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**Review Article**

# Therapeutic Potential Of Hellenia Speciosa In Experimental Amnesia & Neurodegenerative Disorders

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## ABSTRACT

Neurodegenerative disorders (NDDs) and Alzheimer's disease in particular, are a global health threat that involve progressive loss of neurons, cognitive decline and amnesia. No disease-modifying treatments are currently available; those that are available are limited to symptomatic relief, and have side effects. In this review, the therapeutic role of medicinal plants in neuroprotection will be discussed with the emphasis on Hellenia speciosa (syn. A traditionally used rhizomatous herb (Costus speciosus). Hellenia speciosa is rich in phytochemicals including alkaloids, flavonoids, phenolics, steroidal saponins (diosgenin) and terpenoids. Preliminary data show that it possesses strong antioxidant, anti-inflammatory, anticholinesterase, and neuroprotective effects in different experimental models of amnesia, such as scopolamine-, diazepam-, and aluminum chloride-induced models. All these effects occur through several mechanisms, including the activation of Nrf2 and the restoration of SOD, catalase, and GSH, inhibition of acetylcholinesterase, suppression of pro-inflammatory cytokines (TNF-α, IL-1β), and preservation of neuronal architecture in the hippocampus. The plant shows strong anti-amnesic properties and good cognition enhancing activity in the behavioural assays Morris water maze, elevated plus maze and passive avoidance. But standardization, bio-availability improvement and strong clinical validation still remain as challenges. Hellenia speciosa has been identified as a potential multi-target drug against neurodegenerative disorders in this review. Advanced delivery systems, clinical trials and mechanistic elucidation should be the focus of future research to bring the traditional use to evidence-based therapeutics.

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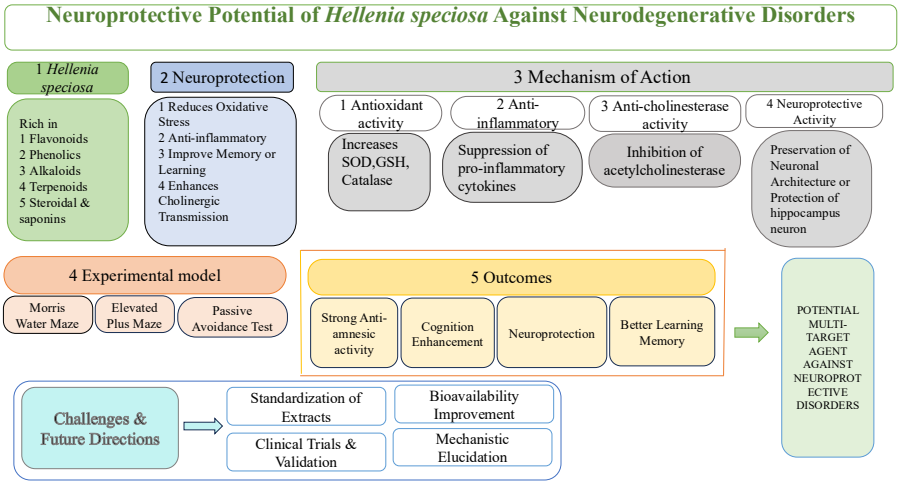


## 1. INTRODUCTION

Neurodegenerative disorders (NDDs) are progressive diseases of the central nervous system that involve the progressive loss and death of neurons. Some examples are Alzheimer's Disease, Parkinson's Disease, Amyotrophic lateral sclerosis and Huntington's Disease. They tend to be linked to abnormal protein aggregations, cell dysfunction, inflammation and neuronal impairment, causing symptoms like memory loss, cognitive decline and motor impairment. These disorders are more common and are having a greater impact on the world's growing aging population (1,2). Amnesia is a memory loss condition characterized by the inability to recall past memories or the loss of memory for the past and the inability to form new memories. It is primarily divided into retrograde amnesia – forgetting of past memories and anterograde amnesia – impaired ability to create new memories. Risk factors include brain injury, stroke, infections, psychological trauma, substance use, and neurodegenerative diseases like Alzheimer's disease. In neurodegenerative illnesses, amnesia frequently is associated with injury to brain regions involved in memory, such as the hippocampus(3,4). The standard treatment for NDDs and memory loss that is associated with them is mostly symptomatic, with little disease-modifying effects and sometimes with side effects. Demand for better, safer, more accessible therapeutic solutions is increasing, especially where multiple pathological mechanisms are involved including oxidative stress, inflammation and protein aggregation (5). Derived from traditional medicine systems, herbal therapeutics are gaining recognition for their multi-target activities and their relatively low toxicity profiles and their antioxidant properties. In contrast to synthetic drugs, a number of plant-derived compounds exhibit neuro- and anti-inflammatory, and cognitive-enhancing properties (6,7). Medicinal plants have been utilized for centuries in different traditional healing methods such as Ayurveda, Traditional Chinese Medicine and in CNS related diseases. Bacopa monnieri, Withania somnifera, Curcuma longa, Centella asiatica, and Ginkgo biloba exhibit neuroprotective effects via several mechanisms, such as cholinesterase inhibition, antioxidant activity, modulation of neurotransmitters, and reduction of neuroinflammation. (8,9). These plants are rich in bioactive secondary metabolites (alkaloids, polyphenols, terpenoids, saponins) which can cross the blood-brain barrier and produce

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Although the pre-clinical findings are promising, a thorough summary of available evidence pertaining to the relationship between medicinal plants and amnesia and overall neurodegenerative effects is necessary. The current treatments are only moderately effective and therefore the need to look into ethnopharmacological knowledge for novel leads. This review is intended to draw a connection between traditional use and scientific validation. This review aims at an evaluation of the therapeutic perspective of medicinal plants for the management of neurodegenerative disorders with emphasis on amnesia. Specific goals are: 1) To summarize the pathophysiological mechanisms of NDDs and amnesia; 2) To document important medicinal plants containing bioactive compounds; 3) To review neuroprotective mechanisms; 4) To discuss the challenges and future directions for the usage of herbs to treat disorders on the CNS.

## 2. Neurodegenerative Disorders

### 2.1 Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder, and the most important cause of dementia, representing 60-80% of cases. It's a condition of gradual cognitive deterioration involving the loss of memory, thinking and behavior (10).

Pathologically, AD is characterized by the presence of extracellular deposits of amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles formed by hyperphosphorylated tau protein. These result in loss of synapses and brain atrophy and neuronal death (particularly in the hippocampus and cortex). The amyloid cascade hypothesis suggests that Aβ deposition leads to the subsequent development of tau pathology, inflammation, and oxidative stress. (11,12) Cognitively, the disease of AD goes from mild cognitive impairment (MCI) to severe dementia. Early signs are short term memory loss, and later signs are language impairments, disorientation, and loss of activities of daily living. Major contributors include genetic factors (such as mutations in the genes APP, PSEN1 and PSEN2 for early onset Alzheimer's disease and the APOE ε4 allele for late onset Alzheimer's disease), and ageing. (13)

### 2.2 Parkinson's Disease

Parkinson's disease (PD) is the second most prevalent neurodegenerative condition, and is mainly a motor disorder. Is characterized by the death of dopaminergic neurons in the substantia nigra pars compacta and the accumulation of α-synuclein into Lewy bodies. (14,15) The major motor symptoms are bradykinesia, resting tremor, rigidity and postural instability. Olfactory dysfunction, sleep disturbances, autonomic dysfunction and cognitive impairment are non-motor symptoms that frequently precede motor signs. Pathogenesis includes misfolding and propagation (prion-like spread) of α-synuclein, mitochondrial dysfunction, oxidative stress and neuroinflammation. (15,16) Genetic (SNCA, LRRK2, PARKIN) mutations only cause 5-10% and most cases are sporadic, meaning they are caused by age and environmental factors such as pesticides. (15)

### 2.3 Dementia

Dementia is not a disease; it is a syndrome of acquired cognitive (mental) decline that interferes with functioning. The most prevalent subtypes are Alzheimer's (60-70%), vascular dementia, dementia with Lewy bodies, frontotemporal dementia and mixed pathologies. (17,18) It is characterized by the progressive deterioration of

memory, executive function, language skills, visuospatial deficits, and behaviour. Dementia is associated with protein pathies and neuronal loss in the context of neurodegenerative disease. There is much overlap, such as Parkinson's disease dementia (PWD) and dementia with Lewy bodies (DLB) share α-synuclein pathology. (19) The prevalence is increasing worldwide, influenced by the ageing population and is causing a substantial socio-economic burden. Diagnosis is dependent on clinical evaluation, neuro-imaging and biomarkers.(20)

### 2.4 Cognitive Dysfunction

Neurodegenerative disorders can manifest as mild cognitive impairment (MCI) or even dementia. Episodic memory is most affected early in AD, related to the involvement of the hippocampus. Initial symptoms in PD are mostly characterized by executive dysfunction, attention deficits, and visuospatial impairments.(19) Disruptive cholinergic, dopaminergic and other neurotransmitter systems, disrupted synapses, and network disconnection may all lead to a cognitive impairment. MCI is often a pre-dementia state and the annual conversion rate to dementia is 10–15% in amnestic types.(21) Risk stratification and monitoring of progression is achieved by neuropsychological testing, biomarkers (CSF Aβ/tau, amyloid-PET) and imaging.

The causes and risk factors are described in section

2.5. Neurodegenerative disorders are polyetiological. Non-modifiable risk factors are strongest with age, genetic predisposition (APOE ε4 for AD and LRRK2/GBA for PD), family history and sex (higher risk of PD in men and higher risk of AD in women). (22,23),

Modifiable risk factors include vascular factors (hypertension, diabetes, obesity, hyperlipidemia), lifestyle (smoking, physical inactivity, poor diet, alcohol), head trauma, hearing loss, depression, and environmental exposures (pesticides, heavy metals, air pollution).(24,25) Protective factors might include higher education, cognitive reserve, Mediterranean diet, exercise and social engagement. Preventable modifiable risk factors contribute to up to 40% of dementia cases. (26)

### 2.6 Pathophysiology of Neurodegeneration

The mechanisms underlying neurodegeneration include gradual neuronal damage, as well as common pathways such as pathological protein aggregation, synaptic dysfunction, network disorders, impaired proteostasis, cytoskeletal changes, energy imbalance, defects in DNA/RNA, inflammatory processes, and neuronal cell death, which can be summarized by the eight hallmarks of the disease.(2) Protein aggregation (Aβ/tau in AD; α-synuclein in PD) wreaks havoc on proteostasis through impairments of the ubiquitin-proteasome system and autophagy-lysosomal systems. There is a failure of energy production and oxidative stress due to mitochondrial dysfunction. Activated microglia and astrocytes increase inflammation, which leads to worsening damage. (27) Combination of these processes results in neuronal populations' selective vulnerability: e.g., hippocampal neurons in AD, nigral dopaminergic neurons in PD. The misfolded proteins are capable of propagating in a manner similar to a prion, which helps to spread the disease. Progression is then further modulated by epigenetic changes and gene-environment interactions. (28)

## 1. Experimental Models of Amnesia

### 3.1 Definition and Classification

The experimental amnesia is related to the memory impairment that can occur in animals due

to pharmacological or chemical manipulation, which mimics certain aspects of human memory impairment, especially in the context of neurodegenerative diseases such as Alzheimer's disease (AD). It is useful for the elucidation of memory mechanisms and screening potential therapeutic agents. (29,30)

There are two categories of amnesia: loss of pre-existing memories (retrograde amnesia) and loss of the ability to create new memories (anterograde amnesia). This anterograde amnesia is the norm in experiments. Others are based on other categories, such as transient and persistent; global and selective; or by the specific agent that causes the action (cholinergic, GABAergic, or neurotoxic). (31,32)

These models have been developed to mimic cognitive impairments by disrupting specific neurotransmitter systems, oxidative stress and/or neuroinflammation, while enabling a controlled investigation of memory processes.

