https://doi.org/10.48047/AFJBS.6.Si2.2024.5312-5324


# Evaluation of *in-vitro* anti-oxidant and neuroprotective property of Bromelain

[1]Tania Michael, [2]Divyan Devasir Sathyaseelan, [3]Lakshmi Thangavelu, [3*]Royapuram Parthasarathy Parameswari

[1]Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamilnadu, India.

[2]Department of General Surgery, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, India.

[3]Centre for Global Health Research, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Thandalam, Chennai, Tamilnadu, India.

***Corresponding author**

**Dr. Royapuram Parthasarathy Parameswari**

Associate Professor

Centre for Global Health Research,

Saveetha Medical College and Hospital

Saveetha Institute of Medical and Technical Sciences

Saveetha University, Thandalam, Chennai, Tamilnadu, India.

Email: paramsarathy@gmail.com; parameswarirp.smc@saveetha.com

Article History
Volume6,IssueSi2,2024
Received:18Apr2024
Accepted:20Jun2024
doi:10.48047/AFJBS.6.Si2.2024.
5312-5324

**Abstract**

**Background:** Bromelain, identified as a cysteine protease enzyme, is widely known for its role in aiding the digestion process by breaking down protein molecules. Extensive research has explored its potential health benefits, including chemoprotection, promotion of wound healing, systemic anti-inflammatory properties, and anticancer effects. Recently, Bromelain has attracted attention for its notable neuroprotective effects. However, additional studies are required to validate its neuroprotective potential. The current investigation focuses on examining the antioxidant capacity of Bromelain, given the crucial role of oxidative stress in the onset of Alzheimer's disease. Moreover, the study assesses the neuroprotective efficacy of Bromelain by examining its impact on acetylcholinesterase enzyme and amyloid beta peptide.

**Materials and Methods**: The free radical scavenging and antioxidant effect of Bromelain was evaluated using *in vitro* DPPH radical scavenging activity, ABTS radical scavenging activity and Xanthine oxidase inhibitory activity. The effect of Bromelain on acetylcholinesterase activity was measured under *in vitro* conditions. The inhibitory potential of Bromelain against synthetic monomer amyloid β peptide was also evaluated to confirm the neuroprotective activity exhibited by Bromelain.

**Results**: The free radical scavenging ability of Bromelain was carried out and compared with ascorbic acid. Bromelain has demonstrated significant quenching of both DPPH and ABTS free radicals. Xanthine oxidase, a major source of reactive oxygen species was also found to be markedly inhibited by Bromelain. The *in vitro* enzyme based acetylcholinesterase activity showed that Bromelain considerably inhibited acetylcholinesterase activity. The Aβ1–42 *in vitro* results showed that Bromelain effectively inhibited the aggregation of amyloid beta plaques.

**Conclusion:** In summary, the results of the present study showed that Bromelain exhibited significant antioxidant and neuroprotective effect which contributes new insights about the compound.

**Keywords:** Neuroprotection; Alzheimer’s disease; Bromelain; free radicals; acetylcholinesterase; amyloid peptide

## Introduction

Neurological diseases are a significant global health concern, and their prevalence can be influenced by various factors, including population aging, lifestyle changes, and improved diagnostic capabilities. Alzheimer's disease (AD) is the most common neurological disorder and key cause of dementia in older adults; it is characterized by lesions including extracellular β-amyloid peptide (Aβ) accumulations, neurofibrillary tangles composed of intra-neuronal abnormally phosphorylated Tau, and neuronal and synaptic losses (1). A European collaborative study of population-based cohorts found that prevalence of AD increased continuously with age, from 0.6% in the 65 to 69 years age group to 22.2% at the age of 90

years and older. Oxidative stress and neurodegeneration are implicated in the pathogenesis of various neurological disorders, including Alzheimer's disease, Parkinson's disease, and stroke (2).

