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Research Article

Antioxidant Activity and Phytochemicals of Locally Consumed Plant Foods from Baguio City, Philippines

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Abstract

In the Philippines, Baguio City - known as the "City of Pines" holds the country's major source of temperate climate vegetables. With increased dietary awareness, the consumption of plant foods rich in antioxidants has become relevant. Twenty-nine methanolic extracts from Baguio-produced plant foods were evaluated for antioxidant potential using DPPH, ferric reduction antioxidant power (FRAP), metal chelation, superoxide anion, nitric oxide, hydroxyl radical scavenging activities, MTT reduction, and phytochemical tests. Fagopyrum tataricum leaves, Vaccinium myrtoides fruit, and Morus alba fruit showed the most effective DPP radical, concentration-dependent reducing power, but low metal chelating activity. Solanum tuberosum tuber (22.86±63.26%) showed effective concentration-dependent chelating activity at 125 µg/mL. Citrus aurantium fruit (26.77±9.24%) and Raphanus raphanistrum root (41.13±0.11%) demonstrated an effective scavenging activity against superoxide anions at 45.5 µg/mL. Significant nitric oxide scavenging activity was observed in some fruits. Brassica oleracea Cab leaves $(54.36 \pm 2.38\%)$ showed the highest inhibitory activity against hydroxyl radicals at 166.7 µg/mL. Phytochemical analyses showed that most plant samples revealed the presence of glycosides, terpenes/terpenoids, and steroids/phytosterols, while few contained phenolic and tannin components. These phytochemicals may explain the dual behavior as an antioxidant or a prooxidant observed. Thus, determining food antioxidant component types and their concentration is necessary to maximize the potential to scavenge oxidants.

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INTRODUCTION

Reactive Oxygen Species (ROS) are normal oxidant by-products of aerobic metabolism¹. These reactive intermediates, which include superoxide anion (O₂•), the hydroxyl radical (OH•), and the singlet oxygen, are derived from the incomplete oneelectron reduction of molecular oxygen². Prolonged exposure to high ROS concentrations may lead to non-specific damage to proteins, lipids, and nucleic acids, often inducing irreversible functional alterations or complete destruction³. Antioxidants have gained importance for years due to their ability to neutralize the pathological effects of increased oxidative concentrations⁴⁵. Though our bodies are equipped with innate antioxidant machinery, such as antioxidant enzymes and vitamins, these may not be enough to protect against the detrimental effects of excess free radicals. Consumption of foods rich in phytochemicals with strong antioxidant properties may help prevent and repair cellular damage to our bodies⁵. Natural antioxidants, particularly in fruits and vegetables, have gained increasing interest among consumers and the scientific community. Fruit and vegetable juices are rich sources of numerous phytochemicals, polyphenols, carotenoids, fiber, vitamins, and minerals⁶⁷. Complex mixtures of these phytochemicals present in fruits and vegetables provide additive and synergistic effects on health promotion⁸. Thus, dietary intake of this food as a source of antioxidants is recommended.

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In the Philippines, Baguio City – known as the "City of Pines" – supplies the Philippines' daily requirements for highland vegetables⁹. Due to the temperate climate and wide land area, Baguio provided a conducive environment to cultivate quality fruits, vegetables, and other agricultural products. The Philippine Department of Agriculture stated that Baguio and neighboring provinces in Cordillera supply 80% of the highland vegetable requirement in major markets of Metro Manila and other lowland provinces¹⁰. Knowing the possible beneficial effects of plant foods cultivated from the country's primary agricultural source of prime vegetables, fruits, and crops is essential. Currently, limited studies are conducted on Baguio's commonly consumed plant foods. Thus, the study aims to determine the phytochemical constituents of some Baguio derived vegetables and evaluate their antioxidant activities *in vitro*.

MATERIALS AND METHODS

Materials

Twenty-six plant samples were collected from local markets of Baguio (shown in **Table I**). Edible plant parts commonly consumed by locals were utilized in the study. All samples gathered were authenticated by the Institute of Biology Herbarium - University of the Philippines, Diliman. 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), sodium nitroprusside (SNP), sulfanilamide, naphthyl ethylenediamine dihydrochloride (NED), potassium ferricyanide [K₃Fe(CN)₆], 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.