### 3.2. Mechanism of memory impairment

It is believed that memory loss in experimental models is due to disruption of the acquisition, consolidation and retrieval of memory. Many of the models involve problems in the cholinergic system, which is essential for encoding and consolidation in the hippocampus and cortex.(33,34)|

Other factors include oxidative stress, neuroinflammation, disrupted proteostasis (e.g., impaired long-term potentiation – LTP), and altered synaptic plasticity. Agents can upregulate acetylcholinesterase (AChE) activity, downregulate choline acetyltransferase (ChAT), upregulate malondialdehyde (MDA) or decrease the BDNF/ERK/CREB pathways. (34,35) The memory consolidation of hippocampal circuits is impaired by excess GABAergic overactivation (e.g., benzodiazepines).

### 3.3 Aluminum Chloride ($AlCl_3$)-Induced Amnesia

$AlCl_3$ is a neurotoxin that is commonly used to cause AD-like pathology and amnesia in rodents. Long-term treatment (usually 70–100 mg/kg, i.p. or oral, for 4–8 weeks) causes cognitive impairment, oxidative damage, cholinergic abnormalities and histopathological alterations similar to those found in AD. (36,37)

These include mitochondria dysfunction, lipid peroxidation, tau hyperphosphorylation, Aβ accumulation and neuroinflammation. $AlCl_3$ enters the brain and localizes in the cortex and hippocampus, where it leads to deficits in spatial and recognition memory as measured in the Morris water maze (MWM) and elevated plus maze (EPM).(36,38)

This model has limited utility for the study of systemic toxicity and variability in dosing but is useful for modelling chronic, progressive neurodegeneration.

### 3.4 Scopolamine-Induced Amnesia

Scopolamine, a non-selective muscarinic receptor antagonist, is the most common pharmacological agent for inducing acute amnesia. When administered to rodents at 0.3–3 mg/kg of i.p. injection, it reliably causes learning and memory deficits which reflect the cholinergic hypothesis of AD. (39,40)

It is an inhibitor of central cholinergic transmission resulting in impaired encoding and consolidation. Other effects include augmentation of oxidative stress, neuroinflammation, decreased BDNF expression and disruption of LTP in the hippocampus. Passive avoidance, Y-maze and

object recognition and MWM tasks all show behavioural impairments. (34,41)

It is a reversible model that has been widely used for screening nootropic and neuroprotective drugs, but it primarily reflects acute cholinergic dysfunction and not complete neurodegenerative disease.

### 3.5 Diazepam-Induced Amnesia

Diazepam is a benzodiazepine that causes amnesia mainly by increasing the activity of GABA_A receptors, which in turn leads to increased inhibitory activity in memory-relevant areas of the brain. In mice/rats: Doses of 1 mg/kg i.p. cause anterograde amnesia (impairment in memory consolidation). (29,42)

It has an inhibitory effect on performance in passive avoidance, EPM and MWM tests with only minor motor effects at lower doses. Mechanisms include modulation of α1/α5 GABA_A subunits, disruption of hippocampal theta rhythms and interference with synaptic plasticity. (43,44)

The model is applicable to examining the role of GABAergic signalling in memory and could be used to test agents that have an effect on the cognitive side effects of benzodiazepines.

The production of animal models for use in memory studies.

### 3.6 Production of Animal Models for Memory Studies.

The primary species is rodents (mice and rats), also because of their well-characterised behaviour and neuroanatomy. The common strains include Swiss albino mice, Wistars and Sprague-Dawley rats. (29)

Behavioral paradigms include:

Elevated Plus Maze (EPM): a spatial memory test that assesses transfer latency.

Cognitive Maps: Spatial learning and reference memory. Learning and Memory: Spatial learning and reference memory.

Passive Avoidance: Assesses fear associated memory.

Y-Maze/Object Recognition: Tests working/recognition memory.(45) Comprehensive evaluation involves the use of biochemical (AChE, oxidative markers) and electrophysiological (LTP) and histological assessment. There are limitations such as species differences and difficulties of complete recapitulation of human multifactorial neurodegeneration. (30)

## 4 Role of Oxidative Stress and Neuroinflammation

The association between oxidative stress and neuroinflammation. Link between oxidative stress and neuroinflammation.

### 4.1 Oxidative Stress in Brain Disorders

Oxidative stress (OS) is defined as an imbalance between the generation of reactive oxygen species (ROS), and the antioxidant defence systems in the brain resulting in damage. In the brain, the high requirement for oxygen, the high concentration of polyunsaturated fatty acids and low antioxidants make it more vulnerable. (46,47)

In neurodegenerative disorders, like Alzheimer's disease (AD) and Parkinson's disease (PD), OS plays a role in neuronal death, protein misfolding, synaptic dysfunction and progression of cognitive decline. It serves as a hub that links amyloid-β (Aβ) accumulation, tau hyperphosphorylation,

mitochondrial dysfunction and neuroinflammation. Early signs of OS can be seen even in the initial stages of dementia, such as mild cognitive impairment (MCI) and can occur before noticeable neurons loss. (47,48)

### 4.2 Free Radical Generation

In the brain, free radicals are formed via several mechanisms, mostly ROS including: superoxide ($O_2^-$), hydroxyl radical ($OH^-$), and hydrogen peroxide ($H_2O_2$). Mitochondrial electron transport chain (ETC) is a significant endogen source especially complexes I and III where leakage of electrons results in the formation of superoxide. (49)

Other important ones are NADPH oxidase (NOX) enzymes, which generate superoxide upon activation of the neuroinflammation, particularly NOX2 in microglia, as well as enzymatic systems such as monoamine oxidase and xanthine oxidase. Dysregulated calcium homeostasis, metal ions (e.g., $Fe^{2+}$, $Cu^{2+}$ via Fenton reaction) and Aβ aggregates accentuate the generation of ROS in pathological states. 10.1523/JNEUROSCI.4468-06.2007, (50)

This vicious cycle is compounded by mitochondrial dysfunction, which reduces production of ATP and increases leakage of ROS, thus creating a vicious cycle in neurodegeneration. (51)

### 4.3 Lipid Peroxidation

The brain is a crucial target tissue for oxidative damage, and ROS cause lipid peroxidation in the neuronal membrane, resulting in the formation of toxic aldehydes like malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE). (52,53)

One of the most reactive products is 4-HNE that becomes part of the adduct with proteins, DNA and phospholipids and causes disruption of enzyme function, disruption of signalling pathways, and protein aggregation, such as tau and α-synuclein. Brain tissue, CSF and plasma from AD and PD patients consistently have higher levels of MDA and 4-HNE, with increased levels as disease severity increases. (54,55)

This process causes the loss of integrity, changes the fluidity and leads to further inflammation and apoptosis, which exacerbates the process of neurodegeneration.

### 4.4 Neuroinflammation and Cytokines

Neuroinflammation involves chronic activation of microglia and astrocytes which contributes to the continuous production of pro-inflammatory cytokines (such as TNF-α, IL-1β, IL-6), chemokines and ROS. Acute inflammation protects neurons; chronic inflammation causes neuronal damage in neurodegenerative diseases.(56,57)

The activated microglia are associated with Aβ plaques and Lewy bodies and produce cytokines that further promote OS and protein pathology in AD and PD. Patient peripheral blood and brains contain elevated levels of the cytokines: TNF-α, IL-1β and IL-6, which are associated with cognitive decline. Anti-inflammatory cytokines such as IL-10 may have a neuroprotective effect, but this balance is frequently tipped towards pro-inflammatory predominance.(58,59)

OS and neuroinflammation are a vicious cycle: ROS activates NF-κB signalling leading to cytokine production, and cytokines induce additional ROS production through NOX and mitochondrial pathways. (60)

### 4.5 Cholinergic Dysfunction

Cholinergic dysfunction, which results in a decrease in acetylcholine (ACh) level and loss of cholinergic neurons in the basal forebrain, is a hallmark of AD and is involved in memory loss. Dysfunctions in cholinergic signalling are caused by oxidative stress and neuroinflammation.(48,61)

ROS and cytokines stimulate acetylcholinesterase (AChE) to decrease the availability of ACh, and to damage cholinergic projections and receptors. In contrast, cholinergic signalling through α7 nicotinic receptors in microglia and astrocytes has anti-inflammatory and antioxidant effects through modulation of Nrf2 pathways and reduction of cytokine production. (62)

This bi-directional relationship drives further exacerbation of OS and inflammation, further cholinergic neuron loss, creating a vicious cycle in cognitive decline.

## 5 Medicinal Plants in Neuroprotection

### 5.1 Herbal drugs used in CNS disorders

Medicinal plants are used in different systems of medicine (Ayurveda, Traditional Chinese Medicine etc.) for central nervous system (CNS) disorders such as neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD) and dementia. Bacopa monnieri, Withania somnifera, Curcuma longa, Centella asiatica, Ginkgo biloba and Panax ginseng are some of the key herbs known to possess a neuroprotective effect.(63,64)

Brahmi (Bacopa monnieri) is a famous Nootropic herb. It has bacosides that have been shown to help enhance memory, decrease the aggregation of amyloid-β, and anti-inflammatory and antioxidant activities. Withania somnifera (Ashwagandha) has adaptogenic, antioxidant, and neurotrophic properties, which help to protect nerve cells from damage caused by stress and maintain cognitive function. (63,65)

Turmeric (Curcuma longa) and its active curcuminoid demonstrate robust anti-amyloid, anti-inflammatory and antioxidant properties, affecting several pathways in AD and PD. Ginkgo biloba standardized extracts (such as EGb 761) help to increase cerebral blood flow, reduce oxidative stress, and inhibit platelet-activating factor. Other candidates for further development are Crocus sativus (saffron), which is used for cognitive function and Centella asiatica for neurite outgrowth and memory. (64,66,67)

The herbs are traditionally utilized as extracts or polyherbal mixes aimed at several pathological features of neurodegeneration.

### 5.2 The benefits of herbal medicine.

Herbal medicines have a number of advantages over conventional synthetic drugs for CNS disorders. They usually operate at multiple targets, targeting oxidative stress, neuroinflammation, protein aggregation, and cholinergic dysfunction all at once, a beneficial effect in view of the complex, multifactorial character of the neurodegeneration. (68,69)

Herbal remedies tend to be safer than many pharmaceutical remedies with less serious side effects, thus, they can be used for a long period of time in ageing population. They are typically easier to obtain and more affordable, especially in resource-poor areas, and are compatible with the cultural practices of traditional medicine. (70,71)

A large number of phytochemicals have pleiotropic effects including antioxidant, anti-apoptotic properties, as well as stimulating expression of tropho- and neurotrophic factors, such as BDNF and promoting neuroplasticity.

Herbal drugs might also facilitate bioavailability in standardized or formulated extracts, and demonstrate synergistic effects in polyherbal blends. (72)

However, various factors such as variability of extract quality, standardization requirement, and the possibility of herb-drug interactions are challenges to be addressed.

### 5.3 Phytochemicals with Neuroprotective Activity

The bioactive secondary metabolites responsible for the neuroprotective effect of medicinal plants are called phytochemicals. The major classes are polyphenols (flavonoids, curcuminoids, stilbenes), alkaloids, terpenoids, saponins and carotenoids. (73,74)

Curcumin (from Curcuma longa) has been found to inhibit aggregation of Aβ, decrease hyperphosphorylation of tau, to suppress inflammation via NF-κB pathway and to activate Nrf2 antioxidant pathway. Bacosides of Bacopa monnieri, have a protective effect on oxidation, inhibit cholinesterase and promote increased synaptic plasticity. The Withania somnifera modulates stress responses, promotes neurite outgrowth and exerts anti-inflammatory effects, all through the action of withanolides (such as withaferin A). (63,74)

Resveratrol (stilbene) is a molecule that activates SIRT1, enhances mitochondria function and decreases oxidative stress. Quercetin and catechins are flavonoids that have free radical scavenging activity along with the ability to regulate signalling pathways, such as PI3K/Akt and MAPK. Anti-apoptotic and vascular protective effects are provided by terpenoids like ginkgolides and ginsenosides. (5,75)

Mechanisms of action of these compounds are frequently similar, including the ability to scavenne ROS and lipid peroxidation, suppress pro-inflammatory cytokines (such as TNF-α and IL-1β), prevent protein misfolding and provide neurotrophic support. (73)

## 6 Plant Profile of Hellenia speciosa

### 6.1 Taxonomy

Hellenia speciosa (J.Koenig) S.R.Dutta is a rhizomatous perennial herb of Costaceae family, order Zingiberales and class Monocots. It was once considered under Costus, Costus speciosus (J)The species (Koenig) Sm., is now classified under Hellenia. (76)

Kingdom: Plantae

Order: Magnoliids, Asterales, Astercae, Asteraceae

Order: Zingiberales

Family: Costaceae

Genus: Hellenia

Species: Hellenia speciosa (J.Flowers drooping, whitish, or tinged with pink.Fruit: flowers drooping, whitish or tinged with pink (basionym: Banksea speciosa J.Koenig).Koenig, 1783).(77)

The genus Hellenia is characterized by features like the branched axillary shoots with leafy branches, woody bracts and the open, showy labellum. (78)

### 6.2 Synonyms and vernacular Names

Hellenia speciosa has many synonyms because taxonomic changes have occurred over the years: Costus speciosus (J.Koenig) Sm., Cheilocostus speciosus (J.Larson) J.D.Specht, Banksea speciosa

J.Koenig, Costus sericeus Blume, Costus hirsutus Blume and some varietal names.(76,79)

Vernacular Names: Ginger is commonly sold in the market under the names Crepe ginger, Spiral ginger, Wild ginger and Malay ginger.

