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Antioxidant Activity and Total Phenolic Content of Underutilized Edible Tree Species of the Philippines

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Abstract: Recently, neglected and underutilized species (NUS) have deservedly come to the forefront of scientific interest because they can contribute to the human nutrition, due to the content of bioactive substances such as antioxidants. Despite the great diversity and rich tradition in use of Philippine NUS, the nutritional properties of many edible plants remain unexplored. The main objective of this study was to evaluate various parts of eleven NUS fruits and vegetables traditionally consumed in the Philippines, namely *Allaeanthus luzonicus*, *Canarium ovatum*, *Dillenia philippinensis*, *Ficus pseudopalma*, *Flacourtia indica*, *Flacourtia inermis*, *Garcinia intermedia*, *Heliotropium arboreum*, *Posoqueria latifolia*, *Stelechocarpus burahol* and *Sterculia quadrifida* for their total phenolic content (TPC) and *in vitro* antioxidant activity (DPPH and ORAC assays). The strongest antioxidant activity obtained in both assays were inflorescence of *A. luzonicus* (DPPH IC_{50} = 91.0 μg/mL, ORAC IC_{50} = 37.9 μg/mL) and fruit of *S. burahol* (DPPH IC_{50} = 253.7 μg/mL, ORAC IC_{50} = 32.2 μg/mL). These two species also had the highest TPC (202.1 and 133.0 μg GAE/mg extract, respectively). For all samples tested, the strong correlation was found between TPC and antioxidant activity. Based on our results, *A. luzonicus* and *S. burahol* have a promising potential as novel antioxidant rich food.

Keywords: birch flower; kepel fruit; non-communicable diseases; free radicals' inhibitors

1. Introduction

It is well known that oxidative stress is involved in the development of various human non-communicable diseases (NCDs), such as cardiovascular conditions and cancer, the incidence of which has been on the rise in recent years [1]. In the Philippines, NCDs account for 68% of all deaths and the probability of premature death (before the age of 70) from one of the four main NCDs (cardiovascular diseases, cancer, chronic respiratory disease, and diabetes) is 29% [2]. Several studies have shown that plant-based diet can reduce the risk of the development of NCDs [3]. This is likely due to the high content of various biologically active compounds collectively called antioxidants, that can inhibit or prevent oxidative injury to vulnerable molecules in living systems. Antioxidants operate on variety of levels, e.g. they can bind with reactive oxygen species and neutralize them. They also act as scavengers by preventing cell and tissue damage from free radicals or help with adaptation to oxidative stress. Several preclinical and clinical studies have already proven the effectiveness of antioxidants in the management of different NCDs, mainly due to their anti-aging, anti-cancer, anti-diabetic, anti-inflammatory, and hepato-, and neuroprotective effects [4,5]. Vitamins, carotenoids, and phenols are the most well-known natural antioxidants [6]. Phenolic compounds are mainly biosynthesized through the shikimic acid pathway from phenylalanine or tyrosine and the hydroxyl group on the benzene ring is responsible for their antioxidant properties [7]. In recent years, many scientific studies have confirmed the strong antioxidant effect of some phenolic compounds, as reviewed by San Miguel-Chávez [8]. Among them, especially flavonoids are highlighted as the most potent plant antioxidants, together with tannins, chalcones, coumarins, and phenolic acids. Several synthetic antioxidants are available today that have certain advantages, mainly their stability. However, consumers have a stronger preference for natural antioxidants especially due to their lower side effects and natural way of their origin [5,8,9].

Neglected and underutilized species (NUS) have been used for centuries for food and other purposes, but their use and importance have diminished over time. Currently, only a fraction of the possible edible species is consumed worldwide, and much of it remains underutilized despite their immense potential. However, with the changing demand for plant and crop traits, NUS are considered as potential future food crops that can contribute to improve nutrition, dietary diversity, and human health. In addition to the NUS ability to boost the diet, their benefits also lie in their environmental friendliness and resistance to harsh conditions and diseases [10]. Among all, no other crops are so positively perceived by society as fruits and vegetables. They are undisputable components of a healthy human diet containing various kind of antioxidants. It is already known that their increased daily intake may reduce the risk of NCD development, which is the number one cause of death worldwide [11]. Given this fact, the WHO recommended consuming at least 400 g of fruit and vegetables to prevent NCDs, whereby fruit should represent one third (meaning approximately 140 g) [12]. However, the most of the world's population is well below this threshold, especially in less developed countries. In recent years, the term "superfruits" has emerged and is receiving increasing attention as a marketing strategy to promote NUS with extraordinary health benefits, precisely in conjunction with their high antioxidant activity. Among the most well-known superfruits rich in antioxidants are the acai berry (*Euterpe oleracea* Mart.) or the goji berry (*Lycium chinense* Mill.), whose various products are now popular worldwide [13,14]. However, there are still a huge number of lesser-known fruits and vegetables, which antioxidant potency has not been evaluated yet.

