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RESEARCH ARTICLE

Comparative analysis of phenolic compounds, antioxidant activity and enzyme inhibitory effects of *Camellia pubicosta* Merr. leaf extracts

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Abstract

This study investigated the phenolic content and various bioactivities of extracts from the leaves of *Camellia pubicosta* Merr., a plant species native to Vietnam. The results revealed that catechin and epicatechin were significantly more abundant in the aqueous methandic extract (ME70) compared to other extracts. The acetone/methanol (AC/ME) and aqueous ethanolic (ET70) extracts demonstrated the most potent antioxidant activity. However, no significant differences in DPPH radical scavenging activity were observed among AC/ME, ME70 and ET70. The ethyl acetate extract exhibited the strongest α-amylase inhibitory activity, whereas AC/ME was the most effective against α-glucosidase. Correlation analysis suggested that phenolic compounds were key contributors to the radical scavenging activities, while flavonoids played a significant role in the α-amylase inhibitory effects of the extracts. These findings enhanced our understanding of the bioactive phenolics present in *C. pubicosta* leaves and their potential health benefits.

Keywords: antioxidant; α-amylase; *Camellia pubicosta*; catechin; HPLC

Introduction

The Theaceae family represents one of the largest plant groups, encompassing 120 to 280 species primarily distributed across East and Southeast Asia. The highest diversity is concentrated in China and Vietnam. Species in this family are small shrubs or trees with vibrant green foliage and brightly colored flowers, usually in yellow, pink, or red shades. The genus *Camellia*, a key family member, stands out for its fruit and seed traits, with large capsules splitting at the top and holding wingless, spherical or polygonal seeds (1, 2). Among the species within the genus *Camellia*, *C. sinensis* is particularly notable for its use in producing tea. Numerous studies have highlighted the potential health-endorsing properties of *Camellia* species, including its role in weight management, cardiovascular health, neurological regulation and cancer prevention. Recent research has also identified its potential in mitigating the progression of Alzheimer's disease (3, 4). These benefits are largely attributed to the high phenolic content in *Camellia* species, particularly catechins, which exert potent antioxidant activity that helps combat oxidative damage caused by free radicals. Key catechins identified in *Camellia* species include epigallocatechin gallate, epigallocatechin, epicatechin gallate, gallocatechin and catechin, which collectively account for 20-30 % of the dry weight (5). Additionally, tannins, derived from flavan-3-ols or flavan-3,4-diols, are also significant components of *Camellia* species (6).

In Vietnam, the Theaceae family comprises 11 genera and approximately 80 species (7). This diversity continues to expand with new species discoveries. *Camellia pubicosta* Merr., a white flowered species, was first described in 1942 and is native to Vietnam (8). The species can be found growing naturally in open forests with poor soils and high humidity, even tolerating areas lacking full shade (9, 10). Despite the rich phenolic profile and potential health benefits reported in the *Camellia* genus, the chemical composition and bioactivities of *C. pubicosta* have not been investigated. *Camellia* species, particularly *C. sinensis*, are widely known to have strong antioxidant activity and carbohydrate hydrolyzing inhibitory effects. We hypothesize that *C. pubicosta* contains phenolics that contribute to significant antioxidant properties and inhibitory activities against carbohydrate digestion. Our study aimed to fill this gap by evaluating phenolic contents in extracts of this plant species. Additionally, the study assessed their antioxidant activity and inhibitory effects on α-amylase and α-glucosidase. The findings provided new insights into the chemical and potential health-endorsing properties of *C. pubicosta,* supporting its potential applications in functional foods and nutraceuticals.

Materials and Methods

Sample collection

Fresh leaves of *Camellia pubicosta* were collected from Ba Vi National Park (21° 04' 24" N, 105° 21' 44" E; altitude 910 m) in Tan Linh Commune, Ba Vi District, Hanoi, Vietnam. A voucher specimen (BV368) was deposited at the Institute of Innovation in Pharmaceutical and Healthcare Food, Thu Dau Mot University, Binh Duong, Vietnam. The collected leaves were then freeze-dried and stored in a refrigerator until further analysis.

