

## From nature's pharmacy: Bioactive compounds of *Pyracantha coccinea* fruits and their health effects

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This study aimed to investigate the biopharmacological and anticancer properties of *Pyracantha coccinea* M. Roem fruits. Fruits were extracted with ethanol and methanol using the Soxhlet extraction method. Total phenolic, flavonoid, antioxidant, and oxidant contents, as well as elemental composition, antimicrobial, and antiproliferative activities of the extracts were determined. Antioxidant capacity values were measured as DPPH 40.65%–80.61%, TAS (Total Antioxidant Status) 8.337 mmol/L, TOS (Total Oxidant Status) 12.510  $\mu\text{mol/L}$ , and OSI (Oxidative Stress Index) 0.150 for the ethanol extract. For the methanol extract, DPPH was 29.38–65.29%, TAS 7.669 mmol/L, TOS 11.253  $\mu\text{mol/L}$ , and OSI 0.147. Total phenolic content was 3.791 mg GAE/g in the ethanol extract and 8.684 mg GAE/g in the methanol extract. The extracts exhibited antimicrobial effects on bacteria and fungi at different concentrations, and their antiproliferative activity increased with concentration. Bioactive compounds such as fumaric acid, catechin, syringic acid, thymoquinone, phloridzin, resveratrol, quercetin, ellagic acid, naringenin, and luteolin were identified. In addition, various elements were detected at different concentrations. As a result, it was found that *P. coccinea* fruits possess a wide range of bioactive compounds and can serve as a natural source for pharmacological research. These findings indicate its potential as a therapeutic agent due to its antioxidant, antimicrobial, and antiproliferative activities. Further clinical studies are recommended to explore these properties in more detail.

**Keywords:** Anticancer, Antimicrobial, Antioxidant, Phenolic, *Scarlet firethorn*

Plants are natural resources that have been widely used by humans throughout history to treat diseases and maintain health<sup>1</sup>. Plants exhibit various biological activities such as anticancer, antitumor, antimicrobial, antiaging, anti-allergic, antioxidant, DNA protective, anti-inflammatory and hepatoprotective (liver protective) thanks to the bioactive compounds they contain<sup>2-4</sup>. The biological activities of plants are due to the phenolic compounds, flavonoids, terpenes, alkaloids and other secondary metabolites they contain. These compounds can reduce oxidative stress by neutralizing free radicals, inhibit the growth of pathogenic microorganisms, and prevent the growth of cancer cells by regulating cell proliferation. Additionally, the elemental content of plants is essential for various biochemical processes in the human body<sup>5,6</sup>.

The *Pyracantha* genus belongs to the Rosaceae family and is a plant group that spreads in temperate, cool, and subtropical climates such as the Mediterranean. *P. coccinea* is an evergreen shrub

species and is known for its small white flowers and vibrant red fruits. Its leaves have serrated edges and are arranged oppositely. Its fruits have a bitter and sour taste and are traditionally used in making jams, jellies, sauces, and marmalade<sup>7,8</sup>. However, the medicinal potential of *P. coccinea* has not yet been fully determined.

The aim of this study was to determine the antioxidant, antimicrobial and antiproliferative activities of *P. coccinea* fruits and to analyze the chemical components of the plant. Antioxidant activity was evaluated in terms of the plant's capacity to neutralize free radicals. Antimicrobial activity was tested against various bacterial and fungal species to investigate the plant's potential in combating infectious diseases. Antiproliferative activity was examined using cell culture models and the plant's potential to inhibit the growth of cancer cells was evaluated. In addition, the chemical properties of the plant, such as phenolic compounds, total phenolic content and elemental analyses, were also examined in detail.

This study aims to provide comprehensive data on the biological activities and chemical content of

*P. coccinea* and to reveal the potential of the plant to be used in pharmacological and therapeutic applications. The findings obtained will contribute to the evaluation of plants as natural drug sources and will form a basis for future research.

