ORIGINAL ARTICLE

The *In Vitro* Antioxidant Activity and Phytochemicals of Locally Consumed Plant Foods from Quezon Province, Philippines

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ABSTRACT

Background. Quezon province has been one of the primary agricultural sources of vegetables and crops in southern Luzon due to its diverse agricultural topography. Having increased dietary awareness, consumption of antioxidant-rich foods has become relevant.

Objectives. Thirteen (13) methanolic extracts of endemic Quezon plant foods were evaluated for phytochemical constituents and antioxidant potential.

Methods. The plant extracts were subjected to in vitro antioxidant assays, which include DPPH [2,2-diphenyl-1-picrylhydrazyl], FRAP [Fluorescence recovery after photobleaching], metal chelation, superoxide, nitric oxide, hydroxyl radical scavenging activities and MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] reduction. Qualitative phytochemical analysis were also employed.

Results. *P. umbellatum*, locally known as kamamba and *C. longa*, locally known as luyang dilaw showed high antioxidant activity using DPPH and MTT assays by 82.46±0.75% and 82.35±1.79% and 78.46±0.71% and 60.97±2.60% at 66.67 µg/mL, respectively. FRAP assay revealed a comparable reducing power with BHT (butylated hydroxytoluene) (93.61±0.56%) at 72.15 µg/mL in *C. longa* (92.49±1.32%), *P. umbellatum* (92.38±0.64%), and *Z. officinale* (90.33±2.06%) (luya), but found with low metal chelating activity. The highest activity against hydroxyl radical were observed in *S. edule* (sayote) (34.30±1.88%), *P. edulis* (pasyonaryo) (33.48±1.34%) and *D. philippinensis* (katmon) (34.71±0.85%) at 45.5 µg/mL. On the other hand, *Z. officinale*, *C. esculenta* (gabi), *V. unguiculata* (sitaw) fruit and *D. philippinensis* showed dual action, as antioxidant and as pro-oxidant, in Superoxide Scavenging and Nitric Oxide assays. Quezon province plant foods contain flavonoids, phenolic compounds, glycosides and coumarins and quinones which may explain their behavior as antioxidant.

Conclusion. The study revealed that different plant foods showed different capacity to scavenge particular oxidants. However, *P. umbellatum* and *C. longa* may be considered promising sources of natural antioxidants.

Key Words: Quezon province, antioxidants, indigenous, plant food

INTRODUCTION

Antioxidants are compounds that have gained importance in years due to their ability to inhibit some pathological effects of increased levels of free radicals or Reactive Oxygen Species (ROS) concentrations.[1-2] ROS or pro-oxidant species are normally generated during cell metabolism, either as bio-products of several enzymes or as a result of the intracellular metabolism of foreign compounds, and by ionizing radiation. Prolonged exposure to high ROS concentrations may highly damage and propagate the oxidative damage by generating organic radical species.[1-4]

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Our bodies are equipped with cellular defenses, such as antioxidant enzymes, vitamins and free radical scavengers, against oxygen toxicity. However, these may not be enough to protect against excess free radicals. Consumption of foods rich in phytochemicals with strong antioxidant property may help prevent and repair cellular damage to our bodies.[1-3,5-6]

Most plants contain phytochemicals that possess antioxidant activity. It is recognized that antioxidant (mainly polyphenolic) compounds from plant extracts can act by either free radical scavenging, singlet oxygen quenching, chelating of transitional metal such as iron, as well as a reducing agent and activator of antioxidative defense enzyme system to suppress radical damage in biological systems.[5-8]

The Philippine archipelago, which hosts very high degree of land and animal endemism, may provide the best plant foods which may exhibit health benefits like antioxidants.[9-10] Quezon province known as the "food basket" of south Luzon - is one of the primary agricultural sources of vegetables and crops due to its diverse agricultural topography. Their contribution as source of food antioxidants can further be substantiated if more studies on their potential are done. Thus, the antioxidant activities and phytochemicals present in each plant sample of some Quezon-derived vegetables were evaluated *in vitro*.

MATERIALS AND METHODS

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), phenazinemethosulfate (PMS), sodium nitroprusside (SNP), sulfanilamide, naphthylethelenediaminedihydrochloride (NED), Folin-Ciocalteu reagent, quercetin, butylated hydroxytoluene (BHT), ethylene diaminetetraacetic acid (EDTA), ascorbic acid, and reduced nicotinamide adenine dinucleotide (NADH) were purchased from Sigma-Aldrich Co. St. Louis, Germany. All other chemicals used were of analytical grade.

Plant samples

Thirteen (13) indigenous and endemic plant foods, namely; *Colocasia esculenta* (gabi) corm, *Displazium esculentum* (pako) leaves, *Raphanus raphanistrum* (labanos) root, *Sechium edule* (sayote) fruit, *Dillenia philippinensis* (katmon) fruit, *Phaseolus vulgaris* (abitsuelas) fruit, *Vigna unguiculata* short (paayap) fruit, *Vigna unguiculata* long (paayap) fruit, *Passiflora. edulis* (passion fruit) fruit, *Capsicum annuum* (siling-haba) fruit, *Curcuma longa* (luyang-dilaw) rhizome, *Zingiber officinale* (luya) rhizome and *Piper umbellatum* (kamamba) leaves, were purchased from provincial market of Quezon province (particularly grown from the towns of Lucban and Sariaya). All samples were authenticated by the Institute of Biology Herbarium, University of the Philippines Diliman.

