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Neuroprotective Potential of Thai Cinnamon *(Cinnamomum bejolghota)* Bark Extracts against Oxidative Stress-Induced Neurotoxicity in SH-SY5Y Neuroblastoma Cells

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Abstract

Neurodegenerative diseases are associated with oxidative stress, which may be alleviated by antioxidant-rich plant compounds such as cinnamon. This study investigates the neuroprotective effects of Thai cinnamon bark *(Cinnamomum bejolghota)* extracts, in both powder and essential oil forms, against H_2O_2-induced neurotoxicity in SH-SY5Y neuroblastoma cells. The extracts were analyzed for their phenolic and flavonoid content as well as their antioxidant activities using DPPH, FRAP, and ABTS assays. Neurotoxicity and neuroprotection were assessed via MTT, LDH, TBARs, and ROS assays. The powder extract exhibited a high phenolic content (1,275.25 ± 24.07 mg GAE/g) and demonstrated stronger antioxidant activity across all assays compared to the oil form. DPPH and ABTS assays revealed IC50 values of 195.80 µg/mL and 35.55 µg/mL, respectively, for the powder extract, while the FRAP assay confirmed its dose-dependent scavenging effects ($p \leq 0.01$). Conversely, the oil extract exhibited low flavonoid content (3.05 ± 3.82 mg QE/g), weaker antioxidant activity, and no significant effects at any tested concentration ($p \leq 0.01$). MTT assays confirmed the safety of powder concentrations (100–1,000 µg/mL) for neurons, whereas oil concentrations as low as 80 µg/mL reduced cell survival to 68.85%. Both extracts displayed neuroprotective properties against oxidative stress, with the powder extract demonstrating superior efficacy. At 1,000 µg/mL, the powder extract significantly reduced LDH activity, ROS levels, and lipid peroxidation ($p \leq 0.01$). These findings suggest that Thai cinnamon powder, when incorporated into food or beverages, may help prevent neurodegeneration caused by oxidative stress. Further studies are needed to optimize its therapeutic potential.

Keywords**:** *Thai cinnamon extract; neurodegenerative disease; antioxidant property; oxidative stress*

1. Introduction

The disruption involves the accumulation of reactive species, including superoxide, hydroxyl radicals, and hydrogen peroxide (Kim et al., 2015; Niedzielska et al., 2016), along with a decrease in antioxidant activity (Singh et al., 2019). Cell death is mediated by sustained intracellular oxidative stress (OS) and increased reactive oxygen species (ROS), which affect the structure and function of the cell membrane. Particularly noteworthy was the impact of malondialdehyde (MDA), the final product of lipid peroxidation, which adversely affects DNA, protein molecules, and contributes to mitochondrial dysfunction (Kim et al., 2015; Niedzielska et al., 2016; Singh et

al., 2019). A recent study reported that decreased in axonal transport (AT) of neurons associated with the etiology of neurodegenerative diseases (ND) was caused by reactive oxygen species (ROS) (Tesco, & Lomoio, 2022). Therefore, targeting the treatment or prevention of ROS-induced neuronal cell dysfunction to diminish OS and/or trigger the antioxidant system could represent potential therapeutic approaches against ND.

A growing body of evidence suggests that phytochemical compositions from plants or herbs play a crucial role in antioxidant activities through various mechanistic pathways investigated by *in vitro*, *in vivo*, and preclinical studies, and have been used in the prevention of degenerative diseases (Shal et al., 2018; Thahira Banu, & Lunghar, 2023; Zhu et al., 2020). The bioactive compounds found in plants include alkaloids, steroids, terpenoids, flavonoids, and polyphenols. These chemical compounds are utilized as food ingredients for functional foods and in the flavoring industry. Interestingly, a single active compound with pharmaceutical activity could potentially be developed into a drug for the treatment or prevention of neurodegenerative diseases.

Cinnamon, belonging to the genus *Cinnamomum* and the Lauraceae family, is derived from the bark or leaves of cinnamon trees and is utilized as an essential oil and flavoring agent. The species include Ceylon cinnamon, also known as true cinnamon or Mexican cinnamon *(Cinnamomum verum* Syn. *C. zeylanicum*), and Cassia cinnamon or Chinese cinnamon *(C. aromaticum).* Both the raw bark and essential oil are used as ingredients in cooking, baking, cosmetics, supplementation, and alternative medicine. Cinnamon primarily consists of bioactive components such as cinnamaldehyde, and eugenol. The previous study reported that the volatile profiles of samples from *C. cassia*, *C. burmannii*, and *C. loureiroi* showed higher levels of cinnamaldehyde, (approximately 81-95%), and the highest eugenol (approximately 2–9%) compared to *C. zeylanicum*, as determined by spectroscopic analysis, demonstrating that cinnamaldehyde is the major component (Sudarsh, & Müller-Maatsch, 2023). Thus, cinnamaldehyde has been recognized for its role in counteracting cellular oxidative stress. Its mechanism involves the neutralization of free radicals by donating hydrogen atoms, thereby preventing cellular toxicity (Durak et al., 2014; Tepe, & Ozaslan, 2020; Rasool et al., 2022). Additionally, cinnamaldehyde stimulates mitochondria to release cytochrome C and enhances levels of anti-apoptotic proteins such as caspase 9, caspase 3, and Bcl-2, contributing to cell survival under oxidative stress conditions (Lv et al., 2017; Singh et al., 2021; Rasool et al., 2022). However, extraction methods can impact the polyphenol composition and antioxidant activity of cinnamon (Muhammad et al., 2021; Jayaprakasha et al., 2007).

