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# Evaluating polyphenol components, lung cancer cell inhibition, and antioxidant activity of *Polyscias* spp.

**Huynh Mi, Le Cong Thang, Nguyen Ngoc Yen Vy, Chim Tung Chi, Tran Le Khai, Do Tan Khang***

Institute of Food and Biotechnology, Can Tho University, Vietnam

* Correspondence to Do Tan Khang <dtkhang@ctu.edu.vn.vn>

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**Abstract.** *Polyscias* spp. is a traditional medicinal species widely used in Vietnam, but its polyphenol composition and related bioactivities have not been thoroughly examined. This study aimed to investigate the phytochemical compounds, total phenolic and flavonoid contents, antioxidant activity, and cytotoxic effect on A549 lung cancer cells from ethanol extracts of the roots, stems, and leaves of *Polyscias fruticosa* and *Polyscias serrata* species. Phytochemical screening reveals the presence of alkaloids, carotenoids, coumarins, flavonoids, polyphenols, quinones, saponins, tannins, and terpenoids in both species. The leaf extract of *P. serrata* has the highest total phenolic and flavonoid contents (47.097 mg GAE/g and 759.17 mg QE/g, respectively). The HPLC analysis indicates that quercetin was identified in *P. fruticosa* root only, while rutin was detected in all samples. The antioxidant activity was evaluated via DPPH and FRAP assays, with $IC_{50}$ values ranging from 1064 to 7417 μg/mL. The *P. serrata* leaf extract exhibited the most potent cytotoxic effect against A549 cells ($IC_{50}$ = 156.42 ± 5.07 μg/mL).

**Keywords:** *Polyscias* spp., polyphenol, flavonoid, antioxidant, A549 lung cancer cells, ethanol extract

## 1 Introduction

Currently, both in Vietnam and globally, the search for safe, user-friendly, and reliable medicinal products remains a top priority for improving the quality of life and decreasing the burden of disease. For centuries, people from diverse countries, cultures, and ethnic backgrounds have made continuous efforts to discover natural remedies by using plant-based extracts. Among the widely recognised medicinal plants, the genus *Polyscias*, belonging to the family Araliaceae [1], has attracted considerable research attention because of its phytochemical richness and significant pharmacological potential.

Lung cancer remains one of the leading causes of cancer-related mortality worldwide, posing a significant challenge to modern medicine [2]. Molecular pathology studies have revealed numerous driver mutations in non-small cell lung cancer, including mutations in EGFR, KRAS, and BRAF, as well as alterations in ALK, ROS1, and RET, along with the dysregulation of the PI3K/AKT/mTOR pathway and epigenetic changes. Furthermore, tumour mutational burden, intratumour heterogeneity, and microenvironmental interactions critically influence treatment response and prognosis [3].

Despite advances in lung cancer molecular biology, research on *Polyscias* species in this context remains limited. Existing studies on *P. fruticosa* have documented the presence of abundant triterpene saponins, flavonoids, and other phenolic compounds [4]. According to previous studies [5, 6], optimised saponin extraction from *P. fruticosa* roots yielded approximately 41.24 mg/g dry material and demonstrated cytotoxic activity against A549 lung

cancer cells, among others [5, 6]. Nonetheless, a thorough comparative examination of *P. fruticosa* and *P. serrata* is required, regarding their roots, stems, and leaves. Besides, the data directly correlating their phytochemical content with the suppression of lung cancer cells are also limited.

Given this background, *P. fruticosa* and *P. serrata* were selected for the present investigation because they are widely used in Vietnamese traditional medicine for tonic and anti-inflammatory purposes and have been reported to contain abundant secondary metabolites with putative anticancer potential [4, 7]. To address the research gap, we prioritised three analytical endpoints. Firstly, we quantified polyphenolic and flavonoid components, as these are strongly associated with antioxidant and anticancer activities. Secondly, we assessed their antioxidant capacity, given the central role of oxidative stress in lung carcinogenesis. Finally, we evaluated their cytotoxic effects on lung cancer cells to provide preliminary evidence of therapeutic relevance. By integrating these endpoints, we aim to establish a molecular foundation for correlating phytochemical richness with biological activity, thereby supporting the potential development of *Polyscias*-derived compounds as adjuncts in the treatment of lung cancer.

