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QUANTITATIVE ESTIMATION OF PHYTO-CHEMICALS IN PATHADI GHRITA THROUGH HPLC METHOD

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ABSTRACT

Introduction: A traditional Ayurvedic remedy called Pathadi Ghrita is recommended for neurological conditions and Medhya (cognitive enhancement). For quality control and scientific validation, standardization and phytochemical characterisation of such polyherbal ghrita formulations are crucial.
Objective: To evaluate the phytochemical profile of Pathadi Ghrita using HPLC Method.
Methods: Pathadi Ghrita was subjected to solvent extraction using water:methanol:acetonitrile followed by RP-HPLC-DAD analysis at 210 nm and 254 nm wavelengths. Chromatographic separation was performed on an ULTRASIL-AR (250 × 4.6 mm) column using water-acetonitrile gradient mobile phase. The chromatographic fingerprint and phytoconstituent distribution were analyzed.
Results: RP-HPLC chromatograms demonstrated 62 peaks at 210 nm and 59 peaks at 254 nm, indicating the presence of multiple phytoconstituents. Terpenoids constituted the major class (58–60%), followed by alkaloids (approximately 30%), glycosides (5–6%), flavonoids (0.3%), phenolic acids (0.25%) and phytosterols (0.18%). Piperine was identified as the predominant alkaloid.
Conclusion: RP-HPLC fingerprinting revealed a rich diversity of bioactive metabolites in Pathadi Ghrita, particularly lipid-soluble terpenoids and piperine. The findings support the therapeutic potential and provide a scientific basis for quality control and standardization of Pathadi Ghrita.

KEYWORDS: Pathadi Ghrita, RP-HPLC, Phytochemical profiling, Piperine, Terpenoids, Ayurvedic formulation.

INTRODUCTION

Ayurveda emphasizes the use of Medhya Rasayana formulations for enhancing intellect, memory, concentration and cognitive performance. Ghrita-based formulations are considered ideal carriers for delivering lipophilic phytoconstituents to the central nervous system because of their Yogavahi property and ability to cross biological membranes[1].
Pathadi Ghrita is a classical Ayurvedic formulation containing Patha (Cissampelos pareira), Vacha (Acorus calamus), Shigru (Moringa oleifera), Haritaki (Terminalia chebula), Shunthi (Zingiber officinale), Maricha (Piper nigrum), Pippali (Piper longum), Saindhav Lavana and Ghrita. These ingredients possess documented antioxidant, neuroprotective, anti-inflammatory and cognitive-enhancing activities[2-6].
Modern analytical techniques such as RP-HPLC provide reliable tools for qualitative and quantitative assessment of phytoconstituents in herbal formulations. Chromatographic fingerprinting serves as an important quality-control parameter and helps establish reproducibility and authenticity of herbal medicines[7].
Therefore, the present study was undertaken to develop an RP-HPLC fingerprint profile of Pathadi Ghrita and characterize its major phytochemical constituents.

MATERIALS AND METHODS

ULTRACHROM INNOVATIVES PVT. LTD.
DEALING HPLC | UHPLC | SPC | GC | CE | FTIR | UV TECHNOLOGIES
Accredited Testing Lab by ISO 9001:2015 and ISO/IEC 17025:2017
Companies DIN. No. 07185651 | PAN No. AACCU0049C | GST No. 27AACCU0049C3ZP | VAT No. 27601439583V

INSTRUMENTAION DETAILS

CHROMATOGRAPHY/SPECTROSCOPY CONDITIONS	DESCRIPTIONS/SPECIFICATIONS
HPLC/TLC/GC/HPTLC Column details	ULTRASIL-AR (250 x 4.6 mm. ID)
Mobile Phase/Eluent Compositions	Water-Acetonitrile variable Concentraion
Temperature	31°C
Wavelength Selected	210 and 254 nm
Flow rate	1 mL/minutes
Injection Volume	20 ul
Other Instruments/Equipments/Accessories	UltraSonicator, Rheodyn Injector, Weighing Balanace (Mettler Toledo), Water Bath
	HPLC-DAD Instrument; SHIMADZU Prominence UFLC 20AD