## Materials and Methods

### Sample collection and preparation

*P. coccinea* fruit samples were collected from Osmaniye Korkut Ata University campus (coordinates 37°02'26.5"N 36°13'17.6"E). Plant samples were identified and stored in the herbarium of Osmaniye Korkut Ata University Biology Department (MS-1513). Collected fruit samples were dried under controlled conditions in a laboratory environment. The drying process was carried out at room temperature (20±2°C, 50±5% humidity) and in the shade to ensure the preservation of bioactive compounds of the fruits. Then, dried fruit samples were turned into a homogeneous powder using a mechanical grinder.

For the extraction process, 10 g of powdered fruit sample was weighed and placed in the Soxhlet extractor. Samples were extracted with 200 mL of ethanol (EtOH) at 50°C for 6-h. Soxhlet extraction method is a classical and reliable method that allows efficient obtaining of bioactive compounds from plant materials. After the extraction process was completed, the solvent was evaporated and the extract was concentrated. For this process, a Buchi R100 rotary evaporator operating at 40°C was used. The same procedure was repeated to obtain the methanol (MeOH) extract.

The crude extracts obtained were stored at +4°C for later analysis and biological activity tests. This storage condition was preferred to preserve the stability of the bioactive compounds of the extracts and to prevent their deterioration. The extraction and storage processes were meticulously carried out to ensure that the pharmacological properties of the plant materials were preserved and reliable results were obtained.

### Antioxidant activity assays

The DPPH assay works by combining a solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) with an antioxidant solution of an antioxidant, which is a molecule that tends to donate hydrogen atoms, to reduce the DPPH radical<sup>9</sup>. DMSO (dimethyl sulfoxide) was used to generate stock solutions comprising extracts at concentrations of 0.25, 0.5,

1 and 2 mg/mL. 160 µL of 0.039% DPPH was mixed with 50 µL of the ready solutions. Next, it was incubated for half an hour. At 517 nm, absorbance was measured following incubation. For every concentration that was specified, these procedures were carried out again. Reference antioxidants were ascorbic acid (AA). Finally, the following formula was used to calculate the percentages of DPPH free radical scavenging:

$$\text{Inhibition \%} = \left[ \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right] \times 100$$

The plant fruit's TAS, TOS and OSI values were ascertained using commercial kits bearing the Rel Assay brand (Rel Assay Diagnostics Kits, Turkey). According to Erel (2004), the TAS value was calibrated using trolox, and the findings were represented as mmol Trolox equivalent/L<sup>10</sup>. According to Erel (2005), the TOS value was calibrated using hydrogen peroxide, and the findings were expressed as µmol H<sub>2</sub>O<sub>2</sub> equivalent/L<sup>11</sup>. The unit of the TOS value and the unit of the TAS value were equalized for determining the OSI value. Next, the percentage was calculated by dividing the TOS value by the TAS value<sup>12</sup>.

### Determination of polyphenolic contents

*P. coccinea* fruit extract dissolved in ethanol was filtered using a filter with a pore size of 0.45 µm. The fruit phenolics of *P. coccinea* were evaluated using a Nexera Shimadzu UHPLC & LC-MSMS (Shimadzu) that was equipped with a column thermostat (CTO-10ASVP), a dual gradient pump, an autosampler (SIL-20AC), and a degasser (DGU-20A3R). A phenolic separation was conducted at 40°C using an Intersil ODS-4 analytical column with dimensions of 3 mm × 100 mm × 2 µm. The mobile phase was composed of a blend of solvents A and B, which comprised of 0.1% formic acid in water and methanol. The mobile phase flowed at a rate of 0.3 mL per minute, and a volume of 2 µL was injected. The conditions were sequentially as follows: 5% B for the initial 4 min, 95% B for the subsequent 7 min, 7 to 7.01 min for the following 7 min, and 5% B for the final 7 min. The identification of mass was conducted using a Shimadzu LCMS-8030, which is a triple tandem quadrupole mass spectrometer. An analysis was conducted on both positive and negative electrospray ionization processes. Nitrogen gas was used for nebulizing at a flow rate of 3 L/min and drying at a flow rate of 15 L/min. The heat block temperature was set at 400°C, the desolvation

temperature at 260°C, and the interface voltage at 4.5 kV. The data was analyzed with the LabSolutions software developed by Shimadzu<sup>13</sup>.