Hindi: Kebu, Keu, Kevu

Sanskrit: Pushkaramula, Jatala

Malayalam: Channakoova, Narunjanna

Tamil: Kostam

Manipuri: Khongban takhelei

Others: Aanakoova (regional), Ting (Pohnpean). (77)

**6.3 Geographical Distribution**

Hellenia speciosa grows in tropical and subtropical Asia, from India and southern China, through Indochina, Malesia, New Guinea, the Solomon Islands, and northeast Queensland Australia). It is especially abundant in the Greater Sunda Islands of Indonesia.(76)

It grows in wet tropical biomes, typically in moist forests, river bank, and shaded, humid environments. It has become naturalised in some areas, such as parts of the West Indies, Hawaii, Mauritius, Réunion, Fiji and Central America. It is widely distributed in India in the Eastern Himalayas, Western Ghats and in the North-East states.(80)

**6.4 Morphological Characteristics**

Hellenia speciosa is an erect, occasionally branched, herbaceous perennial growing up to 2–3 meters tall from a stout, creeping, tuberous rhizome. Stem is spirally twisted at the base; bases of leaves spirally sheath the stem.(80,81)

Flowers: Spikelets borne in a terminal panicle, 1.5-5 cm long, bearing numerous small, lateral spirally arranged flowers.Fruits: Spikelets, terminal, 1.5-5 cm long, with many small flowers, lateral and spirally arranged. Upper surface is glabrous, often lower surface is pubescent or silky.(81)

Inflorescence: Terminal, spike-like with crowded, overlapping woody bracts. Flowers are large, white or cream with a prominent crinkled (crepe-like) labellum which is yellow at the centre. The flower has only one stamen and inferior ovary. Fruits are in capsules containing many black seeds.(82)

The main medicinal part of the rhizome is aromatic, fleshy and tuberous. The plant is a rhizomatous geophyte adapted to humid tropical environment.

**6.5 Traditional and Ethnomedicinal uses**

Hellenia speciosa is a plant of great medicinal value especially in the Ayurveda system and roots of the plant are used as bitter, astringent, acrid, cooling, purgative, anthelmintic, depurative and febrifuge medicine.(76,83)

According to Ayurvedic medicine, the rhizome is used for making medications for inflammatory conditions, diabetes, anemia, intestinal worms, skin diseases, asthma and bronchitis. It is also used as an expectorant and tonic. (77),

In other ethnomedicinal practices:

It is used in traditional Malay medicine for high fever, small pox, purgative and in ritual against evil spirits.

Mizo traditional medicine employs it for kidney and urinary problems.In various Indian folk systems it is used for digestive disorders, respiratory ailments and metabolic disorders.

(84,85) The plant is also recorded in historical documents, such as the Kama Sutra, for cosmetic use. Traditional applications of the plant include use in medicine for diabetes, inflammation, anti-oxidative and anti-microbial activities have garnered the drug interest of modern pharmacology. (77)

## 7 Phytochemical Constituents of Hellenia speciosa

### 7.1 Alkaloids

The Hellenia speciosa (syn. is a source of alkaloids, nitrogen containing secondary metabolites, which were reported in different extracts of the plant. Costus speciosus). The presence of these has been consistently confirmed by qualitative phytochemical screening of plants, rhizomes and whole plant extracts.(86,87)The Ethanolic and Methanolic rhizome extracts have been subjected to GC-MS analysis, revealing the presence of several alkaloids as bioactive compounds. These are responsible for the antimicrobial, antioxidant and neuroprotective properties of the plant. Common alkaloids found are pharmacological active derivatives such as cholinesterase inhibitors and anti-inflammatory. (85)

While the level of alkaloids is comparatively low compared to steroids and terpenoids, their presence further justifies the traditional use of the plant in CNS-related diseases.

### 7.2 Flavonoids

One of the most prominent phenolic secondary metabolites found in Hellenia speciosa is the flavonoids. Plentiful reported in leaf, rhizome and aerial part extract. The ethanolic and methanolic extracts of the plants revealed a high amount of flavonoids based on qualitative tests and quantitative estimations.(88)

Some of the major flavonoids are responsible for the antioxidant, anti-inflammatory and neuroprotective effects of the plant, which are possible to achieve by free radical scavenging and metal chelating effects. Flavonoids have been found in the different solvent extracts and the concentration is generally higher in the leaves.

These compounds have been associated with the plant's effects on oxidative stress and neuroinflammation, which could be of interest for neurodegenerative studies.

### 7.3 Phenolic Compounds

Hellenia speciosa is rich in phenolic compounds, such as simple phenolics and tannins. The levels of phenolics, which contribute to the significant antioxidant activity, were obtained from phytochemical screening of various parts (rhizome, leaves and stems). (86,89)

Other phenolic derivatives have been identified via GC-MS profiling, in addition to the tocopherols and related compounds. The phenolic compounds demonstrate high free radical scavenging, reducing and metal-chelating properties which match the traditional utilization of this plant in medicine for inflammation and diabetes. (85)

The higher TPC of the polar extracts is indicative of their potential for reducing oxidative stress related to CNS disorders.

### 7.4 Glycosides

The major bioactive constituents of Hellenia speciosa are glycosides, especially the steroidal and cardiac glycosides. The rhizome contains particularly the steroidal saponins and their

glycosides including diosgenin glycosides (dioscin and prosapogenins).(90)

The glycosides are confirmed in the phytochemical analysis of various solvent extracts. These compounds are used to make steroidal medications and help explain the hypoglycemic, anti-inflammatory and neuroprotective properties of this plant. Other significant glycosides found in tubers and roots are sitosterol-β-D-glucoside. (91)

The very important traditional therapeutic uses of the plant are related to the glycosidic fraction.

### 7.5 Terpenoids

One of the major classes of bioactive compounds in Hellenia speciosa is the terpenoids, which include mono-, sesqui-, and diterpenes. They are also prevalent with the use of steroids.(77)

The GC-MS analysis of the extracts of the rhizome has identified a wide variety of terpenoids, volatile oils and other related compounds. These are responsible for the aromatic qualities of the rhizome and have various pharmacological properties such as antioxidant, antimicrobial and anti-inflammatory.(85,92)

Terpenoids help the plant's neuroprotective properties by regulating oxidative stress and inflammatory pathways.

### 7.6 Other Bioactive Constituents

Besides the main classes, Hellenia speciosa has steroids (e.g., diosgenin, 5-α-stigmast-9(11)-en-3-β-ol), fatty acids, tocopherols, proteins, amino acids, vitamins and carbohydrates. It is believed that steroids, particularly steroidal saponins are the most abundant and pharmacologically important constituents.(77,90)

Quinones, coumarins, tannins and other volatile oils are also reported. All these add to the pleiotropic pharmacological profile of the plant such as anti-diabetic, anti-microbial and CNS-protective properties.,(85)

## 8 Pharmacological Activities of Hellenia speciosa.

### 8.1 Antioxidant Activity

Hellenia speciosa (syn. Costus speciosus has significant antioxidant activity, mainly due to its high levels of phenolic compounds, flavonoids and steroidal saponins. Rhizome and leaves of A. sphaerocarpa have been shown to exhibit superior free radical scavenging activity through various assays including DPPH, hydroxyl radical and nitric oxide scavenging assays.(81,93)

The methanolic rhizome extract frequently possesses a better activity than the standards used, such as ascorbic acid and quercetin, because of its higher total phenolic and flavonoid content. This antioxidant activity assists in reducing oxidative stress, which plays a crucial role in neurodegenerative diseases. In vitro tests demonstrate dose dependent metal-chelating and reducing power.(81,94)

### 8.2 Anti-inflammatory Activity

The plant exhibits significant anti-inflammatory activity as shown by its ability to inhibit protein denaturation, to decrease paw oedema in K\C models and to suppress pro-inflammatory mediators. These effects are mainly attributed to the sesquiterpenes found in the rhizome, including costunolide.(95,96)

Both ethanolic and methanolic extracts have an ability to suppress the COX and LOX pathways and lower levels of TNF-α and IL-6. The anti-inflammatory properties are confirmed by in vivo

experiments, which demonstrate considerable inhibition of granuloma formation and paw swelling(91,97)

These activities are associated with NF-κB signalling downregulation and modulation of the oxidative stress pathways.

### 8.3 Neuroprotective Activity

The neuroprotective activity of Hellenia speciosa is of good interest, and is mainly attributed to its antioxidant and anti-inflammatory properties. ROS scavenging action of the plant prevents the oxidative damage of the neuronal cells. Methanolic extracts have been shown to have anticholinesterase activity that helps in maintaining acetylcholine levels in brain.(77,94)Cold immobilization stress models have been used to study the effect of extracts on brain neurotransmitters and monoamine oxidase activity, which could be beneficial in stress-related neurodegeneration. Diosgenin and other steroidal compounds have been found to have neurotrophic and membrane-stabilizing properties.(98)

### 8.4 Anti-amnesic Activity

Some promising data exists for Hellenia speciosa's anti-amnesic properties. It has anticholinesterase and antioxidant properties indicating potential therapeutic use in models of scopolamine- and aluminum-induced amnesia. The extracts of rhizome may have beneficial effects on memory retention and retrieval, because of antioxidant activity and increased cholinergic transmission.The rhizome extracts may have beneficial effects on memory retention and retrieval by reducing oxidative stress and enhancing cholinergic transmission. (94)

Protective effects against amyloid-induced toxicity and synaptic dysfunction are likely mediated by phytocompounds, such as bacoside-like saponins and flavonoids; some dedicated amnesia reversal studies are appearing.

### 8.5 Cognitive Enhancing Activity

The multi-target activity of Hellenia speciosa on the oxidative stress, inflammation, and cholinergic system makes it a valuable nootropic agent. Extracts enhance behavior models' learning and memory parameters via raising BDNF-like neurotrophic support and boosting synaptic plasticity(77).