Table I. List of plant foods used with sample shortened names and parts used

Plant sample	English name/local name	Parts used	Specimen No.
Beta vulgaris L.	Sugar beets/redbeet	Root	NPL-032
Daucus carota subsp. Sativus (Hoffm.) Arcang.	Carrots/karot	Root	NPL-033
Lactuca sativa L. (Iceberg)	Iceberg lettuce/letsugas iceberg	Leaves	NPL-034
Lactuca sativa L. (Romaine)	Romaine lettuce/letsugas iceberg	Leaves	NPL-035
Lactuca sativa L. (Green Ice)	Green ice lettuce/letsugas green ice	Leaves	NPL-036
Lactuca sativa L. (Deep Red)	Deep red lettuce/pulang letsugas	Leaves	NPL-037
Brassica oleracea L. (cabbage)	Cabbage/repolyo	Leaves	NPL-038
Brassica oleracea L. (red cabbage)	Red cabbage/pulang repolyo	Leaves	NPL-039
Brassica oleracea L. (broccoli)	Broccoli/brokoli	Flower buds	NPL-040
Brassica oleracea L. (cauliflower)	Cauliflower/koliplor	Flower buds	NPL-041
Brassica rapa L. (pechay baguio)	Chinese cabbage/petchay Baguio	Leaves	NPL-042
Brassica rapa L. (flowering pechay)	Chinese cabbage/petchay Baguio	Flower	NPL-043
Nasturtium officinale R.Br	Watercress/tonghoy	Leaves	NPL-044
Raphanus raphanistrum subsp. sativus (L.) Domin	Red radish / pulang labanos	Fruit	NPL-045
Sechium edule (Jacq.) Sw.	Chayotte/sayote	Fruit	NPL-046
Morus alba L.	Mulberry/moras	Fruit	NPL-049
Fagopyrum tataricum (L.) Gaertn.	Buckwheat/malabato	Leaves	NPL-050
<i>Fragaria x ananassa</i> (Duchesne ex Weston) Duchesne ex	Strawberry/presa	Fruit	NPL-051
Rozier			
Citrus x aurantium L.	Sweet orange/Sagada oranges	Fruit	NPL-052
Solanum melongena L.	Eggplant/talong	Fruit	NPL-053
Capsicum annuum L.	Bell pepper	Fruit	NPL-054
Solanum lycopersicum L.	Tomato/kamatis	Fruit	NPL-055
Solanum tuberosum L.	Potato/patatas	Tuber	NPL-057
Cucurbita maxima Duschesne	Squash/kalabasa	Fruit	NPL-058
Solanum nigrum L.	Deadly nightshade/lubi-lubi or amti	Leaves	NPL-059
Vaccinium myrtoides	Blueberry/ayusip	Fruit	NPL-060

Methods

Sample preparation and extraction

All collected leaves, fruits, and roots were washed with distilled water, chopped, and cut into smaller pieces. Samples were dried through air-drying and lyophilization. Air-drying was employed on all leaf samples. On the other hand, samples with high water content were freeze-dried using Martin Christ Freeze Dryer Beta 2-8 LSC. All dried samples were kept at 4°C in sealed containers until ready for extraction. The dried samples were extracted with methanol at room temperature for 24 hours. The extract was then separated from the residue by filtration. The residue was re-extracted twice. The methanol

extract 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.

Phytochemical screening

The secondary metabolites were determined in each plant sample using various tests^{11,12}. The presence of reducing sugars in all plant samples was detected using Molisch, Fehling's, and Benedicts tests; proteins using Ninhydrin and Biuret tests; 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.

Antioxidant assays

DPPH scavenging activity: As much as $10 \,\mu$ L of standard and test compounds at different concentrations were loaded into a 96-well microplate. Afterward, 140 μ L of 6.85×10^{-5} M DPPH was added to each well. The microplate was incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm¹³.

Ferric reduction antioxidant power (FRAP) assay: As much as 70 μ L of standard and test compounds at different concentrations were mixed with 176.5 μ L of 0.2 M sodium phosphate buffer (pH=7.4) and 176.5 μ L of 1% [K₃Fe(CN)₆]. The mixture was incubated at 50°C for 20 minutes. After incubation, the reaction mixtures were acidified with 176.5 μ L of 10% trichloroacetic acid and were centrifuged at 650x for 10 minutes. An aliquot of 273 μ L of the supernatant was added to 273 μ L of deionized water. Finally, 55 μ L of 0.1% FeCl₃ was added to this solution. The absorbance was measured at 700 nm¹⁴.

Metal chelating activity: In a 96-well microplate, different concentrations of the 20 μ L methanol extracts were loaded, and 100 μ L of 0.2 mmol/L FeCl₂ was added to each well. Afterward, 40 μ L of 5 mmol/L ferrozine was added. The reaction mixture was incubated at room temperature for 10 minutes. The absorbance was measured at 562 nm¹⁵.

Superoxide scavenging activity: As much as 10 μ L of standard and test compounds at different concentrations were loaded into a 96-well microplate. Then, 100 μ L 468 μ M NADH, 100 μ L 156 μ M NBT, and 50 μ L 60 μ M PMS were added into each well. Five-minute incubation was done at room temperature. The absorbance was measured at 560 nm¹⁶.

Nitric oxide scavenging assay: 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 30 minutes, 0.5 mL of the incubated solution was added with 1 mL of Griess reagent (0.33% sulfanilamide in 20% glacial acetic acid, 0.5 mL, and 0.1% NED, 1 mL) and was incubated for 30 minutes at room temperature. The absorbance was measured at 546 nm¹⁴.

MTT assay: Stock solutions of test compounds and extracts were prepared in DMSO (250-1000 μ g/mL). The MTT (1 mg/mL) was dissolved in water. An aliquot of 190 μ L of MTT solution in water and 10 μ L of test compounds or extracts in DMSO were vortexed in a capped glass vial (2 mL) for 1 minute. As much as 200 μ L DMSO was added, and the solution was vortexed again. The reaction mixture was then incubated at 37°C for six hours, 200 μ L of the reaction mixture was pipetted to a 96-well cell culture plate, and the absorbance was measured at 570 nm¹⁷.

Hydroxyl radical scavenging assay: The reaction mixture in a final volume of 1.0 mL contained 100 μ L of 2-deoxy 2-ribose (28 mM in 20 mM KH₂PO₄ buffer, pH 7.4), 500 μ L of the extract at various concentrations in buffer, 200 μ L of 1.04 mM EDTA and 200 μ M FeCl₃ (1 : 1, v/v), 100 μ L of 1.0 mM hydrogen peroxide (H₂O₂), and 100 μ L of 1.0 mM ascorbic acid. Test samples were kept at 37°C for an hour. The free radical damage imposed on the substrate, deoxyribose, was measured using the thiobarbituric acid test. As much as 1 mL of 1% thiobarbituric acid (TBA) and 1 mL of 2.8% trichloroacetic acid (TCA) was added to the test samples and incubated at 100°C for 20 minutes. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer¹⁸.

