
Published online 05 December 2021

ABSTRACT

Antioxidants of plant origin reduce or inhibit oxidative stress-induced tissue damage in gastric ulcers. Plants' edible and non-edible parts have been investigated as potential sources of natural antioxidants. The present study aims at investigating the antioxidant potentials of aqueous extracts of Irish potato tubers, Avocado pear seeds, and unripe pawpaw and plantain fruits used traditionally in ulcer treatment. *In vitro* anti-oxidative potential of the plants was assessed using their inhibitory effects on H_2O_2-induced lipid peroxidation, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl free radicals generation. The plants' phenolic profile and total antioxidant capacity (TCA) were assessed using gas chromatography equipped with flame ionization detector and phosphomolybdenum method respectively. Total phenolic compounds content ranged from 598.80 µg/g in Irish potato through 171.44 µg/g and 123.11 µg/g in avocado pear and pawpaw respectively to 77.14 µg/g in the plantain. Irish potato (149 ± 13.02 mg AAE/g) and avocado pear (146.60 ± 4.53 mg AAE/g) had significantly ($p<0.05$) higher TCA than pawpaw (53.31 ± 7.16 mg AAE/g) and plantain (35.55 ± 3.13 mg AAE/g). Generally, Irish potato and avocado pear had the lowest values of 50% inhibitory concentrations (IC_{50}) against OH free radicals and lipid peroxidation, while Irish potato and pawpaw had the lowest IC_{50} values against DPPH radical. Irish potato and avocado pear were observed to have highest phenolic compounds distribution and TCA values, which translated to their higher *in vitro* antioxidant potency. The observed rich TCA and phenolic contents, and high radical scavenging abilities showed that the plants possess potent antioxidant properties.

***Keywords*:** Lipid peroxidation, Irish potato, Avocado pear, Pawpaw, Plantain, Gastric ulcer.

Introduction

The existence of free radicals generated from normal and abnormal aerobic metabolic reactions of the body has remained a challenge to the healthy wellbeing of humans. This is because free radicals contain highly reactive unpaired electron species that can elicit cell damage.[1] Reactive nitrogen and oxygen species induced by oxygen and nitrogen radicals respectively, and other free radical-facilitated reactions have been associated with lipid peroxidation leading to some degenerative or pathological processes. Lipid peroxidation is the oxidative degradation of membrane lipids. Oxidative stress-induced tissue damage is implicated as a cause and consequence of aging, coronary heart disease, diabetes, cancer, gastric ulcer, and many other human diseases.[2-4] It has been suggested that antioxidants use is the most effective means of neutralizing and or reducing free radical generation and its effect.[2]

Antioxidants are a collection of compounds with the potential to delay, reduce, or inhibit oxidative reactions of biological macromolecules.[5] Antioxidants can be obtained naturally via diet, endogenously, and synthetically. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are known antioxidants of synthetic origin. They are suspected to have mutagenic or toxic effects.[5] Thus, the search for naturally derived antioxidants has continued to increase.

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Citation Igwe CU, Onyeagoro EE, Morah AC, Nwaogu LA, Iheme CI, Onwuliri VA. Phenolic Profile and *In Vitro* Antioxidant Potential of Four Medicinal Plants. Trop J Nat Prod Res. 2021; 5(11):2037-2042. doi.org/10.26538/tjnpr/v5i11.24

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Polyphenols and vitamin contents of vegetables and fruits have been suggested as the basis for the antioxidant activity of many plants. Among these, the polyphenols were proposed to elicit the highest antioxidant activity levels.[6] Evaluation of antioxidant activities of microalgae, medicinal plants, spices, fruits, and vegetables has indicated that many of them are rich in natural antioxidants.[7,8] Epidemiological studies have shown that fruits, vegetables, and less processed staple foods offer the best protection against the development of oxidative stress-induced diseases.[5,6]

