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Phytochemical Composition, Antioxidant, and Acetylcholinesterase Inhibitory Activities of *Pluchea pteropoda* Extract

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ABSTRACT

Pluchea pteropoda (Asteraceae) has been traditionally employed in folk medicine; however, its phytochemical characteristics and biological activities remain insufficiently defined. This study aimed to elucidate the phytochemical composition, antioxidant properties, and acetylcholinesterase (AChE) inhibitory potential of leaf and root extracts prepared using 45% and 70% ethanol. Four extracts of 45% ethanol extract of leaves (L45), 70% ethanol extract of leaves (L70), 45% ethanol extract of roots (R45), and 70% ethanol extract of roots (R70) were obtained and subjected to qualitative phytochemical screening, quantitative determination of total phenolic and flavonoid contents, DPPH radical scavenging and FRAP reducing power assays, as well as AChE inhibition assessment via Ellman's method. Data were analyzed using regression-based IC_{50} estimation and reported as the mean of triplicate measurements. Leaf extracts exhibited substantially higher phenolic (up to 52.89 mg GAE/g) and flavonoid contents (20.62 mg QE/g) compared to root extracts. Antioxidant performance varied according to solvent strength: L45 demonstrated the most potent DPPH scavenging activity (IC_{50} = 194.8 µg/mL), whereas L70 showed superior reducing capability. AChE inhibitory activity followed the order L70 > L45 > R70 > R45, although L70 remained approximately 16 times less potent than galantamine. Collectively, the findings indicate that *P. pteropoda* possesses noteworthy antioxidant potential and moderate AChE inhibition, suggesting its promise as a natural source for the development of phytotherapies targeting oxidative stress and neurodegenerative disorders.

Keywords: *Pluchea pteropoda*, phytochemicals, antioxidants, flavonoids, acetylcholinesterase, Alzheimer's disease.

Introduction

Reactive oxygen species (ROS) include superoxide anion ($\cdot O_2$-), hydroxyl radical (·OH), alkoxyl radical (RO·), peroxyl radical (ROO·), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and many reactive nitrogen compounds [1]. The imbalance between ROS generation and endogenous antioxidant capacity causes oxidative stress, damaging proteins, membrane lipids, DNA, and low-density lipoproteins [1,2]. Oxidative stress is closely related to many chronic diseases, such as cancer, diabetes, cardiovascular disease, Parkinson's, Alzheimer's, and aging. [3,4]. The human body has enzymatic and non-enzymatic systems to reduce ROS, but in many pathological cases, the amount of ROS exceeds the control capacity. Therefore, supplementing antioxidants from outside, especially natural sources, is necessary. Antioxidants from vegetables, fruits, and herbs are popular because they are safe and have fewer side effects than synthetic ones [3]. Recent findings also support the strong DPPH and FRAP antioxidant activities of herbal extracts, such as those reported for *Anacardium occidentale* stem bark [5].

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In modern medicine, many natural antioxidants have been proven, such as curcumin (turmeric), resveratrol (grapes), *Ginkgo biloba* extract, green tea, aged garlic, melatonin, and vitamins C and E [6]. In Alzheimer's patients, vitamin E has been shown to slow the progression of the disease, making it an essential part of many doctors' treatment regimens [6]. Okolie *et al.* demonstrated strong antioxidant and lipid peroxidation inhibitory effects of the root extract of *Dennettia tripetala* [7]. This reinforces the trend of researching and exploiting medicinal herbs as a source of antioxidants to prevent or support the treatment of oxidative stress-based diseases. Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive decline, especially memory. One of the essential pathogenesis mechanisms is the decline of acetylcholine (ACh) in the brain. Therefore, the current mainstream therapy is to inhibit the enzyme acetylcholinesterase (AChE), prevent ACh hydrolysis, and maintain the concentration of this neurotransmitter, thereby improving memory [2]. However, classic AChE inhibitors such as tacrine, donepezil, rivastigmine, or galantamine have numerous side effects, including liver toxicity, nausea, and digestive disorders [2,8]. This opens up the research direction of using herbal medicines to inhibit AChE as a safer alternative. Herbal medicines present several advantages. They are derived from abundant natural sources and are relatively inexpensive. In addition, they commonly contain multiple bioactive constituents, including flavonoids, alkaloids, and polyphenols, which may exert synergistic pharmacological effects. Moreover, compared with synthetic drugs, herbal preparations are generally associated with lower risks of severe toxicity during long-term use. However, herbal medicines also present essential limitations. Their pharmacological activity is often weaker than that of modern drugs; most preparations have not yet been standardized to ensure a consistent content of active ingredients, and large-scale clinical studies validating their efficacy and safety are limited. Therefore, while herbal medicines hold great potential as complementary or alternative therapies, further efforts are needed to enhance their standardization,