Antioxidants play a crucial role in mitigating the damaging effects of oxidative stress on neuronal cells. Bromelain, a mixture of proteolytic enzymes derived from pineapple (*Ananas comosus*), has gained attention for its diverse pharmacological properties (3). Bromelain contains a combination of proteases, peroxidases, phosphatases, glucosidases, cellulases, and other enzymes, which collectively contribute to its biological activities (4, 5). Proteases in bromelain may play a role in clearing protein aggregates, which are a hallmark of several neurodegenerative diseases, including Alzheimer's and Parkinson's. Studies have suggested that Bromelain may exhibit antioxidant properties by scavenging free radicals and modulating cellular redox status. Furthermore, its ability to influence various cellular processes raises the intriguing possibility of neuroprotection (6). The neuroprotective property of substances like Bromelain is of significant importance due to its potential to safeguard the health and function of nerve cells and the nervous system. While its anti-inflammatory and anticancer activities have been widely explored, there is a paucity of research regarding its potential antioxidant and neuroprotective effects (7, 8).

*In-vitro* assessments are fundamental for elucidating the underlying mechanisms of Bromelain's antioxidant and neuroprotective effects. These experiments allow for a controlled environment where specific parameters can be measured and analysed. Understanding the in-vitro actions of Bromelain on neuronal cells and oxidative stress markers is crucial before progressing to in-vivo studies. Therefore, this study aims to evaluate the in-vitro antioxidant potential of Bromelain using established assays, such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, ABTS radical scavenging and Xanthine oxidase inhibitory assays. Additionally, we seek to explore the neuroprotective effects of Bromelain using acetylcholinesterase enzyme and amyloid β peptide by in vitro enzyme inhibitory assay systems. The outcomes of this research could provide valuable insights into the therapeutic potential of Bromelain for neurodegenerative disorders and contribute to the development of novel antioxidant interventions.

## Materials and Methods

### Chemicals and reagents

2,2- diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Xanthine, acetylthiocholine iodide, acetylcholine enzyme (0.3U/ml)

were procured from Sigma-aldrich, USA. Ascorbic acid was purchased from TCI chemicals, India. Donepezil hydrochloride was purchased as a tablet from a local pharmacy. All other chemicals, reagents and solvents used were of analytical grade and purchased from SRL chemicals, India.

**In vitro antioxidant activity**

**DPPH Free Radical Scavenging activity assay**

The antioxidant potential of the extract was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the method described by Koleva et al., 2002 (9). A 0.004% DPPH solution was prepared in 100 ml of methanol. Subsequently, 10µL of Bromelain at varying concentrations (10µM, 20µM, 40µM, 80µM, 160µM, and 320µM) was added to 190µL of the DPPH solution in a 96-well plate. After thorough vortexing, the reaction mixture was incubated for 20 minutes at 37°C. Methanol, the solvent used to prepare the DPPH solution, served as the control blank. The reduction in absorbance of the test mixture, due to the quenching of DPPH free radicals, was measured at 517 nm. The experiments were conducted in triplicate, and the percentage inhibition was calculated. Ascorbic acid was used as the standard reference in this study. The DPPH radical scavenging activity of the Bromelain was calculated by the below equation:

% Scavenging effect = (Absorbance of control–Absorbance of sample) × 100/Absorbance of control.

**ABTS radical scavenging assay**

The ABTS radical scavenging activity was measured following the procedure described by González-Palma et al., 2016 (10). A 7mM solution of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was prepared in water. To generate the ABTS radical cation (ABTS·+), the ABTS stock solution was reacted with potassium persulfate at a final concentration of 2.45mM and the mixture was left in the dark at room temperature for 12-16 hours before use. The resulting ABTS·+ solution was then diluted with water until it reached an absorbance of 0.70 (±0.02) at 734 nm. For the assay, 0.07mL of Bromelain at various concentrations (10-320µM) was added to 3mL of the ABTS radical solution. After incubating for 6 minutes, the absorbance was recorded at 734 nm using a spectrophotometer. Ascorbic acid was used as the reference standard in this experiment.

