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# NEUROPROTECTIVE AND MEMORY-ENHANCING PROPERTIES OF EXTRACTS OF *POLYSCIAS GUILFOYLEI* (W. BULL) L.H. BAILEY AND *POLYSCIAS FILICIFOLIA* (C. MOORE EX E. FOURN.) L.H. BAILEY CULTIVATED IN VIETNAM

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**Summary**

Ethanolic extracts of leaves, stems, and roots of *Polyscias guilfoylei* L.H. Bailey (*P. guilfoylei*) and *Polyscias filicifolia* (Merr.) L.H. Bailey (*P. filicifolia*) collected from several regions of Vietnam were screened for neuroprotective activity against glutamate toxicity in HT22 mouse hippocampal neuronal cells. Extracts from leaves and roots of *P. filicifolia* and leaves of *P. guilfoylei* exhibited pronounced neuroprotective effects in HT22. Among active samples, extracts from *P. guilfoylei* leaves and from *P. filicifolia* roots cultivated in Nam Dinh province were subsequently evaluated for their ability to enhance short-term memory in a *Drosophila melanogaster* transgenic human amyloid precursor protein (hAPP) model of Alzheimer's disease. This finding highlights the difference between the two species and suggests that extracts of *P. guilfoylei* leaves and *P. filicifolia* roots are good candidates for further studies in rodent models to define bioactive metabolites and related mechanisms for neuroprotection of Alzheimer's and related dementias.

**Keywords:** *Polyscias guilfoylei; Polyscias filicifolia; HT22 cell line; Neuroprotective; Memory-enhancing; hAPP transgenic Drosophila.*

## 1. Introduction

*Polyscias* species are traditional tonic herbs with a long history of use and wide distribution across various regions of Vietnam. Among them, *Polyscias fruticosa* (*P. fruticosa*) (L.) Harms has been most widely used and exploited for its health-promoting and cognitive-enhancing effects [1],[2],[3],[4],[5]. The root of this species is the most frequently used tissue and is listed as *P. fruticosa* (L.) Harms in the Vietnamese Pharmacopoeia V [6]. Nevertheless, the roots of two other species, *P. guilfoylei* and *P. filicifolia*, are often used interchangeably as tonic herbs. Recently, studies of the botanical characteristics of *P. guilfoylei* cv. *quinquefolia* have been conducted [7]. In addition, analyses of genetic polymorphisms using the RAPD technique [8] and rbcL gene sequencing [9],[10] have contributed valuable data for distinguishing among *Polyscias species*. Ìcâ

Morphological characteristics that distinguish these species include: *P. fruticosa*: The leaves are large and finely alternated, typically bipinnate to tripinnate, measuring 20 - 40 cm in length, with sharply serrated and sometimes lobed leaflets; *P. filicifolia*: The compound leaves bear 11 - 13 leaflets, which are lanceolate in shape with large, deeply incised serrations; *P. guilfoylei*: The compound leaves bear seven leaflets, which typically have a whitish marginal band [1].

With the aim of assessing whether *P. guilfoylei* and *P. filicifolia* cultivated in Vietnam possess neuroprotective properties, we conducted a study of the memory-enhancing activities of extracts of these species in a transgenic *Drosophila melanogaster* expressing the human beta amyloid precursor protein, a well-recognized model [11].

## 2. Materials and Methods

### *2.1. Materials*

Leaves, stems, and roots of 3-year-old *P.*

*filicifolia* (C. Moore ex E. Fourn.) L.H. Bailey and *P. guilfoylei* (W. Bull) L.H. Bailey were collected from cultivated populations in seven provinces of Vietnam (**Table 1**). The study sampling areas represent major raw material supply regions across Vietnam and represent the Northern Delta, Northern Mountains and Midlands, Central, Central Highlands, and Southern Delta. Each sample was randomly collected from plants in five different locations within each cultivation area, and these were then pooled into a composite sample. The *P. filicifolia* and *P. guilfoylei* were identified by Dr. Tran Van On, Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy, and deposited as voucher specimens HNIP/18684/22 and HNIP/18686/22, respectively. All samples were extracted with ethanol 70%. The plant parts were separately categorized as roots, stems, and leaves. Leaves were washed and drained, while stems and roots were washed and cut into pieces 2-5 mm thick. The samples were then dried at 60°C for 24 hours, crushed into coarse powder, and stored in tight plastic bags. The extracts were obtained under reflux using a condenser following the summarized procedure as follows: 100 g of material was extracted with 70% ethanol at a ratio of 1:10 (w/v), performed 3 times, each time for 1 hour. The combined extracts were then pooled, the solvent was recovered, and the residue was concentrated in a water bath to obtain a thick extract. The extraction yields are presented in **Table 1**.

