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## SYNERGISTIC NEUROPROTECTION BY BROMELAIN AND MYRCENE IN PRECLINICAL ALZHEIMER'S DISEASE MODELS

**Nalla Manoj Kanna**

Research Scholar, Sunrise University, Alwar Rajasthan

**Dr. Parag Ramchandra Patil**

Professor, Sunrise University, Alwar Rajasthan

### ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and neuronal loss. Despite advances in understanding its pathophysiology, effective therapies remain limited. Bromelain, a proteolytic enzyme derived from pineapple, and myrcene, a naturally occurring monoterpene, have demonstrated neuroprotective and anti-inflammatory properties individually. This study explores the synergistic neuroprotective potential of bromelain and myrcene in preclinical Alzheimer's disease models. Using in vitro neuronal cultures and in vivo AD rodent models, we evaluated cognitive function, amyloid-beta ( $A\beta$ ) aggregation, oxidative stress markers, and neuroinflammatory responses. The combination of bromelain and myrcene demonstrated enhanced neuroprotection compared to individual treatments, reducing  $A\beta$  accumulation, attenuating oxidative stress, and improving memory performance. These findings suggest a promising combinatorial therapeutic strategy for mitigating AD pathology.

**Keywords:** Bromelain, Myrcene, Neuroprotection, Alzheimer's disease, Oxidative stress

## I. INTRODUCTION

Alzheimer's disease (AD) remains one of the most devastating neurodegenerative disorders of the aging population, marked by progressive memory loss, cognitive impairment, and a steady decline in functional independence. Globally, AD affects millions of individuals, and its prevalence continues to rise with increasing life expectancy. Despite extensive scientific efforts, current therapeutic strategies offer only symptomatic relief and do not adequately target the complex underlying mechanisms of the disease. The multifactorial nature of AD—which encompasses amyloid-beta ( $A\beta$ ) deposition, tau hyperphosphorylation, oxidative stress, mitochondrial dysfunction, synaptic loss, and neuroinflammation—has prompted the search for multi-target therapeutic agents. This shift has fueled scientific interest in natural compounds due to their broad pharmacological actions, low toxicity, and potential to modulate several pathological pathways simultaneously.

Among natural molecules gaining attention, bromelain and myrcene stand out as promising candidates for neuroprotection. Bromelain is a proteolytic enzyme complex derived from *Ananas comosus* (pineapple) and has been extensively studied for its anti-inflammatory, antioxidant, anti-thrombotic, and immunomodulatory properties. Traditionally recognized for its role in reducing inflammation and promoting tissue repair, bromelain has more recently demonstrated potential therapeutic benefits within the central nervous system. Its anti-inflammatory effects, particularly the reduction of pro-inflammatory cytokines and modulation of immune cell activation, are relevant to AD pathology, where chronic neuroinflammation plays a central role. Furthermore, bromelain's proteolytic activity may influence protein aggregation pathways, potentially contributing to the reduction of amyloid-beta accumulation.

Myrcene, a naturally occurring monoterpene found in plants such as hops, lemongrass, basil, and cannabis, is widely recognized for its antioxidant, analgesic, anti-inflammatory, and anxiolytic effects. Its lipophilic nature allows efficient penetration across the blood–brain barrier, making it particularly suitable for neurological applications. Recent studies suggest that myrcene can mitigate oxidative stress, protect neuronal membranes against lipid peroxidation, and enhance endogenous antioxidant defenses. Additionally, myrcene has been shown to modulate neurotransmitter systems and exert neuroprotective effects in models of neurotoxicity and excitotoxicity. These properties align closely with therapeutic objectives in AD, where oxidative stress and neuronal vulnerability substantially contribute to disease progression.

Although bromelain and myrcene have each demonstrated individual neuroprotective benefits, the potential synergistic effects of combining these compounds remain largely unexplored in the context of Alzheimer's disease. Synergistic combinations of natural compounds can offer significant therapeutic advantages, including enhanced efficacy, improved bioavailability, and greater modulation of multiple pathological pathways. In diseases as complex as AD, such combinatorial strategies may outperform single-agent therapies by simultaneously addressing several molecular targets. Bromelain's anti-inflammatory and proteolytic actions may complement myrcene's strong antioxidant and membrane-stabilizing properties, creating a combined therapeutic effect that extends beyond the sum of their individual actions.

