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Research Article

Mastakam Yoga

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Antioxidant and Neuroprotective Potential of Mastakam Yoga via HRMS Analysis: A study on Acorus calamus & Centella asiatica

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Introduction: Globally, the prevalence of neurodegenerative disorders like Dementia, Parkinson's disease, and Alzheimer's disease is increasing, highlighting the need for effective natural neuroprotective and antioxidant agents. In Ayurveda, herbs like Centella asiatica (Mandukparni) and Acorus calamus (Vacha) are well known for their neuroprotective and memory-boosting properties. In this study, a unique herbal formulation called Mastakam Yoga (MSY) was prepared with hydroalcoholic extracts of these two herbs in equal proportion. MSY is suggested to have synergistic antioxidant and neuroprotective benefits. The objective of this study is to discover bioactive components in Mastakam Yoga using High-Resolution Mass Spectrometry and assess their role in neuroprotective and antioxidant activities.

Materials and Methods: Mastakam Yoga (MSY) was formulated by combining an equal amount of hydroalcoholic extracts of Acorus calamus and Centella asiatica. The formulation was analysed using High-Resolution Mass Spectrometry (HRMS) for phytochemical profiling. The compounds were then examined through existing research to check for known antioxidant or neuroprotective qualities. Emphasis was placed on compounds known for antioxidant, anti-inflammatory, and neuroprotective effects.

Results: Several bioactive substances, including kynurenic acid, betaine, gabapentin, nootkatone, vanillin, and scopoletin, were found by HRMS analysis of Mastakam Yoga. These chemicals are linked to neuroprotective mechanisms that include oxidative stress inhibition, synaptic plasticity enhancement, and neuroinflammation control.

Discussion: The findings suggest that MSY has strong potential as a natural antioxidant and neuroprotective agent. However, further in vivo and clinical trials are essential to validate these findings and examine the potential therapeutic use of MSY in treating cognitive and neurodegenerative diseases.

Keywords: Mastakam Yoga, Neuroprotective, Kynurenic acid, Vanillin, High-Resolution Mass Spectrometry

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Siddharth Shankar, Post Graduate Scholar, Department of Rasa Shastra Evam Bhaishajya Kalpana, Faculty of Ayurveda, IMS, BHU, Varanasi, Uttar Pradesh, India. Email: sidharthbhu98@gmail.com	Shankar S, Singh A, Chouhan P, Gautam DNS, Antioxidant and Neuroprotective Potential of Mastakam Yoga via HRMS Analysis: A study on Acorus calamus & Centella asiatica. J Ayu Int Med Sci. 2025;10(6):104-115. Available From https://jaims.in/jaims/article/view/4455/	

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Introduction

The most complex organ in the human body is the brain, composed mainly of neurons and neuroglial cells. Neurons transmit electrical and chemical signals, whereas neuroglia cells, especially astrocytes and microglia, are essential for maintaining neuronal health and reacting quickly to physiological stress or injury. Dysfunction in these supportive cells can lead to significant neurological impairments.**[1]** Glial cells are triggered in cases of acute neuronal damage and, depending on the severity of the injury, may either start the removal of permanently injured neurons or accelerate repair processes.**[2]** An imbalance between oxidants and antioxidants in the body leads to oxidative stress, which is a significant cause of such neuronal damage. This stress results from the excessive oxidation of biomolecules by reactive oxygen species (ROS) and reactive nitrogen species (RNS), which include molecules such as hydrogen peroxide, hydroxyl radicals, superoxide anions, nitric oxide, and peroxynitrite.**[3,4]** DNA mutations, reduced protein function, and lipid oxidation can all occur under oxidative stress, which ultimately compromises the cell membrane and causes cell death.**[5]** Antioxidants, both synthetic and natural, are essential in the fight against oxidative stress. These substances have anti-inflammatory, antibacterial, anti-cancer, cardioprotective, and neuroprotective properties and neutralize ROS and RNS even at low doses.**[6,7]** Their mechanisms include scavenging free radicals, chelating metal ions, interrupting free radical chain reactions, restoring other antioxidants, and modulating enzyme function.**[8,9]** Defending the central nervous system against acute injuries like stroke and trauma, as well as chronic neurodegenerative diseases like Alzheimer's, Parkinson's, epilepsy, and various types of dementia,is known as neuroprotection.**[10]** Understanding that neuronal damage following ischemic events often unfolds over time highlights the opportunity for timely therapeutic intervention to prevent or reduce further injury. Currently, several neuroprotective drugs aimed at treating acute stroke have progressed to Phase III clinical trials. These include calcium channel blockers, NMDA receptor inhibitors, lubeluzole, CDP-choline, the antioxidant tirilazad, anti-ICAM 1 antibody, GM-1 ganglioside, clomethiazole, sodium channel blocker fosphenytoin, and piracetam.**[11]**