Chemicals

Extraction solvents (HPLC grade) used in the study, including methanol, ethanol and ethyl acetate, were purchased from Fisher Scientific (Pennsylvania, USA). Phenolic acid analytical standards (purity > 99 %) were obtained from Sigma-Aldrich (St. Louis, USA). Flavonoid analytical standards (purity > 99 %) were procured from Chengdu Biopurify Phytochemicals (Sichuan, China). ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid))and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich and Alfa Aesar (Massachusetts, USA), respectively. Acarbose was obtained from the National Institute of Drug and Quality Control (Hanoi, Vietnam).

Preparation of crude extracts

The leaf samples (10 g) were extracted using 150 mL of acetone/methanol (6: 4, v/v), 70 % methanol, 70 % ethanol, or 100 % ethyl acetate in a pre-cleaned Erlenmeyer flask. The mixtures were vigorously shaken on an orbital shaker (IKA HS-260, Germany) at 120 rpm at room temperature for 24 hr, then filtered through Whatman filter paper. The filtrates were evaporated under reduced pressure and the resulting residues (AC/ME, ME70, ET70 and EA, respectively) were used for phenolic analysis and bioactivity determination.

Phenolics and bioactivities

Phenolics in the extracts were determined using a high-performance liquid chromatography. Antioxidant activity of the extracts was evaluated using ABTS and DPPH radical scavenging assays. Additionally, the inhibitory effects of the extracts α-amylase and α-glucosidase activities were assessed. Determination of phenolic and flavonoid contents, individual phenolics and bioactivities is described in the Supplementary data (Sections S1-S6).

Statistical analysis

All measurements were carried out in triplicate and the results are presented as mean ± standard deviation. The data were analyzed using one-way analysis of variance (ANOVA) to compare phenolic concentrations and bioactivities among the extracts. Tukey's HSD test was applied to determine significant differences between means, with statistical significance set at $p < 0.05$. All statistical analyses were conducted using the XLSTAT software package (version 2016, Addinsoft, France).

Results and Discussion

Phenolic content

The results revealed that all the monitored phenolic compounds were detected in the *C. pubicosta* leaf extracts (Table 1). Gallic acid was present in all extracts, with the highest concentration found in AC/ME (1.90 ± 0.05 mg/g) and the lowest in EA (0.25 ± 0.00 mg/g). Similarly, ferulic acid was detected in all extracts, with AC/ME and ME 70 containing higher amounts than the others. Chlorogenic acid was most abundant in ME 70 (0.88 ± 0.01 mg/g) but was not detected in EA**.** Among the monitored phenolics**,** epicatechin was the most abundant, with concentrations ranging from 1.00 mg/g (EA) to 6.13 mg/g (ME 70)**.** Catechin was also present at high levels, particularly in AC/ME (3.56 ± 0.07 mg/g) and ME 70 (3.75 ± 0.25 mg/g), approximately twice the amount found in ET 70. However, catechin was not detected in EA. EGCG was found in higher concentrations in ET 70**,** though, like catechin, it was undetectable in EA. Meanwhile, quercetin and kaempferol were detected in all extracts, with comparable concentrations across different solvents.

While individual phenolic profiling provided insight into specific compounds, TPC offered a broader perspective on the overall phenolic abundance in the extracts. The results showed that ME 70 and AC/ME exhibited significantly higher TPC than ET 70 and EA. As seen in Table 1, ME 70 contained 192.43 ± 2.70 mg GAE/g, which was four times higher than EA, indicating that aqueous methanol and acetone/methanol were the most effective solvents for phenolic extraction. Conversely, EA was the richest extract in flavonoids, with a TFC of 96.21 ± 3.38 mg QE/g, whereas ME 70 had the lowest flavonoid content.