Plant sample preparation and extraction

Approximately, 1-2 kilos of each plant food were collected. All collected leaves, fruits and roots were washed, cut into smaller pieces and were dried through air-drying and lyophilization. Air-drying was employed to all leaf samples. Samples with high water content (such as fruits), on the other hand, were freeze-dried (Martin Crist Freeze dryer BETA 2-8 LSC). The dried samples were extracted thrice with methanol (AR) at room temperature within twenty-four (24) hours. The pooled methanol extract collected was concentrated using a rotary evaporator (Buchi Rotavapor R-200) at 40°C. Extracts were kept in tightly-sealed bottles at 4°C until use.

Antioxidant assays

DPPH Radical Scavenging Assay[11]

To evaluate the scavenging activity of the methanol extracts, the change of optical density of DPPH radical was monitored. Ten microliter (10 µL) of Ascorbic Acid standard and test compounds at different concentrations were loaded into a 96-well microplate. Afterwards, 140 µL of 6.85 x 10^{-5} M DPPH was added into each well. The microplate was incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm.

Ferric Reduction Antioxidant Power[12]

Reducing power of methanol extracts was measured using ferric reducing antioxidant power (FRAP) assay. Seventy microliter (70µL) of butylated hydroxytoluene (BHT) standard and test compounds at different concentrations were mixed with 176.5 µL of 0.2 M sodium phosphate buffer (pH=7.4) and 176.5 µL of 1% $[K_3Fe(CN)_6]$. The mixture was incubated at 50°C for 20 min. After incubation, the reaction mixtures were acidified with 176.5 µL of trichloroacetic acid (10%) and were centrifuged at 650 x g for 10 minutes. An aliquot of 273 µL of the supernatant was added to 273 µL of deionized water. Finally, 55 µL of $FeCl_3$ (0.1%) was added to this solution. The absorbance was measured at 700 nm.

Metal Chelating Activity[13]

Chelation of ferrous ions was performed. [11] In a 96-well microplate, different concentrations of the methanol extracts (20 µL) were loaded and 100 µL of 0.2 mmoles/L $FeCl_2$ was added in each well. Afterwards, 40 µL of 5 mmoles/L ferrozine was added. The reaction mixture was incubated at room temperature for 10 minutes. The absorbance was measured at 562 nm.

Superoxide Anion Scavenging Activity[14]

This method was measured by the reduction of NBT according to a previously reported method. Ten microliter (10 µL) of standard and test compounds at different concentrations were loaded into a 96-well microplate. Then, 100 µL 468 µM NADH, 100 µL 156 µM (NBT)

and 50 µL 60 µM of PMS were added into each well. Five-minute incubation was done at room temperature. The absorbance was measured at 560 nm.

Nitric Oxide Scavenging Assay [12]

Griess reaction was carried out in microscale volumes. Sodium nitroprusside (10 mM, 2 mL) in phosphate buffer saline was incubated with test compounds in different concentrations at room temperature for 150 minutes. After thirty minutes, 0.5 mL of the incubated solution was added with one (1) mL of Griess reagent (0.33% sulfanilamide in 20% glacial acetic acid, 0.5 mL and 0.1% NED, 1.0 mL) and was incubated for 30 minutes at room temperature. The absorbance was measured at 546 nm.

Hydroxyl Radical Scavenging Assay [15]

The decomposing effect of methanol extracts on hydroxyl radicals was determined by the assay of malondialdehyde chromogen formation due to 2-deoxyribose degradation. The reaction mixture in a final volume of 1mL contained 100 µL of 2-deoxy 2- ribose (28 mM in 20 mM KH_2PO_4 buffer, pH 7.4), 500 µL of the extract at various concentrations in buffer, 200 µL of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1, v/v), 100 µL of 1.0 mM hydrogen peroxide (H_2O_2) and 100 µL of 1.0 mM ascorbic acid.

Test samples were kept at 37˚C for one (1) hour. The free radical damage imposed on the substrate, deoxyribose was measured using the thiobarbituric acid test. One (1) mL of 1% thiobarbituric acid (TBA) and 1 mL 2.8% trichloroacetic acid (TCA) were added to the test samples and was incubated at 100˚C for 20 min. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer.

MTT Reduction Assay [16]

Reducing abilities of methanol extracts were measured using MTT Assay. Stock solutions of test compounds and extracts were prepared in DMSO (250-1000 µg/mL). The MTT (1 mg/mL) was dissolved in water. An aliquot of 190 µL of MTT solution in water and 10 µL of test compounds or extracts in DMSO were vortexed in a capped glass vial (2 mL) for 1 min. To this was added DMSO (200 µL), and the solution was vortexed again. The reaction mixture was then incubated at 37˚C for 6 hours, 200 µL of the reaction mixture was pipetted to a 96-well cell culture plate, and the absorbance was measured at 570 nm.

Phytochemical Screening[17-19]

The secondary metabolites such as reducing sugars, proteins, alkaloids, glycosides, steroids and phytosterols, terpenes and terpenoids, anthraquinones, saponins, polyphenols, flavonoids, and tannins were determined in each plant sample using preliminary and confirmatory tests.

The presence of reducing sugars in all plant samples were detected using Molisch, Fehling's and Benedicts tests; proteins using ninhydrin and biuret test; alkaloids using Mayer's, Wagner's, Hager's and Dragendorff's tests; glycosides using Modified Borntranger's and Keller Killiani tests; steroids using Liebermann-Burchard test; terpenes and terpenoids using Salkowski's test; quinones using sulfuric acid test; anthraquinones using hydrochloric acid Test; flavonoids using alkaline reagent and Shinoda tests; polyphenols using ferric chloride test; tannins using ferric chloride and gelatin tests; and saponins using Froth Test.

Statistical Analysis

Results were expressed as mean±SD. The statistical analysis was performed using one-way ANOVA. The differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Several *in vitro* assays were carried out for evaluating antioxidant activities of plant foods because antioxidant test models vary in different respects. Different procedures use different oxidants or radicals to inhibit. Thus, antioxidant activity must not be based on a single antioxidant assay alone. Several antioxidant assays were performed in all extracts of plant foods collected from Quezon Province, particularly from the towns of Lucban and Sariaya, where most of the plant foods in Quezon are harvested.