Stomach ulcers occur when stomach acid erodes the lining of the digestive tract.[9] Common causes include the effects of the bacteria *Helicobacter pylori*, anti-inflammatory pain relievers, histamines, oxidative stress, and other stress factors.[8] Treatment of proven uncomplicated peptic ulcer disease has changed dramatically in recent years. Discontinuation of the use of non-steroidal anti-inflammatory drugs (NSAIDs) and treatment of infections due to *H. pylori* are key elements for the successful management of the disease. However, the complete cure of the disease has remained prolonged and elusive. A recent screening of plants for potential anti-ulcer activity has revealed many components including flavonoids and phenolic compounds with ulcer healing and anti-ulcer properties.[5] Thus, the current research was designed to assess the *in vitro* anti-oxidative potentials of *Solanum tuberosum* (Irish potato) tubers, *Persea americana* (avocado pear) seeds, unripe *Carica papaya* (pawpaw), and unripe *Musa paradisiaca* (plantain) fruits, commonly used in southern Nigeria for the management of suspected ulcers of the stomach. Earlier studies have shown that fresh or dried parts of these plants are used individually but mostly in duplicate or triplicate combinations soaked and/or boiled in water or soaked in alcohol for peptic ulcer treatment.[10] Kayode and his colleagues reported that among 62 plant species implicated in the treatment of gastric ulcers in Nigeria, that *Carica papaya* and *Musa paradisiaca* had the highest prevalence rates (40% each) of use.[10]

The unripe fruit of *Carica papaya* (pawpaw) is employed in the treatment of several ailments by traditional medical practitioners. Areas of application reported included but were not limited to, the

treatment of sore throat, asthma, sickle cell anaemia, wounds, ulcers, boils, malaria, fever, pain, tonsillitis, indigestion, dyspepsia, jaundice, and cancer.[11] Intake of unripe pawpaw extract has been linked with the anti-ulcer effect.[12] Similarly, unripe *Musa paradisiaca* (plantain) fruit is reported to be among the traditional herbal remedies for peptic ulcer disease. A natural flavonoid from unripe plantain pulp, leucocyanidin, protects erosion of the gastric mucosa.[13] Dried unripe plantains have been shown to possess anti-ulcerogenic activity and an effective prophylactic and healing agents for the treatment of aspirin-induced ulcers.[14]

Persea americana (avocado pear) has several medicinal and health benefits.[15] The skin of the avocado is used as an antibiotic, for ridding the intestinal tract of parasites, and as a remedy for dysentery. In powdered form, the seeds have been used as a treatment for dandruff, while pieces of the seed are placed in tooth cavities as a toothache palliative. When the pulp of avocado is added to the diet, it helps to reduce cholesterol levels.[15] In the same vein, *Solanum tuberosum* (Irish potato) has been described to have stomach and intestines-soothing effects as well as anti-inflammatory activity.[16-17] These properties were attributed to potatoes due to their rich content of potassium, magnesium, and vitamins such as beta-carotene, thiamine, riboflavin, niacin, ascorbic acid, among others, which may have significant roles in the use of Irish potatoes in the treatment of stomach ulcers.[18] Furthermore, the roughage in potatoes has been suggested to reduce the chances of ulcers by prevention of constipation and acid formation. The reported anti-ulcerogenic effects of these plants coupled with their traditional use in stomach ulcer treatment motivated the present study. Thus, the study is aimed at assessing the antioxidant potentials of aqueous extracts of Irish potato tubers, Avocado pear seeds, unripe pawpaw, and unripe plantain fruits used traditionally in ulcer treatment.

Materials and Methods

Collection of Plant Samples

Freshly harvested tubers of Irish potato, seeds of avocado pear, and unripe fruits of pawpaw and plantain were purchased or sourced in August 2019 from Ihiagwa town, Owerri West Local Government Area of the State of Imo in Nigeria. Prof. J. C. Obiefuna, a plant taxonomist, of the Crop Science Department, Federal University of Technology Owerri (FUTO), Nigeria validated the plant samples. The plants' reference specimens with identification numbers IMSUH 0256 to 0259 respectively were deposited at the herbarium of Imo State University Owerri, Nigeria.

Each plant was first thoroughly washed under running water. The Irish potato tubers and unripe fruits of pawpaw and plantain were carefully peeled. The plant samples were sliced into tiny flat chips and oven-dried for 30 min at 60 ^{o}C to achieve fast and uniform drying to constant weights, and then milled into fine flours.