Data analysis

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

RESULTS AND DISCUSSION

DPPH scavenging activity

One mechanism by which antioxidants inhibit oxidation is by quenching reactive species through hydrogen or electron donation¹⁹. The DPPH assay is one of the most popular and frequently employed methods among antioxidant assays. DPPH is a stable free radical with a deep purple color and a strong absorption around 517 nm²⁰. Increased concentration of antioxidants may result in a lighter solution²¹.

Methanol extracts of *F. tataricum*, *L. sativa* GI, *V. myrtoides*, and *M. alba* have exhibited good concentration-dependent inhibitory activity against DPP radicals. *Fagopyrum tataricum* showed the most effective DPP radical inhibition (74.53±1.59%) at 66.67 µg/mL. Relatively good inhibitions were also observed in *L. sativa* GI (69.86±1.59%), *V. myrtoides* (66.10±4.43%), and *M. alba* (63.25±0.05%) at the same concentration. In addition, all these plant samples had demonstrated >50% scavenging even at 33.33 µg/mL dose. Similar findings have been observed in other studies. *Fagopyrum tataricum* exhibited significant antioxidant activity with IC₅₀ of 159.51±1.29 µg/mL²². *Vaccinium myrtoides* fruit exhibited the highest antioxidant activity as indicated by its 92.35±0.69% DPPH radical scavenging activity among Baguio fruit and fruit wines⁹. Moreover, ethanolic leaf extracts of *V. myrtoides* (20.85 µg/mL) from Ifugao showed higher DPP inhibition than ascorbic acid (21.56 µg/mL)²³. Significant increase in scavenging activity was observed in *M. alba* with 3.48%, 8.28%, 16.63%, and 25.84% inhibitions between 100-1000 µg/mL²⁴.

Different DPP inhibitory activities were observed between *L. sativa* and *B. oleracea* sample varieties. The four varieties of *L. sativa* exhibited DPPH inhibition at 66.67 µg/mL in increasing order: *L. sativa* RL (10.79±2.74%), *L. sativa* I (17.99±2.36%), *L. sativa* DR (33.18±5.56%), and *L. sativa* GI (69.86±1.59%), respectively. Similar findings showed that red and darker green types of lettuce possess higher antioxidant activity than the green type of lettuce. Red coral lettuce possessed the lowest EC₅₀ value, followed by green coral, iceberg, butterhead, and romaine with 303.56±11.3, 775.55±43.7, 3991.67±174.7, 4230.13±401.5, and 4485.41±784.4 µg/mL, respectively²⁵. In addition, red varieties of lettuce were significantly higher than those of green lettuce²⁶. Likewise, four varieties of *B. oleracea* showed different activities at the highest concentration. *B. oleracea* RCab was revealed to be the most active extract, and *B. oleracea* Cab (5.96±8.82%) was the least. Methanolic extracts of *B. oleracea* varieties exhibited varying DPP radical inhibitions in the order: red cabbage > green cabbage > broccoli > cauliflower²⁷.

Some plant samples showed moderate scavenging activities. Moderate scavenging activities of extracts were observed in the following plant samples in increasing order: *B. oleracea* RCab, *F. anannasa, L. sativa* DR, *B. vulgaris,* and *S. nigrum,* with inhibitions of 29.99±3.38%, 32.92±2.06%, 33.18±5.56%, 34.28±1.67% and 47.54±0.93% at 66.67 μ g/mL, respectively. Methanol leaf extract of *S. nigrum* significantly inhibited DPP radicals in a concentration-dependent manner with IC₅₀ of 165 μ g/mL²⁸. In contrast, low scavenging activities (DPPH inhibition <25%) were demonstrated by other extracts, even at the highest concentration. No DPPH inhibition was observed for *C. aurantium*. The overall results are presented in **Figure 1**.

FRAP assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, antioxidant capacity measures the reduction of the ferric ion (Fe³⁺) to the ferrous ion (Fe²⁺) by donor electrons in the sample²⁹. The activity is monitored by measuring the formation of Perls' Prussian blue complex at 700 nm³⁰. Methanol extracts *of F. tataricum, L. sativa* GI, *V. myrtoides,* and *M. alba* have exhibited the highest concentration-dependent reducing ability among Baguio plant foods. *Fagopyrum tataricum* showed the most effective iron reduction (72.44±1.99%) at 38.2 µg/mL. Comparable reductions to that of *F. tataricum* were also observed in *L. sativa* GI (71.60±1.00%), *V. myrtoides* (71.80±1.80%), and *M. alba* (71.77±1.94%). As mentioned earlier, the plant possessed reducing abilities based on other studies. Maximum activity was exhibited by methanol extract of *F. tataricum*, greater than the gallic acid standard at 200 µg/mL³¹. Wild Blueberries (*Vaccinium* sp.) extract of some wild and cultivated Blueberries from Romania showed iron reduction, with Wild Type 1 (73.71±3.2 µM Fe²⁺/g) being the most potent³². Mulberry variety (S-1708; *Morus* sp.) exhibited reducing activity of 4107.22±97.6 µM/Fe(II) mg and 3540.60 µM/Fe(II) mg in methanol and ethanol solvents, respectively³³.