DPPH Radical Scavenging Capacity

The antioxidant compounds present in the medium convert DPPH$^{\cdot}$ radical to a more stable DPPH molecular product by donating an electron or a hydrogen atom.[20] As shown in Figure 1, the methanol extracts of *P. umbellatum* L. (*kamamba*) leaves and *C. longa* L. (*luyang-dilaw*) rhizome scavenged DPPH$^{\cdot}$ radical by 82.46±0.75% and 82.35±1.79%, respectively at 66.67 µg/mL which were comparable with ascorbic acid activity (85.64±0.76%). These results are congruent with previous studies which reported that *P. umbellatum* methanol extract exhibited high levels of DPPH$^{\cdot}$ scavenging activity.[21-23] The Quezon province variety of *C. longa* rhizome, on the other hand, exhibited lower DPPH$^{\cdot}$ radical scavenging capacity of 52.26% and 75.48% at 250 µg/mL and 500 µg/mL, respectively than that of the Indian variety with DPPH$^{\cdot}$ radical inhibition of 78.42% at 300 µg/mL.[24] Both *P. umbellatum* (*kamamba*) leaves and *C. longa* (*luyang-dilaw*) had demonstrated >50% scavenging activity even at the lowest concentration.

An endemic plant species, *D. philippinensis* (*katmon*) fruit, showed a lower scavenging activity (ranging from 11.52 to 38.69%) than *P. umbellatum* leaves and *C. longa* rhizome but higher activity than *P. edulis* (passion fruit) by 25%. All other commonly consumed plant foods in Quezon exhibited ~10% DPPH radical scavenging activity except for *V. unguiculata* short (*paayap* short) and *P. vulgaris* (abisuelas) fruit. *Vigna unguiculata* (paayap long) fruit showed a minimal activity at 66.67 µg/mL. Observed DPPH$^{\cdot}$ radical scavenging

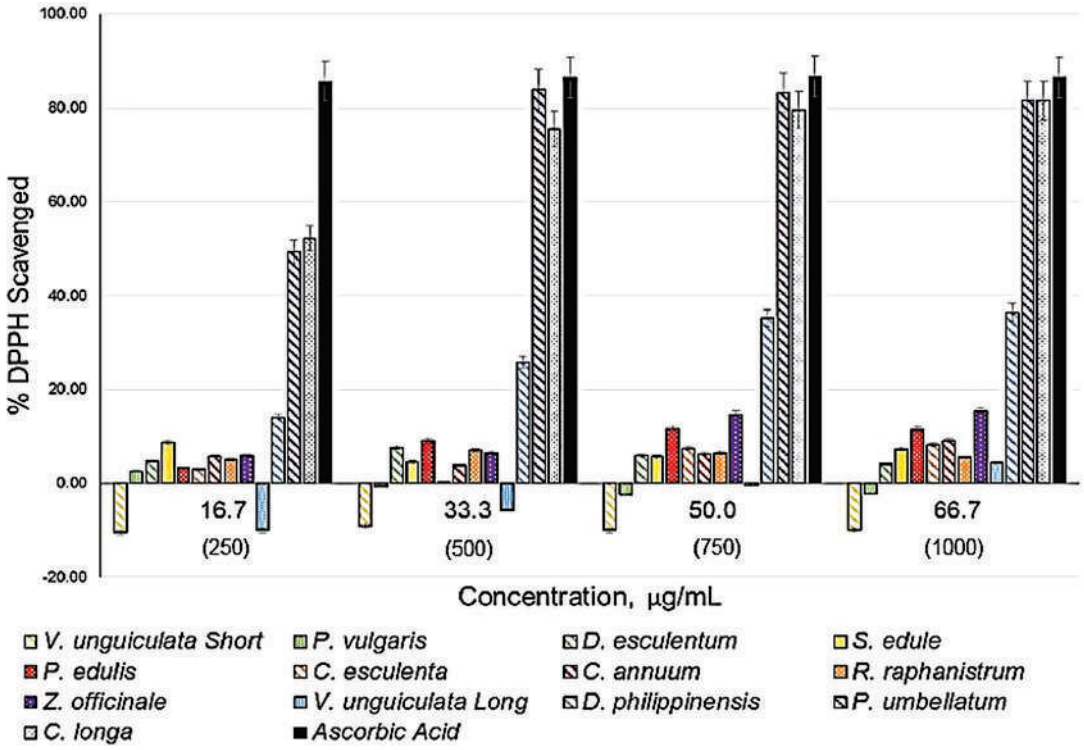

Figure 1. ***DPPH Scavenging Assay of Quezon Plant Foods.*** *Piper umbellatum* L. (Kamamba) leaves and *Curcuma longa* L. (Luyang-dilaw) rhizome showed comparable inhibitory activity with ascorbic acid against DPP radical at 66.67 µg/mL. *D. philippinensis* (katmon) fruit showed an effective concentration-dependent scavenging activity. Other commonly consumed plant foods in Quezon exhibited ~10% scavenging activity.

activities may be attributed to the phytochemicals present in each plant sample. Phytochemical analysis showed that *C. longa* contains flavonoids while *D. philippinensis* and *P. umbellatum* contain phenolic compounds (Table 1). Phenolic and flavonoid compounds widely exist in plants and have been a major contributor to the antioxidant activity.[25]

Ferric Reducing Antioxidant Power (FRAP) Assay

Another mechanism of antioxidant action is through electron donation. The reducing or antioxidant capacity is determined based on the ability of the reductants (or antioxidants) present in the extract to reduce ferric (III) ions to ferrous (II) ions.[19] Among the 13 plant foods harvested in Quezon, *C. longa, P. umbellatum,* and *Z. officinale* were observed to have highly significant reducing power by 92.49±1.32%, 92.38±0.64% and 90.33±2.06%, respectively at 50.9µg/mL which were comparable with the reference standard, butylated hydroxytoluene (BHT) (93.61±0.56%) (Figure 2).