Extraction of Phenolic Compounds

Briefly, to 20 g of each plant sample in a labeled test tube, 10 ml of KOH (50 %m/v) and 15 ml ethanol were added. The reaction mixtures were incubated for 60 min in a temperature-controlled (60 ^{0}C) water bath, and then dispensed into a separatory funnel. Each reaction tube was sequentially and respectively washed with 20, 10, 10, and 3 ml of ethanol, cold water, hot water, and hexane. The extracts obtained were combined and then washed thrice with 10 ml of 10 % solution of ethanol in water. The resultant solution was dried with anhydrous sodium sulphate and the remaining solvent was removed completely by evaporation. The extract obtained was dissolved in pyridine (1000 µl), mixed and exactly 200 µl pipetted into an ampoule ready for quantification of phenolic compounds.

Quantitation of Phenolic Compounds

The quantitation of the plants' phenolic compounds was accomplished with the aid of a gas chromatograph equipped with a flame-ionization indicator. Separation was achieved with a RESTEK (MXT-1, 15 m x 250 µm x 0.15 µm) column at an injector temperature of 280 ^{0}C. The sample (2 µl) was injected at a velocity flow rate of 30 cm/s. The carrier gas, helium (5.0 pas) flowed at the rate of 3 ^{0}C min^{-1} for 5 minutes, while the flame-ionization detector functioned at 320 ^{0}C.

The concentrations (µg/g) of the phenolic compounds were resolved from the ratio of the mass and area of the internal standard to the area of the identified compounds.

Preparation of Plant Extracts for Antioxidant Analyses

Each sample (50 g) was weighed into a conical flask (50 mL), made up with distilled water, and labeled. A magnetic stirrer was used to strongly mix the solution and then kept at room temperature for 24 hours with intermittent stirring. Each extract was later subjected to two stages of filtration. Coarse filtration was carried out using a muslin sieve, whereas fine filtration was carried out with a Whatman filter. After filtration, the final extracts obtained were labeled and stored in a refrigerator at 4 ^{o}C for further analysis.

Total Antioxidant Capacity

The total antioxidant capacity (TAC) of the extracts was determined using the phosphomolybdenum method.[19] Briefly, 0.4 mL of each extract was pipetted into 4 mL of phosphomolybdenum reagent in a test tube. Each reaction tube was corked and allowed to stand for 90 min in a water bath at 95ºC. Thereafter, the solution was cooled and its absorbance read at 695 nm. A standard curve prepared with the ascorbic acid standard was used for estimation of each sample TAC and presented as mg equivalent of ascorbic acid (AAE) per gram of sample extract.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of the extracts was assessed as previously described.[20] Each sample extract was mixed in distilled water at concentrations of 0 - 2000 µg/mL, and then 0.75 mL of each sample solution was pipetted into 1.5 mL of 0.01 mg/mL methanol solution of DPPH (Fluka Chemie, Switzerland). The mixtures were incubated at room temperature for 15 minutes and their absorbance read at 517 nm against methanol blank. Catechin was used as standard.

Hydroxyl Radical Scavenging Activity

The potentials of the plants to scavenge for hydroxyl radical were determined by assessing the competition reactions between the plant extracts and deoxyribose for hydroxyl radicals produced by a Fe^{3+}/ascorbate/EDTA/H_2O_2 system.[21] Varying concentrations of each extract (0 – 3000 µg/ml) were added to a reagent mixture made up of deoxyribose (2.8 mM), KH_2PO_4/KOH buffer (20 mM, pH 7.4), H_2O_2 (1 mM) and 0.1 mM each of ascorbic acid, EDTA, and $FeCl_3$ (0.1 mM) to a final volume of 1.0 mL. Each reaction solution was incubated at 37**º**C for 1 hour, and the degradation of deoxyribose was assessed as thiobarbituric acid reactive substances (TBARS).[22] Briefly, a mixture of 20% acetic acid (1.5 mL; pH 3.5), 8.1% sodium dodecyl sulphate (0.2 mL) and 0.8% thiobarbituric acid (1.5 mL) was heated (100 ^{o}C) for 1 hour. Thereafter, the solution was allowed to cool and extra trichloroacetic acid (2 mL) was added. The solution mixture was thoroughly mixed, centrifuged for 10 min at 3000 g, and then the absorbance measured at 532 nm. The TBARS concentration was estimated with the aid of the molar extinction coefficient of malondialdehyde (MDA). The hydroxyl radical scavenging activity of each extract measured as the deoxyribose degradation inhibition was deduced as[23]:

