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HIGH-RESOLUTION MASS SPECTROMETRIC PROFILING OF *ACORUS CALAMUS:* EXPLORING ITS NEUROPROTECTIVE PROPERTIES

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ABSTRACT

Neurological disorders are emerging as a major global health concern, leading to increased attention on neuroprotective agents for their potential in preventing or mitigating neuronal damage. *Acorus calamus (Vacha)*, a traditional medicinal herb, has demonstrated promising neuroprotective effects in preclinical experiments. This study employs high-resolution mass spectroscopy (HRMS) to profile the constituents of *Acorus calamus (Vacha)*, focusing on identifying compounds with neuroprotective activity. The study mainly focuses on seven compounds (Nicotinamide, Betaine, Tranexamic acid, Anthranilic acid, Syringic acid, Fraxetin, Trans-anethole) with neuroprotective activity, suggesting that Vacha can modulate oxidative stress, inflammation, and neurotransmitter systems. These bioactive compounds bolster antioxidative defence, alleviate neuroinflammation, and preserve neuronal integrity, emphasizing their therapeutic value in neurodegenerative diseases. Their synergistic actions contribute to cognitive resilience and neurological health, positioning Acorus calamus as a promising natural remedy. This study advances our knowledge of the molecular constituents of Acorus calamus (Vacha) and underscores its potential as a neuroprotective agent.

KEYWORDS: HRMS, Neuro-protective, Anti-oxidant, *Vacha*, Nootropic.

INTRODUCTION

Neurodevelopmental disorders are a group of behavioural and cognitive conditions that emerge during early development, resulting in significant challenges in acquiring and performing intellectual, motor, language, and social skills. In 2019, approximately one in every eight individuals worldwide—equivalent to 970 million people—were affected by a mental disorder, with anxiety and depression being the most prevalent. By 2020, the COVID-19 pandemic had significantly exacerbated these conditions, leading to an estimated 26% increase in anxiety disorders and a 28% rise in major depressive disorders within a single year. Neuronal damage can result from a variety of interconnected causes that disrupt normal brain function. Key contributors include excitotoxicity, mitochondrial dysfunction, protein misfolding and aggregation, and neuroinflammation, as seen in many neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD).

Reactive oxygen species (ROS), including superoxide, nitric oxide, hydrogen peroxide, and the extremely reactive hydroxyl radicals, are produced in excess and lead to oxidative damage to lipids, proteins, and DNA in neurons. Brain tissue is vulnerable to oxidative injury because of its high oxygen consumption, comparatively low antioxidant levels, and poor regeneration capacity.[1]

Excitotoxicity refers to neuronal injury resulting from the overactivation of excitatory neurotransmitters, mainly glutamate receptors, particularly NMDA and AMPA receptors, leading to increased intracellular calcium levels. Elevated calcium triggers a cascade of harmful events, including mitochondrial dysfunction, oxidative stress, and activation of proteases and lipases, which damage cellular components.[2]

Impaired mitochondrial function disrupts ATP production, increases ROS, and initiates damage to cellular components like lipids, proteins, and DNA, triggering neuronal injury and apoptosis.[3]

Misfolded proteins, such as amyloid-β in Alzheimer's, α-synuclein in Parkinson's, and huntingtin in Huntington's disease, tend to aggregate into toxic oligomers and insoluble fibrils. These abnormal aggregates disrupt cellular functions, impair proteasomal and autophagic degradation pathways, and trigger inflammatory and apoptotic responses.[4]

Microglia-mediated neurotoxicity is a significant contributor to neurodegenerative diseases. Microglia, the

brain's resident immune cells, become chronically activated in response to neuronal injury, misfolded proteins, or environmental toxins. Once activated, they release pro-inflammatory cytokines, reactive oxygen species (ROS), and nitric oxide, creating a toxic environment that damages neurons.[5] These mechanisms often interact, creating a vicious cycle that exacerbates neuronal injury and contributes to progressive neurodegeneration.

Neuroprotection is the term used to describe methods and systems that protect the nervous system from injury and degeneration to maintain the structure and functionality of neurons. The pathogenic mechanisms that cause neurodegeneration, such as excitotoxicity, oxidative stress, mitochondrial dysfunction, protein misfolding, and neuroinflammation, are inhibited by neuroprotective drugs.

Neuroprotective compounds, such as antioxidants (Compounds like vitamin E, flavonoids, and glutathione), reduce oxidative stress and protect neuronal cells.[6] Anti-inflammatory agents suppress harmful microglial activation, inflammasome activation, and inhibit the production of cytokines.[7] Calcium channel blockers help prevent excitotoxicity by regulating calcium influx into neurons,[8] And mitochondrial stabilizers (Coenzyme Q10 and creatine) improve mitochondrial function and energy production, and can reduce neuronal damage by interrupting these harmful cascades.[9]

Prominent Ayurvedic authorities like *Acharya Charaka* and *Acharya Sushruta* have extensively documented *Vacha*'s role in addressing cognitive deficits and enhancing mental clarity in their classical texts (Agnivesh, 2015).

It has been utilized to strengthen *Medha* (intellectual grasp and knowledge retention), *Buddhi* (cognitive abilities), *Smriti* (memory recall), and *Sandnya* (conscious awareness), thereby supporting overall neurological and cognitive health.

Acorus calamus Linn. (Acoraceae), commonly referred to as *Vacha* in Sanskrit, is a perennial, aromatic herb with significant medicinal applications. Extensively utilized in traditional therapeutic practices, particularly within *Ayurvedic* and *Chinese* medicinal systems, it is recognized for its diverse pharmacological properties and therapeutic potential. *Vacha* functions as a potent nervine tonic, exerting a stabilizing and soothing influence on the central nervous system. It is widely recognized for its neuroprotective, anti-inflammatory, and antioxidant properties, making it beneficial for conditions like anxiety, insomnia, epilepsy, and even Alzheimer's disease. Its neuroprotective and anxiolytic properties contribute to the alleviation of stress, anxiety, restlessness, and sleep disturbances, including insomnia. By modulating excessive neural excitability, *Vacha* plays a crucial role in mitigating hyperactive states that may predispose individuals to heightened anxiety and epileptic episodes, thereby supporting overall neurological health. Additionally, *Vacha* is known to support speech clarity and mental alertness.