effectiveness, and clinical validation. However, the trend of using medicinal herbs to inhibit AChE is growing strongly, especially species containing flavonoids, phenolics, and alkaloids, which are common in many medicinal plants [9,10].

The Pluchea genus (Asteraceae) comprises approximately 80 species, primarily found in tropical regions. Many species are used in folk medicine to treat inflammation, fever, liver disease, and neurological disorders [11]. Chemical studies have shown that this genus is rich in sesquiterpenoids, flavonoids, triterpenoids, alkaloids, and tannins [12,13]. This group of compounds has many biological activities: antioxidants, antibacterial, liver protection, pain relief, and especially AChE inhibition [11,14]. In Vietnam, *Pluchea indica* (Asteraceae) has been extensively studied, and found to demonstrate a potent antioxidant activity, contains flavonoids and phenolic acids, and exhibits AChE inhibitory effects. Tea made from *P. indica* leaves has also been clinically tested, and found to improve blood sugar and lipid levels. Meanwhile, *Pluchea pteropoda* is a native species commonly found in the Mekong Delta and other regions, but this species is less studied [15].

Oxidative stress and neurodegeneration are two significant challenges of modern medicine. There is currently no cure for Alzheimer's disease, and modern medicines have numerous toxicity-based limitations [8]. Meanwhile, medicinal herbs with antioxidant and AChE inhibitory activities have demonstrated potential for prevention and supportive treatment [3,11,12]. *Pluchea pteropoda* is a native species rich in polyphenol and flavonoid compounds, and has been initially proven to have antioxidant and AChE inhibitory activities [12]. However, research on its antioxidant and AchE medicinal attributes are insufficient in literature.

Accordingly, the present study was undertaken to provide additional scientific evidence on the chemical composition and biological activities of *Pluchea pteropoda*, and to evaluate its potential role in the prevention or supportive treatment of Alzheimer's disease. Furthermore, the outcomes of this research are intended to serve as a scientific basis for the development of medicinal preparations from indigenous plants, thereby contributing to both the conservation and rational utilization of Vietnamese medicinal plants [15,16]. The specific objectives of this study was to extract and analyze the main chemical constituents of the leaves and roots of *P. pteropoda*, to evaluate the antioxidant activity of its extracts using DPPH and FRAP models, and to determine their *in vitro* acetylcholinesterase (AChE) inhibitory activity.

Materials and Methods

Plant materials

Fresh specimens of *Pluchea pteropoda* were collected in GreenHerb farm (10.676600779096017, 106.63407645993439) at Ho Chi Minh City, Vietnam, on September 14, 2024. The plant was taxonomically authenticated at the Center for Ginseng and Medicinal Materials, Ho Chi Minh City according to document number 25-2024/TTSDL. The aerial parts (i.e. the leaves including the young stems) and roots were separated, washed, shade-dried, ground into fine powder, and stored in airtight containers until use.

Preliminary phytochemical screening

Preliminary phytochemical analysis was performed using standard chemical tests to detect the presence of flavonoids (Mg/HCl test), saponins (frothing test), alkaloids (Dragendorff's reagent), carotenoids (chloroform + H_2SO_4), tannins (gelatin-salt test), coumarins (NaOH + chloroform), triterpenoids (Salkowski's test), polyphenols ($FeCl_3$), organic acids (Na_2CO_3), polysaccharides (Lugol's reagent), anthocyanidins (HCl/NaOH), anthranoids (alkaline test), and reducing sugars (Fehling's test) [8,17,18].