**Xanthine oxidase inhibitory activity**

The XO inhibitory activity was measured spectrophotometrically following the method described by (11). Fresh substrate and enzyme solutions were prepared for the assay. The assay mixture was composed of 50µL of various concentrations of Bromelain (10-320µM), various

concentrations of Ascorbic acid (10-320µM), 35µL of 0.1mM phosphate buffer (pH=7.5), and 30µL of enzyme solution (0.01 units/mL of XO in 0.1mM phosphate buffer, pH=7.5), and was prepared immediately before use. The reaction was initiated by adding 60µL of substrate solution (150mM of Xanthine in 0.1mM phosphate buffer) after incubating the mixture for 30 minutes at 25°C. The formation of uric acid was monitored by measuring the absorption at 295 nm at 25°C, and the initial rate was calculated. A blank was prepared in the same way. One unit of XO was defined as the amount of enzyme required to produce 1 mmol of uric acid per minute at 25°C. XO inhibitory activity was expressed as the percentage inhibition of XO, calculated using the formula (1-B/A) × 100, where A and B are the activities of the enzyme without and with different concentrations of Bromelain and Ascorbic acid, respectively. $IC_{50}$ values were determined from the average data of three replicates. Ascorbic acid was used as a reference standard.

***In vitro* acetylcholinesterase (AChE) inhibition assay (12)**

The AChE inhibitory activities of Bromelain and the standard Donepezil hydrochloride were evaluated at concentrations ranging from 10-320µM and 10-320µg/ml, respectively. To prepare these solutions, 200µl of each concentration of Bromelain and Donepezil hydrochloride were dissolved in 0.05M tris base. In brief, 200µl of acetylthiocholine iodide (15mM), 1000µl of DTNB (3mM), and 200µl of Bromelain or Donepezil at various concentrations were mixed and incubated for 15 minutes at 30ºC. The mixture's absorbance was monitored spectrophotometrically at 412nm, measured 10 times at 13-second intervals. Subsequently, 200µl of AChE solution (0.3U/ml) were added to initiate the reaction, and the absorbance was recorded. A control sample containing all components except Bromelain or Donepezil was also prepared. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

IA (%) = (Activity of Control – Activity of Test)/ Activity of Control x 100

**Assessment of Aβ (1–42) Concentration**

**Preparation of Aβ solution**

The Aβ solution was prepared following the method described by reference (14). In brief, synthetic β-Amyloid Peptide 1-42 (Aβ1-42) (PP69, Sigma Merck, USA) was dissolved in 0.1% ammonia solution to achieve a final concentration of 250µM. This solution was then sonicated in ice-cold water for a total of 5 minutes (1 minute at a time, repeated 5 times) to prevent pre-aggregation. For the preparation of the working Aβ solution, aliquots of the initial Aβ solution were diluted to a concentration of 25µM in a buffer containing 50mM phosphate (pH 7.5) and 100mM NaCl.

**Thioflavin T fluorescence assay**

The thioflavin T (ThT) fluorescence assay was conducted as described in reference (13). An 8µL solution of Aβ was combined with various concentrations of Bromelain (10-320µM) and Donepezil (10-320µg/ml). This mixture was then added to 1.6mL of ThT solution, which contained 5µM ThT and 50mM NaOH-glycine buffer (pH 8.5). The samples were incubated at 37°C, and the fibrillogenesis rate was monitored using ThT fluorescence assays. ThT fluorescence levels of the samples were measured with a Biotek Synergy H4 hybrid multi-mode reader (USA), with excitation and emission wavelengths set at 446 nm and 490 nm, respectively.

**Statistical analysis**

Data were analysed using GraphPad prism (version 7.0). The results were expressed as Mean±SEM and the $IC_{50}$ values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means of bromelain and standards at ***p<0.001, **p<0.01 and *p<0.05 level of significance. The means were compared with standards groups using the Holm-Sidak Test.