## 2 Materials and methods

### 2.1 Plant material collection and extraction

#### a) Sample collection and processing

Mature specimens of *Polyscias* spp. (approximately 8–10 years old) were collected from the Hoa An Pharmaceutical Conservation Cooperative in Soc Trang Province. Healthy, disease-free, and vigorously growing individuals were selected for the study. The plant material was separated into roots, stems, and leaves. The samples were dried in an oven at 50 °C until a constant weight was achieved, and then they were reweighed to determine the moisture loss. The dried material was then ground into a fine powder for subsequent analytical procedures.

#### b) Preparation of free phenolics

The powdered roots, stems, and leaves of *P. fruticosa* (L.) Harms and *P. serrata* Balf. were placed in cloth bags and soaked in 96% ethanol at a ratio of 1:10 (w/v) for 72 hours at ambient temperature. The mixture was filtered through Whatman filter paper and evaporated at 50 °C to obtain the extract. The final extracts were stored at 4 °C for further experiments.

#### c) Preparation of bound phenolics

Bound phenolics were extracted from the residues remaining after free phenolic extraction, following the previously described method with slight modifications [5]. The residues were treated with 50 mL of 4 M NaOH and stirred at 60 °C for 4 hours. The resulting mixture was centrifuged at 5,000 rpm for 10 minutes and then filtered through Whatman paper. The filtrate was acidified to pH 2.0 with 37% hydrochloric acid and subsequently extracted five times with ethyl acetate. The ethyl acetate supernatant was collected and evaporated to dryness with a rotary evaporator. The final extract was stored at 4 °C for subsequent analyses.

### 2.2 Phytochemical screening and quantification of phenolics and flavonoids

#### a) Identification of bioactive compounds in extract with spectrophotometric method

The bioactive compounds were identified following the method proposed by Harborne and Dey [6] with some modifications. A volume of 200 μL of the 1 mg/mL extract was placed into a 96-well microplate. Then, spectrophotometric

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measurements were conducted at the specific wavelengths listed in Table 1. Methanol was used as a control.

**Table 1.** Specific wavelengths used to qualitatively characterise compounds in extracts

| Compound | Wavelength (nm) |
|---|---|
| Polyphenols | 215 |
| Coumarin | 255 |
| Tannins | 265 |
| Flavonoids | 300 |
| Carotenoids | 450 |
| Saponins | 545 |
| Alkaloids | 550 |
| Terpenoids | 230 |

**b) Determination of total phenolic content in extracts**

The total phenolic content was determined following the method developed by Yadav and Agarwala [7], with modifications. A modified Folin-Ciocalteu assay was used to quantify the free phenolic compounds in the plant extracts. A gallic acid standard curve was established, and the total phenolic content in the extract samples was determined from the recorded absorbance (Abs) values. The results were obtained from the standard curve, and the phenolic content was determined as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

**c) Determination of total flavonoid content in extracts**

The flavonoid content in the free phenolic compounds of the plant extracts was determined with the aluminum chloride colourimetric method proposed by Djeridane et al. [8], with certain modifications. A standard curve was constructed by using quercetin, and the flavonoid concentration in the samples was calculated from the recorded absorbance values by means of the standard curve equation. The flavonoid content was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

**d) Bioactive compound quantification**

High-performance liquid chromatography (HPLC) analysis was conducted following a validated procedure with slight modifications [9]. The analysis was performed with a Shimadzu-C system (Model LC-2030 Plus, Shimadzu, Japan). Chromatographic separations were accomplished by using a SUPELCOSIL LC-18-S Reversed-Phase Column (5 μm particle size, L × I.D. 25 cm × 4.6 mm). Silica gel was used as the stationary phase, and the mobile phase was a mixture of acetonitrile and 2% acetic acid.