Extraction procedure

Powdered samples of leaves and roots (250 g each) were macerated separately with 2.5 L of 45% or 70% ethanol at room temperature for 3 days (w/v ratio 1:10). After filtration, the extracts were concentrated under reduced pressure using a rotary evaporator, yielding four crude extracts i.e. 45% ethanol extract of leaves (L45), 70% ethanol extract of leaves (L70), 45% ethanol extract of roots (R45), and 70% ethanol extract of roots (R70). The extracts were weighed to determine yield and stored at 4 °C until analysis [8].

Evaluation of Moisture content

The moisture content of the plant powder was determined using a moisture analyzer (Model MB23, OHAUS, USA). Briefly, 5.0 g of the plant powder was accurately weighed and dried at 105 °C until a constant weight was obtained. The moisture content was calculated based on the weight loss and expressed as a percentage (%).

Total phenolic content (TPC)

The Folin Ciocalteu method was used to estimate the TPC [19]. Briefly, 0.5 mL of extract solution was mixed with 5.0 mL of Folin–Ciocalteu reagent (10%). After 5 minutes, 4.0 mL sodium carbonate (1 M) was added. The mixture was incubated at 45 °C for 15 minutes, and absorbance was measured at 765 nm. The UV-Vis absorption spectra were recorded on a UV-1800 spectrophotometer (Shimadzu Corporation, Japan). Gallic acid (50–250 µg/mL) was used to prepare the calibration curve. Results were expressed as mg gallic acid equivalents per g dry extract (mg GAE/g).

Total flavonoid content (TFC)

TFC was measured via the aluminum chloride colorimetric method [20]. The extract was mixed with 1.5 mL of methanol and 0.1 mL of sodium nitrite (0.5 M). After 5 minutes, 0.1 mL aluminum chloride (10%) was added, followed by 0.1 mL sodium acetate (1 M). The mixture was diluted to 4.5 mL with distilled water, incubated at room temperature for 30 minutes, and the absorbance was recorded at 432 nm. Quercetin (20–100 µg/mL) was used for calibration, and results were expressed as mg quercetin equivalents per g dry extract (mg QE/g).

DPPH radical scavenging assay

The antioxidant capacity of the extracts were evaluated using the DPPH method with slight modifications [21,22]. A 0.6 mM DPPH solution was prepared in methanol. Extracts (at concentrations of 50, 100, 200, 400 and 800 µg/mL) were mixed with DPPH solution by combining 0.5 mL of extract solution with 0.5 mL of 0.6 mM DPPH, and the volume was adjusted to 3 mL with methanol. The mixtures were incubated in the dark at room temperature for 30 minutes, and absorbance was recorded at 517 nm. Ascorbic acid served as the positive control. The radical scavenging activity was calculated using equation 1:

$$\%\,\text{Inhibition} = \frac{A-B}{A}.100\% \qquad equation\ 1$$

Where A is the absorbance of the control, B is the absorbance of the test sample with DPPH solution. IC_{50} values, the concentration required to inhibit 50% DPPH radicals, were obtained from dose–response curves.

Reducing power assay

The reducing ability of extracts were assessed according to the ferricyanide method [23]. Each test consisted of 1.0 mL of extract (at concentrations of 20, 100, 200, 300, 400 and 500 µg/mL) mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 minutes, followed by the addition of 2.5 mL of trichloroacetic acid (10%) and centrifugation at 3000 rpm for 10 minutes. Supernatants (2.5 mL) were mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%). After 5 minutes, absorbance was measured at 700 nm. Ascorbic acid (20–500 µg/mL) was used as a standard. Increased absorbance (ΔOD) indicated stronger reducing power.

Acetylcholinesterase inhibition assay

AChE inhibition was measured using Ellman's colorimetric method [24,25]. Reaction mixtures contained 150 µL Tris–HCl buffer (pH 8.0), 30 µL 5-5'-dithiobis-2-nitrobenzoic acid (DTNB), 60 µL AChE enzyme solution, and 30 µL extract or galantamine (positive control). After incubation at 25 °C for 15 minutes, 30 µL of acetylthiocholine iodide (ATCI) was added to initiate the reaction. Absorbance was measured at 415 nm after 10 minutes. The inhibition percentage was calculated via equation 2:

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$$\%\,\text{Inhibition} = \frac{A-B}{A}.100\% \qquad \textit{equation 2}$$