#### Total phenolic content (TPC)

TPC was measured using the Folin-Ciocalteu (FC) spectrophotometric technique. The specimens were ready for analysis after being diluted 20 times with EtOH. In order to calculate the TPC, distilled water was added to the FC reagent to dilute it 1:9, and a 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was made. To test tubes, 0.4 mL of the sample was introduced for examination. On it were placed 1.6 mL of Na<sub>2</sub>CO<sub>3</sub> and 2 mL of FC reagent solution. The mixture was vortexed and incubated for one hour under closed conditions. The absorbance was then measured at a wavelength of 765 nm. It was examined three times. The standard was gallic acid. Gallic acid equivalents per gram (mg GAE/g) of the extracts studied was the TPC of the *P. coccinea* fruit extract<sup>14</sup>.

#### Antimicrobial activity assays

The agar dilution method, which is advised by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on antibacterial Susceptibility Testing (EUCAST), was used to test the plant extracts' antibacterial activity in MeOH and EtOH. MeOH and EtOH extracts' minimum inhibitory concentrations (MIC) were measured in relation to common bacterial and fungal strains. Bacterial strains included *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *Acinetobacter baumannii* ATCC 19606 strains. The strains of fungi used were *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135, and *C. glabrata* ATCC 90030. American culture collections provided the bacterial and fungal strains. Mueller Hinton Broth was used for preculturing bacterial strains, while RPMI 1640 Broth was used for preculturing fungal strains. The turbidity of the bacteria and fungi was adjusted to the 0.5 McFarland standard in order to obtain a standardized vaccine. Distilled water was used to create all dilutions, and extracts ranging in concentration from 25 to 400 µg/mL were evaluated. The presence of colonies was seen as a sign of growth, and their absence as a sign of restraint. Furthermore, control plates were included in every study series. The minimum inhibitory concentration (MIC) was defined as the lowest dilution that inhibits the growth of bacteria and fungi<sup>15,16</sup>.

#### Elemental analyses

A sample of 0.3 g of *P. coccinea* fruit was obtained. It is mixed with 2 mL HNO<sub>3</sub> and 3 mL H<sub>2</sub>O<sub>2</sub>. Using the wet combustion approach, a temperature and pressure program was implemented in the closed system microwave (Berghoff) solubilizer. Twenty-five milliliters of pure water were added to the clear mineralized solution that resulted. The technique comprising the components to be studied was chosen, and the standards were initially introduced to the device, following the tuning solution's performance evaluation of the ICP-MS device (Agilent 7500a model)<sup>17</sup>. The element levels of Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Se, and Zn were measured in solubilized samples.

#### Antiproliferative activity assays

MeOH and EtOH extracts from the plant fruit were tested for cell viability in A549 cells using the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide). Cells were separated from the plant using 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) after it was 70-80% confluent. They were separated and then placed on plates. The transplant was followed by a 24-h incubation period. Following the incubation procedure, the cells were cultured for a full day with extracts diluted at varied concentrations (25, 50, 100, and 200 µg/mL). The growth medium used in the controls was not FCS-supplemented. Supernatants were dissolved in growth media and replaced with 1 mg/mL MTT (Sigma) following a 48-h incubation period. After that, the mixture was incubated at 37 °C until a purple precipitate formed. The MTT that the cells had ingested was combined with DMSO (Sigma-Aldrich, MO, USA) to solubilize the supernatants. After that, an Epoch spectrophotometer (BioTek Instruments, Winooska, VT) was used to measure the plates at 570 nm<sup>18</sup>.

## Results

#### Antioxidant activity and total phenolic contents

In this study, the antioxidant capacity of *P. coccinea* fruits was investigated in detail. The results are presented in Table 1 and Table 2.