Four bioactive compounds were quantified in this assay. The methanol standard solution for each compound was prepared at the following concentrations: quercetin (0, 20, 100, 150, and 200 ppm); rutin (0, 20, 40, 60, 80, 150, and 200 ppm); kaempferol (20, 40, 60, 100, and 150 ppm). The working solutions were prepared by dissolving the extract in methanol and transferring them to vials for subsequent analysis.

A reverse-phase HPLC assay was used for quantifying quercetin, rutin, and kaempferol. The assay was carried out with an isocratic elution with a flow rate of 1.3 mL/minute, a column temperature of 35 °C, a mobile phase of acetonitrile and 2% v/v acetic acid (pH 2.60) (40:60 v/v), and a detection wavelength of 370, 350, and 367 nm, respectively. The injection volume of each solution was 20 μL.

### 2.3 Determination of extract antioxidant activity

**a) 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging assay**

The antioxidant activity was evaluated by means of the DPPH radical scavenging assay, with slight modifications from a previously described method [10]. A 100 μg/mL DPPH solution was prepared in methanol. Gallic acid at concentrations of 1, 2, 3, 4, and 5 μg/mL, as well as extract samples at various concentrations, were also prepared in methanol. In a 96-well plate, 100 μL of each sample or gallic acid at different concentrations was mixed with 100 μL of the DPPH solution. After 30-minute incubation in the dark at ambient temperature, the absorbance was measured with a spectrophotometer at a wavelength of 517 nm. Methanol was used as a control.

**b) Reducing power assay**

The reducing power of the extract was assessed according to the method proposed by Ghasemzadeh et al. [11], with modifications. 100 μL of each sample at different concentrations was mixed with 250 μL of a 0.2 M phosphate buffer (pH 6.6) and 250 μL of 1% potassium ferricyanide $K_3[Fe(CN)_6]$. The mixture was then incubated at 50 °C for 30 minutes. After incubation, 250 μL of 10% trichloroacetic acid ($C_2HCl_3O_2$) was added, and the solution was centrifuged at 4,000 rpm for 10 minutes to obtain a supernatant. Next, 250 μL of the supernatant was combined with 250 μL of distilled water and 50 μL of a 0.1% ferric chloride $FeCl_3$ solution. 200 μL of the final mixture was transferred into a 96-well microplate, and the absorbance was measured at 700 nm with a UV-visible spectrophotometer. The standard curve (a positive control) was established with gallic acid at concentrations of 20, 40, 60, 80, and 100 μg/mL, serving as a positive control, while methanol was used as a negative control.

### 2.4 MTT-Based assessment of the antiproliferative activity

The anti-lung cancer activity of the sample was evaluated at the Applied Biochemistry Laboratory, Institute of Biochemistry, Vietnam Academy of Science and Technology.

## 3 Results and discussion

### 3.1 Identification of bioactive compounds in extracts

The qualitative analysis of bioactive compounds was performed with a spectrophotometer at specific wavelengths for the ethanol extracts of *P. fruticosa* and *P. serrata*. The experimental results indicate the presence of various phytochemicals in both extracts.

The qualitative results show that both *P. fruticosa* and *P. serrata* contain numerous biological compounds such as alkaloids, carotenoids, coumarins, flavonoids, polyphenols, quinones, saponins, tannins, and terpenoids. Among these compounds, *P. serrata* has a higher carotenoid content than *P. fruticose*, and it also possesses beneficial compounds such as tannins and saponins. Certain differences exist among studies because of the differences in extraction methods, solvent concentrations, and detection limits. In general, *P. serrata* has a richer active ingredients and higher medicinal potential.

A study conducted in 2023 [12] reported that the ethanol extract from the leaves of *P. fruticosa* had phenolics, flavonoids, alkaloids, saponins, coumarins, and quinones but without tannins. In contrast, our ethanol extracts from the roots, stems, and leaves of *P. fruticosa* had low or undetectable levels of alkaloids and saponins, while tannins were present in all three parts. These differences may be due to variations in extraction methods, solvent concentrations, or detection thresholds.