EXPERIMENTAL DETAILS: METHODOLOGY

Exactly 1 mL of Pathadi Ghrita was taken and dissolved in 6 ml of acetonitrile-methanol-water (3:2:1, v/v). It was kept overnight for 12 hours at room temperature in air tight HPLC grade container and then put in water bath at 55°C for 10 minutes. Furthermore, the sample was ultra-sonicated for 10 minutes in order to accelerate the solid-liquid extraction and then filtered through 0.45 um nylon filter. Freshly prepared sample of 20 ul was injected into the HPLC-DAD instrument to investigate medicinal phytoconstituents from Pathadi Ghrita.

All chromatographic conditions have been mentioned in above table and HPLC results were submitted separately along with the HPLC reports.

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Fig.No.01:HPLC Report Of Pathadi Ghrita(Instrumentation Details & Methodology)

Sample Collection

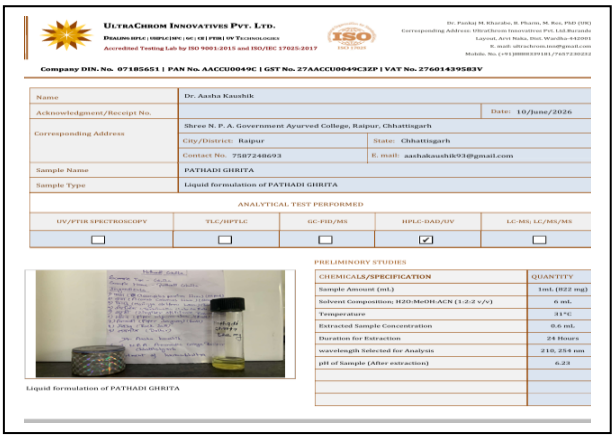
Pathadi Ghrita was prepared according to classical Ayurvedic procedures,Ghrita was analysed in liquid form and submitted for chromatographic evaluation at UltraChrom Innovatives Pvt. Ltd., Wardha, Maharashtra.

Sample Preparation

Exactly 1 mL of Pathadi Ghrita was dissolved in 6 mL of acetonitrile:methanol:water (3:2:1 v/v). The sample was kept overnight in an airtight container and subsequently heated in a water bath at 55°C for 10 minutes. Ultrasonication was carried out for 10 minutes followed by filtration through a 0.45 µm nylon membrane filter. Twenty microliters of the filtrate were injected into the HPLC system.

Instrumentation

- Instrument: SHIMADZU Prominence UFLC HPLC-DAD
- Column: ULTRASIL-AR (250 × 4.6 mm)
- Mobile phase: Water–Acetonitrile
- Flow rate: 1.0 mL/min
- Detection wavelengths: 210 nm and 254 nm
- Injection volume: 20 µL
- Temperature: 31°C



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Name	Dr. Aasha Kaushik
Acknowledgment/Receipt No.	Date: 10/June/2026
Corresponding Address	Shree N. P. A. Government Ayurved College, Raipur, Chhattisgarh
City/District: Raipur	State: Chhattisgarh
Contact No. 7587248693	E. mail: aashakaushik93@gmail.com
Sample Name	PATHADI GHRITA
Sample Type	Liquid formulation of PATHADI GHRITA

ANALYTICAL TEST PERFORMED

UV/FTIR SPECTROSCOPY	TLC/HPTLC	GC-FID/MS	HPLC-DAD/UV	LC-MS/LC/MS/MS
☐	☐	☐	☑	☐

Liquid formulation of PATHADI GHRITA

PRELIMINORY STUDIES

CHEMICALS/SPECIFICATION	QUANTITY
Sample Amount (mL)	1mL (822 mg)
Solvent Composition; H2O-MeOH-ACN (1:2:2 v/v)	6 mL
Temperature	31°C
Extracted Sample Concentration	0.6 mL
Duration for Extraction	24 Hours
wavelength Selected for Analysis	210, 254 nm
pH of Sample (After extraction)	6.23