The traditional brain tonic benefits correspond to the modern understanding of enhancing cognitive function via neurotransmitter systems and lowering neuroinflammation. Polyherbal formulas with the plant demonstrate synergistic cognitive effects.(99)

### 8.6 Other reported pharmacological effects are as follows:

In addition to the above, Hellenia speciosa has been noted to have anti-diabetic (hypoglycemic through diosgenin) antimicrobial, antifungal, anticancer, hepatoprotective, and nephroprotective properties. It also exhibits pain relieving, anti-pyretic, and anti-hyperlipidemic activity.(81,94)

Recent studies also show that it has antiviral activity against adenoviruses and other infectious diseases. In addition its estrogenic, anthelmintic and larvicidal properties broaden its therapeutic range.(85)

## 9 Mechanism of neuroprotective action

### 9.1 Reduction of oxidative Stress

Hellenia speciosa (syn. The antioxidant activity is the main mechanism of the neuroprotective effect of Costus speciosus. The plant's high

concentration of phenolics and flavonoids has an effective scavenging effect on free radicals like DPPH, hydroxyl and superoxide radicals and also enhances the endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).(100),(81)

Rhizome extracts in methanol and ethanol reduce the level of lipid peroxidation (mdha) and restore the reduced glutathione (GSH) level in oxidative stress models. Bioactive compounds, such as costunolide, eremanthin and diosgenin, have been shown to inhibit generation of mitochondrial ROS and to activate the Nrf2 pathway, increasing the expression of cytoprotective genes like heme oxygenase-1 (HO-1). This decrease in oxidative damage helps prevent apoptosis of hippocampal and cortical neurons, which is important in Alzheimer's and other neurodegenerative conditions.(101,102)

### 9.2 Acetylcholinesterase Inhibition

Acetylcholinesterase (AChE) inhibition is one of the main actions of Hellenia speciosa that made it useful for treating the neuroprotective and anti-amnesic effects. Alkaloids found in the rhizome show significant anticholinesterase activity, thus raising the level of acetylcholine in the synaptic cleft and improving the cholinergic neurotransmission.(81,94)

Various solvent extracts have been shown to exhibit a dose-dependent inhibition of AChE in vitro, which is similar to the effect of known inhibitors. Such a mechanism may be especially important in reversing cholinergic deficits present in experimental models of amnesia (such as induced by scopolamine) and in Alzheimer's disease. The plant helps maintain levels of acetylcholine, which helps to consolidate memory and stimulate cognitive function, and limits excitotoxic stress.(94)

### 9.3 Anti-inflammatory Mechanism

Hellenia speciosa has multiple mechanisms of action in modulating neuroinflammation. The NF-κB nuclear translocation inhibitory activity and suppression of pro inflammatory cytokines (TNF-α, IL-1β, IL-6) and enzymes (iNOS, COX-2) by sesquiterpene lactones (costunolide and dehydrocostus lactone) have been demonstrated. (96,99)

The rhizome phytocompounds are found to be TLR-4 signalling modulators, which decrease the activation of microglial cells and the release of inflammatory mediators. Diosgenin, a significant steroidal saponin, also decreases the inflammation induced by LPS in microglial models. This anti-inflammatory effect reduces the vicious cycle of oxidative stress and chronic neuroinflammation, thus preventing neurons from being damaged by cytokines.(95)

### 9.4 Neurotransmitter Modulation

The plant has an effect on the central neurotransmitter systems, which gives it a neuroprotective profile. Under stressful conditions, alcohol extracts from the rhizomes of Hellenia speciosa influence levels of norepinephrine, dopamine and serotonin in the brain. They also are inhibitors of monoamine oxidases (MAO), thereby preventing the overproduction of monoamines.(98)

The modulation of catecholamines and indoleamines is balanced, which aids in reducing the neuronal damage caused by stress and may contribute to mood regulation, along with cognitive function. Such effects are beneficial for treating neuropsychiatric symptoms of neurodegenerative diseases.

### 9.5 Protection Against Neuronal Damage

Hellenia speciosa directly protects the neurons by stabilizing their membrane, inhibiting apoptosis and inducing pathways of cell survival. Diosgenin and related steroidal compounds modulate neuronal signaling, decrease excitotoxicity and increase neurotrophic support.

Ethanolic leaf extract has been found to reduce the neuronal loss, maintain the architecture of the hippocampus, and improve the behavioral outcomes in the aluminum chloride-induced Alzheimer's models. All the antioxidant, anti-inflammatory and anticholinesterase properties would work together to prevent protein aggregation, synaptic loss, and mitochondrial dysfunction, providing multi-target protection against progressive neurodegeneration.(103) DOI: 10.35629/4494-090411981202

## 10 Behavioral Assessment in Experimental Animals

### 10.1 Morris Water Maze Test

The Morris water maze (MWM) is one of the most popular behavioral tasks for examining spatial learning and reference memory in rodents, including amnesia and models of neurodegenerative diseases. It is developed by Richard Morris, and it uses distal visual cues and the aversion of rodents to water in order to be developed.(104)

Procedure: Animals are introduced into an opaque pool of water (22-25°C) that is 120-150 cm in diameter with an invisible escape platform 1-2 cm below the surface of the water. Training consists of 4–6 trials per day for 4–5 days and the escape latency (time to reach the platform) is measured. The last day of the probe trial (with platform removed) evaluates memory retention through the time spent in the target quadrant and platform crossings.(105,106)

Impaired animals display an increase in escape latency and decrease in the preference for the target quadrant in Alzheimer's and amnesia models (such as scopolamine or $AlCl_3$). This test is very sensitive to the dysfunction of the hippocampus; it is often applied to determine the effectiveness of treating a neuroprotective agent(107,108)

### 10.2 Elevated Plus Maze

The Elevated Plus Maze (EPM) is mainly an anxiety assessment test, but is also modified to assess spatial memory and learning, particularly transfer latency. It is in the form of a plus, consisting of two open arms and two enclosed arms (50 cm × 10 cm) raised 50–70 cm above the ground(109,110)

The procedure: An animal is positioned at the center facing an open arm. Time spent and entries into open vs. closed arms (for anxiety) and transfer latency, (time to move from open to enclosed arm) are measured. Transfer time for memory versions is recorded day 1 (acquisition) and retested after 24 hours (retention). The lower the transfer latency on retention day, the better the memory.(111)

This test can be useful in the study of amnesia as it reveals cognitive defects caused by benzodiazepines, scopolamine or aging. Easy, quick and sensitive to nootropic interventions.(112)

### 10.3 Passive Avoidance Test

Passive Avoidance Test (PAT) or Step-through/Step-down avoidance measures emotional (fear-motivated) memory and learning. Based on the rodent's preference of dark environments and aversion to foot shock. (113)

Apparatus: Light compartment is connected to a dark shock compartment. Acquisition Trial –

Animal is shocked at the dark chamber entrance when it is mildly shocked (0.5-1 mA). Step-through latency (time to re-enter the dark chamber) is measured 24 hours after training with no shock. The longer the delay, the better the memory will remain. (114,115)

The test is very susceptible to amnesic agents (such as scopolamine and diazepam) and has been widely employed as a screening tool for anti-amnesic and neuroprotective agents in Alzheimer's models.

### 10.4 Y-Maze Test

The Y-Maze Test is used to measure spontaneous alternation behavior, indicative of short-term spatial working memory, which is the tendency of the rodent to explore new arms. The apparatus is a three arm (120° apart) Y maze(116,117)

Procedure: The animal is held in one arm and given some time to freely explore for 5–8 minutes. Alternation is noted when the animal goes through three different arms in order (e.g., ABC, CAB). The percentage of spontaneous alternation is determined by:

[(Number of alternations) / (Total arm entries – 2)] × 100. (118,119)

Impaired alternation is a sign of working memory impairments, often found in dysfunction of either the hippocampus or the prefrontal cortex. The test is fast, not training dependent and also commonly used to screen cognitive enhancers.

### 10.5 Actophotometer Test

The Actophotometer (photoactometer) is used to quantify locomotor activity to help identify general CNS stimulant or depressant effects, and exclude motor impairments which might interfere with the interpretations of cognitive test results. (120)

The procedure involves the use of infrared beams or photocells to count beam breaks as the animal moves about in a cage. The baseline activity is measured for 5–10 minutes prior and following the administration of the drug. High counts are suggestive of CNS stimulation, low counts are suggestive of sedation or motor impairment.(121)

This test is used in studies that effect neuroprotection and anti-amnesic effects to ensure that any improvements in memory tasks are not related to changes in locomotion. It is crucial in the validation of the specificity of the effects of herbal extracts or test compounds.(122)

## 11 Biochemical parameters in neuroprotection studies

### 11.1 Acetylcholinesterase (AChE)

Acetylcholine esterase (AChE) is an important enzyme of the cholinergic system that breaks down acetylcholine into choline and acetate at the synaptic clefts. Increased AChE activity may be associated with decreased levels of acetylcholine, which play a role in cognitive impairment in Alzheimer's disease and experimental amnesia models.

AChE inhibition is a key therapeutic target in neuroprotection studies. Reduced AChE activity in the hippocampus or cerebral cortex has been shown to be a marker of better cholinergic function and memory enhancement. The estimation is often done by the spectrophotometric method that measures the rate of production of thiocholine at 412 nm, developed by Ellman.

The significant AChE inhibitory effect of Hellenia speciosa extract has been found in both scopolamine and $AlCl_3$ induced models of anti-

amnesic activity. Test compounds with AChE activity reduction are considered to be neuroprotective and cognitive enhancing.

### 11.2 Superoxide Dismutase (SOD)

Dismutation enzyme superoxide dismutase (SOD) is a front line antioxidant enzyme that converts superoxide radicals ($O_2^-$) into hydrogen peroxide and oxygen. Reduction in SOD activity is a characteristic of oxidative stress in neurodegenerative diseases.

SOD activity levels are determined in experimental studies by nitroblue tetrazolium reduction inhibition methods and/or by pyrogallol autoxidation methods. The restoration of SOD activity with the use of neuroprotective agents suggests improvement in cellular protection against reactive oxygen species (ROS).

Medicinal plant extracts generally significantly increase the amount of SOD in the hippocampus and cortex of amnesic subjects, which thus protect the cells from oxidative damage.

### 11.3 Reduced Glutathione (GSH)

Reduced glutathione (GSH) is the most important intracellular non-enzymatic antioxidant which eliminates free radicals and helps to maintain cellular redox homeostasis. Low levels of GSH are correlated with high neuronal susceptibility in Alzheimer's disease, Parkinson's disease, and in toxin-induced amnesia.

normally, the content of GSH is measured by Ellman's reagent (DTNB) which forms a yellow coloured complex that can be measured at 412 nm. The greater GSH levels following treatment indicate enhanced antioxidant status and neuroprotection.

In the experimental models, many phytochemicals from plants such as Hellenia speciosa replenish GSH level in brain homogenates, which helps to reduce lipid peroxidation and protein oxidation.

### 11.4 Catalase Activity

Catalase is a peroxisomal enzyme that catalyses the conversion of hydrogen peroxide ($H_2O_2$) to water and oxygen, thus preventing the formation of highly reactive hydroxyl radicals. In neurodegenerative diseases, the decrease in the activity of catalase worsens the damage caused to the membranes and DNA of neurons.

The activity of enzymes is usually determined by the breaking down of $H_2O_2$ at 240 nm. Catalase activity is considered to be a strong antioxidant indicator of neuroprotection in the treated groups.

In studies using extracts of the rhizome of Hellenia speciosa and other medicinal plants, there has been a significant increase in catalase activity in the brain of animals treated with aluminum chloride and scopolamine.

### 11.5 Malondialdehyde (MDA)

Malondialdehyde (MDA) is a stable final product of peroxidation of polyunsaturated fatty acids, and a commonly used indicator of brain oxidative stress and lipid membrane damage. There is a consistent increase in MDA levels reported in AD patients and in experimental models of amnesia.

The most common method for estimation of MDA is the thiobarbituric acid reactive substances (TBARS) that measure pink colored chromogen at 532nm. Decreased levels of MDA after therapeutic interventions suggests decreased oxidative damage and protection of membranes.

Herbal neuroprotective agents such as Hellenia speciosa can effectively reduce MDA

concentrations in brain tissues in relation to the behavioral outcomes in memory tests.

The role of inflammation markers such as TNF-α in diabetes.

Tumor necrosis factor-alpha (TNF-α) is a key pro-inflammatory cytokine that is up-regulated in neuroinflammatory diseases, alongside other cytokines IL-1β and IL-6. Neuroinflammation is a key process in the pathogenesis of neurodegenerative diseases, leading to neuronal death and cognitive decline.

The quantification of these biomarker is carried out with ELISA kits in brain homogenates or serum. Reductions in the levels of cytokines such as TNF- α following treatment indicate anti-inflammatory and neuroprotective effects. The NF-κB pathway modulation is commonly assessed as a secondary endpoint.

Medicinal plants that have been found to reduce TNF-α and inflammatory markers in rodent models have a multi-target neuroprotective effect by interrupting the oxidative stress-inflammation cycle.