In this study, the DPPH radical scavenging activities of ethanol and methanol extracts of *P. coccinea* fruits were compared. The results obtained revealed that the extracts showed lower activity compared to the standard antioxidant

Table 1 — DPPH radical scavenging activities of *Pyracantha coccinea* extracts

Concentration (mg/mL)	0.25	0.5	1	2
Ascorbic acid (%)	82.25±1.13	90.93±1.38	94.43±0.47	96.48±0.46
EtOH	40.65±2.41	58.99±0.86	74.29±1.96	80.61±1.04
MeOH	29.38±0.97	46.35±1.68	55.51±2.80	65.29±1.65

[Values are presented as means ± SD (n=3)]

Table 2 — Antioxidant and total phenolic properties of *Pyracantha coccinea* fruit extracts

	TAS (mmol/L)	TOS (μmol/L)	OSI (TOS/(TAS×10))	TPC (mg/g)
EtOH	8.337±0.155	12.510±0.371	0.150±0.007	3.791±0.067
MeOH	7.669±0.155	11.253±0.142	0.147±0.003	8.684±0.494

[Values are presented as means ± SD (n=3)]

Table 3 — Phenolic contents of fruit of *Pyracantha coccinea*

Analytes	Retention time	m/z	Amount (ppm)
Fumaric acid	0.809	115.20>71.10	64.17
Catechin	2.532	291.00>139.10	265.44
Syringic acid	3.001	199.10>140.10	0.62
Thymoquinone	3.337	165.00>137.00	0.3
Phloridzin	3.594	435.10>273.10	2.7
Resveratrol	3.606	229.00>135.00	1.09
Ellagic acid	3.681	301.10>228.90	0.41
Quercetin	3.891	301.10>150.90	2.94
Naringenin	3.952	271.00>150.90	0.5
Luteolin	4.069	285.00>150.90	0.7

Ascorbic acid. However, it was observed that the ethanol extract exhibited higher antioxidant activity compared to the methanol extract. This indicates that the solvent used in the extraction process has a significant effect on the solubility and extraction efficiency of plant compounds. One of the main objectives of this study was to evaluate the total phenolic content (TPC) of ethanol and methanol extracts from *P. coccinea* fruits. The results obtained are presented in Table 2. In the present study, methanol extract showed higher TPC than ethanol extract, indicating that methanol is a more effective solvent for phenolic extraction.

#### Phenolic contents

In this study, the presence of various bioactive compounds such as fumaric acid, phloridzin, catechin, syringic acid, quercetin, thymoquinone, resveratrol, ellagic acid, naringenin and luteolin were detected in *P. coccinea* fruits. The results determined are displayed in Table 3.

#### Antimicrobial activity

A study was done to examine the effects of ethanol and methanol extracts derived from *P. coccinea* fruit on various bacterial and fungal strains. The results collected are displayed in Table 4. In this study, the antimicrobial activities of ethanol and methanol extracts obtained from *P. coccinea* fruits against

various bacterial and fungal species were evaluated. Both extracts inhibited microbial growth at different concentrations, with ethanol extract exhibiting stronger activity. This suggests that the choice of solvent significantly influences the solubility and extraction efficiency of bioactive compounds. In our study, it was determined that the ethanol extract of *P. coccinea* fruits showed the highest inhibitory effect against *A. baumannii* at a concentration of 25 μg/mL. Ethanol extract showed inhibitory activity against *C. glabrata* at 50 μg/mL concentration, and against *E. coli*, *S. aureus*, *C. albicans*, *S. aureus* MRSA, *C. krusei* and *P. aeruginosa* at 100 μg/mL concentration. Ethanol extract showed inhibitory activity against all tested microorganisms at 200 μg/mL concentration. It was determined to be effective against *E. faecalis* at this concentration. Methanol extract was tested against various microorganisms at different concentrations. Inhibitory effects were observed against *A. baumannii* at 50 μg/mL concentration, *S. aureus*, *E. coli*, *S. aureus* MRSA and *C. glabrata* at 100 μg/mL concentration, *P. aeruginosa*, *C. albicans* and *C. krusei* at 200 μg/mL concentration. Its effect on *E. faecalis* was observed only at 400 μg/mL. These findings show that *P. coccinea* fruit extracts are effective against both gram-positive and gram-negative bacteria. Their antifungal activity also suggests potential utility against fungal pathogens.