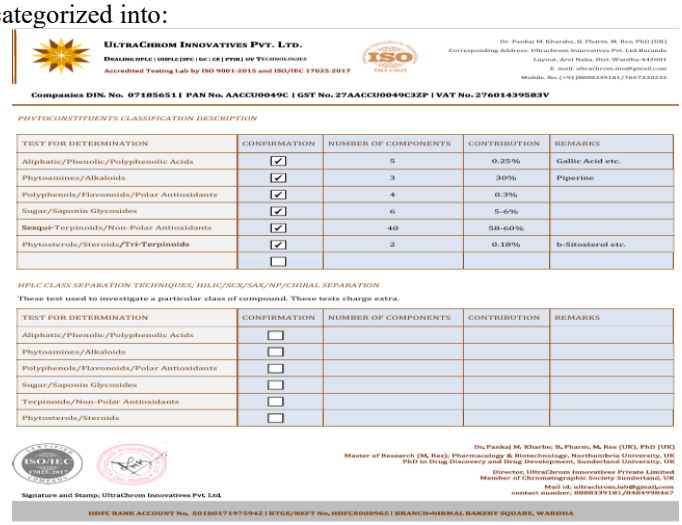
Fig.No.02:HPLC Report Of Pathadi Ghrita(Instrumentations)

Chromatographic Conditions

Chromatographic analysis was performed using a Shimadzu Prominence UFLC HPLC-DAD system equipped with an ULTRASIL-AR column (250 × 4.6 mm). Water-acetonitrile gradient mobile phase was employed at a flow rate of 1 mL/min. Detection was carried out at 210 nm and 254 nm wavelengths. The column temperature was maintained at 31°C.

Phytochemical Classification

Detected compounds were categorized into:



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PHYTOCONSTITUENTS CLASSIFICATION DESCRIPTION

TEST FOR DETERMINATION	CONFIRMATION	NUMBER OF COMPONENTS	CONTRIBUTION	REMARKS
Aliphatic/Phenolic/Polyphenolic Acids	☑	5	0.25%	Gallic Acid etc.
Phytoamines/Alkaloids	☑	3	30%	Piperine
Polyphenols/Flavonoids/Polar Antioxidants	☑	4	0.3%	
Sugar/Saponin Glycosides	☑	6	5-6%	
Sesqui-Terpinoids/Non-Polar Antioxidants	☑	40	58-60%	
Phytosterols/Steroids/Tri-Terpinoids	☑	2	0.18%	b-Sitosterol etc.
	☐			

HPLC CLASS SEPARATION TECHNIQUES; HILIC/SCX/SAX/NP/CHIRAL SEPARATION

These test used to investigate a particular class of compound. These tests charge extra.

TEST FOR DETERMINATION	CONFIRMATION	NUMBER OF COMPONENTS	CONTRIBUTION	REMARKS
Aliphatic/Phenolic/Polyphenolic Acids	☐			
Phytoamines/Alkaloids	☐			
Polyphenols/Flavonoids/Polar Antioxidants	☐			
Sugar/Saponin Glycosides	☐			
Terpinoids/Non-Polar Antioxidants	☐			
Phytosterols/Steroids	☐			

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Fig.No.03:HPLC Report Of Pathadi Ghrita(Detected Compounds)

based on chromatographic behavior and reference spectral data.

