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Comparative study of *Pentanema verbascifolium* extracts: Phytochemical composition, antioxidant potential, and enzyme inhibition across plant parts

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ABSTRACT

Plants are invaluable sources of bioactive compounds with diverse therapeutic potential, yet the specific roles of various plant parts remain underexplored. This study aimed to assess the chemical composition, antioxidant activity, and enzyme inhibitory properties of methanol extracts from different parts (flower, leaf, stem, and root) of *Pentanema verbascifolium* obtained via ultrasound-assisted extraction. Chemical profiling revealed significant variability in phytochemical distribution, with leaves exhibiting the highest total phenolic (94.38 mg GAEs/g) and flavonoid contents (67.54 mg REs/g). Chlorogenic acid, a dominant compound, was most abundant in leaves (9739 μg/g), correlating strongly with their superior antioxidant activity. Among the antioxidant assays, leaf extracts showed the highest reducing power (Cupric Reducing Antioxidant Capacity-CUPRAC: EC_{50} 0.60 mg/mL; Ferric reducing antioxidant power-FRAP: EC_{50} 0.26 mg/mL) and radical scavenging ability (2,2-Diphenyl-1-picrylhydrazyl-DPPH: IC_{50} 1.35 mg/mL; ABTS: IC_{50} 1.28 mg/mL), supported by their highest Relative Antioxidant Capacity Index (RACI) score (1.26). Root extracts displayed notable phosphomolybdenum activity (EC_{50} 0.33 mg/mL) and the strongest tyrosinase inhibition (IC_{50} 1.16 mg/mL). Enzyme inhibition assays highlighted leaf and flower extracts as potent α-amylase inhibitors (IC_{50} 2.5 mg/mL), while α-glucosidase inhibition was comparable across extracts (IC_{50} 1.1 mg/mL). Correlation analyses identified phenolics, especially chlorogenic acid, as primary contributors to antioxidant activity, whereas flavonoids played a lesser role. These findings underscore the functional diversity of *P. verbascifolium* extracts, particularly the leaves, as rich sources of phenolics with potential therapeutic applications. Future studies should focus on isolating and characterizing individual bioactive compounds and evaluating their synergistic effects to enhance the understanding of their pharmacological potential.

1. Introduction

Traditional medicine relies extensively on natural products, particularly plants, for therapeutic applications. Various medical systems, including Ayurveda, Unani, and traditional Chinese medicine, have long utilized plant-derived bioactive compounds to treat numerous ailments (Birhan et al., 2024; Rondilla et al., 2021). The pharmacological potential of these medicinal plants is largely attributed to their secondary metabolites, which exhibit antioxidant, anti-inflammatory, and enzyme inhibitory properties (Twaij & Hasan, 2022). Scientific validation of these traditional remedies is crucial for identifying bioactive compounds that may serve as leads for drug development.

Oxidative stress plays a significant role in the pathogenesis of chronic diseases such as atherosclerosis, diabetes, neurodegenerative disorders, and cancer (Forman & Zhang, 2021; Reddy, 2023). Plant-derived antioxidants can mitigate oxidative stress, reducing cellular damage and improving disease outcomes (Bai et al., 2022; Liu et al., 2024). Consequently, research on plant metabolites with antioxidant potential remains a focal point in pharmaceutical and nutraceutical studies (Liu et al., 2025).

Enzyme inhibition is another critical area of research, particularly concerning neurodegenerative diseases, hyperpigmentation, and diabetes. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors play a key role in managing Alzheimer's disease by enhancing cholinergic function and reducing β-amyloid aggregation (Adewusi et al., 2010). Similarly, tyrosinase inhibitors have therapeutic relevance in hyperpigmentation disorders and food preservation (Zolghadri et al., 2019). In diabetes management, α-amylase and α-glucosidase inhibitors

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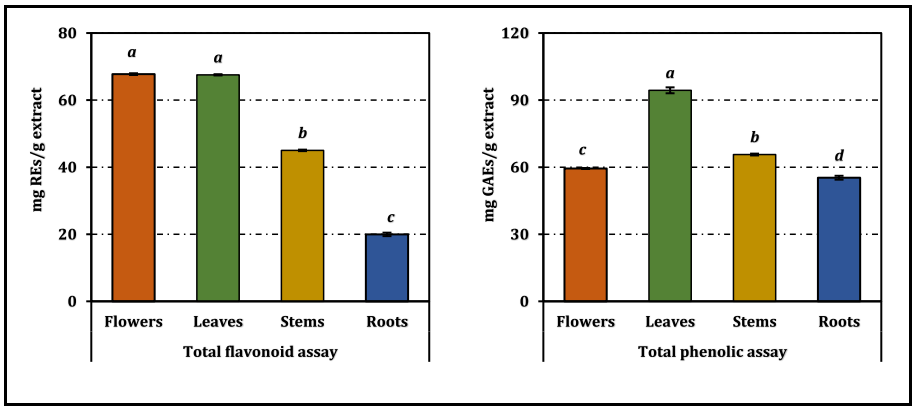

Fig. 1. Total flavonoid and phenolic contents of *Pentanema verbascifolium* extracts. REs and GAEs: Rutin and gallic acid equivalents, respectively. Values indicated by the same superscripts (*a–d*) are not different from the honestly significant difference after Tukey's hoc test at 5 % significance level.

regulate postprandial glucose levels, making plant-derived polyphenols valuable candidates for natural therapeutics (Nair et al., 2013).

This study investigates the chemical composition and bioactivities of *Pentanema verbascifolium* (Willd.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., a plant with limited scientific documentation. Our research was guided by the hypothesis that different parts of *P. verbascifolium* may exhibit significant bioactive properties, including antioxidant potential and enzyme inhibitory effects, due to their phytochemical composition. This hypothesis was based on previous studies highlighting the presence of phenolic compounds and flavonoids in related species, which have demonstrated pharmacological relevance in oxidative stress-related disorders. Given the role of oxidative stress and enzyme dysregulation in conditions such as Alzheimer's disease, diabetes, and hyperpigmentation, we specifically evaluated the inhibitory effects of *P. verbascifolium* extracts on acetylcholinesterase, tyrosinase, and α-amylase enzymes. Methanol extracts from its flowers, leaves, roots, and stems were obtained using ultrasound-assisted extraction and analyzed for their total phenolic and flavonoid content, chromatographic composition, and bioactivity. To ensure consistency in antioxidant evaluation, Relative Antioxidant Capacity Index (RACI) analysis was applied, and statistical correlations between RACI values and antioxidant assays were determined. Furthermore, correlations between phenolic compounds and antioxidant activity were assessed. To our knowledge, this is the first comprehensive study on the chemical and biological properties of *P. verbascifolium*, providing valuable insights for future pharmacological and nutraceutical applications.

2. Material and method

2.1. Plant material

P. verbascifolium was collected in full bloom on June 1, 2024, from limestone formations in the Akseki district of Antalya, Turkey (36°54′32″N, 31°53′28″E) at an altitude of 1600 m. The identification of the plant was confirmed by Dr. Bedrettin Selvi, and a voucher specimen (GOPU 9527) has been deposited in the Herbarium of the Faculty of Arts and Sciences at Tokat Gaziosmanpaşa University.