The solvent polarity played an important role in phenolic extraction. Aqueous methanol and ethanol**,** being polar solvents, effectively dissolved a broad spectrum of polar and moderately polar compounds, including catechins. Their efficiency stemmed from their ability to break down plant cell

Table 1. Phenolic contents in the extracts of *C. pubicosta* leaves

Phenolics*	AC/ME	ME70	ET70	EA
Gallic acid	1.90 ± 0.05 a	1.68 ± 0.02 b	0.76 ± 0.06 c	0.25 ± 0.00 d
Chlorogenic acid	0.78 ± 0.07 b	0.88 ± 0.01 a	0.64 ± 0.00 c	n.d.
Ferulic acid	0.56 ± 0.01 a	0.56 ± 0.00 a	0.53 ± 0.00 b	0.53 ± 0.00 b
Catechin	3.56 ± 0.07 a	3.75 ± 0.25 a	1.70 ± 0.49 b	n.d.
Epicatechin	5.24 ± 0.00 b	6.13 ± 0.07 a	2.06 ± 1.12 c	1.00 ± 0.05 c
EGCG	1.09 ± 0.10 b	1.17 ± 0.02 b	1.64 ± 0.02 a	n.d.
Quercetin	1.20 ± 0.00 a	1.20 ± 0.01 a	1.17 ± 0.01 b	1.20 ± 0.00 a
Kaempferol	0.37 ± 0.00 a	0.36 ± 0.00 b	0.34 ± 0.00 d	0.35 ± 0.00 c
TPC (mg GAE/g)	176.77 ± 0.52 b	192.43 ± 2.70 a	148.88 ± 0.52 c	46.37 ± 3.15 d
TFC (mg QE/g)	49.18 ± 0.96 b	16.10 ± 0.68 d	26.75 ± 0.03 c	96.21 ± 3.38 a

*: Concentrations of individual phenolics are shown as mg/g extract. AC/ME, ME 70, ET 70 and EA denote the extracts obtained with acetone/methanol (6 : 4, v/v), aqueous methanol (70 %, v/v), aqueous ethanol (70 %, v/v) and ethyl acetate (100 %). Different letters (a, b, c, d) denote significant differences in the phenolic content among the extracts ($p < 0.05$).

walls, facilitating enhanced extraction. Additionally, these solvents are widely used for phenolic extraction due to their capacity to preserve bioactive properties. In contrast, ethyl acetate was less effective in extracting phenolics from *C. sinensis* pollen compared to methanol, ethanol and water, which aligned with the lower TPC observed in the EA extract in this study (11). Moreover, phenolics in these extracts could have interacted with other compound classes, including alkaloids, triterpenoids and polysaccharides, which may have influenced extraction yields and consequently impacted TPC and TFC.

The presence of phenolic compounds in *Camellia* species is well-documented, highlighting their importance in both plant defense and potential health benefits. For instance, a diverse range of phenolics has been identified in *C. nitidissima, C. euphlebia* and *C. insularis* (12), many of which have demonstrated antioxidant, antimicrobial and anti-inflammatory properties. Several of these compounds were also detected in the *C. pubicosta* samples analyzed in this study, further supporting the widespread occurrence of phenolics across different *Camellia* species. Among these bioactive compounds, catechins are particularly significant, as they are well known to be the major phenolic constituents in tea leaves (*C. sinensis*), contributing to tea's antioxidant activity and health -promoting effects. Interestingly, catechins are also abundant in yellow camellias, reinforcing their broader functional role in the *Camellia* genus. A previous study on six yellow *Camellia* species reported total catechin levels ranging from 0.3 to 1.1 mg/g in crude leaf suggesting a potential variability in catechin content across different species and growing conditions (13). Beyond their well-recognized antioxidant properties, catechins serve crucial ecological and physiological roles in *Camellia* plants. They have been suggested to play a role in plant defence mechanisms, acting as deterrents against herbivores and pathogens by inhibiting microbial growth and reducing palatability. Additionally, catechins may contribute to cold tolerance, helping plants survive under environmental stress conditions by stabilizing cell membranes and reducing oxidative damage (14, 15). These functional properties highlight the ecological significance of catechins in plant adaptation while also reinforcing their potential value for nutraceutical and pharmacological applications.