## 12 Histopathological Evaluation

### 12.1 Histology of Brain Tissue

Brain tissue analysis is crucial to investigate structural changes in neurodegenerative models as well as to measure the neuroprotective activity of medicinal plants like Hellenia speciosa. Brain histology is directly examining the integrity of the brain, the shape of the cells and the structure of the tissues in parts that are essential to the memory and mental capacities of the brain, such as the hippocampus (CA1, CA3, Dentate Gyrus) and cerebral cortex(123,124)

The animals are usually perfused transcardially in experimental studies with saline followed by neutral buffered formalin 10%. The brains are removed, post-fixed, transferred through graded alcohols and cleared in xylene and then embedded in paraffin wax. The serial coronal or sagittal sections are obtained by cutting with a rotary microtome (5-7 µm thick). Key areas are determined based on stereotaxic coordinates (for rats: Paxinos and Watson atlas). Behavioral and biochemical results are complemented by histological results that provide evidence of neuroprotection at the cellular level. (125)

### 12.2 Hematoxylin and Eosin Staining

Hematoxylin and Eosin (H&E) staining is the standard staining method used in routine brain tissue histopathological evaluation. Hematoxylin is a basic dye which stains the nucleic acids in the nuclei blue-purple, and eosin is an acidic dye which stains cytoplasmic proteins, extracellular matrix and myelin pink-red, giving excellent contrast(126,127)

Sandard protocol (Paraffin sections): Dip in xylene (2 changes, 5–10 min).

Rehydrate using a graded series of ethanol (100% to 95% to 70% to distilled water).

Stain in Harris' or Mayer's hematoxylin for 3-5 minutes, and then bluing in alkaline solution.

Eosin Y (1-3 min) (counterstain). Dehydrate, clear in xylene and mount in DPX or like medium. (128) Using this technique, neuronal cell bodies, nuclei, cytoplasm and the surrounding neuropil are clearly visible. For neuroprotective assays, H&E stained sections are evaluated by a blinded pathologist with light microscopy (10×-40×) with regard to parameters including neuronal density, pyknosis, vacuolation, and inflammation.(129)

### 12.3 Histopathological changes in Neurodegeneration:

Pathohistological changes in experimental amnesia and neurodegenerative models ($AlCl_3$, scopolamine or rotenone-induced) include the presence of neurons with eosinophilic (red) appearance of acutely degenerated cells, chromatin condensation and pyknotic nuclei, along with neuronal loss, shrinkage and cytoplasmic vacuolization(129,130)

In the Hippocampus and Cortex in diseased animals, there is a significant loss of neurons particularly in the CA1 and CA3, along with a rise in perineuronal vacuolation and gliosis. Chronic $AlCl_3$ models can show neurofibrillary tangle like structures and plaque like deposits. Such changes are associated with behavioural testing of memory loss.(131) Extracts from Hellenia speciosa and other neuroprotective agents frequently lead to substantial preservation of neuronal architecture such as decreased pyknotic neurons, restoration of cellular density, minimal vacuolation and better layering of the hippocampus. The plant's anti-oxidant, anti-inflammatory and anti-apoptotic properties are confirmed by such improvements(36,125)

The quantitative analysis can be done by counting viable neurons per field or by stereological analysis (such as optical fractionator) for unbiased estimation of the number of total neurons in any specific region.(123)

## 13 Current therapies and limitations.

### 13.1 Synthetic Drugs used in Alzheimer's disease.

The current pharmacological treatment of Alzheimer's disease (AD) consists mainly of symptomatic drugs and more recently disease-modifying drugs. Cholinesterase inhibitors (ChEIs) and the NMDA receptor antagonist memantine are the mainstay symptomatic drugs.

Cholinesterase inhibitors: Donepezil, rivastigmine, and galantamine are drugs used for mild to moderate AD. They enhance the levels of acetylcholine, which is released when it is not broken down, and have a small effect on cognition, global functioning, and ADLs. Donepezil is also used to treat severe AD.

NMDA Receptor Antagonist: Memantine is indicated, alone or in combination with donepezil, for moderate to severe AD. It acts on the regulation of glutamate excitotoxicity and offers neuroprotective effects.

Several anti-amyloid monoclonal antibody drugs have been approved since 2021 – these are called Disease-Modifying Therapies or DMTs. Aducanumab (Aduhelm), lecanemab (Leqembi) and donanemab (Kisunla) target amyloid-β plaques. These are the first drugs proven to be clinically beneficial in slowing clinical decline by reducing amyloid burden (amyloid PET imaging).

Other agents being investigated and/or in use in an off-label manner are anti-tau agents, anti-inflammatories, and repurposed agents (e.g., metformin and semaglutide).

### 13.2 Limiting and side effects

Although synthetic therapies are widely used in the treatment of AD, there are many limitations. Cholinesterase inhibitors do not stop disease progression and only offer limited and short-term symptomatic benefit (usually 6–12 months). As the neurodegenerative process progresses, their effectiveness is reduced.

The most common Adverse Effects of ChEIs are nausea, vomiting, diarrhea, anorexia, bradycardia

and muscle cramps. Rivastigmine has a higher gastrointestinal intolerance. The side effects of memantine might include dizziness, headache, confusion, and constipation.

Anti-amyloid monoclonal antibodies have significant side effects, such as amyloid-related imaging abnormalities (ARIA) that affect the brain, including cerebral edema (ARIA-E) and microhemorrhages (ARIA-H). In some patients they may cause very serious neurological problems. They are also costly, not widely available and must be given intravenously every six to eight weeks and genetic testing is required (APOE ε4 carriers are at higher risk).

There is no single treatment at the moment that is able to reverse or halt the neurodegenerative process. Poor translation of promising agents from preclinical models to the clinic has been a problem in many clinical trials for AD, stressing the complexity and multifactorial nature of the disease.

### 13.3 The need for alternative herbal remedies.

Because of the drawbacks of synthetic drugs, a search for safer, multi-target and cost-effective alternatives has grown even more intense. Herbal medicines have been found to have multi-modal mechanisms such as antioxidant, anti-inflammatory, anti-amyloid, anti-tau and neuroprotective properties and are generally safer for chronic use.

Preliminary studies in preclinical models have shown the potential of medicinal plants like Hellenia speciosa, Bacopa monnieri, Withania somnifera, and Curcuma longa in regulating oxidative stress, cholinergic function and neuro-inflammation. The synergistic effects are commonly observed with these phytotherapies attributed to the presence of various phytochemicals (flavonoids, terpenoids, alkaloids and steroidal saponins).

Herbal methods can work on several pathological hallmarks, which means that there may be other effects apart from symptomatic relief - disease-modifying effects. They tend to be more affordable, more acceptable in many populations. But there are still issues to overcome, including standardisation, bioavailability and stringent clinical validation. They need to be evaluated in well-designed randomized controlled trials to determine their efficacy and safety and integrate them into AD mainstream practice.

Novel treatment strategies are needed to overcome the increasing worldwide burden of dementia. Ethnopharmacological knowledge, when associated with modern scientific studies, is a valuable route towards more effective and comprehensive neuroprotective therapies.

## 14 Future perspectives and research opportunities.

### 14.1 Scope for clinical studies

While preclinical studies on Hellenia speciosa (syn. Although Costus speciosus has shown promising effects in terms of neuroprotective, antioxidant, anti-inflammatory and anti-amnesic activities, there is a lack of human clinical evidence. Randomized, double-blinded and placebo-controlled trials (RCTs) should be considered for future clinical studies to assess the safety and efficacy of standardized rhizome extracts in patients with mild cognitive impairment (MCI) and early-stage Alzheimer's disease (AD) (5,132)

The key outcome measures should encompass cognitive evaluations (such as MMSE, ADAS-Cog), biomarker analysis (CSF Aβ/tau,

neuroimaging) and quality of life indices. Dosing studies (e.g., 200–800 mg/day) and long-term safety tests (6–12 months) are necessary. Escalation and/or combination trials with other treatments (e.g., donepezil) may consider synergistic effects. Since traditional formulation is very well known, and the safety profile is very good in pre-clinical studies, there is a logical progression towards Phase I/II studies that will investigate the bioavailability and penetration of the BBB in the plant. (7)

### 14.2 Challenges in Herbal Drug Development

There are some challenges for the development of herbal medicines for neurodegenerative disorders. However, standardization still remains a significant challenge because there is great variation in the phytochemical make up depending on geographical location, harvest time and extraction processes. As a result of this there is a lack of consistency in therapeutic outcomes between studies.(133)

The bioavailability of important phytoconstituents (steroidal saponins, flavonoids) is limited, making them less available in the central nervous system. The process of moving towards market approval is hindered by regulatory hurdles, such as strict adherence to quality control, toxicity testing, and Good Manufacturing Practices (GMP) standards. Perhaps most importantly, there are concerns about possible herb-drug interactions and the requirement for strong long-term safety data. Besides, the complexity of herbal products results in the inability to identify the exact mechanism of action and active component of the product to patent and accept it for regulation.(5)

### 14.3 Future Research Directions

Several interesting lines of research are suggested for Hellenia speciosa. Advanced techniques such as HPLC-MS and NMR techniques can be used to isolate and characterize specific bioactive lead compounds (diosgenin derivatives, costunolide) which can be used for structure-activity relationship studies to help develop semi-synthetic analogs. (85)

The use of nanotechnology-based delivery systems (liposomes, nanoparticles, solid lipid nanoparticles) is worth exploring to improve bioavailability and deliver targeted drugs to the brain. The multi-omics approaches (genomics, proteomics, metabolomics) and network pharmacology can give deeper insights into multi-target mechanisms. Large population, multi-center clinical trials with patient stratification based on biomarkers are needed now. (134)

Further directions encompass study of synergistic polyherbal formulations, long-term toxicity study, long-term teratogenicity study, and study of the preventive potential in the high-risk population. The combination of artificial intelligence and machine learning in virtual screening of phytocompounds could speed up the drug discovery process. Lastly, sustainable cultivation and conservation measures for Hellenia speciosa need to be supported to sustain a raw material supply.(135)

Overall, Hellenia speciosa has a significant potential for use as a multi-target neuroprotective agent. Traditional knowledge, when coupled with modern scientific validation through careful research, may result in the development of new and safe therapeutic approaches for neurodegenerative diseases.

## REFERENCES

1. Lamptey RNL, Chaulagain B, Trivedi R, Gothwal A, Layek B, Singh J. A Review of the Common Neurodegenerative Disorders:

Current Therapeutic Approaches and the Potential Role of Nanotherapeutics. Int J Mol Sci [Internet]. 2022 Feb 6;23(3):1851. Available from: https://www.mdpi.com/1422-0067/23/3/1851