2.2. Methanol extraction

Plant samples were air-dried in a shaded, well-ventilated environment to prevent moisture retention. The dried material was separated into flowers, leaves, stems, and roots, each of which was pulverized into a fine powder. Ultrasound-assisted extraction was performed using methanol as the solvent, with a plant-to-solvent ratio of 1:20 (w/v). Extraction was carried out for 1 h in a sonication bath, and the resulting methanol extracts were concentrated under reduced pressure using a rotary evaporator. Extracts were stored at 4 °C for a maximum of two weeks. Extraction yields were calculated as 5.72 %, 6.42 %, 5.00 %, and 3.25 % for flowers, leaves, stems, and roots, respectively.

2.3. Determination of the phenolic compositions of the extracts

Total phenolic and flavonoid contents of the extracts were assessed spectrophotometrically following the method described by Zengin et al. (2017). Phytochemical profiling of the extracts was performed using a previously validated chromatographic method (Cittan & Çelik, 2018). Detailed analytical parameters are provided in Tables S1 and S2 of the supplementary materials.

2.4. Biological activity

Antioxidant activities of the extracts were evaluated using the phosphomolybdenum assay, ferrous ion chelating assay, reducing power assays (CUPRAC and FRAP), and radical scavenging assays (DPPH and ABTS), following the methodologies described by Kocak et al. (2016) and Sarikurkcu et al. (2020). Enzyme inhibitory activities were assessed using cholinesterase (AChE and BChE), α-amylase, α-glucosidase, and tyrosinase inhibitory assays, based on the protocol described by Ozer et al. (2018). Supplementary materials include comprehensive descriptions of the methods employed.

2.5. Statistical analysis

Results were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA, followed by Tukey's post-hoc test, with *p*-values less than 0.05 considered significant. Analyses were performed using SPSS version 26.0.

To account for variations in the mechanisms underlying different antioxidant assays, direct result comparisons were standardized using the RACI. The RACI values were computed by subtracting the mean assay values from the raw data and dividing by the standard deviation, allowing for meaningful cross-assay comparisons. Pearson correlation analysis was further applied to evaluate relationships between RACI values and individual assay results, providing a comprehensive understanding of antioxidant potential (Sun & Tanumihardjo, 2007).

3. Results and discussion

3.1. Chemical composition

The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts demonstrate a significant variation among different plant parts (Fig. 1). Leaf extracts exhibit the highest TPC (94.38 mg GAEs/g),

Table 1
Concentration (μg/g extract) of selected phenolic compounds in *P. verbascifolium* extracts determined by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC–ESI–MS/MS).

Compounds	Flowers	Leaves	Stems	Roots
Chlorogenic acid	2839 ± 34 ^{d}	9739 ± 129 ^{a}	8363 ± 41 ^{b}	5996 ± 12 ^{c}
Apigenin	1923 ± 27 ^{b}	2406 ± 31 ^{a}	1547 ± 17 ^{c}	134 ± 9 ^{d}
Apigenin 7-glucoside	2022 ± 37 ^{b}	2245 ± 1 ^{a}	1445 ± 2 ^{c}	35.7 ± 0.7 ^{d}
3-Hydroxybenzoic acid	222 ± 4 ^{a}	230 ± 3 ^{a}	181 ± 2 ^{b}	39.2 ± 0.4 ^{c}
4-Hydroxybenzoic acid	220 ± 5 ^{a}	230 ± 3 ^{a}	183 ± 2 ^{b}	36.7 ± 0.6 ^{c}
Kaempferol	414 ± 10 ^{a}	150 ± 4 ^{b}	65.7 ± 0.1 ^{c}	39.9 ± 1.5 ^{d}
Vanillin	86.5 ± 6.7 ^{c}	111 ± 2 ^{ab}	123 ± 5 ^{a}	95.0 ± 0.2 ^{bc}
Luteolin 7-glucoside	128 ± 2 ^{a}	110 ± 1 ^{b}	52.8 ± 0.4 ^{c}	nd
2,5-Dihydroxybenzoic acid	136 ± 1 ^{a}	77.6 ± 6.4 ^{b}	144 ± 5 ^{a}	49.9 ± 1.8 ^{b}
Protocatechuic acid	133 ± 2 ^{b}	76.1 ± 0.9 ^{c}	140 ± 1 ^{a}	51.0 ± 1.0 ^{d}
Syringic acid	87.4 ± 2.3 ^{b}	74.4 ± 2.1 ^{c}	104 ± 5 ^{a}	90.0 ± 2.8 ^{b}
Ferulic acid	50.1 ± 3.3 ^{c}	67.0 ± 1.4 ^{b}	76.5 ± 2.2 ^{a}	54.6 ± 1.2 ^{c}
Luteolin	186 ± 27 ^{a}	60.0 ± 0.1 ^{b}	46.2 ± 3.8 ^{c}	nd
Caffeic acid	79.1 ± 3.3 ^{a}	44.2 ± 0.4 ^{c}	47.4 ± 1.7 ^{c}	62.0 ± 1.7 ^{b}
Pinoresinol	21.8 ± 2.0 ^{b}	7.34 ± 0.38 ^{c}	20.8 ± 0.4 ^{b}	46.5 ± 1.5 ^{a}
Rosmarinic acid	21.3 ± 1.8 ^{a}	5.91 ± 0.45 ^{c}	8.48 ± 0.35 $^{b\ c}$	11.7 ± 0.6 ^{b}
Taxifolin	89.0 ± 0.2 ^{a}	5.75 ± 0.14 ^{c}	5.10 ± 0.02 ^{c}	7.02 ± 0.26 ^{b}
Gallic acid	6.23 ± 0.10 ^{b}	5.79 ± 0.08 ^{b}	20.4 ± 0.3 ^{a}	nd
Quercetin	14.8 ± 0.8	nd	nd	nd
Pyrocatechol	nd	nd	nd	nd
Verbascoside	nd	nd	nd	nd
p-Coumaric acid	nd	nd	nd	nd
Sinapic acid	nd	nd	nd	nd
Eriodictyol	nd	nd	nd	nd
Hyperoside	nd	nd	nd	nd
(−)-Epicatechin	nd	nd	nd	nd
(+)-Catechin	nd	nd	nd	nd
2-Hydroxycinnamic acid	nd	nd	nd	nd
3,4-Dihydroxyphenylacetic acid	nd	nd	nd	nd
Hesperidin	nd	nd	nd	nd

The values indicated by the same superscripts within the same row are not different according to the Tukey's honestly significant difference post hoc test at 5 % significance level. nd: Not detected.

significantly surpassing stem (65.66 mg GAEs/g), flower (59.41 mg GAEs/g), and root extracts (55.29 mg GAEs/g). A similar trend is observed for TFC, with leaf and flower extracts showing comparable, statistically indistinguishable values (67.54 and 67.75 mg REs/g, respectively), while stem (45.01 mg REs/g) and root (19.96 mg REs/g) extracts contain considerably lower amounts.

This pattern indicates that the aerial parts (leaves and flowers) are the primary contributors to the plant's phenolic and flavonoid profiles, possibly due to their direct exposure to environmental stressors like UV radiation, which often triggers phenolic compound biosynthesis as a protective mechanism.