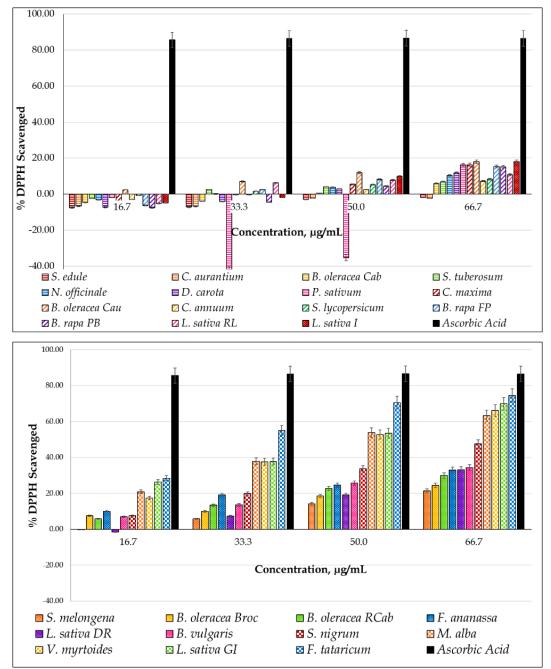


Figure 1. DPPH scavenging assay of Baguio plant foods.

Interestingly, other plant samples showed high reducing abilities. Relatively high reducing power of extracts was observed in the following plant samples in increasing order: *B. oleracea* RCab, *B. vulgaris, L. sativa* DR, and *B. rapa* FP, with an inhibition of 71.92±3.46%, 72.74±3.41%, 74.32±0.33% and 74.65±2.83% at 50.9 µg/mL, respectively. In contrast, low-reducing activities (iron reduction <25%) were demonstrated by other extracts, even at the highest concentration. Moreover, no iron reduction was observed for *C. maxima*. Reducing power between sample varieties of *L. sativa* was assessed. Varieties of *L. sativa* exhibited varying iron reduction at 50.9 µg/mL in increasing order: *L. sativa* I (37.06±8.80%), *L. sativa* RL (41.84±2.38%), *L. sativa* DR. (74.32±0.33%) and *L. sativa* GI (76.46±2.21%), respectively. Strong reducing capacity exhibited by plant samples may indicate greater antioxidant activity³⁴. These results infer that dark-colored lettuces have higher FRAP values than the typical green varieties³⁵. The overall results are presented in **Figure 2**.

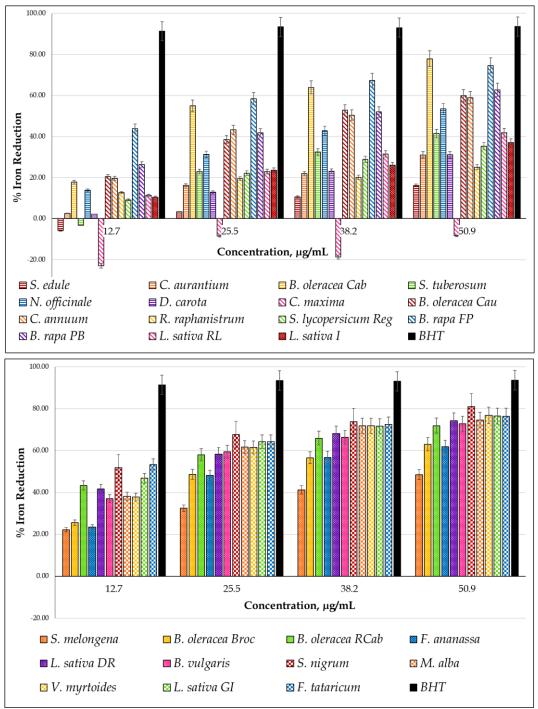


Figure 2. Reducing Power of Baguio plant foods.

Metal chelating activity

Metals, such as Fe and Cu, and ultra-trace elements, Co and Ni, act as cofactors that assist enzymes in catalyzing biochemical reactions efficiently³⁶. Transition metals become toxic at elevated tissue concentrations. Excess transition metals can initiate hydroxyl radical production through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^+ + OH^-$)³⁷. This process hastens the rate of lipid peroxidation by the continuous generation of peroxy radicals (LOO•) to form lipid hydroperoxides (LOOH) – the main primary products of lipid peroxidation³⁸. Chelation therapy is the preferred medical treatment for reducing the toxic effects of metals³⁹. The use of metal chelators can improve the symptoms of metal overload. Synthetic chelators have posed severe side effects; hence, using natural plant foods with chelating ability has been getting more attention⁴⁰.

In metal chelation, a decrease in the absorbance of the red-violet Fe^{2+} /ferrozine complex is monitored⁴¹. Low metal chelating activity was observed from Baguio plant foods. Most plant samples were <20%, even at the highest concentration. *S.*

tuberosum showed the highest effective concentration-dependent chelating activity with inhibitions 6.30±3.82%, 16.76±3.82%, and 22.86±63.26% at 62.5, 93.8, and 125 µg/mL, respectively. However, lower chelation was observed in Baguio-grown *S. tuberosum* compared with other studies. Benguet varieties, Igorota (EC₅₀= 11.0±3.2 µg/mL) and Ganza (EC₅₀= 14.7±3.2 µg/mL) exhibited effective chelating activity⁴². Purple-colored potatoes had a higher chelating effect than white and yellow potato varieties from Korea⁴³. Conversely, no iron chelation was observed for *D. carota* and *C. annuum*. The overall results are presented in **Figure 3**.