**Results**

**DPPH radical scavenging assay**

Bromelain was prepared at varying concentrations of 10µM, 20µM, 40µM, 80µM, 160µM and 320µM. The different concentrations of Bromelain was tested for its potential against DPPH radical formation. The results demonstrated significant inhibition of DPPH radicals by Bromelain which is comparable to Standard Ascorbic acid. The lowest inhibition was found at 10µM with inhibitory percentage of 0.85% while ascorbic acid exhibited 11% inhibition at the same concentration. However, a maximum of 71.8% inhibition was observed at maximum bromelain concentration of 320µM.

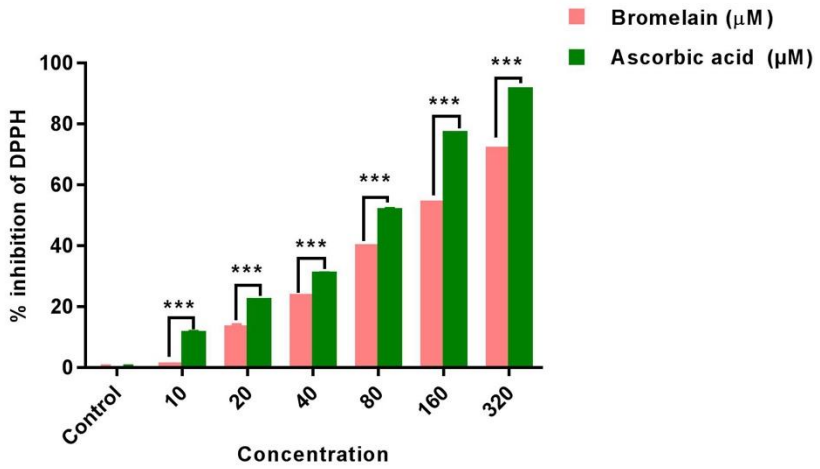

**Figure 1: Bar graph showing % inhibition of DPPH free radicals by different concentrations of Bromelain compared with standard Ascorbic acid. Two-way ANOVA was used to assess differences between means of bromelain and standards at ***p<0.001, **p<0.01 and *p<0.05 level of significance. The means were compared with standards groups using the Holm-Sidak Test.**

**ABTS radical scavenging assay**

Bromelain at all its tested concentrations of 10-320μM has exhibited marked quenching of ABTS radicals (Figure 2). The ABTS quenching ability of Bromelain was compared to Standard Ascorbic acid. Maximum inhibition of 77.85% and 92.78% was observed for Bromelain and ascorbic acid respectively at the highest concentration of 320μM.

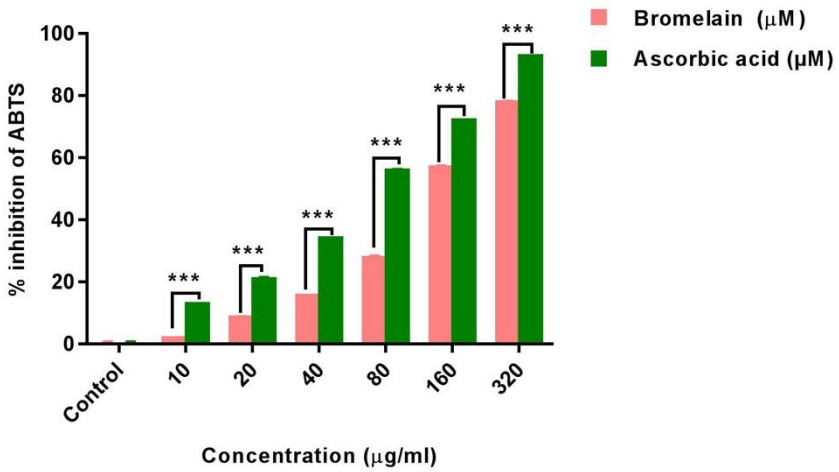


**Figure 2: Bar graph showing % inhibition of ABTS free radicals by different concentrations of Bromelain compared with standard Ascorbic acid. Two-way ANOVA was used to assess differences between means of bromelain and standards at ***p<0.001, **p<0.01 and *p<0.05 level of significance. The means were compared with standards groups using the Holm-Sidak Test.**

**Xanthine oxidase inhibitory effect of Bromelain**

Xanthine oxidase, major source of reactive oxygen species was found to be significantly inhibited to a maximum percentage of 81.64% by Bromelain at 320μM (Figure 3).