Where A is the absorbance of the control, and B is the absorbance of the test sample. IC_{50} values were obtained by regression analysis of dose–response curves. A graph of the form as een in equation 3 was established when determining the AChE inhibition capacity (%) of a series of 5 concentrations of extract (the L45, R45, and L70 extracts at 31.25, 62.5, 125, 250 and 500 µg/mL, while the R70 extract at 62.5, 125, 250, 500 and 1000 µg/mL) and the positive control where y is the AChE percentage inhibition and x is the sample concentration. From equation 3, given that y is equal to 50, we can estimate the value of x i.e. the IC_{50}:

$$y = a.ln(x) + b \qquad \textit{equation 3}$$

Statistical analysis
All experiments were conducted in triplicate (n=3), and the results were presented as mean ± standard deviation (SD). The IC_{50} values were estimated using regression analysis. Data were analyzed using Microsoft Excel 2019 software (Microsoft Corp., Redmond, WA, USA).

Results and Discussion

Phytochemical composition
Qualitative screening indicated that *P. pteropoda* leaves contain major bioactive groups, including flavonoids, tannins, triterpenoids, carotenoids, and organic acids. Ethanol extracts were particularly rich in saponins, flavonoids, and tannins, all of which are associated with analgesic and anti-inflammatory effects. The *P. pteropoda* roots were characterized by flavonoids, saponins, and polysaccharides, which are also known for their diverse pharmacological activities. Overall, the phytochemical profile of *P. pteropoda* was comparable to other species in the genus Pluchea, including *P. indica*, *P. dioscoridis*, *P. carolinensis*, *P. lanceolata*, and *P. odorata* [11].
Phenolic compounds of the *Pluchea* genus (flavonoids, tannins, alkaloids, sesquiterpenoids, triterpenoids) have been reported to possess antioxidant, hepatoprotective, anti-inflammatory, and neuroprotective properties[13,26]. In particular, *P. indica* which is rich in flavonoids with potent antioxidant [27,28]and AChE inhibitory activities [28]. A recent review further highlighted its diverse bioactive constituents (flavonoids, phenolic acids, tannins, alkaloids, and saponins), supported by preclinical evidence of antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, and lipid-lowering effects, with preliminary clinical trials showing that *P. indica* tea improved blood glucose and lipid levels in subjects at risk of diabetes [29].

Extraction yields
The moisture content of *P. pteropoda* powder was 10.58% for leaves and 9.11% for roots. From 250 g of each material extracted with 2.5 L of ethanol, the yields obtained were 25.25 g for the L45 extract with a moisture of 2.78%, 21.16 g for the L70 extract with a moisture of 4.64%, 31.29 g for the R45 extract with a moisture of 6.53%, and 17.87 g for the R70 extract with a moisture of 7.11%.

Total phenolic content (TPC)
Gallic acid was used as a standard, from which a calibration curve was constructed at 765 nm with the equation y = 0.0059x - 0.0249 (R^2 = 0.9941). Based on the calibration curve, the total polyphenol content of *P. pteropoda* was determined, with the L70 extract achieving the highest value (52.89 ± 0.47 mg GAE/g), which is higher than that of the L45 extract (48.81 ± 0.30 mg GAE/g). In comparison, the root extracts had significantly lower TPC content (R45 and R70: 29.30 ± 0.35 mg GAE/g) (Table 1).
This difference in TPC reflects the biological characteristics, where leaves are usually richer in phenolics due to their protective role against UV rays and oxidative stress [30]. Compared with *P. indica* (68.37 ± 10.61 mg GAE/g) [31], the polyphenol content of *P. pteropoda* was lower but not significant, which is consistent with previous reports [32]. Compared with two other Asteraceae species, *P. pteropoda* was equivalent to *Cladanthus arabicus* (L.) Cass but lower than *Bubonium imbricatum* Cav., although the AChE inhibitory activity of *C. arabicus* was higher [33]. In addition, the polyphenol content of *Artemisia copa* Phil. was only 36.21 ± 0.32 mg GAE/g and lower than that of *P. pteropoda*, but still exhibited both antioxidant and AChE inhibitory activities [34].