Acorus calamus demonstrates strong antioxidant activity, which plays a crucial role in neutralizing harmful free radicals within the body. Numerous scientific studies have emphasized the plant's capacity to combat oxidative stress, underscoring its therapeutic potential. The DMSO (Dimethyl Sulfoxide) extract of *Acorus calamus* displayed strong antioxidant, anti-inflammatory, and antibacterial properties, with effectiveness comparable to, and in some instances surpassing, that of standard chemical agents. These results support the traditional use of *A. calamus* in alternative medicine and provide a scientific basis for its therapeutic potential.[10]

The bioactive constituents contribute to the herb's neuroprotective, antioxidant, and antidepressant properties, with evidence indicating its modulation of neurotransmitter pathways, including GABAergic, dopaminergic, and serotonergic systems.[11]

This study aims to highlight the key compounds involved in neuroprotection, providing valuable insights into their mechanisms and potential therapeutic applications. Given the growing prevalence of neurological disorders, particularly neurodegenerative diseases, neuroprotection has become a crucial focus of research. By uncovering novel bioactive molecules in *Vacha*, this study can pave the way for innovative treatment strategies and advancements in neurological health.

MATERIALS AND METHODS

Authentication of *Vacha* (*Acorus calamus*): The Raw material *Vacha* for the analysis by liquid chromatography-mass spectrometry was collected from the local market, Gola-Deena Nath Varanasi, Uttar Pradesh. That was later authenticated in the Department of Botany, Institute of Science, Banaras Hindu University, with accession No. of *Acorus calamus* L. Acora. 2025/01.

Method Employed for HRMS Analysis: The sample preparation for high-resolution mass spectrometry (HRMS) analysis of *Vacha* (*Acorus calamus* Linn.) began with the addition of 100 mg of the sample to 1.5 mL of solvent (methanol: water; 80:20) followed by homogenization using an Eppendorf Thermomixer (Eppendorf SE) at 750 rpm for 30 minutes at 25 °C. The homogenized sample was then subjected to centrifugation at 3500 rpm for 10 minutes at 25 °C, and the resulting supernatant was filtered using a 0.22 µm polytetrafluoroethylene (PTFE) syringe filter. A 4 µL aliquot of the filtrate was injected into a C18 reverse-phase high-performance liquid chromatography (RP-HPLC) column (Hypersil GOLD™, Thermo Fisher Scientific; particle size: 1.9 µm, dimensions: 2.1 mm ×

100 mm). The reversed-phase chromatographic separation employed a gradient elution method, transitioning from a high aqueous phase (+0.1% formic acid) to a highly organic phase (methanol + 0.1% formic acid). The liquid chromatography gradient parameters were as follows: 0–6 minutes, 5% methanol; 6–10 minutes, 30% methanol; 10–20 minutes, 50% methanol; 20–25 minutes, 90% methanol; 25–27 minutes, 90% methanol; 27–30 minutes, 5% methanol. The flow rate was maintained at 300 µL/min, with a column oven temperature of 40°C. The optimized sample of *Vacha* was analyzed for metabolomics using a Thermo Fisher Scientific Orbitrap Eclipse Tribrid mass spectrometer coupled with a nano-liquid chromatography and ultra-high-pressure liquid chromatography system (Dionex Ultimate 3000 Rapid Separation Liquid Chromatography, RSLC). A heated electrospray ionization (HESI) source was utilized for sample introduction into the mass spectrometer. The Orbitrap analyzer operated at a resolution of 60,000 for both positive and negative ion modes, covering a mass range (m/z) of 100–1,000. The system parameters included a 35% RF lens, a 25% normalized automatic gain control (AGC) target, and an intensity threshold of 2.0e5 for MS-OT (master scan). To acquire data-dependent MS/MS (ddMS2) spectra using higher collisional dissociation (HCD), selection parameters included quadrupole isolation mode with a 1.5 m/z isolation window, HCD activation type with collision energies of 30%, 45%, and 60%, a resolution of 15,000, and a 20% normalized AGC target. The raw data obtained from the mass analyzer were processed using Compound Discoverer 3.3.2.31 (Thermo Fisher Scientific) with default parameters and online databases. The selected workflow—Natural Product Unknown ID—conducted untargeted food research without statistical analysis. This approach facilitated retention time alignment, unknown compound detection, and grouping across all samples. It also predicted elemental compositions for all detected compounds and eliminated chemical background interference through blank sample filtration. Compound identification was carried out using mzCloud (HighChem LLC, Bratislava, Slovakia) based on ddMS2 and DIA data, ChemSpider (Royal Society of Chemistry, Cambridge, UK) through exact mass or formula matching, and local database searches against mass lists with or without retention time information. Spectral similarity searches were performed in mzCloud for ddMS2 compounds, and spectral distance scoring was employed for ChemSpider and mass list matches, ensuring accurate compound identification and characterization.[12]

RESULTS

The total ion chromatogram (TIC) represents the cumulative ion intensity detected over time, serving as a crucial analytical tool for assessing sample composition and identifying various compounds. This graphical depiction of ion detection during chromatographic separation provides valuable insights into the molecular constituents present within the sample. The TIC of the components present in *Acorus calamus* is depicted in Figure 1.