The LC-ESI-MS/MS analysis highlights the presence of diverse phytochemicals in the extracts, with notable variations in their concentrations across different plant parts (Table 1). Chlorogenic acid is most abundant in the leaf extract (9739 μg/g), followed by the stem (8363 μg/g), root (5996 μg/g), and flower extracts (2839 μg/g), demonstrating its dominance in leaves and aligning with their high total phenolic content (TPC). Apigenin and its glucoside are also most concentrated in leaves (2406 μg/g and 2245 μg/g, respectively), with flower extracts containing slightly lower but still significant amounts (1923 μg/g and 2022 μg/g), whereas their levels in roots are minimal. Kaempferol is found at the highest concentration in flowers (414 μg/g), with decreasing levels in stems (65.7 μg/g) and roots (39.9 μg/g). Vanillin is slightly more abundant in stems (123 μg/g) compared to leaves and roots (111 μg/g and 95 μg/g, respectively). Luteolin and its glucoside are predominantly localized in flowers and leaves, with luteolin being undetectable in roots, emphasizing the specificity of flavonoid distribution. Meanwhile, roots display relatively higher levels of certain less abundant compounds such as pinoresinol (46.5 μg/g) and syringic acid (90.0 μg/g) compared to aerial parts.

The chemical compositions of the extracts show distinct patterns. Leaf extracts are enriched with phenolic acids like chlorogenic acid and 4-hydroxybenzoic acid, as well as flavonoids such as apigenin and kaempferol, making them the most phenolic-rich fraction, supported by their high TPC and total flavonoid content (TFC). While flower extracts have slightly lower TPC than leaves, they are rich in flavonoids like luteolin and apigenin, indicating their metabolic specialization. Stem extracts, with intermediate phenolic content, are particularly rich in vanillin and ferulic acid. Root extracts, having the lowest TPC and TFC, still stand out for their higher concentrations of unique compounds such as pinoresinol and syringic acid, reflecting the distinct metabolic roles of roots.

Methanol, as a moderately polar solvent, proves effective in extracting a broad range of phenolic acids and flavonoids. The observed distribution patterns suggest that the solvent's polarity aligns well with the polar nature of phenolic acids (e.g., chlorogenic acid) and glycosylated flavonoids (e.g., apigenin 7-glucoside). Nevertheless, differences in compound concentrations among extracts are also influenced by the intrinsic chemical composition and specific metabolite localization in plant tissues.

The high TPC in leaf extracts strongly correlates with their elevated chlorogenic acid and flavonoid levels, supporting the contribution of these compounds. In contrast, root extracts, with their lower TPC and TFC, contain limited amounts of these bioactive compounds. Overall, the findings highlight the chemical diversity of *P. verbascifolium*, with leaves emerging as the richest source of phenolic and flavonoid compounds. The compositional differences among plant parts reflect their functional specialization and underscore the effectiveness of methanol for extracting polar phytochemicals. Future research could explore the biological activities of individual compounds to uncover their potential applications.

This study presents the first comprehensive analysis of the chemical composition of methanol extracts obtained from the flower, leaf, stem, and root parts of *P. verbascifolium* using ultrasound-assisted extraction. A thorough literature review revealed no prior reports detailing the phytochemical profile of this species, making our findings a novel addition to the body of knowledge surrounding the genus Pentanema.

While the chemical compositions of certain *Pentanema* species have been documented, the current study expands the scope of knowledge by examining multiple plant parts and employing advanced extraction techniques. For instance, *P. divaricatum* was reported to contain 4α,5α-epoxy-10α,14H-1-epi-inuviscolide, a sesquiterpene lactone with notable antifungal and antibacterial properties (Momen-Roknabadi et al., 2008). Similarly, the methanol extract of *P. confertiflorum* root bark demonstrated significant total phenolic (TPC: 76.7 mg GAE/g), flavonoid (TFC: 86.89 mg QE/g), and antioxidant activities (Birhan et al., 2024). These findings suggest a potential correlation between the phenolic and flavonoid contents of *Pentanema* species and their bioactivities.

Moreover, Pei et al. (2024-a) isolated five new sesquiterpenoids, including stereoisomers, from *P. britannicum* flowers, highlighting the genus's chemical diversity. Our study complements these findings by providing insights into the chemical constituents of *P. verbascifolium*,

Table 2
Antioxidant activities of *Pentanema verbascifolium* extracts.

Assays	Flowers	Leaves	Stems	Roots	Trolox	EDTA
Phosphomolybdenum (EC_{50}: mg/mL)	0.44 ± 0.002 ^{d}	0.35 ± 0.004 ^{b}	0.39 ± 0.002 ^{c}	0.33 ± 0.011 ^{b}	0.23 ± 0.014 ^{a}	–
CUPRAC reducing power (EC_{50}: mg/mL)	1.03 ± 0.011 ^{d}	0.60 ± 0.027 ^{b}	0.82 ± 0.021 ^{c}	0.84 ± 0.016 ^{c}	0.14 ± 0.006 ^{a}	–
FRAP reducing power (EC_{50}: mg/mL)	0.43 ± 0.010 ^{d}	0.26 ± 0.006 ^{b}	0.34 ± 0.003 ^{c}	0.41 ± 0.005 ^{d}	0.045 ± 0.0004 ^{a}	–
DPPH radical (IC_{50}: mg/mL)	2.54 ± 0.020 ^{d}	1.35 ± 0.008 ^{b}	1.85 ± 0.071 ^{c}	2.43 ± 0.090 ^{d}	0.22 ± 0.0031 ^{a}	–
ABTS radical cation (IC_{50}: mg/mL)	2.07 ± 0.006 ^{d}	1.28 ± 0.049 ^{b}	1.88 ± 0.030 ^{c}	2.22 ± 0.042 ^{e}	0.20 ± 0.004 ^{a}	–
Ferrous ion chelating (IC_{50}: mg/mL)	6.78 ± 0.981 ^{c}	1.33 ± 0.081 ^{a}	1.53 ± 0.028 ^{ab}	3.19 ± 0.093 ^{b}	–	0.018 ± 0.0001 ^{a}

TEs and EDTAEs mean trolox and ethylenediaminetetraacetic acid (disodium salt) equivalents, respectively. Values indicated by the same superscripts (*a-d*) are not different from the honestly significant difference after Tukey's hoc test at 5 % significance level.