**Table 1.** List of herbal samples used in the study

| Sample | Vietnamese name | Taxonomic name | Plant material | Collection Location | Extraction yield (%) |
|---|---|---|---|---|---|
| M1 | Đinh lăng lá ráng, Đinh lăng ráng | *P. filicifolia* | Leaves | Hai An, Ninh Binh (Hai An, Hai Hau, Nam Dinh before July 1st 2025) | 33.09 |
| M2 | | | Leaves | Buon Ma Thuot, Dak Lak (Thang Loi, Buon Ma Thuot, Dak Lak before July 1st 2025) | 31.40 |
| M3 | | | Leaves | Hoa Hiep, Dak Lak (Hoa Hiep Nam, Dong Hoa, Phu Yen before July 1st 2025) | 31.29 |
| M4 | | | Leaves | Binh Minh, Ninh Binh (Binh Minh, Kim Son, Ninh Binh before July 1st 2025) | 33.69 |
| M5 | | | Leaves | Dong Hung Thuan, Ho Chi Minh city (Tan Thoi Nhat, District 12, Ho Chi Minh before July 1st 2025) | 30.47 |
| M6 | Đinh lăng lá trổ, Đinh lăng trổ | *P. guilfoylei* | Leaves | Hai An, Ninh Binh (Hai An, Hai Hau, Nam Dinh before July 1st 2025) | 37.54 |
| M7 | | | Leaves | Cu Jut, Lam Dong (Tam Thang, Cu Jut, Dak Nong before July 1st 2025) | 36.34 |
| M8 | | | Leaves | Hoa Hiep, Dak Lak (Hoa Hiep Nam, Dong Hoa, Phu Yen before July 1st 2025) | 34.52 |
| M9 | | | Leaves | Dao Xa, Phu Tho (Thach Dong, Thanh Thuy, Phu Tho before July 1st 2025) | 53.87 |
| M10 | | | Leaves | Dong Hung Thuan, Ho Chi Minh city (Tan Thoi Nhat, District 12, Ho Chi Minh, before July 1st 2025) | 31.70 |
| M11 | Đinh lăng lá ráng, Đinh lăng ráng | *P. filicifolia* | Stems | Hai An, Ninh Binh (Hai An, Hai Hau, Nam Dinh before July 1st 2025)) | 14.68 |
| M12 | | | Stems | Buon Ma Thuot, Dak Lak (Thang Loi, Buon Ma Thuot, Dak Lak before July 1st 2025) | 14.30 |

| Sample | Vietnamese name | Taxonomic name | Plant material | Collection Location | Extraction yield (%) |
|---|---|---|---|---|---|
| M13 | | | Stems | Hoa Hiep, Dak Lak (Hoa Hiep Nam, Dong Hoa, Phu Yen before July 1st 2025) | 11.45 |
| M14 | | | Stems | Binh Minh, Ninh Binh (Binh Minh, Kim Son, Ninh Binh before July 1st 2025) | 14.61 |
| M15 | | | Stems | Dong Hung Thuan, Ho Chi Minh city (Tan Thoi Nhat, District 12, Ho Chi Minh before July 1st 2025) | 16.74 |
| M16 | Đinh lăng lá trổ, Đinh lăng trổ | *P. guilfoylei* | Stems | Hai An, Ninh Binh (Hai An, Hai Hau, Nam Dinh before July 1st 2025) | 23.27 |
| M17 | | | Stems | Cu Jut, Lam Dong (Tam Thang, Cu Jut, Dak Nong before July 1st 2025) | 20.49 |
| M18 | | | Stems | Hoa Hiep, Dak Lak (Hoa Hiep Nam, Dong Hoa, Phu Yen before July 1st 2025) | 21.27 |
| M19 | | | Stems | Dao Xa, Phu Tho (Thach Dong, Thanh Thuy, Phu Tho before July 1st 2025) | 20.59 |
| M20 | | | Stems | Dong Hung Thuan, Ho Chi Minh city (Tan Thoi Nhat, District 12, Ho Chi Minh before July 1st 2025) | 21.10 |
| M21 | Đinh lăng lá ráng, Đinh lăng ráng | *P. filicifolia* | Roots | Hai An, Ninh Binh (Hai An, Hai Hau, Nam Dinh before July 1st 2025) | 17.66 |
| M22 | | | Roots | Buon Ma Thuot, Dak Lak (Thang Loi, Buon Ma Thuot, Dak Lak before July 1st 2025) | 17.02 |
| M23 | | | Roots | Hoa Hiep, Dak Lak (Hoa Hiep Nam, Dong Hoa, Phu Yen before July 1st 2025) | 19.86 |
| M24 | | | Roots | Binh Minh, Ninh Binh (Binh Minh, Kim Son, Ninh Binh before July 1st 2025) | 15.32 |
| M25 | | | Roots | Dong Hung Thuan, Ho Chi Minh city (Tan Thoi Nhat, District 12, Ho Chi Minh before July 1st 2025) | 15.20 |
| M26 | Đinh lăng lá trổ, Đinh lăng trổ | *P. guilfoylei* | Roots | Hai An, Ninh Binh (Hai An, Hai Hau, Nam Dinh before July 1st 2025) | 24.39 |
| M27 | | | Roots | Cu Jut, Lam Dong (Tam Thang, Cu Jut, Dak Nong before July 1st 2025) | 19.43 |
| M28 | | | Roots | Hoa Hiep, Dak Lak (Hoa Hiep Nam, Dong Hoa, Phu Yen before July 1st 2025) | 26.28 |
| M29 | | | Roots | Dao Xa, Phu Tho (Thach Dong, Thanh Thuy, Phu Tho before July 1st 2025) | 25.28 |
| M30 | | | Roots | Dong Hung Thuan, Ho Chi Minh city (Tan Thoi Nhat, District 12, Ho Chi Minh before July 1st 2025) | 23.28 |

Illustrative images of the two *Polyscias* species are shown in **Fig. 1**.