The Philippines, consisting of more than 7000 islets, is probably the most biologically diverse country in the world with the endemism rate of plants estimated to be 39%; however, for certain taxa, it can be even higher [15,16]. Although it is home to an incredible range of species, rice and cereals comprise almost half of the total one-day per capita food intake, while fruits and vegetables lag behind [17]. Furthermore, fruit consumption in the Philippines has declined significantly over the past twenty-five years, from 188 g in 1996 to 93 g in 2021, far below the recommended daily intake of fruit, and ranking them 42nd within the group of 165 countries in terms of fruit consumption per capita in 2021 [18]. Low fruit and vegetable intake can be linked, among other things, to a lack of knowledge about their benefits and recommended daily intake, but also to socio-economic factors and the high price of imported crops [19]. The situation could be improved by increased awareness of locally available species and their inclusion in the daily diet. Nowadays, several local NUS crops and their products are already well established in Philippine markets, e.g. purple yam (*Dioscorea alata* L.; also known as ube), a root vegetable rich in essential vitamins and minerals and excellent source of phenolics antioxidants (mainly anthocyanins) [20], or pili nut (*Canarium ovatum* Engl.) which fruit pulp and seed are edible and contain a considerable amount of essential nutrients, antioxidants, and unsaturated fatty acids [21]. Despite their probably nutritional, health, economic and ecological importance, many Philippine plant species remains NUS as result of the lack of comprehensive botanical investigation and systematic scientific studies of their nutritional, biological and chemical properties. Moreover, due to massive deforestation many species are often threatened with extinction, which can have an adverse effect not only on the ecosystem but also on human well-being [22,23]. Thus, the main aim of the present study was to provide a comprehensive overview of the antioxidant potential and total phenolic content (TPC) of selected underutilized tree species traditionally consumed as a fruit or vegetable in the Philippines. By uncovering the antioxidant activity and phenolic content of these NUS, we aim to not only enrich the knowledge on biological

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effects and chemical composition of Philippine traditional fruits and vegetables, but also raise awareness about their health beneficial properties.

2. Materials and Methods

2.1. Plant Materials

Based on traditional uses of fruits and vegetables in Filipino cuisine [24–27], 11 tree species (Figure 1) have been chosen for the evaluation of their antioxidant potential and TPC. The plant samples were collected in May 2017, in 5 collection sites, namely the garden of the Institute of Plant Breeding (IPB) of the University of the Philippines Los Banos (UPLB), Dr. Coronel Fruit Conservation Farm, the Mount Makiling Forest (all Laguna province), Sambawan Island (Biliran province); and on the markets in Metropolitan Manila province. Fresh plant material was air-dried in shady place for several days and then stored in paper backs until the use. Plant samples were identified and authenticated by local expert Dr. Pablito M. Magdalita from the IPB UPLB, and ethnobotany experts, Prof. Ladislav Kokoska, and Dr. Johana Rondevaldova, from the Faculty of Tropical AgriSciences of the Czech University of Life Sciences, Prague (CZU), using the handbook "Important and Underutilized Edible Fruits of the Philippines" [24]. Voucher specimens are deposited in the herbarium of the Department of Botany and Plant Physiology of the Faculty of Agrobiology, Food and Natural Resources of the CZU. The Ethnobotanical data including scientific names of the species, family, local name, plant part tested, voucher specimen number, and traditional edible use are summarized in Table 1. The complete scientific names and authorities have been checked in Plants of the World Online [28].