Cinnamon extract was fractionated using solvents such as n-hexane, ethyl acetate, n-butanol, and water, revealing that the water fraction exhibited the strongest antioxidant capacity correlated with total phenolic content (Lee et al., 2023). The essential oil of cinnamon showed weak antioxidant activity, despite its main bioactive compound being inactive in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and Ferric reducing antioxidant power (FRAP) assays (Tepe, & Ozaslan, 2020). Both non-digested and digested cinnamon extracts, subjected to simulated gastrointestinal digestion, exhibited similar bioactive constituents, although the digested extract notably demonstrated increased antiradical properties (Durak et al., 2014). The antioxidant properties of the phenolic compounds mentioned above were investigated *in vitro*. Additionally, from a pharmacological perspective, while a single chemical may act on one target site, many phytochemical compounds can act on one or multiple targets to influence physiological processes through synergistic or antagonistic actions (Briskin, 2000). It is important to consider that the observed activity in the human organism may differ.

Currently, cinnamon is globally commercialized as a dietary supplement, with health benefits including anti-inflammatory, antifungal, and anti-HIV properties, as well as improvements in heart function, wound healing, immune enhancement, and anticancer effects. Additionally, it has shown effectiveness in neuroprotection by reducing oxidative stress associated with ND. Nonetheless, its cellular mechanism of action remains poorly understood. Previous research has demonstrated the impact of cooking and *in vitro* digestion on the antioxidant capacity and anti-inflammatory activity of cinnamon. Despite having a higher total phenolic content than when consumed raw, the amount tested after digestion showed a decrease in total phenolic content but an increase in the % inhibition of anti-inflammatory activity (Baker et al., 2013). This finding aligns with studies on the antioxidant and anti-inflammatory effects of cinnamon *(C. verum)* bark extract. Following *in vitro* digestion simulation, there is a reduction in total polyphenol content, particularly tannins, while other bioactive compounds like cinnamic acid are preserved. Functionally, the digested extract maintains its antioxidant and anti-inflammatory effects at the cellular level (Pagliari et al., 2023).

Thailand is home to a diverse range of herbs, which are rich in phenolic compounds and flavonoids, contributing to their medicinal properties (Pasakawee et al., 2018). Thai cinnamon bark (*C. bejolghota*) is commonly used as a spice and for its medicinal properties. It contains high levels of bioactive compounds, including phenols and flavonoids, which enhance its therapeutic potential (Liu et al., 2023b). Recognized under the Traditional Home Medicines Act, cinnamon is used for various medicinal purposes, including the relief of flatulence, constipation, dizziness, and pain (Royal Thai Government Gazette, 2013). Despite its established use, the therapeutic potential of Thai cinnamon, particularly in the treatment of neurodegenerative diseases, remains underexplored.

2. Objectives

The current research aims to explore the neuroprotective effects of Thai cinnamon bark (*C. bejolghota*) by quantitatively analyzing the phytochemical content of both its powder and essential oil forms, against H_2O_2-induced neurotoxicity in SH-SY5Y neuroblastoma cells.

3. Materials and Methods

3.1 Materials

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), DPPH, 2',7'-dichlorofluorescein diacetate (DCFH-DA), 2-thiobarbituric acid (TBA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), Dulbecco's modified Eagle's medium (DMEM)/F12, Folin–Ciocalteu reagent (FCR), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), retinoic acid, trichloroacetic acid (TCA), β-nicotinamide adenine dinucleotide, reduced dipotassium salt (NADH), was purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), trypsin/EDTA, penicillin-streptomycin and phosphate-buffered saline (PBS) were purchased from Gibco (Grand Island, NY).

3.2 Preparation of Cinnamon Extract

Thai cinnamon extract from the bark of *C. bejolghota*, in both powder and essential oil forms, was obtained from Chakkrawatherb Factory in Bangkok, Thailand. The essential oil was extracted using steam distillation. Ten milligrams of the extract and essential oil were dissolved in DMSO, then placed in an ultrasonic bath at 80 kHz and 30°C for 1 hour. The mixture was then manually stirred for 5 minutes until a homogeneous solution was obtained (Muhammad et al., 2021), and subsequently diluted with 1 mL of DMSO to achieve a 5% concentration, which was further diluted to 0.5% for use in assays. The solution was filtered through a 0.22 μm filter before being applied to the culture. Quantitative phytochemical analysis was conducted to assess total phenolic and flavonoid content (Wongon, & Limpeanchob, 2020).

3.3 Cell Culture Preparation

Human SH-SY5Y neuroblastoma cells (American Type Culture Collection [ATCC], Rockville, MD) were cultured in DMEM/F12 supplemented with 10% FBS and 0.01% penicillin-streptomycin at 37°C in a humidified incubator with 95% air and 5% CO_2. Cells were seeded into 96-well plates at a density of 2×10^4 cells/well and allowed to attach for 24 hours. Subsequently, the cells were differentiated by incubation in a low serum culture medium containing 2% FBS and 10 μM retinoic acid for 6 days to induce neuronal differentiation. Before treatment with experimental compounds, the cells were cultured in serum-free media for 24 hours to standardize cellular conditions. To ensure consistency and reproducibility, cells used in all experiments were within passages 10 to 30. This practice was adopted to minimize variability in cellular responses across experiments (Yamchuen et al., 2014).