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**Table 2.** Results determined presence of phytochemicals in ethanol extract of *P. fruticosa*

| Compound | Wavelength (nm) | Root | Stem | Leaf |
|---|---|---|---|---|
| Alkaloids | 550 | – | – | – |
| Carotenoids | 450 | – | – | + |
| Coumarin | 255 | + | + | + |
| Flavonoids | 300 | + | + | + |
| Polyphenols | 215 | + | + | + |
| Quinone | 260 | + | + | + |
| Saponins | 545 | – | – | – |
| Tannins | 265 | + | + | + |
| Terpenoids | 230 | + | + | + |

*(Note: "+" indicates presence, "–" indicates absence.)*

**Table 3.** Results determined presence of phytochemicals in ethanol extract of *P. serrata*

| Compound | Wavelength (nm) | Root | Stem | Leaf |
|---|---|---|---|---|
| Alkaloids | 550 | – | – | + |
| Carotenoids | 450 | – | – | + |
| Coumarin | 255 | + | + | + |
| Flavonoids | 300 | + | + | + |
| Polyphenols | 215 | + | + | + |
| Quinone | 260 | + | + | + |
| Saponins | 545 | – | – | + |
| Tannins | 265 | + | + | + |
| Terpenoids | 230 | + | + | + |

*Note: "+" indicates presence, "–" indicates absence.*

### 3.2 Total phenolic and flavonoid compounds in plant extracts

**Total phenolic content in free extracts of *Polyscias* spp.**

The total phenolic content in the free extracts of *Polyscias* spp. was determined by means of a gallic acid standard curve at various concentrations (Table 4).

The results show that the phenolic content in the root, stem, and leaf parts of *Polyscia serrata* Balf is higher than that in the corresponding parts of *P. fruticosa* (L.) Harms. Overall, the phenolic content increased in the order roots < stems < leaves for both species. The total phenolic content in the leaf extract of the *P. serrata* Balf species (47.097 ± 0.018 mg GAE/g) was the highest among all the extracts. In contrast, the lowest total phenolic content was observed in the root extract of the *P. fruticosa* (L.) Harms species (16.336 ± 0.0028 mg GAE/g)

Compared with *P. fruticosa*, *P. serrata* exhibited the phenolic content approximately 2 to 3 times higher. This difference may be attributed to factors such as solvent polarity, the quantity of compounds extracted in the crude extract from each plant part, plant growth duration, and the sample type [13].

**Table 4.** Total phenolic content in free extracts of *Polyscias* spp.

| Species | Total phenolic content | | |
|---|---|---|---|
| | Root | Stem | Leaf |
| *P. fruticosa* (L.) Harms | $16.336^{C} \pm 0.0028$ | $16.445^{C} \pm 0.0026$ | $16.663^{C} \pm 0.0026$ |
| *Polyscia serrata* Balf | $27.967^{B} \pm 0.0370$ | $31.119^{B} \pm 0.0360$ | $47.097^{A} \pm 0.0180$ |

Note: Mean values followed by different superscript letters (A, B, C) within the same row or column are significantly different at $p < 0.05$ according to Tukey's HSD test.

**Total flavonoid content in free extracts of *Polyscias* spp**

The total flavonoid content in the free extracts of *Polyscias* spp. was determined by means of the quercetin standard curve at various concentrations (Table 5).

The results indicate that the flavonoid content in the root, stem, and leaf parts of *P. serrata* Balf was higher than that of the corresponding parts of *P. fruticosa* (L.) Harms. In general, the flavonoid content in the root, stem, and leaf increased in this order for both species. The total flavonoid content in the leaf extract of *P. serrata* Balf (759.17 ± 65.21 mg GAE/g) was the highest among all extracts, whereas the root extract of *P. fruticosa* (L.) Harms exhibited the lowest flavonoid content (16.336 ± 0.0028 mg GAE/g).