C. longa (*luyang dilaw*) grown in Nigeria and Bangladesh and *P. umbellatum* (kamamba) variety in Pennsylvania, USA were reported with significant reducing power.[23,26-27] *Z. officinale (luya)* rhizome showed significant ferric ion reduction (76.24% reduction) even at the lowest concentration of 12.79 µg/mL, which agrees with the reducing potencies of reference standards as shown: Trolox > BHT > Ginger > α-tocopherol.[28-29]

Potential plant materials, *D. esculentum (pako)* leaves and *D. philippinensis (katmon)* fruit, showed effective reductive activity of 70.56±6.6% and 68.36±11.6% at 50.9 µg/mL and 36.77±12.8% and 44.53±23.1% at 12.7 µg/mL, respectively. Methanol extract of *D. esculentum* manifested significant reducing power (0.98) which exceeded even that of ascorbic acid (0.772) at a concentration of 200 µg/mL.[30]

The following plant foods, *V. unguiculata* (*paayap* short), *P. vulgaris* (*abitsuelas*) fruit, *V. unguilata* (*paayap,* long), *C. annuum* (*siling haba*) fruit, *R. raphanistrum* (*labanos*) root and *P. edulis* (*passion fruit*) exhibited nearly 50% reducing capacity at 50.9 µg/mL, whereas *C. esculenta* (*gabi*) corm and *S. edule* (*sayote*) have minimal reducing ability to convert ferric ion to ferrous form.

Metal Chelation Assay

It has been known that excess amount of some transition metals like iron generate hydroxyl radicals through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH\bullet$) and accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals.[31] Antioxidants also exhibit this activity by forming insoluble complexes with metals that catalyze lipid oxidation.[32]

Table 1. Qualitative analysis of methanolic extracts of Quezon plant foods

Plant Sample	Flavonoids	Coumarins	Reducing Sugars	Proteins	Saponins	Glycosides	Alkaloids	Terpenes/ Terpenoids	Tannins	Phenolics	Steroids and Phytosterol	Quinones	Anthraquinones
C. esculenta corm	+	+	+	-	-	+	-	-	-	-	-	-	-
D. esculentum leaves	-	+	+	+	-	+	-	+	+	+	-	-	-
R. raphanistrum root	+	+	+	-	-	+	-	+	-	-	+	+	-
S. edule fruit	+	+	+	-	-	-	-	+	-	-	+	+	-
D. philippinensis fruit	-	+	+	-	-	+	-	+	+	+	+	+	-
P. vulgaris fruit	+	+	+	-	-	-	-	-	-	-	+	+	-
V. unguiculata (Short) fruit	+	+	-	-	+	+	+	+	+	+	+	+	-
V. unguiculata (Long) fruit	-	+	+	-	-	-	-	+	+	+	+	+	-
P. edulis fruit	+	+	+	-	-	+	-	+	-	-	+	+	-
C. annuum fruit	-	+	+	-	-	+	-	+	-	-	+	+	-
C. longa rhizome	+	+	+	-	-	+	-	+	-	-	+	+	+
Z. officinale rhizome	+	+	+	-	-	+	-	+	-	-	+	+	-
P. umbellatum leaves	+	+	+	+	-	+	-	+	+	+	-	+	-

+' represents presence of the phytoconstituent; '-'represents absence of the phytoconstituent.

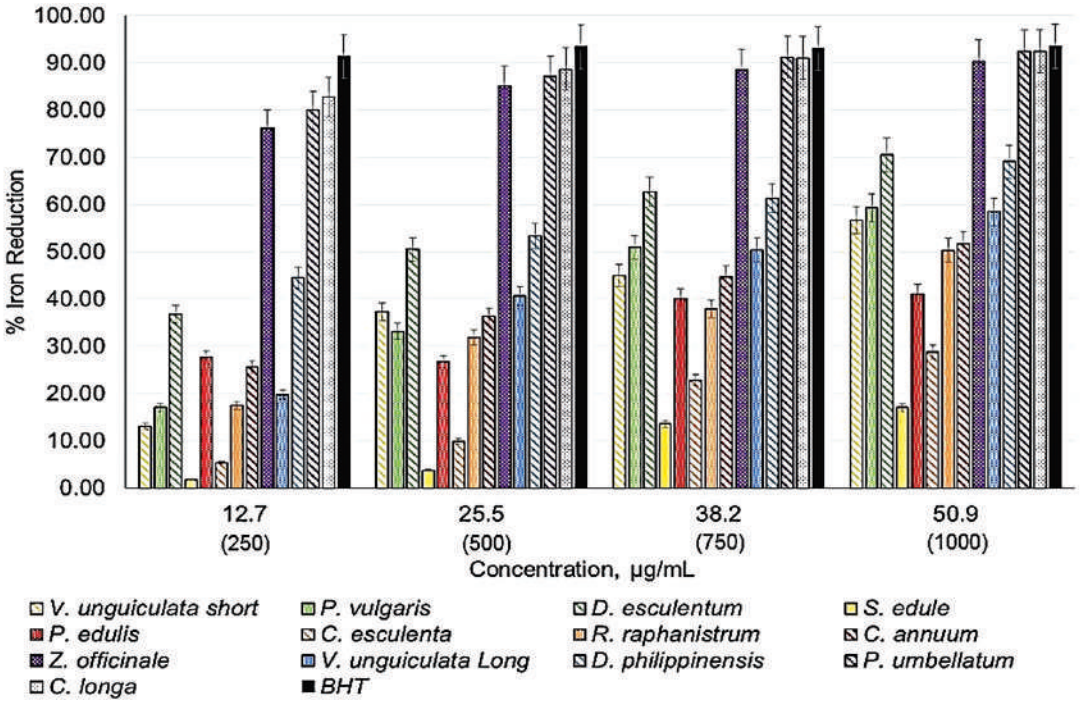

Figure 2. ***Reducing Power of Quezon Plant Foods.*** *C. longa* (Luyang-dilaw) rhizome (Kamamba) leaves and *Curcuma longa* L. (Luyang-dilaw) rhizome were observed to have highly significant reducing power. *S. edule* (sayote) fruit have minimal reducing ability to convert ferric ion to ferrous form.