$$\%OH\ Radical\ Scavenging\ = 100 - \frac{MDAtest}{MDAcontrol} \times 100$$

Lipid Peroxidation Inhibition Effect

Inhibition of lipid peroxidation ability of the plant extracts was evaluated via incubation of varying concentrations (0 - 2000 µg/mL) of the extracts in hydrogen peroxide (5 mM) treated homogenate of rabbit brain.[24]

The Biochemistry Departmental Ethics Committee, FUTO approved the study protocol with the ethical reference number FUTO/BCH/DEC/XXI/03/02. Utmost and humane care was fully adopted in the handling of the animals.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Phosphate-buffered saline (10 %w/v) was used to homogenize the whole rabbit brain. Mixtures of the homogenate (200 µL), plant extract (0 – 2000 µg/mL), and hydrogen peroxide (5 mM) were incubated for one hour, and the TBARS concentrations were measured as described above for hydroxyl radical scavenging activity.[22] The extent of TBARS formed upon treatment with the extract was deduced from the measure of the difference observed between the extract-treated test and the control, while inhibition of lipid peroxidation was deduced from:

$$\%\ Inhibition\ = 100 - \frac{Test}{Control} \times 100$$

Statistical Analysis

GraphPad Prism-based (version 5.3; USA) one-way analysis of variance (ANOVA) and Turkey posthoc test were used to analyze the data generated. Significant extrapolations were adjudged at a 95 % confidence level.

Results and Discussion

Ulceration in the stomach ensues due to perturbation in the normal mucosal stability instigated by either diminished resistance or enhanced aggression on the mucosa.[25] Most peptic ulcers occur in the duodenum, while gastric ulcers of the stomach are rare. The gastric mucosa is persistently exposed to many potentially harmful chemical and infectious agents. Common among these are acids, drugs, bile acids, pepsin, food ingredients, and microbial products from mainly *H. pylori.* The presence of these agents elicits a reduction in gastric motility and flow of blood in the gastric region, as well as increased production and secretion of pepsin and gastric acid. They do also cause inhibition of cell growth and proliferation as well as the diminished synthesis of prostaglandins.[26] Another cause of enhanced aggression leading to ulceration is oxidative damage elicited by free radicals. Aggressive free radical-induced gastric mucosal damage has been linked with an upsurge in oxidative stress.[27]

Hence, treatment of stomach ulcers with drugs is aimed at stimulation of mucosal defenses or blockage of aggressive factors associated with ulcer induction. In counteracting aggressive factors leading to ulcer, treatment over the years have included the use of antioxidants. Mechanism of antioxidants' actions has been reported to include scavenging of free radicals, metal catalysts chelation, antioxidant enzymes activation, tocopherol radicals reduction, and oxidases inhibition.[28,29] Antioxidants have also been suggested to inhibit oxidation-induced breakage of DNA strands. The presence and effect of reactive nitrogen and oxygen radical species have been correlated with DNA strand breakages leading to degenerative diseases. Plant compounds responsible for these antioxidant effects are mainly flavonoids, monophenols, and polyphenols, among others. [30] Some of which have been reported in appreciable quantities in the present plants under study.