3.4 Quantitative Phytochemical Content Analysis

3.4.1 Total Phenolic Content Determination (Folin-Ciocalteu Method)

In a 96-well plate, Thai cinnamon bark extract, in both powder and essential oil forms, was added. Each form was dissolved in 5% DMSO (20 μL) and mixed with FCR (100 μL) and a solution containing 15 g/L sodium carbonate (80 μL). The mixture was then subjected to incubation at 50°C for 5 minutes, followed by an additional 30-minute incubation period at room temperature. Absorbance readings were taken at 750 nm. Gallic acid acted as the reference phenolic compound, and the total phenolic content was quantified and expressed in terms of gallic acid equivalents (GAE) (Wongon, & Limpeanchob, 2020).

3.4.2 Total Flavonoid Content Determination

The total flavonoid content in cinnamon extract from the bark, in both powder and essential oil forms, was determined using the aluminum chloride colorimetric method. The extract, dissolved in 5% DMSO (20 μL), was mixed with 95% ethanol (60 μL), 4% $AlCl_3$ (10 μL), 0.4 M CH_3COOK (10 μL), and distilled water (100 μL) in 96-well plates. Following a 40-minute incubation at room temperature, absorbance

was measured at 415 nm. Quercetin was employed as the standard flavonoid, and the total flavonoid content was calculated and expressed as quercetin equivalents (QE) (Wongon, & Limpeanchob, 2020).

3.5 Antioxidant Activities *In vitro* Assay

3.5.1 DPPH Radical Scavenging Capacity Assay

The free radical scavenging activities of the cinnamon extract from the bark, in both powder and essential oil forms dissolved in 5% DMSO, were assessed using the DPPH assay. A DPPH solution (180 µL) prepared at a concentration of 0.2 mM in methanol was mixed with 20 µL of the test substances (cinnamon raw and essential oil in 5% DMSO). The absorbance of the reaction mixture was measured at 510 nm using a microplate reader after a suitable incubation period. The following equations were used to express the results as the percent inhibition of DPPH scavenging activity. These calculations provide a quantitative measure of the antioxidant potential of the test samples based on their ability to neutralize free radicals (Yamchuen et al., 2017a). The percent inhibition of DPPH scavenging activity reflects the extent to which the sample neutralizes DPPH radicals. The formula used to calculate the percent inhibition of DPPH scavenging activity was determined by subtracting the mean absorbance of the sample from the mean absorbance of the control, dividing the result by the mean absorbance of the control, and then multiplying by 100 (Panchakul et al., 2022).

3.5.2 FRAP Assay

The reducing activities of the cinnamon extract from the bark, in both powder and essential oil forms dissolved in 5% DMSO, were evaluated by their ability to reduce Fe^{3+} to Fe^{2+} using the FRAP assay. FRAP reagent (180 µL), composed of 3 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3$ in a ratio of 10:1:1, was mixed with 20 µL of the test substances (cinnamon extract or essential oil forms). The absorbance of the reaction mixture was measured at 595 nm using a microplate reader. The absorbance data was expressed as the concentration of Fe^{2+}, reflecting the reducing power of the test samples (Yamchuen et al., 2017a).

3.5.3 ABTS Scavenging Assay

ABTS solution (180 µL) was prepared in the dark and allowed to stand at room temperature for 12-16 hours before testing. Subsequently, it was mixed with 20 µL of the test substances (both powder and essential oil forms in 5% DMSO). The absorbance of the reaction mixture was then measured at 734 nm using a microplate reader after a suitable incubation period. The results were expressed as the percentage of radical scavenging activity, calculated based on the decrease in absorbance of the ABTS solution due to the antioxidant properties of the test samples (Duangjai et al., 2024). The percent inhibition of ABTS scavenging activity was calculated by subtracting the mean absorbance of the sample from that of the control, dividing the result by the mean absorbance of the control, and then multiplying by 100 (Panchakul et al., 2022).

3.6 Investigation of Neuronal Cell Toxicity by MTT Assay

After SH-SY5Y neuroblastoma cells underwent differentiation, they were exposed to both cinnamon bark extract in its powder and essential oil forms. The 2-hour pre-treatment with cinnamon extract was chosen based on previous studies showing its effectiveness in allowing sufficient interaction time for the test substance to exert neuroprotective effects, optimizing the potential of cinnamon extract to mitigating oxidative damage (Yamchuen et al., 2017a). H_2O_2 was then introduced at a concentration of 200 µM, and the cells were incubated for an additional 24 hours. Cell viability was assessed using the MTT assay, with 5 mg/mL MTT solution in PBS added to each well for 2 hours. Subsequently, the medium was carefully aspirated, and 200 µL of DMSO (1:1) was added to dissolve the formazan crystals (Yamchuen et al., 2017a).

3.7 Investigation of Neuroprotective Effect in Neuronal Cells Using Antioxidant Assays

3.7.1 Determination of Lactate Dehydrogenase Activity (LDH)

After the completion of the treatment period, culture media were collected to assess cell toxicity. LDH, a soluble cytosolic enzyme, serves as a marker for cell membrane integrity. To measure LDH activity, culture media (100 µL) were mixed with NADH solution (100 µL) in the presence of sodium pyruvate and monitored using a microplate reader. The decrease in absorbance at 340 nm correlates directly with the increasing quantity of LDH released from cells into the culture media. The results were normalized by the total number of viable cells and presented as a percentage of the control cells (Yamchuen et al., 2017b).