#### Elements

The elemental composition of *P. coccinea* fruits was determined by analytical evaluation, and the results are presented in Table 5. Among the detected elements, magnesium (67.09 ± 1.03 ppm) and calcium (39.55 ± 1.40 ppm) were found in the highest concentrations, indicating that these minerals are the predominant components of the fruit. Iron was also present at a relatively high level (22.73 ± 0.60 ppm), suggesting a potential nutritional contribution. Manganese (4.45 ± 0.42 ppm) and zinc (1.36 ± 0.05

Table 4 — Antimicrobial activity of ethanol and methanol extract of *Pyracantha coccinea* fruit

	1	2	3	4	5	6	7	8	9
EtOH	100	100	200	100	100	25	50	100	100
MeOH	100	100	400	100	200	50	100	200	200

[\*(1) *S. aureus*, (2) *S. aureus* MRSA, (3) *E. faecalis*, (4) *E. coli*, (5) *P. aeruginosa*, (6) *A. baumannii*, (7) *C. glabrata*, (8) *C. albicans*, (9) *C. krusei* \*400, 200, 100, 50, 25 µg/mL extract concentrations]

Table 5 — Some chemical elements of fruit of *Pyracantha coccinea*

Analytes	Amount (ppm)
Ca	39.55±1.40
Co	0.18±0.01
Cr	0.26±0.03
Cu	0.43±0.01
Fe	22.73±0.60
Mg	67.09±1.03
Mn	4.45±0.42
Mo	0.07±0.00
Se	0.001±0.000
Zn	1.36±0.051

[Values are presented as means ± SD (n=3)]

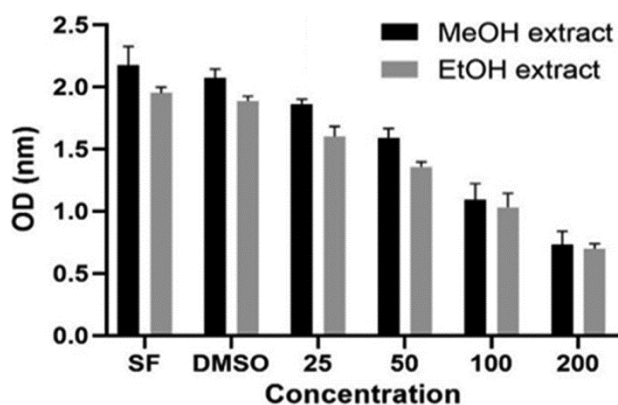


Fig. 1 — Antiproliferative effects of ethanol and methanol extracts of *Pyracantha coccinea* fruits on A549 cells. [SF: The group not treated with chemicals, only kept in the medium; DMSO: The group in which the medium and DMSO were applied; the group in which the extract was applied at 25, 50, 100 and 200 µg/mL concentrations]

ppm) were detected in moderate amounts, while copper ( $0.43 \pm 0.01$  ppm), chromium ( $0.26 \pm 0.03$  ppm), and cobalt ( $0.18 \pm 0.01$  ppm) were observed at trace levels. The lowest concentrations were measured for molybdenum ( $0.07 \pm 0.00$  ppm) and selenium ( $0.001 \pm 0.000$  ppm).

#### Antiproliferative activity

We conducted a study to investigate the impact of ethanol and methanol extracts from *P. coccinea* fruit on the A549 lung cancer cell line. The results obtained are displayed in Fig. 1.

The findings of our study showed that ethanol (EtOH) extracts of *P. coccinea* fruits exhibited a stronger biological effect compared to methanol

(MeOH) extracts. However, this difference diminished as the concentration increased. Both extracts demonstrated a dose-dependent increase in antiproliferative activity, especially at higher concentrations. This suggests that the bioactive compounds found in *P. coccinea* fruits increase their effect in direct proportion to the concentration.