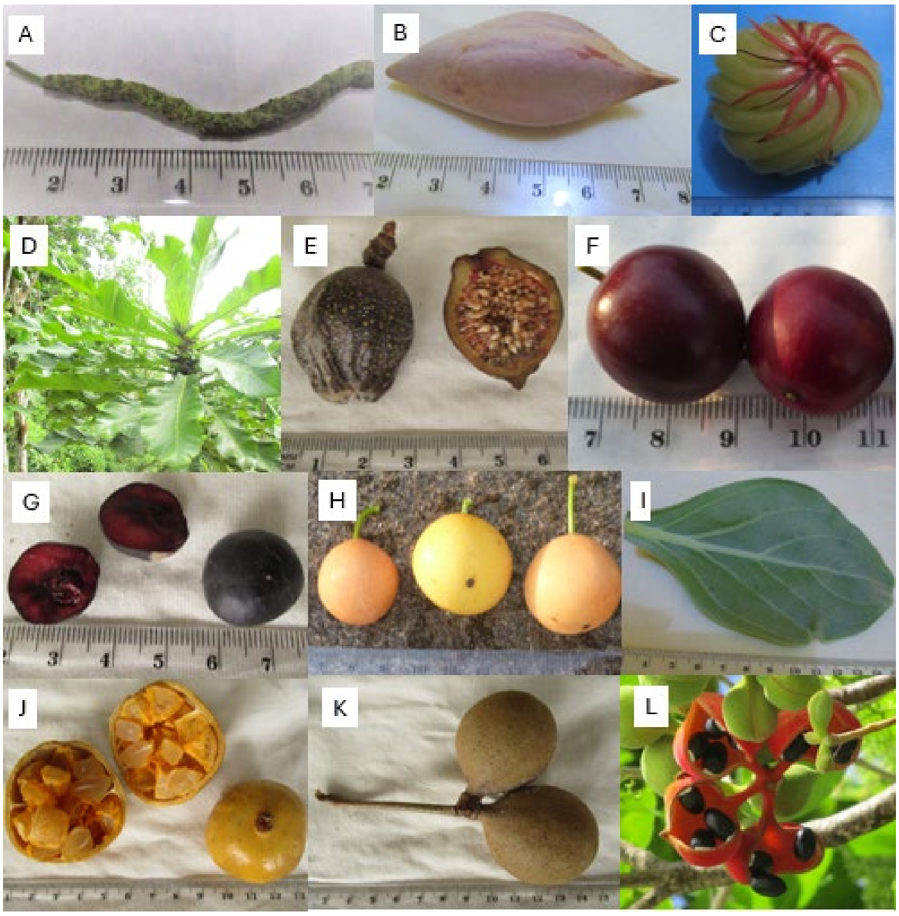

Figure 1. Photographs of collected neglected and underutilized edible tree species of the Philippines. Footnotes: A: *Allaeanthus luzonicus* inflorescence, B: *Canarium ovatum* seed, C: *Dillenia philippinensis* fruit pulp, D: *Ficus pseudopalma* leaves, E: *Ficus pseudopalma* fruit, F: *Flacourtia indica* fruit, G: *Flacourtia inermis* fruit, H: *Garcinia intermedia* fruit, I: *Heliotropium arboreum* leaf, J: *Posoqueria latifolia* fruit, K: *Stelechocarpus burahol* fruit, L: *Sterculia quadrifida* fruit with seeds.

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Table 1. Ethnobotanical data on neglected and underutilized edible tree species of the Philippines.

Scientific name of the species	Family	Local name	VSN	Collection site	Plant part used	Traditional edible uses [references]
Allaeanthus luzonicus (Blanco) Fern.-Vill., syn. *Broussonetia luzonica* (Blanco) Burr.	Moraceae	Baeg, himbabao	NA	Market in Manila, Metropolitan Manila Province	inflorescence	Inflorescence is cooked and eaten as vegetable [24,25]
Canarium ovatum Engl.	Burseraceae	Pili	NA	Garden of the Institute of Plant Breeding, Los Banos, Laguna Province	seed	Seeds are eaten as nuts [24,26]
Dillenia philippinensis Rolfe	Dilleniaceae	Katmon	02492KBFR8	Mount Makiling Forest, Los Banos, Laguna Province	fruit pulp	Fruit is acid and eaten raw as fruit or used as souring agent [24,26]
Ficus pseudopalma Blanco	Moraceae	Niog-niogan	02493KBFR9	Dr. Coronel Fruit Conservation Farm, Los Banos, Laguna Province	young leaves	Young leaves eaten as vegetable, raw or cooked [24,26]
					fruit pulp with seeds	Fruit eaten raw or cooked, taste like rhubarb [24,26]
Flacourtia indica (Burm.f.) Merr.	Salicaceae	Kakai, palutan	02494KBFRA	Garden of the Institute of Plant Breeding, Los Banos, Laguna Province	whole fruit	Tasty fruits eaten fresh or processed into jellies and jams [24,27]
Flacourtia inermis Roxb.	Salicaceae	Lovi-lovi, batoko plum	02495KBFRB	Garden of the Institute of Plant Breeding, Los Banos, Laguna Province	whole fruit	Acidic fruits eaten fresh or processed into jellies and jams [24,26,27]
Garcinia intermedia (Pittier) Hammel	Clusiaceae	Berba, waika plum	02496KBFRC	Garden of the Institute of Plant Breeding, Los Banos, Laguna Province	fruit pulp	Fruit eaten raw, with sweet to sour taste, or processed into jams, jellies and drinks [24]
Heliotropium arboreum (Blanco) Mabb., syn. *Argusia argentea* (L.f.) Heine	Boraginaceae	Kapal-kapal, salakapo	02511KBFR0	Sambawan Island beach, Biliran Province	leaves	Leaves with parsley-like taste, eaten as vegetable raw in salads or cooked [25]