3.7.2 Determination of Lipid Peroxidation by TBA Reactive Substances (TBARs) Assay

Following the completion of the treatment period, 100 µL of TBARs reagent was added directly to each well. The TBARs reagent contained 0.4% TBA, 1.4% TCA, and 8% HCl in a ratio of 1:2:1. The plate was then incubated at 90°C for 1 hour to allow the reaction between the TBARs reagent and lipid peroxides. After incubation, the plate was cooled to room temperature and the contents were transferred to new 96-well black plates. The fluorescence intensity, indicative of lipid peroxidation levels, was measured using a microplate reader with excitation at 535 nm and emission at 595 nm. Data normalization by the number of viable cells was performed to ensure accuracy and consistency across different treatment conditions, accounting for potential variations in cell density (Yamchuen et al., 2014).

3.7.3 Determination of Intracellular ROS

Intracellular ROS levels were measured as described previously (Yamchuen et al., 2014). SH-SY5Y cells were incubated with 10 µM DCFH-DA in serum- and phenol red-free DMEM/F-12 medium for 30 minutes. After this incubation period, the culture medium was discarded. The cells were then treated with different concentrations of raw cinnamon extract and essential oil forms dissolved in 5% DMSO. The fluorescence resulting from DCFH-DA oxidation by intracellular ROS was measured using a microplate reader. The excitation wavelength was set to 485 nm, and the emission wavelength was set to 535 nm. To ensure accuracy and consistency, the data from each experiment were normalized to the number of viable cells (Yamchuen et al., 2014).

3.8 Statistical Analysis

The data from all experiments were expressed as mean ± standard deviation (SD) from at least three independent experiments. Statistical analyses were conducted using analysis of variance (ANOVA) followed by the least significant difference (LSD) test for multiple group comparisons. For two-group comparisons, the dependent samples t-test is employed to assess the sample means from two related groups. Statistically significant differences were identified with p-values ≤ 0.01.

4. Results

4.1 Phytochemical of Cinnamon Bark in Powder and Essential Oil Forms

This study conducted a quantitative phytochemical analysis of Thai cinnamon bark *(C. bejolghota)* in its powder and essential oil forms. The powder form exhibited a significant presence of phenolic compounds, averaging approximately 1,275.25 mg GAE/g. Conversely, the oil showed only trace amounts of flavonoids, with an average concentration of 3.05 mg QE/g. Notably, phenolic compounds were undetectable in the oil form, while flavonoids were undetectable in the powder form due to results falling below the standard value. This suggests a contrast in phytochemical composition between raw cinnamon bark in powder form, which is rich in phenolic compounds, and its essential oil form, which contains minimal flavonoids (Table 1).

4.2 Antioxidant Activities *In vitro Study*

The study investigated the antioxidant properties of Thai cinnamon extract from the bark in its powder form, demonstrating dose-dependent free radical scavenging and metal ion reduction abilities as evaluated through DPPH, FRAP, and ABTS assays, as shown in Figures 1, 2, and 3, respectively. Conversely, cinnamon oil exhibited only trace antioxidant activity. The results of each assay were explained as follows.

Table 1 Quantitative phytochemical analysis of Thai cinnamon bark extract

Thai cinnamon extract from the bark	Mean ± SD	
	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)
Powder form	1,275.25 ± 24.07	NA
Essential oil form	NA	3.05 ± 3.82

Note: NA denotes that the value is not applicable because it falls below the standard.

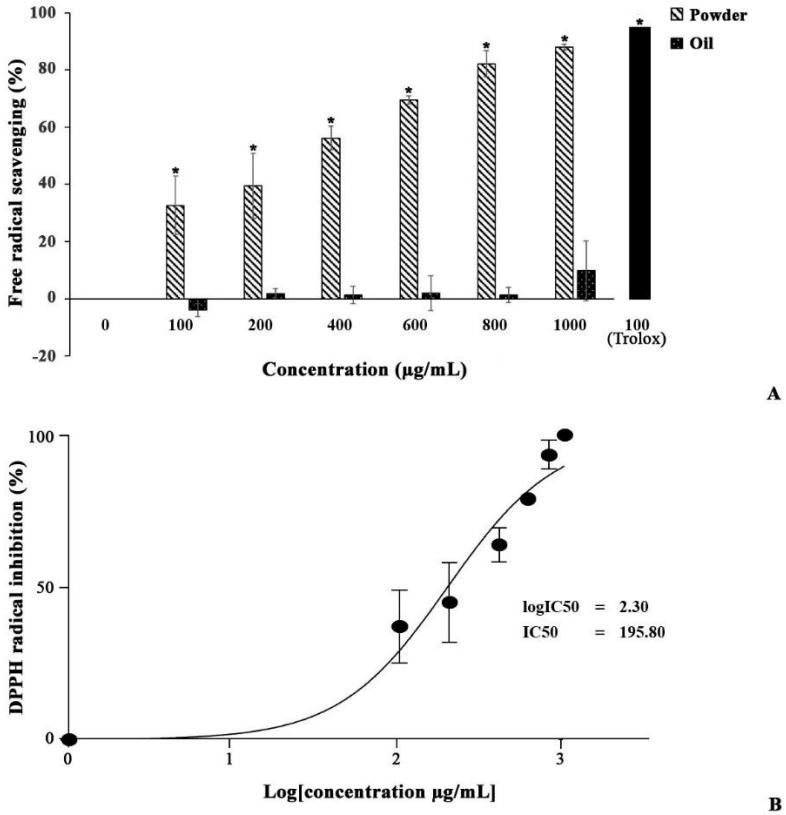

Figure 1 Free radical scavenging activity of powder (A) and oil (B) forms of cinnamon extracted from the bark measured by DPPH assay. Each form was tested at concentrations of 100 to 1,000 µg/mL (A), with Trolox serving as a control. The IC50 value for powder form was determined to be 195.8 µg/mL (B). The data are presented as means ± SD from three experiments. *indicates statistically significant differences at any concentration level ($p \leq 0.01$)

