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Phytochemical screening, antioxidant potential, and nutritional analysis of five *Polyscias* species: An *in vitro* and *in vivo* study

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ABSTRACT

Introduction: Species of the genus *Polyscias* (Araliaceae) have long been used in traditional medicine because of their anti-inflammatory, antibacterial, and antioxidant properties. However, information on the phytochemical composition, antioxidant activity, and nutritional value of several *Polyscias* species remains limited. This study investigated the phytochemical profiles, antioxidant potential, and nutritional composition of extracts from five *Polyscias* species: *P. fruticosa*, *P. filicifolia*, *P. serrata*, *P. balfouriana*, and *P. ilicifolia*.

Methods: Phytochemical screening and quantitative analyses were performed to determine total phenolic and flavonoid contents. Antioxidant activity was assessed using DPPH, ABTS, and ferric-reducing assays. Nutritional composition, including moisture, protein, lipid, carbohydrate, and energy content, was also analyzed. The protective effects of *P. fruticosa* extract against oxidative stress induced by paraquat and hydrogen peroxide were evaluated in *Drosophila melanogaster*.

Results: *P. fruticosa* contained the highest levels of phenolics and flavonoids and exhibited stronger antioxidant capacity in the ABTS and ferric-reducing assays than the other tested species. *In vivo* experiments showed that *P. fruticosa* extract improved the survival and lifespan of *D. melanogaster* under oxidative stress conditions. Nutritional analysis indicated that *P. balfouriana* had the highest energy content (74.99±1.56 kcal), whereas *P. ilicifolia* showed the highest protein content (4.25%).

Conclusion: The findings demonstrate the therapeutic and nutritional potential of *Polyscias* species, particularly *P. fruticosa*, as promising natural sources for functional food and nutraceutical applications.

Implication for health policy/practice/research/medical education:
The strong antioxidant activity of *Polyscias* species, especially *Polyscias fruticosa*, highlights their potential for development as functional foods and nutraceuticals to support the prevention of oxidative stress–related diseases. These findings support further research, including clinical studies, to confirm their safety, efficacy, and therapeutic applications in humans.

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Introduction

The relationship between diet and health has been well established by numerous studies, leading to growing interest in functional foods and nutraceuticals (1). Finding reliable, safe treatments to improve quality of life remains a persistent goal. For centuries, diverse cultures have utilized plant extracts and natural remedies to treat various ailments (2). Plants naturally synthesize a variety of secondary metabolites, including phenolic compounds and carotenoids, which hold considerable value in the pharmaceutical and food industries (3). The concentration of these bioactive compounds is influenced by factors such as agricultural practices, environmental conditions, genetic traits, and stress (4).

The Araliaceae family is a significant taxonomic group within the plant kingdom, encompassing 43 genera and 1,400 species, and is extensively used in conventional and contemporary phytotherapeutic procedures. These bioactive compounds are known for their broad spectrum of biological activities, which include anti-proliferative,

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anti-inflammatory, anti-parasitic, and antidiabetic effects. Additionally, they have demonstrated significant therapeutic potential in modulating the functions of the nervous and cardiovascular systems (5).

One underexplored plant genus in the Araliaceae family is *Polyscias*, known for its phytochemical diversity and therapeutic potential (6). There are roughly 116 species of the genus that are frequently used as ornaments (7). Typically grown as perennial shrubs, *Polyscias* species are native to tropical Pacific and Southeast Asian regions (8). In traditional medicine, *Polyscias* roots have been used as febrifuges, anti-dysentery agents, diuretics, and treatments for neuralgia and rheumatism. The leaves have been employed as tonics, antibacterials, anti-inflammatories, and digestive aids (9). For example, *Polyscias fulva* has been used in traditional medicine to address venereal infections, fever, malaria, mental illnesses, and obesity (10). *Polyscias fruticosa* is recognized for its anti-inflammatory and tonic properties (11). Traditional uses of specific *Polyscias* species highlight their diverse therapeutic applications (6). For instance, *Polyscias cumingiana* has stimulant and antiviral properties; *Polyscias fruticosa* is noted for its stimulant, vulnerary, and emetic qualities (12); *Polyscias scutellaria* leaves are used for their stomachic, astringent, disinfectant, and ulcer-healing properties (13). Other species, such as *Polyscias samoensis*, have been used as poultices for skin conditions, while *Polyscias fulva* roots and bark decoctions have been used as aphrodisiacs, tonics, treatments for inflammation, and for managing obesity (14,15).

Notwithstanding the increasing scholarly interest in the genus, comparative investigations into the phytochemical composition and biological activities of diverse *Polyscias* species remain limited. This study addresses this lacuna by evaluating the phytochemical profiles and biological properties of five *Polyscias* species: *Polyscias fruticosa*, *P. filicifolia*, *P. serrata*, *P. balfouriana*, and *P. ilicifolia*. This study provides the first integrated evaluation combining phytochemical profiling, *in vitro* antioxidant assays, *in vivo* validation, and nutritional analysis across multiple *Polyscias* species, thereby addressing the current lack of comprehensive comparative studies within this genus.