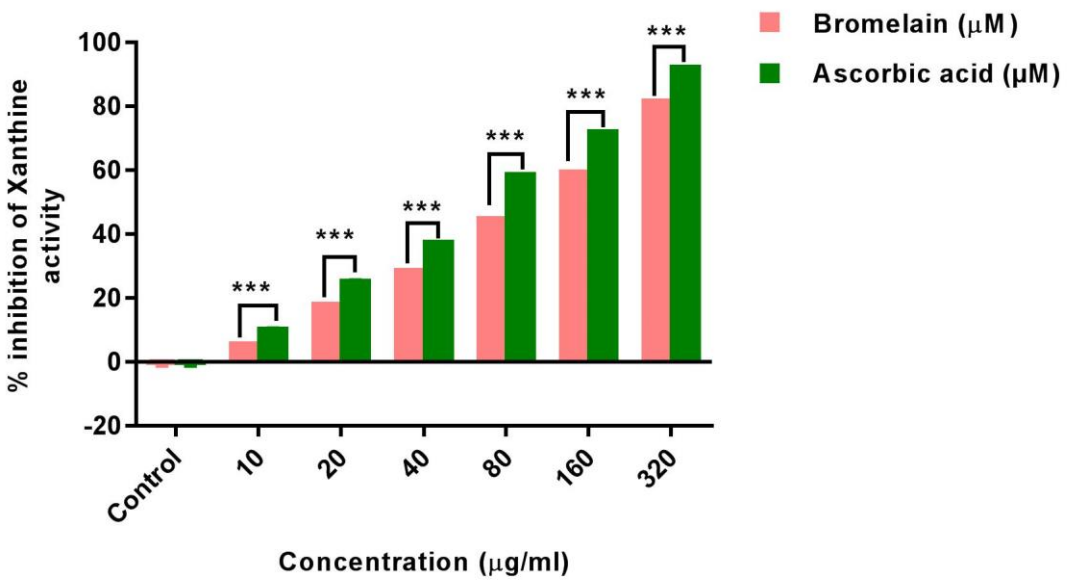


**Figure 3: Bar graph showing % inhibition of Xanthine oxidase by different concentrations of Bromelain compared with standard Ascorbic acid. Two-way ANOVA was used to assess differences between means of bromelain and standards at ***p<0.001, **p<0.01 and *p<0.05 level of significance. The means were compared with standards groups using the Holm-Sidak Test.**

**In vitro acetylcholinesterase inhibitory activity of Bromelain**

Bromelain exhibited considerable and dose-dependent decrease in the activity of acetylcholinesterase under *in vitro* conditions. A minimum inhibition of 2.33% to a maximum inhibition of 76.24% was observed for Bromelain from 10μM to 320μM. The acetylcholinesterase inhibition potential was compared with standard cholinesterase inhibitor Donepezil hydrochloride.

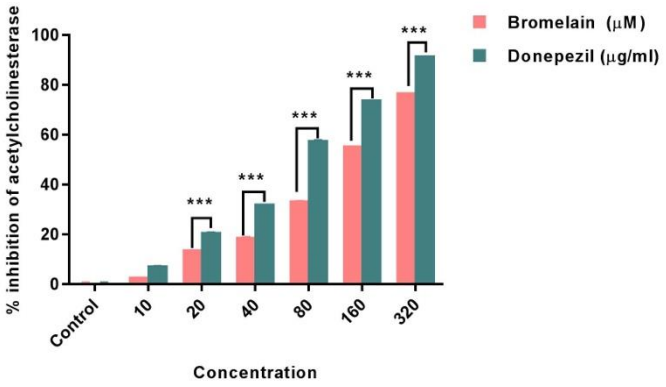


**Figure 4: Bar graph showing % inhibition of acetylcholinesterase by different concentrations of Bromelain compared with standard Donepezil. Two-way ANOVA was used to assess differences between means of bromelain and standards at ***p<0.001,**