Ayurveda, a traditional system of medicine with roots in ancient India, derives its name from the Sanskrit, meaning "the science of life." It promotes a holistic approach to health with an emphasis on reestablishing equilibrium between the body, mind, intellect, and spirit. According to this system, certain herbs that are known to improve cognition and rejuvenation are categorized as *Medhyarasayanas*, a term composed of the words *Medhya* (intellect or cognition) and *Rasayana* (rejuvenation or revitalization). The most widely recognized of these herbs that improve cognition include *Vacha*, *Mandukparni*, *Shankhapushpi*, *Brahmi*, *Jatamansi*, *Jyotishmati*, and *Ashwagandha*. A new herbal blend identified as *Mastakam Yoga* (MSY) was created with inspiration from this ancient tradition. The term *Mastakam* refers to the head or skull in Sanskrit, while *Yoga* implies a combination or formulation. MSY primarily features two well-known *Medhyarasayana* herbs: *Mandukparni* (*Centella asiatica*) and *Vacha* (*Acorus calamus*), both recognized for their neuroprotective, antioxidant, and memory-enhancing effects. *Acorus calamus* Linn., commonly called *Vacha* or sweet flag, belongs to the *Acoraceae* family and is a tall, perennial marsh plant. Originally from Central Asia and Eastern Europe, this plant is now found all over the world in wetland habitats,including swamps and riverbanks.**[12]** There are various common names for the plant, including cinnamon sedge, sweet flag, flag root, myrtle grass, myrtle sedge, sweet cane, sweet rush, pine root, vaca, and sweet sedge. In *Ayurveda*, the rhizomes are especially valued for their role in promoting brain and nervous system health. Numerous biological activities, such as antioxidant, anti-inflammatory, antimicrobial, anticonvulsant, memory-enhancing, sedative, central nervous system depressant, acetylcholinesterase inhibition, cardiovascular, diuretic, immunosuppressive, genotoxic, and mutagenic effects, have been demonstrated by contemporary pharmacological research on *Acorus calamus*.**[13]** *Centella asiatica* (Linn.), also known as Indian Pennywort, is a slowly spreading perennial herb belonging to the *Apiaceae* family, recognized for its low, creeping growth habit. It usually reaches a height of 15 cm and grows best in damp, dark areas like riverbanks and rice paddies, especially in sandy loam soils.**[16]** The whole plant is utilized in traditional medicine due to its wide range of therapeutic benefits. It is particularly valued for its ability to promote long life,

Boost mental performance, stabilize blood pressure & stimulate nervous system. Despite its significant role in supporting brain health, *Centella asiatica* has been classified as threatened & endangered species by International Union for Conservation of Nature (IUCN).**[17]** Pharmacologically, it exhibits various beneficial properties, including anti-inflammatory, neuroprotective, anxiolytic, sedative, cognitive-enhancing, anti-epileptic & wound-healing activities. **[18]**

Materials and Methods

Authentication And Preparation of *Mastakam Yoga* (MSY)

The ingredients needed to formulate *Mastakam Yoga* (MSY) were purchased for High-Resolution Mass Spectrometry (HRMS) analysis from Gola-Deena Nath local market, Varanasi, Uttar Pradesh, and authenticated at Department of Botany, Institute of Science, Banaras Hindu University. The samples were given following accession codes: *Vacha* (*Acorus calamus* L.) – Acora. 2024/01 and *Mandukparni* (*Centella asiatica* L. Urb.) – Apia. 2024/01. *Churna* (powder) was made from raw components after authentication, and each one was then extracted separately using a hydroalcoholic solvent. *Vacha* and *Mandukparni's* resultant extracts had different, distinctive smells and looked pale and dark brown, respectively. The dried, solvent-free extracts of *Vacha* and *Mandukparni* were combined in equal parts using a ceramic mortar and pestle to create *Mastakam Yoga* (MSY). After 15 minutes of trituration, mixture was homogenous and produced a dark brown product with a distinct scent.

Method Employed for HRMS Analysis

For High-Resolution Mass Spectrometry (HRMS) analysis of *Mastakam Yoga* (MSY), 100 mg of the formulation was mixed with 1.5 ml of a methanol-water solvent system (80:20) and homogenized using an Eppendorf Thermomixer at 750 rpm for 30 minutes at 25 °C. The homogenate was centrifuged at 3,500 rpm for 10 minutes, and the resulting supernatant was filtered through a 0.22 µm polytetrafluoroethylene (PTFE) syringe filter. A 4 µl aliquot of the filtrate was then injected into a C18 reverse-phase high-performance liquid chromatography (RP-HPLC) column (Hypersil GOLD™, Thermo Fisher Scientific; 1.9 µm particle size, 2.1 mm × 100 mm).

Chromatographic separation was carried out using gradient that began with an aqueous phase containing 0.1% formic acid & transitioned to highly organic phase (methanol with 0.1% formic acid). Gradient schedule was as follows: 5% methanol from 0–6 minutes, 30% from 6–10 minutes, 50% from 10–20 minutes, 90% from 20–27 minutes & then returning to 5% from 27–30 minutes. Flow rate was maintained at 300 µL/min, with column oven temperature of 40°C. Optimized MSY sample was analyzed for metabolomic profiling using Thermo Fisher Scientific Orbitrap Eclipse Tribrid mass spectrometer coupled with Dionex Ultimate 3000 Rapid Separation Liquid Chromatography (RSLC) system. Heated electrospray ionization (HESI) source introduced sample into mass spectrometer following chromatographic separation. Orbitrap analyzer operated at resolution of 60,000 in both positive & negative ion modes across mass-to-charge (m/z) range of 100–1,000. Key settings included 35% RF lens, 25% normalized automatic gain control (AGC) target, & minimum intensity threshold of 2.0e5 for MS-OT scans. For data-dependent MS2 (ddMS2) with higher-energy collisional dissociation (HCD), parameters included quadrupole isolation (1.5 m/z window), HCD activation at 30%, 45%, & 60% collision energies, Orbitrap resolution at 15,000, & 20% normalized AGC target. Raw data were processed using Compound Discoverer 3.3.2.31 (Thermo Fisher Scientific) through Natural Product Unknown ID workflow, which included both online & local database searches, focusing on untargeted metabolomic analysis without statistical evaluation. Software performed retention time alignment, unknown compound detection & sample-wide grouping, while predicting elemental compositions & filtering out background noise using blanks. Compound identification involved spectral matching with mzCloud (HighChem LLC) for ddMS2 & DIA data, exact mass or formula searches through ChemSpider (Royal Society of Chemistry), & local database comparisons using mass lists with or without retention time data. Spectral similarity scoring was applied via mzCloud, & spectral distance scoring was used for ChemSpider & mass list identifications.**[19]**

Results

The overall intensity of all detected ions throughout time is represent by total ion chromatogram (TIC).