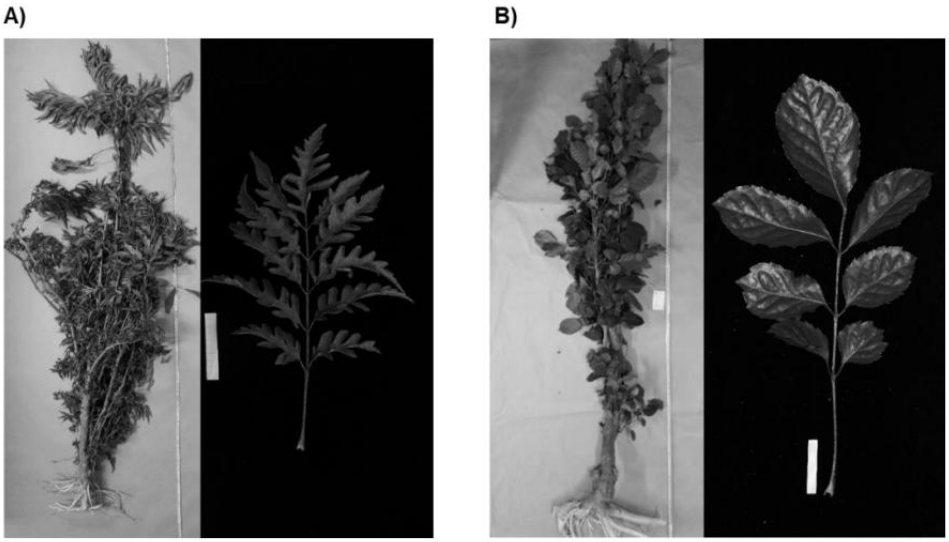



**Fig. 1.** Illustrative images of *Polyscias filicifolia* (C. Moore ex E. Fourn.) L.H. Bailey (A) and *Polyscias guilfoylei* (Bull) L.H. Bailey (B)

*2.2. Cell lines and experimental animals*

- HT22 cell lines (mouse hippocampal neuronal cells) were obtained from the Korea Cell Line Bank (KCLB, Seoul, South Korea).

- Elav-GFP (Elav) strain of *Drosophila melanogaster* with the *Elav-GFP* gene complex was used as the control group.

- UAS-Gal4>hAPP strain (hAPP, hAPP64385): a *Drosophila* transgenic strain expressing the human amyloid precursor protein (*hAPP*) was used as a model for Alzheimer's disease (AD) [11].

All *Drosophila* strains were kindly provided by Prof. Masamitsu Yamaguchi, Kyoto Institute of Technology, Kyoto University, Japan.

*2.3. Chemicals*

- Neuroprotective screening assays: Dulbecco's Modified Eagle Medium (DMEM; Cytiva, USA), Fetal Bovine Serum (FBS-Standard; Canada), Dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA; Lot #SHBC3313V), Monosodium glutamate (MSG; Sigma-Aldrich, USA; Lot #BCCB9683), N-acetylcysteine (NAC; Sigma-Aldrich, USA; CAS 616-91-1), Calcein AM (Life Technologies Corp., USA; Lot #2505979), and Penicillin-Streptomycin (Gibco, Thermo Fisher Scientific, USA; Lot #24418502).

- Odor-based memory assay: *n*-amyl acetate (AM) and *l*-octanol (OCT), purchased from Sigma-Aldrich, USA.

*2.4. Experimental methods*

2.4.1. Screening for neuroprotective effects in HT22 cells:

The experiment was conducted according to the protocol of Selvaraj *et al.* [12]. Briefly, HT22 cells were cultured in DMEM supplemented with 10% FBS and 1% Penicillin-Streptomycin, incubated at 37°C with 5% $CO_2$ until reaching 70 - 80% confluence. The cells were harvested, counted, and seeded into 96-well plates at a density of $3 \times 10^3$ cells/well, followed by incubation at 37°C/5% $CO_2$ for 24 hours. Sample extracts (at 11, 33, and 100 µg/mL), positive control (*N*-acetylcysteine, 1 mM), or vehicle control (0.5% DMSO) were added. After 24 hours of incubation, glutamate (5 mM) was treated in each well. The cells were incubated for an additional 24 hours before being stained with 1 µM Calcein AM-containing media for 10 minutes at room temperature. The number of viable cells was assessed using the Operetta CLS High-Content Analysis System and Harmony 3.5.1 software. The percentage cell viability was calculated using the formula:

$$\text{Cell survival rate (\%)} = \frac{\text{Viable cells in sample}}{\text{Viable cells in 0.1\% DMSO control)}} \times 100$$

Samples were considered to possess neuroprotective activity against glutamate toxicity if cell viability was about 80% compared to the DMSO (vehicle control) group. Each experiment was performed in triplicate.