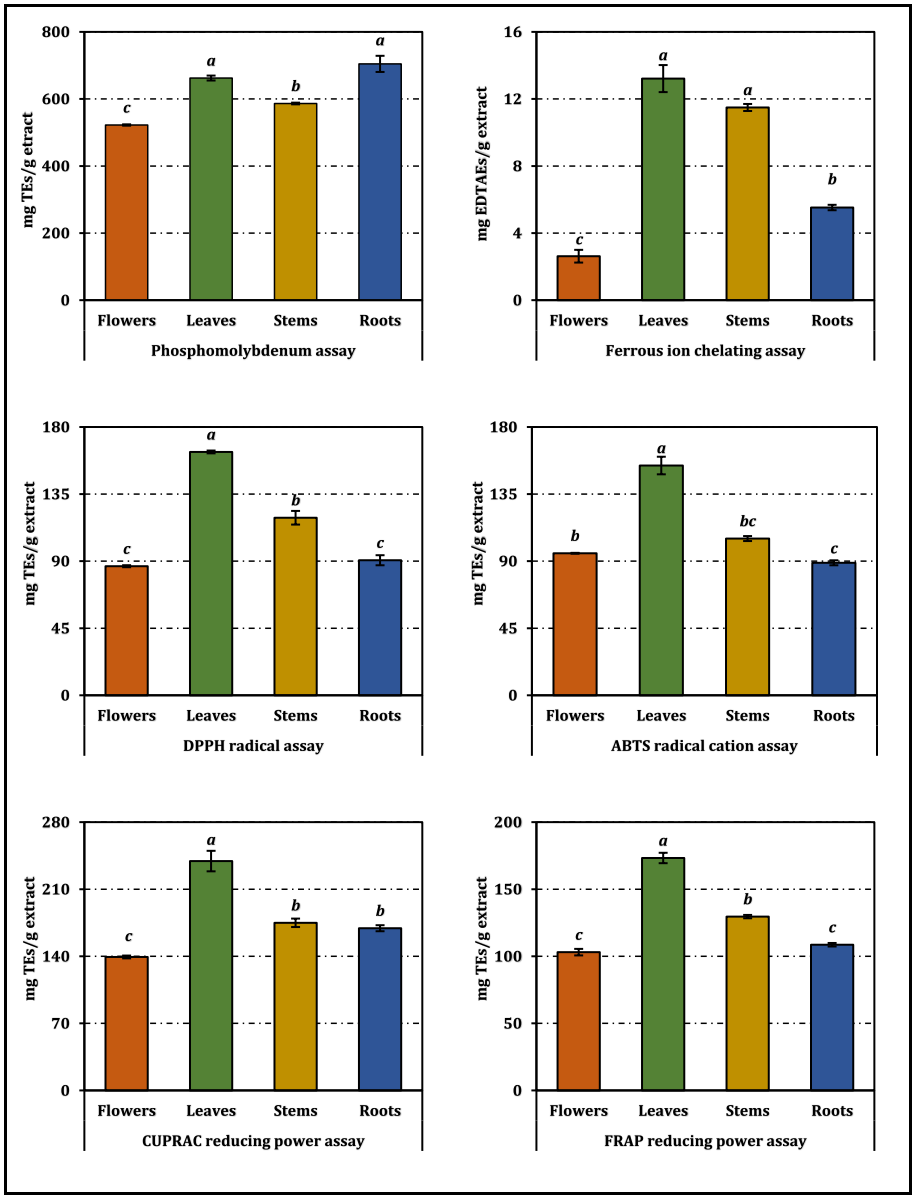

Fig. 2. Antioxidant activity of *Pentanema verbascifolium* extracts. TEs and EDTAEs, trolox and ethylenediaminetetraacetic acid (disodium salt) equivalents, respectively. Values indicated by the same superscripts (*a–d*) are not different from the honestly significant difference after Tukey's hoc test at 5 % significance level.

which could potentially harbor similarly unique sesquiterpenoids or other bioactive compounds. Additionally, *P. kurdistanicum* essential oil was found to be rich in oxygenated sesquiterpenes, such as 14-hydroxy-(Z)-caryophyllene and terpinen-4-ol (Safar et al., 2022). These studies collectively underscore the genus's significant phytochemical potential, aligning with our findings on *P. verbascifolium*.

Recent advancements in natural compound extraction have introduced innovative methodologies that enhance both efficiency and sustainability. For instance, the study by Trampetti et al. (2019) highlighted the effectiveness of solvent-based extraction techniques in obtaining bioactive metabolites from *Cistanche phelypaea*, demonstrating the influence of solvent type on antioxidant and enzyme inhibitory properties.

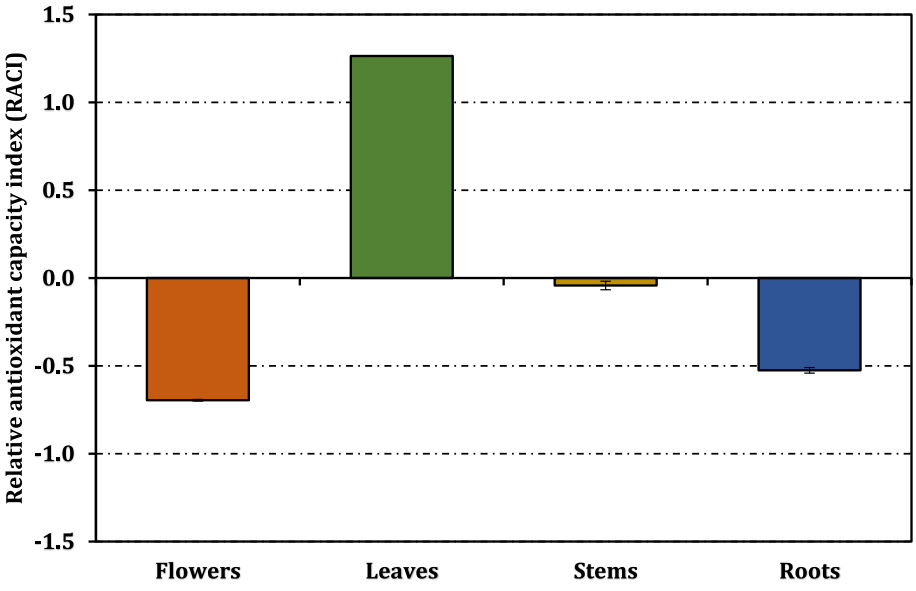

Fig. 3. Relative antioxidant capacity index of *Pentanema verbascifolium* extracts.

Additionally, Marinaccio, Zengin, Bender, Dogan, et al. (2024) explored ultrasound-assisted extraction (UAE) in combination with deep eutectic solvents (DES) for the recovery of lycopene from tomato skin waste, illustrating a green and efficient approach. Another study by the same group investigated the direct enrichment of extra virgin olive oil (EVOO) with lycopene using UAE, showcasing the potential of food-grade solvents for bioactive compound extraction (Marinaccio, Zengin, Bender, Cichelli, et al., 2024). These studies underline the importance of optimizing solvent selection and extraction parameters to enhance the yield and bioactivity of phytochemicals. In light of these advancements, our study contributes to the growing body of knowledge by comparing the phytochemical composition, antioxidant potential, and enzyme inhibitory activities of *P. verbascifolium* extracts obtained through different solvent-based extraction methods.

The current research offers a unique contribution by employing ultrasound-assisted extraction, a technique known for its efficiency in isolating thermally sensitive compounds. This methodological advancement likely enhanced the yield and preservation of bioactive constituents in our study compared to conventional methods employed in previous investigations.

From a phytochemical perspective, the chemical composition of *P. verbascifolium* offers a valuable point of comparison to other *Pentanema* species. While oxygenated sesquiterpenes, phenolic acids, and flavonoids have been the primary compounds of interest in the genus, the absence of data on *P. verbascifolium* highlights the novelty of our findings. The detailed chemical profiling of its flower, leaf, stem, and root parts provides a foundational dataset for future studies, including bioactivity assessments and potential industrial applications.

In summary, this study represents the first step in elucidating the phytochemical landscape of *P. verbascifolium*, offering new insights into the chemical diversity of the genus *Pentanema*. Our findings not only fill a critical gap in the literature but also lay the groundwork for further exploration of the bioactive properties of this species and its potential applications in pharmacology, agriculture, and other fields.