Testing the free radical scavenging activity of both powder and essential oil forms using the DPPH assay at concentrations ranging from 100 to 1000 µg/mL revealed that the powder form exhibited activity proportional to the concentration, ranging from 32.59% to 87.94%. This result was statistically significant at all concentration levels ($p \leq 0.01$) (Figure 1A). However, cinnamon oil showed relatively little free radical scavenging activity, with values less than 9.70%, and no statistically significant difference even at 1,000 µg/mL. The IC50 of the powder form was determined to be 195.8 µg/mL (Figure 1B).

In the FRAP assay at concentrations of 15-125 µg/mL, the powder form exhibited a significant dose-dependent effect with statistically significant differences at each level ($p \leq 0.01$) (Figure 2A), while cinnamon oil showed no metal ion reduction effect and no significant differences at any concentration level ($p \leq 0.01$) (Figure 2B).

Additionally, the ABST assay, which measures radical scavenging activity, was used to test antioxidant activity at concentrations of cinnamon powder ranging from 6.25 to 200 µg/mL, determining the IC50 value at 35.55 µg/mL. The results indicated that the powder form exhibited radical scavenging activity in a concentration-dependent manner, with statistically significant differences at each level ($p \leq 0.01$) (Figure 3A). For cinnamon oil, tested at concentrations of 50-1,000 µg/mL, the results showed relatively little radical scavenging effect and no statistically significant differences at any concentration level ($p \leq 0.01$) (Figure 3B).

Therefore, the results from all three assays, using Trolox as a positive control, confirm that the powder form has better antioxidant activity than oil form.

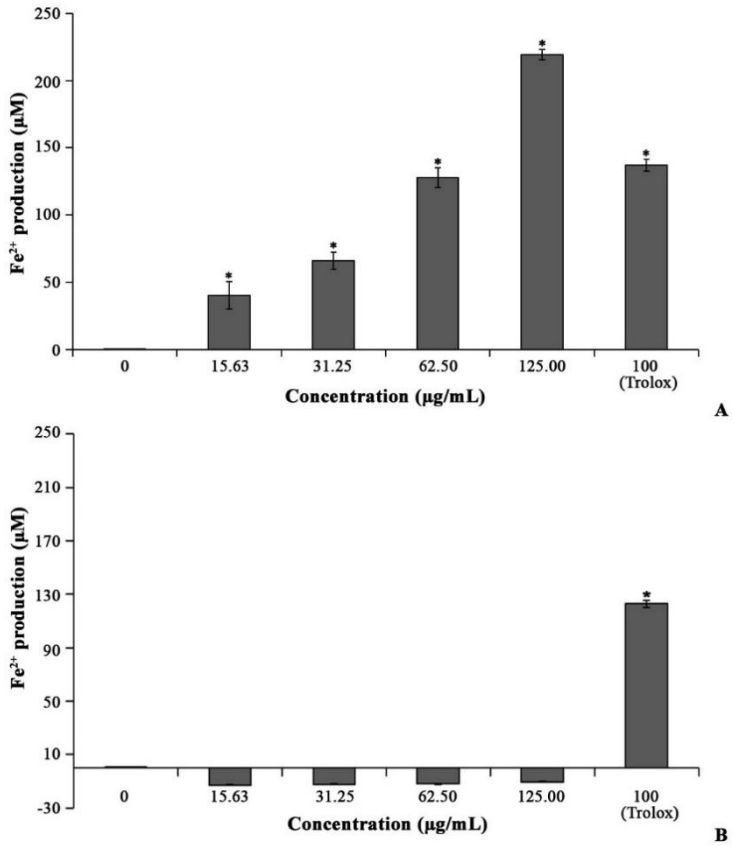

Figure 2 Metal ion reduction of powder (A) and essential oil (B) forms of cinnamon extracted from the bark measured by FRAP assay. Each form was tested at concentrations of 15-125 µg/mL, with Trolox (100 µM) serving as a control. The data are presented as means ± SD from three experiments. *indicates statistically significant differences at any concentration level ($p \leq 0.01$)

4.3 Effect of Cinnamon Powder and Oil on Neuronal Cell Toxicity

The neurotoxicity of both cinnamon powder and essential oil was assessed using the MTT assay. Powder concentrations ranging from 100 to 1,000 µg/mL showed no neuronal toxicity (Figure 4A). However, essential oil concentrations of 5-80 µg/mL did not significantly decrease cell viability, except at 80 µg/mL (Figure 4B), indicating potential neurotoxicity at higher essential oil concentrations. Further testing on SH-SY5Y neurons confirmed the safety of powder concentrations up to 1,000 µg/mL, while essential oil concentrations between 5 and 40 µg/mL exhibited no toxicity (Figure 4B). Thus, it is recommended to use lower concentrations of cinnamon extract for neuroprotective purposes, and non-toxic doses were applied in subsequent studies. The concentrations of cinnamon powder and oil were 1,000 µg/mL and 20 µg/mL, respectively.