****p<0.01 and *p<0.05 level of significance. The means were compared with standards groups using the Holm-Sidak Test.**

### *In vitro* inhibition of Aβ42 by Bromelain

The results of synthetic β-Amyloid Peptide 1-42 aggregation assay showed that Bromelain effectively inhibited the aggregation of Aβ42. The % inhibition of aggregation was compared to standard Donepezil. The inhibitory activity was found to be promising.

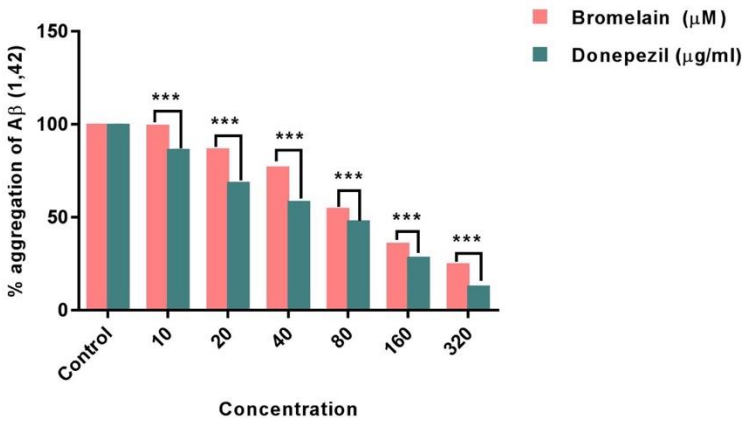


**Figure 5: Bar graph showing % inhibition of aggregation of amyloid beta peptide by different concentrations of Bromelain compared with standard Donepezil. Two-way ANOVA was used to assess differences between means of bromelain and standards at ***p<0.001, **p<0.01 and *p<0.05 level of significance. The means were compared with standards groups using the Holm-Sidak Test.**

## Discussion

Neuroprotective drugs are substances aimed at safeguarding the nervous system, particularly the brain, against injury, deterioration, or disease. They are pivotal in halting or delaying the advancement of neurological disorders and ailments (14, 15). Many such conditions, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, and stroke, involve the damage or degeneration of nerve cells (16, 17). A promising therapeutic approach involves targeting acetylcholinesterase and amyloid β42 (Aβ42) oligomers, potentially offering effective neuroprotective strategies for both the prevention and treatment of Alzheimer's disease (18). Against this backdrop, the current study investigates Bromelain, the active component found in pineapples, to assess its potential antioxidant and neuroprotective effects.

Oxidative stress is a primary factor in the development of diseases such as Alzheimer's and Parkinson's, and it is implicated in various neurological disorders (19, 20). Addressing the complex nature of neurodegenerative conditions like Alzheimer's disease (AD) would benefit from additional actions such as scavenging free radicals and exhibiting antioxidant properties, alongside the primary targeted effects (21). Neuroprotective drugs with antioxidant properties can neutralize harmful free radicals and reduce oxidative damage to nerve cells (22). By slowing down the progression of neurological disorders or preventing further damage, these neuroprotective drugs can enhance the quality of life for individuals affected by these conditions (23). Bromelain's free radical scavenging property was demonstrated through radical scavenging assays exhibited by significant inhibition of DPPH, ABTS and Xanthine oxidase. Therefore, it can be concluded that bromelain possesses antioxidant properties, which can be utilized to prevent or slow down the progression of neurological diseases (16).