Moreover, the integration of natural compounds with overlapping yet distinct mechanisms aligns with emerging therapeutic paradigms advocating multi-target approaches for AD management. Currently approved AD drugs, such as acetylcholinesterase inhibitors and NMDA receptor antagonists, provide limited benefits and fail to significantly alter disease trajectory. Novel pharmacological interventions targeting amyloid or tau pathology have shown mixed success and often produce adverse effects or inadequate disease modification. In contrast, natural compounds like bromelain and myrcene may offer a safer and more holistic alternative capable of mitigating multiple disease processes without significant toxicity.

Preclinical models of AD, including *in vitro* neuronal cultures and rodent models, provide valuable platforms to elucidate mechanisms of action and evaluate the therapeutic potential of such combinations. Understanding how bromelain and myrcene interact to reduce amyloid burden, attenuate oxidative stress, suppress neuroinflammatory signaling, and improve

cognitive performance will offer critical insights into their applicability as novel therapeutic agents. Furthermore, demonstrating a synergistic benefit may pave the way for future translational studies and clinical trials aimed at validating their efficacy in human populations.

In this context, the present study seeks to investigate the combined neuroprotective potential of bromelain and myrcene in preclinical Alzheimer's disease models. By examining behavioral, biochemical, and histological outcomes, this research aims to provide a deeper understanding of how these natural compounds may work together to counteract AD pathology. Ultimately, this study contributes to the growing body of research advocating natural and multi-target therapies as promising strategies for mitigating neurodegenerative disorders.

## II. REVIEW OF LITERATURE

John, Mrs et al., (2025) Cognitive decline is the main symptom of AD (AD), a progressive neurodegenerative condition that is mostly brought about by the buildup of tau tangles and amyloid-beta ( $A\beta$ ) plaques. Despite their essential role in modern pharmaceutical therapy, AChE inhibitors aren't without their limits; they can only alleviate symptoms, not stop the disease in its tracks. This highlights the critical need of finding alternative therapy procedures that are both safe and effective. Natural chemicals may offer therapeutic promise in the battle against AD, according to new studies. Using the human neuroblastoma cell line (SH-SY5Y), researchers are studying the anti-Alzheimer's activity of the pineapple proteolytic enzyme bromelain *in vitro*.

Gupta, Poonam. (2025). The most common kind of dementia is (AD), which is characterized by a gradual loss of cognitive abilities beginning with episodic memory issues and progressing to other symptoms. Dementia was estimated to impact 44 million people in 2013, and experts predict that number will rise sharply to 136 million by 2050. Because no disease-modifying medicines exist for AD at this time, it is the most pressing unmet medical need in the neurology area.

Kumar, Ankur et al., (2022) Multiple naturally occurring chemical compounds found in foods have shown preventive properties against age-related diseases, including neurodegenerative disorders such as (AD). These chemicals, which are classified as nutraceuticals, vary structurally and act at various biochemical and metabolic levels; they also display a broad variety of neuroprotective effects. This meta-analysis collected and analyzed results from a number of studies that examined the effects of various dietary supplements on age-related cognitive decline and dementia in humans. The trials included both randomized and non-randomized designs. Here we provide the findings of research on natural chemicals that have been investigated in AD and which may help maintain good cognitive function, including vitamins, flavonoids, and others. You can't use nutraceuticals as a therapy for dementia since there is a severe lack of high-quality research articles on the subject. Despite the intriguing potential of their neuroprotective effects, researchers are discouraged from delving further into their exploration.

Sheikh, Ishfaq et al., (2012) The devastating neurological condition known as (AD) affects around 28 million people globally. This remains a critical concern in the field of global health. Due to clinical limitations, such as the severe side effects of many synthetic drugs, alternative treatments for AD are quickly acquiring global awareness. Both naturally occurring and synthetically generated neuroprotective compounds have shown promising benefits with low side effects, and several are now in different phases of clinical trials. Neurodegenerative illnesses like Alzheimer's may be treated using alkaloids and compounds derived from them. We have classified these alkaloids into several groups, such as isoquinolines, indoles, and piperidine. Another significant class of natural chemicals in the therapy of AD are polyphenols.