It is an essential analytical instrument for determining the different compounds present in a sample and interpreting its composition. The TIC offers important information about the components of the sample by graphically representing the ions found during chromatographic separation. The TIC for the components of *Mastakam Yoga* is shown in Figure.**[1]**

Antioxidant and Neuroprotective Properties of the Phytochemical Components of *Mastakam Yoga*

HRMS analysis led to the identification of 4164 phytochemical constituents in the *Mastakam Yoga* (Equal combination of *Acorus calamus* and *Centella asiatica*). Among those phytochemical components, the following components, namely Kynurenic acid, Kaempferol, Betaine, Nootkatone, Gabapentin, Vanillin, and Scopoletin, exhibit neuroprotective and antioxidant activity as per the following references.

Kynurenic acid (KYNA)

Kynurenic acid (KYNA) is an endogenous tryptophan (Trp) metabolite with neuroprotective properties. Martos et al. (2022) investigated the role of KYNA (Fig. 2) in the passive avoidance cognitive test in mice, with a particular emphasis on memory consolidation, retention, and retrieval functions, which was administered via the intracerebroventricular (i.c.v.) route. A high dose (40 µg/2 µL) markedly reduced the memory performance of mice, whereas a low dose 0.5 µg/2 µL enhanced the memory consolidation by prolonging avoidance latency.

The result demonstrated that a low dose of KYNA had a positive effect on a specific memory aspect within the domain of cognition. This effect was at least partially mediated by four neurotransmission systems: serotonergic, dopaminergic, α- and β-adrenergic, and opiate systems**[20]**.

Lugo-Huitrón et al. (2011) demonstrated that the ability of KYNA to scavenge for •OH and O2•− is greater than its effectiveness against ONOO−, and it also diminishes the generation of lipid peroxidation (LP) as well as intracellular and extracellular ROS induced by FeSO4 and 3-NPA (3-nitropropionic acid). It demonstrates KYNA's antioxidant and ROS scavenging capabilities. KYNA was about ten times more potent than GSH (Glutathione-Endogenous antioxidants) at scavenging O2•−.**[21]**

Kaempferol (KF)

Sánchez et al. (2007) studied the neuroprotective effects of KF (Fig. 3) against brain damage in a MCAO model using Wistar rats. The administration of KF intravenously at micromolar doses (10–15 µmol/L of blood) 30 minutes before a 60-minute middle cerebral artery occlusion and right after reperfusion was found to greatly reduce brain damage. Additionally, KF treatment reduced metalloproteinase activation, offered protection against nitrosative-oxidative stress, and diminished apoptotic cell death.**[22]**

Li etal. (2019) reported that KF treatment significantly reduced cerebral infarct volume, attenuated inflammation and blood–brain barrier (BBB) disruption, and improved neurological outcomes in rats, with mechanisms involving reduced pro-inflammatory cytokine production, inhibition of iNOS and COX-2 expression, decreased NF-κB p65 phosphorylation, and reduced MMP-3 expression.**[23]**

Betaine

In the study (Y. Zhang and J. Jia 2023), Betaine treatment effectively decreased the levels of IL-1, IL-18, and TNF-α without affecting cell viability in BV2 microglial cells. The results showed that Betaine (Fig. 4) suppressed the activation of the NLRP3 inflammasome and the NF-κB pathway in BV2 cells treated with AβO.**[24]** Ibi et al. (2019), A selective inhibitor of GAT2/BGT-1, NNC 05–2090 suppressed the preventive effects of betaine on amyloid β peptide (Aβ)-induced cognitive impairment without affecting MDA levels. It suggests that betaine is transported through GAT2/BGT-1 and prevents cognitive impairment in Aβ25–35 injected mice.**[25]**

Nootkatone

Nootkatone (NKT) combined with Schisandrin (SCH) exhibited neuroprotective properties by influencing PI3K/AKT signaling pathway, resulting in reduction of inflammation, apoptosis, and autophagy. The NKT + SCH treatment activated PI3K/AKT/Gsk-3β/mTOR cascade. Furthermore, there was a reduction in levels of inflammation-associated proteins such as NF-κB, IKK, IL-1β, IL-6, and TNF-α. The lowered levels of cleaved-Caspase3 and LC3-II provided additional evidence for suppression of apoptotic and autophagic pathways.**[26]**

He et al. (2018), Alzheimer's disease (AD) model of mice induced by intracerebroventricular (i.c.v.) injection of Aβ1–42oligomers. AD mice received daily intracerebroventricular (i.c.v.) injections of NKT (Fig. 5) at dosages of 0.02 mg/kg and 0.20 mg/kg, or a vehicle (PBS), into the lateral ventricle for five consecutive days. The administration of NKT resulted in decreased levels of malondialdehyde (MDA), amyloid-beta (Aβ), and acetylcholinesterase (AChE) in the brain, while simultaneously raising the levels of glutathione peroxidase (GSH-Px) and improving histopathological characteristics in the hippocampus. These findings highlight the therapeutic potential of NKT in an Aβ1–42-induced Alzheimer's disease mouse model, suggesting its anti-oxidative and anti-AChE properties, along with its ability to inhibit Aβ accumulation.**[27]**