Materials and Methods

Plant materials and extractions

Five *Polyscias* species (*P. fruticosa, P. filicifolia, P. serrata, P. balfouriana,* and *P. ilicifolia*) were collected from Can Tho city, Vietnam, and taxonomically authenticated (Figure 1). Voucher specimens (PS-CT601–605) were deposited at the Department of Biology, Can Tho University. Leaves were air-dried at 25–30 °C until constant weight and ground using a DFY-600 grinder (Linda Machinery, China) to obtain a uniform powder. For extraction, 100 g of dried powder was macerated in 1 L of 98% ethanol at room temperature (25 ± 2 °C) for 5–7 days with manual stirring every 18 h. The extraction was repeated three times, and

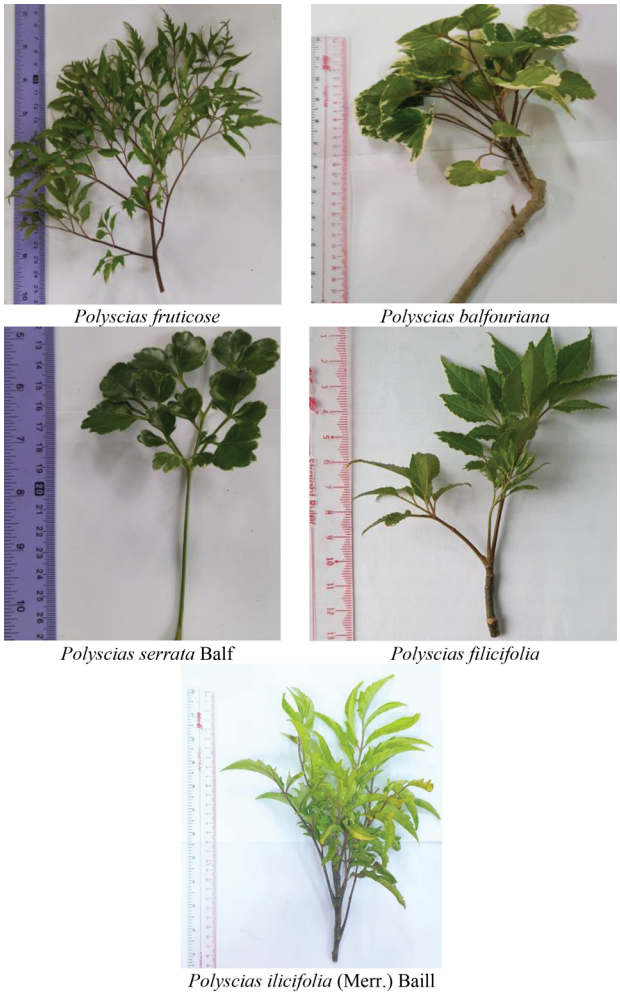

Figure 1. Five *Polyscias* species collected in Can Tho city, Vietnam.

the combined filtrates were passed through Whatman No. 1 filter paper and concentrated under reduced pressure at 40 °C using a rotary evaporator. Extracts were stored at −20 °C until analysis.

Screening and quantitative analysis of phytochemicals

Preliminary phytochemical screening was performed according to previously published methods to detect major secondary metabolites, including alkaloids, flavonoids, phenolics, saponins, and quinones (16-18). Because the phytochemical constituents were subsequently quantified, only the quantitative results were considered for further analysis and discussion.

Total phenolic contents

The total phenolic content (TPC) was quantified using a modified Folin–Ciocalteu assay (19). Briefly, 250 µL of extract (100 µg/mL) was mixed with 250 µL Folin–Ciocalteu reagent and 250 µL of 10% Na_2CO_3, incubated at 40 °C for 30 min, and the absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Gallic acid (0–140 µg/mL, $R^2 > 0.99$) was used as the calibration standard. Results were expressed as mg gallic acid equivalents per

gram of dry extract (mg GAE/g). All experiments were performed using three independent extract preparations (biological replicates), and each measurement was conducted in triplicate (technical replicates). Results are expressed as mean ± standard deviation (SD).

Total flavonoid contents

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method (20). The reaction involved sequential addition of 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH, followed by absorbance measurement at 510 nm. Quercetin (0–500 µg/mL, $R^2 > 0.99$) was used as the calibration standard. Results were expressed as mg quercetin equivalents per gram of dry extract (mg QE/g). All experiments were performed using three independent extract preparations (biological replicates), and each measurement was conducted in triplicate (technical replicates). Results are expressed as mean ± SD.

In vitro antioxidant activity

Antioxidant activity was evaluated using ABTS, DPPH, and reducing power assays. For the ABTS assay, $ABTS^{\bullet+}$ solution (7 mM ABTS and 2.45 mM $K_2S_2O_8$) was incubated in the dark for 12–16 h and diluted to an absorbance of 0.70 ± 0.05 at 734 nm. A total of 10 µL extract was mixed with 990 µL $ABTS^{\bullet+}$ solution, incubated for 6 min at room temperature, and the absorbance was measured at 734 nm (21).

For the reducing power assay, 500 µL extract was mixed with 0.2 M phosphate buffer (pH 6.6) and 1% $K_3Fe(CN)_6$, incubated at 50 °C for 20 min, followed by the addition of 10% trichloroacetic acid and centrifugation at 3,000 rpm for 10 min. The supernatant was reacted with 0.1% $FeCl_3$, and absorbance was measured at 700 nm (22).