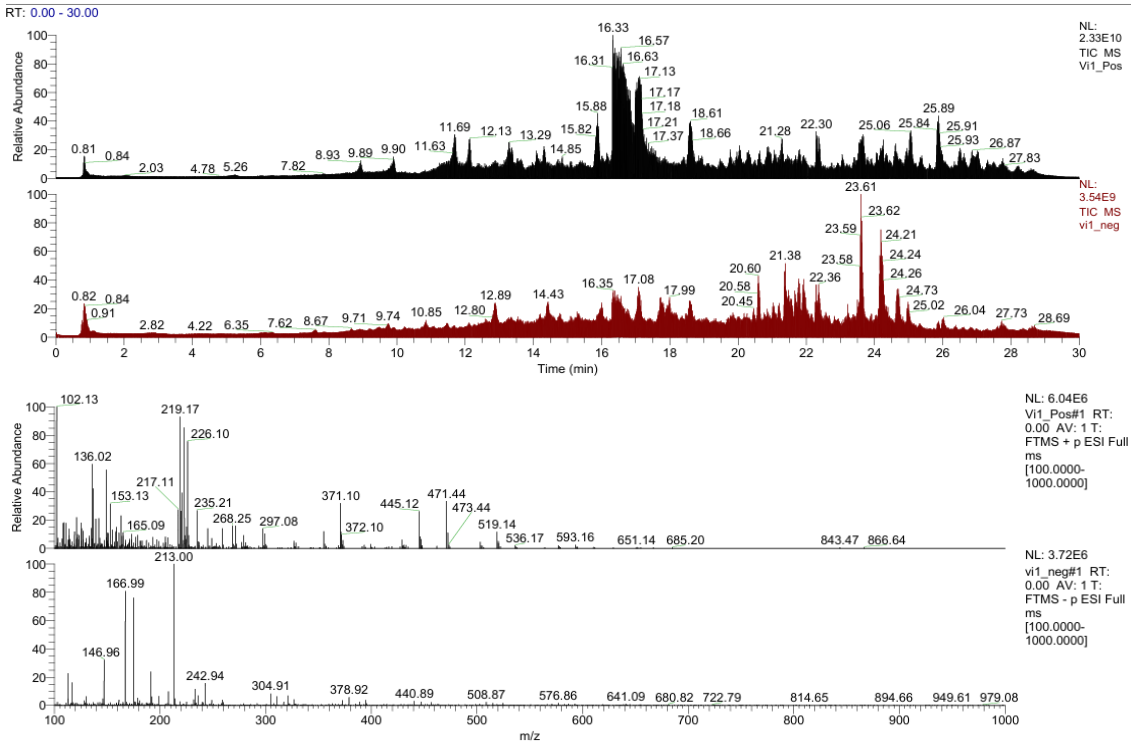

Figure 1: Total ion chromatogram of *Vacha* (*Acorus calamus*) obtained by UHPLC HRMS analysis of the *Vacha* sample in positive and negative ion modes.

UHPLC-HRMS: Ultra-High-Performance Liquid Chromatography-High Resolution Mass Spectrometry. A standard ion chromatogram serves as a crucial reference for identifying and quantifying ions present in the analyzed sample. In the case of *Vacha* (*Acorus calamus*), the neuroprotective components and detected ions were systematically quantified and characterized by evaluating their retention times and peak intensities, thereby facilitating a comprehensive assessment of their molecular profiles.

Neuroprotective activities of the phytochemical constituents found in *Vacha*

High-resolution mass spectrometry (HRMS) analysis of *Acorus calamus* identified a total of 4257 phytochemical constituents. Among these, specific bioactive compounds—including Nicotinamide, Betaine, Tranexamic acid, Anthranilic acid, Syringic acid, Fraxetin, and Trans-anethole demonstrated neuroprotective properties, as evidenced by the referenced studies mentioned below.

NICOTINAMIDE

Nicotinamide, a form of vitamin B3, is a promising neuroprotective phytoconstituent due to its role in cellular metabolism and neuronal survival. Nicotinamide holds promise as a therapeutic agent for a range of CNS diseases due to its involvement in fundamental cellular processes vital for neuronal survival and function.[13]

Nicotinamide provides neuroprotection against MPTP-induced damage in a Parkinson's disease mouse model by reducing oxidative stress and neuroinflammation. This effect is mediated through the activation of the Nrf2/HO-1 antioxidant pathway and the inhibition of the TLR4 inflammatory pathway. Additionally, nicotinamide treatment has been found to reverse Parkinson 's-like pathologies by improving dopamine transporter levels and motor function.[14]

The study by Klaidman, Mukherjee, and Adams (2001) investigated the effects of oxidative stress on brain pyridine nucleotides and the potential neuroprotective role of nicotinamide. The researchers administered t-butyl hydroperoxide (t-BuOOH) to mice, leading to rapid oxidation of NADPH and gradual depletion of NAD+ across various brain regions. This depletion was associated with increased DNA fragmentation, suggesting activation of a cell death pathway involving poly (ADP-ribose) polymerase (PARP), NAD+, and ATP depletion. Administration of nicotinamide, a precursor for NAD+, elevated NAD+ levels and mitigated DNA fragmentation, indicating its potential as a therapeutic strategy for neurodegenerative conditions.[15]

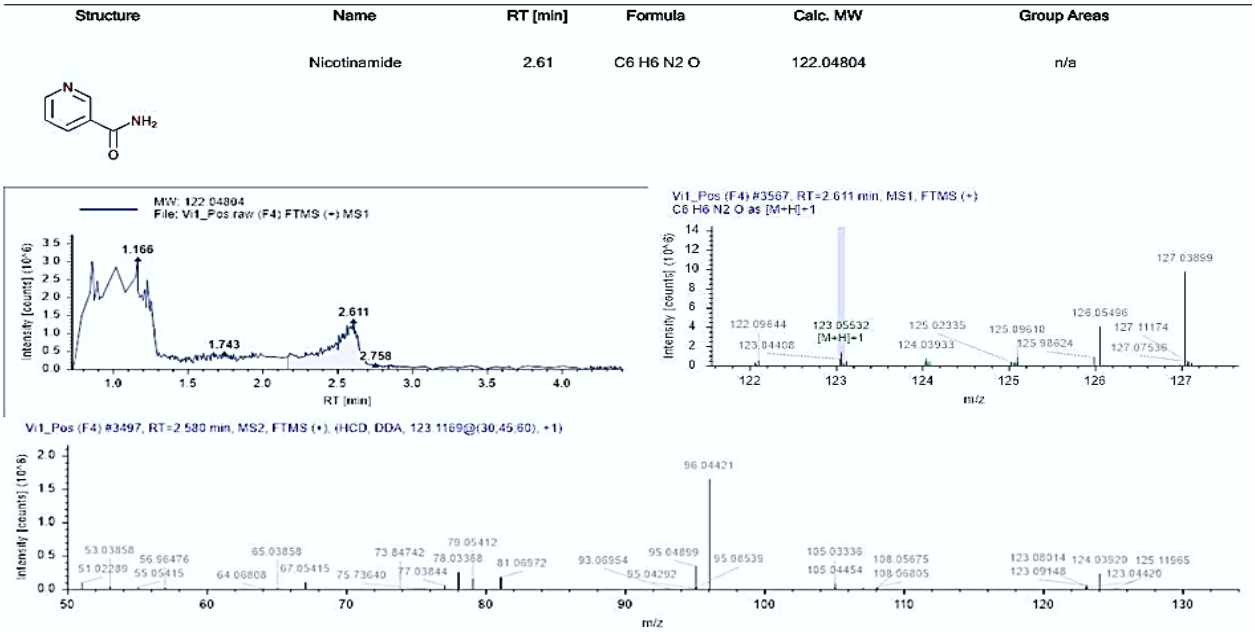

Figure 2: Standard ion chromatogram of Nicotinamide.