Table 1: TPC and TFC of *P. pteropoda* extracts

Extract	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
L45	48.81 ± 0.30	15.77 ± 0.04
L70	52.89 ± 0.47	20.62 ± 0.27
R45	29.30 ± 0.35	10.54 ± 0.06
R70	23.62 ± 0.38	10.72 ± 0.16

Total flavonoid content (TFC)
Quercetin was used as a standard to construct a graph of the relationship between concentration and optical density, thereby determining the flavonoid content. The quercetin standard curve was constructed as y = 0.0089x – 0.0477, with a correlation coefficient (R^2) of 0.9977, representing the relationship between concentration and optical density (ΔOD). Similar to the trend of polyphenol content, the flavonoid content in the extracts from *P. pteropoda* was highest in L70 extract (20.62 ± 0.27 mg QE/g extract), followed by L45 (15.77 ± 0.04 mg QE/g extract). In comparison, the lowest values were observed in R70 (10.72 ± 0.16 mg QE/g extract) and R45 (10.54 ± 0.06 mg QE/g extract) (Table 1).
Flavonoids represent this genus's most diverse and widespread compounds, and numerous structures have been isolated and identified [11,35]. Phenolic compounds of the *Pluchea* genus (flavonoids, tannins, alkaloids, sesquiterpenoids, triterpenoids) have been reported to possess antioxidant, hepatoprotective, anti-inflammatory, and neuroprotective properties [13,26]. In particular, *P. indica* which is rich in flavonoids with potent antioxidant [27,28]and AChE inhibitory activities [28]. A recent review further highlighted its diverse bioactive constituents (flavonoids, phenolic acids, tannins, alkaloids, and saponins), supported by preclinical evidence of antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, and lipid-lowering effects, with preliminary clinical trials showing that *P. indica* tea improved blood glucose and lipid levels in subjects at risk of diabetes [29].

DPPH radical scavenging of P. pteropoda extracts
The results showed that all four extracts from the *P. pteropoda* can scavenge DPPH radicals, in which L45 extract showed the most vigorous activity with IC_{50} = 194.796 µg/mL, followed by R45 (IC_{50} = 348.036 µg/mL), while L70 and R70 had lower activity with IC_{50} of 408.453 µg/mL and 450.717 µg/mL, respectively (Figure 1).

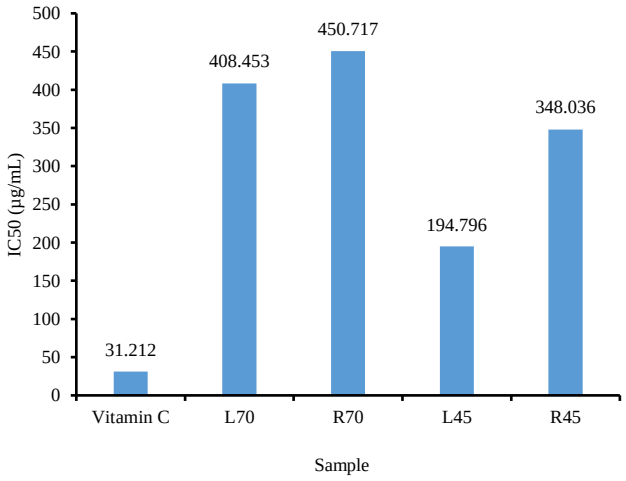

Figure 1: DPPH radical scavenging activity of *P. pteropoda* extracts

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Although the IC_{50} values of the extracts were still high compared to the positive control for Vitamin C (IC_{50} = 31.212 µg/mL), the results still showed the antioxidant potential of both the roots and leaves of *P. pteropoda*. Notably, the 45% ethanol extracts (L45, R45) had more potent activity than the 70% ethanol extracts (L70, R70), suggesting that the 45% ethanol as solvent can extract compounds with free radical neutralizing ability, such as flavonoids and polyphenols, more effectively. Thus, the presence of Phytochemicals in the extract may not be the deciding factor for the antioxidant capacity; instead, the activity of the compounds within it determines the total activity of the extract.