For the DPPH assay, extracts were incubated with 1000 µg/mL DPPH solution at 30°C for 30 min in the dark, and absorbance was recorded at 517 nm. Trolox was used as a positive control, and EC_{50} values were calculated using nonlinear regression (23). All experiments were performed using three independent extract preparations (biological replicates), and each measurement was conducted in triplicate (technical replicates). Results are expressed as mean ± SD.

In vivo antioxidant activity

Determination of resistance to paraquat (PQ) exposure

The fly stock used in this study was Canton-S (CS) wild-type *Drosophila melanogaster* (Bloomington Drosophila Stock Center, USA). Flies were maintained on a standard cornmeal–yeast–agar medium at 25 ± 1°C, 60–70% relative humidity, under a 12:12 h light–dark cycle. Male flies (48 h post-eclosion) were collected under light CO_2 anesthesia and allowed to recover for 24 h prior to treatment. Flies were randomly assigned to three groups: (i) extract-supplemented group (1 mg/mL extract incorporated into food), (ii) positive control (gallic acid, 0.05 mg/mL), and (iii) untreated control (standard diet). The composition of the diet and volume of food per vial (10 mL) were kept constant across treatments. Food was replaced every 48 h to maintain stability of bioactive compounds (24).

After 20 days of feeding, flies were starved for 5 h in empty vials containing moist filter paper to prevent dehydration, then transferred to vials containing Whatman No. 1 filter paper soaked with 1 mL of freshly prepared 20 mM paraquat solution in 5% sucrose. Each vial contained 10 flies, with five independent replicates per group (n = 50). Vials were maintained under the same environmental conditions as described above. Mortality was recorded at 4 h intervals until all flies died. Mean lifespan, median (50%) survival time, and maximum lifespan (mean survival of the longest-living 10% of flies) were calculated. Survival curves were constructed using Kaplan–Meier analysis to assess resistance to paraquat-induced oxidative stress.

Determination of resistance to hydrogen peroxide-induced oxidative stress

The hydrogen peroxide resistance assay was performed using the same fly strain, age, and rearing conditions as described above (25). Flies were maintained on their respective diets (control, 1 mg/mL extract, or 0.05 mg/mL gallic acid) for 5 days prior to the assay. Ten flies per vial (five replicates) were transferred to empty vials and starved for 6 h with access to moist filter paper. Subsequently, flies were exposed to oxidative stress by transferring them to vials containing Whatman No. 1 filter paper saturated with 1 mL of freshly prepared 10% (v/v) H_2O_2 in 5% (w/v) sucrose solution. The filter paper and H_2O_2 solution were replaced every 24 h to ensure consistent exposure. Flies were maintained at 25 °C, and mortality was recorded at 12 h intervals until all individuals died. Survival parameters, including mean, median, and maximum lifespan, were calculated, and Kaplan–Meier survival analysis was performed. This assay allowed quantitative assessment of the protective effect of *Polyscias* extracts against hydrogen peroxide-induced oxidative stress.

Estimation of proximate composition

Proximate composition was determined using fresh leaf material of the five *Polyscias* species, not the concentrated ethanolic extracts. Moisture content was measured by oven drying at 105°C to constant weight. Ash content was determined by incineration at 500 °C for 5 h, lipid content by Soxhlet extraction using petroleum ether (60–80 °C) for 6–8 h, protein content by the micro-Kjeldahl method (N × 6.25), and carbohydrate content by difference were determined according to AOAC methods (26,27). Energy values were calculated using Atwater factors (4, 9, and 4 kcal/g for protein, fat, and carbohydrates, respectively) (26,27). All nutritional parameters are therefore expressed on a fresh weight basis.

Statistical analysis

Statistical analysis was performed using Minitab 16. All experiments were performed using three independent extract preparations (biological replicates), and each measurement was conducted in triplicate (technical replicates). Results were expressed as mean ± SD. Differences among groups were assessed using one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant. Graphs were generated using Microsoft Excel 2016.

Results

Qualitative phytochemical compounds

Table 1 indicates the presence of components of the extract from 5 species: *P. fruticosa, P. filicifolia, P. serrata, P. balfouriana, and P. ilicifolia*. The results showed that phenolics, flavonoids, alkaloids, saponins, and quinones (except for *P. ilicifolia*) were all present in the tested extract. The tannin compound was only found in *P. ilicifolia*.

Total polyphenol and flavonoid contents

Table 2 summarises TPC and TFC in five plant extracts of the *Polyscias* genus. The TPC values varied widely, ranging from 109.93 to 316.09 mg GAE/g dry extract weight. The decreasing TPC in plant extract was in the following order: *P. fruticosa* > *P. filicifolia* > *P. serrata* > *P. balfouriana* > *P. ilicifolia*. *P. fruticosa* exhibited the highest phenolic content at 316.09 (mg GAE/g dry extract weight), while the lowest TPC was observed in *P. ilicifolia* with a value of 109.93 (mg GAE/g dry extract weight).

