Physicochemical Standardization And Lc–Ms-Based Phytochemical Profiling Of Ajeya Ghrita: A Classical Ayurvedic Formulation

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ABSTRACT
Ajeya Ghrita is a classical Ayurvedic polyherbal formulation described in Ashtanga Sangraha and Sushruta Samhita for the management of toxic conditions (Visha). The present study aimed to prepare Ajeya Ghrita according to classical Sneha Kalpana principles and scientifically standardize it through physicochemical evaluation and Liquid Chromatography–Mass Spectrometry (LC-MS) analysis. The formulation was prepared using authenticated raw drugs and analyzed in an AYUSH-approved laboratory. Organoleptic and physicochemical parameters demonstrated acceptable quality, stability, and microbial safety. LC-MS profiling identified several bioactive phytoconstituents, including ellagic acid, bornyl acetate, licarin A, barbatic acid, kanakugiol, trimethoxychalcone, aphidicolin, isorhamnetin 3-O-glucuronide, agrimol B, and convallotoxin. These compounds are reported to possess antioxidant, anti-inflammatory, neuroprotective, and cholinesterase inhibitory activities. The findings provide scientific evidence supporting the phytochemical composition and quality of Ajeya Ghrita, thereby contributing to its standardization and validating its potential therapeutic application in toxicological and neurodegenerative disorders...

INTRODUCTION
Ayurveda, the ancient Indian system of medicine, emphasizes prevention and management of diseases through holistic therapeutic approaches. Agada Tantra, one among the eight branches of Ayurveda, deals with toxicology and the management of various types of Visha (poisons). Classical Ayurvedic texts describe Gara Visha as cumulative or artificial toxins that gradually produce harmful effects in the body. In the modern era, prolonged exposure to pesticides, herbicides, industrial pollutants, and synthetic chemicals can be correlated with the concept of Gara Visha due to their chronic toxic manifestations.

Ajeya Ghrita is a classical polyherbal ghrita formulation mentioned in Ashtanga Sangraha and Sushruta Samhita in the context of Vishachikitsa. The formulation contains several medicinal herbs possessing Vishaghna, Rasayana, antioxidant, and anti-inflammatory properties. Ingredients such as Madhuka (*Glycyrrhiza glabra*), Haridra (*Curcuma longa*), Manjishta *(Rubia cordifolia*), Tagara (*Valeriana wallichii*), and Sariva (*Hemidesmus indicus*) are known for their therapeutic efficacy in combating oxidative stress and toxic manifestations. Processed in Go Ghrita, the formulation facilitates enhanced absorption and delivery of bioactive phytoconstituents due to the unique properties of Sneha Kalpana.

In recent years, scientific validation and standardization of Ayurvedic formulations have gained significant importance. Advanced analytical techniques such as Liquid Chromatography–Mass Spectrometry (LC-MS) provide accurate phytochemical profiling and identification of bioactive compounds present in herbal formulations. LC-MS is a highly sensitive analytical technique capable of detecting polar, non-volatile, thermolabile, and high molecular weight compounds that are difficult to identify by conventional methods. The technique helps in characterization of phytoconstituents such as flavonoids, alkaloids, phenolics, glycosides, terpenoids, and fatty acids responsible for the pharmacological activity of formulations.

Despite the classical importance and therapeutic utility of Ajeya Ghrita, limited scientific data are available regarding its LC-MS phytochemical profiling. Therefore, the present study was undertaken to prepare Ajeya Ghrita according to classical Ayurvedic principles and to analyse its phytochemical constituents using LC-MS analysis for scientific standardization and validation of the formulation.

MATERIALS AND METHODOLOGY

Collection and Authentication of Raw Drugs

All the raw drugs required for the preparation of Ajeya Ghrita were procured from authenticated Ayurvedic raw drug suppliers and identified in the Department of Dravyaguna. The raw materials were cleaned properly, dried under suitable conditions, and powdered separately for pharmaceutical processing.

Preparation of Ajeya Ghrita

Ajeya Ghrita was prepared according to the reference mentioned in Astanga Sangraha Uttara Sthana following the classical Sneha Kalpana method. The powdered ingredients were converted into Kalka and processed with Go Ghrita and prescribed quantity of water under mild to moderate heat with continuous stirring until attainment of Sneha Siddhi Lakshanas such as Phena Nasha and Varti formation. The prepared Ghrita was filtered through a clean cotton cloth and stored in airtight stainless-steel containers for further analysis.

Organoleptic and Physicochemical Evaluation

The prepared Ajeya Ghrita was evaluated for organoleptic parameters including color, odor, appearance, taste, and consistency. Physicochemical parameters such as specific gravity, acid value, saponification value, iodine value, refractive index, peroxide value, and rancidity tests were carried out using standard procedures in an AYUSH-approved ASU Drug Testing Laboratory.

LC-MS Analysis

LC-MS analysis of Ajeya Ghrita was carried out for phytochemical profiling and identification of bioactive constituents. The analysis was performed using a High-Performance Liquid Chromatography system coupled with Mass Spectrometry equipped with an Electrospray Ionization (ESI) source.