4.4 Neuroprotective Effect of Cinnamon Powder and Essential Oil in Neuronal Cell Cultures

The neuroprotective effects of cinnamon powder and oil were investigated in neuronal cell cultures. Pre-treatment with 1,000 µg/mL of powder and 20 µg/mL of oil reduced LDH activity, intracellular ROS levels, and lipid peroxidation caused by 200 µM H_2O_2. The powder improved cell viability more than the essential oil. Both forms reduced oxidative stress and improved neuronal survival, with powder showing slightly greater results. H_2O_2 at 200 µM induced 48.67% cell death, but pre-treatment with cinnamon forms significantly reduced LDH activity, ROS, and lipid peroxidation compared to H_2O_2 alone ($p \leq 0.01$) (Table 2).

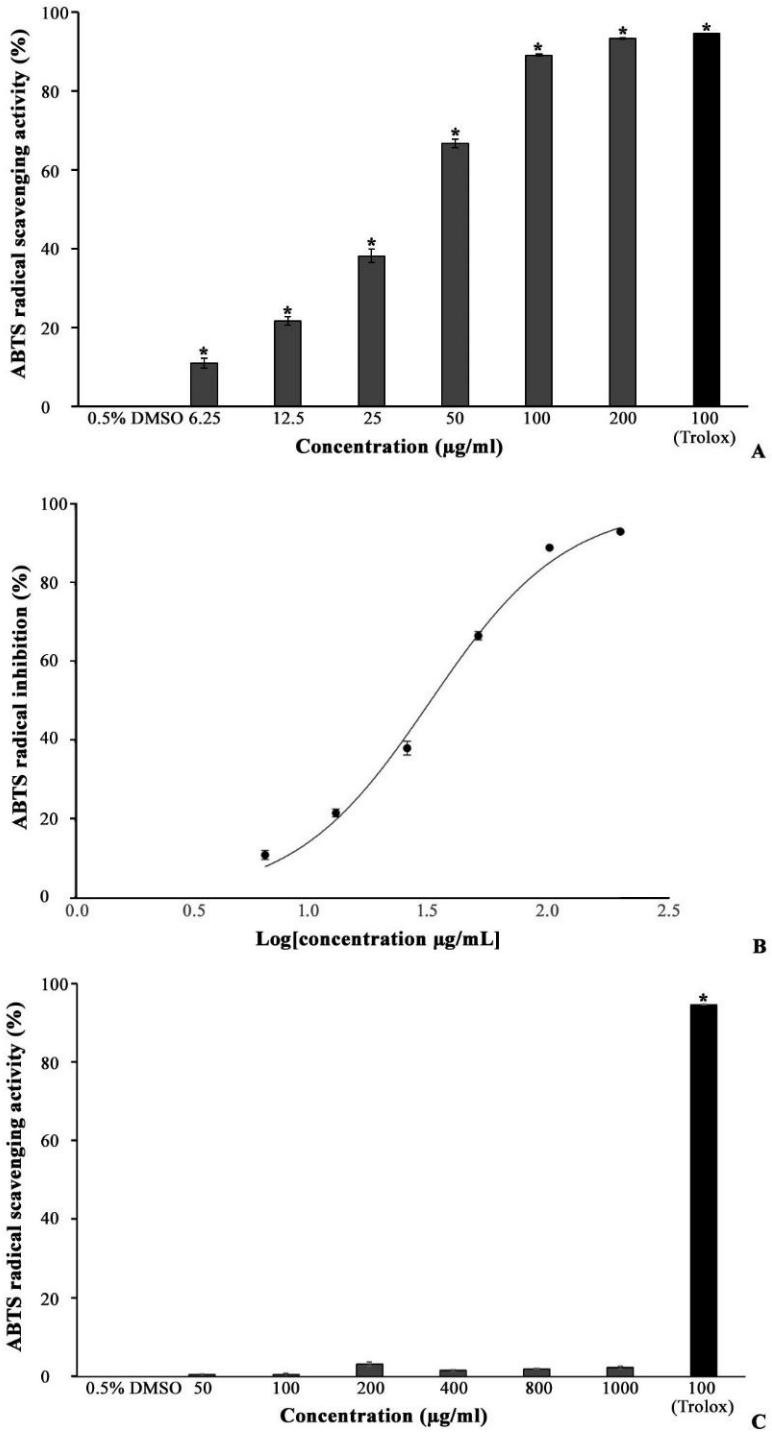

Figure 3 Free radical scavenging activity of powder and essential oil forms of cinnamon extracted from the bark measured by ABTS assay. The powder form was tested at concentrations of 6.25-200 µg/mL (A) and the IC50 value for powder form was determined to be 35.55 µg/mL (B), while oil form was tested at concentrations of 50-1,000 µg/mL (C). DMSO and Trolox were used as negative and positive controls, respectively. The data are presented as means ± SD from three experiments. * indicates statistically significant differences at any concentration level ($p \leq 0.01$)