Given their diverse bioactivities, further research into the catechin composition of different *Camellia* species, particularly lesser-known varieties like *C. pubicosta*, could provide valuable insights into their potential uses in functional foods, pharmaceuticals and natural plant-based preservatives. Understanding the environmental and genetic factors influencing catechin biosynthesis may also help optimize their production for both plant resilience and human health benefits.

Antioxidant activity

In this study, ABTS and DPPH radical scavenging assays were employed to assess the antioxidant activity of *C. pubicosta* extracts, with results presented in Fig. 1 & 2. Among the extracts, acetone/methanol (AC/ME) and aqueous ethanol (ET 70) exhibited the strongest ABTS radical scavenging capacity, with IC_{50} values of 162.85 ± 3.21 µg/mL and 180.72 ± 15.28 µg/mL, respectively, followed by ME 70 (IC_{50} = 276.08 ± 9.78 µg/mL). In contrast, the ethyl acetate extract (EA) demonstrated the weakest activity (IC_{50} = 3395.66 ± 80.44 µg/mL). However, when compared to ascorbic acid (IC_{50} = 50.24 ± 2.17 µg/mL), all extracts showed lower ABTS scavenging ability. Regarding DPPH radical scavenging activity, no significant differences were observed among AC/ME, ME 70 and ET 70, with IC_{50} values ranging from 54.96 to 74.84 µg/mL. However, EA exerted considerably weaker DPPH radical scavenging ability compared to the other extracts. Like the ABTS results, ascorbic

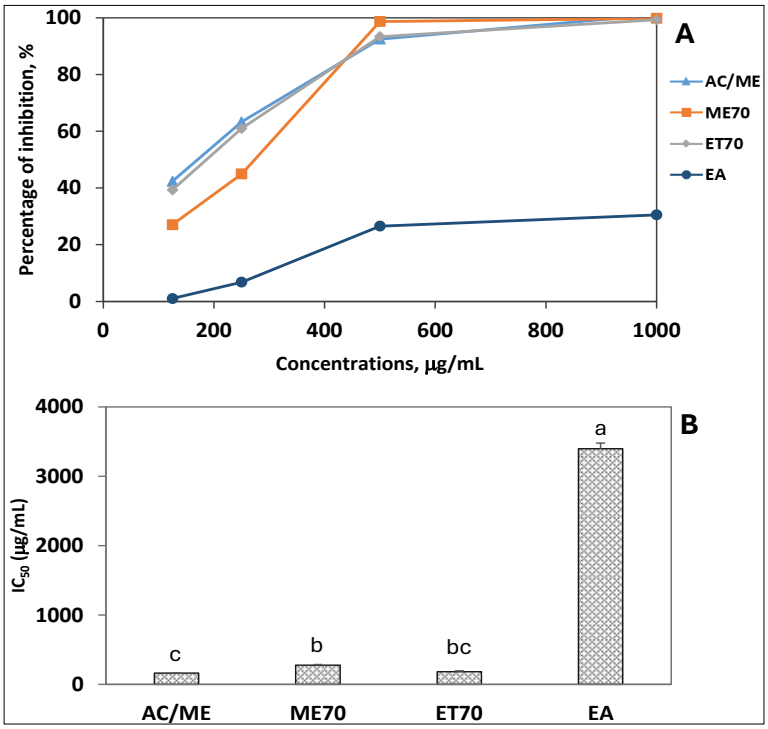

Fig. 1A. ABTS radical scavenging activity of the *C. pubicosta* leaf extracts. AC/ME, ME 70, ET 70 and EA stand for the extracts obtained with acetone/methanol (6: 4, v/v), aqueous methanol (70 %, v/v), aqueous ethanol (70 %) and ethyl acetate (100 %).