2. Wilson DM, Cookson MR, Van Den Bosch L, Zetterberg H, Holtzman DM, Dewachter I. Hallmarks of neurodegenerative diseases. Cell [Internet]. 2023 Feb;186(4):693–714. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867422015756
3. Anna Giorgi. Amnesia [Internet]. 2023 [cited 2026 May 29]. Available from: https://www.medicalnewstoday.com/articles/9673#type
4. Mayo Clinic Staff. Amnesia - Symptoms and Causes [Internet]. 2025 [cited 2026 Jun 5]. Available from: https://www.org/diseases-conditions/amnesia/symptoms-causes/syc-20353360.
5. Nahar L, Charoensup R, Kalieva K, Habibi E, Guo M, Wang D, et al. Natural products in neurodegenerative diseases: recent advances and future outlook. Front Pharmacol [Internet]. 2025 Mar 19;16. Available from: https://www.frontiersin.org/articles/10.3389/fphar.2025.1529194/full
6. Iriti M, Vitalini S, Fico G, Faoro F. Neuroprotective Herbs and Foods from Different Traditional Medicines and Diets. Molecules [Internet]. 2010 May 14;15(5):3517–55. Available from: https://www.mdpi.com/1420-3049/15/5/3517
7. Nagori K, Nakhate KT, Yadav K, Ajazuddin, Pradhan M. Unlocking the Therapeutic Potential of Medicinal Plants for Alzheimer's Disease: Preclinical to Clinical Trial Insights. Futur Pharmacol [Internet]. 2023 Nov 13;3(4):877–907. Available from: https://www.mdpi.com/2673-9879/3/4/53
8. Kumar V. Potential medicinal plants for CNS disorders: an overview. Phyther Res [Internet]. 2006 Dec;20(12):1023–35. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ptr.1970
9. Egbuna C, Rudrapal M. Phytochemical Drug Discovery for Central Nervous System Disorders: Biochemistry and Therapeutic Effects [Internet]. Egbuna C, Rudrapal M, editors. Phytochemical Drug Discovery for Central Nervous System Disorders: Biochemistry and Therapeutic Effects. Wiley; 2023. 1–442 p. Available from: https://onlinelibrary.wiley.com/doi/book/10.1002/9781119794127
10. Lui, Forshing; Tsao JW. Alzheimer Disease. In StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499922
11. Zheng, Qiuyang; Wang X. Alzheimer's disease: insights into pathology, molecular mechanisms, and therapy [Internet]. [cited 2026 Jun 5]. Available from: https://doi.org/10.1093/procel/pwae026 10.3389/fmed.2024.1474043
12. Safiri S, Ghaffari Jolfayi A, Fazlollahi A, Morsali S, Sarkesh A, Daei Sorkhabi A, et al. Alzheimer's disease: a comprehensive review of epidemiology, risk factors, symptoms diagnosis, management, caregiving, advanced treatments and associated challenges. Front Med [Internet]. 2024 Dec 16;11. Available from: https://www.frontiersin.org/articles/10.3389/fmed.2024.1474043/full
13. Pan Y, Cho H, Lou Q, Zhang W, Asken B, Song Q. Genetic risk in Alzheimer's disease. npj Syst Biol Appl [Internet]. 2026 Feb 14;12(1):39. Available from: https://www.nature.com/articles/s41540-026-00665-8

14. Antonina Kouli, Kelli M. Torsney and W-LK. Parkinson's Disease: Pathogenesis and Clinical Aspects [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK536722
15. Morris HR, Spillantini MG, Sue CM, Williams-Gray CH. The pathogenesis of Parkinson's disease. Lancet [Internet]. 2024 Jan;403(10423):293–304. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673623014782
16. Jankovic J, Tan EK. Parkinson's disease: etiopathogenesis and treatment. J Neurol Neurosurg Psychiatry [Internet]. 2020 Aug;91(8):795–808. Available from: https://jnnp.bmj.com/lookup/doi/10.1136/jnnp-2019-322338
17. Tadi. PDECSP. Major Neurocognitive Disorder (Dementia). In: StatPearls [Internet]. 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557444
18. Chin KS. Pathophysiology of dementia. Aust J Gen Pract [Internet]. 2023 Aug 1;52(8):516–21. Available from: https://www1.racgp.org.au/ajgp/2023/august/pathophysiology-of-dementia
19. Aarsland D, Batzu L, Halliday GM, Geurtsen GJ, Ballard C, Ray Chaudhuri K, et al. Parkinson disease-associated cognitive impairment. Nat Rev Dis Prim [Internet]. 2021 Jul 1;7(1):47. Available from: https://www.nature.com/articles/s41572-021-00280-3
20. Moroz OF, Kravchenko VI, Kushch BO, Zholos A V. Dementia and neurodegenerative diseases: What is known and what is promising at the cellular and molecular level. Basic Clin Pharmacol Toxicol [Internet]. 2024 Sep 30; Available from: https://onlinelibrary.wiley.com/doi/10.1111/bcpt.14087
21. Morais RF, Pires R, Jesus T, Lemos R, Duro D, Lima M, et al. Cognitive impairment in neurodegenerative diseases: A trans-diagnostic approach using a lesion-symptom mapping analysis. Neuroscience [Internet]. 2025 May;573:214–27. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0306452225002362
22. Mayo Clinic Staff. Alzheimer's disease – Symptoms and causes [Internet]. 2026. Available from: https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447
23. Altoida. Top Risk Factors for Neurodegenerative Disease. 2021; Available from: https://altoida.com/blog/top-risk-factors-for-neurodegenerative-disease
24. Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. Front Cell Neurosci [Internet]. 2015 Apr 10;9. Available from: http://journal.frontiersin.org/article/10.3389/fncel.2015.00124/abstract
25. UC Davis Health. Neurodegenerative Diseases. 2026; Available from: https://health.ucdavis.edu/alzheimers-research/conditions/neurodegenerative-diseases
26. Alzheimer Society of Canada. Risk factors for dementia. 2023; Available from: https://alzheimer.ca/en/about-dementia/how-can-i-reduce-risk-dementia/risk-factors-dementia
27. Tesco, Giuseppina; Lomoio S. Pathophysiology of neurodegenerative diseases: An interplay among axonal transport failure, oxidative stress, and inflammation? Semin Immunol [Internet]. 2022; Available from:

https://pmc.ncbi.nlm.nih.gov/articles/PMC9807734/

28. Bloomingdale P, Karelina T, Ramakrishnan V, Bakshi S, Véronneau‐Veilleux F, Moye M, et al. Hallmarks of neurodegenerative disease: A systems pharmacology perspective. CPT Pharmacometrics Syst Pharmacol [Internet]. 2022 Nov 17;11(11):1399–429. Available from: https://ascpt.onlinelibrary.wiley.com/doi/10.1002/psp4.12852
29. Rapaka D, Adiukwu PC, Bitra VR. Experimentally induced animal models for cognitive dysfunction and Alzheimer's disease. MethodsX [Internet]. 2022;9:101933. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2215016122003107
30. More S, Kumar H, Cho D-Y, Yun Y-S, Choi D-K. Toxin-Induced Experimental Models of Learning and Memory Impairment. Int J Mol Sci [Internet]. 2016 Sep 1;17(9):1447. Available from: https://www.mdpi.com/1422-0067/17/9/1447
31. Elsevier. Amnesia - an overview [Internet]. 2022 [cited 2026 Jun 5]. Available from: https://www.sciencedirect.com/topics/neuroscience/amnesia
32. Contributors W. Amnesia [Internet]. 2026 [cited 2026 Jun 5]. Available from: https://en.wikipedia.org/wiki/Amnesia
33. Yadang FSA, Nguezeye Y, Kom CW, Betote PHD, Mamat A, Tchokouaha LRY, et al. Scopolamine-Induced Memory Impairment in Mice: Neuroprotective Effects of Carissa edulis (Forssk.) Valh (Apocynaceae) Aqueous Extract. Int J Alzheimers Dis [Internet]. 2020 Aug 31;2020:1–10. Available from: https://www.hindawi.com/journals/ijad/2020/6372059/
34. Lu C, Wang Y, Xu T, Li Q, Wang D, Zhang L, et al. Genistein Ameliorates Scopolamine-Induced Amnesia in Mice Through the Regulation of the Cholinergic Neurotransmission, Antioxidant System and the ERK/CREB/BDNF Signaling. Front Pharmacol [Internet]. 2018 Oct 12;9. Available from: https://www.frontiersin.org/article/10.3389/fphar.2018.01153/full
35. Investigating the Mechanisms Involved in Scopolamine-Induced Memory Degradation. Arch Razi Inst [Internet]. 2023 Nov 14;555–64. Available from: https://archrazi.areeo.ac.ir/article_130309.html
36. Khan K, Emad NA, Sultana Y. Inducing Agents for Alzheimer's Disease in Animal Models. J Explor Res Pharmacol [Internet]. 2024 Jul 16;000(000):000–000. Available from: https://www.xiahepublishing.com/2572-5505/JERP-2023-00028
37. Abo El-Magd NF, Ramadan NM, Eraky SM. Liraglutide attenuates aluminum chloride-induced Alzheimer's disease in rats by modulating the oxLDL/LPA/LPAR1 pathway. Commun Biol [Internet]. 2026 Feb 11;9(1):262. Available from: https://www.nature.com/articles/s42003-026-09531-z
38. Al-Amin MM, Chowdury MIA, Saifullah ARM, Alam MN, Jain P, Hossain M, et al. Levocarnitine Improves AlCl3-Induced Spatial Working Memory Impairment in Swiss albino Mice. Front Neurosci [Internet]. 2019 Mar 26;13. Available from: https://www.frontiersin.org/article/10.3389/fnins.2019.00278/full
39. Biolabs C. Scopolamine Induced Rodent Amnesia Model. 2026; Available from: https://www.creative-biolabs.com/drug-discovery/therapeutics/scopolamine-induced-rodent-amnesia-model.htm

40. Charles River Laboratories. Scopolamine-Induced Amnesia - AD Model [Internet]. 2026. Available from: https://www.criver.com/products-services/discovery-services/pharmacology-studies/neuroscience-models-assays/alzheimers-disease-studies/scopolamine-induced-amnesia-model
41. Eshaghi Ghalibaf MH, Rajabian A, Parviz M, Akbarian M, Amirahmadi S, Vafaee F, et al. Minocycline alleviated scopolamine-induced amnesia by regulating antioxidant and cholinergic function. Heliyon [Internet]. 2023 Feb;9(2):e13452. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2405844023006659X
42. Reddy N, Sultanpur C, Saritha V. Evaluation of nootropic activity of Curcuma longa leaves in diazepam and scopolamine-induced amnesic mice and rats. Int J Basic Clin Pharmacol [Internet]. 2015;714–9. Available from: http://www.ijbcp.com/index.php/ijbcp/article/view/829
43. Kaplan K, Hunsberger HC. Benzodiazepine-induced anterograde amnesia: detrimental side effect to novel study tool. Front Pharmacol [Internet]. 2023 Sep 14;14. Available from: https://www.frontiersin.org/articles/10.3389/fphar.2023.1257030/full
44. Gamzu ER. Animal Model Studies of Benzodiazepine-Induced Amnesia. In: Benzodiazepine Receptor Ligands, Memory and Information Processing [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 1988. p. 218–29. Available from: http://link.springer.com/10.1007/978-3-642-73288-1_16
45. Rajangam J, Lavanya O. Effect of Rosuvastatin on learning and memory in scopolamine induced amnesia in Mice. Trends Med [Internet]. 2018;18(2). Available from: http://www.oatext.com/effect-of-rosuvastatin-on-learning-and-memory-in-scopolamine-induced-amnesia-in-mice.php
46. Teleanu DM, Niculescu A-G, Lungu II, Radu CI, Vladâcenco O, Roza E, et al. An Overview of Oxidative Stress, Neuroinflammation, and Neurodegenerative Diseases. Int J Mol Sci [Internet]. 2022 May 25;23(11):5938. Available from: https://www.mdpi.com/1422-0067/23/11/5938
47. Zhang J, Zhang Y, Wang J, Xia Y, Zhang J, Chen L. Recent advances in Alzheimer's disease: mechanisms, clinical trials and new drug development strategies. Signal Transduct Target Ther [Internet]. 2024 Aug 23;9(1):211. Available from: https://www.nature.com/articles/s41392-024-01911-3
48. Song T, Song X, Zhu C, Patrick R, Skurla M, Santangelo I, et al. Mitochondrial dysfunction, oxidative stress, neuroinflammation, and metabolic alterations in the progression of Alzheimer's disease: A meta-analysis of in vivo magnetic resonance spectroscopy studies. Ageing Res Rev [Internet]. 2021 Dec;72:101503. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1568163721002506
49. Chandimali N, Bak SG, Park EH, Lim H-J, Won Y-S, Kim E-K, et al. Free radicals and their impact on health and antioxidant defenses: a review. Cell Death Discov [Internet]. 2025 Jan 24;11(1):19. Available from: https://www.nature.com/articles/s41420-024-02278-8
50. Tarafdar A, Pula G. The Role of NADPH Oxidases and Oxidative Stress in Neurodegenerative Disorders. Int J Mol Sci [Internet]. 2018 Nov 30;19(12):3824. Available from: https://www.mdpi.com/1422-0067/19/12/3824