3.2. Antioxidant activity

The antioxidant activities of *P. verbascifolium* extracts were evaluated using six distinct assays, each targeting different antioxidant mechanisms. Significant differences in activity were observed among the extracts, as presented in Table 2 and Fig. 2. Below, the results of each assay are summarized, followed by an integrative analysis using the RACI to identify the most potent extract.

In the phosphomolybdenum assay, which assesses total antioxidant capacity, root extracts demonstrated the highest activity (EC_{50}: 0.33 mg/mL), statistically comparable to leaf extracts (EC_{50}: 0.35 mg/mL). Stem (EC_{50}: 0.39 mg/mL) and flower extracts (EC_{50}: 0.44 mg/mL) displayed significantly lower activities. As expected, the reference standard, trolox, exhibited much stronger activity (EC_{50}: 0.23 mg/mL), underscoring the relative differences between the plant extracts.

The CUPRAC reducing power assay identified leaf extracts as the most effective (EC_{50}: 0.60 mg/mL), followed by stem (EC_{50}: 0.82 mg/mL) and root (EC_{50}: 0.84 mg/mL) extracts, which exhibited statistically similar activity. Flower extracts were the least potent in this assay (EC_{50}: 1.03 mg/mL). Trolox, with an EC_{50} of 0.14 mg/mL, showed substantially greater activity compared to all plant extracts.

In the FRAP reducing power assay, a similar trend was observed. Leaf extracts displayed superior antioxidant activity (EC_{50}: 0.26 mg/mL), followed by stem (EC_{50}: 0.34 mg/mL) and root extracts (EC_{50}: 0.41 mg/mL). Flower extracts (EC_{50}: 0.43 mg/mL) again exhibited the weakest activity. The benchmark compound trolox (EC_{50}: 0.045 mg/mL) demonstrated far greater activity than any of the extracts.

Radical scavenging activities were evaluated using the DPPH and ABTS assays. In the DPPH assay, leaf extracts showed the highest scavenging capacity (IC_{50}: 1.35 mg/mL), followed by stem (IC_{50}: 1.85 mg/mL), flower (IC_{50}: 2.54 mg/mL), and root (IC_{50}: 2.43 mg/mL) extracts. The ABTS assay produced similar results, with leaf extracts (IC_{50}: 1.28 mg/mL) being the most effective, followed by stem (IC_{50}: 1.88 mg/mL), flower (IC_{50}: 2.07 mg/mL), and root (IC_{50}: 2.22 mg/mL) extracts. In both assays, trolox showed superior activity (IC_{50}: 0.22 mg/mL and 0.20 mg/mL, respectively).

The ferrous ion chelating assay, which evaluates metal ion-binding capacity, revealed that leaf extracts had the strongest activity (IC_{50}: 1.33 mg/mL), followed by stem (IC_{50}: 1.53 mg/mL) and root (IC_{50}: 3.19 mg/mL) extracts. Flower extracts exhibited significantly weaker chelating activity (IC_{50}: 6.78 mg/mL). The reference standard EDTA displayed the strongest activity (IC_{50}: 0.018 mg/mL), emphasizing the relatively limited chelating ability of the plant extracts.

Given the variability in assay principles, direct comparisons between the results of different assays are scientifically inappropriate. To provide a standardized comparison, RACI values were calculated for each extract (Fig. 3). The calculated RACI values were as follows: flower (−0.70), leaf

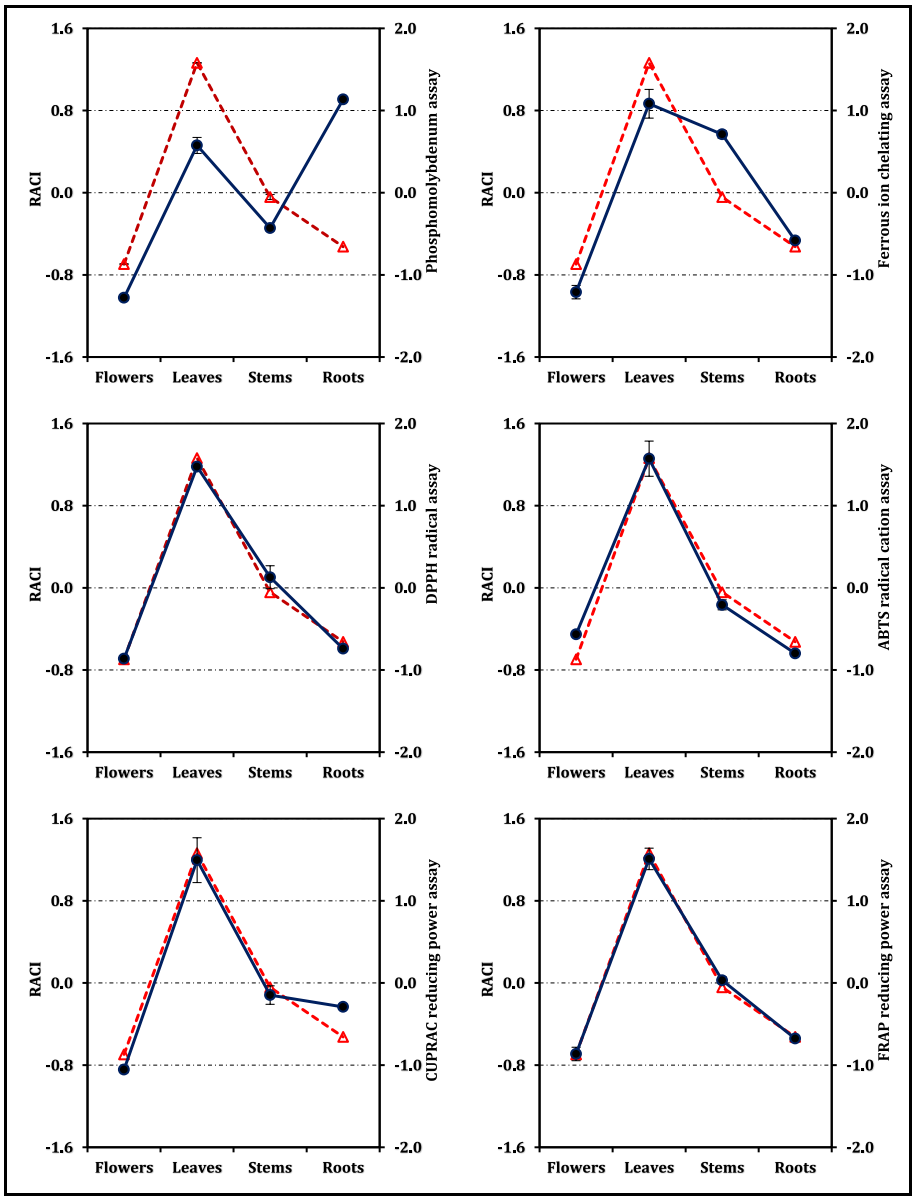

Fig. 4. Correlation between the RACI (dashed red line with triangle) and antioxidant activity (solid dark blue line with circle).

(1.26), stem (−0.04), and root (−0.53).

Based on the RACI analysis, leaf extracts demonstrated the highest antioxidant capacity, with a positive RACI value of 1.26. This observation aligns with their elevated phenolic and flavonoid content, as determined in prior analyses. These bioactive compounds, such as chlorogenic acid, apigenin, and kaempferol, likely contribute to the leaf extracts' robust performance across various antioxidant mechanisms, including free radical scavenging, reducing power, and metal ion chelation.