Quezon plant foods which exhibited significant reducing power (FRAP values), showed a significant low metal chelating activity. Iron chelation of all plant samples were <20% even at the highest concentration (Figure 3). The highest value demonstrated by *D. esculentum (pako)* leaves was 16.04±1.91% at the 125 µg/mL. *C. longa* showed rather consistent chelation of 10.40±2.13% to 12.13±0.55% in all doses. On the other hand, no iron chelation was observed for *R. raphanistrum (labanos)*. Chelating agents, which form σ-bonds with metals, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of metal ions.[33]

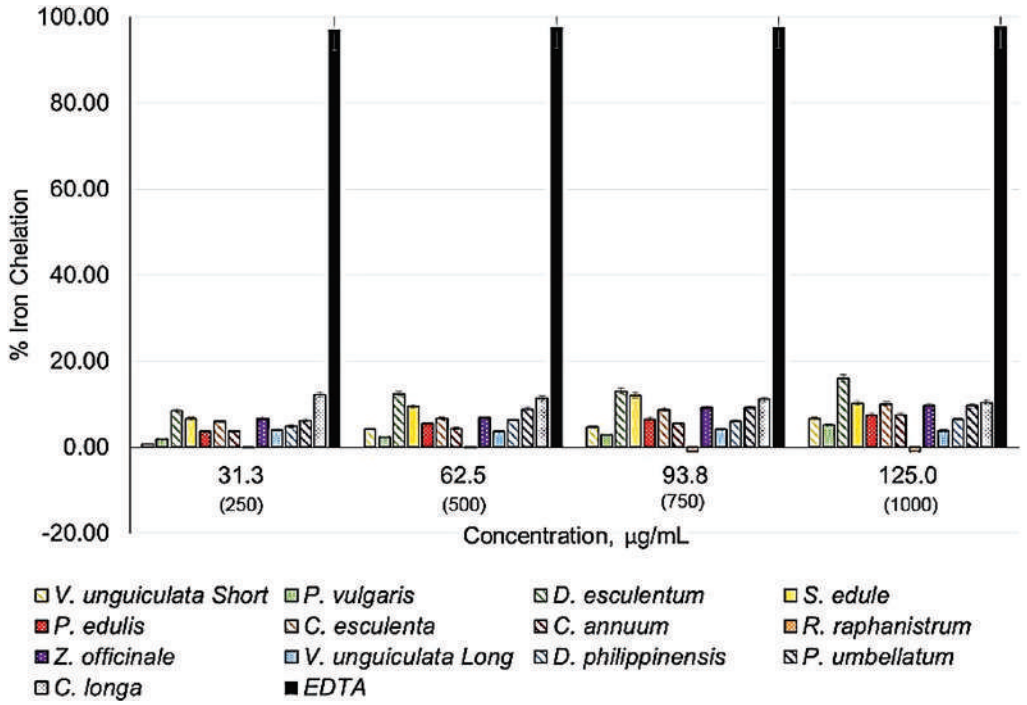

Figure 3. ***Metal chelating Activity of Quezon Plant Foods.*** *D. exculentum* (pako) leaves demonstrated the highest chelation at the 125 µg/mL. *C. longa* (Luyang-dilaw) showed rather consistent chelation at all doses. No iron chelation was observed for *R. raphanistrum* (labanos).

Superoxide Radical Scavenging Capacity

Superoxide radical ($O_2^{-\cdot}$) is highly reactive that initiates free radical formation of hydrogen peroxide, hydroxyl radicals, or singlet oxygen and peroxynitrite in living systems.[34]

The Superoxide Scavenging Activity of Quezon plant foods showed a different antioxidant profile. It was observed that as the dose increased, their scavenging ability decreased and stimulated superoxide radical ($O_2^{-\cdot}$) production, as shown in Figure 4. It was *V. unguiculata Short (paayap)* fruit, *P. vulgaris*, *C. esculenta (gabi) corm, C. annuum (siling-haba), Z. officinale (luya), V. unguiculata (paayap)* short and *D. philippinensis (katmon)* fruit which showed scavenging activity by 8.27±1.29%, 6.22±0.94%, 14.91±7.97%, 10.61±2.56%, 8.59±0.13%, 8.27±1.29% and 2.79±6.47% at 250 µg/mL, respectively. *Z. officinale (luya)* showed a significant change in activity from 250 µg/mL (8.59%) to 1000 µg/mL (-65.31%). *C. esculenta (gabi)* corm *(14.91% to -43.92%), V. unguiculata Short (paayap)* fruit *(8.27% to -52.46%)* and *D. philippinensis (katmon)* fruit (2.79% to -9.36%) exhibited the same pattern of activity. It was only *P. vulgaris* and *C. annuum* which sustained scavenging capacity till 1000 µg/mL.