51. Zong Y, Li H, Liao P, Chen L, Pan Y, Zheng Y, et al. Mitochondrial dysfunction: mechanisms and advances in therapy. Signal Transduct Target Ther [Internet]. 2024 May 15;9(1):124. Available from: https://www.nature.com/articles/s41392-024-01839-8
52. Barrera G, Pizzimenti S, Daga M, Dianzani C, Arcaro A, Cetrangolo GP, et al. Lipid Peroxidation-Derived Aldehydes, 4-Hydroxynonenal and Malondialdehyde in Aging-Related Disorders. Antioxidants [Internet]. 2018 Jul 30;7(8):102. Available from: https://www.mdpi.com/2076-3921/7/8/102
53. Li Y, Zhao T, Li J, Xia M, Li Y, Wang X, et al. Oxidative Stress and 4-hydroxy-2-nonenal (4-HNE): Implications in the Pathogenesis and Treatment of Aging-related Diseases. Fernandes ES, editor. J Immunol Res [Internet]. 2022 Mar 23;2022:1–12. Available from: https://www.hindawi.com/journals/jir/2022/2233906/
54. Zhang X, Hou L, Guo Z, Wang G, Xu J, Zheng Z, et al. Lipid peroxidation in osteoarthritis: focusing on 4-hydroxynonenal, malondialdehyde, and ferroptosis. Cell Death Discov [Internet]. 2023 Aug 29;9(1):320. Available from: https://www.nature.com/articles/s41420-023-01613-9
55. McGrath LT. Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde. QJM [Internet]. 2001 Sep 1;94(9):485–90. Available from: https://academic.oup.com/qjmed/article-lookup/doi/10.1093/qjmed/94.9.485
56. Tansey MG, Wallings RL, Houser MC, Herrick MK, Keating CE, Joers V. Inflammation and immune dysfunction in Parkinson disease. Nat Rev Immunol [Internet]. 2022 Nov 4;22(11):657–73. Available from: https://www.nature.com/articles/s41577-022-00684-6
57. Neuroinflammation as a Link in Parkinson's and Alzheimer's Diseases: A Systematic Review and Meta-Analysis. aging Dis [Internet]. 2024; Available from: https://www.aginganddisease.org/EN/10.14336/AD.2024.1174
58. Dhapola R, Kumari S, Sharma P, Paidlewar M, Vellingiri B, Medhi B, et al. Cytokine associated neuroinflammation in Parkinson's disease: Molecular pathways, therapeutic targets, and translational insights. Cytokine Growth Factor Rev [Internet]. 2026 Apr;88:1–17. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1359610126000018
59. Antwi MH, Bockarie A, Osei GN, Donkor DM, Simpong DL. Cytokines and immune biomarkers in neurodegeneration and cognitive function: A systematic review among individuals of African ancestry. Alzheimer's Dement [Internet]. 2025 Jul 25;21(7). Available from: https://alz-journals.onlinelibrary.wiley.com/doi/10.1002/alz.70514
60. Dash UC, Bhol NK, Swain SK, Samal RR, Nayak PK, Raina V, et al. Oxidative stress and inflammation in the pathogenesis of neurological disorders: Mechanisms and implications. Acta Pharm Sin B [Internet]. 2025 Jan;15(1):15–34. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2211383524004040
61. Chen Z-R, Huang J-B, Yang S-L, Hong F-F. Role of Cholinergic Signaling in Alzheimer's Disease. Molecules [Internet]. 2022 Mar 10;27(6):1816. Available from: https://www.mdpi.com/1420-3049/27/6/1816

62. Gamage R, Wagnon I, Rossetti I, Childs R, Niedermayer G, Chesworth R, et al. Cholinergic Modulation of Glial Function During Aging and Chronic Neuroinflammation. Front Cell Neurosci [Internet]. 2020 Oct 15;14. Available from: https://www.frontiersin.org/article/10.3389/fncel.2020.577912/full
63. Ghosh S, Singha PS, Ghosh D. Neuroprotective compounds from three common medicinal plants of West Bengal, India: a mini review. Explor Neurosci [Internet]. 2023 Dec 26;2(6):307–17. Available from: https://www.explorationpub.com/Journals/en/Article/100630
64. Moise G, Jîjie A-R, Moacă E-A, Predescu I-A, Dehelean CA, Hegheș A, et al. Plants' Impact on the Human Brain—Exploring the Neuroprotective and Neurotoxic Potential of Plants. Pharmaceuticals [Internet]. 2024 Oct 7;17(10):1339. Available from: https://www.mdpi.com/1424-8247/17/10/1339
65. Valotto Neto LJ, Reverete de Araujo M, Moretti Junior RC, Mendes Machado N, Joshi RK, dos Santos Buglio D, et al. Investigating the Neuroprotective and Cognitive-Enhancing Effects of Bacopa monnieri: A Systematic Review Focused on Inflammation, Oxidative Stress, Mitochondrial Dysfunction, and Apoptosis. Antioxidants [Internet]. 2024 Mar 25;13(4):393. Available from: https://www.mdpi.com/2076-3921/13/4/393
66. Herbal Approaches to the Treatment of a Neurodegenerative Disease [Internet]. [cited 2026 Jun 12]. Available from: https://medizinonline.com/en/herbal-approaches-to-the-treatment-of-a-neurodegenerative-disease
67. Khazdair MR, Anaeigoudari A, Hashemzehi M, Mohebbati R. Neuroprotective potency of some spice herbs, a literature review. J Tradit Complement Med [Internet]. 2019 Apr;9(2):98–105. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2225411018300026
68. Puri V, Kanojia N, Sharma A, Huanbutta K, Dheer D, Sangnim T. Natural product-based pharmacological studies for neurological disorders. Front Pharmacol [Internet]. 2022 Nov 7;13. Available from: https://www.frontiersin.org/articles/10.3389/fphar.2022.1011740/full
69. Islam A, Mishra A, Ahsan R, Fareha S. Phytopharmaceuticals and Herbal Approaches to Target Neurodegenerative Disorders. Drug Res (Stuttg) [Internet]. 2023 Sep 12;73(07):388–407. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/a-2076-7939
70. Sharifi-Rad M, Lankatillake C, Dias DA, Docea AO, Mahomoodally MF, Lobine D, et al. Impact of Natural Compounds on Neurodegenerative Disorders: From Preclinical to Pharmacotherapeutics. J Clin Med [Internet]. 2020 Apr 8;9(4):1061. Available from: https://www.mdpi.com/2077-0383/9/4/1061
71. Di Paolo M, Papi L, Gori F, Turillazzi E. Natural Products in Neurodegenerative Diseases: A Great Promise but an Ethical Challenge. Int J Mol Sci [Internet]. 2019 Oct 18;20(20):5170. Available from: https://www.mdpi.com/1422-0067/20/20/5170
72. Ratheesh G, Tian L, Venugopal JR, Ezhilarasu H, Sadiq A, Fan T-P, et al. Role of medicinal plants in neurodegenerative diseases. Biomanufacturing Rev [Internet]. 2017 Dec 24;2(1):2. Available from: http://link.springer.com/10.1007/s40898-017-0004-7

73. Kumar Gp, Khanum F. Neuroprotective potential of phytochemicals. Pharmacogn Rev [Internet]. 2012;6(12):81. Available from: https://www.phcogrev.com/PharmacognRev-6-12-81
74. Ayaz M, Mosa OF, Nawaz A, Hamdoon AAE, Elkhalifa MEM, Sadiq A, et al. Neuroprotective potentials of Lead phytochemicals against Alzheimer's disease with focus on oxidative stress-mediated signaling pathways: Pharmacokinetic challenges, target specificity, clinical trials and future perspectives. Phytomedicine [Internet]. 2024 Feb;124:155272. Available from: https://linkinghub.elsevier.com/retrieve/pii/S094471132300630X
75. Naoi M, Shamoto-Nagai M, Maruyama W. Neuroprotection of Multifunctional Phytochemicals as Novel Therapeutic Strategy for Neurodegenerative Disorders: Antiapoptotic and Antiamyloidogenic Activities by Modulation of Cellular Signal Pathways. Future Neurol [Internet]. 2019 Jan 25;14(1). Available from: https://www.tandfonline.com/doi/full/10.2217/fnl-2018-0028
76. Contributors W. Hellenia speciosa [Internet]. 2026 [cited 2026 Jun 12]. Available from: https://en.wikipedia.org/wiki/Hellenia_speciosa
77. Qin Y, Wang N, Pan H, Lei X, Li X. Hellenia speciosa: A comprehensive review of traditional applications, phytonutrients, health benefits and safety. Food Chem [Internet]. 2025 Feb;465:142003. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814624036537
78. Chen J, Zeng S, Zeng L, Nguyen KS, Yan J, Liu H, et al. Parahellenia, a new genus segregated from Hellenia (Costaceae) based on phylogenetic and morphological evidence. Plant Divers [Internet]. 2022 Jul;44(4):389–405. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2468265922000178
79. National Tropical Botanical Garden. Hellenia speciosa - Plant Detail - Tropical Plants Database [Internet]. [cited 2026 Jun 12]. Available from: https://ntbg.org/database/plants/detail/hellenia-speciosa
80. Ken Fern. Hellenia speciosa [Internet]. 2024. Available from: https://tropical.theferns.info/viewtropical.php?id=Hellenia+speciosa
81. Sohrab S, Mishra P, Mishra SK. Phytochemical competence and pharmacological perspectives of an endangered boon—Costus speciosus (Koen.) Sm.: a comprehensive review. Bull Natl Res Cent [Internet]. 2021 Dec 4;45(1):209. Available from: https://bnrc.springeropen.com/articles/10.1186/s42269-021-00663-2
82. Visible) FU or GN (if. Facebook Group Post [Internet]. Available from: https://www.facebook.com/groups/325781850773723/posts/3408248582527019/
83. State Medicinal Plants Board Kerala (SMPB Kerala). Hellenia speciosa (J.Koenig) S.R.Dutta [Internet]. Available from: https://smpbkerala.in/herbal-data/hellenia-speciosa.asp
84. Wikipedia Contributors. Hellenia speciosa [Internet]. 2026. Available from: https://en.wikipedia.org/wiki/Hellenia_speciosa
85. Alnhhas S, Bouback T, Albeshri A, Alsahafi T, Al-Sarraj F, Al-Sulaimany FA, et al. Antiviral activity of Hellenia speciosa (J. Koenig) S.R. Dutta rhizome metabolites against human adenovirus: insights from molecular docking and in vitro studies. Front Pharmacol [Internet]. 2026 Jan 13;16. Available from:

https://www.frontiersin.org/articles/10.3389/fphar.2025.1687104/full

86. SHAIKH SS, BAWAZIR AS, YAHYA BA. Phytochemical, Histochemical and In Vitro Antimicrobial Study of Various Solvent Extracts of Costus speciosus (J. Koenig) Sm. and Costus pictus D. Don. Turkish J Pharm Sci [Internet]. 2022 Apr 29;19(2):145–52. Available from: https://turkjps.org/articles/doi/tjps.galenos.2021.02058
87. Delarosa A, Hendrawan RP, Halimah E. Screening of Costus speciosus and Determination of Antioxidant Potential Using DPPH Method: A Review. European J Med Plants [Internet]. 2023 Jul 25;34(7):17–28. Available from: https://journalejmp.com/index.php/EJMP/article/view/1146 No Title.
88. M; B, BB; K, LR S. Analysis of Primary and Secondary Metabolite Profile of Costus speciosus (Koen Ex. Retz.) Sm. Rhizome [Internet]. 2014. Available from: https://www.scholarsresearchlibrary.com/articles/analysis-of-primary-and-secondary-metabolite-profile-of-costus-speciosuskoen-exretz-sm-rhizome.pdf
89. University KA. Hellenia speciosa [Internet]. Available from: http://medicinalplants.kau.in/mobile/plant_new/144
90. Agrawal S, Kochar N, Chandewar A. Review on Costus speciosus a Medicinal Plant. Res J Pharm Technol [Internet]. 2018;11(4):1697. Available from: http://www.indianjournals.com/ijor.aspx?target=ijor:rjpt&volume=11&issue=4&article=080
91. R R. GC-MS Analysis of Bioactive Compounds in Ethanolic Leaf Extract of Hellenia speciosa (J.Koenig) S.R. Dutta. Appl Biochem Biotechnol [Internet]. 2022 Jan 11;194(1):176–86. Available from: https://link.springer.com/10.1007/s12010-021-03742-2
92. J; N, M; B, M N. In-vitro Evaluation of Antioxidant Activity and Phenolic Content of Costus speciosus (Koen) J.E. Sm. 2010; Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3863442/
93. A Review on the Therapeutic and Medicinal Activities of Costus speciosus. Available from: https://pharmacophorejournal.com/article/a-review-on-the-therapeutic-and-medicinal-activities-of-costus-speciosus
94. Al-Attas AAM, El-Shaer NS, Mohamed GA, Ibrahim SRM, Esmat A. Anti-inflammatory sesquiterpenes from Costus speciosus rhizomes. J Ethnopharmacol [Internet]. 2015 Dec;176:365–74. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378874115302270
95. Raj A, Chakravorty A, Luktuke S, Santra S, Das S, Sahoo S, et al. Anti-inflammatory Potential of Costus speciosus rhizome Bioactive Phytochemicals: A Combined GC-MS and Computational Approach Targeting TLR-4 Signaling. Curr Comput Aided Drug Des [Internet]. 2025 May;21(3):285–301. Available from: https://www.eurekaselect.com/235356/article
96. Thakur; S, Sharma M. Phytochemical Profiling and In vitro Evaluation of Biological Activities of Costus speciosus (J. Koenig) SM. 2025; Available from: https://www.ijpsjournal.com/article/Phytochemical+Profiling+and+In+vitro+Evaluation+of+Biological+Activities+of+Costus+speciosus+J+Koenig+SM
97. N; V, RL K. Effect of Costus speciosus and Wedelia chinensis on Brain Neurotransmitters and Enzyme Monoamine Oxidase Following Cold Immobilization Stress. 2009; Available