The phenolic content of the studied plants ranged from a total of 598.80 µg/g in Irish potato through 171.44 µg/g and 123.11 µg/g in avocado pear and pawpaw respectively to 77.14 µg/g in plantain (Table 1). The Table shows significantly higher contents of anthocyanins (271.20 ± 3.90 µg/g), epicatechin (78.33 ± 3.19 µg/g), catechin (118.30 ± 3.76 µg/g), and naringin (62.78 ± 2.19 µg/g) in Irish potato when compared to the other studied antiulcer plants. Thus, Irish potato and avocado pear had a significantly higher distribution of phenolic compounds than pawpaw and plantain fruits with anthocyanins, catechin, and epicatechin being the most prominent polyphenols. On the other hand, anthocyanidins and rutin were generally found to be among the least occurring phenolic compounds in the studied plants. The comprehensive antioxidant potential of a sample is measured as total antioxidant capacity (TAC), giving a measure of the entire antioxidant capacity of all antioxidants in the biological sample. Thus, TAC levels depict the synergistic interactions of antioxidant molecules of a biological sample. It reflects the potential of the antioxidants present in the sample to neutralize free radicals.[31] In the present study, TAC of the plants varied significantly ($p<0.05$) from Irish potato (149.90 ± 13.02 mg AAE/g) through avocado pear (146.60 ± 4.53 mg AAE/g) to unripe pawpaw (53.31 ± 7.16 mg AAE/g) and plantain (35.55 ± 3.13 mg AAE/g) fruits (Figure 1). This observation is in tandem with the earlier discussed higher distribution of sampled phenolic compounds in Irish potato and avocado pear in comparison with the other two plant samples. The production and accretion of phenolic compounds in plant parts are influenced by the environmental and chemical conditions under which the plant grows as well as the plant type and genotype.[32] The results indicate greater content of phenolic molecules and consequently greater antioxidant potential and stronger anti-ulcerative capacity in Irish potato and avocado pear than unripe pawpaw and plantain fruits.

Figure 2A shows concentration-dependent DPPH radical scavenging activities of the antiulcer plants. At the highest concentration (2000 µg/ml), pawpaw and Irish potato had higher DPPH radical scavenging activities of 69.47 % and 67.86 % respectively, than avocado pear (58.67 %) and plantain (53.38 %).

Table 1: Phenolic compounds (µg/g) of aqueous extracts of the antiulcer plants

Phenolic Compound (µg/g)	Irish Potato	Plantain	Pawpaw	Avocado Pear
Rutin	0.49 ± 0.05^{c}	0.23 ± 0.03^{a}	1.90 ± 0.08^{b}	0.38 ± 0.08^{ac}
Anthocyanins	271.20 ± 3.90^{b}	22.14 ± 2.02^{a}	10.06 ± 1.96^{a}	55.73 ± 2.47^{c}
Anthocyanidins	0.51 ± 0.06^{a}	0.60 ± 0.04^{a}	0.60 ± 0.05^{a}	0.60 ± 0.04^{a}
Epicatechin	78.33 ± 3.19^{c}	32.75 ± 1.81^{a}	10.58 ± 2.31^{b}	10.61 ± 2.32^{b}
Catechin	118.30 ± 3.76^{b}	ND	66.22 ± 2.98^{a}	66.17 ± 2.93^{a}
Kaempferol	30.42 ± 1.91^{c}	1.66 ± 0.45^{a}	7.55 ± 1.70^{b}	6.72 ± 1.25^{ab}
Flavones	36.70 ± 1.76^{b}	2.42 ± 0.29^{a}	2.45 ± 0.33^{a}	2.46 ± 0.30^{a}
Naringenin	0.07 ± 0.01^{b}	8.53 ± 1.24^{a}	8.63 ± 1.22^{a}	12.44 ± 1.17^{c}
Naringin	62.78 ± 2.19^{b}	ND	6.34 ± 1.10^{a}	7.56 ± 0.82^{a}
Tannin	ND	8.81 ± 0.90^{a}	8.78 ± 0.91^{a}	8.77 ± 0.91^{a}
TOTAL	598.80	77.14	123.11	171.44

The values presented are mean ± standard deviation of triplicate determinations. The values bearing different superscript letters per row are statistically significant ($p<0.05$). ND, indeterminate.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

However, the 50 % inhibitory concentration (IC_{50}) values deduced from the figure for the aqueous extracts ranged from 310.61, 596.31, 753.04 to 773.65 µg/ml for Irish potato, pawpaw, plantain, and avocado pear respectively, which were generally higher than that of the standard, tannic acid (117.10 µg/ml).