This difference in DPPH radical scavenging of *P. pteropoda* extracts can be explained biologically because leaves often synthesize many bioactive compounds, especially flavonoids and polyphenols, which are associated with antioxidant capacity. At the same time, roots mainly take on the function of absorption and transport, so they contain less of these compounds [36]. Compared with other species in the genus *Pluchea*, the DPPH radical scavenging ability of *P. pteropoda* was still lower, specifically compared with the ethanol leaf extracts of *P. sagittalis* (IC_{50} = 33.9 µg/mL) [37], *P. indica* (IC_{50} = 21.53 µg/mL) [38], and *P. lanceolata* (IC_{50} = 80.60 ± 0.53 µg/mL) [39].

These are all species with long-standing applications in traditional medicine i.e. *P. sagittalis* is commonly used in South America for the treatment of inflammation, wounds, and digestive disorders [37]; *P. indica* is used as a medicinal tea in Southeast Asia, with experimental evidence showing anti-inflammatory, wound healing, glucose/lipid regulating, and anti-cancer (*in vitro*) effects [38]; while *P. lanceolata* plays a vital role in Indian Ayurvedic medicine and received attention from modern research due to its antioxidant and anti-cancer cytotoxic effects (*in vitro*) [39]. Although its DPPH radical scavenging activity is lower than that of the above species, the plant still exhibits antioxidant potential due to its high content of flavonoids, tannins, and carotenoids, which contain hydroxyl groups (-OH), thereby participating in the hydrogen atom donation mechanism to neutralize DPPH free radicals [40]. Plant species such as *Stachytarpheta jamaicensis* have demonstrated even lower DPPH IC_{50} values (16–33 µg/mL), highlighting the variability of antioxidant strength among medicinal plants [41].

Reducing power of P. pteropoda extracts

The ability of the extracts to reduce Fe^{3+} to Fe^{2+} from the plant decreased in the order L70 > R70 > L45 > R45. At a concentration of 400 µg/mL, L70 extract gave the highest antioxidant activity, reaching about 0.41times that of ascorbic acid; followed by R70 extract (i.e. about 0.28 times), L45 extract (about 0.20 times), and the lowest was R45 extract (about 0.17 times) as seen in Figure 2. These results indicate that the leaves, particularly when extracted with 70% ethanol, are a rich source of compounds with strong reducing properties. The significant differences between the extraction solvents reflect the critical role of polarity in the recovery of antioxidant compounds.

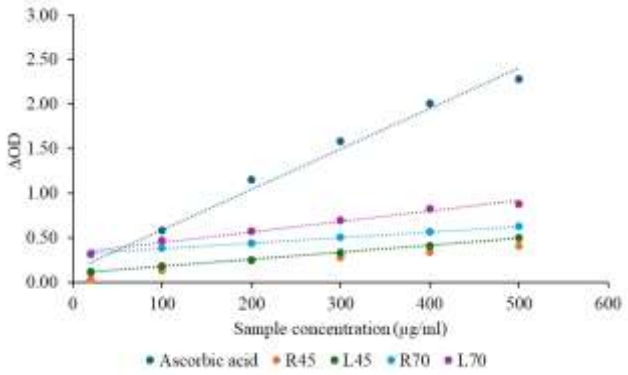

Figure 2: Reducing power of *P. pteropoda* extracts

For reducing power of *P. pteropoda* leaves, 70% ethanol extract was much more effective than 45% ethanol extract, indicating that the main compounds responsible for the reducing ability were of intermediate polarity. The 70% ethanol extract has the optimal polarity, suitable for effectively dissolving and extracting poorly soluble compounds in very polar (45%) or hypopolar solvents. These compounds are likely to be phenolic and flavonoid glycosides [42]. This finding is also consistent with the quantitative results, which show that the 70% ethanol extract from the leaves has the highest polyphenol and flavonoid content. When compared to the positive control of ascorbic acid, a common antioxidant. It is evident that the reducing ability of the crude extracts remains limited. However, this is consistent with the general characteristics of many species of the genus *Pluchea*. In *P. indica*, studies have shown that polar solvents, such as methanol and ethanol, extract high polyphenol and flavonoid contents, resulting in the most vigorous DPPH free radical scavenging activity and iron ion reduction ability [42]. Thus, the results in *P. pteropoda* are consistent with those of the same genus and strengthen the hypothesis of correlation between phenolic and flavonoid contents and reducing capacity.