Gabapentin

Neuroprotective effects of gabapentin in experimental spinal cord injury using the clip compression method. Following trauma, the levels of lipid peroxidation in tissues showed that animals treated with gabapentin had improved outcomes compared to the trauma group. These results were no better than the methylprednisolone group. Treatment groups demonstrated better ultrastructural findings than the trauma group. The results of the high-dose (200 mg/kg) gabapentin group were significantly better neuroprotective than the low-dose(30 mg/kg) gabapentin group.**[28]** Yan et al. (2019) showed that dose-dependent (75 and 150 mg/kg) pretreatment with Gabapentin (GBP) protected against cerebral ischemia-reperfusion injury via activation of the PI3K/Akt/mTOR pathway, which provided a neuroprotective function by inhibiting oxidative stress-related neuronal autophagy. This neuroprotection by GBP (Fig. 6) was significantly inhibited by 10 μM LY294002 (PI3K inhibitor).**[29]**

Vanillin

Dhanalakshmi et al.'s (2015) study explored neuroprotective effects of vanillin, a natural phenolic compound, against rotenone-induced neurotoxicity in SH-SY5Y neuroblastoma cells. The findings showed that pretreatment with 100 nM vanillin substantially improved cell viability, restoring it to about 84% of control levels. This protective effect was linked to decreased apoptosis, stabilization of mitochondrial membrane potential, & decrease in production of reactive oxygen species (ROS). Expression of impor. apoptotic proteins, including increased levels of antiapoptotic Bcl-2 & decreased levels of proapoptotic Bax, caspase-3, caspase-8, & caspase-9. Vanillin (Fig. 7) also prevented activation of JNK and p38 mitogen-activated protein kinase (MAPK) signaling pathways, which are linked to cell death caused by rotenone.**[30]**

Scopoletin

The study conducted by Malik et al. (2011) showed antioxidant properties of scopoletin, a natural coumarin molecule, by using a variety of standard assays, using α-Tocopherol as a reference antioxidant. The findings indicated that scopoletin displayed considerable free radical scavenging activity across several measures. Scopoletin efficiently eliminated harmful free radicals from body, exhibiting 63.79% activity in DPPH radical scavenging, 70.21% in hydrogen peroxide scavenging, 68.98% in superoxide radical scavenging, 39.97% in hydroxyl radical scavenging, and 38.61% in ferrous ion (Fe^{2+}) chelation, respectively. The majority of tests proved that scopoletin was slightly less efficient than α-tocopherol.**[31]** The findings suggest that scopoletin (Fig. 7) has strong antioxidant qualities and could be used as an alternative medicine to lessen harm caused by oxidative stress in biological systems.

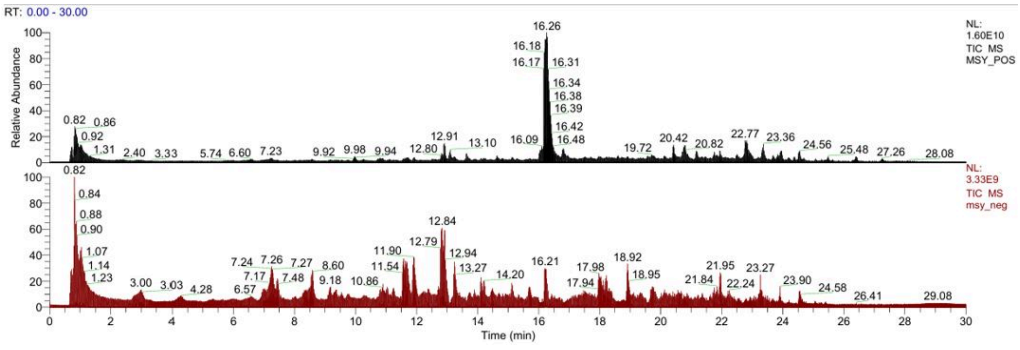


Figure 1 (Part A): Total ion chromatogram of *Mastakam Yoga* obtained through HRMS analysis of the *Mastakam Yoga* sample in both positive and negative ion modes

(HRMS: High Resolution Mass Spectrometry) *

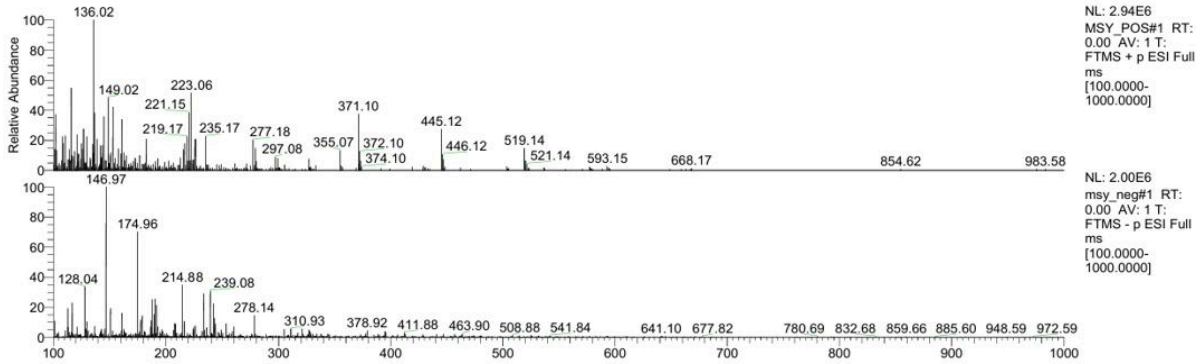


Figure 1 (Part B): Total ion chromatogram of *Mastakam Yoga* obtained through HRMS analysis of the *Mastakam Yoga* sample in both positive and negative ion modes.