BETAINE

Betaine, an essential phytoconstituent, offers neuroprotection by reducing oxidative stress and inflammation. It supports cognitive health through neurotransmitter regulation and neuronal defence mechanisms.[16]

The study by Hashim et al. (2024) assessed the neuroprotective effects of betaine against copper oxide nanoparticle (CuO-NP)-induced neurotoxicity in albino rats. Rats were divided into four groups: a control group, a CuO-NP-treated group, a betaine-treated group, and a combined betaine and CuO-NP-treated group. CuO-NP exposure led to increased malondialdehyde (MDA) levels, decreased glutathione (GSH) levels, and altered expression of key proteins such as acetylcholinesterase (AChE), nuclear factor erythroid 2-related factor 2 (Nrf-2), and superoxide dismutase (SOD). Histopathological

examination revealed neuronal degeneration and vacuolation. Betaine administration mitigated these effects by reducing MDA levels, enhancing GSH levels, and modulating protein expressions, thereby offering neuroprotection against CuO-NP-induced toxicity.[17]

The study by Rahmani et al. (2019) investigated the neuroprotective effects of betaine in a 6-hydroxydopamine (6-OHDA)-induced hemi-Parkinsonism model using male Wistar rats. Betaine was administered orally at doses of 50, 100, and 200 mg/kg for two weeks, both before and after 6-OHDA injection. The 200 mg/kg dose significantly reduced plasma homocysteine levels and improved motor asymmetry, as measured by apomorphine-induced rotation tests. Additionally, it decreased malondialdehyde concentrations and neuronal loss in the substantia nigra pars compacta, suggesting betaine's potential as an antioxidant and neuroprotective agent in Parkinson's disease models.[18]

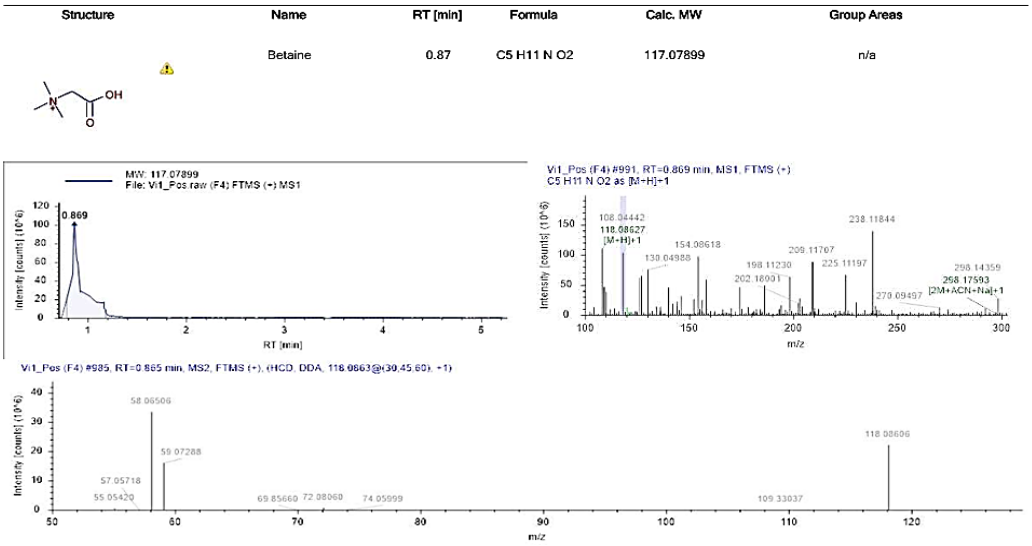

Figure 3: Standard ion chromatogram of Betaine.

TRANEXAMIC ACID

Tranexamic acid (TXA) is primarily known for its antifibrinolytic properties, but research suggests it may have neuroprotective effects, particularly in cases of traumatic brain injury (TBI). Offer neuroprotection in traumatic brain injury (TBI), with its primary benefit potentially stemming from limiting the progression of bleeding within the brain (intracranial haemorrhage or ICH). This effect seems to be more significant when TXA is given soon after the injury (within 3 hours) and possibly in individuals with less severe TBI.[19]

Tranexamic acid suppressed the secretion of the inflammatory cytokines by aging M1-type macrophages, thereby improving age-related memory and learning abilities. Administration of tranexamic acid decreased the concentrations of interleukin (IL)-1β and tumor necrosis factor-α, while it increased the levels of IL-10 and transforming growth factor-α in the brain.[20]

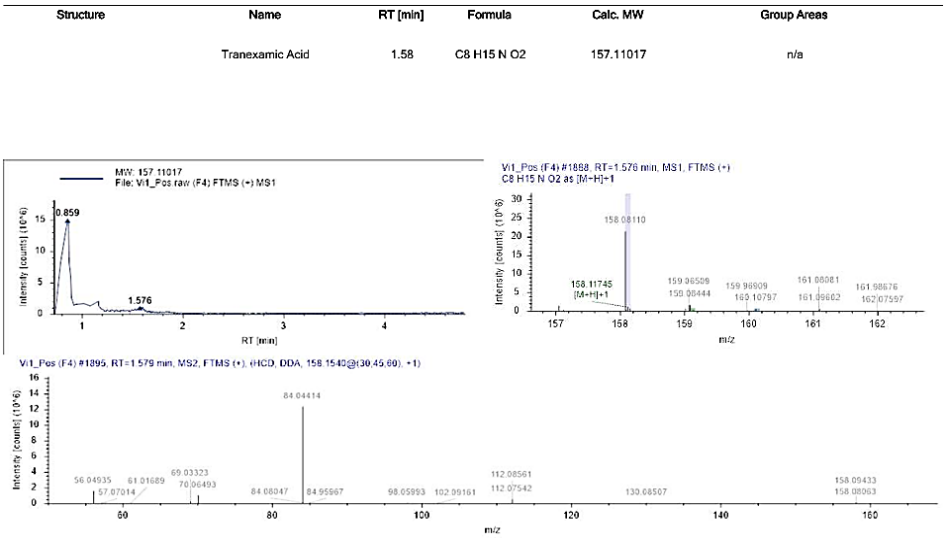

Figure 4: Standard Ion Chromatogram of Tranexamic Acid.