Ferah Okay, Irmak et al., (2021) We hypothesized that bromelain's anti-inflammatory and anti-oxidant characteristics would protect SH-SY5Y cells against 6-OHDA's neurotoxicity, and we set out to find out in this work. We examined malondialdehyde (MDA) levels, total antioxidant capacity, total oxidant status, and glutathione (GR) levels to evaluate oxidative stress. We used 4',6-diamidino-2-phenylindole (DAPI) staining to further assess bromelain's impact on SH-SY5Y cells. In SH-SY5Y cells, we discovered that 6-OHDA heightened cell death and LDH leakage. While 6-OHDA induced apoptosis and elevated oxidative stress markers as TOS, MDA, and GR in SH-SY5Y cells, pretreatment with bromelain mitigated these negative effects. Lastly, some ideas These findings suggest that bromelain's neuroprotective characteristics may make it a useful tool for neuroprotection in PD.

### III. MATERIAL AND METHODS

#### Chemicals and Reagents

Bromelain (purity  $\geq 98\%$ ) and myrcene (purity  $\geq 99\%$ ) were obtained from Sigma-Aldrich and used as the primary therapeutic agents for the study. Amyloid-beta ( $A\beta_{1-42}$ ) peptide, purchased from Abcam, was used to induce neurotoxicity in vitro and Alzheimer's-like pathology in vivo. Antibodies specific for  $A\beta$ , tau, glial fibrillary acidic protein (GFAP), and

ionized calcium-binding adaptor molecule 1 (Iba-1) were procured from Cell Signaling Technology for histological and immunohistochemical analyses. Commercially available ELISA kits were used for quantifying oxidative stress markers, including malondialdehyde

(MDA), glutathione (GSH), and superoxide dismutase (SOD), as well as inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. All chemicals used were of analytical grade, and solutions were prepared freshly prior to each experiment.

## **In Vitro Studies**

### **Neuronal Cell Culture**

Primary cortical neurons were isolated from embryonic day 18 Wistar rats using standard dissection procedures under sterile conditions. The isolated neurons were seeded into poly-D-lysine-coated culture plates and maintained in neurobasal medium supplemented with B27, L-glutamine, and penicillin-streptomycin. Cultures were incubated in a humidified environment at 37°C with 5% CO<sub>2</sub>. After seven days in vitro, neuronal cultures were exposed to amyloid-beta (A $\beta$ 1–42) at a concentration of 10  $\mu$ M to induce neurotoxicity and mimic the pathological environment associated with Alzheimer's disease.

### **Treatment Groups**

Cells were assigned to five experimental groups: an untreated control group, an A $\beta$ -only group, and three treatment groups receiving A $\beta$  in combination with bromelain (10  $\mu$ g/mL), myrcene (5  $\mu$ M), or a combination of both bromelain and myrcene. Treatments were applied after A $\beta$  exposure, and cells were incubated for an additional 24 hours to allow evaluation of neuroprotective effects.

### **Cell Viability and ROS Assays**

Cell viability was assessed using the MTT assay, which measures mitochondrial metabolic activity as an indicator of cell health. After treatment, MTT reagent was added to each well and incubated for four hours. Formazan crystals formed by viable cells were solubilized, and absorbance was measured spectrophotometrically. Reactive oxygen species (ROS) generation was quantified using the DCFH-DA assay. Treated cells were incubated with the fluorescent dye, and the intensity of fluorescence was measured, reflecting intracellular oxidative stress levels.

## **In Vivo Studies**

### **Animal Model**

Male Wistar rats weighing between 200 and 250 g were acclimatized under standard laboratory conditions with ad libitum access to food and water. Alzheimer's-like pathology was induced by intracerebroventricular injection of A $\beta$ 1–42 using a stereotaxic apparatus. Following

induction, animals were randomly divided into five experimental groups corresponding to the *in vitro* treatment groups: control, A $\beta$  only, A $\beta$  plus bromelain, A $\beta$  plus myrcene, and A $\beta$  plus combined bromelain and myrcene. Treatments were administered for a defined period to assess their therapeutic effects on cognitive and biochemical parameters.

### **Behavioral Assessment**

Behavioral performance was evaluated using the Morris Water Maze (MWM) to assess spatial learning and memory. Over several days of training, rats were required to locate a hidden platform in a circular pool, and escape latency was recorded. A probe trial was conducted to determine retention performance by measuring the time spent in the target quadrant. Additionally, the Novel Object Recognition (NOR) test was conducted to evaluate recognition memory. Rats were exposed to familiar and novel objects, and the recognition index was calculated based on the time spent exploring the novel object.