**Table 5.** Total flavonoid content in free extracts of *Polyscias* spp.

| Species | Total flavonoid content | | |
|---|---|---|---|
| | Root | Stem | Leaf |
| *P. fruticosa* (L.) Harms | $348.33^{B} \pm 9.46$ | $398.33^{B} \pm 49.05$ | $493.33^{B} \pm 101.25$ |
| *P. serrata* Balf | $489.17^{B} \pm 18.76$ | $502.50^{B} \pm 43.80$ | $759.17^{A} \pm 65.21$ |

Note: Mean values followed by different superscript letters (A, B, C) within the same row or column are significantly different at $p < 0.05$ according to Tukey's HSD test.

### 3.3 HPLC-based quantification

**Quercetin quantification**

The quercetin standard curve was built by identifying the peak area of standard solutions with the concentrations of 0, 20, 100, 150, and 200 µg/mL from the chromatogram. It is linear in the 0–200 µg/mL range; the regression equation is $y = 31419 \times x + 264047$ with $R^2$ being 0.9469. This coefficient of correlation reveals a closed linearity. This standard curve was used to quantify the level of quercetin in the extracts.

According to the results, quercetin was exclusively found in the roots of *P. fruticosa* (L.) Harms. Other plant parts, namely the leaves and stems of *P. fruticosa* (L.) Harms, and the roots, stems, and leaves of *P. serrata* Balf show no detectable presence of quercetin. According to the quercetin standard curve equation ($y = 31419 \times x + 264047$), the quercetin content in the root extract of *P. fruticosa* (L.) Harms is 34.28 µg/mL.

**Rutin quantification**

The rutin standard curve was built over a concentration range from 20 to 200 µg/mL. The result show two distinct peak regions: the first region with the major peak eluting at 1.7 minutes and the second region with the highest peak at 2.44 minutes. The peak heights increased proportionally with the concentration. Notably, in the second region, the main peak split into two closely spaced peaks, with the taller one observed at 2.44 minutes.

The occurrence of two peaks complicated the determination of the exact retention time for rutin, as one of them was likely attributable to the solvent. Nevertheless, the second zone was believed to belong to rutin, according to the retention time and the relative peak height. This assignment is supported by the fact that rutin has a much larger molecular size than methanol, resulting in a longer retention time. Furthermore, at 350 nm, rutin exhibits stronger absorbance than methanol, contributing to a higher peak intensity.

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The standard curve for rutin was constructed from the peak areas at the retention time of 2.44 minutes for standard solutions at concentrations of 20, 40, 60, 80, and 150 µg/mL. The regression equation has a form $y = a \times x + b$, where $x$ represents the rutin concentration, and $y$ represents the corresponding peak area.

**Table 6.** Rutin concentration (µg/mL) from root, stem, and leaf extracts

| | Root | Stem | Leaf |
|---|---|---|---|
| *P. fruticosa* (L.) Harms | 321.58 | 430.52 | 263.92 |
| *P. serrata* Balf | 261.76 | 262.25 | 163.71 |

According to Table 6, for *P. fruticosa* (L.) Harms, the stem extract exhibited the highest rutin content at 430.52 µg/mL, followed by the root (321.58 µg/mL), and lastly the leaf (263.92 µg/mL). For *P. serrata* Balf, all three plant parts show relatively high rutin concentrations, with levels decreasing in the order of stem (262.25 µg/mL), root (261.76 µg/mL), and leaf (163.71 µg/mL).

**Kaempferol quantification**

The standard curve for rutin was constructed by measuring the peak areas at concentrations of 0, 20, 40, 60, 100, and 150 µg/mL. The linear regression equation is $y = 11745 \times x + 181791$, where $x$ represents the kaempferol concentration, and $y$ represents the corresponding peak area, with $R^2$ being 0.9703. This coefficient of correlation reveals a closed linearity. The standard curve was used to quantify the kaempferol level in the extracts. According to the HPLC chromatogram, kaempferol was observed at 367 nm at about 6.3 minutes.