DPPH, ABTS, and FR radical scavenging activities

The DPPH, ABTS, and FR radical scavenging activities were demonstrated by the EC_{50} values (half-maximal effective concentration) presented in Table 3. In the DPPH assay, the extracts showed relatively weak radical-scavenging activity, with all EC_{50} values exceeding 1200

Table 1. Qualitative phytochemical compounds in five *Polyscias* species extracts

Extracts	Phenolics	Flavonoids	Tannins	Alkaloids	Saponins	Quinones
P. fruticosa	+	+	-	+	+	+
P. balfouriana	+	+	-	+	+	+
P. serrata	+	+	-	+	+	+
P. ilicifolia	+	+	+	+	+	-
P. filicifolia	+	+	-	+	+	+

Note: + present; - absent.

Table 2. Total phenolic and flavonoid contents in five *Polyscias* species extracts

Extracts	Total phenolic (mg GAE/g dry extract weight)	Total flavonoid (mg QE/g dry extract weight)
P. fruticosa	316.09 ± 2.63^{a}	242.05 ± 4.61^{a}
P. balfouriana	159.06 ± 9.24^{d}	217.91 ± 1.00^{b}
P. serrata	237.14 ± 2.63^{c}	141.30 ± 3.60^{e}
P. ilicifolia	109.93 ± 4.02^{e}	177.61 ± 10.14^{c}
P. filicifolia	268.72 ± 4.56^{b}	138.26 ± 6.18^{c}

Note: Values are presented as mean ± standard deviation (SD) of three independent replicates (n = 3). Within each column, the means followed by different superscript letters differ significantly at 0.05 probability level ($P < 0.05$), according to the Tukey test. GAE/g: gallic acid equivalent per gram of dry extract; QE/g: quercetin equivalent per gram of dry extract

Table 3. EC_{50} (half maximal effective concentration) values of the antioxidant capacity of five species of the *Polyscias* genus

Sample	EC_{50} value (µg/mL)		
	DPPH	ABTS	FR
P. fruticosa	1319.49 ± 4.84^{d}	147.12 ± 1.62^{e}	68.80 ± 1.56^{d}
P. balfouriana	4755.17 ± 21.29^{a}	321.60 ± 1.15^{b}	248.48 ± 9.38^{b}
P. serrata	2478.12 ± 8.97^{c}	370.66 ± 0.92^{a}	124.46 ± 1.57^{c}
P. ilicifolia	2626.39 ± 4.23^{b}	187.62 ± 6.36^{c}	342.05 ± 2.56^{a}
P. filicifolia	1227.32 ± 3.19^{e}	177.50 ± 1.94^{d}	122.92 ± 1.51^{c}
Gallic acid	3.60 ± 0.30^{f}	0.44 ± 0.01^{f}	0.68±0.03^{e}

Note: Values are presented as mean ± standard deviation (SD) of three independent replicates (n = 3). Within each column, means followed by different superscript letters differ significantly at the 0.05 probability level ($P < 0.05$), according to Tukey test. DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); FR: ferric-reducing assay.

μg/mL. Within this range, the activity followed the order: *P. filicifolia* > *P. fruticosa* > *P. serrata* > *P. ilicifolia* > *P. balfouriana*.

In the ABTS assay, *P. fruticosa* exhibited the strongest radical-scavenging activity among the tested species, whereas *P. serrata* showed the weakest activity. A similar trend was observed in the ferric-reducing assay, in which *P. fruticosa* demonstrated the highest reducing capacity. Lower EC_{50} values indicate stronger antioxidant activity.

Protective effect of *Polyscias fruticosa* against paraquat-induced mortality in *Drosophila melanogaster*

Surviving flies were counted every 4 h post-exposure, and we plotted survival percentage graphs for each 4 h of paraquat (PQ) co-exposure across different feedings. The group experiments were divided into 3 groups: PQ-induced flies, PQ-induced flies treated with *P. fruticosa* extract, and gallic acid (positive control). Figure 2 indicates that PQ significantly increased fly mortality, with averages of 50% and 91.6% at 12 h and 24 h, respectively. Meanwhile, the treatment of *P. fruticosa* extract provided significant protection against PQ-induced toxicity, with 16% of flies surviving at 24 h, compared with 8.3% survival being observed for PQ-exposed flies. Interestingly, the supplement diet of *P. fruticosa* extract enhanced the survival time of flies up to 32 h, compared to 28 h in PQ-induced flies. The positive control elevated the survival of flies to 48 h. All of the flies were dead at 28 h, 36 h, and 52 h in the PQ-induced flies, *P. fruticosa,* and the positive control, respectively. The result suggested that *P. fruticosa* could also improve the survival of flies pretreated with PQ.

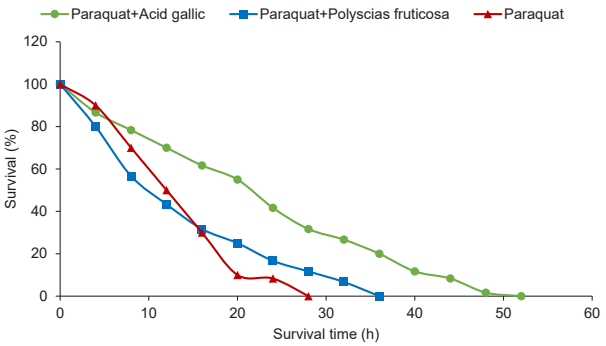

Figure 2. Protective effect of *Polyscias fruticosa* extract on survival of *Drosophila melanogaster* under paraquat-induced oxidative stress. Data are presented as mean ± SEM (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *P. fruticosa*-treated groups showed significantly higher survival compared to the paraquat-only group (*$P<0.05$ vs Paraquat induced flies, ANOVA, Tukey's test).