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Posoqueria latifolia (Rudge) Schult.	Rubiaceae	Not known	02502KBFR0	Dr. Coronel Fruit Conservation Farm, Los Banos, Laguna Province	fruit pulp	Fleshy raw aril eaten as fruit [24]
Stelechocarpus burahol (Blume) Hook.f. & Thomson	Annonaceae	Kepel	02499KBFRF	Dr. Coronel Fruit Conservation Farm, Los Banos, Laguna Province	fruit pulp	Fruit pulp is eaten as fresh fruit [24]
Sterculia quadrifida R.Br.	Malvaceae	Red-fruited kurrajong	02519KBFR8	Sambawan Island beach, Biliran Islands province	seeds	Seeds are eaten raw or roasted as nuts [24]

NA: not available; VSN: voucher specimen number.

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2.2. Chemicals and Reagents

2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), fluorescein sodium salt (FL), and gallic acid were purchased from Sigma-Aldrich (Prague, Czech Republic). Dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent, and methanol were bought from Penta (Prague, Czech Republic). Na_2CO_3 and KH_2PO_4 salts were obtained from Erba Lachema (Brno, Czech Republic), while K_2HPO_4 was from Lach-Ner (Neratovice, Czech Republic).

2.3. Preparation of Extracts

Air-dried plant material was grounded to fine powder using electric mills GM100 (Retsch, Germany) and Tissue Lyser II. (Retsch, Germany) and 1 g of powder was extracted in 30 mL of 100 % methanol and left for 24 h to shake in a GFL3005 shaker (Burgwedel, Germany). Subsequently, the extract was filtered and concentrated to dryness by Rotary evaporator R-200 (Büchi, Switzerland) in a vacuum at 40 °C. Dry residue was dissolved in DMSO to a stock concentration of 51,200 µg/mL and stored at -20 °C until further analyses. In total, 12 extracts were prepared and the yield of dry residues is part of Table 2.

2.4. Antioxidant Activity Evaluation

Confirming antioxidant activity by more than one method can offer a more complete evaluation of the total antioxidant capacity of individual plants [29]. Thus, we examined antioxidant activity by two methods varying based on the mode of action, the DPPH representing electron atom transfer reaction and oxygen radical absorbance capacity (ORAC) representing hydrogen transfer assay [30]. Trolox was used as a positive control for both assays. Results were calculated by Gen 5 software (BioTek, Winooski, USA) and expressed as mean values of half maximal inhibitory concentration (IC_{50}) with standard deviation (±SD) in µg/ml.

2.4.1. DPPH Assay

The method based on Sharma and Bhat [31] was used to assess of extracts' ability to inhibit DPPH radical. A two-fold serial dilution of each extract and Trolox was performed in methanol via automatized pipetting platform Freedom Evo 100, equipped with a four-channel liquid handling arm (Tecan, Mannedorf, Switzerland) in 96- well microtiter plates. Subsequently, 75 µL of methanol and 25 µL of 1 mM methanol solution of DPPH was added into each tested well of microtiter plate to create a final volume of 200 µL. The final tested concentrations ranged from 0.125 to 256 µg/mL. Plates were incubated for 30 min in the dark at room temperature, and then absorbance was measured spectrophotometrically at 517 nm using Cytation 3 Multimode Reader (BioTek,Winooski, USA).