The hydroxyl radical scavenging activities of the aqueous extracts of the antiulcer plants are shown in Figure 2B. Generally, increasing concentrations of the sample extracts led to greater inhibitory activity against hydroxyl free radical with the highest extract concentration (2000 µg/ml) of Irish potato, avocado pear, plantain, and pawpaw eliciting 62.83, 57.22, 52.63, and 40.26 % inhibitory effects respectively. The 50 % inhibitory concentration (IC_{50}) of avocado pear (481.89 µg/ml) was equivalent to that of the standard, catechin (485.32 µg/ml), while those of Irish potato, plantain, and pawpaw were higher at 722.19, 1804.13, and 2618.90 µg/ml respectively.

Figure 2C shows that increasing concentrations of the aqueous extract of the antiulcer plants led to greater inhibitory activity against lipid peroxidation. At the highest concentration of 2000 µg/ml, avocado pear, Irish potato, and pawpaw had higher lipid peroxidation inhibitory effects at 60.53 %, 55.27 %, and 53.51 % respectively, than plantain (37.50 %). The 50 % inhibitory concentration (IC_{50}) of avocado pear (93.80 µg/ml) was similar to the observed IC_{50} of the standard, catechin (65.00 µg/ml), while those of Irish potato, pawpaw, and plantain were higher at 534.52, 891.19, and 2666.67 µg/mL respectively. The results in Figure 2 showed that the DPPH and hydroxyl free radicals scavenging and the lipid peroxidation inhibitory activities of the plants studied followed a similar logistic dose-response model. A similar *in vitro* free radical scavenging pattern was observed by Alisi *et al.* for honey samples, which the authors ascribed to the existence of different substances in the samples with anti-oxidative properties.[33] These results suggest that as the concentrations of the sample extracts increase, the content of the antioxidant substances increase with a concomitant increase in the radical scavenging/inhibitory effects. Although the observed lipid peroxidation inhibitory and radical scavenging effects of the plants were generally lower than those of the standards used, extracts of the plants elicited significant free radical inhibitory potentials. Our results showed that DPPH and hydroxyl free radicals scavenging and lipid peroxidation inhibitory efficiency measured as IC_{50} values of the aqueous extracts varied between the samples, with the aqueous extract of Irish potato being generally the most efficient followed by unripe avocado pear and pawpaw fruit extracts.

DPPH is an exogenous molecule that has a proton-free radical which absorbs light at 517 nm. When DPPH is exposed to proton radical scavengers, its light absorption potential is reduced considerably. Thus, its measurement is applied *in vitro* to monitor and quantify antioxidant radical scavengers in biological samples. On the other hand, hydroxyl free radical has been implicated as a major causative agent of ulceration of gastric mucosa due to its oxidative effect. It elicits oxidative damage by enhancing the peroxidation of membrane lipids and oxidation of proteins and DNA molecules.[32] Hence, the observed *in vitro* efficiency of these plant extracts in DPPH and hydroxyl free radicals scavenging and lipid peroxidation inhibition indicates their potential in peptic ulcer management, and may explain their tradomedical application in the treatment of stomach ulcers generally.

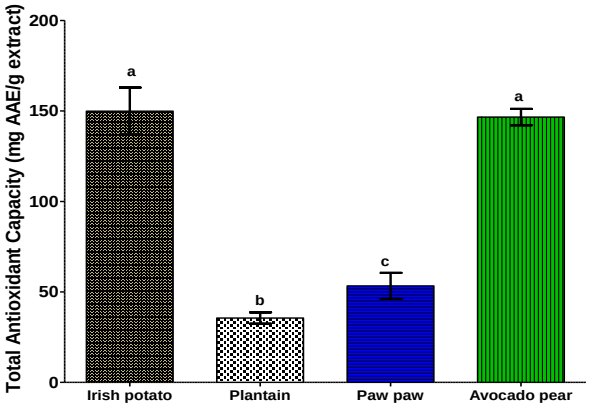

Figure 1: Total antioxidant capacities (mg AAE/g extract) of aqueous extracts of the antiulcer plants. Bars are mean ± standard deviation of triplicate determinations. Bars bearing similar alphabet letters are not statistically significant (p>0.05).