Resistance to hydrogen peroxide (H_2O_2)-induced oxidative stress in flies

The effect of *P. fruticosa* on the oxidative stress resistance of fruit flies is shown in Figure 3. *P. fruticosa* supplementation increased survival time in the hydrogen peroxide challenge test compared to the control group. *P. fruticosa* supplementation significantly increased the survival of fruit flies when compared to the untreated control. In the *P. fruticosa*-treated flies, the mean, the mean maximum, and the median survival were 22.06±0.46 h (3.46% increase, $P<0.05$), 31.66±1.52 h (3.46% increase, $P<0.05$), and 19.33±0.57 h (2.33 % increase, $P<0.05$), respectively.

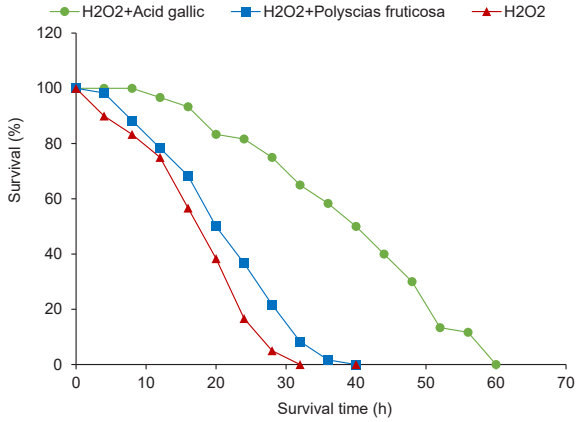

Figure 3. Effect of *Polyscias fruticosa* extract on survival of *Drosophila melanogaster* under H_2O_2-induced oxidative stress. Data are presented as mean±SEM (n=5). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test ($P<0.05$). *P. fruticosa* treatment significantly increased mean, median, and maximum survival compared to the control group (*$P<0.05$ vs H_2O_2 group, ANOVA, Tukey's test).

Nutritional composition of five different species of *Polyscias* extracts

The nutritional analysis of five *Polyscias* species was conducted using fresh leaf materials and revealed significant variation in composition (Table 4). The moisture content ranged from 79.59% in *P. balfouriana* to 84.07% in *P. ilicifolia*, consistent with the high water content of fresh plant tissues. Ash content, reflecting mineral composition, was the highest in *P. balfouriana* (2.17%) and the lowest in *P. filicifolia* (1.47%). Crude protein content varied among species, with *P. ilicifolia* showing the highest value (4.25±0.01%) and *P. filicifolia* the lowest (3.43±0.07%). Lipid content was low across all samples, ranging from 0.32% in *P. fruticosa* to 0.88% in *P. serrata*. Carbohydrate content was the highest in *P. filicifolia* (14.50±0.96%) and the lowest in *P. ilicifolia* (9.17±0.16%). Accordingly, energy content ranged from 57.01 kcal in *P. ilicifolia* to 74.99±1.56 kcal in *P. balfouriana*. These results indicate that each *Polyscias* species exhibits a distinct nutritional profile when assessed on a fresh weight basis.

Discussion

The phytochemical screening of the extracts from five *Polyscias* species has revealed the presence of various bioactive compounds, underscoring their potential for diverse medicinal applications. As detailed in Table 1, *P. fruticosa*, *P. filicifolia*, *P. serrata*, and *P. balfouriana* contained phenolic, flavonoid, alkaloid, saponin, and

Table 4. Proximate composition of five *Polyscias* species

Extracts	Moisture (%)	Ash content (%)	Crude protein (%)	Lipid (%)	Carbohydrate (%)	Energy (kcal/100 g)
P. fruticosa	83.16 ± 0.16	1.67 ± 0.00	3.76 ± 0.00	0.32 ± 0.01	11.09 ± 0.17	62.26 ± 0.62
P. balfouriana	79.59 ± 0.39	2.17 ± 0.01	3.49 ± 0.01	0.40 ± 0.01	14.35 ± 0.41	74.99 ± 1.56
P. serrata	81.23 ± 0.20	1.99 ± 0.01	3.54 ± 0.00	0.88 ± 0.01	12.37 ± 0.22	71.54 ± 0.82
P. ilicifolia	84.07 ± 0.14	2.14 ± 0.03	4.25 ± 0.01	0.37 ± 0.00	9.17 ± 0.16	57.01 ± 0.60
P. filicifolia	80.25 ± 0.89	1.47 ± 0.00	3.43 ± 0.07	0.36 ± 0.01	14.5 ± 0.96	74.93 ± 3.54

Values are expressed as mean ± SD (n = 3). No significant differences were observed among species ($P > 0.05$).

quinone compounds. The rich and diverse phytochemical profile of *Polyscias* species supports their traditional medicinal use and suggests significant potential for modern therapeutic applications. The consistent presence of these bioactive compounds across species suggests a valuable source of natural antioxidants, anti-inflammatory agents, and other pharmacologically active compounds (6).