In contrast, flower, stem, and root extracts exhibited negative RACI values, indicating comparatively lower antioxidant activities. Among these, stem extracts (−0.04) showed the least negative value, reflecting an intermediate antioxidant capacity, possibly due to their moderate phenolic content. Root extracts (−0.53) displayed reduced antioxidant potential, consistent with their lower concentrations of total phenolics and flavonoids but potentially influenced by the presence of distinctive compounds such as syringic acid and pinoresinol. Flower extracts (−0.70) had the weakest antioxidant capacity, as evidenced by their poor performance in most assays.

The high correlation between the antioxidant activity values and the RACI values (Fig. 4) supports the validity of using RACI as a comprehensive measure of antioxidant capacity. Leaf extracts consistently exhibited superior activity across the assays, with the exception of the phosphomolybdenum assay, where root extracts displayed comparable efficacy. The high activity of the root extract in the phosphomolybdenum test may be related to the differences in phytochemical composition, especially the high concentration of pinoresinol, the presence of other unstudied phytochemicals, and the unique mechanism of the test method.

Overall, these results highlight the variability in antioxidant activity among different parts of *P. verbascifolium*. The superior performance of leaf extracts underscores their potential as a valuable source of bioactive compounds, warranting further exploration for health-related applications. Conversely, the unique activity patterns observed in root extracts suggest the presence of specialized phytochemicals that may merit targeted investigation.

This study represents the first comprehensive evaluation of the antioxidant activities of methanol extracts obtained from the flower, leaf, stem, and root parts of *P. verbascifolium* using ultrasound-assisted extraction. A thorough literature review revealed that no prior

Table 3
Enzyme inhibition activity of *Pentanema verbascifolium* extracts.

Samples	AChE inhibition (IC_{50}: mg/mL)	BChE inhibition (IC_{50}: mg/mL)	Tyrosinase inhibition (IC_{50}: mg/mL)	α-Amylase inhibition (IC_{50}: mg/mL)	α-Glucosidase inhibition (IC_{50}: mg/mL)
Flowers	1.38 ± 0.069 ^{b}	3.30 ± 0.118 ^{c}	1.25 ± 0.012 ^{d}	2.55 ± 0.011 ^{b}	1.10 ± 0.002 ^{a}
Leaves	1.38 ± 0.004 ^{b}	3.65 ± 0.187 ^{c}	1.26 ± 0.004 ^{d}	2.52 ± 0.025 ^{b}	1.10 ± 0.029 ^{a}
Stems	1.42 ± 0.013 ^{b}	2.10 ± 0.038 ^{b}	1.20 ± 0.003 ^{c}	2.72 ± 0.079 ^{c}	1.20 ± 0.016 ^{b}
Roots	1.41 ± 0.009 ^{b}	1.97 ± 0.050 ^{b}	1.16 ± 0.001 ^{b}	2.82 ± 0.010 ^{c}	1.10 ± 0.002 ^{a}
Galanthamine	0.0028 ± 0.0001 ^{a}	0.0031 ± 0.0001 ^{a}	–	–	–
Kojic acid	–	–	0.075 ± 0.006 ^{a}	–	–
Acarbose	–	–	–	0.92 ± 0.004 ^{a}	1.25 ± 0.028 ^{b}

GALAEs, ACEs, and KAEs mean galanthamine, acarbose and kojic acid equivalents, respectively. na: not active. Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's hoc test at 5 % significance level.

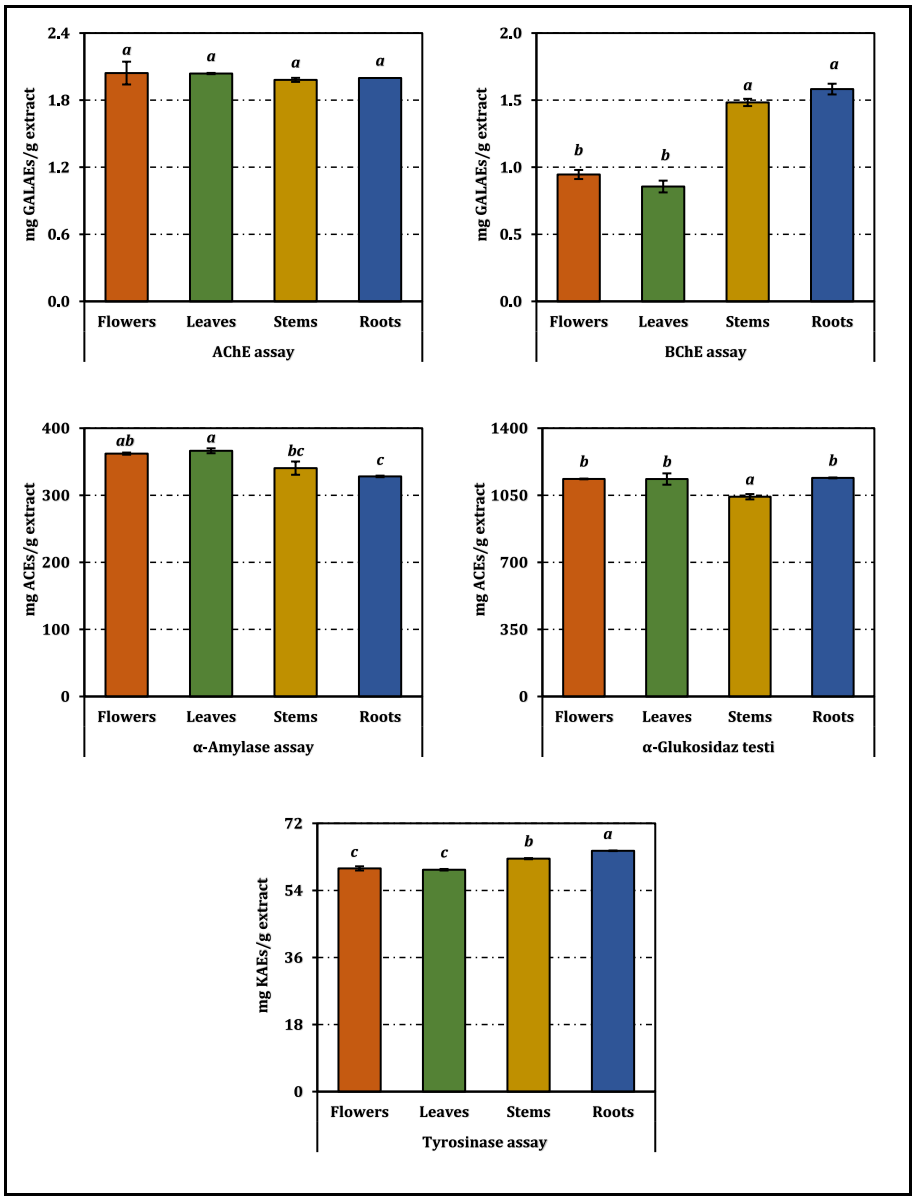

Fig. 5. Enzyme inhibition activity of *Pentanema verbascifolium* extracts. ACEs, GALAEs and KAEs mean acarbose, galanthamine and kojic acid equivalents, respectively. Values indicated by the same superscripts (*a–d*) are not different from the honestly significant difference after Tukey's hoc test at 5 % significance level.

research has investigated the antioxidant potential of this species, making the presented findings novel and a significant contribution to the scientific literature.