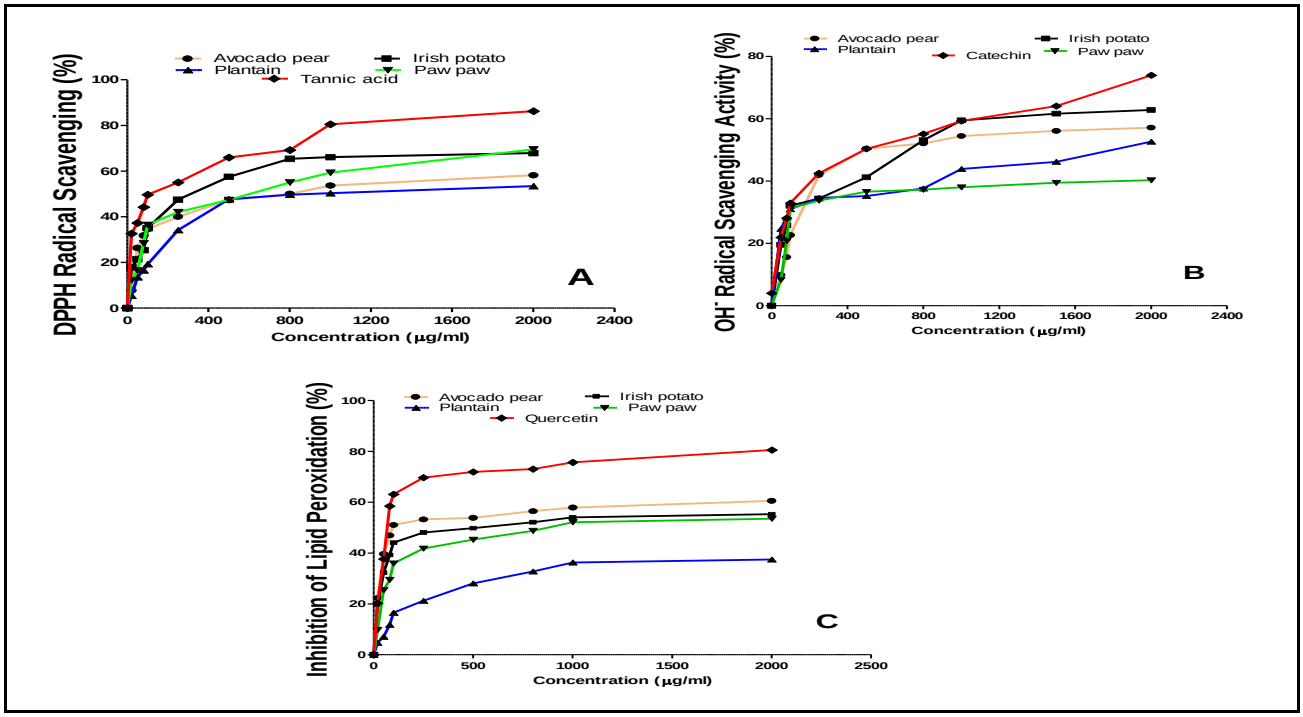

Figure 2: Free radical scavenging properties of aqueous extracts of the antiulcer plants and standards (tannic acid, catechin and quercetin). DPPH radical (A), hydroxyl radical (B) and inhibition of lipid peroxidation (C).

Conclusion

In conclusion, aqueous extracts of Irish potato tuber, avocado pear seed, and unripe fruits of pawpaw, and plantain have appreciable contents of phenolic compounds, eliciting significant antioxidant activity against DPPH and hydroxyl free radicals and lipid peroxidation. Irish potato tuber and avocado pear seed samples showed significantly greater contents of the phenolic compounds and showed stronger antioxidant activities than unripe fruits of pawpaw and plantain.

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.

Acknowledgements

The authors are grateful to Mr. Anthony Nnani and Dr. Emeka Asiwe for all the technical support they graciously offered during the study.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

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