RESULTS

Chromatographic Fingerprint

The RP-HPLC chromatogram of Pathadi Ghrita showed a complex phytochemical pattern with:

- 62 detectable peaks at 210 nm

- 59 detectable peaks at 254 nm

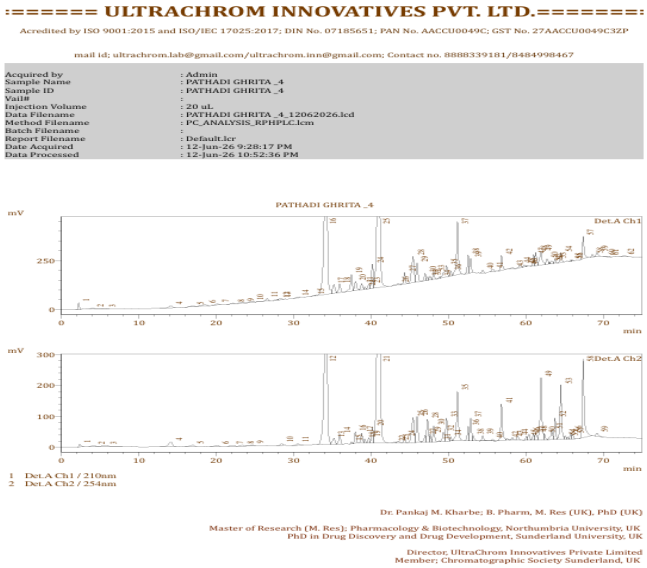

Fig.No.04:HPLC Report Of Pathadi Ghrita(Wide Range Of Phytoconstituents)

indicating the presence of a wide range of phytoconstituents.

Table No.01: Phytoconstituents Of Pathadi Ghrita

S.NO.	Phytochemical Class	Contribution (%)	Components
1.	Phenolic acids	0.25	5
2.	Alkaloids	30	3
3.	Flavonoids	0.30	4
4.	Glycosides	5–6	6
5.	Terpenoids	58–60	40
6.	Phytosterols	0.18	2

Major Peaks

The chromatogram exhibited two dominant peaks around retention times 34 – 41 minutes, contributing approximately 30.94% and 29.02% of the total area respectively, indicating the abundance of major bioactive constituents.

Identified Marker Compounds

The study identified:

- Gallic acid
- Vanillic acid
- Piperine
- β-Sitosterol

among the detectable phytochemical constituents. Piperine represented the predominant alkaloidal constituent.

DISCUSSION

The RP-HPLC profile revealed that Pathadi Ghrita is predominantly composed of lipophilic phytoconstituents. This observation is expected because ghrita selectively extracts and retains lipid-soluble metabolites during the pharmaceutical processing of herbal drugs.

The low proportion of phenolic acids and flavonoids observed in the chromatogram may be attributed to their polar nature and limited partitioning into the lipid matrix of ghrita. Similar findings have been reported in studies evaluating medicated ghee formulations where hydrophilic constituents are present in relatively lower concentrations[8].

Piperine, detected as the major alkaloid, is well known for its bioavailability-enhancing, antioxidant, anti-inflammatory and neuroprotective properties. It enhances absorption of phytochemicals and may contribute significantly to the Medhya action of Pathadi Ghrita[9].

Terpenoids represented the largest phytochemical group (58–60%). Terpenoids derived from Acorus calamus, Zingiber officinale, Piper longum and Moringa oleifera have demonstrated antioxidant, anti-inflammatory and cognitive-enhancing activities in experimental studies[10–12].

The presence of phytosterols, glycosides, phenolic acids and flavonoids further contributes to the pharmacological diversity of the formulation. These compounds collectively provide antioxidant and neuroprotective effects that may support cognitive functions and neuronal health[13].