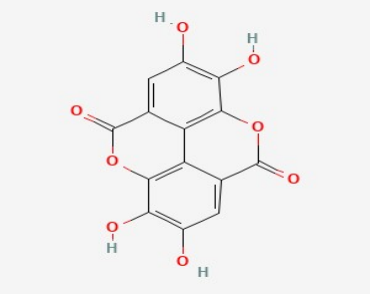
For sample preparation, a measured quantity of Ajeya Ghrita was dissolved in methanol, sonicated, and filtered through a 0.22 µm membrane filter before analysis. Chromatographic separation was achieved using a reverse-phase C18 column under optimized gradient conditions using suitable analytical-grade solvents as mobile phase. Mass spectrometric detection was performed in both positive and negative ionization modes over an appropriate mass-to-charge (m/z) scan range.

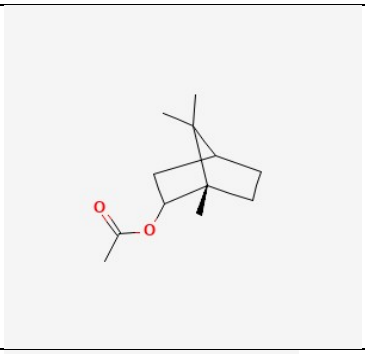
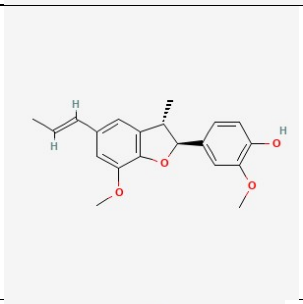
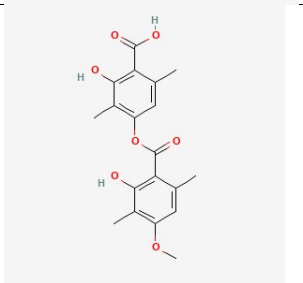
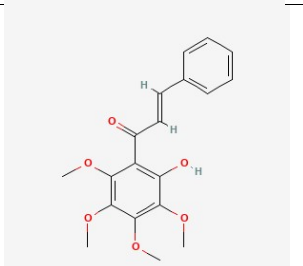
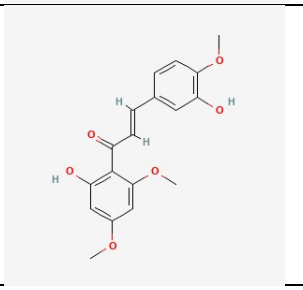
The compounds detected were identified based on retention time, molecular ion peaks, fragmentation patterns, and comparison with standard spectral databases and published literature. The identified phytoconstituents were analyzed for their probable pharmacological activities including antioxidant, anti-inflammatory, and neuroprotective properties.

RESULTS-

Ajeya Ghrita was obtained as a dark brown, semi-viscous formulation. The physicochemical analysis showed an acid value of 0.677, saponification value of 243.64, refractive index of 1.460, specific gravity of 0.950, viscosity at 37 °C of 51.11 cSt, iodine value of 32.51, and peroxide value of 1.07. The total aerobic microbial count was $<10^3$ CFU/ml and the total yeast and mould count was $<10^2$ CFU/ml,

Tab no.1 - Phytoconstituents identified in MS Analysis

Compound	RT (min)	Formula	Structure
Ellagic acid	6.31	C□□H□O□	

Bornyl acetate	9.24	C□□H□□O□	
Licarin A	8.13	C□□H□□O□	
Barbatic acid	11.97	C□□H□□O□	
Kanakugiol	11.06	C□□H□□O□	
2',3-Dihydroxy-4,4',6'-trimethoxychalcone	10.51	C□□H□□O□	

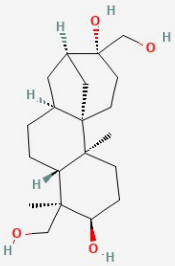
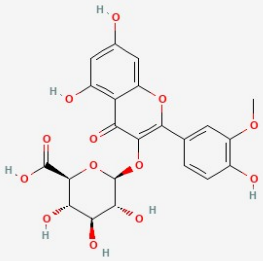
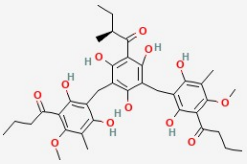
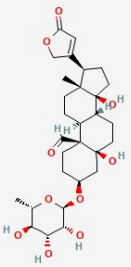
Aphidicolin	11.36	C□□H□□O□	
Isorhamnetin 3-O-glucuronide	1.36	C□□H□□O□□	
Agrimol B	12.68	C□□H□□O□□	
Convallotoxin	12.93	C□□H□□O□□	