2.4.2. Behavioral assays in the hAPP transgenic D. melanogaster:

2.4.2.1. *Drosophila* culturing and extract treatment:

Flies were cultured on standard media (containing 5% dry yeast, 5% sucrose, 1% agar, 3% powdered milk, 0.5% propionic acid and 0.4% sodium benzoate) and at laboratory temperature 25 ± 1°C, a relative humidity of 50%, a day/night period 12 h/12 h (7:00 to 19:00). Behaviour tests were carried out between 9:00 and 18:00. In this study, two genetic crosses were performed to obtain different fly strains for behavioural assessment. For the physiological controls group, Virgin male flies of Elav were crossed with virgin females of Elav, followed by a ratio of 10 males: 10 females in the bottle containing standard media for 4-5 days to produce the first generation (F1) offspring, which served as physiological controls. For pathological and treatment groups, male flies of hAPP were crossed with virgin females of Elav, followed by a ratio of 10 males: 10 females in the bottle containing standard media with or without extract, at a concentration of 2 and 4 mg/ml. The crosses were performed for 4-5 days to produce the first generation (F1) offspring expressing hAPP, which served as *D. melanogaster* for AD to evaluate the effect of the extract.

2.4.2.2. Evaluation of short-term memory of larvae:

The assay was performed according to Gerber *et al.* (2017) [13]. The procedure is illustrated in **Fig. 2**. Each experiment was repeated six times with approximately 144 third-stage larvae per group.

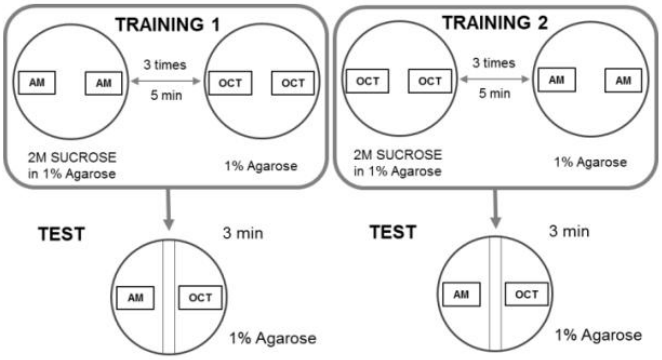



**Fig. 2.** Scheme of the experimental procedure of short-term memory in larvae

At 3 min, the number of larvae on the AM side and on the OCT side was counted. The preference (PREF) of the larvae in each dish (AM and OCT), and the Learning Index (LI) are calculated as:

$$\mathrm{PREF}_{AM} = \frac{(N_{AM} - N_{OCT})}{N_{Total}} \qquad \mathrm{PREF}_{OCT} = \frac{(N_{OCT} - N_{AM})}{N_{Total}}$$

$$\mathrm{LI} = \frac{(\mathrm{PREF}_{AM} - \mathrm{PREF}_{OCT})}{2}$$

Evaluation Criteria:

- LI > 0 indicates learning and memory ability.
- LI ≈ 0 indicates no learning or memory.
- LI < 0 indicates aversive learning behavior (often observed when larvae associate stimuli with punishment, e.g., quinidine or high-salt conditions).

2.4.2.2. Evaluation of the crawling ability of larvae

This assay followed the method described by Nichols *et al.* (2012) [14]. Briefly, third-stage larvae were collected and washed twice with

distilled water to remove food traces. 4 larvae were transferred to a 100 mm petri dish containing 1.5% agar, and the movements of the larvae were recorded using a digital camera for 1 minute. The crawling trajectories and average movement speed were analyzed using ImageJ software. The total number of larvae in each study group was about 32 (each experiment was conducted 8 times, with 4 larvae per time).