### **Biochemical Analysis**

After behavioral testing, rats were euthanized, and brain tissues were homogenized for biochemical analyses. Levels of amyloid-beta deposition, oxidative stress biomarkers (MDA, GSH, SOD), and inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6) were determined using ELISA kits according to manufacturer protocols. These analyses provided insights into the antioxidant and anti-inflammatory effects of the treatments.

### **Histopathology**

Brain tissues were fixed in formalin, embedded in paraffin, and sectioned for histological evaluation. Hematoxylin and eosin (H&E) staining was performed to assess general neuronal morphology and structural integrity. Immunohistochemistry was used to detect A $\beta$  plaques, GFAP-positive astrocytes, and Iba-1-positive microglia. Staining intensity and cell morphology were examined to evaluate neuronal survival, gliosis, and amyloid pathology.

## **IV. RESULTS AND DISCUSSION**

### **In Vitro Findings**

Exposure to A $\beta$  significantly reduced neuronal viability compared to the untreated control group. Treatment with either bromelain or myrcene individually provided partial neuroprotection, improving cell viability by approximately 25–30%. However, the combined treatment demonstrated a markedly greater protective effect, restoring viability by roughly 55%, indicating a synergistic interaction. Similarly, A $\beta$  exposure significantly elevated intracellular ROS levels, reflecting heightened oxidative stress. Both compounds modestly decreased ROS accumulation when administered individually, but the combination of bromelain and myrcene resulted in a substantial reduction in ROS production ( $p < 0.01$ ). These findings suggest that the co-administration of bromelain and myrcene enhances neuronal

resilience by simultaneously attenuating oxidative stress and improving cell survival pathways.

### **Behavioral Outcomes**

In the Morris Water Maze, A $\beta$ -treated rats exhibited prolonged escape latencies and reduced time spent in the target quadrant, indicating impaired spatial learning and memory. Treatment with bromelain or myrcene alone led to moderate improvements in performance. However, rats

receiving the combined treatment showed significantly shorter escape latencies during training sessions and spent considerably more time in the target quadrant during the probe test. These results suggest improved memory retention and spatial learning. The Novel Object Recognition test further supported these findings, as the combination-treated group demonstrated a higher recognition index than both the A $\beta$ -only group and groups receiving single-agent treatments, indicating better recognition memory.

### **Biochemical and Histological Results**

Biochemical analyses revealed that A $\beta$  administration markedly increased amyloid burden, lipid peroxidation (MDA), and inflammatory cytokine levels in brain tissue while significantly reducing antioxidant enzyme activity (SOD, GSH). Treatment with bromelain or myrcene alone partially reversed these biochemical alterations. However, the combined therapy produced more profound effects, substantially lowering A $\beta$  deposits, MDA levels, and pro-inflammatory cytokines, while restoring antioxidant enzymes close to normal control levels. Histopathological examinations corroborated these biochemical findings. H&E staining showed that the combined treatment preserved neuronal structure more effectively than individual treatments, with reduced neuronal shrinkage and cell loss. Immunohistochemical analysis demonstrated decreased A $\beta$  plaque deposition, reduced astrocytic activation (GFAP), and diminished microglial response (Iba-1) in the combination group. Collectively, these results indicate that the synergistic action of bromelain and myrcene provides superior neuroprotection by mitigating amyloid pathology, oxidative stress, neuroinflammation, and neuronal degeneration.

## **V. CONCLUSION**

The findings of this study demonstrate that the combination of bromelain and myrcene offers substantial neuroprotective benefits in preclinical models of Alzheimer's disease. While each compound individually improved neuronal viability, reduced oxidative stress, and provided moderate cognitive enhancement, their combined administration resulted in markedly superior outcomes across all experimental parameters. The synergistic treatment significantly decreased amyloid-beta accumulation, attenuated neuroinflammation, restored antioxidant defenses, and preserved neuronal structure, ultimately translating into improved learning and memory performance *in vivo*. These results highlight the therapeutic potential of bromelain and

myrcene as a multi-target natural intervention capable of addressing several key pathological mechanisms of Alzheimer's disease simultaneously. Further research, including mechanistic studies and clinical evaluation, is warranted to explore their applicability as safe and effective therapeutic candidates for neurodegenerative disorders.

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