2.4.2. ORAC Assay

The method previously described by Ou et al. [32] and slightly modified by Tauchen et al. [33] and Rondevaldova et al. [34] was used for the evaluation of the ability of the extracts to protect FL from AAPH degradation. Two-fold serial dilution of each extract was prepared in phosphate buffer (75 mM, pH 7.0) in black 96-well microtiter plates using the automated pipetting platform Freedom EVO 100, equipped with a four-channel liquid handling arm (Tecan, Mannedorf, Switzerland). Afterwards, 150 µL of FL (48 nM) was added to each well (except for the outer wells of the plate that were filled with 200 µl of distilled water for better thermal mass stability and not used for evaluation). After 10 min of incubation at 37 °C in Memmert Incubator IF110plus (Memmert, Schwabach, Germany), the reaction was started by adding of 25 µL of freshly prepared AAPH (153 mM). Control (FL with AAPH in phosphate buffer) and blank (FL in phosphate buffer) were part of each microtiter plate. Plates were subsequently incubated for 90 minutes, and the fluorescence changes were measured by Cytation 3 Multimode Reader (BioTek, Winooski, USA) with excitation and emission wavelengths set at 485 and 528 nm, respectively.

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2.5. Determination of TPC

To estimate TPC, a slightly modified method previously described by Singleton et al. [35] was used. The method was adjusted for 96-well microtiter plates. Initially 100 µl of each extract was mixed with 25 µl of pure Folin-Ciocalteu reagent and submitted to orbital shaking at approximately 500 rpm for 10 min. The reaction was started by adding 75 µl of 12% Na_2CO_3. The plates were kept in the dark for 2 h at 37 °C. Absorbance was measured at 700 nm by Cytation 3 Multimode Reader using Gen5 software (BioTek Instruments, Winooski, USA). Fourteen concentration levels of gallic acid (two-fold dilution in the range from 0.015625 to 126 µg/ml) were used to create the standard calibration curve. The results were expressed as mean values ± SD as gallic acid equivalents (µg GAE/mg extract).

2.6. Statistics and Calculations

All experiments were performed as three independent tests each carried out in triplicate. Linear correlation coefficients (r) between DPPH, ORAC and TPC were established using Pearson product-moment correlation in Microsoft Excel 365 (Microsoft, Redmond, WA, USA). The degree of the correlation was interpreted using the guide of Evans [36] as follows (for the absolute value of r): 0-0.19 very weak, 0.20–0.39 weak, 0.40–0.59 moderate, 0.60–0.79 strong, 0.80-1 very strong. The mean values of each analysis (DPPH, ORAC, and TPC) for each plant species were visualized as a heatmap using Euclidean distance for clustering with pheatmap v 1.0.12 in R [37].

3. Results

In total, twelve extracts prepared from eleven NUS of edible trees of the Philippines were evaluated for their antioxidant potential by two different assays (DPPH and ORAC). Moreover, as phenolics are often strongly associated with antioxidant potential, TPC was also determined. To the best of our knowledge, this is the first report on any antioxidant activity and TPC of *Allaeanthus luzonicus* inflorescences, *Canarium ovatum* seeds, *Ficus pseudopalma* leaves, *F. pseudopalma* pulp with seeds, *Garcinia intermedia* fruits, *Heliotropium arboreum* leaves, *Posoqueria latifolia* arils, and *Sterculia quadrifida* seeds. Antioxidant activity represented by values of IC_{50} and contents of total phenolics determined for tested parts of NUS of edible trees of the Philippines are shown in the Table 2.

Table 2. Antioxidant activity and total phenolic content of neglected and underutilized edible tree species of the Philippines.