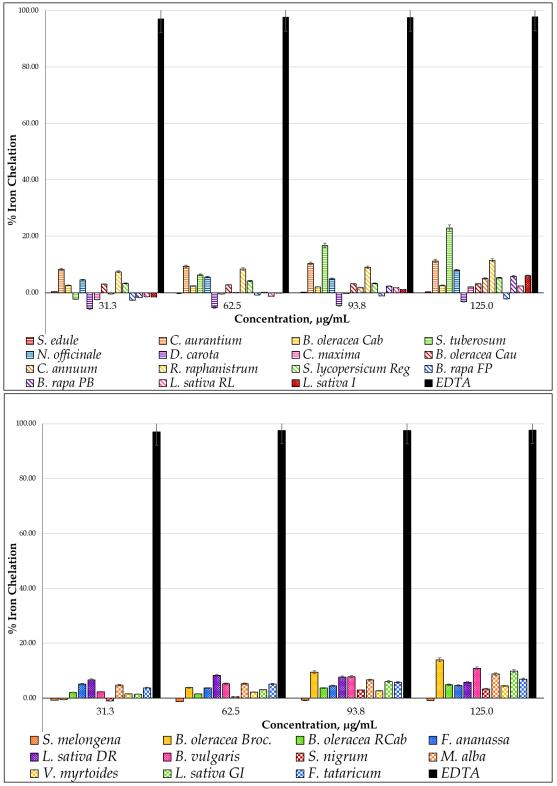


Figure 3. Metal chelating activity of Baguio plant foods.

Superoxide scavenging activity

Superoxide $(O_2 \bullet^-)$ is a free radical with a short biological lifespan produced by the one-electron reduction of molecular oxygen⁴⁴. Superoxide anion initiates free radical formation of other reactive oxygen species in living systems⁴⁵. It can also react with nitric oxide and form peroxynitrite⁴⁶. Thus, knowing the right food which will provide good superoxide scavenging properties may help lower the risk of many degenerative diseases. The overall results are presented in **Figure 4**.

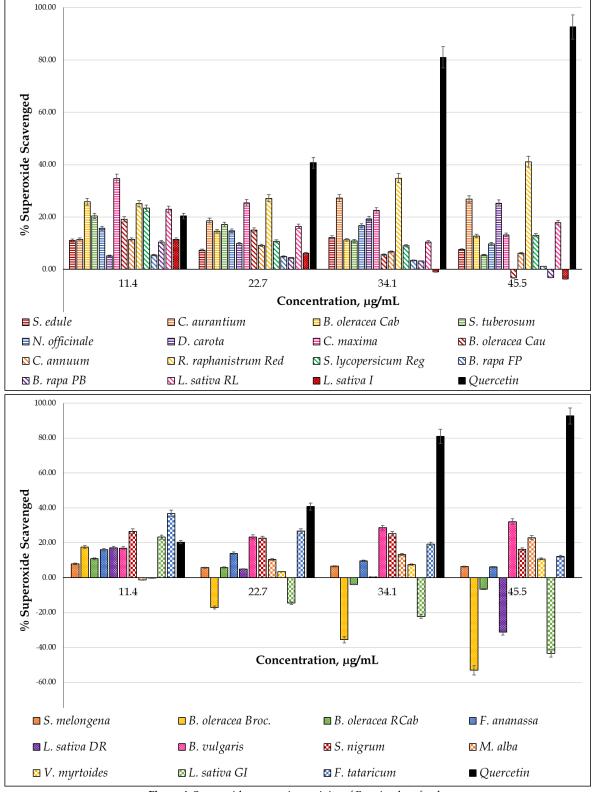


Figure 4. Superoxide scavenging activity of Baguio plant foods.

In this assay, a decrease in absorbance at 560 nm indicates the scavenging of superoxide radicals⁴⁷. Methanol extracts of Baguio plant foods showed inhibitions of superoxide radicals. Some extracts showed the highest superoxide radical scavenging activity at the lowest concentration. Greater than 20% superoxide scavenging activity was observed at 11.4 μ g/mL dose in the following plant samples in increasing order: *L. sativa* RL (22.94±2.51%), *L. sativa* GI (23.20±8.44%), *S. lycopersicum* Reg (23.35±2.70%), *B. oleracea* Cab (25.87±0.47%), *S. nigrum* (26.57±3.98%), *C. maxima* (34.61±2.21%), and *F. tataricum* (36.81±1.70%). *Citrus aurantium* and *R. raphanistrum* demonstrated an effective scavenging activity against superoxide anions. However, it was observed that as the dose of some extracts increases, their scavenging ability decreases. *Fagopyrum tataricum* showed a significant change in activity from the lowest concentration with 36.81±1.70% (at 11.4 μ g/mL) superoxide scavenging capacity to a 12.15±0.46% (at 45.5 μ g/mL). The same pattern of activity was demonstrated by *L. sativa* GI (23.20 to -43.41%), *S. lycopersicum* Reg (23.35 to 13.03%), *B. oleracea* Cab (25.87 to 12.73%), *S. nigrum* (26.57 to 16.26%) and *C. maxima* (34.61 to 13.15%). Similar findings were reported in some Quezon Province plant food extracts that exhibited a decrease in superoxide scavenging activity and stimulation of superoxide radical production as the dose was increased.5 *Ficus odorata*, an endemic medicinal plant in the Philippines, has shown a concentration-dependent stimulation of O2•-48. With these reported results demonstrated the dual action, as an antioxidant and as a prooxidant, of some plant foods studied. Therefore, natural antioxidants have been shown to act as prooxidants under certain conditions⁵.