The results showed dual action, as antioxidant and as prooxidant, among plant foods studied. It was reported that natural antioxidants like polyphenols, flavonoids, carotenoids can also act as prooxidants.[35-38] Polyphenols with low oxidation potentials (Epa) exhibit antioxidant activity, while those with high Epa values act as prooxidants. This characteristic could describe a dual action of phenolic compounds, where high-Epa polyphenols exist in some extracts that simultaneously exhibit antioxidant and prooxidant activities.[39] These plant foods contain flavonoids, phenolic compounds, glycosides and quinones (Table 1) as described above.

Nitric Oxide Scavenging Assay

Nitric oxide (NO) is a highly reactive compound synthesized in many cells of the body from arginine by nitric oxide synthase. It reacts with superoxide extremely rapidly and generates an oxidant and nitrating agent – peroxynitrite which is a powerful oxidant.[40] However, NO possesses a dual role. It may either induce cancer progression or halt cancer growth and act as therapeutic agents depending on the level of NO in the blood. At lower concentrations, NO aids in angiogenesis, but on the contrary, higher levels of NO tend to be cytotoxic to cancer cell by the formation of peroxynitrite, which acts as an inducer of apoptosis and other toxic species during immune surveillances.[8,41]

The methanol extracts of *P. vulgaris, D. esculentum, S. edule, P. edulis* and *D. philippinensis* exhibited antioxidant activity by moderately scavenging NO (at an average of 35%), while *C. esculenta, R. raphanistrum, C. annuum, Z. officinale, V. unguiculata* Long and *C. longa* (at an average of 24%) at 250 µg/mL (Figure 5). As the concentration was increased to 1000 µg/mL, *Z. officinale, R. raphanistrum* and *C. esculenta*

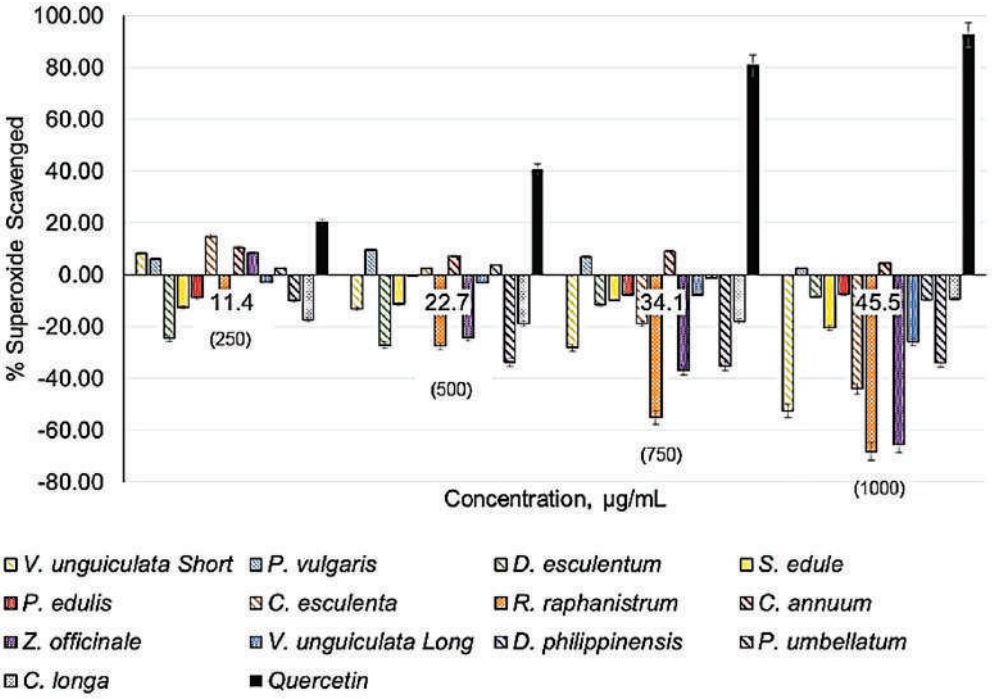

Figure 4. ***Superoxide Scavenging Activity of Quezon Plant Foods.*** *C. esculenta* (gabi) corm, *C. annuum* (siling-haba) fruit, *Z. officinale* (luya) rhizome, *V. unguiculata* Short (paayap) fruit and *D. philippinensis* (katmon) fruit inhibited superoxide anions at 250 µg/mL. Other plant foods behaved oppositely.