Species	Extraction yield (%)	Antioxidant assay /mean IC_{50} ± SD (µg/ml)		TPC (µg GAE/ mg extract)
		DPPH	ORAC	
Allaeanthus luzonicus	12.0	253.7±13.1	32.2±12.9	133.0±4.6
Canarium ovatum	10.2	>256	>256	23.2±4.1
Dillenia philippinensis	21.5	>256	>256	18.2±1.7
Ficus pseudopalma leaves	7.8	>256	122.2±8.1	16.1±0.7
F. pseudopalma pulp with seeds	7.2	>256	106.7±25.2	18.6±0.9
Flacourtia indica	57.5	>256	203.7±25.4	45.9±1.1
Flacourtia inermis	38.1	>256	236.6±14.6	25.6±1.0
Garcinia intermedia	26.1	>256	>256	17.8±1.9
Heliotropium arboreum	11.1	>256	>256	16.7±4.1
Posoqueria latifolia	20.1	>256	166.7±21.9	19.6±0.8
Stelechocarpus burahol	12.3	91.0±12.6	37.9±8.7	202.1±6.7
Sterculia quadrifida	13.1	>256	152.3±9.4	55.6±2.4
Trolox		9.2±2.5	7.9±1.2	

Footnotes: DPPH: 2,2-diphenyl-1-picrylhydrazyl; GAE: gallic acid equivalent; IC_{50}: half maximal inhibitory concentration; ORAC: oxygen radical absorbance capacity; SD: standard deviation; TPC: total phenolic content.

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In both antioxidant assays, the most active extracts were *A. luzonicus* inflorescence (IC_{50} for DPPH and ORAC at 253.7 µg/ml and 32.2 µg/ml, respectively) and *Stelechocarpus burahol* fruit (IC_{50} for DPPH and ORAC at 91.0 µg/ml and 37.9 µg/ml, respectively). These two species were also highest in respective TPCs (133.0 and 202.1 µg GAE/mg extract) while the other samples showed much lower TPC values ranging from 16.1 to 55.6 µg GAE/mg extract. No other species could inhibit DPPH even at the highest concentration tested (256 µg/ml). A moderate antioxidant effect was exhibited by *F. pseudopalma* leaves, *F. pseudopalma* pulp with seeds, *P. latifolia* arils, and *S. quadrifida* seeds in the ORAC assay, with IC_{50} ranging from 106.7 to 166.7 µg/ml, whereas other species showed only weak or no effect ($IC_{50} \geq 203.7$ µg/ml). In accordance with the widely accepted view that phenolics are highly active antioxidants, we found a very strong correlation between ORAC and TPC, and DPPH and TPC ($r=0.920$ and $r=0.825$, respectively, both significant at $p<0.05$).

Based on the results of DPPH and ORAC assays and TPC analysis, the heatmap visualizes the hierarchical clustering of antioxidant properties in plant species studied, highlighting the differences in antioxidant properties among the NUS of edible trees of the Philippines and providing a visual representation of their potential health benefits (Figure 2). The dendrogram groups the species into two major clusters: one with generally higher antioxidant effects, including *A. luzonicus* and *S. burahol*, and a second including all the other species tested showing lower antioxidant properties. *S. burahol* exhibits high values across all three assessments, indicating its strong antioxidant potential and high phenolic content. *A. luzonicus* shows high ORAC but low DPPH values, with medium TPC. Species present in the first subcluster of the second cluster such as *Ficus pseudopalma* (both pulp with seeds and leaves), *Flacourtia indica, P. latifolia*, and *S. quadrifida*, display moderate values for ORAC, while having no activity in DPPH and low levels of TPC, suggesting that other compounds than phenolics could be responsible for their antioxidant effect. Species occurring in the second subcluster of the second cluster, including *C. ovatum*, *Dillenia philippinensis*, *G. intermedia*, *H. arboreum*, and *Flacourtia inermis*, generally exhibit almost no antioxidant potential with very low TPC.