Acetylcholinesterase inhibition of P. pteropoda extracts

The AChE inhibitory activity of the extracts from *P. pteropoda* decreased in the order L70 > L45 > R70 > R45. This can be explained by the fact that the content of bioactive compounds in the leaves are usually higher than that in the roots, as has been noted in many previous studies [36,43,44]. Compared with the galantamine control, the IC_{50} ratios of the extracts L70, L45, R70, and R45 were 16.08, 21.90, 43.61, and 46.92, respectively (Figure 3).

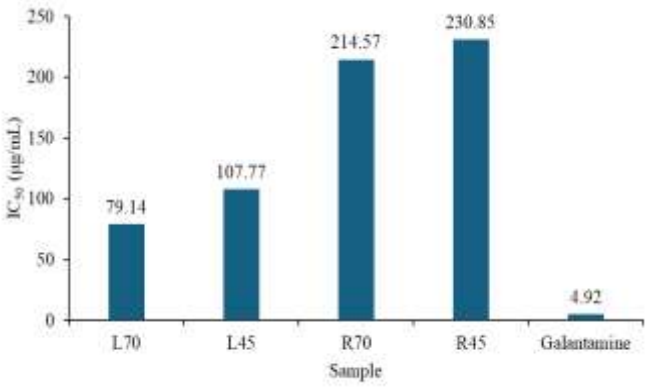

Figure 3: AChE inhibition activity of *P. pteropoda* extracts

AChE inhibition activity of L70 extract was not quite different from that of the hot water extract of *Artemisia copa* (i.e. IC_{50} of extract/IC_{50} of galantamine = 15.07), a species of the Asteraceae family used in folk medicine to support the treatment of memory loss [34]. However, compared with *Artemisia campestris*, a popular medicinal herb used in the treatment of neurological diseases, the AChE inhibitory activity of *P. pteropoda* was significantly lower [45]. At the same time, extracts and fractions from *Carpolobia lutea*, a medicinal herb that supports neurological treatment, also showed IC_{50} values equivalent to those of *P. pteropoda* [43]. Several publications have demonstrated that when the IC_{50} of AChE inhibitory activity is ≤ 100 µg/mL, the extract is considered to have potential for further research in the treatment of dementia [46,47]. For example, methanol extracts from 20 plant species used in traditional Indian medicine (Ayurveda) have identified many species, such as *Emblica officinalis*, *Nardostachys jatamansi*, *Nelumbo nucifera*, *Punica granatum*, and *Rauvolfia serpentina*, as having AChE inhibitory activity with IC_{50} < 100 µg/mL [47]. Similarly, methanol extracts and fractions from several other species have also been found to have potential with IC_{50} < 100 µg/mL [46]. Compared to some preparations from *Ginkgo biloba*, a typical medicinal herb with AChE inhibitory activity, with an IC_{50} ranging from 15 to 60 µg/mL [48], the L70 extract of the plant can also be considered remarkable. Overall, the above results suggest that the plant is a potential medicinal source that warrants further investigation to support its use in treating memory loss.

Conclusion

This study demonstrated that *Pluchea pteropoda* possesses notable antioxidant and acetylcholinesterase (AChE) inhibitory activities, associated with its considerable levels of phenolics and flavonoids.

Among the four extracts, the L70 showed the highest phytochemical content (TPC = 52.89 mg GAE/g, TFC = 20.62 mg QE/g). The L45 exhibited the highest DPPH inhibitory activity, with an IC_{50} value of 194.796 µg/mL. In the reducing power assay, L70 displayed the highest activity, reaching 0.41 times that of ascorbic acid at a concentration of 400 µg/mL. All extracts demonstrated AChE inhibition, with activity decreasing in the order L70 > L45 > R70 > R45.

The established findings provide a strong scientific foundation for the traditional use of *Pluchea pteropoda* and warrant a clear trajectory for future research to translate its in vitro potential into viable therapeutic applications. The next crucial step must involve isolating and structurally elucidating the most potent bioactive compounds. These steps must include assessment of cytotoxicity and *in vivo* safety profiles, as well as critical studies on the bioavailability of the isolated phenolic compounds to ensure they can reach their target sites effectively. Confirmation of efficacy should then be sought through *in vivo* animal models of oxidative stress and cognitive impairment to validate the potential of standardized *P. pteropoda* preparations as a phytotherapy for neurodegenerative disorders.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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