Nitric oxide scavenging activity

Nitric oxide (NO) is an important cell signaling molecule. At lower concentrations, NO aids in angiogenesis⁴⁹. Nitric oxide plays vital roles in humans, which include dilating blood vessels, raising blood supply and lowering blood pressure, regulating platelet aggregation, signaling molecules between neurons, and killing bacteria^{50,51}. However, NO also possesses a dual role. As a free radical, nitric oxide readily reacts with other radical species and the metal centers of metalloproteins⁵². Moreover, when nitric oxide is near superoxide, peroxynitrite (ONOO⁻) may spontaneously form⁴⁴. In this assay, the Griess reagent can estimate nitric oxide formed¹⁴. Nitric oxide scavengers compete with oxygen, producing reduced nitric oxide. Most of the Baguio plant foods exhibited high nitric oxide scavenging ability. Greater than 50% superoxide scavenging activity was observed in *S. lycopersicum* Reg (75.43±0.71%), *B. vulgaris* (65.21±0.37%), *C. aurantium* (64.10±0.57%), *S. melongena* (62.20±0.84%), *V. myrtoides* (57.69±1.47%), *R. raphanistrum* (56.70±2.64%), *L. sativa* GI (56.02±1.01%), *S. tuberosum* (53.77±3.43%), *M. alba* (51.96±0.60%), *C. annuum* (50.84±0.88%), at 16.7 µg/mL dose. Observed activities were comparable to gallic acid (56.93±6.35%) at the same concentration. However, some of the Baguio extracts were seen to behave as prooxidants as the dose was increased to 66.7 µg/mL.

Lactuca sativa GI (Green Ice Lettuce) exhibited a significant change in NO scavenging ability from $56.02\pm1.00\%$ (at 16.7 µg/mL) to $-87.33\pm2.50\%$ (at 66.7 µg/mL). The same pattern of activity was demonstrated by *B. oleracea* Broc (35.74 to -45.58%), *B. rapa* FP (11.73 to -27.58%), *S. nigrum* (29.51 to -69.25%), *L. sativa* RL (33.67 to -57.57%), *N. officinale* (47.09 to -47.27%), and *S. edule* (18.96 to -24.39%). The overall results are presented in **Figure 5**.

MTT assay

MTT was developed as an antioxidant assay utilizing the redox reaction to screen natural product extracts or purified compounds¹⁷. Methanol extracts of *S. tuberosum*, *B. oleracea* Cab, *B. vulgaris*, *S. nigrum*, and *L. sativa* GI have exhibited the highest concentration-dependent reducing ability among Baguio plant foods. *Brassica oleracea* Cab showed the most effective MTT reduction (48.89±0.431%) at 66.7 µg/mL. Good reductions were also observed in *B. vulgaris* (45.79±1.35%), *L. sativa* GI (43.73±0.81%), *S. nigrum* (41.47±2.04%), and *S. tuberosum* (31.97±1.18%) at the same concentration. In addition, all these plant samples had extended their potency showing >20% scavenging even at 16.7 µg/mL. In contrast, low-reducing activities (iron reduction <25%) were demonstrated by other extracts, even at the highest concentration. Moreover, no MTT reduction was observed for *S. melongena*, *F. annanasa*, *M. alba*, *V. myrtoides*, and *F. tataricum*. The overall results are presented in **Figure 6**.

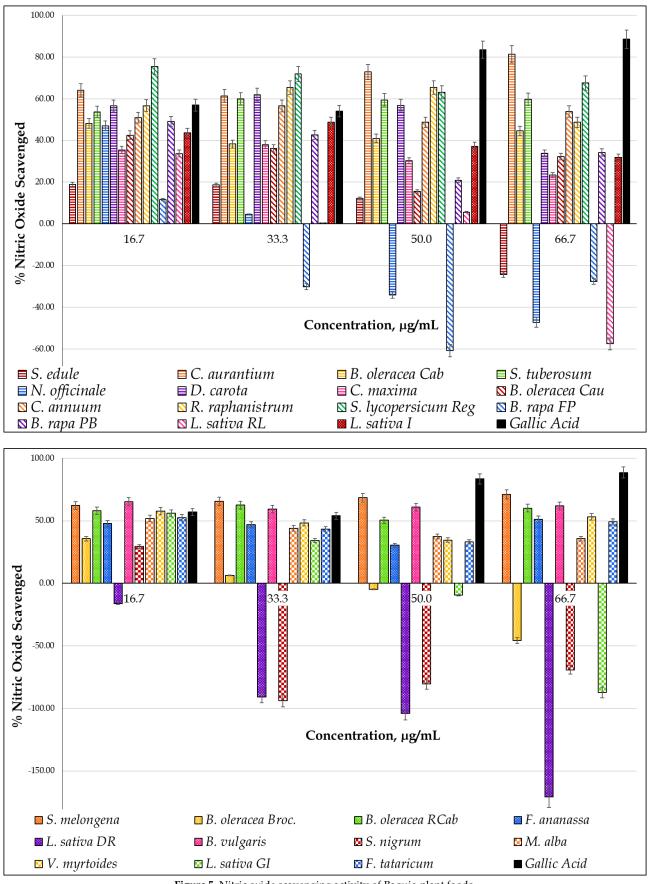


Figure 5. Nitric oxide scavenging activity of Baguio plant foods.