ANTHRANILIC ACID

Anthranilic acid, also known as 2-aminobenzoic acid, is an aromatic amine and a key intermediate in the tryptophan-kynurenine metabolic pathway, which has significant implications in neurological and psychiatric disorders.[21] Anthranilic acid derivatives hold promise for neuroprotection due to their ability to modulate NMDA receptors, exert antioxidant effects, and possess anti-inflammatory properties.[22]

The study by Prasher and Sharma (2021) provides a comprehensive review of anthranilic acid derivatives, highlighting their diverse pharmacological activities. These derivatives exhibit anti-inflammatory, antimicrobial, antiviral, and anticancer properties. Notably, certain compounds demonstrate neuroprotective effects by modulating key pathways involved in neurodegeneration. Additionally, transition metal complexes of these derivatives show promise in managing metabolic disorders like diabetes and obesity by regulating α-glucosidase activity. The review underscores the therapeutic potential of anthranilic acid derivatives and their analogues in drug development.[23]

The 2023 review by Shaw, Hess, and Weimer explores the role of microbial-derived tryptophan metabolites, particularly anthranilic acid and its derivatives, in neurological health and disease. Tryptophan, an essential amino acid, is metabolized via three primary pathways: the serotonin pathway, the kynurenine pathway, and the indole pathway. Anthranilic acid, produced through the kynurenine pathway, serves as a precursor to several neuroactive compounds, including quinolinic acid and NAD^+. The balance between these metabolites is crucial; alterations can influence neuroinflammation and neurotransmission, potentially contributing to conditions like Alzheimer's disease, depression, and schizophrenia. The gut microbiome plays a significant role in modulating these pathways, highlighting the importance of the gut-brain axis in neurological health.[24]

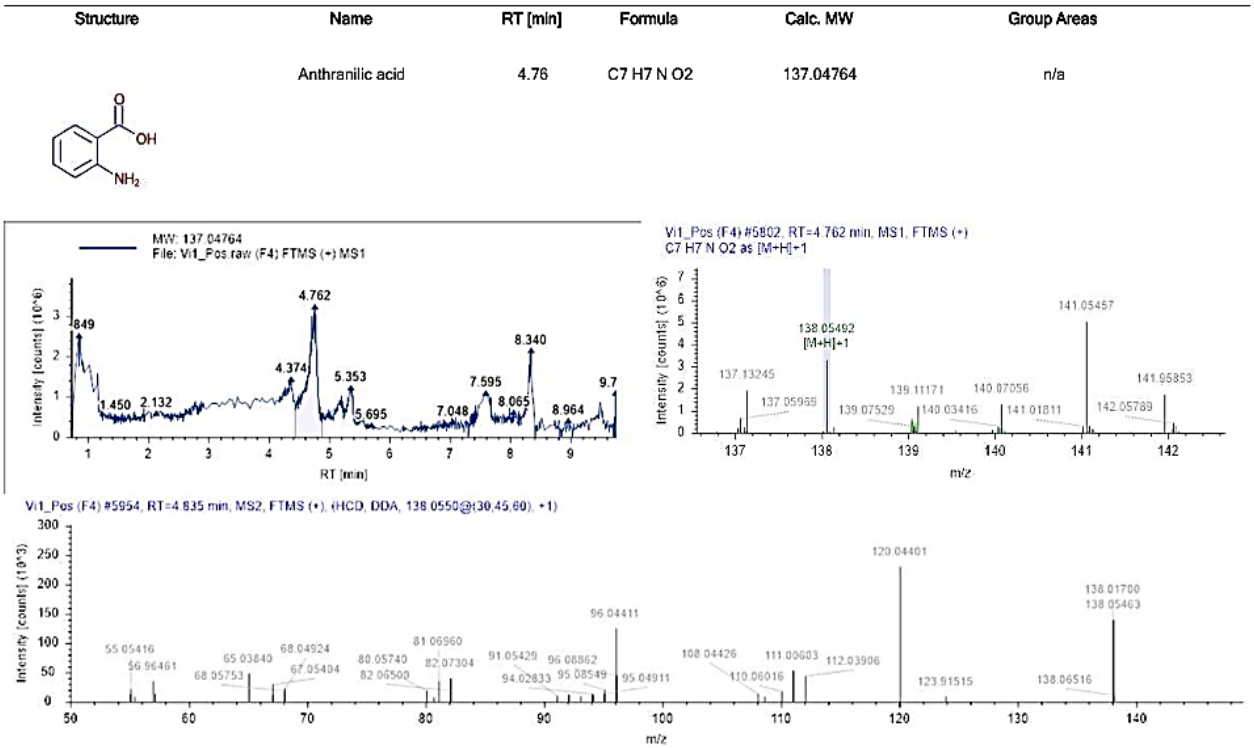

Figure 5: Standard ion chromatogram of Anthranilic acid.

SYRINGIC ACID

Syringic acid, a natural phenolic compound, exhibits antioxidant and anti-inflammatory properties. It has shown protective effects against cerebral ischemic injury and traumatic brain injury-induced cognitive dysfunction by modulating oxidative stress pathways and inflammatory responses.[25]

The study by Zhao et al. (2020) investigated the neuroprotective effects of syringic acid (SA) in a rat model of Alzheimer's disease (AD) induced by aluminium chloride ($AlCl_3$). Rats were administered $AlCl_3$ (100 mg/kg) intraperitoneally for 60 days to induce AD-like symptoms. Subsequently, they received low (25 mg/kg) and high (50 mg/kg) doses of SA for 30 days. Behavioral assessments, including the Morris water maze, Y-maze, elevated plus maze, and open field test, revealed that SA supplementation significantly improved memory, learning, and locomotor activity in AD rats. Biochemical analyses indicated that SA treatment reduced acetylcholinesterase activity, nitric oxide levels, and malondialdehyde concentrations, while enhancing antioxidant enzyme activities. Western blotting showed decreased expression of pro-inflammatory markers such

as NF-κB, IL-1β, IL-6, TNF-α, and COX-2 in the hippocampus and cortex. Histopathological examination revealed that SA administration alleviated neuronal degeneration induced by $AlCl_3$. These findings suggest that SA exerts neuroprotective effects through its antioxidant and anti-inflammatory properties, offering potential therapeutic benefits for AD management.[26]