The HPLC analysis at 367 nm reveals no detectable kaempferol in the root, stem, and leaf of *P. fruticosa* (L.) Harms and *P. serrata* Balf extracts. The absence of a peak at this retention time of the kaempferol standard indicates that the compound is either present at very low concentrations or absent in the tested samples. Additionally, this may be attributed to an inefficient extraction process, which could have resulted in inadequate recovery or loss of kaempferol during sample preparation.

In the chromatograms of the crude extracts from the tested samples, one or more peaks were observed at a retention time of approximately 2 to 5 minutes earlier than that of the kaempferol standard. These earlier peaks are presumed to correspond to other phytochemical constituents present in the samples. The presence of these signals may result from the complexity of the crude extracts, which were not fully purified, leading to overlapping peaks or potential interference with the detection of kaempferol.

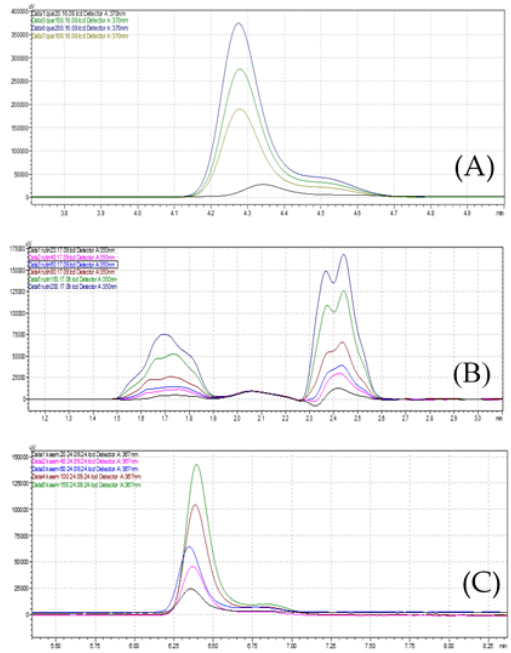


**Fig. 1.** HPLC chromatograms of standard phenolic compounds: (A) Quercetin; (B) Rutin; (C) Kaempferol

Moreover, kaempferol can interact with the constituents of the stationary phase in various ways, leading to the separation of isomeric or molecular forms of kaempferol throughout its passage down the column. This often occurs when the stationary phase is not completely

homogeneous or when the chromatographic conditions are not optimal.

### 3.4 Antioxidant activity in the extract

#### DPPH radical scavenging

Gallic acid, used as a positive control, exhibited strong antioxidant activity with an $IC_{50}$ value of 2.689 μg/mL, indicating its high potency in scavenging free radicals. The antioxidant activity of *Polyscias* extracts was evaluated under the same conditions, with a concentration range from 500 to 2500 μg/mL. The $IC_{50}$ values of the root, stem, and leaf extracts of both *P. fruticosa* and *P. serrata* are presented in Table 7.

**Table 7.** $IC_{50}$ values of extracts determined with DPPH assays

| Species | $IC_{50}$ | | |
|---|---|---|---|
| | Root | Stem | Leaf |
| *P. fruticosa* (L.) Harms | 1534.30 | 1453.20 | 2312.90 |
| *P. serrata* Balf | 1153.79 | 1380.65 | 1064.39 |

Among the extracts of *P. fruticosa*, the one of stem exhibited the highest radical scavenging capacity, with the lowest $IC_{50}$ value at 1453.2 μg/mL, followed by the root extract with an $IC_{50}$ of 1534.3 μg/mL, and the leaf extract at 2312.9 μg/mL. In contrast, the leaf extract of *P. serrata* demonstrated the strongest antioxidant activity among all the samples tested, with an $IC_{50}$ of 1064.39 μg/mL. The root and stem extracts of *P. serrata* also exhibited moderate antioxidant activity, with $IC_{50}$ values of 1153.79 μg/mL and 1380.65 μg/mL, respectively.

