

Liquid Chromatography–Mass Spectrometry Study Focusing on Neuroprotective Effect of Compounds Obtained from *Bacopa monnieri* Juice

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Abstract

Introduction: According to Ayurvedic literature, *Brahmi* is highly recommended for the management of psychiatric problems since it acts on the brain. In traditional Ayurvedic medicine, *Brahmi*, a herb is recommended for the management of various psychological disorders such as *Alakshmi* (inauspicious condition), *Unmada* (insanity), and *Apasmara* (epilepsy). Drug quality is a basic necessity in this day and age, and analytical testing can help combat impurities or subpar pharmaceuticals. **Materials and Methods:** Compound analysis of *Brahmi Juice* (*Swarasa*) using liquid chromatographic mass spectrometry (LC-MS). **Results:** LC-MS analysis shows major compound such as L-Phenylalanine, Phenylacetylene, DL-Arginine, Azelaic acid, Erucamide, D-(+)-Pyroglutamic acid, L-Glutamic acid, Valine, Choline, L-Isoleucine, DL-Tryptophan, L-(-)-Methionine, and Beta-Ionone which play a significant role in neuroprotection. **Conclusion:** The study showed the presence of compounds rich in neurotransmitters which also act as neurotonics having their specific neuroprotective effect in conditions such as Alzheimer’s Disease, Parkinsonism, and Dementia. By employing a wide array of testing procedures, manufacturers are able to ensure compliance with regulatory requirements and supply consumers with a better product that effectively delivers *Brahmi*’s therapeutic benefits.

Key words: Analytical, Ayurveda, Brahmi, liquid chromatographic mass spectrometry, Swarasa

INTRODUCTION

Herb “*Brahmi* (*Bacopa monnieri*)” used for treating psychiatric problems is a well-known Ayurvedic traditional medicine. Based on research, most psychiatric disorders can benefit from the widespread internal and external use of *Brahmi*,^[1] the word “*Brahmi*” comes from the name of the mythological creator of the Hindu pantheon, “*Brahma*.” Since the brain is the center of creative activity in the human body, *Brahmi* refers to substances that promote brain health. The *Charak Samhita* has the earliest direct mention of *Brahmi*’s ability to improve memory. *Brahmi* is recommended as a treatment for mental disorders (retardation) that result in insanity.^[2] Nowadays, a drug’s traits are more crucial in the treatment of illness. A high-quality medicine is more valuable in today’s world. To produce pharmaceuticals of high quality, we must choose raw materials of high quality and follow good manufacturing, storage,

and other procedures. Sometimes, even after doing all of these procedures, the properties of that specific medication are unknown until analysis. Many analytical tests with standard values are mentioned to overcome this. A prepared medication can be regarded as high grade if it passes certain tests.^[3] These tests encompass both qualitative and quantitative analyses of its constituents, contaminants, and physical characteristics. To find and measure any compounds in the formulation, pesticide residue analysis uses techniques such as liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry.^[4] These qualities

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shed light on the stability, uniformity, and acceptance of the formulation by the consumer.^[5] To accomplish the goal, for the first time, we carried out an liquid chromatography-mass spectrometry (LC-MS) analysis of *Brahmi* juice (*Swarasa*).

MATERIALS AND METHODS

Methodology of Brahmi juice extraction

Juice was prepared in Ayurvedic Pharmacy of IMS, BHU with the help of End Runner. First, Brahmi plant material was cut in to small pieces, then crushed in End Runner till formation *Kalka* (Bolus), after that *Kalka* was pressed in clean cloth and juice is obtained.^[6]

Chromatographic conditions

Application Mode: CAMAG Linomat 5 – Applicator

Filtering System: Whatman filter paper No. 1

Stationary Phase: MERCK - TLC/HPTLC Silica gel 60 F254 on aluminum sheets

Application (Y axis) Start Position: 10 mm development

End Position: 80 mm from plate base

Sample Application Volume: 10 µL

Standard Application Volume: 7.5 µL

Distance Between Tracks: 20 mm

Development Mode: CAMAG TLC Twin Trough Chamber 2 of 15

Chamber Saturation Time: 30 min

Mobile Phase: Chloroform: acetone:water (14:2:0.03 v/v)

Pre-chromatographic washing: Pre-chromatographic derivatization was done by diethyl ether

visualization: at 366 nm (after derivatization)

Spray reagent: 1% LLP in hexane

Derivatization mode: CAMAG - Dip tank for about 1 min

Drying Mode, Temp, and Time: TLC Plate Heater Preheated at 100 ± 50 C for 3 min

Methodology of LC-MS for compound analysis of Brahmi Ghrita

Instrument: High-resolution Accurate Mass Spectrometry System

Model: Orbitrap Eclipse Tribrid Mass Spectrometer

Make: Thermo Fisher Scientific

For Small Molecule UHPLC: DionexUltiMate 3000 RS

UHPLC System Solvent Composition for Small Molecule

Solvent A: 100% Water + 0.1% Formic Acid

Solvent B: 80% Acetonitrile + 0.1% Formic Acid

Solvent C: 100% Methanol + 0.1% Formic Acid

Column Detail: Hypersil GOLD™ C18 Selectivity HPLC Column

Particle size 1.9 µm, Diameter 2.1 mm, Length 100 mm.