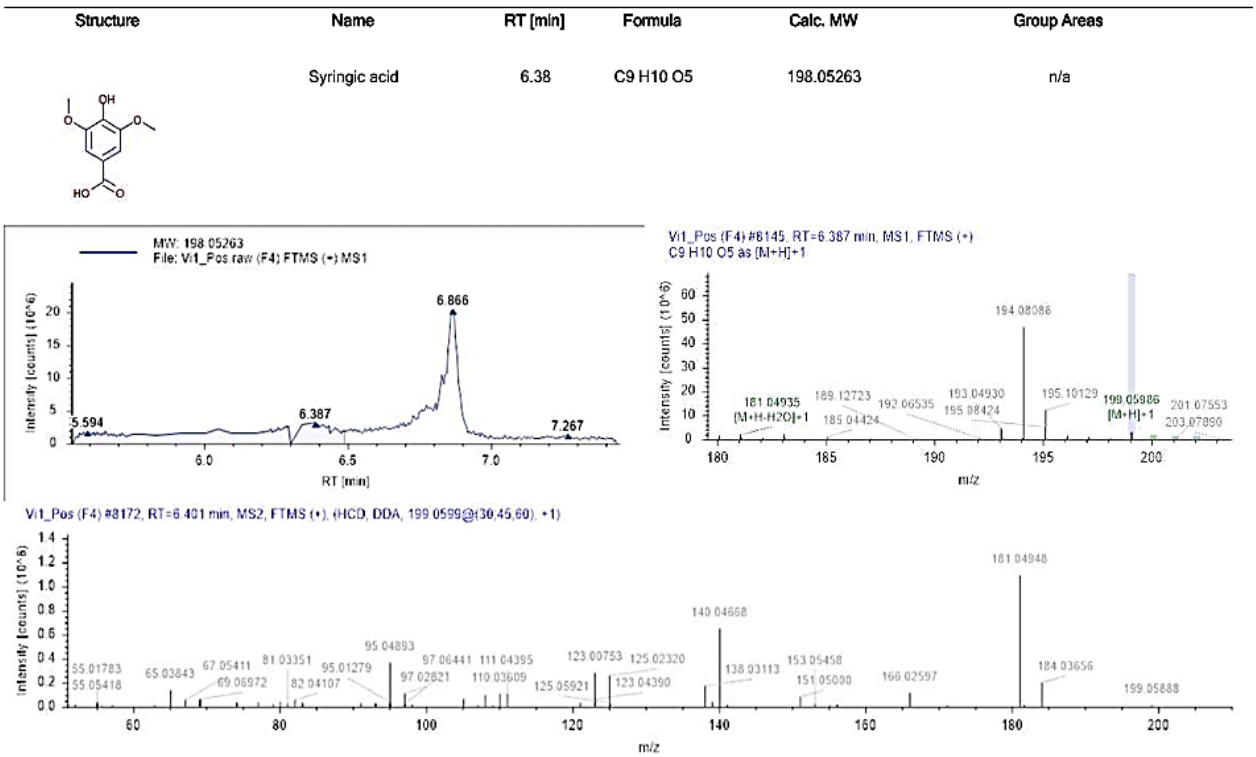

Figure 6: Standard ion chromatogram of Syringic acid.

FRAXETIN

Fraxetin is a naturally occurring coumarin derivative found in various plant species, notably in the Fraxinus (ash tree) genus. It has attracted attention in biomedical research due to its antioxidant, anti-inflammatory, and neuroprotective properties. Fraxetin showed protective effects against rotenone-induced neurotoxicity in SH-SY5Y neuroblastoma cells by modulating antioxidant enzymes and enhancing stress response proteins. It reduced MnSOD and catalase activity while increasing HSP70 expression, suggesting its potential to strengthen cellular defenses and mitigate oxidative stress compared to myricetin and N-acetylcysteine.[27]

Oral administration of fraxetin (20–60 mg/kg) significantly reduced anxiety-related responses in both the open field test and elevated plus-maze. Moreover, at the highest dose, fraxetin effectively restored dopamine levels, specifically in the frontal cortex. Fraxetin readily crosses the blood-brain barrier, maintaining permeability, and its antioxidant activity may well have potential in neurodegenerative disorders that stem from oxidative stress.[28]

The study by Ahmed et al. (2023) investigated the effects of fraxetin, a natural coumarin derivative known for its antioxidant and neuroprotective properties, on behavioral and neurochemical alterations induced by chronic unpredictable stress (CUS) in mice. Acute oral administration of fraxetin at doses of 20–60 mg/kg significantly alleviated depression-like behaviors in the forced swim test and reduced anxiety in both the open field and elevated plus-maze tests. Additionally, fraxetin improved memory deficits observed in the Y-maze test. Biochemical analyses revealed that higher doses of fraxetin decreased elevated serum corticosterone levels and reversed stress-induced reductions in serotonin levels in the frontal cortex, hippocampus, and striatum. Furthermore, fraxetin increased noradrenaline levels in the striatum and restored dopamine levels in the frontal cortex at the highest dose. These findings suggest that fraxetin may offer therapeutic potential for managing anxiety, depression, and cognitive impairments associated with chronic stress.

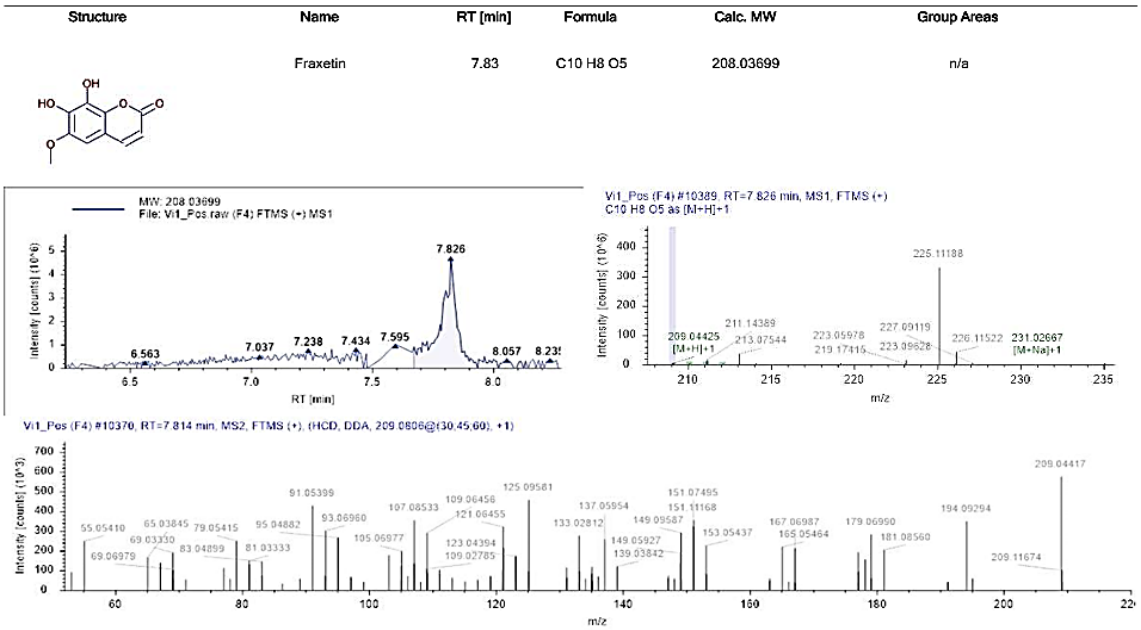

Figure 7: Standard ion chromatogram of Fraxetin.