Fig. 1B. IC_{50} values (mg/mL) of the extracts. Error bars denote standard deviation of the means. Different letters (a, b) indicate significant differences in the bioactivity among the extracts ($p < 0.05$).

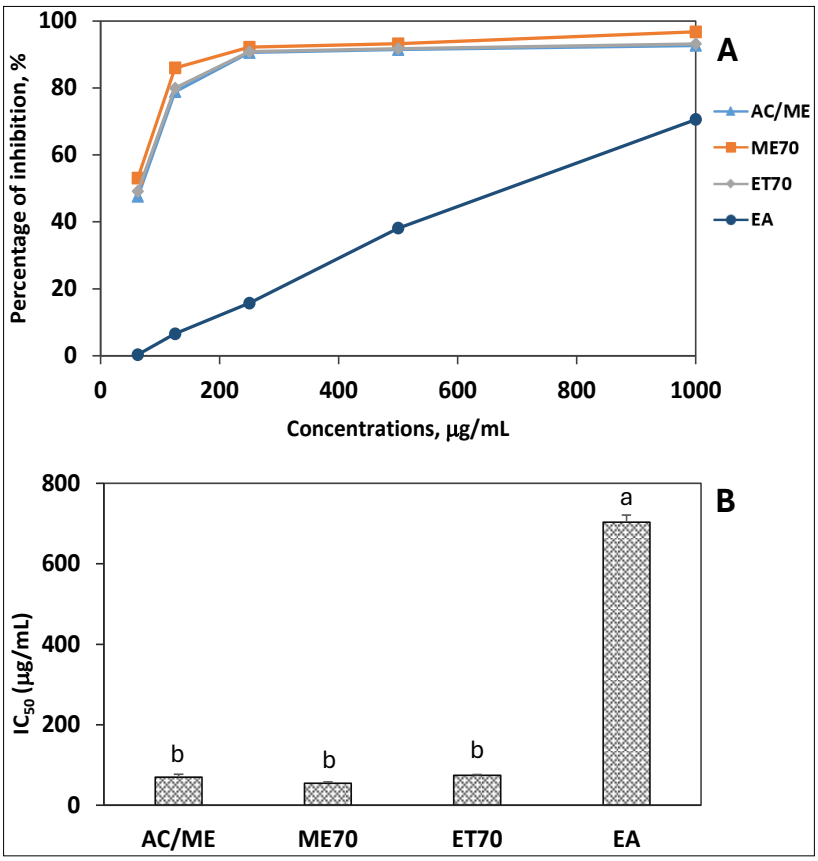

Fig. 2A. DPPH radical scavenging activity of the *C. pubicosta* leaf extracts. AC/ME, ME 70, ET 70 and EA stand for the extracts obtained with acetone/methanol (6: 4, v/v), aqueous methanol (70 %, v/v), aqueous ethanol (70 %) and ethyl acetate (100 %).

Fig. 2B. IC_{50} values (mg/mL) of the extracts. Error bars denote standard deviation of the means. Different letters (a, b) indicate significant differences in the bioactivity among the extracts ($p < 0.05$).

acid remained the most potent antioxidant, outperforming all tested extracts. Despite these differences, both assays confirmed a dose-dependent increase in radical scavenging activity as extract concentrations increased (Fig. 1A & 2A).