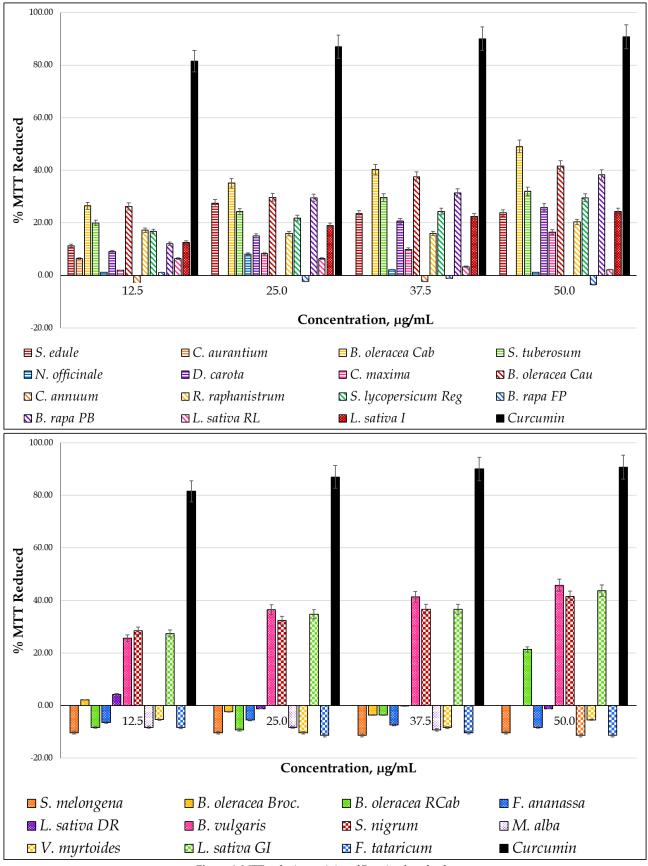


Figure 6. MTT reducing activity of Baguio plant foods.

Hydroxyl radical scavenging assay

Hydroxyl radicals are highly reactive and short-lived molecules. These molecules can react with lipids, polypeptides, proteins, and DNA, especially thiamine and guanosine bases⁵³. The high reactivity of hydroxyl radicals may result in reduced disulfide bonds in fibrinogen, resulting in abnormal folding⁵⁴. Therefore, it is vital to remove hydroxyl radicals. Baguio plant foods showed scavenging ability against hydroxyl radicals. Among these plant foods, *B. oleracea* Cab showed the highest inhibitory activity against hydroxyl radicals at 166.7 µg/mL by 54.36±2.38%. Antioxidant activity and polyphenol content of *B. oleracea* varieties, it was revealed that methanolic extracts of *B. oleracea* varieties exhibited varying hydroxyl radical inhibitions in the order: red cabbage > green cabbage > broccoli > cauliflower²⁷. Comparable scavenging to that of *B. oleracea* Cab was also observed in *C. maxima* (53.26±0.82%), *C. annuum* (48.38±2.14%), *L. sativa* RL (48.37±0.819%), and *L. sativa* GI (46.18±1.2%) at the same concentration. In addition, varieties of *L. sativa* exhibited different nitric oxide radical scavenging activities. The four varieties of *L. sativa* activity at 166.7 µg/mL in decreasing order: *L. sativa* RL > *L. sativa* GI > *L. sativa* I > *L. sativa* DR. These conformed with results using various Serbian lettuce varieties⁵⁵. Among the three plant varieties, the Neva variety possessed the highest hydroxyl scavenging activity (IC₅₀=87.56±1.05 µg/mL), followed by Emerald, then Vera⁵⁴. Other commonly consumed plant foods in Baguio exhibited ~30-40% scavenging activity at 166.7 µg/mL. The overall results are presented in **Figure 7**.

Phytochemical analysis

All Baguio plant samples contained reducing sugars and quinone compounds. Terpenes and terpenoids were found in all extracts except *B. rapa* FP, *N. officinale*, and *S. nigrum*. Only *L. sativa* Green GI, *L. sativa* DR, *B. oleracea* RCab, *F. tataricum*, and *V. myrtoides* revealed the presence of phenolic and tannin compounds. Alkaloids were found in *D. carota*, *L. sativa* DR, *B. oleracea* RCab, *R. raphanistrum*, *C. aurantium*, and *S. lycopersicum* Reg. A few plant samples contained flavonoid and saponin compounds. Among all samples tested, *N. officinale* possessed the least phytochemicals present. The overall results are presented in **Table II**.

Many phytochemicals support the body's innate antioxidant machinery. Phytochemicals: polyphenols, flavonoids, anthocyanins, and carotenoids are the major contributors to their antioxidant properties⁵⁶. Polyphenols, which include flavonoids, stilbenes, lignans, and phenolic acids, are chemical substances characterized by aromatic rings with one or more hydroxyl groups. These compounds react with free radicals, resulting in the delocalization of the gained electron and stabilization of the aromatic nucleus through resonance. This, in turn, stops the free radical chain reaction⁵⁷. Carotenoids, which exhibit a characteristic, symmetrical tetraterpene skeleton found in colored pigments of plant foods, are effective "radical-trapping antioxidants" and one of the most efficient singlet oxygen quenchers⁵⁸. Other phytochemicals such as terpenes and terpenoids, alkaloids, and saponin compounds were also reported for antioxidant activities⁵⁹⁻⁶¹.

However, several studies reported that phenolic antioxidants can also act as prooxidants under certain conditions, like high concentrations of transition metal ions, alkali pH, and the presence of oxygen molecules⁶²⁶⁴. Large molecular weight phenolics, such as hydrolysable and condensed tannins, have little or no prooxidant properties compared to simple phenol. Polyphenols with low oxidation potentials (Epa) exhibit antioxidant activity, while those with high Epa values act as prooxidants⁵. 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.