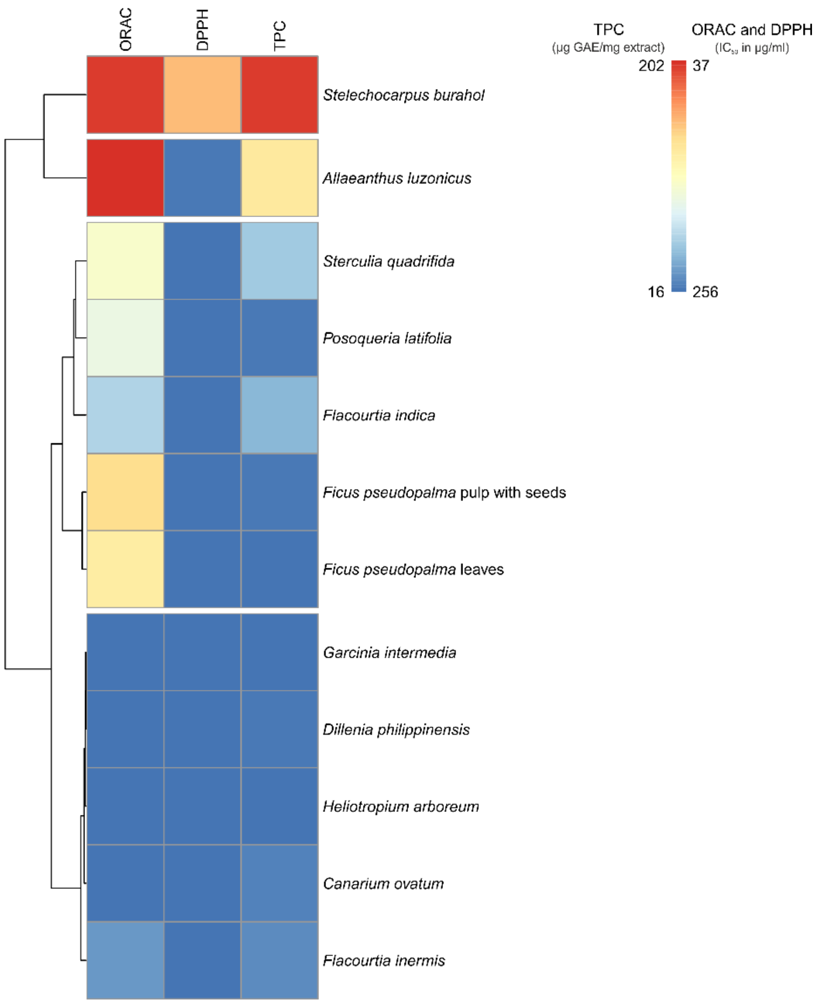

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Figure 2. Hierarchical clustering of antioxidant activities in various plant species. The color gradient represents the intensity of antioxidant activity and total phenolic content. DPPH: 2,2-diphenyl-1-picrylhydrazyl; GAE: gallic acid equivalent; IC_{50}: half maximal inhibitory concentration; ORAC: oxygen radical absorbance capacity; TPC: total phenolic content.

4. Discussion

Among all NUS tested in this study, the fruit of *S. burahol*, a tree commonly known as kepel that is naturally found and cultivated in South-East Asia, produced the most potent antioxidant properties. Ripe fruit with juicy pulp is eaten fresh and used in traditional medicine as a diuretic or to prevent kidney inflammation. Interestingly, its consumption gives a pleasant fragrance to body excretions such as urine and sweat [27]. Several studies have already evaluated the antioxidant activity of *S. burahol*, but to the best of our knowledge, this is the first study conducted using ORAC assay. Herlina et al. [38] reported strong inhibitory activity of the fruit methanol extract on DPPH with IC_{50} 7.5 µg/ml and TPC of 58.3 µg GAE, which is in accordance with our study showing also quite strong ability to inhibit DPPH radical corresponding with one of the highest TPC from all tested samples. Similarly, Sundari et al. [39] observed that ethyl acetate and methanol extracts obtained from fruit flesh of this species produced very strong antioxidant effect against DPPH (IC_{50} 19.3 and 12.0 µg/ml, respectively). Subsequent chemical analysis revealed that one of the main constituents is epigallocatechin gallate, a polyphenolic antioxidant naturally occurring in tea. Moreover, the study of Ismail et al. [40] confirmed the beneficial effect of *S. burahol* fruit extract *in vivo* on oxidative stress in the serum, liver, heart and brain of high-fat diet-fed rats.

A. luzonicus, an endemic tree of the Philippines naturally occurring in lowland thickets and forests, is the species that showed the second strongest antioxidant properties among all samples tested in this study. Its inflorescences are consumed as a favorite indigenous vegetable mainly in Northern Philippines in various traditional Filipino dishes such as 'pinakbet' (vegetable stew) or 'bulanglang' (vegetable soup) [41]. According to Antonio and Galacgac [42], this species is seasonal in availability with distinct flowering season from June to March; however, off-season varieties producing flowers in other months also exist. The genus *Allaeanthus* contains only four accepted species and is very closely related to the genus *Broussonetia*; moreover, *A. luzonicus* is more commonly known by the synonym *Broussonetia luzonica* [28,43]. In the present study, the antioxidant potential of its inflorescences was analyzed for the first time and the results showed interesting antioxidant potential in both DPPH and ORAC assays. On the other hand, there are several studies focused on the antioxidant activity of other species from genus *Broussonetia*, as reviewed by Wang et al. [44]. For example, Sun et al. [45] described *B. papyrifera* fruits as a promising species to prevent oxidation and its antioxidant activity was positively correlated with their TPC. Previous phytochemical investigation of species in genus *Broussonetia* revealed that *B. kazinoki*, *B. papyrifera*, and *B. zeylanica* contain alkaloids and phenols, mainly phenolic acids and flavonoids (many of them are prenylated) belonging to the diphenylpropane, chalcone, flavan, flavanone, flavone, flavonol, and aurone classes [46]. Some of these compounds such as 3,4-dihydroxybenzoic acid, dihydroconiferyl alcohol, ferulic acid, and curculigoside C have demonstrated ability to inhibit DPPH with IC_{50} ranging between 39.5-65.6 µM [47].