The analysis of TPC and TFC across five species of the *Polyscias* genus has revealed significant variability within and between species. The observed range of TPC values, from 109.93 to 316.09 mg GAE/g dry extract weight, demonstrates a considerable disparity in phenolic content, with *P. fruticosa* exhibiting the highest phenolic content and *P. ilicifolia* the lowest. Similarly, the TFC analysis displayed notable variation, ranging from 138.26 to 242.05 mg QE/g dry extract weight, with *P. fruticosa* again showing the highest flavonoid content. The high level of TPC and TFC in *P. fruticosa* may be attributed to genetic factors that enhance biosynthetic pathways or adaptation to environmental stimuli conducive to compound production. *P. fruticosa* might possess specific genes that confer an enhanced ability to produce secondary metabolites, or it might be more efficiently utilizing available resources under certain environmental conditions to maximize compound synthesis (28). Since environmental conditions such as altitude, UV exposure, and soil characteristics were consistent across the sampling site, these factors are unlikely to account for the higher levels observed in *P. fruticosa* (29). Genetic variation is a primary driver of the differences observed in secondary metabolite profiles. Each species' unique genetic makeup influences the activities of enzymes involved in the phenolic biosynthetic pathway, resulting in varying levels of phenolic compounds (30). Interestingly, the ranking of TFC did not precisely mirror that of TPC, indicating that the regulatory mechanisms and environmental influences on these secondary metabolites are complex and possibly independent (29). These findings are supported by primary phytochemical studies on *Polyscias* species. Huan et al isolated oleanane-type triterpenoid saponins from *P. fruticosa*, confirming the presence of bioactive saponin compounds with potential pharmacological activity (9). The relatively high polyphenol and flavonoid contents observed in *P. fruticosa* may contribute to its antioxidant behavior; however, this effect should be interpreted alongside the contribution of saponins and other constituents rather than assuming a direct correlation. Similarly, LC–MS/MS-based analyses of *P. filicifolia* have identified oleanolic triterpenoid saponins and related metabolites (31). Despite its lower TFC, the presence of these non-flavonoid compounds may explain its comparatively higher DPPH activity, suggesting that antioxidant capacity is not solely dependent on flavonoid concentration. In *P. serrata*, NMR-based studies have reported compounds such as uracil, uridine, adenosine, indole-3-carboxylic acid, koaburside, and randianin (32), indicating a chemically diverse profile. The moderate TPC and TFC values observed in this species may therefore reflect contributions from multiple classes of phenolic and non-phenolic compounds. Although *P. balfouriana* and *P. ilicifolia* lack detailed LC-MS/HPLC characterization, their moderate TPC and TFC values suggest the presence of similar polyphenolic compounds. Given their taxonomic relation to other *Polyscias* species, they likely contain structurally related bioactive compounds that warrant further phytochemical investigation.

The evaluation of antioxidant activities among five *Polyscias* species using DPPH, ABTS, and ferric-reducing (FR) assays has revealed significant differences in their radical-scavenging capabilities. *P. filicifolia* demonstrated the most potent antioxidant activity with an EC_{50} of 1,227.32 μg/mL for DPPH. *P. fruticosa* in both ABTS and RF methods with EC_{50} values of 147.12 and 68.80 μg/mL, respectively. The phytochemical screening of five *Polyscias* species revealed a consistent presence of phenolics, flavonoids, alkaloids, saponins, and quinones, indicating a strong biochemical basis for antioxidant activity. These compounds are known to regulate redox balance by donating electrons, stabilizing radicals, and chelating metals, thereby limiting the generation and propagation of reactive oxygen species (ROS) (33-35). In addition, polyphenols can modulate endogenous antioxidant systems, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which collectively maintain cellular redox homeostasis (35,36). The marked variation in TPC and TFC among species suggests differential regulation of secondary metabolite biosynthesis, likely influenced by key enzymes of the phenylpropanoid pathway, such as phenylalanine ammonia-lyase and chalcone synthase (30).

Although *P. fruticosa* exhibited the highest TPC and TFC, this did not consistently translate into stronger antioxidant activity across all assays. In particular, the relatively weak

DPPH scavenging activity observed across all extracts (EC_{50} > 1200 μg/mL) suggests that a direct correlation between total metabolite content and antioxidant capacity should be interpreted with caution. The antioxidant assays (DPPH, ABTS, and FR) reflect electron transfer and reducing mechanisms, and the observed differences among species highlight the assay-dependent nature of antioxidant evaluation. While *P. fruticosa* and *P. filicifolia* showed comparatively lower EC_{50} values in ABTS and FR assays, these results do not necessarily indicate uniformly strong antioxidant capacity. Moreover, the relatively higher DPPH activity of *P. filicifolia* despite its lower TFC suggests that non-flavonoid compounds, including other phenolics, saponins, and alkaloids, may contribute significantly to radical-scavenging activity (33,34). The *in vitro* antioxidant results, therefore, provide only a partial indication of biological activity. Although *P. fruticosa* demonstrated comparatively stronger performance in ABTS and reducing power assays, its *in vivo* protective effects in *D. melanogaster* should not be attributed solely to its higher TPC and TFC. Instead, these effects likely arise from a combination of factors, including the presence of diverse bioactive compounds, their bioavailability, and their ability to modulate endogenous antioxidant systems (34). Importantly, this chemical antioxidant potential translated into biological activity in the *D. melanogaster* model, where *P. fruticosa* significantly improved survival under paraquat-induced oxidative stress (Figure 2). This consistency between *in vitro* and *in vivo* findings suggests that the bioactive compounds are not only effective radical scavengers in chemical systems but are also bioavailable and functionally active in living organisms.