Overall, the *P. serrata* extracts had more potent antioxidant effects than those of *P. fruticosa*. However, all extracts had significantly higher $IC_{50}$ values than pure gallic acid. This finding is within expectation as these extracts are crude mixtures containing various compounds and potential impurities, whereas gallic acid is a purified standard substance.

Antioxidant activity is expressed through the $IC_{50}$ value, where the compounds or samples with $IC_{50}$ values below 50 μg/mL are considered to have strong activity, and values between 50–100 μg/mL indicate moderate activity [14]. In this study, the high $IC_{50}$ values suggest that the antioxidant activity of *Polyscias* extracts is relatively low. However, the $IC_{50}$ values ranging from 200 to 1000 μg/mL are still considered to reflect low antioxidant potential but may nonetheless indicate promising antioxidant properties [15].

#### Fe3+ reduction

This technique evaluates the ability of an antioxidant to reduce ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$). Results were assessed via absorbance values.

**Table 8.** A0.5 values of extracts and gallic acid

| **Gallic acid** | **$16.43^{D} \pm 0.11$** | | |
|---|---|---|---|
| **Species** | **Sample** | | |
| | **Root** | **Stem** | **Leaf** |
| *P. fruticosa* (L.) Harms | $5659^{B} \pm 68.00$ | $5499^{B} \pm 482.77$ | $3063^{C} \pm 41.32$ |
| *P. serrata* Balf | $4638^{B} \pm 347.53$ | $7417^{A} \pm 761.24$ | $5499^{B} \pm 364.14$ |
| *p* (Interaction between the plant variety and part) | | | <0.05 |

Note: $A_{0.5}$ is the extract concentration (μg/mL) required to reach an absorbance of 0.5 at a wavelength of 700 nm. Mean values followed by different superscript letters (A, B, C) within the same row or column are significantly different at $p < 0.05$ according to Tukey's HSD test.

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The absorbance *A* was directly proportional to the concentration of the extract, which was a common trend for the three plant parts of *P. fruticosa* and *P. serrata*. This indicates that the ability to reduce $K_3Fe(CN)_6$ to $K_4Fe(CN)_6$ increased with concentration. However, there are significant differences in the reduction capacity among the parts of the two plants. For *P. fruticosa*, the reduction capacity decreased in the order root > stem > leaf, with corresponding $A_{0.5}$ values of 5659 ± 68.5499 ± 482.77, and 3063 ± 41.32 g/mL respectively (Table 8). In contrast, for *P. serrata*, the reduction capacity decreased in the order stem > leaf > root, with corresponding $A_{0.5}$ values of 7417 ± 761.24, 5499 ± 364.14, and 4638 ± 347.53 g/mL, respectively. Both plants exhibited significantly weaker reducing activity compared with gallic acid, with an $A_{0.5}$ of 16.43 ± 0.11. These results indicate an interaction between the two factors: plant variety and parts in the iron-reducing capacity, with *p* being less than 0.05.

Additionally, the results show a low correlation between the flavonoid content in the quantitative assay and the antioxidant capacity in the iron-reducing reaction. Specifically, the flavonoid content in the two plant types increased in the order root < stem < leaf. However, in this experiment, the order of antioxidant capacity changed as previously described.

**a) Antiproliferation activity against lung cancer cells**

The extracts were compared with ellipticine, which served as a positive control. According to Table 9, the $IC_{50}$ values of the four extracts were generally higher than that of ellipticine ($IC_{50}$ = 0.44 ± 0.02 μg/mL).

**Table 9.** $IC_{50}$ values and antiproliferative activity of extracts against A549 lung cancer cells (μg/mL)

| Extracts | | Percentage inhibition of A549 lung cancer cells by concentration (μg/mL) | | | | $IC_{50}$ (μg/mL) |
|---|---|---|---|---|---|---|
| | | 4 | 16 | 64 | 256 | |
| *P. fruticosa* (L.) Harms | Leaf | 0 | 5 | 16 | 81 | 164.43 ± 4.18 |
| | Root | 0 | 0 | 7 | 23 | >256 μg/mL |
| *P. serrata* Balf | Leaf | 0 | 5 | 18 | 84.5 | 156.42 ± 5.07 |
| | Root | 0 | 0 | 8 | 25 | >256 μg/mL |
| Ellipticine | | | | | | 0.44 ± 0.02 |