(HRMS: High Resolution Mass Spectrometry) *

Structure	Name	RT [min]	Formula	Calc. MW	Group Areas
	Kynurenic acid	6.57	C10 H7 N O3	189.04221	n/a

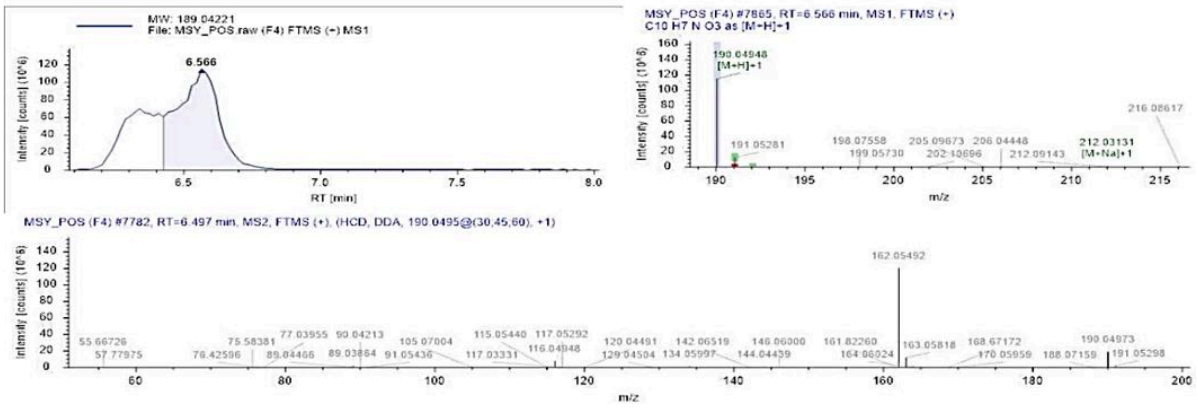


Figure 2: Standard ion chromatogram of Kynurenic Acid.

Structure	Name	RT [min]	Formula	Calc. MW	Group Areas
	Kaempferol	14.72	C15 H10 O6	286.04692	n/a

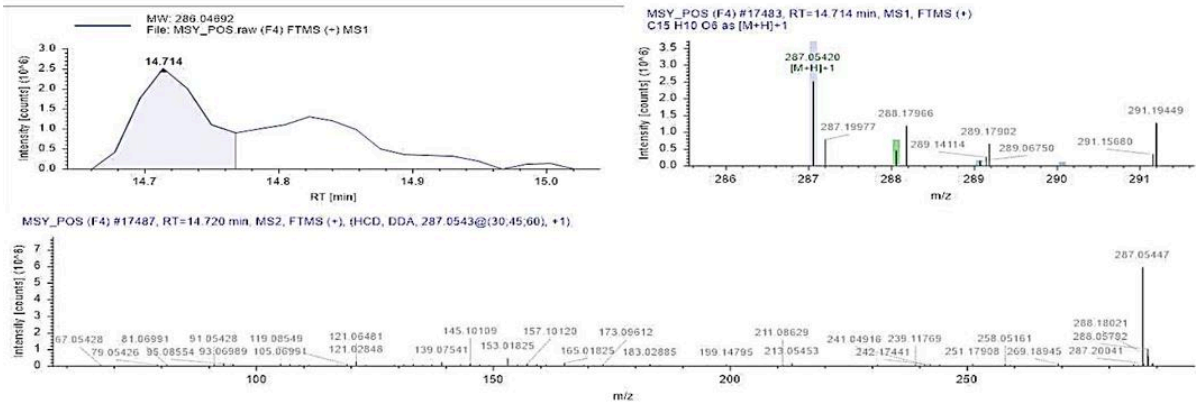
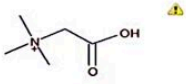


Figure 3: Standard ion chromatogram of Kaempferol.

Structure	Name	RT [min]	Formula	Calc. MW	Group Areas
	Betaine	0.82	C5 H11 N O2	117.07894	n/a

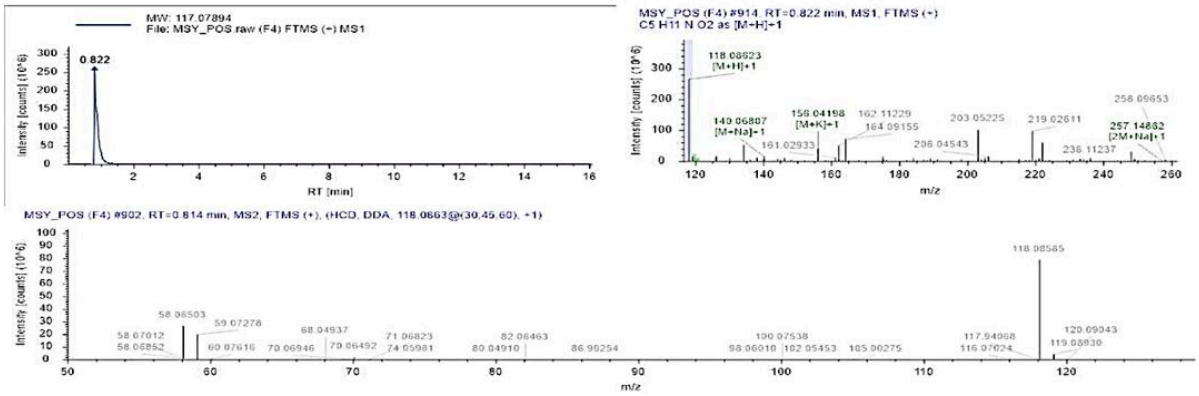
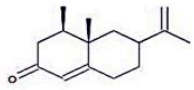


Figure 4: Standard ion chromatogram of Betaine.