Quantitative chromatographic analyses identified chlorogenic acid, apigenin, and apigenin 7-glucoside as the major constituents of these extracts. These compounds are well-documented for their potent antioxidant properties, as supported by previous studies. For instance, chlorogenic acid has been demonstrated to possess substantial free radical scavenging activity, along with DNA-protective effects, due to its ability to neutralize reactive oxygen species (Xu et al., 2012). The

antioxidant activity of chlorogenic acid is further influenced by its structural features, including the number and position of hydroxyl groups, which enhance its reactivity with free radicals (Marinova et al., 2011).

Similarly, apigenin and its derivative, apigenin 7-glucoside, have been reported to exhibit significant antioxidant effects. Density functional theory (DFT) calculations have shown that the antioxidant capacity of apigenin is related to its electronic properties, including the dissociation energy of its hydroxyl groups and its polarizability (Kandasamy & Rathinam, 2011). Additionally, apigenin 7-glucoside has demonstrated anti-inflammatory and free radical scavenging activities *in vivo*, which are likely linked to its antioxidant mechanisms (Fuchs & Milbradt, 1993).

The results of this study align with these findings, suggesting that the high antioxidant activities observed in *P. verbascifolium* extracts may be attributed to the synergistic or individual contributions of these bioactive compounds. This research not only highlights the antioxidant potential of *P. verbascifolium* for the first time but also provides insights into the role of its major constituents in modulating oxidative stress. Consequently, the data presented herein contribute valuable knowledge to the growing body of literature on plant-derived antioxidants and their applications in combating oxidative damage.

3.3. Enzyme inhibitory activity

All extracts exhibited similar AChE inhibitory activity, with IC_{50} values ranging from 1.38 to 1.42 mg/mL (Table 3 and Fig. 5). Flower and leaf extracts shared identical activity levels, while root and stem extracts showed minor differences that were not statistically significant. In contrast, BChE inhibition showed greater variability, with root and stem extracts displaying significantly lower IC_{50} values (2.10 and 1.97 mg/mL, respectively), indicating stronger inhibitory potential compared to flower and leaf extracts. Reference compound Trolox demonstrated superior efficacy in both assays, underscoring the modest inhibition exhibited by the plant extracts.

Root extracts showed the highest tyrosinase inhibition (IC_{50}: 1.16 mg/mL), followed by stem (IC_{50}: 1.20 mg/mL), flower (IC_{50}: 1.25 mg/mL), and leaf extracts (IC_{50}: 1.26 mg/mL), with all differences statistically significant. However, none of the plant extracts approached the potent activity of kojic acid, the standard reference.

Leaf and flower extracts exhibited stronger α-amylase inhibition (IC_{50}: 2.52 and 2.55 mg/mL, respectively) compared to stems and roots, while α-glucosidase inhibition was comparable among flower, leaf, and root extracts (IC_{50}: 1.10 mg/mL) but slightly weaker in stems (IC_{50}: 1.20 mg/mL). Interestingly, the inhibitory activity of these extracts was comparable to that of acarbose, the reference inhibitor, for α-glucosidase (IC_{50}: 1.25 mg/mL).

Root extracts emerged as the most promising, with consistent activity across BChE, tyrosinase, and α-glucosidase inhibition assays, and strong α-glucosidase inhibition comparable to other parts. Their diverse bioactivity suggests the presence of unique phytochemicals. Flower and leaf extracts showed modest activity, while stem extracts performed moderately across most assays. These findings emphasize the need for further exploration of the bioactive compounds in the root extracts of *P. verbascifolium*.

This study provides novel insights into the enzyme inhibitory activities of methanol extracts from different parts (flowers, leaves, stems, and roots) of *P. verbascifolium* using ultrasound-assisted extraction. A thorough literature review confirmed that the inhibitory potentials of this plant against cholinesterase, tyrosinase, α-amylase, and α-glucosidase enzymes had not been previously investigated. Consequently, these findings represent significant contributions to the existing body of knowledge and establish a new reference point for future research into the bioactivities of this plant.

Quantitative chromatographic analyses identified chlorogenic acid, apigenin, and apigenin 7-glucoside as the primary bioactive components of the extracts. These compounds are widely recognized for their multifaceted biological activities, including antioxidant, enzyme inhibitory, and neuroprotective properties, as corroborated by existing literature.

Chlorogenic acid has demonstrated dual antioxidant and cholinesterase inhibitory effects in various models. Kwon et al. (2010) showed that it significantly inhibits acetylcholinesterase activity both *in vitro* and *in vivo*, improving cognitive performance in scopolamine-induced amnesia models and mitigating oxidative stress in the hippocampus and frontal cortex. Similarly, Oboh et al. (2013) reported that chlorogenic acid and its precursor, caffeic acid, inhibit acetylcholinesterase and butyrylcholinesterase activities in a dose-dependent manner while preventing oxidative damage induced by pro-oxidants. This highlights its potential as a multitarget agent against neurodegeneration.

Apigenin further enhances the neuroprotective profile of the extracts. Álvarez-Berbel et al. (2022) revealed that apigenin not only inhibits acetylcholinesterase activity but also reduces amyloid-beta aggregation, a pathological hallmark of Alzheimer's disease (AD). Its ability to bind to the peripheral anionic site of acetylcholinesterase underscores its role in modulating amyloid-beta fibril formation and alleviating oxidative stress. Apigenin 7-glucoside, though less potent than its aglycone counterpart, has also shown inhibitory effects on cholinesterase enzymes (Sezen Karaoğlan et al., 2023) and targets key AD-related proteins (İstifli & Sarıkürkcü, 2021).

The tyrosinase inhibitory activity of *P. verbascifolium* extracts marks another significant finding. Chlorogenic acid has been identified as a key contributor to tyrosinase inhibition in natural product studies. Oh et al. (2019) demonstrated its role in enhancing antioxidant and tyrosinase inhibitory activities in *Cudrania tricuspidata* fruit extracts. Similarly, de Freitas et al. (2016) noted its high tyrosinase inhibition in *Morus nigra* leaf extracts, highlighting its potential for use as a skin-whitening agent.

While apigenin 7-glucoside's role in tyrosinase inhibition remains less clear, Bouzaiene et al. (2016) provided indirect evidence of its effects on enzymatic pathways related to melanogenesis, suggesting its potential as a modulator of tyrosinase activity. These findings position *P. verbascifolium* as a promising candidate for dermatological and cosmetic applications.

The study also highlights the α-amylase and α-glucosidase inhibitory activities of *P. verbascifolium* extracts, which are crucial for managing carbohydrate metabolism disorders. Chlorogenic acid exhibited strong inhibitory effects on these enzymes, as reported by Wang et al. (2022), who demonstrated increased inhibitory activity with the lipophilicity of its derivatives. Zheng et al. (2020) provided mechanistic insights, showing that chlorogenic acid acts as a mixed-type inhibitor of porcine pancreatic α-amylase by binding through hydrogen bonding and altering the enzyme's secondary structure.

Apigenin and apigenin 7-glucoside also contribute to these bioactivities. Witkowska-Banaszczak et al. (2020) reported that apigenin 7-glucoside effectively inhibits α-amylase activity, suggesting its therapeutic potential in managing hyperglycemia and related metabolic disorders. These findings indicate that the combination of chlorogenic acid, apigenin, and apigenin 7-glucoside in *P. verbascifolium* extracts may exert synergistic effects, enhancing their overall enzyme inhibitory potential.