The root extract of *P. fruticosa* showed 7% and 23% inhibitory effects at the respective concentrations of 64 μg/mL and 256 μg/mL. Similarly, the root extract of *P. serreta* Balf also demonstrated an increasing percentage of inhibition from concentrations of 64 to 256 μg/mL; however, at 256 μg/mL, the maximum inhibition was 25%. Neither of these root extracts attained half maximal inhibitory concentration ($IC_{50}$ > 256 μg/mL), indicating that perhaps the bioactive compounds in the root extracts were not specific for this cell line, or the antiproliferative capacity was present but inadequate. On the other hand, the *P. fruticosa* (L.) Harms's leaf extract exhibited a notable cytotoxic effect against A549 human lung carcinoma cells, with a percentage of inhibition increasing from 0 to 81% when the concentration of the extract increased from 4 to 256 μg/mL ($IC_{50}$ = 164.43 ± 4.18 μg/mL). Meanwhile, the *P. serrata* Balf's leaf extract was considered to have the best cytotoxic activity against A549 lung cancer cells among the four extracts, reaching 84.5% of the cell inhibition when $IC_{50}$ = 156.42 ± 5.07 μg/mL, which exhibits a potential herbal source for anticancer treatment in the future.

Compared with previous studies on the inhibition of A549 lung cancer cells, our extracts demonstrated a modest level of cytotoxic activity. For example, a study on the cytotoxicity capacity of *Caryota mitis*'s leaf and fruit extracts against A549 lung and Hep-G2 liver cancer cells [16] reported lower inhibition against the A549 cells than both the *P. fruticosa* (L.) Harms and *P. serrata* Balf. leaf and root extracts evaluated in the present study. This also suggests that the *Polyscias* genus possesses more relevant bioactive compounds for targeting lung carcinoma cells. Moreover, at very low doses (50 μg/mL), essential oils derived from *P. guilfoylei* have shown significant anticancer activity against Dalton's Lymphoma Ascites (DLA), suggesting potential effects of other *Polyscias* species against various cancer cell lines [17]. Significantly, although the highest inhibition observed in our investigation (84.5% for *P. serreta* Balf) was slightly lower than that of the saponin-enriched root extracts of *P. Fruticosa* [18], methodological differences likely explain this discrepancy. While our extracts were crude ethanol extracts, the previous research applied ultrasound-assisted extraction to enrich specific saponin fractions, which are known to exhibit stronger targeted anticancer effects. This implies that the effectiveness of *P. fruticosa* leaf and root extracts may be improved by means of further fractionation and extraction adjustment.

Collectively, these findings suggest additional evidence of the anticancer potential of *P. fruticosa* (L.) Harms and *P. serreta* Balf, even though exhibiting moderate cytotoxic effects. In the future, research focusing on compound isolation and standardised extraction methods is necessary to further clarify the therapeutic value of this species.

## 4 Conclusions

The ethanol extracts of *P. fruticosa* and *P. serrata* contained abundant phenolic and flavonoid compounds. Notably, the *P. serrata* leaf extract exhibited the highest total phenolic (47.097 mg GAE/g) and flavonoid (759.17 mg QE/g) contents. The HPLC analysis detected rutin in all extracts and quercetin only in *P. fruticosa* roots. The *P. serrata* leaf extract exhibited the strongest antioxidant activity ($IC_{50}$ = 1064.39 μg/mL) and cytotoxicity against A549 lung cancer cells ($IC_{50}$ = 156.42 ± 5.07 μg/mL), indicating its potential as a natural antioxidant and anticancer source.

Further research should focus on isolating and characterising the active constituents, clarifying their molecular mechanisms in cancer inhibition, and conducting *in vivo* studies in animal models to evaluate safety, dosage, and pharmacological efficacy before potential clinical applications.

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