Structure	Name	RT [min]	Formula	Calc. MW	Group Areas
	Nootkatone	20.95	C15 H22 O	218.16663	n/a

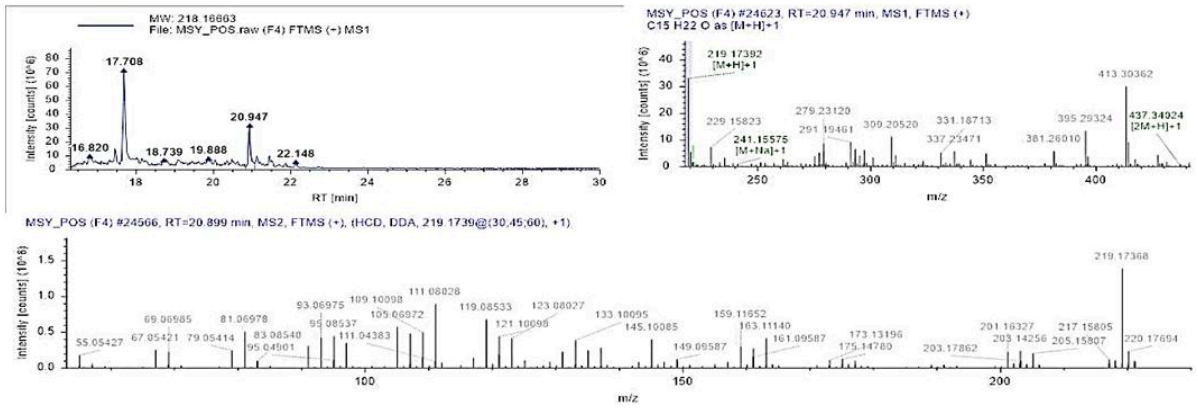
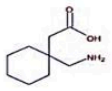


Figure 5: Standard ion chromatogram of Nootkatone.

Structure	Name	RT [min]	Formula	Calc. MW	Group Areas
	Gabapentin	4.16	C9 H17 N O2	171.12576	n/a

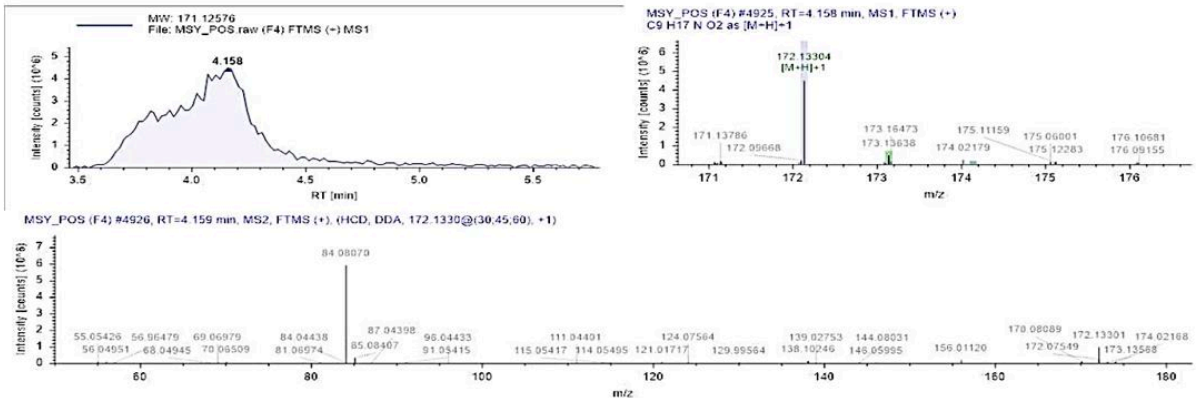


Figure 6: Standard ion chromatogram of Gabapentin.

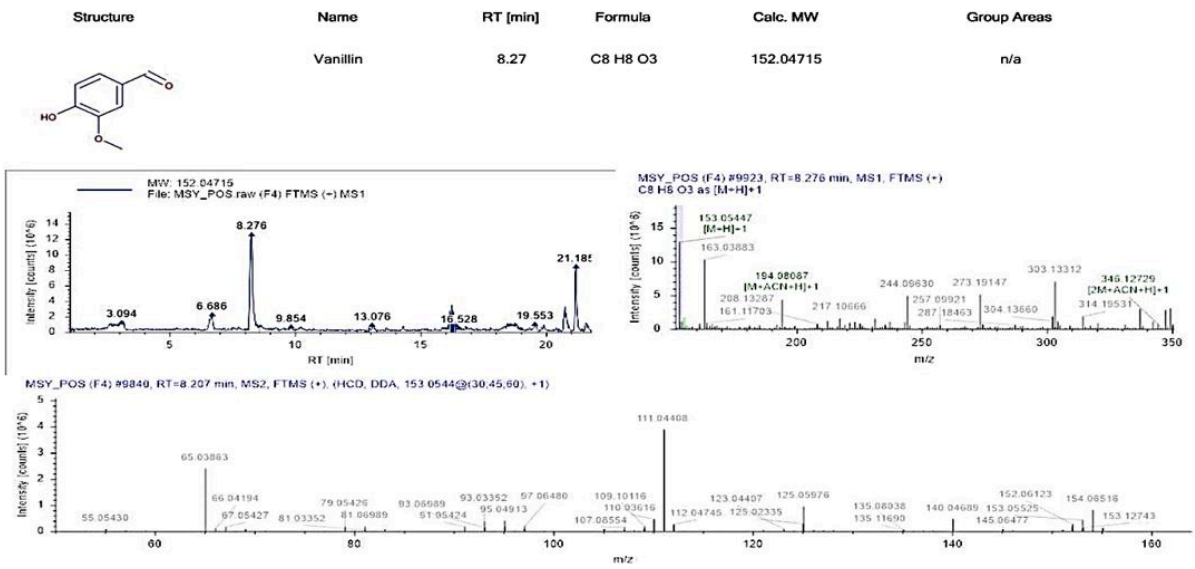


Figure 7: Standard ion chromatogram of Vanillin.