Table no – 2 Primary mechanism identified compound in Ajeya Ghrita

Compound	Primary mechanism	Disease Target
Bornyl acetate	Aβ inhibition, NARC-1/PCSK-9, AChE, anti-neuroinflammation	Alzheimer's, MS, neuroinflammation
Ellagic acid	AChE/BACE-1 inhibition, Nrf2/HO-1, tau dephosphorylation, anti-Aβ	Alzheimer's, Parkinson's, Huntington's
Kanakugiol	MAO-A/B inhibition, GABA-B activation, anti-excitotoxic, BBB-permeable	Alzheimer's, Parkinson's, depression
Trimethoxy chalcone	NLRP3 suppression, Nrf2 activation, BuChE inhibition	Neuroinflammation, Alzheimer's
Isorhamnetin 3-O-glucuronide	IL-6 suppression in Aβ-microglia, p-JNK/p38/NF-κB inhibition	Alzheimer's, neuroinflammation
Licarin A	BACE-1 inhibition, AChE inhibition, TNF-α/p38 MAPK suppression	Alzheimer's, neuroinflammation
Barbatic acid	AChE inhibition, BDNF/NGF upregulation, neurotrophic activity	Alzheimer's, neurodegeneration
Aphidicolin	DNA-pol α inhibition preventing neuronal cell cycle re-entry	Alzheimer's (early-stage)

DISCUSSION

The present study was undertaken to prepare Ajeya Ghrita according to the classical Sneha Kalpana method and to scientifically standardize the formulation through physicochemical evaluation and LC-MS analysis. The prepared formulation was obtained as a dark brown, semi-viscous ghrita, indicating proper completion of the pharmaceutical process and uniform incorporation of the herbal constituents into the lipid base.

The physicochemical parameters demonstrated the quality and stability of the formulation. The acid value (0.677) indicated minimal hydrolysis and low free fatty acid content, reflecting good storage stability. The peroxide value (1.07) suggested negligible oxidative deterioration, confirming that the ghrita remained resistant to rancidity. The saponification value (243.64) reflected the presence of short- and medium-chain fatty acids, which may enhance digestion and facilitate the absorption of lipid-soluble phytoconstituents. The iodine value (32.51) indicated moderate unsaturation of fatty acids, contributing to both stability and therapeutic efficacy. The specific gravity (0.950), refractive index (1.460), and viscosity (51.11 cSt at 37°C) were consistent with the standard characteristics of a properly prepared medicated ghrita. Furthermore, the total aerobic microbial count ($<10^3$ CFU/ml) and total yeast and mould count ($<10^2$ CFU/ml) confirmed that the formulation was microbiologically safe and complied with acceptable quality standards.

LC-MS analysis revealed the presence of several biologically active phytoconstituents, providing scientific evidence for the therapeutic potential of Ajeya Ghrita. Ellagic acid has established antioxidant, anti-inflammatory, and neuroprotective activities through inhibition of oxidative stress, amyloid-β aggregation, and neuronal damage. Bornyl acetate possesses anti-neuroinflammatory and neuroprotective properties and has been reported to maintain blood–brain barrier integrity. Licarin A exhibits acetylcholinesterase (AChE) and BACE-1 inhibitory activity along with suppression of inflammatory mediators, suggesting its potential role in neuroprotection. Kanakugiol has demonstrated MAO-A/B inhibitory activity, GABA-B receptor modulation, and protection against neuronal excitotoxicity. Isorhamnetin 3-O-glucuronide has been shown to suppress IL-6 and NF-κB signaling, thereby reducing neuroinflammation. Trimethoxychalcone exhibits antioxidant and anti-inflammatory effects through activation of the Nrf2 pathway and inhibition of the NLRP3 inflammasome. Barbatic acid possesses neurotrophic and acetylcholinesterase inhibitory activity, while Aphidicolin has been reported to prevent neuronal cell-cycle re-entry, a mechanism associated with early neurodegenerative changes. The identification of Agrimol B and Convallotoxin further supports the diverse phytochemical composition of the formulation, although their therapeutic role in Ajeya Ghrita requires further investigation.

The presence of multiple phytoconstituents with complementary antioxidant, anti-inflammatory, cholinesterase inhibitory, and neuroprotective activities suggests that the therapeutic efficacy of Ajeya Ghrita may result from the synergistic action of these bioactive compounds. Thus, the present LC-MS profiling not only validates the phytochemical richness of the formulation but also provides a scientific basis for its traditional use in Vishachikitsa. The findings contribute to the quality standardization of Ajeya Ghrita and establish a foundation for future pharmacological and clinical studies exploring its potential in toxicological and neurodegenerative disorders.

CONCLUSION

The present study successfully prepared and standardized Ajeya Ghrita according to classical Ayurvedic principles. Physicochemical and microbiological evaluation confirmed the quality, stability, and safety of the formulation. LC-MS analysis identified several bioactive phytoconstituents with established antioxidant, anti-inflammatory, and neuroprotective activities, providing scientific evidence for its therapeutic potential. The findings support the standardization of Ajeya Ghrita and establish a scientific basis for its traditional use, while encouraging further pharmacological and clinical studies

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