Analysis

All analyses were conducted using the online database “Compound Discoverer 3.3.2.31” with its default settings. An untargeted metabolomics workflow was employed, utilizing molecular networks, online databases, and mzLogic. Chemical identification was based on fragment patterns from ChemSpider (using formula or precise mass) and mzCloud (ddMS2).

RESULTS

Compound analysis by LC-MS

By performing LC-MS analysis, we found 2749 compounds [Table 1], out of which the compounds that play role in neuroprotection are presented in Table 1.

DISCUSSION

Similar to other Ayurvedic herbs, Brahmi derives its medicinal properties from particular botanical constituents.^[7] In herbal formulations, contaminants such as pesticides, heavy metals, and microbiological infections can present major health hazards. To determine and measure these impurities and guarantee that *Brahmi* is safe to consume, analytical testing is used.^[8] Global regulatory agencies set strict standards for the potency and security of herbal remedies. To prove compliance with these restrictions and enable the lawful distribution and sale of *Brahmi* in both domestic and international markets, analytical data are necessary.^[5] Analytical testing provides important information about the chemical composition and properties of *Brahmi*. Regular testing produces analytical data that can also be used to guide ongoing research and development projects that try to improve the method or develop new uses for Brahmi in the medical field. Ayurvedic medicine is constantly improving and innovating owing to this iterative approach.

Compounds from LC-MS having neuroprotective property

Identifying compounds responsible for the treatment of neurological diseases or associated mechanisms requires a thorough understanding of their pharmacological properties and mechanisms of action. While several compounds listed

may have potential therapeutic effects, some may be more directly relevant to neurological conditions.

The total ions chromatogram and extracted ion chromatograms for some of the significantly identified drugs together with the base peak chromatogram of the sample acquired by negative and positive ion mode along their molecular structure and ion chromatogram are supporting this study.^[9] Here are some compounds from the list that have been studied or implicated in neurological health [Figure 1].

L-phenylalanine

Neurotransmitters including dopamine, norepinephrine, and epinephrine, which are essential for brain function and mood control, are derived from L-phenylalanine.^[10] It is also a part of the treatment for phenylketonuria, a hereditary condition that affects the metabolism of neurotransmitters.

DL-arginine

Arginine is a precursor to nitric oxide, a signaling molecule involved in vasodilation and neurotransmission. It has been studied for its potential neuroprotective effects in conditions such as ischemic stroke and Alzheimer’s disease.^[11]

L-glutamic acid

The central nervous system’s main excitatory neurotransmitter is glutamate. While glutamate modulators are being researched for their potential as treatments for a variety of neurological conditions, excessive glutamate signaling can also lead to neurotoxicity.^[12]

DL-tryptophan

Serotonin, a neurotransmitter important in mood regulation, sleep-wake cycles, and cognitive function, is derived from tryptophan. Its possible antidepressant effects and significance in treating neurological disorders like anxiety and depression have been researched.^[13]

L-(-)-methionine

An important amino acid, methionine is involved in methylation processes and the creation of neurotransmitters. Its neuroprotective qualities and possible advantages in ailments including Parkinson’s disease and age-related cognitive decline have been studied in various researches.^[14]

D-(+)-pyroglutamic acid D-(+)-

Pyroglutamic acid, sometimes referred to as 5-oxoproline, is a metabolite that plays a role in the brain’s antioxidant defence systems and glutathione metabolism. Research indicates that pyroglutamic acid may have neuroprotective effects by promoting the synthesis of glutathione, scavenging free radicals, and reducing the damage that oxidative stress causes to neurons.^[15]

Azelaic Acid

Azelaic acid is a dicarboxylic acid that possesses antibacterial and anti-inflammatory characteristics. Although its direct contribution to neurological disorders is not well understood, its anti-inflammatory properties may have consequences for neuroinflammatory disorders such as multiple sclerosis or neurodegenerative diseases.^[16]

Table 1: Results of isolated compounds in Brahmi juice

Name	Calc. MW	m/z	RT (min)	Area (Max.)
L-Phenylalanine	165.07883	166.08611	1.578	10561069992
Phenylacetylene	102.04691	120.08073	1.579	4565038628
DL-Arginine	174.11139	175.1187	0.406	3115604901
Azelaic acid	188.10394	187.09666	12.978	2584071035
Erucamide	337.334	338.34128	27.303	557172648
D-(+)-Pyroglutamic Acid	129.04245	130.04976	0.396	529943221.7
L-Glutamic acid	147.05247	146.0452	0.846	516581231.8
Valine	117.07887	118.08616	0.433	414187851
Choline	103.09979	104.10706	0.39	337116272.1
L-Isoleucine	131.09457	132.10184	0.574	310377312.1
DL-Tryptophan	204.08972	188.07047	3.163	283504547.7
L-(-)-Methionine	149.05091	150.05817	0.464	278705141.3
Beta-Ionone	192.15122	175.14795	13.828	257690188.2

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