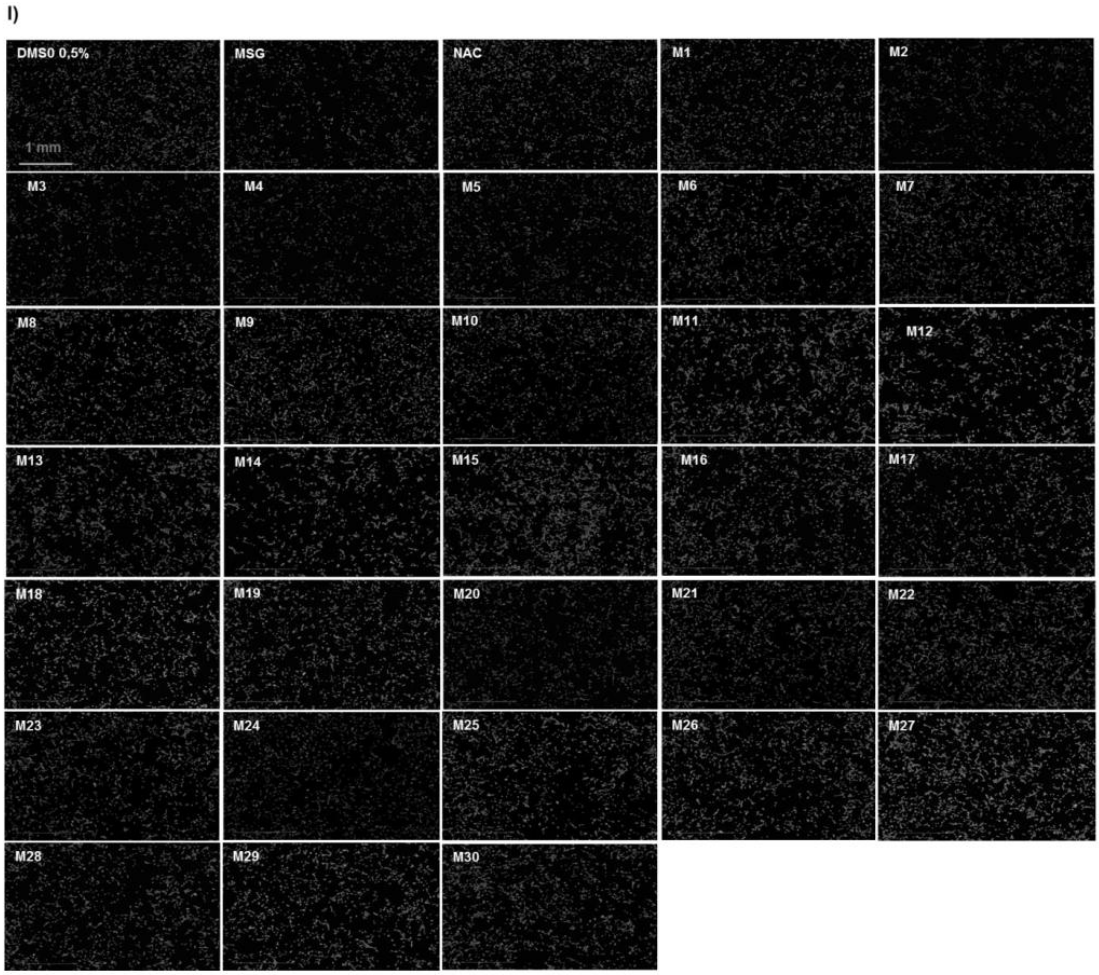
*2.5. Statistical analysis*

Statistical analyses were performed using SPSS software v23. Larval locomotion data were processed with ImageJ, followed by statistical analysis with SPSS v23. The data obtained in the odor-taste learning assay were expressed as the median with 25 and 75 percentiles and analyzed using the Mann-Whitney U test. The data obtained from the larvae crawling assay and from the neuroprotective effect experiments in HT22 cells were expressed as the mean ± S.E.M. and analyzed by One-Way ANOVA followed by either LSD or Dunnett's T3 post hoc test. Differences with $p < 0.05$ were considered significant.

**3. Results**

*3.1. Screening of neuroprotective effects of extracts in HT22 cells*

The neuroprotective effects of extracts derived from the leaves, stems, and roots of *P. filicifolia* and *P. guilfoylei* were screened in HT22 neuronal cells cultured in media containing glutamate [12]. Images of viable cells were captured after staining with 1 µM calcein-AM. Representative images of surviving HT22 cells treated with 0.5% DMSO or the extracts (at a concentration of 33 µg/mL) are shown in **Fig. 3**.



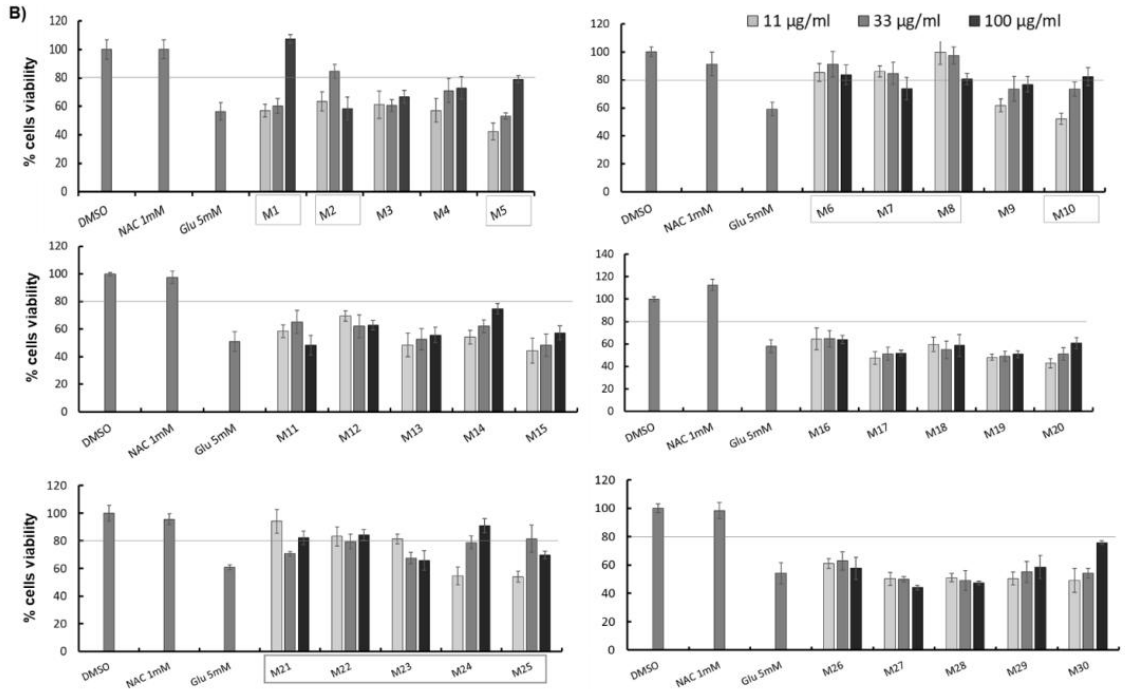



**Fig. 3.** Neuroprotective effects of the extracts on HT22 cells
*A) Representative images illustrating the effects of the extracts (33.3 µg/mL) on HT22 cell morphology;*
*B) Effects of the extracts on HT22 cell viability.*