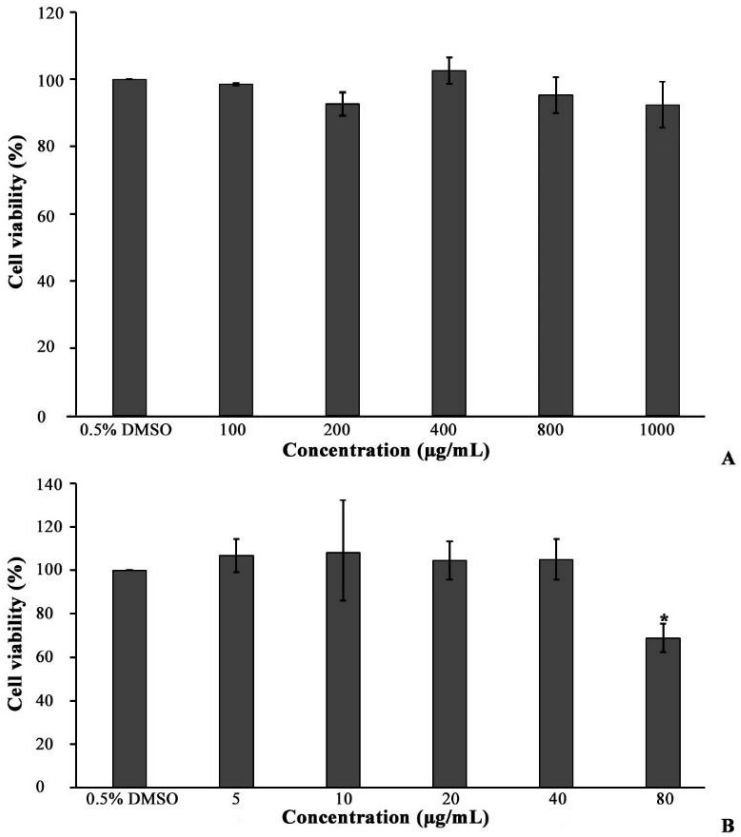

Figure 4 The toxicity of powder and essential oil forms of cinnamon bark extract on neuronal cell viability after 24 hours treatment measured by MTT assay. The powder form was tested at concentrations of 100-1,000 µg/mL (A), while the essential oil form was tested at concentrations of 5-80 µg/mL (B). DMSO (0.5%) served as a control for both forms. The data are presented as means ± SD from three experiments. * indicates statistically significant differences at any concentration level ($p \leq 0.01$)

Table 2 The neuroprotective effect in neuronal cells using antioxidant assays and cell viability

Treatment	Activity in neuronal cell culture (% of control)			
	LDH (Mean ± SD)	**Lipid peroxidation** (Mean ± SD)	**Intracellular ROS** (Mean ± SD)	**Cell Viability** (Mean ± SD)
Control group				
Untreated	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
H_2O_2 (200µM)	446.43 ± 47.91*	181.39 ± 14.50*	185.815 ± 5.66*	48.67 ± 2.69*
Trolox (100 µM)	161.82 ± 42.84**	98.47 ± 6.66**	91.35836 ± 3.84**	64.20 ± 3.81**
Cinnamon extract				
Powder (1,000 µg/mL)	172.39 ± 35.58**	57.39 ± 1.57**	55.13201 ± 2.37**	67.61 ± 5.04**
Essential Oil (20 µg/mL)	190.55 ± 51.39**	109.19 ± 8.90**	103.0471 ± 7.50**	50.32 ± 3.65

Note: Before H_2O_2 (200µM) treatment, SH-SY5Y neuroblastoma cells were pre-treated with its CM raw (1,000 µg/mL) and oil forms (20 µg/mL). LDH activities were assessed by the LDH assay, Lipid peroxidation was assessed by the TBAR assay, intracellular ROS level was assessed using the DCFH-DA assay, and cell viability was assessed by MTT assay. The data was calculated as percent of control or untreated cells and are presented as mean ± SD from at least three independent experiments. Trolox as positive control. (* $p \leq 0.01$ vs. control, ** $p \leq 0.01$ vs. cinnamon extract).

5. Discussion

The quantitative phytochemical analysis of Thai cinnamon bark revealed high concentrations of phenolic compounds (1,275.25 ± 24.07 mg GAE/g) in the powder form, while the essential oil contained only trace amounts of flavonoids (3.05 ± 3.82 mg QE/g). These results highlight the considerable difference in the phytochemical profiles of the two forms of cinnamon, with the powder exhibiting a higher potential for antioxidant activity. These findings are consistent with previous research on cinnamon cultivars such as *C. cassia* and *C. verum*, which showed variations in phenolic and flavonoid content, depending on species and extraction methods (Liu et al., 2023a; Wijewardhana et al., 2019). For example, *C. verum* from Sri Lanka had much lower total phenolic content (18.94 ± 0.46 mg GAE/100g) compared to Thai cinnamon, reinforcing the idea that the source and extraction method strongly influence the bioactive properties of cinnamon bark.

Variations in phenolic and flavonoid content can be attributed to various factors, including plant species, growing regions, and environmental conditions. Abiotic factors like temperature, sunlight, and drought have been shown to influence secondary metabolite production, which is particularly valuable for medicinal plants (Thakur et al., 2019). For instance, the slight phytochemical differences found in cinnamon species grown in varying climates in Bali, Indonesia (Darmadi et al., 2024), suggest that growing conditions can significantly affect the composition of bioactive compounds.