There are not many neuroprotective drugs available on the market, primarily because few compounds can cross the blood-brain barrier (BBB). Overcoming the BBB is a significant challenge for drugs and therapeutic agents (24). Previous research has demonstrated that a combination of Bromelain and Donepezil effectively reversed spatial learning and memory deficits induced by AlCl3 and D-galactose. Moreover, it reduced cognitive impairment by enhancing cholinergic activity and synaptic plasticity while decreasing oxidative damage, neuroinflammation, and Aβ 1–42 aggregation (6). These findings suggest that Bromelain can cross the BBB. Therefore, the present study investigated the anti-Alzheimer's effects of Bromelain on acetylcholinesterase activity and amyloid beta plaque formation in an in vitro assay. The results of this study showed that Bromelain has promising neuroprotective effects, as evidenced by significant inhibition of acetylcholinesterase and amyloid beta plaque formation.

The use of natural compounds in disease treatment has garnered increasing attention in recent years due to several associated advantages. These compounds, often derived from plants, fruits, vegetables, or other natural sources, typically have a long history of human consumption and are generally considered safe with lower toxicity compared to some synthetic drugs. For instance, natural compounds like Bromelain exhibit neuroprotective properties by reducing oxidative stress, inflammation, and apoptosis (25). These effects are particularly significant in the context of neurodegenerative diseases such as Alzheimer's and Parkinson's. Additionally, natural compounds often possess characteristics that enhance their ability to cross the blood-brain barrier, allowing them to reach the brain more effectively (26). This improved bioavailability contributes to their therapeutic potential in neurological diseases. Further

research using mouse models and identifying the mechanisms of action of Bromelain is crucial to validate the reported effects of Bromelain presented in this study.

**Conclusion**

In summary, our research suggests that Bromelain, commonly found in pineapple and widely consumed, has the potential to delay the progression of Alzheimer's disease (AD). These findings provide valuable insights that could aid in the development of novel preventive treatments for AD.

**CONFLICT OF INTEREST**

There is no conflict of interest.

**ACKNOWLEDGMENT**

We extend our gratitude to Saveetha dental college and hospital for their constant support and encouragement and hereby helping in successful completion of this research work.

**References**

1. Risacher SL, Saykin AJ. Neuroimaging and other biomarkers for Alzheimer's disease: the changing landscape of early detection. Annual review of clinical psychology. 2013 Mar 28;9:621-48.
2. Love, S. and Jenner, P. (1999) Oxidative Stress in Neurological Disease.
3. Balls, A.K. (1941) Protein-digesting Enzymes of Papaya and Pineapple.
4. Mulia, R.N. (2012) Bromelain Enzyme Protection During Pineapple Juice Extraction. LAP Lambert Academic Publishing.
5. Ota S, Moore S and Stein WH: Preparation and chemical properties of purified stem and fruit bromelains. Biochemistry. 3:180–185. 1964.
6. Kumar R, Kumar R, Sharma N, Khurana N, Singh SK, Satija S, Mehta M, Vyas M. Pharmacological evaluation of bromelain in mouse model of Alzheimer’s disease. Neurotoxicology. 2022 May 1;90:19-34.
7. Epuri, V., Prathap, L., Reddy, V., Rajesh, B. and Ramesh, B., 2023. Cytoprotective and anti-inflammatory effect of ethanolic extract of Withania somnifera root in combination with doxycycline in rotenone induced cytotoxicity in neuroblastoma cell lines. *Biomedicine*, *43*(01), pp.469-476.
8. Murugan, S.K., Bethapudi, B., Raghunandhakumar, S., Purusothaman, D., Nithyanantham, M., Mundkinajeddu, D. and Talkad, M.S., 2022. A flavonoid rich standardized extract of Glycyrrhiza glabra protects intestinal epithelial barrier function and regulates the tight-junction proteins expression. BMC Complementary Medicine and Therapies, 22(1), p.38.
9. Koleva II, van Beek TA, Linssen JPH, de Groot A, Evstatieva LN. Screening of plant

extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 2002 Jan-Feb;13(1):8–17.