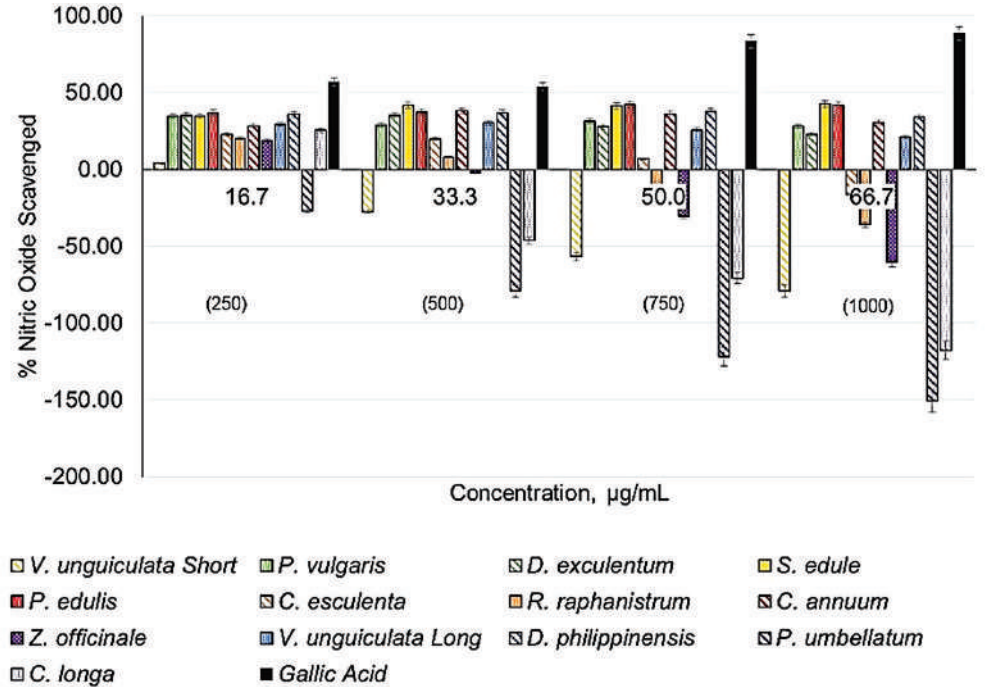

Figure 5. ***Nitric Oxide Scavenging Activity of Quezon Plant Foods.*** Methanol extracts of *P. vulgaris* (abisuelas) fruit, *D. exculentum* (pako) leaves, *S. edule* (sayote) fruit, *P. edulis* (passion fruit) fruit and *D. philippinensis* (katmon) fruit exhibited moderately scavenging of NO• at 250 µg/mL. *P. umbellatum* (kamamba) leaves did not show any scavenging activity against NO•. *S. edule* (sayote) and *P. edulis* (passion fruit) seemingly have consistent NO-scavenging ability from the 250-1000 µg/mL.

were observed to behave as prooxidant. *P. umbellatum* (*kamamba*) leaves did not show any scavenging activity against NO while *C. longa* and *V. unguiculata* (short) lost their anti-NO activity when the concentration was raised to 500 µg/mL. *S. edule* and *P. edulis* seemingly have consistent NO-scavenging ability from the 250-1000 µg/mL.

MTT Assay

This antioxidant assay utilizes the redox reaction between MTT and selected natural product extracts or purified compounds was found comparable with the lipid peroxidation inhibitory assay.[16]

P. umbellatum and *C. longa* methanol extracts showed good reducing ability on MTT at 66.67 µg/mL by 78.46±0.71% and 60.97±2.60%, respectively. Both samples had demonstrated >40% scavenging activity even at the lowest concentration. The activity profile of *C. longa* in this study agreed with other studies while *Z. officinale*, on the other hand, showed increasing reducing activity with 20.87±0.31%, 26.62±0.37%, 31.05±0.79% and 34.05±0.72% reductions at 16.67 µg/mL, 33.33 µg/mL, 50.00 µg/mL, and 66.67 µg/mL, respectively. Except for *D. philippinensis*, other commonly consumed plant foods in Quezon exhibited ~20% scavenging activity. *P. edulis* however, showed a reversed reaction at 50 µg/mL. (Figure 6) Since this assay was similar with lipid peroxidation assay, *P. umbellatum* and *C. longa* may be a good natural alternative antioxidant in the prevention of lipid peroxidation.

Hydroxyl Radical Scavenging Assay

Hydroxyl radical is the most reactive free radical formed from the reaction of superoxide anion and hydrogen peroxide in the presence of metal ions (such as copper and iron). In living systems, these molecules are the major active oxygen species causing lipid peroxidation.

The methanol extracts of *S. edule* (*sayote)*, *P. edulis* (*passion fruit*) and *D. philippinensis* (*katmon*) showed the highest activity against hydroxyl radical in all concentrations with 34.30±1.88%, 33.48±1.34% and 34.71±0.85% at 45.5 µg/mL, respectively (Figure 7). Juice from *P. edulis* fruit grown in Columbia showed dose-dependent hydroxyl radical scavenging with 95.7% at 1 mg/mL.[42] *Dillenia indica* L. *(Indian catmon)* (IC_{50} = 51.82 µg/ml), a related species of *D. philippinensis* (*katmon*), fruit extract exhibited significant scavenging activity on hydroxyl radical comparable with ascorbic acid (IC_{50} = 50.44 µg/ml).[43] Potential plant materials, *C. longa* (*luyang-dilaw*) and *P. vulgaris (abitsuelas)* fruit, showed scavenging activity of 27.71±2.95% and 28.10±0.63% at 45.5 µg/mL and 22.33±4.59% and 25.22±1.78% at 11.4 µg/mL, respectively. The following plant foods, *C. esculenta* (*gabi*), *V. unguiculata* (paayap long), *C. annuum (siling haba*) and *Z. officinale (luya*) exhibited 10.75 to 17.78% scavenging activity at 45.5µg/mL whereas *R. raphanistrum* (*labanos*) has the least scavenging capacity against hydroxyl radical.