The majority of leaf extracts exhibited neuroprotection above the 80% threshold in HT22 cells (7 out of 10 samples, including 3 extracts from *P. filicifolia* leaves and 4 extracts from *P. guilfoylei* leaves). None of the stem extracts from *P. filicifolia* or *P. guilfoylei* exhibited neuroprotective effects on HT22 cells. The root extracts of *P. guilfoylei* showed no neuroprotective activity, but all of the root extracts from *P. filicifolia* exhibited neuroprotective activity. In summary, eight extracts obtained from the leaves and roots of *P. filicifolia* demonstrated neuroprotective activity: M1 (100 µg/mL); M2 (33 µg/mL); M5 (100 µg/mL); M21 (11 µg/mL, 100 µg/mL); M22 (11, 33, 100 µg/mL); M23 (11 µg/mL); M24 (33, 100 µg/mL); and M25 (33 µg/mL) and four leaf extracts from *P. guilfoylei* exhibited neuroprotective activity: M6 (11, 33, 100 µg/mL); M7 (11, 33 µg/mL); M8 (11, 33, 100 µg/mL); and M10 (100 µg/mL) (**Fig. 3**).

Based on these results, two extracts were selected for further evaluation of memory-enhancing effects in the transgenic *D. melanogaster hAPP* model of Alzheimer's Dementia: M6 (leaf extract of *P. guilfoylei* cultivated in Nam Dinh) and M21 (root extract of *P. filicifolia* cultivated in Nam Dinh). These samples were chosen due to the neuroprotective benefits in the HT22 cell line screening model and the availability of sufficient, high-quality, and stable raw materials for subsequent studies.

*3.2. Effects of M6 and M21 extracts on the behavior of hAPP transgenic D. melanogaster modeling Alzheimer's disease*

3.2.1. Evaluation of short-term memory in D. melanogaster larvae:

The odor-taste learning assay was conducted to assess the short-term memory of third-stage transgenic *D. melanogaster* hAPP larvae treated with the two selected extracts (M6 and M21) at concentrations of 2 mg/mL and 4 mg/mL. The *D. melanogaster* with (*Elav-hAPP*) exhibited a significantly lower learning index (LI), approximately 57%, compared to the

physiological control group ($p < 0.05$), confirming previous studies indicating the expression of hAPP diminishes short-term learning and memory functions [15].

The transgenic *Drosophila* hAPP larvae treated with M21 at both concentrations of 2 and 4 mg/mL exhibited a significantly higher learning index (LI) compared to the control *D. melanogaster* hAPP group ($p < 0.001$). Similarly, *hAPP* transgenic larvae treated with M6 at a concentration of 2 mg/mL also demonstrated significantly elevated LI relative to the control *D. melanogaster* hAPP pathological group ($p < 0.05$). However, treatment with M6 at 4 mg/mL did not exhibit statistically significant improvement in the learning index ($p > 0.05$).

These findings indicate that M21 (at concentrations of 2 and 4 mg/mL) and M6 (at a concentration of 2 mg/mL) effectively improve short-term memory impairment in *hAPP* transgenic *Drosophila* larvae (**Fig. 4**).

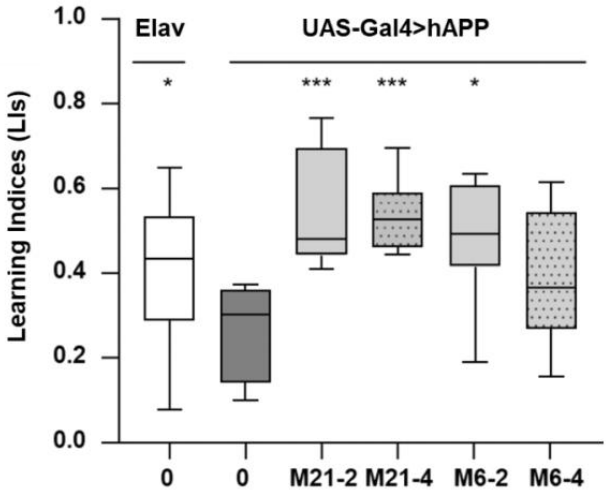



**Fig. 4.** Effects of the extracts on odor-taste learning in third-instar *hAPP* transgenic *Drosophila* larvae
Results are presented as box-and-whisker plots (n = 6 per group). *$p < 0.05$; ***$p < 0.001$ compared to the disease control group. Elav (physiological control group): wild-type *Drosophila*; UAS-Gal4>hAPP: *hAPP* transgenic *Drosophila*; M21-2, M21-4, M6-2, M6-4 (mg/mL): *hAPP* transgenic *Drosophila* treated with M21 or M6 at concentrations of 2 and 4 mg/mL, respectively.