#### Discussion

The body has antioxidant defense mechanisms to counterbalance the harmful effects of ROS. These mechanisms neutralize ROS through enzymatic antioxidants (such as superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidants (such as vitamins C and E, glutathione, and phenolic compounds)<sup>19-21</sup>. However, when antioxidant defense systems are inadequate or ROS production is excessive, oxidative stress occurs. Oxidative stress is known to contribute to the development of chronic diseases due to the accumulation of damage at the cellular level. These diseases include serious health problems such as cancer, cardiovascular diseases, neurodegenerative disorders (such as Alzheimer's and Parkinson's), diabetes, and multiple sclerosis<sup>22,23</sup>. Additional antioxidants obtained from the diet play a crucial role in mitigating the effects of oxidative stress. Plants exhibit strong antioxidant properties thanks to the phenolic compounds, flavonoids, and other bioactive molecules they contain. Therefore, determining the antioxidant capacity of plant sources is of great importance for the prevention and treatment of oxidative stress-related diseases<sup>24</sup>.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is a widely used method to evaluate the free radical scavenging ability of natural antioxidants<sup>25</sup>. In the literature, the DPPH activity values of ethanol and methanol extracts of *P. coccinea* fruits were reported as 78.73% and 97.43%, respectively<sup>25</sup>. Similarly, in a different study, it was reported that the methanol extract of *P. coccinea* fruits showed 94.21% DPPH activity<sup>26</sup>. In this study, it was determined that as the concentration of the extracts increased, their antioxidant activities also increased. This finding shows that the capacity of the bioactive compounds in

the extracts to neutralize free radicals increased depending on the concentration. It is thought that the differences in the obtained results may be due to the ecological and environmental conditions of the regions where the plant was collected. Factors such as soil structure, climate, altitude where the plant grows and harvest time can significantly affect the bioactive compound content of the plants and therefore the antioxidant capacity. Therefore, it is expected that there will be variations between the results obtained in different studies.

There are no previous reports in the literature on the TAS, TOS and OSI values of *P. coccinea*. This study is the first to evaluate these parameters. The ethanol extract of *P. coccinea* fruits used in our study exhibited higher TAS, TOS and OSI values compared to the methanol extract. These findings indicate that ethanol extract contains more antioxidant and oxidant compounds. In the literature, the TAS, TOS and OSI values of various plants were investigated using Rel Assay kits. For example, the TAS value for *Mentha longifolia* subsp. *longifolia* was reported as 3.628 mmol/L, the TOS value as 4.046  $\mu\text{mol/L}$  and the OSI value as 0.112<sup>27</sup>. For *Dittrichia graveolens*, the TAS value was determined as 6.933 mmol/L, TOS value as 12.535  $\mu\text{mol/L}$  and OSI value as 0.181<sup>28</sup>. For *Ferulago platycarpa*, the TAS value was recorded as 5.688 mmol/L, TOS value as 15.552  $\mu\text{mol/L}$  and OSI value as 0.273<sup>29</sup>. For *Rumex scutatus*, the TAS value was reported as 8.656 mmol/L, TOS value as 4.951  $\mu\text{mol/L}$  and OSI value as 0.057<sup>30</sup>. For *Helianthemum salicifolium*, the TAS value was reported as 9.490 mmol/L, TOS value as 14.839  $\mu\text{mol/L}$  and OSI value as 0.157<sup>31</sup>. In comparison, TAS values of both ethanol and methanol extracts of *P. coccinea* were higher than those of *M. longifolia* subsp. *longifolia*, *D. graveolens*, and *F. platycarpa*, but lower than those of *R. scutatus* and *H. salicifolium*. TAS values are considered as an indicator of total antioxidant compounds found in natural products<sup>32</sup>. In this context, it is understood that the antioxidant potential of *P. coccinea* fruits is comparable to many plants known for their antioxidant properties in the literature. While TOS values measure the presence of oxidant active compounds in natural products, OSI values reflect the capacity of antioxidant molecules to prevent the formation of oxidant compounds<sup>32</sup>. In our study, it was determined that TOS and OSI values of ethanol and methanol extracts of *P. coccinea* were higher than