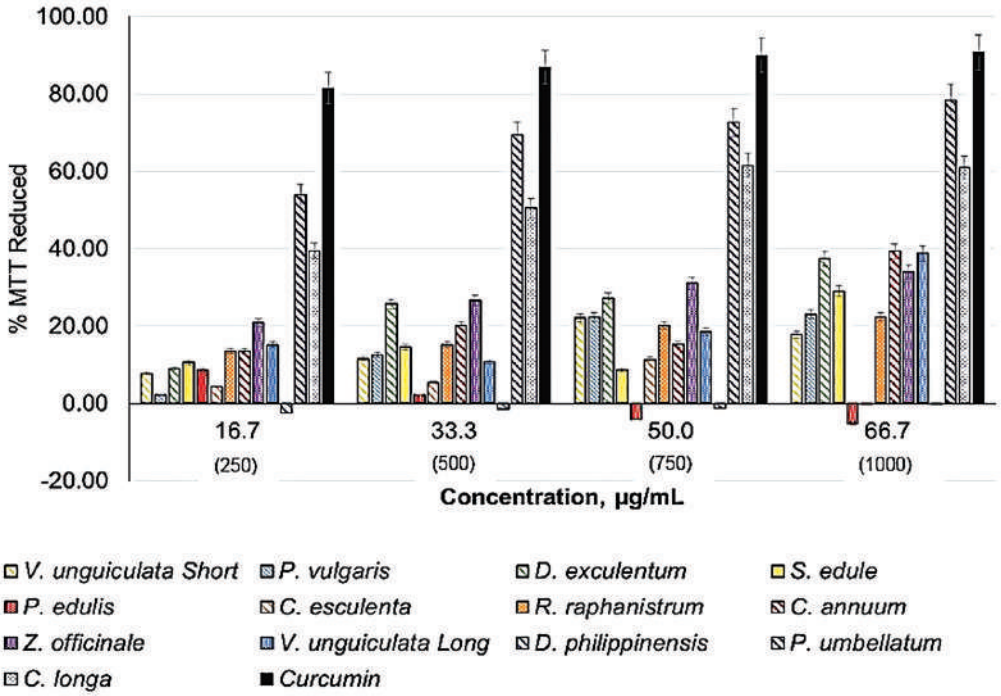

Figure 6. ***MTT Reducing Activity of Quezon Plant Foods.*** Methanol extracts of *P. umbellatum* (kamamba) leaves and *C. longa* (Luyang-dilaw) rhizome showed good reducing ability on MTT and demonstrated >40% scavenging even at the lowest concentration. Other commonly consumed plant foods in Quezon exhibited ~20% scavenging activity.

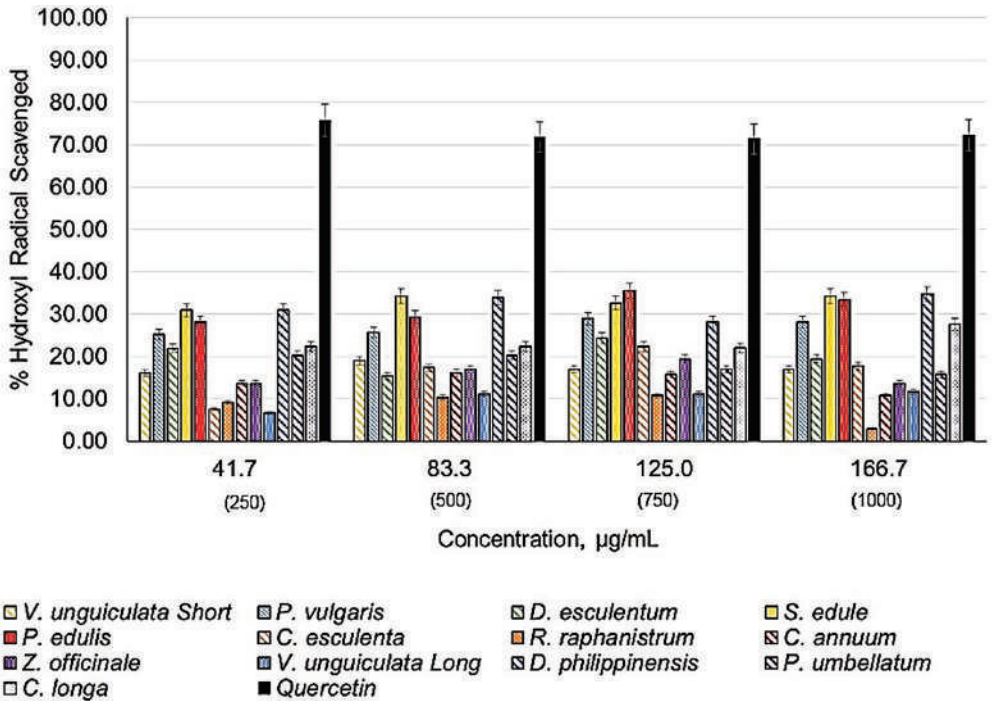

Figure 7. ***Hydroxyl Radical Scavenging Assay of Quezon Plant Foods.*** Methanol extracts of *S. edule* (sayote) fruit, *P. edulis* (passion fruit) fruit and *D. philippinensis* (katmon) fruit showed the highest activity against hydroxyl radical in all concentrations. *R. raphanistrum* (labanos) root has the least scavenging capacity against hydroxyl radical.

Phytochemical Analysis

Phytochemical screening showed that Quezon plant foods seemed to have similar phytochemical constituents (Table 1). All plant samples contain coumarin constituents. Flavonoids were found in all extracts except *D. esculentum*, *D. philippinensis*, *V. unguiculata* long and *C. annuum*. *D. esculentum*, *D. philippinensis*, *V. unguiculata* short, *V. unguiculata* long, and *P. umbellatum* revealed the presence of phenolic and tannin compounds. Only *P. umbellatum* and *D. esculentum* possess proteins while saponins and alkaloids were only found present in *V. unguiculata* short. *C. longa* solely possess anthraquinone compounds. In contrast, terpenoids were not present in *P. vulgaris* and *C. esculenta* while steroidal compound was absent in *D. esculentum*, *C. esculenta* and *P. umbellatum* extracts. Among all sample tested, *P. umbellatum*, *D. philippinensis*, *C. longa* and *Z. officinale* contained several phytochemical constituents based on qualitative phytochemical screening. *C. esculenta*, on the other hand, possessed flavonoids, coumarins and reducing sugars only.

In conclusion, the study revealed that different plant foods showed different capacity to scavenge particular oxidants. However, *P. umbellatum* and *C. longa* were observed to have the highest ferric reducing antioxidant power and thus considered good natural alternatives in the prevention of lipid peroxidation. The findings of this study may help determine what and how much of these plant foods should be properly consumed.

Statement of Authorship

All authors participated in conceptualizing, plant and data collection and processing of data. All authors approved the final version submitted.

Author Disclosure

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