3.2.2. Evaluation of locomotor activity in *Drosophila* larvae:

The crawling assay was performed by measuring the distance moved by *D. melanogaster* larvae over 45 seconds, from which the average locomotor speed was calculated. Each experiment was repeated at least eight times, with four larvae per trial in each experimental group. After analyzing the distance moved by the larvae within 45 seconds, the average locomotor speed was determined. Representative images of larval crawling tracks over 45 seconds are shown in **Fig. 5A** and were analyzed by ImageJ software version 1.53 (**Fig. 5B**).

Observation of the larval crawling tracks in **Fig. 5A** revealed that *hAPP* transgenic *D. melanogaster* larvae frequently exhibited a circular crawling pattern, whereas larvae in the M21 and M6 treatment groups at both 2 mg/mL and 4 mg/mL concentrations tended to move in a more linear trajectory.

The locomotor speed of *hAPP* transgenic *D. melanogaster* larvae was approximately 13% lower than that of the physiological control group (Elav) ($p < 0.01$), confirming the suitability of this model for assessing locomotor function [15]. Notably, only M21 (at a concentration of 2 mg/mL) and M6 (at a concentration of 4 mg/mL) produced a significant improvement in locomotor

speed compared to the disease control group, with $p < 0.001$ and $p < 0.05$, respectively.

These results demonstrate that M21 at a concentration of 2 mg/mL and M6 at a concentration of 4 mg/mL are effective in improving locomotor function impaired in *hAPP* transgenic *Drosophila* larvae.

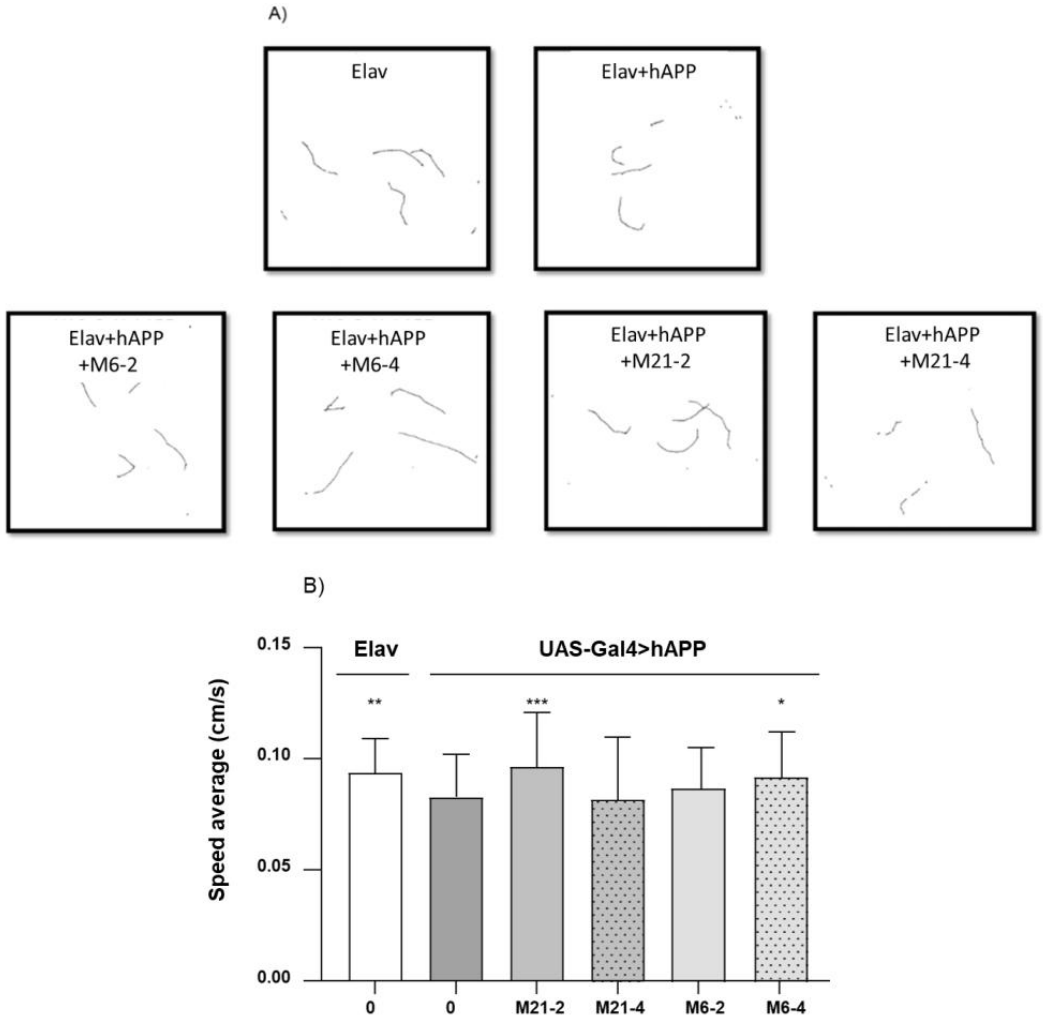



**Fig. 5.** Effects of the extracts on locomotor activity in third-instar *hAPP* transgenic *Drosophila* larvae
**p < 0.05; ** p < 0.01; *** p < 0.001 compared to the disease control group.*
*A) Representative images of larval crawling tracks in each experimental group;*
*B) Locomotor speed of larvae in the different experimental groups.*

## 4. Discussion

In this study, we screened extracts of *P. guilfoylei* and *P. filicifolia* cultivated in Vietnam for neuroprotection of neuronal HT22 cells exposed to glutamate. Nguyen et al. (2024) demonstrated that the ethanol extract of dried leaves of *P. guilfoylei* significantly exhibits neuroprotective effects against glutamate-induced oxidative stress in HT22 neuronal cells and attenuated brain cell death in an *in vivo* ischemic brain injury model [16]. We employed a hAPP transgenic *Drosophila* model expressing human amyloid precursor protein (hAPP) to assess the memory-enhancing effects of *P. guilfoylei* and *P. filicifolia*.