In our study, the powder form of Thai cinnamon demonstrated potent antioxidant activity in both the DPPH (IC50 = 195.80 µg/mL) and ABTS (IC50 = 35.55 µg/mL) assays, surpassing other cinnamon species, including *C. verum* from Thailand (IC50 = 120-140 µg/mL in DPPH and IC50 = 150-160 µg/mL in ABTS) (Pasakawee et al., 2018). This is in line with previous studies demonstrating that phenolic compounds and flavonoids are responsible for the strong antioxidant properties of cinnamon. The significant antioxidant activity observed in the FRAP assay further supports these findings (Saranya et al., 2017). However, the essential oil of Thai cinnamon exhibited weaker radical scavenging activity across all assays, consistent with other reports that pure cinnamon oil showed little to no antioxidant effects (Tepe, & Ozaslan, 2020).

The neuroprotective effects of cinnamon were also examined in SH-SY5Y cells. The powder form of cinnamon showed no neurotoxic effects in concentrations ranging from 100 to 1,000 µg/mL, suggesting its safety at these levels. However, the essential oil form exhibited significant neurotoxic effects at concentrations as low as 5 µg/mL, with marked reductions in cell viability at 80 µg/mL. Despite the neurotoxicity of the essential oil, both forms of cinnamon exhibited protective effects against H_2O_2-induced oxidative stress, indicating that the phenolic compounds and flavonoids in cinnamon may mitigate oxidative damage by neutralizing ROS and maintaining cellular integrity.

This study's findings align with previous research, which found that cinnamon powder exhibits minimal cytotoxicity in various cell types (Singh et al., 2021; Lv et al., 2017). Additionally, studies on cinnamon oil, such as those by Bahramsoltani et al., (2023), have demonstrated its potential neuroprotective effects in neurodegenerative diseases. These results support the hypothesis that the neuroprotective properties of cinnamon may be linked to its ability to reduce oxidative stress, a key factor in the pathogenesis of neurodegenerative diseases.

Previous research has described the molecular pathways involved in neuronal protection. Cinnamaldehyde, a key compound in cinnamon extracts, has been shown to enhance Bcl-2 expression, inhibit Bax expression, and decrease the LC3-II/LC3-I ratio, suggesting its protective role against glutamate-induced oxidative stress and apoptosis in PC12 cells (Lv et al., 2017). Similarly, trans-cinnamaldehyde suppresses H_2O_2-induced growth inhibition and DNA damage by blocking abnormal ROS accumulation. It prevents apoptosis by preserving mitochondrial membrane potential, inhibiting cytochrome c release, increasing the Bcl-2/Bax expression ratio, and reducing caspase-3 activity (Choi, 2021). These findings demonstrate trans-cinnamaldehyde's ability to protect cells from mitochondria-mediated apoptosis caused by oxidative stress. While our study did not directly measure these molecular markers, we propose that similar mechanisms may underlie the observed neuroprotective effects.

These findings indicate that the phenolic and flavonoid content in both powdered and essential oil forms of cinnamon may provide neuroprotective effects against H_2O_2-induced neurotoxicity in SH-SY5Y neuroblastoma cells. Phenols are well-known for their antioxidant properties, which include scavenging free radicals, reducing oxidative stress, and protecting cellular components from damage caused by reactive free radicals (Liu et al., 2023a). In contrast, flavonoids enhance cellular resilience by modulating signaling pathways involved in oxidative stress, inflammation

and apoptosis (Zahra et al., 2024). Thus, phenolic acids and flavonoids in cinnamon have been shown to suppress ROS production, decrease lipid peroxidation, and maintain mitochondrial integrity. The study's findings suggest that these bioactive compounds can mitigate oxidative damage, improve neuronal survival, and protect against neurodegenerative processes linked to excessive ROS and cellular homeostasis disruption. Future studies should investigate the mechanisms of cinnamon's neuroprotective effects, focusing on its pure bioactive chemical compounds. Research should also explore optimal dosages, synergistic effects between powder and essential oil forms, and the long-term impact of cinnamon in animal models of neurodegeneration to assess its therapeutic potential.

6. Conclusion

Thai cinnamon, a staple in Thai traditional medicine and cuisine, holds significant promise for therapeutic applications owing to its rich phenolic and flavonoid content. This study investigated the neuroprotective potential of Thai cinnamon bark *(C. bejolghota)* extracts in powder and essential oil forms against H_2O_2-induced neurotoxicity in SH-SY5Y neuroblastoma cells. Quantitative phytochemical analysis revealed substantially high phenolic content in the powder form compared to the essential oil form, indicating the potency of the powder extract. Variability in phenolic and flavonoid content among different cinnamon sources underscores the importance of factors like plant species, growth conditions, and extraction methods. The powder extract exhibited superior antioxidant activity, as evidenced by its high scavenging activities in the DPPH, FRAP and ABTS assays. In contrast, the essential oil extract displayed weaker radical inhibition across all assays, suggesting differences in antioxidant efficacy between the two forms. Furthermore, the powder extract demonstrated neuroprotective effects against oxidative stress-induced neurotoxicity, while the essential oil extract exhibited neurotoxic effects at higher concentrations. These findings demonstrate the safety and efficacy of powdered cinnamon as a neuroprotective agent.

In conclusion, both powdered and essential oil forms of Thai cinnamon exhibit potential in alleviating neurodegenerative conditions. Promoting powdered cinnamon as a functional food and using essential oil for Thai massage may help prevent nerve cell degeneration due to oxidative stress. Further research on mechanisms and optimal delivery methods could maximize Thai cinnamon's therapeutic benefits.

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