In summary, the study revealed that phytochemicals from these plant foods exhibited various degrees and types of antioxidant components and activities. For example, some plant foods, *F. tataricum, V. myrtoides*, and *M. alba*, possess higher or equal antioxidant activity than known standards. The various composition of phytochemicals in plant foods exerts different "strength" against oxidants. Reports have shown that exogenous antioxidants may show prooxidant activities, especially when administered at high doses. Despite these drawbacks, food-based secondary metabolites hold promising avenues for health benefits. Further work may be done to elucidate these results *in vivo* and identify compounds responsible for these activities. Moreover, continuous studies on phytochemical mechanisms and interactions may be done to establish the most significant impact of antioxidant systems on alleviating chronic diseases.

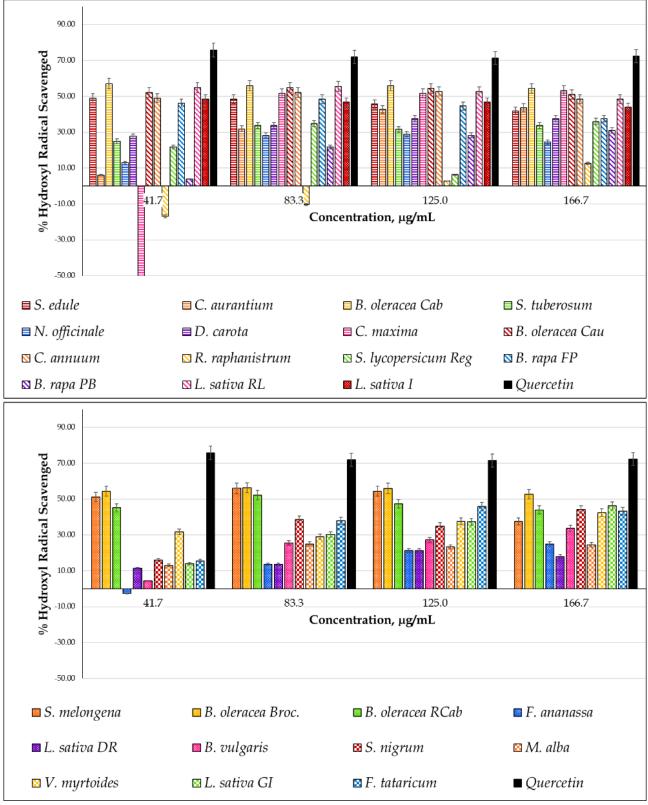


Figure 7. Hydroxyl radical scavenging activity of Baguio plant foods.

Plant Sample	Flavonoids	Coumarins	Reducing sugars	Proteins	Saponins	Glycosides	Alkaloids	Terpenes/ Terpenoids	Tannins	Phenolics	Steroids and phytosterol	Quinones	Anthraquinones
B. vulgaris	-	-	+	+	+	+	-	+	-	-	-	+	-
D. carota	-	+	+	+	+	+	+	+	-	-	+	+	+
L. sativa I	-	+	+	+	-	-	-	+	-	-	-	+	+
L. sativa RL	+	+	+	+	-	-	-	+	-	-	-	+	-
L. sativa GI	-	+	+	+	-	+	-	+	+	+	+	+	-
L. sativa DR	-	+	+	-	-	+	+	+	+	+	+	+	-
B. oleracea Cab	+	+	+	+	-	-	+	+	-	-	-	+	-
B. oleracea RCab	+	+	+	+	-	+	+	+	+	+	+	+	-
B. oleracea Broc	-	+	+	-	-	+	-	+	-	-	+	+	+
B. oleracea Cau	-	+	+	+	-	+	-	+	-	-	+	+	-
B. rapa PB	-	+	+	+	+	+	-	+	-	-	-	+	+
B. rapa FP	-	+	+	+	-	+	-	-	-	-	+	+	-
N. officinale	-	+	+	-	-	-	-	-	-	-	+	+	-
R. raphanistrum	+	+	+	+	-	+	+	+	-	-	+	+	+
S. edule	+	+	+	-	+	+	-	+	-	-	+	+	-
M. alba	+	+	+	-	-	+	-	+	-	-	+	+	+
F. tataricum	-	+	+	-	-	-	-	-	+	+	-	+	-
F. ananassa	+	+	+	+	-	+	-	+	-	-	+	+	+
C. aurantium	+	+	+	+	-	+	+	+	-	-	+	+	-
S. melongena	-	+	+	-	-	-	-	+	-	-	+	+	-
C. annuum	-	+	+	+	-	+	-	+	-	-	-	+	-
S. lycopersicum Reg	-	+	+	+	+	+	+	+	-	-	-	+	-
S. tuberosum	-	+	+	+	+	-	-	+	-	-	-	+	-
C. maxima	-	-	+	+	+	-	-	+	-	-	+	+	+
S. nigrum	-	+	+	+	+	-	-	-	-	-	-	+	-
V. myrtoides	+	+	+	-	-	+	-	+	+	+	+	+	+

Table II.	Phytochemical	profile of methanolic	extracts of Baguio plant foods
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CONCLUSION

In conclusion, the study revealed that Baguio plant foods contained various phytochemicals and showed promising antioxidant capacities. The findings of this study may provide information that consuming Baguio-cultivated plant foods are beneficial and may be applied in the management of various free radical-linked diseases.

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AUTHORS' CONTRIBUTION

PRB performed laboratory experiments and wrote the paper. **RCC** performed laboratory experiments; **GFY** supervised the flow, conceptualized, and advised ideas, and edited the manuscript.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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