Mechanistically, paraquat induces oxidative stress by generating superoxide radicals, which are detoxified by SOD into hydrogen peroxide, which is then degraded by CAT and GPx (37). The enhanced survival observed in *P. fruticosa*-treated flies indicates that the extract likely supports this enzymatic antioxidant defense system, either by upregulating enzyme activity or by reducing oxidative burden, thereby preserving cellular function (38,39). The improved survival observed in *P. fruticosa*-treated flies suggests a potential role in supporting these defense pathways; however, this effect should be interpreted as a combined outcome of multiple biochemical mechanisms rather than a direct consequence of total polyphenol or flavonoid content alone.

Consistent with the *in vitro* antioxidant results and paraquat model findings, *P. fruticosa* supplementation significantly enhanced resistance to H_2O_2-induced oxidative stress. This enhanced resistance to oxidative stress is indicative of *P. fruticosa*'s potent antioxidant properties, primarily due to its high polyphenol and flavonoid content (40). As shown in Figure 3, *P. fruticosa*-treated flies exhibited a mean survival time of 22 h, representing a 3.46% increase over the control group, which was statistically significant ($P < 0.05$). This improvement in mean survival time underscores the efficacy of *P. fruticosa* extract in mitigating the deleterious effects of H_2O_2-induced oxidative stress. Furthermore, maximum and median survival times also showed significant enhancements of 3.46% and 2.33%, respectively ($P < 0.05$), reinforcing the overall positive impact of *P. fruticosa* on oxidative stress resistance. The antioxidant properties of *P. fruticosa* are likely due to its ability to scavenge free radicals and enhance cellular antioxidant defenses. This relationship is supported by the *D. melanogaster* model, in which *P. fruticosa* improved survival under paraquat- and H_2O_2-induced oxidative stress. Paraquat generates superoxide radicals that are detoxified by SOD into hydrogen peroxide, which is subsequently decomposed by CAT and GPx. The improved survival suggests that *P. fruticosa* enhances this enzymatic cascade, potentially by upregulating antioxidant enzyme expression or preserving enzyme activity (35,36). Such modulation of SOD, CAT, and GPx has been widely reported for polyphenol-rich extracts and represents a key mechanism beyond direct ROS scavenging (34).

Under H_2O_2-induced stress, the increased survival time further indicates enhanced peroxide detoxification, primarily mediated by CAT and GPx. The observed improvements in lifespan metrics suggest that *P. fruticosa* maintains intracellular redox balance through both direct and indirect antioxidant mechanisms. Polyphenols and flavonoids not only neutralize ROS but also activate cellular defense pathways, including the Nrf2-regulated antioxidant response, which controls the expression of detoxifying and antioxidant enzymes (34,41).

Overall, the integration of phytochemical composition, *in vitro* assays, and *in vivo* outcomes supports a mechanistic framework in which *Polyscias* extracts, particularly *P. fruticosa*, mitigate oxidative stress through complementary pathways involving radical scavenging, metal chelation, and modulation of enzymatic antioxidant defenses.

The nutritional analysis of fresh leaf material from five *Polyscias* species demonstrates significant variation in their macronutrient compositions, suggesting diverse dietary and medicinal applications. This analysis provides crucial insights into the potential utilization of these species in various nutritional and therapeutic contexts. All *Polyscias* species exhibit high moisture content, ranging from 79.59% in *P. balfouriana* to 84.07% in *P. ilicifolia*. This high moisture content can impact the preservation and extraction processes of these fresh leaf materials. High moisture levels may require specific storage conditions and drying techniques to maintain the stability and longevity of the bioactive compounds in the extracts. The ash content, which reflects the mineral composition, varies significantly among the species. *P. balfouriana* has the highest ash content at 2.17%, indicating a richer mineral profile, while *P. filicifolia* has the lowest at 1.47%. These differences suggest that different *Polyscias* species could