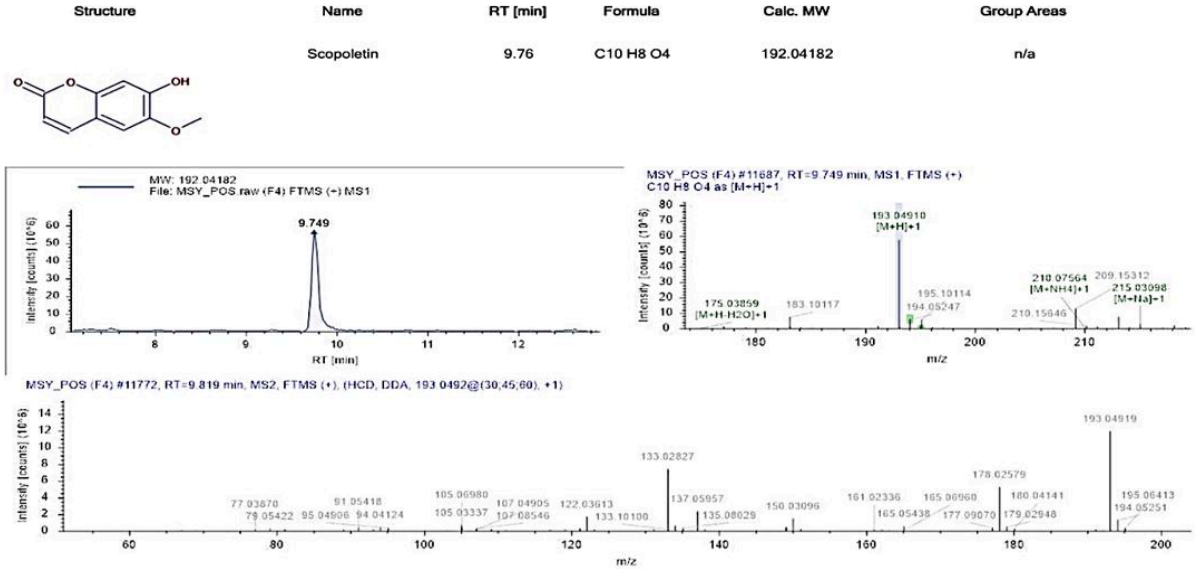


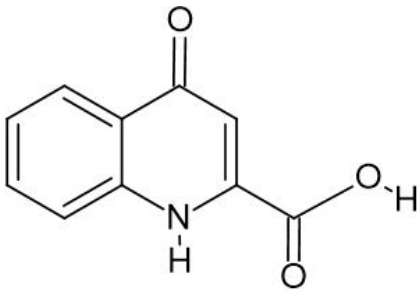
Figure 8: Standard ion chromatogram of Scopoletin.

Discussion

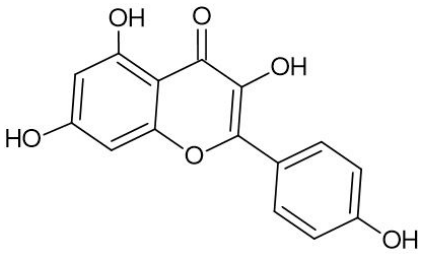
Ayurveda (the Indian traditional system of medicine) emphasizes the use of plants, either independently or in combination. The term "*Medhyarasayana*" in *Ayurveda* describes a class of preparations intended to improve memory and intelligence. The herbs used in these compositions are chosen for their special therapeutic effects (Prabhava) on the brain. The term "*Medhya*" denotes intellect or retention, while "*Rasayana*" signifies rejuvenative therapies that, when practiced regularly, promote nourishment, health, immunity, and longevity**[32]**. These include *Aindri/Bramhi* (*Bacopa monniera*), *Jyothishmati* (*Celastrus panniculata*), *Kushmanda* (*Benincasa hispida*), *Vacha* (*Acorus calamus* Linn.), *Mandukaparni* (*Centella asiatica* Linn.), *Yastimadhu* (*Glycirrhiza glabra* Linn.), *Guduchi* (*Tinospora cordifolia* (Wild) Miers), *Shankhapushpi* (*Convolvulus pleuricaulis* Chois), and *Jatamamsi* (*Nardostachys jatamamsi*). Following that, we created an entirely novel formulationcalled *Mastakam Yoga* (MSY), which contains two herbal extracts: *Vacha* (*Acorus calamus* Linn.) and *Mandukaparni* (*Centella asiatica* Linn.). The raw data from the HRMS were processed by the default parameters of "Compound Discoverer 3.3.2.31" using online databases for comprehensive evaluation. Through high-resolution mass spectrometry (HRMS) analysis, we identified a total of 4,164 phytochemical constituents in *Mastakam Yoga*, underscoring the formulation's significant chemical diversity & potential therapeutic benefits.