TRANS-ANETHOLE

Trans-anethole (TA), a phenylpropanoid compound predominantly found in anise and fennel, is introduced as a potential neuroprotective agent. The neuroprotective effects of TA may stem from its ability to mitigate excitotoxicity, suppress oxidative stress, and regulate mitochondrial function, collectively contributing to neuronal preservation.[29]

Trans-anethole exerts a neuroprotective effect against OGD/R-induced neuronal injury, primarily by mitigating excitotoxicity, oxidative stress, and mitochondrial dysfunction. Given the diverse pathological mechanisms contributing to ischemic neuronal damage, the multi-targeted action of trans-anethole highlights its potential as a promising therapeutic candidate for the treatment of ischemic stroke.[30]

The study by Ryu et al. (2014) investigated the neuroprotective effects of trans-anethole in cortical neuronal cells subjected to oxygen-glucose deprivation/reoxygenation (OGD/R), an in vitro model of ischemic stroke. Trans-anethole significantly reduced neuronal injury by mitigating intracellular calcium overload through NMDA receptor activation. Additionally, it decreased reactive oxygen species (ROS) production, likely due to its peroxyl radical scavenging activity, as assessed by an oxygen radical absorbance capacity assay. Furthermore, trans-anethole attenuated mitochondrial membrane depolarization, indicating its potential to protect mitochondrial function. These findings suggest that trans-anethole may offer therapeutic benefits in ischemic stroke by targeting multiple pathways involved in neuronal injury.

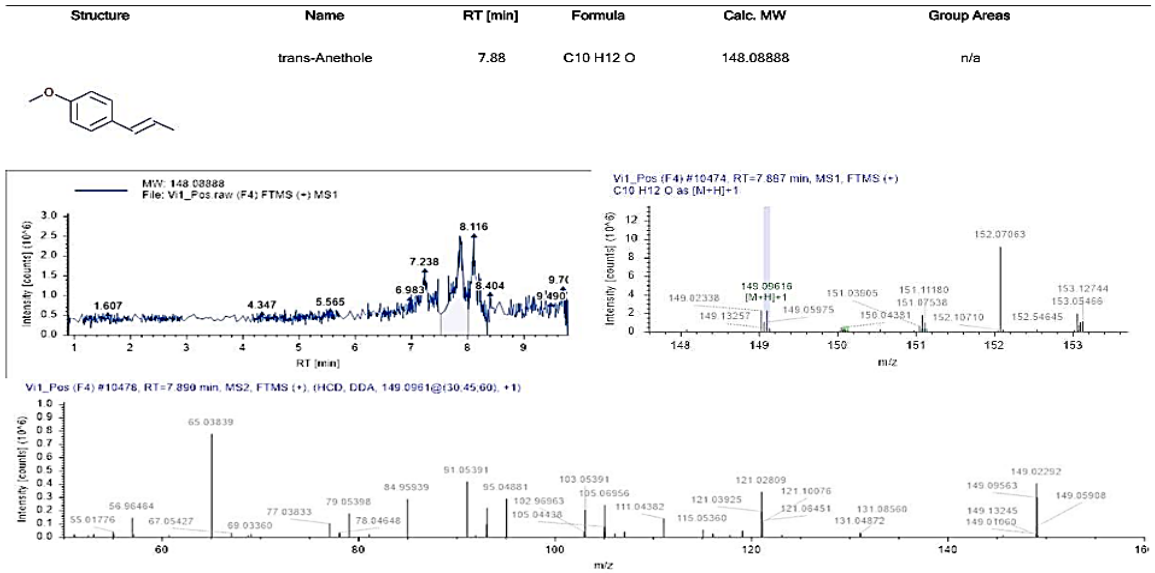

Figure 8: Standard ion chromatogram of Trans-anethole.

DISCUSSION

In Ayurvedic medicine, numerous medicinal herbs are recognized for their cognitive-enhancing and neuroprotective properties. *Acorus calamus* Linn. (*Vacha*) is particularly esteemed for its ability to improve cognitive function, enhance memory retention, and promote mental clarity. To ensure the quality, safety, and efficacy of herbal formulations, rigorous standardization is essential for advancing scientific validation.

Liquid chromatography combined with high-resolution mass spectrometry (LC-HRMS) is a powerful tool used to accurately identify secondary metabolites in unrefined plant extracts. Phytochemical investigations of *Vacha* (*Acorus calamus*) have revealed its neuroprotective properties, which are mainly associated with its ability to neutralize free radicals. This activity highlights its effectiveness in counteracting oxidative stress, reinforcing its potential in promoting neurological health and its therapeutic value. High-resolution mass spectrometry has confirmed the presence of nicotinamide, a neuroprotective compound in *Acorus calamus*, whose protective effects stem from its capacity to alleviate oxidative stress and minimize neuronal injury. Cells possess various internal antioxidant defenses, both enzymatic and nonenzymatic, that protect against damage triggered by oxidative stress.

High-resolution mass spectrometry has confirmed the presence of nicotinamide, a neuroprotective compound in *Acorus calamus*, whose protective effects via enzymatic and non-enzymatic antioxidant defenses protect against damage triggered by oxidative stress by reducing oxidative DNA damage, suppressing glial activation, and modulating apoptotic pathways. Behavioural assays revealed that NAM treatment significantly improved motor function in MPTP-induced neurotoxicity in Parkinson's disease (PD) mice.[31]

Pharmacokinetic studies indicate that betaine accumulation in plasma follows a dose-dependent pattern. Betaine treatment restored the I/R injury by increasing the level of 3-mercaptopyruvate sulfurtransferase (Mpst) and methionine sulfoxide reductases b1 and b2 (Msrb1 and Msrb2), as well as expression of Nos2 (which generates nitric oxide and promotes oxidative stress).[32]

In 6-hydroxydopamine (6-OHDA)-induced hemi-Parkinsonism model, it decreased malondialdehyde concentrations and neuronal loss in the specific area (substantia nigra pars compacta), suggesting a potent antioxidant and neuroprotective agent.[18]