10. González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Téllez-Téllez M, Gupta VK, Díaz-Godínez G, et al. Evaluation of the Antioxidant Activity of Aqueous and Methanol Extracts of Pleurotus ostreatus in Different Growth Stages. Front Microbiol. 2016 Jul 12;7:1099.
11. Bustanji Y. In Vitro Xanthine Oxidase Inhibition by Selected Jordanian Medicinal Plants. Jordan Journal of Pharmaceutical Sciences. 2011, Vol. 4, No. 1. 2011. 16 p.
12. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961 Jul;7:88–95.
13. Miyazaki H, Okamoto Y, Motoi A, Watanabe T, Katayama S, Kawahara SI, et al. Adzuki bean extract reduces amyloid-β aggregation and delays cognitive impairment in models of Alzheimer's disease. Nutr Res Pract. 2019 Feb;13(1):64–9.
14. Arumuganainar, D., Yadalam, P.K., Alzahrani, K.J., Alsharif, K.F., Alzahrani, F.M., Alshammeri, S., Ahmed, S.S., Vinothkumar, T.S., Baeshen, H.A. and Patil, S., 2023. Inhibitory effect of lupeol, quercetin, and solasodine on Rhizopus oryzae: A molecular docking and dynamic simulation study. *Journal of Infection and Public Health*, *16*(1), pp.117-124.
15. Parameswari, R.P. and Lakshmi, T., 2022. Microalgae as a potential therapeutic drug candidate for neurodegenerative diseases. *Journal of Biotechnology*, *358*, pp.128-139.
16. Ferah Okkay, I. et al. (2021) 'Neuroprotective effect of bromelain in 6-hydroxydopamine induced in vitro model of Parkinson's disease', Molecular biology reports, 48(12), pp. 7711–7717.
17. Veeraraghavan, V.P., Jayaraman, S., Rengasamy, G., Mony, U., Ganapathy, D.M., Geetha, R.V. and Sekar, D., 2021. Deciphering the role of microRNAs in neuroblastoma. *Molecules*, *27*(1), p.99.
18. Sancesario GM, Nuccetelli M, Cerri A, Zegeer J, Severini C, Ciotti MT, Pieri M, Martorana A, Caltagirone C, Nistico R, Bernardini S. Bromelain degrades Aβ1-42 monomers and soluble aggregates: an in vitro study in cerebrospinal fluid of Alzheimer's disease patients. Current Alzheimer Research. 2018 Jun 1;15(7):628-36.
19. Foret, M. (2022) Investigating the Early Role of Oxidative Stress in Alzheimer's Disease: Insights from a Transgenic Model of the Amyloid Pathology and Fluorescence Imaging Methods.

20. Mohanraj, K.G., 2022. Antioxidant Activity Of L-Theanine On Cadmium Induced Oxidative Stress Mediated Neurodegeneration-An In Vivo Analysis. *Journal of Population Therapeutics and Clinical Pharmacology*, *29*(02), pp.123-130.
21. Nwidu LL, Elmorsy E, Aprioku JS, Siminialayi I, Carter WG. In vitro anti-cholinesterase and antioxidant activity of extracts of Moringa oleifera plants from Rivers State, Niger Delta, Nigeria. Medicines. 2018 Jul 5;5(3):71.
22. Martin, C.R. and Preedy, V.R. (2020) Oxidative Stress and Dietary Antioxidants in Neurological Diseases. Academic Press.
23. Shiew, P.S. (2012) Enzymatic, Antioxidant Activities and Stability of Bromelain-polyphenol Precipitate in Pineapple Juice.
24. Mishra, K. et al. (2023) 'Recent Advancements in Nanocarrier-assisted Brain Delivery of Phytochemicals Against Neurological Diseases', Neurochemical research, 48(10), pp. 2936–2968.
25. Wu, W. et al. (2023) 'Research Progress on Natural Plant Molecules in Regulating the Blood-Brain Barrier in Alzheimer's Disease', Molecules , 28(22). Available at: https://doi.org/10.3390/molecules28227631.
26. Ayaz, M. et al. (2020) Natural Products-Based Drugs: Potential Therapeutics against Alzheimer's Disease and other Neurological Disorders. Frontiers Media SA.