Numerous components among these phytochemical constituents have neuroprotective and antioxidant characteristics. As a result, we have emphasized a few essential components, including kynurenic acid, betaine, kaempferol, nootkatone, gabapentin, vanillin, and scopoletin. Kynurenic acid improves memory in low doses byinfluencing NMDA receptors, regulating neurotransmission via the serotonergic, dopaminergic, cholinergic, and opiate systems, and balancing brain excitation and inhibition**[20]**. Furthermore, independent of NMDA and nicotinic receptors, KYNA decreases the production of reactive oxygen species (ROS) and lipid peroxidation in brain regions exposed to $FeSO_4$ and 3-nitropropionic acid.**[21]** Kaempferol's protective effects are region-specific; it reduces brain damage by 70–80% in the striatum, 40–50% in the hippocampus and surrounding caudal striatum, and over 90% in the neocortex.**[22]** Modifying the NF-κB pathway, enhancing neurological outcomes, and maintaining the integrity of the blood-brain barrier also guards against cerebral ischemia/reperfusion injury.**[23]** Betaine reduces inflammation, neuronal degeneration, and glial activation in Alzheimer's disease via blocking the NLRP3 inflammasome and NF-κB pathways.**[24]** Its antioxidant properties, which are independent of GAT2/BGT-1 transporters, also lower MDA levels in the brain and hippocampus and prevent cognitive impairments.**[25]** SCH and NKT markedly reduced amyloid-β secretion by activating the PI3K/AKT/GSK-3β/mTOR signaling pathway, decreasing inflammatory markers, reducing levels of cleaved caspase-3, and increasing LC3-II expression, suggesting inhibition of apoptosis and autophagy.**[26]** In an Alzheimer's disease (AD) mouse model, NKT exerts neuroprotective effects through its antioxidative properties and anti-AChE activities.**[27]** Gabapentin shows greater neuroprotective effects at high-dose (200 mg/kg) than the low dose (30 mg/kg), and methylprednisolone in early injury phases.**[28]** Its neuroprotection is linked with activation of the PI3K/Akt/mTOR signaling pathway, which decreases oxidative stress and autophagic activity in a dose-dependent manner.**[29]** Vanillin pretreatment significantly improved cell viability, reduced reactive oxygen species (ROS) production & preserved mitochondrial membrane potential by activation of p38 & JNK MAPK signaling pathways.**[30]** Scopoletin exhibits antioxidant properties by influencing oxidative stress pathways, probably involving mitochondrial & cytochrome P450 systems.**[31]**

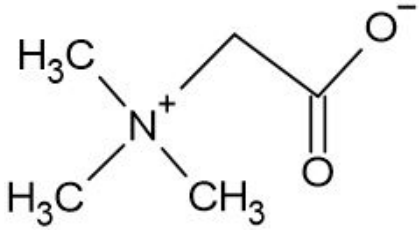
The pharmacological activity of bioactive compounds is intricately linked to their molecular structure, which influences their interaction with biological targets. The antioxidant activity of the specified compounds is governed by Structural features such as the presence and position of hydroxyl groups, conjugated double bonds, aromatic rings, and electron-donating substituents play vital roles in stabilizing free radicals and preventing oxidative damage. Additionally, functional groups capable of modulating neurotransmitter systems, molecular conformation, blood-brain barrier permeability, antioxidant moieties, and anti-inflammatory functionalities determine their neuroprotective efficacy. Hence, the molecular structure of the important components found in *Mastakam Yoga* is depicted in Figure 8.



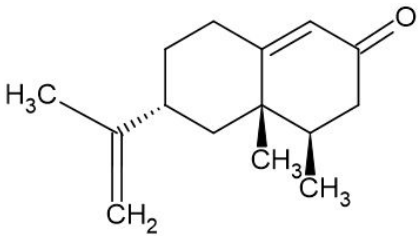
Kynurenic Acid



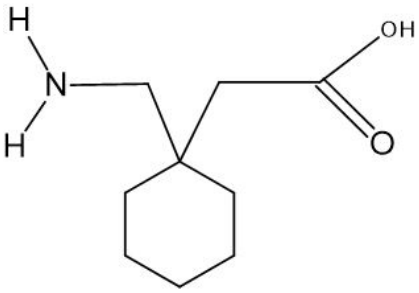
Kaempferol



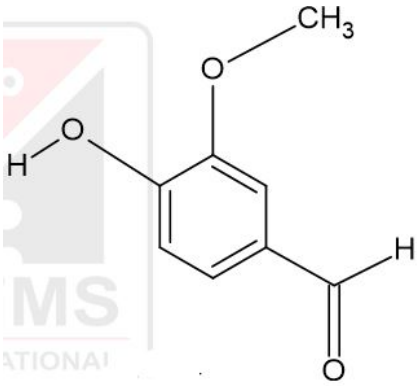
Betaine



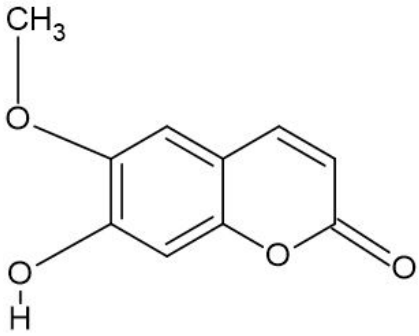
Nootkatone



Gabapentin



Vanillin



Scopoletin

Figure 8: Molecular structure of isolated components from *Mastakam Yoga* possessing neuroprotective and antioxidant properties as identified through High-Resolution Mass Spectrometry analysis. (The authors have created these images)

Conclusion

This study aimed to analyse the neuroprotective and antioxidant properties of MSY, a new *Ayurvedic* formulation composed of equal amounts of hydroalcoholic extracts of *Mandukparni* and *Vacha*. In *Ayurveda*, both herbs are widely known for strengthening nerves, improving memory, and boosting the brain. HRMS was used to examine the phytochemical components of MSY toprove its efficacy scientifically.

Many bioactive compounds, such as kynurenic acid, betaine, gabapentin, nootkatone, and vanillin, were detected by the HRMS data. These phytochemicals are extensively reported in scientific literature for their antioxidant and neuroprotective effects. The presence of these compounds in MSY provides support to the traditional*Ayurvedic* concept that these plants might enhance brain and memory function. Additionally, the study emphasises MSY's potential as a natural antioxidant and neuroprotective agent that may aid in the management or prevention of acute and chronic neurodegenerative diseases. Nevertheless, this study is solely based on phytochemical analysis and review of previously published data. Further research is required to fully comprehend the advantages and safety of MSY. Future in vivo research and carefully planned clinical trials on humans are necessary to validate its effects, determine the appropriate dosage, and examine how it works in the human brain. The study concludes by offering encouraging preliminary evidence that *Mastakam Yoga*, which was created using *Ayurvedic* herbs, might provide a safe and effective herbal remedy to promote brain health. It promotes a closer connection between conventional wisdom and contemporary science through further research and validation.

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