In the contrast to our findings, *D. philippinensis*, *F. indica* and *F. inermis* showed antioxidative properties in previous studies. For example, Barcelo [48] mentioned *D. philippinensis* fruit as the most active out of 31 edible wild fruits from Benguet province, Philippines. On the other hand, he detected one of the lowest TPC, being in accordance with our results. The difference in results of antioxidant activity between the present study and the study of Barcelo can be caused by fruit processing before evaluation, as in our study we evaluated methanol extract prepared from air-dried plant material, however, Barcelo extracted fresh samples directly. Several previous studies rate various *Flacourtia* species quite positively for their antioxidant properties. For example, Alakolanga et al. [49] showed moderate antioxidant activity of *F. inermis* in the DPPH assay with an IC_{50} value of 66.2 µg/ml. Similarly, Perera et al. [50] reported an IC_{50} for DPPH assay of 89 µg/ml and TPC of 8.1 mg GAE/g for fruit of *F. indica*. In addition, Selim et al. [51] found that *F. indica* fruit extract can significantly

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prevent kidney dysfunction in rats caused by oxidative stress. Ripe fruits of *F. indica* and *F. inermis* have previously been identified as rich sources of phenolic antioxidants such as quercetin, rutin, and esculin [49]. However, our study showed no antioxidant activity of these two species in the DPPH assay and only weak activity for ORAC, along with relatively low TPC content. These differences are probably due to the different method of processing the plant material (fresh fruit vs. extract prepared from dried plant material) or different extraction solvents used (ethanol vs. methanol).

Despite the recognized importance of NUS for food and nutrition security, biodiversity conservation, and environmental and socioeconomic benefits, these crops are still not sufficiently utilized in agricultural systems [52]. Incorporating underutilized tree species with antioxidant properties, such as *A. luzonicus* and *S. burahol*, into agroforestry systems in the Philippines, can bring many benefits, as they will contribute to the healthy diets of local populations, and will increase sustainability of agricultural production in the region [10]. *S. burahol* is already well established on some plantations in Indonesia, especially on the island of Java. However, its cultivation remains limited to home gardens in the Philippines [53]. *A. luzonicus* is an indigenous tree that thrives all around the Philippines, but it is consumed only in some parts of Luzon Island [42]. Therefore, appropriate production and processing technologies should be developed for successful introduction of antioxidant-rich food products derived from *A. luzonicus* and *S. burahol* to the market in the Philippines.

5. Conclusions

In conclusion, this study comprehensively evaluates the antioxidant potential and TPC of extracts from eleven NUS of edible trees from the Philippines, many of which were analyzed here for the first time. Our findings reveal significant differences in antioxidant activity among species, with the inflorescence of *A. luzonicus* and the fruits of *S. burahol* showing the highest antioxidant potential in both DPPH and ORAC assays and the highest TPC values. These results underline the potential of these species, especially *A. luzonicus* and *S. burahol*, as promising sources of natural antioxidants. The strong correlation between TPC and antioxidant activity in tested species supports the widely held view that phenolic compounds are key contributors to antioxidant properties. This study increases the knowledge of the antioxidant potential of Philippine NUS, and highlights their potential health benefits. Further research should be focused on the detailed determination of nutritional composition of *A. luzonicus* and *S. burahol*, especially on the isolation and characterization of specific bioactive compounds responsible for their antioxidant activity, but also on the verification of their safety profile regarding the possible content of antinutrients. In the future, the key findings of this study could lead to the discovery of new antioxidants and development of novel food and agricultural products with antioxidant properties.

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