Tranexamic acid suppresses inflammatory cytokine production by blocking plasmin activity, thereby exerting protective effects against amyloid-β accumulation. Actively reduces chronic inflammation, thereby mitigating age-related neurodegeneration. This suppression helps preserve cognitive function, potentially preventing the decline in memory and learning abilities associated with aging.[20]

Anthranilic acid plays a key role in neuroprotection through its involvement in the kynurenine pathway, which regulates tryptophan metabolism and influences neurological health. Alterations in the levels of tryptophan-derived metabolites, including anthranilic acid, have been associated with neurological diseases such as Alzheimer's, depression, and schizophrenia. Shaw, Hess, and Weimer's (2023) study highlights that microbial-derived metabolites can influence brain health through direct and indirect pathways, affecting mood, cognition, and behavior.[21]

Syringic acid pretreatment in spinal cord ischemia/reperfusion injury has demonstrated neuroprotective effects by reducing oxidative stress and neuronal degeneration. It exerts antioxidative properties, scavenging free radicals and minimizing cellular damage. Additionally, it mitigates inflammatory responses, preserving neural integrity and function, thereby enhancing recovery post-injury.[25]

Studies indicate that fraxetin mitigates chronic unpredictable stress (CUS)-induced behavioral deficits, reduces depression-like symptoms, and improves memory performance. It also restores altered neurotransmitter levels, including serotonin, noradrenaline, and dopamine, suggesting its role in managing anxiety and depression.[28]

Trans-anethole has demonstrated the ability to support nerve regeneration and motor recovery in experimental models. A dose-dependent effect was observed, with the highest recovery rate seen at 250 mg/kg. Morphometric analysis revealed that fiber density and myelin sheath thickness were significantly greater in trans-anethole-treated groups compared to the controls. These findings indicate its potential therapeutic role in alleviating nerve damage and managing inflammation-induced sciatica.[29]

Despite the promising neuroprotective effects of *Acorus calamus*, several limitations should be acknowledged. While seven antioxidant and neuroprotective compounds were identified, their potential interactions with other bioactive constituents in *Vacha* remain unexplored, which could influence their overall efficacy. Additionally, individual variability in brain development, shaped by genetic predisposition and health status, was not considered, potentially affecting the applicability of these findings across different populations. Furthermore, the mechanisms underlying the neuroprotective actions of these compounds require more extensive investigation, as the current study provides only preliminary insights. Future research should aim to elucidate these molecular pathways, explore compound interactions, and validate findings through comprehensive in vivo and clinical studies to establish *Acorus calamus* as a reliable neurotherapeutic agent.

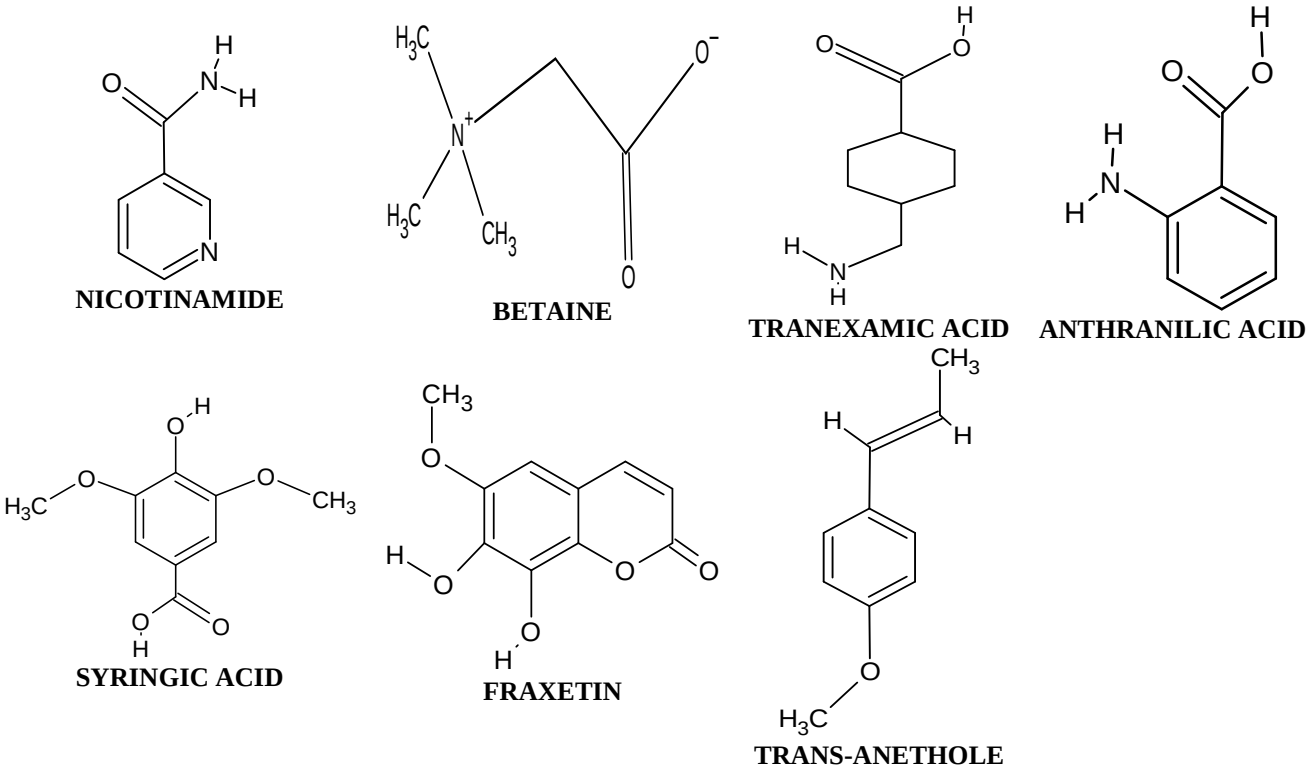

Figure 9: Molecular structure of isolated components from *Acorus calamus* possessing neuroprotective potential as identified through high-resolution mass spectrometry analysis.

CONCLUSIONS

In conclusion, the study on *Acorus calamus* identifies seven key phytoconstituents with significant neuroprotective potential. These bioactive compounds contribute to antioxidative defense, mitigate neuroinflammation, and support neuronal integrity, highlighting their therapeutic relevance in neurodegenerative disorders. While the findings underscore *Acorus calamus* as a promising neuroprotective agent, further studies are required to explore synergistic interactions, optimize bioavailability, and validate clinical efficacy. Advancing research in this area could pave the way for its integration into neurotherapeutic interventions.

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