those of *M. longifolia* subsp. *longifolia* and *R. scutatus*, but lower than *D. graveolens*, *F. platycarpa* and *H. salicifolium*. These results indicate that *P. coccinea* has a significant capacity in terms of inhibition of oxidant compounds. These findings highlight the significant antioxidant potential of *P. coccinea*. The findings support the usability of *P. coccinea* in pharmacological and therapeutic applications. However, further *in vitro* and *in vivo* studies are recommended to fully evaluate this potential.

The total phenolic content of *P. coccinea* fruit extracts has been reported with values ranging from 1.31 to 199.6 mg GAE/g in various studies conducted by different researchers<sup>25,26,33-35</sup>. This wide range can be explained by the variations resulting from factors such as the geographical region where the plant grows, harvest time, extraction method and solvent used. The obtained values were consistent with those in the literature, supporting the reliability of the data and confirming that *P. coccinea* is a rich source of phenolics. Phenolic compounds are important secondary metabolites that are largely responsible for the biological activities of plants. These compounds, known for their antioxidant, antimicrobial, anti-inflammatory and antiproliferative properties, increase the pharmacological potential of plants<sup>36</sup>. Therefore, the high phenolic content of *P. coccinea* fruits makes it possible to evaluate this plant as a natural therapeutic agent. In conclusion, this study revealed that *P. coccinea* fruits have a rich content of phenolic compounds. The findings obtained confirm the presence of phenolic compounds, which form the basis of the antioxidant and other biological activities of this plant. These results suggest potential uses in pharmacological and nutraceutical applications. However, in order to fully evaluate this potential, it is recommended that further studies be conducted in which the structural analyses and biological activities of phenolic compounds are examined in more detail.

Plants synthesize various bioactive chemicals during their metabolic processes. These compounds are called secondary metabolites and although they are not essential nutrients, they are of great medical importance. Secondary metabolites play a role in the defense mechanisms of plants and also exhibit various therapeutic effects on human health, such as antioxidant, antimicrobial, anti-inflammatory and antiproliferative<sup>37-39</sup>. Therefore, the identification of bioactive compounds in plants has great potential for pharmacological research.

In the literature, the presence of compounds such as epicatechin, caffeic acid, rutin, chlorogenic acid, p-hydroxybenzoic acid, syringic acid, vanillin, hyperoside, isoquercitrin, quercetin, gallic acid, protocatechuic acid, catechin, epicatechin gallate and kaempferol has been reported in *P. coccinea* fruits<sup>7,26,33,35</sup>. In contrast, our study detected fumaric acid, thymoquinone, phloridzin, resveratrol, ellagic acid, naringenin, and luteolin for the first time in this species. Such variation may be attributed to differences in geographical origin, climate, soil composition, and extraction methods.

Fumaric acid is a compound known for its antioxidant and anti-inflammatory properties. Thymoquinone stands out with its strong antioxidant and anticancer effects<sup>40</sup>. Phloridzin is of interest especially due to its potential effects in the treatment of diabetes<sup>41</sup>. Resveratrol is known for its protective effects on cardiovascular diseases and cancer<sup>42</sup>. While ellagic acid is known for its antimicrobial and anticancer properties<sup>43</sup>. The identification of these compounds further highlights the therapeutic potential of *P. coccinea*. Given this bioactive composition, *P. coccinea* fruits can be considered a promising natural source for drug development. The findings support the potential use of this plant in both pharmacological and nutraceutical applications. However, further in vitro and in vivo studies are needed to clarify the biological roles of these compounds and support clinical translation.