Comparisons with previous studies highlight variations in *Camellia* species' antioxidant capacities. For instance, an ethanolic extract from *C. quephongensis* leaves exhibited an ABTS IC_{50} value of 57.88 µg/mL, comparable to ascorbic acid (16). Meanwhile, a study on *C. fascicularis* reported that its leaf extracts displayed lower ABTS and DPPH scavenging potential than ascorbic acid (17). Additionally, methanolic extracts from *C. sinensis*, a well-known antioxidant-rich species, demonstrated radical scavenging activity on par with ascorbic acid (18). The antioxidant activity observed in *Camellia* plants is largely attributed to catechins, which are abundant phenolics known for their strong free radical scavenging properties. Our previous study found that catechins presented higher ABTS and DPPH scavenging potential than ascorbic acid, suggesting their significant role in the antioxidant capacity of *Camellia* leaf extracts (16). Catechins are widely distributed in fruits, vegetables and herbs, particularly in green tea, walnuts and berries, all of which possess high antioxidant activity (19, 20).

Enzyme inhibitory effects

The α-amylase inhibitory potential of *C. pubicosta* extracts was evaluated, using acarbose as a reference standard. As shown in Table 2, all extracts exhibited a dose-dependent inhibition of α-amylase. However, at 500 µg/mL, no inhibitory activity was detected in any of the extracts. Among the tested extracts, EA demonstrated the strongest inhibition, followed by ME 70, while the remaining extracts exhibited minimal enzyme inhibition, with IC_{50} values approaching 4000 µg/mL. When compared to acarbose (IC_{50} = 88.80 ± 4.87 µg/mL), all *C. pubicosta* extracts showed significantly weaker inhibitory activity. Previous studies have reported α-amylase inhibitory effect of *Camellia* species. For instance, a 70 % ethanolic extract of *C. sinensis* leaves exhibited moderate inhibitory effects (IC_{50} = 1540 µg/mL), though still weaker than acarbose (21). Similarly, a study on five *Camellia* species from Vietnam found that they either had weak inhibitory activity or no effect on α-amylase (22). Ethyl acetate extracts of leaves from other *Camellia* species have also been reported to exhibit strong anti-α-

Table 2. Inhibitory effects of the extracts and acarbose on α-amylase

Samples	Percentage of inhibition, %				IC_{50} (µg/mL)
	500 µg/mL	1000 µg/mL	2000 µg/mL	4000 µg/mL	
AC/ME	n.i.	4.57 ± 0.69	18.34 ± 0.21	50.86 ± 0.63	3948.04 ± 45.16 a
ME70	n.i.	5.23 ± 0.23	30.26 ± 0.15	66.89 ± 0.64	3147.36 ± 22.65 b
ET70	n.i.	n.i.	13.71 ± 1.25	50.06 ± 1.14	3993.68 ± 49.24 a
EA	n.i.	13.05 ± 0.47	39.93 ± 0.69	89.27 ± 0.58	2432.20 ± 19.03 c

n.i.: no inhibition

Different letters (a, b, c) indicate significant differences in the bioactivity among the extracts ($p < 0.05$).

amylase activity compared to extracts prepared using other solvents (16, 23). As a semi-polar solvent, ethyl acetate selectively extracts flavonoids, including galloylated catechins. This group of compounds are well-known α-amylase inhibitors due to their ability to form non-covalent complexes with the enzyme, thereby reducing its activity (24). In the present study, EA was found to contain the highest flavonoid content (Table 1), which likely contributed to its strongest α-amylase inhibitory effect among the tested extracts.

The results in Table 3 presented the α-glucosidase inhibitory effects of different *C. pubicosta* extracts at various concentrations (125 - 1000 µg/mL) and their IC_{50} values. All the extracts (except EA) exhibited a dose-dependent inhibitory effect on α-glucosidase, where inhibition increased with higher extract concentrations. The acetone/methanol extract showed the highest inhibition at all the tested concentrations, reaching 45.50% at 1000 µg/mL, followed by ME 70 (30.22 %) and ET70 (26.13 %). The ethyl acetate extract (with the highest TFC) exhibited no inhibition at any concentration, indicating that its composition lacks effective α-glucosidase inhibitors. This discrepancy may be because not all flavonoids possess α-glucosidase inhibitory properties; the specific flavonoid subclasses, structural features (e.g., glycosylation pattern, hydroxylation) and degree of polymerization strongly influence bioactivity. None of the extracts reached an IC_{50} below 1000 µg/mL, suggesting weak inhibitory activity against α-glucosidase compared to standard inhibitors like acarbose.