offer varied mineral-based health benefits. Minerals are critical in various bodily functions, including bone health, enzymatic activities, and overall metabolic processes (42). Protein content also shows considerable variation, with *P. ilicifolia* having the highest protein content at 4.25±0.01%, making it a valuable dietary protein source. In contrast, *P. filicifolia* has the lowest protein content at 3.43±0.07%. The high protein content in *P. ilicifolia* could be particularly beneficial for protein supplementation and enrichment in diets, especially in populations with higher protein requirements or deficiencies. The lipid content across all species is relatively low, with *P. serrata* having the highest lipid content at 0.88±0.01% and *P. fruticose* the lowest at 0.32%. Low lipid content is advantageous for health supplements, as it reduces caloric density and the risk of adverse health effects associated with high-fat intake. This makes *Polyscias* fresh leaf material suitable for formulations aimed at weight management and cardiovascular health (42). *P. filicifolia* has the highest carbohydrate content at 14.5±0.96%, enhancing its energy-providing capacity, whereas *P. ilicifolia* has the lowest carbohydrate content at 9.17±0.16%. Carbohydrates are a primary source of energy, and the high carbohydrate content in *P. filicifolia* makes it suitable for energy-boosting supplements and foods (43). The overall energy content reflects these macronutrient variations, with *P. balfouriana* being the most calorie-dense species at 74.99±1.56kcal and *P. ilicifolia* the least at 57.01 kcal. The high energy content of *P. balfouriana* renders it a suitable candidate for products designed to provide rapid and sustained energy, potentially beneficial in sports nutrition and for individuals with elevated energy requirements (43).

These findings highlight the potential for *Polyscias* species to be tailored to specific dietary needs. *P. filicifolia* and *P. balfouriana*, with their high carbohydrate and energy content, are ideal for energy supplements, while *P. ilicifolia*, with its high protein content, is suitable for protein enrichment. The consistently low lipid content across all species is beneficial for health-conscious formulations, aligning with current dietary recommendations that emphasize lower fat intake to reduce the risk of chronic diseases. The observed antioxidant capabilities of *P. fruticosa* suggest a significant therapeutic potential for mitigating oxidative stress-related diseases. This is particularly important given the compound's capacity to enhance cellular defenses and reduce oxidative damage through mechanisms such as free radical scavenging and upregulation of endogenous antioxidant enzymes. Future research should focus on exploring the specific health benefits and roles of these nutritional components in functional foods and nutraceuticals. Studies could investigate the bioavailability of these nutrients and their effects on human health, potentially leading to the development of targeted dietary interventions and therapeutic products.

Several limitations should be considered in interpreting the findings of this study. First, the phytochemical evaluation was mainly based on preliminary qualitative screening and total phenolic/flavonoid estimation, while comprehensive identification and quantification of individual bioactive constituents using advanced analytical techniques such as HPLC, LC–MS/MS, or GC–MS were not conducted for all *Polyscias* species. Consequently, the specific compounds responsible for the observed antioxidant activities remain unclear. Second, the antioxidant potential was assessed primarily through *in vitro* chemical assays (DPPH, ABTS, and ferric-reducing assays), which may not fully represent the complexity of antioxidant mechanisms under physiological conditions. Although the *D. melanogaster* model provided supportive *in vivo* evidence, extrapolation of these findings to mammals or humans should be approached with caution. Third, the molecular mechanisms underlying the protective effects, including the modulation of endogenous antioxidant enzymes and redox-related signaling pathways, were not directly investigated. In addition, plant materials were collected from a single geographical location and sampling period; therefore, possible environmental, seasonal, and developmental influences on phytochemical composition were not evaluated. Finally, toxicological assessment and long-term safety evaluation of the extracts were beyond the scope of this study. Further studies involving detailed metabolomic profiling, mechanistic investigations, mammalian models, and safety assessments are necessary to validate the pharmacological and nutraceutical potential of *Polyscias* species.

Conclusion

This study provides a comparative evaluation of five *Polyscias* species, demonstrating that *P. fruticosa* consistently exhibits the highest phenolic and flavonoid contents and the strongest antioxidant activity across ABTS and reducing power assays, with corresponding protective effects in *D. melanogaster* under paraquat- and H_2O_2-induced oxidative stress. In contrast, *P. filicifolia* showed superior DPPH scavenging activity, indicating assay-dependent differences in antioxidant mechanisms. The integration of *in vitro* and *in vivo* results strengthens the evidence that polyphenol-rich extracts, particularly from *P. fruticosa*, contribute to oxidative stress resistance through both radical scavenging and biological effects. Nutritional profiling further differentiates species-specific value, with *P. balfouriana* and *P. filicifolia* characterized by higher energy and carbohydrate content, and *P. ilicifolia* by higher protein levels. Overall, these findings identify *P. fruticosa* as the most promising candidate for functional applications, while highlighting interspecies variability that is critical for targeted use. Future work should focus on compound-level characterization and bioavailability to establish clearer structure–activity relationships and translational potential.

Authors' contribution

Conceptualization: Truong Thi Phuong Thao.
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Methodology: Truong Thi Phuong Thao.
Project administration: Tran Thanh Men.
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Supervision: Tran Thanh Men.
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Visualization: Truong Thi Phuong Thao.
Writing–original draft: Truong Thi Phuong Thao.
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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Declaration of AI-assisted tools in the writing procedure

The authors used Grammarly solely for English language editing and grammar checking during manuscript preparation. No AI software was used for data collection, data analysis, scientific interpretation, or generation of the manuscript's intellectual content. All research content, conclusions, and final manuscript approval remain the sole responsibility of the authors.

Ethical considerations

All authors observed ethical issues (including plagiarism, violations, falsification of data, falsification of double publication or submission, and redundancy). The study protocol was reviewed and approved by the guidelines of Can Tho University (the ethical approval code is CTU-AEC24120, dated 20th May, 2024).

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