Screening results of 30 extracts derived from

*P. filicifolia* and *P. guilfoylei* demonstrated that all root extracts of *P. filicifolia* exhibited neuroprotective effects on HT22 cells. However, no root extracts from *P. guilfoylei* or stem extracts from either species showed any protective effect on HT22 cells. This suggests that, regardless of cultivation region, the roots of *P. filicifolia* consistently accumulate certain bioactive compounds responsible for neuroprotective activity. In contrast, the roots of *P. guilfoylei* lack such activity under the experimental conditions conducted. Currently, there are no published reports on the chemical composition present in the stems or roots of the two species, *P. filicifolia* and *P. guilfoylei*. The current research findings may suggest that the neuroprotective compounds are present in the leaves and roots of the *P. filicifolia* species (possibly belonging to the polysaccharide or saponin group). However, in the *P. guilfoylei* species, these compounds may be concentrated only in the leaves and not distributed in the roots. Further in-depth research is required to confirm this.

Our results showed that M21 (2 and 4 mg/mL) and M6 (2 mg/mL) improved memory in the hAPP transgenic *D. melanogaster*. However, only M21 at 2 mg/mL and M6 at 4 mg/mL improved locomotor performance. These findings suggest that the memory and locomotor enhancing effects of the two samples do not fully overlap and may depend on different effective concentrations or differences in their chemical compositions. Further chemical analyses of each species are therefore needed to clarify these effects.

Behavioral assays in hAPP transgenic *D. melanogaster* larvae indicated that extract M6 (ethanol extract of *P. guilfoylei* leaves) at a concentration of 4 mg/mL improved motor dysfunction in hAPP larvae, a model of Alzheimer's disease. This finding is consistent with *in vivo* results from a cerebral ischemia mouse model, where the ethanol extract of *P. guilfoylei* leaves demonstrated a neuroprotective effect on the central nervous system against ischemic damage [16]. Furthermore, chemical studies of *P. guilfoylei* leaves have shown them to be rich in oleanolic acid, which possesses anti-inflammatory and neuroprotective effects, potentially contributing significantly to the improvement of motor behavior via anti-neurodegenerative mechanisms [17].

As such, the research and development of anti-aging and neuroprotective agents, as well as therapeutics for memory impairment caused by aging or neurological disorders, remains a global priority. The current study demonstrates that both the leaves of *P. guilfoylei* and the roots of *P. filicifolia* exhibit considerable potential for the development of therapeutic agents comparable to those derived from *P. fruticosa* in treating central nervous system disorders (Alzheimer's disease, stroke due to cerebral ischemia) and motor neuron diseases (Parkinson's disease). These findings pave the way for new directions in the development of natural neuroprotective products and brain health supplements.

Moreover, the results are of significant importance in advancing the medicinal plant resources of the *Polyscias* genus in Vietnam. In particular, *P. guilfoylei*, with its fast growth rate, dense foliage, and high adaptability to adverse environmental conditions, represents a promising candidate for further research and development of new neuroprotective products.

**5. Conclusion**

Screening for neuroprotective effects on HT22 cells of 30 extracts from *P. filicifolia* and *P. guilfoylei* revealed that all root extracts of *P. filicifolia* collected from different regions (Nam Dinh, Dak Lak, Phu Yen, Ninh Binh, and Ho Chi Minh City) exhibited protective effects against glutamate-induced cytotoxicity in HT22 cells. In addition, 70% of the leaf extracts from both *P. filicifolia* and *P. guilfoylei* demonstrated neuroprotective activity, including three leaf extracts of *P. guilfoylei* collected from Nam Dinh (M1), Dak Lak (M2), and Ho Chi Minh City (M5), and four leaf extracts of *P. filicifolia* collected from Nam Dinh (M6), Dak Nong (M7), Phu Yen (M8), and Ho Chi Minh City (M10). Conversely, extracts from the stems of *P. guilfoylei*, stems of *P. filicifolia*, and roots of *P. guilfoylei* did not

show neuroprotective effects in the HT22 cells.

Evaluation of selected extracts (root extract of *P. filicifolia* and leaf extract of *P. guilfoylei*) for behavioral improvement in *D. melanogaster* transgenic for human APP (Alzheimer's disease model) demonstrated that the root extract of *P. filicifolia* (M21) at concentrations of 2 and 4 mg/mL, and the leaf extract of *P. guilfoylei* (M6) at 2 mg/mL, significantly improved short-term memory deficits in the transgenic Alzheimer's model. Furthermore, extract M21 (2 mg/mL) and extract M6 (4 mg/mL) improved locomotor impairments, as evidenced by improved crawling performance in *hAPP* transgenic *D. melanogaster* larvae.

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