from: https://www.researchgate.net/publication/41394574_Effect_of_Costus_speciosus_and_Wedelia_chinensis_on_Brain_Neurotransmitters_and_Enzyme_Monoamine_Oxidase_Following_Cold_Immobilization_Stress

98. El-Far A, Shaheen H, Alsenosy A, El-Sayed Y, Al Jaouni S, Mousa S. Costus speciosus: Traditional uses, phytochemistry, and therapeutic potentials. Pharmacogn Rev [Internet]. 2018;12(23):120. Available from: https://www.phcogrev.com/PharmacognRev-12-23-120
99. MA; V, NC S. Screening of Costus speciosus extracts for antioxidant activity. 2008; Available from: https://www.researchgate.net/publication/5672648_Screening_of_Costus_speciosus_extracts_for_antioxidant_activity
100. Gheraibia S, Belattar N, Diab KA, Hassan ME, El-Nekeety AA, Abdel-Aziem SH, et al. Costus speciosus extract protects against the oxidative damage of zearalenone via modulation of inflammatory cytokines, Nrf2 and iNOS gene expression in rats. Toxicon [Internet]. 2022 Jul;214:62–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0041010122001301
101. Elsevier. Cheilocostus speciosus [Internet]. Available from: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/cheilocostus-speciosus
102. No Title.
103. Othman MZ, Hassan Z, Che Has AT. Morris water maze: a versatile and pertinent tool for assessing spatial learning and memory. Exp Anim [Internet]. 2022;71(3):21–0120. Available from: https://www.jstage.jst.go.jp/article/expanim/71/3/71_21-0120/_article
104. Cyagen. Understanding the Morris Water Maze in Neuroscience [Internet]. 2025. Available from: https://www.cyagen.com/cyagen-lab-notes/water-maze-experiment-neurobehavioral-test
105. NCBI Bookshelf [Internet]. National Center for Biotechnology Information (NCBI); Available from: https://www.ncbi.nlm.nih.gov/books/NBK5219/
106. Curdt N, Schmitt FW, Bouter C, Iseni T, Weile HC, Altunok B, et al. Search strategy analysis of Tg4-42 Alzheimer Mice in the Morris Water Maze reveals early spatial navigation deficits. Sci Rep [Internet]. 2022 Mar 31;12(1):5451. Available from: https://www.nature.com/articles/s41598-022-09270-1
107. Sánchez CQ, Schmitt FW, Curdt N, Westhoff AC, Bänfer IWH, Bayer TA, et al. Search Strategy Analysis of 5xFAD Alzheimer Mice in the Morris Water Maze Reveals Sex- and Age-Specific Spatial Navigation Deficits. Biomedicines [Internet]. 2023 Feb 17;11(2):599. Available from: https://www.mdpi.com/2227-9059/11/2/599
108. Danduga RCSR, Kola PK. Elevated Plus Maze for Assessment of Anxiety and Memory in Rodents. In 2024. p. 93–6. Available from: https://link.springer.com/10.1007/978-1-0716-3662-6_8
109. Sharma AC, Kulkarni SK. Evaluation of learning and memory mechanisms employing elevated plus-maze in rats and mice. Prog Neuro-Psychopharmacology Biol Psychiatry [Internet]. 1992 Jan;16(1):117–25. Available from: https://linkinghub.elsevier.com/retrieve/pii/0278584692900146

110. Morales-Delgado N, Popović N, De la Cruz-Sánchez E, Caballero Bleda M, Popović M. Time-of-Day and Age Impact on Memory in Elevated Plus-Maze Test in Rats. Front Behav Neurosci [Internet]. 2018 Dec 6;12. Available from: https://www.frontiersin.org/article/10.3389/fnbeh.2018.00304/full
111. Conduct Science. Elevated Plus Maze [Internet]. Available from: https://maze.conductscience.com/portfolio/elevated-plus-maze
112. Elsevier. Passive Avoidance Test [Internet]. Available from: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/passive-avoidance-test
113. San DIEGO Instrument. What is a Passive Avoidance Test? [Internet]. [cited 2026 Jun 14]. Available from: https://sandiegoinstruments.com/what-is-a-passive-avoidance-test/
114. Neurofit. Passive avoidence [Internet]. [cited 2026 Jun 14]. Available from: https://www.neurofit.com/tech-anim-passiveavoidance.html
115. Prieur E, Jadavji N. Assessing Spatial Working Memory Using the Spontaneous Alternation Y-maze Test in Aged Male Mice. BIO-PROTOCOL [Internet]. 2019;9(3). Available from: https://bio-protocol.org/e3162
116. Kraeuter A-K, Guest PC, Sarnyai Z. The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. In 2019. p. 105–11. Available from: http://link.springer.com/10.1007/978-1-4939-8994-2_10
117. Kim J, Kang H, Lee Y-B, Lee B, Lee D. A quantitative analysis of spontaneous alternation behaviors on a Y-maze reveals adverse effects of acute social isolation on spatial working memory. Sci Rep [Internet]. 2023 Sep 7;13(1):14722. Available from: https://www.nature.com/articles/s41598-023-41996-4
118. Cygen. Y-Maze Behavioral Task in Neuroscience Research [Internet]. [cited 2026 Jun 14]. Available from: https://www.cyagen.com/cyagen-lab-notes/y-maze-behavioral-task-neuroscience-research
119. Bhosale U, Yegnanarayan R, Prachi P, Zambare M, Somani RS. Study of CNS depressant and behavioral activity of an ethanol extract of Achyranthes Aspera (Chirchita) in mouse model. Ann Neurosci [Internet]. 2011 Jul 20;18(2). Available from: http://www.annalsofneurosciences.org/journal/index.php/annal/article/view/339
120. Slide share. Effect of Drugs on Locomotor Activity Using Actophotometer [Internet]. Available from: https://www.slideshare.net/slideshow/expt-11-effect-of-drugs-on-locomotor-activity-using-actophotometer/245405842
121. Akshayraj Vanrajbhai C, Thorat VM, Patel VS, Patil KP, Chawla LL. Evaluation of Anxiolytic Activity of Angiotensin Receptor Blockers Using Actophotometer Test in Wistar Rats. Cureus [Internet]. 2024 Sep 20; Available from: https://www.cureus.com/articles/282540-evaluation-of-anxiolytic-activity-of-angiotensin-receptor-blockers-using-actophotometer-test-in-wistar-rats
122. Yamaguchi H, Shen J. Histological Analysis of Neurodegeneration in the Mouse Brain. In 2013. p. 91–113. Available from: http://link.springer.com/10.1007/978-1-62703-383-1_8
123. Kang J, Watanabe H, Shen J. Protocols for assessing neurodegenerative phenotypes in Alzheimer's mouse models. STAR Protoc [Internet]. 2021 Sep;2(3):100654. Available from:

https://linkinghub.elsevier.com/retrieve/pii/S2666166721003610

124. Kavuri S, Sivanesan S, Howell MD, Vijayaraghavan R, Rajadas J. Studies on Parkinson's-Disease-Linked Genes, Brain Urea Levels and Histopathology in Rotenone Induced Parkinson's Disease Rat Model. World J Neurosci [Internet]. 2020;10(04):216–34. Available from: https://www.scirp.org/journal/paperinformation?paperid=104471

125. Biosystem L. H&E Staining Overview: A Guide to Best Practices [Internet]. Available from: https://www.leicabiosystems.com/en-in/knowledge-pathway/he-staining-overview-a-guide-to-best-practices/

126. Anisha Bhola. A Colorful Journey with Hematoxylin and Eosin Staining [Internet]. 2023. Available from: https://gloneuro.org/neuro-techniques/a-colorful-journey-with-hematoxylin-and-eosin-staining/

127. Program U of CSD (UCSD) MP. Hematoxylin and Eosin (H&E) Staining Protocol [Internet]. Available from: https://mousepheno.ucsd.edu/hematoxylin.shtml

128. Umer H, Sharif A, Khan HM, Anjum SMM, Akhtar B, Ali S, et al. Mitigation of Neuroinflammation and Oxidative Stress in Rotenone-Induced Parkinson Mouse Model through Liposomal Coenzyme-Q10 Intervention: A Comprehensive In-vivo Study. Inflammation [Internet]. 2025 Jan 21;48(5):2874–95. Available from: https://link.springer.com/10.1007/s10753-025-02237-0

129. Kanan DD, Aydemir I, Alizade A, Ebrahimi S, Aksu F. Investigation of Behavioral Changes and Histopathological Changes in the Brain in Alzheimer's Modeled Mice with Aluminium Chloride (AlCl3). Cyprus J Med Sci [Internet]. 2025 Jun 4;34–7. Available from: https://cyprusjmedsci.com/articles/investigation-of-behavioral-changes-and-histopathological-changes-in-the-brain-in-alzheimers-modeled-mice-with-aluminium-chloride-alcllesssubgreater3lesssubgreater/doi/cjms.2024.2024-123

130. A comparative study of scopolamine, D-Galactose and AlCl3-induced Alzheimer's like disease by enhancing hippocampal neurodegeneration and memory impairment in male rats. ZANCO J PURE Appl Sci [Internet]. 2023 Jun 14;35(3). Available from: https://zancojournal.su.edu.krd/index.php/JPAS/article/view/637

131. Zieneldien T, Kim J, Cao C. The Multifaceted Role of Neuroprotective Plants in Alzheimer's Disease Treatment. Geriatrics [Internet]. 2022 Feb 26;7(2):24. Available from: https://www.mdpi.com/2308-3417/7/2/24

132. Goyal R, Mittal P, Gautam RK, Kamal MA, Perveen A, Garg V, et al. Natural products in the management of neurodegenerative diseases. Nutr Metab (Lond) [Internet]. 2024 May 16;21(1):26. Available from: https://nutritionandmetabolism.biomedcentral.com/articles/10.1186/s12986-024-00800-4

133. Gregory J, Vengalasetti Y V., Bredesen DE, Rao R V. Neuroprotective Herbs for the Management of Alzheimer's Disease. Biomolecules [Internet]. 2021 Apr 8;11(4):543. Available from: https://www.mdpi.com/2218-273X/11/4/543

134. Ashwini Pingle; Gayatri Gaikwad; Amol Darwade; Ganesh Darunte; Kunal Gaikwad. Medicinal Plants in Alzheimer's Disease Management. 2025;29(1). Available

from: https://www.ijsrtjournal.com/article/Medicinal+Plants+in+Alzheimers+Disease+Management.

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