Correlation analysis

In this study, Pearson correlation analysis was used to assess the relationship between phenolic and flavonoid contents and the bioactivities. Table 4 presents the correlation coefficients between TPC and TFC with different bioactivities of the *C. pubicosta* extracts, including free radical scavenging activities and α-amylase inhibitory potential. Total phenolic content showed a strong positive correlation with ABTS (r = 0.805) and DPPH (r = 0.984), indicating that extracts with higher TPC exhibited greater antioxidant activity. This finding is consistent with previous research demonstrating that phenolic compounds in extracts of *Camellia* species and other plants contribute significantly to free radical scavenging activity (20, 25, 26). Total flavonoid content was negatively correlated with both ABTS (r = - 0.700) and DPPH (r = - 0.942), suggesting that flavonoids may not be the primary contributors to antioxidant activity in these extracts. This unexpected negative relationship could be due to variability in flavonoid composition, where some flavonoids exert weaker antioxidant activity compared to other phenolics (e.g., phenolic acids, tannins).

Table 4. Correlation coefficients representing the relationships between total phenolic/flavonoid contents and bioactivities of the extracts

Bioactivities	TPC	TFC
ABTS	**0.805**	-0.700
DPPH	**0.984**	-0.942
Anti-α-amylase	-0.785	**0.722**

Values in bold are different from zero with a significance level $p < 0.01$.

Total phenolic content was negatively correlated with α-amylase inhibition (r = -0.785), indicating that extracts with higher TPC were less effective in inhibiting α-amylase. However, TFC showed a positive correlation with α-amylase inhibition (r = 0.722), suggesting that flavonoids are more effective α-amylase inhibitors than other phenolic groups. As discussed earlier, many flavonoids, such as galloylated catechins, have been reported to inhibit α-amylase by binding to the enzyme's active site or altering its conformation.

Conclusion

This study highlights the phenolic composition and bioactivities of *C. pubicosta* leaf extracts, demonstrating their potential as natural antioxidants and enzyme inhibitors. The extract from aqueous methanol was richest in catechin and epicatechin levels, while those from acetone/methanol and aqueous ethanol showed the strongest ABTS radical scavenging activity. The ethyl acetate extract displayed the highest α-amylase inhibition, likely due to its flavonoid content, whereas the acetone/methanol extract was the most effective against α-glucosidase. Correlation analysis confirmed that phenolics contribute to antioxidant activity, while flavonoids influence α-amylase inhibition. Future research should explore the specific mechanisms behind these bioactivities, evaluate its effectiveness and safety *in vivo* models and investigate potential synergistic effects with other natural compounds. These studies will deepen our insight into its therapeutic potential and facilitate its advancement into practical pharmaceutical or nutraceutical products.

Authors' contributions

NTN carried out bioassays and drafted the manuscript. PMT and TNTL participated in the design of the study, performed the statistical analyses and drafted the manuscript. TSH performed

Table 3. Inhibitory effects of the extracts and acarbose on α-glucosidase

Samples	Percentage of inhibition (%)				IC_{50} (µg/mL)
	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL	
AC/ME	23.34 ± 1.10	28.53 ± 1.68	36.76 ± 0.88	45.50 ± 1.72	> 1000
ME70	9.98 ± 0.38	16.58 ± 0.26	21.87 ± 0.52	30.22 ± 1.78	> 1000
ET70	4.99 ± 0.70	13.42 ± 0.69	18.85 ± 1.50	26.13 ± 1.66	> 1000
EA	n.i.	n.i.	n.i.	n.i.	n.d.

n.i.: no inhibition

a sample collection and species identification. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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