Taken together, this study offers the first comprehensive account of the enzyme inhibitory activities of *P. verbascifolium*, highlighting the roles of chlorogenic acid, apigenin, and apigenin 7-glucoside in these bioactivities. These findings underscore the plant's potential as a natural source for therapeutic agents targeting neurodegenerative diseases, skin disorders, and metabolic conditions. The use of ultrasound-assisted extraction further emphasizes the sustainability and efficiency of this approach in natural product research.

Future investigations should focus on isolating individual compounds, exploring their synergistic effects, and validating their efficacy through *in vivo* studies and clinical trials. Molecular docking analyses could provide deeper insights into the mechanisms underlying their

Table 4
Correlations among phenolic compounds and assays.

	TAP	DPPH	ABTS	CUPRAC	FRAP	FICA
DPPH radical	0.297					
ABTS radical cation	0.233	0.965				
CUPRAC reducing power	0.547	0.946	0.908			
FRAP reducing power	0.341	0.995	0.966	0.964		
Ferrous ion chelating	0.349	0.903	0.778	0.837	0.881	
Total flavonoid	−0.604	0.436	0.578	0.230	0.425	0.156
Total phenolic	0.219	0.969	0.993	0.922	0.976	0.778
Chlorogenic acid	0.529	0.873	0.735	0.873	0.863	0.977
Apigenin	−0.503	0.628	0.714	0.411	0.609	0.408
Apigenin 7-glucoside	−0.584	0.536	0.639	0.310	0.517	0.306
3-Hydroxybenzoic acid	−0.646	0.494	0.582	0.247	0.469	0.301
4-Hydroxybenzoic acid	−0.645	0.501	0.584	0.250	0.474	0.313
Kaempferol	−0.769	−0.285	−0.080	−0.425	−0.284	−0.568
Vanillin	0.146	0.626	0.434	0.533	0.588	0.876
Luteolin 7-glucoside	−0.653	0.312	0.477	0.115	0.304	0.010
RACI	0.363	0.991	0.977	0.968	0.997	0.864

Data show the Pearson Correlation Coefficients between the parameters. TAP: Total antioxidant power by phosphomolybdenum method. 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH): ABTS and DPPH radical scavenging activities, respectively. CUPric Reducing Antioxidant Capacity (CUPRAC) and ferric reducing antioxidant power (FRAP): CUPRAC and FRAP reducing power potential; respectively. FICA: Ferrous ion chelating activity. RACI: Relative antioxidant capacity index.

bioactivities, paving the way for optimized applications in pharmaceuticals, nutraceuticals, and cosmetics.

3.4. Correlations among phenolic compounds and assays

The correlations between the chemical composition and the antioxidant activities of *P. verbascifolium* extracts were evaluated using Pearson's correlation coefficients, as presented in Table 4. These analyses reveal distinct relationships between phenolic and flavonoid compounds and the outcomes of various antioxidant assays, highlighting the compounds contributing to the extracts' bioactivity.

Total phenolic content (TPC) exhibited strong positive correlations with most antioxidant assays, particularly with ABTS (r = 0.993), DPPH (r = 0.969), FRAP (r = 0.976), and CUPRAC (r = 0.922). This robust relationship underscores the critical role of phenolics in radical scavenging and reducing power mechanisms, suggesting that the higher phenolic content in certain extracts enhances their overall antioxidant capacity. Additionally, the correlation between TPC and ferrous ion chelating activity (FICA; r = 0.778) implies that phenolics also contribute to metal ion chelation, albeit to a lesser extent.

Chlorogenic acid demonstrated substantial correlations across multiple assays, including FICA (r = 0.977), CUPRAC (r = 0.873), and DPPH (r = 0.873), indicating its versatility in promoting antioxidant mechanisms. This compound's contribution to reducing power and radical scavenging is particularly notable, given its alignment with phenolic-dependent bioactivities. Similarly, vanillin displayed a significant correlation with FICA (r = 0.876) and moderate associations with CUPRAC (r = 0.533) and FRAP (r = 0.588), suggesting its potential role as a metal ion chelator and reducing agent.

In contrast, total flavonoid content (TFC) showed weaker and inconsistent correlations with the assays, with moderate positive correlations for DPPH (r = 0.436) and ABTS (r = 0.578) but negligible or negative associations with CUPRAC (r = 0.230) and FICA (r = 0.156). This indicates that while flavonoids may contribute to radical scavenging, their overall impact on antioxidant capacity appears less pronounced than that of phenolics. Among specific flavonoids, apigenin and its glucoside exhibited moderate correlations with ABTS (r = 0.714 and 0.639, respectively) and DPPH (r = 0.628 and 0.536, respectively), highlighting their role in radical scavenging. However, kaempferol showed consistently negative correlations across all assays, suggesting it may not significantly contribute to the observed antioxidant activities.

The RACI values, which integrate the results of all antioxidant assays, demonstrated exceptionally strong correlations with FRAP (r = 0.997), DPPH (r = 0.991), ABTS (r = 0.977), and CUPRAC (r = 0.968), further supporting the pivotal role of phenolics in determining the extracts' comprehensive antioxidant performance.

In summary, the data suggest that phenolic compounds, particularly total phenolics and chlorogenic acid, play a dominant role in the antioxidant properties of *P. verbascifolium* extracts. Flavonoids, while contributing to specific radical scavenging activities, appear less influential overall. These findings highlight the importance of phenolic composition in enhancing the bioactivity of natural extracts and provide a framework for understanding the chemical basis of antioxidant mechanisms in this plant.

4. Conclusions

This study highlights the significant role of phenolic compounds, especially chlorogenic acid and total phenolic content, in the antioxidant and enzyme inhibitory activities of *P. verbascifolium* extracts. Leaf extracts emerged as the most bioactive, attributed to their high phenolic and flavonoid levels and diverse phytochemical profiles. Chlorogenic acid strongly correlated with assays such as radical scavenging and metal ion chelation, underscoring its central role.

Flavonoids showed specific but less pronounced effects, with variability in their contributions warranting further investigation. The distinct compound distribution across plant parts suggests part-specific bioactivities, like the metal-chelating abilities of root extracts driven by syringic acid and pinoresinol.

While valuable insights were gained, limitations include the lack of *in vivo* studies and unclear molecular mechanisms of enzyme inhibition. Future research should explore bioavailability, pharmacokinetics, and advanced metabolomic analyses to elucidate compound interactions and mechanisms. Expanding enzyme panels and incorporating molecular docking could enhance mechanistic understanding.

In conclusion, *P. verbascifolium*, particularly its leaves, shows promise as a source of natural antioxidants and enzyme inhibitors. Addressing these gaps could further validate its therapeutic potential and expand its medicinal applications.

Declaration of competing interest

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

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The experimental design, execution, and data analysis were principally carried out by the author. To improve the interpretation of findings and enhance the academic quality of the manuscript, artificial intelligence tools were utilized as auxiliary aids. The outputs produced by AI were thoroughly assessed and validated by the author to confirm their consistency with the research goals and adherence to the highest standards of scientific integrity.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2025.106607.

Data availability

Data will be made available on request.

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