Today, combating diseases caused by microorganisms has become increasingly difficult. The main reasons for this difficulty include the potential side effects of antimicrobial drugs and the problem of drug resistance that develops due to the uncontrolled use of these drugs. Antibiotic resistance, in particular, has become one of the biggest threats facing modern medicine. This has led scientists to discover new and effective antimicrobial agents<sup>44,45</sup>. In this context, plants stand out as a natural solution source. In the literature, it has been reported that *P. coccinea* fruit extracts are effective against microorganisms such as *C. albicans*, *E. coli*, *E. faecalis*, *S. aureus* and *P. aeruginosa* using the disk diffusion method<sup>34</sup>. These results show that the antimicrobial properties of *P. coccinea* are due to the phenolic compounds and other bioactive molecules it contains. In conclusion, this study revealed that *P. coccinea* fruits exhibited antimicrobial activity against traditional bacterial and fungal species. The

results support its potential as a natural antimicrobial agent, but further in vitro and in vivo studies are necessary to assess its clinical relevance.

In the literature, it has been reported that *P. coccinea* fruits contain different levels of elements such as selenium (Se), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), zinc (Zn), silver (Ag), phosphorus (P) and sodium (Na)<sup>26</sup>. These essential minerals play key roles in human metabolism<sup>46</sup>. For example, magnesium is critical for energy metabolism and muscle functions, while zinc is necessary for the immune system and cell growth. Iron plays a role in vital processes such as oxygen transport and DNA synthesis<sup>47</sup>. In our study, magnesium (Mg) was identified as the most abundant element, with a concentration of 67.09 ppm. Variations in elemental levels compared to the literature may be attributed to environmental and ecological factors, such as soil mineral content, climate, altitude, and the plant's uptake capacity. These factors can significantly influence the elemental profile of plant materials. In this context, *P. coccinea* appears to possess a rich mineral composition and can be considered a natural dietary source of essential elements. The findings obtained show that this plant contains essential minerals necessary for human health and has the potential to be used as a food supplement. In addition, considering the biological activities and therapeutic effects of these elements, it can be suggested that *P. coccinea* fruits can be a valuable source for pharmacological research. In conclusion, this study provides valuable data on the elemental composition of *P. coccinea* fruits. The findings support its potential use as both a natural mineral supplement and a candidate for pharmacological applications.

Currently, there is a rising tendency in both the number of deaths and cases of cancer. Specialized therapy approaches tailored to specific cancer types are currently being developed. Supplements containing natural compounds with anticancer properties are utilized based on these therapy strategies. It is crucial to examine the anticancer properties of plants in this particular situation<sup>48</sup>. In the literature, the effects of *P. coccinea* on various cancer cell lines have been investigated. For example, it has been reported that the methanol extract of *P. coccinea* fruits is effective on the Hela cell line (cervical cancer)<sup>5</sup>. In our study, a significant antiproliferative effect was observed on the A549 lung cancer cell line,

indicating potential anticancer properties. These findings suggest that *P. coccinea* fruits may have natural anticancer properties. This study revealed that *P. coccinea* fruits have a promising effect especially on lung cancer cells. The findings obtained support the potential of this plant to be evaluated as a natural anticancer agent. However, it is recommended that further in vitro and in vivo studies be conducted to fully understand the mechanisms of these effects and to transfer them to clinical applications.

### Conclusion

The aim of this study was to evaluate the antioxidant, antimicrobial, and antiproliferative activities of ethanol and methanol extracts obtained from *P. coccinea* fruits. In addition, mineral composition, total phenolic content, and phenolic profile were analyzed. The results demonstrated that *P. coccinea* fruits possess notable antioxidant capacity, exhibit effective antimicrobial activity against human pathogens, and show significant antiproliferative effects on the A549 lung cancer cell line. The extracts were also found to contain high levels of phenolic compounds and essential minerals. These findings support the use of *P. coccinea* as a natural source for pharmacological applications. Further in vitro and in vivo studies are recommended to better understand its therapeutic potential and promote its contribution to human health.

### Conflict of interest

The authors declare that they have no conflicts of interest.

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