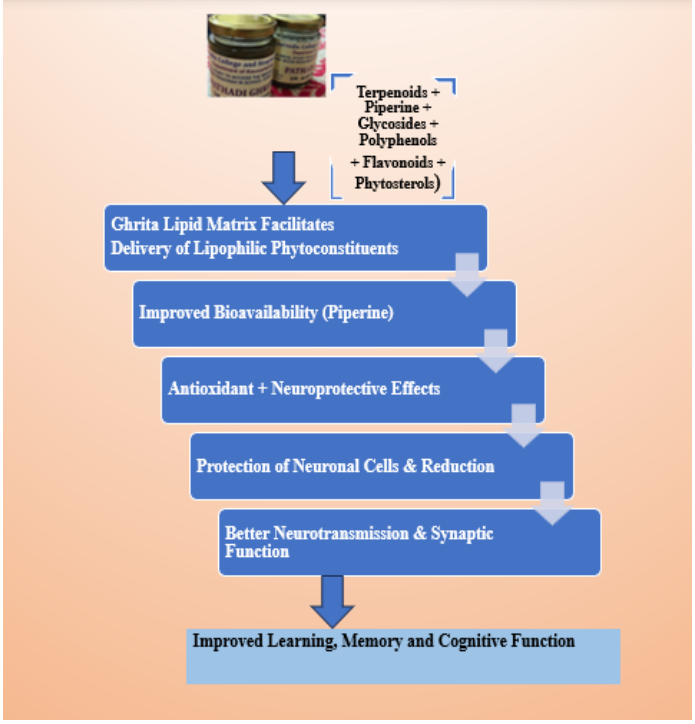

Fig.No05. Machanism and action of Pathadi Grita Contents

Thus, the chromatographic fingerprint generated in the present study can serve as a reference standard for future quality-control investigations and batch-to-batch consistency evaluation of Pathadi Ghrita.

CONCLUSION

RP-HPLC analysis established a characteristic chromatographic fingerprint of Pathadi Ghrita. The formulation demonstrated the presence of 62 phytochemical peaks with terpenoids constituting the major fraction, followed by piperine-rich alkaloids and glycosides. The predominance of lipid-soluble constituents supports the traditional Ayurvedic concept of ghrita as an efficient carrier for neuroactive compounds. The generated fingerprint can be utilized for standardization, quality assurance and future pharmacological investigations of Pathadi Ghrita.

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SPECIFIC PHYTOCONSTITUENTS DESCRIPTION

TEST FOR DETERMINATION	NUMBER OF COMPONENTS	CONTRIBUTION %
Aliphatic/Phenolic/Polyphenolic Acids	Gallic Acid, Vanillic Acid etc	0.25%
Phytoamines/Alkaloids	Piperine etc.	30%
Polyphenols/Flavonoids/Polar Antioxidants	Detected	0.3%
Sugar/Saponin Glycosides	Detected	5-6%
Terpinoids/Non-Polar Antioxidants	Detected	58-60%
Phytosterols/Steroids	B-Sitosterol	0.18%

RISULTS DISCUSSION; CONCLUSION

1. As observed from HPLC analysis, the Ghrita base used for making this formulation has prevented the extraction of polar acid-base strength phytoconstituents like phenolic acids, phytoamines and flavonoids from selected herbs used for making the Pathadi Ghrita. Although they are released in selected extracting solvents; Water:Methanol:Acetonitrile (1:2:3, v/v), but their amount are quite low. Thatswhy the expression of such polar water-methanol soluble acids, alkaloids (Except Piperine) and flavonoids are detected in very less amount in HPLC analysis.
2. Among the alkaloid; Piperine (a neutral phytoamine) is the most prominent alkaloid was detected at 40 minutes in HPLC analysis and it contributes almost 29%. Few Glycosides, almost 5-6% have been detected.
3. Most importantly, as it is a mixture of several Herbal species; *Cissampelas pareira, Acorus calamus, Moringa oleifera, Terminalia chebula, Zingiber officinale, Piper nigrum, Piper longum* most of the hydrophobic (lipid soluble) constituents were detected in HPLC analysis. Terpinoids is the main class of lipid soluble metabolites that has been predominantly observed in HPLC analysis. There are almost 40 such terpinoids have been identified which collectively contributes roughly around 60%. Few Phytosterols (plant based steroids) have also been investigated.

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Fig.No.06:HPLC Report Of Pathadi Ghrita

Future Scope

The HPLC fingerprint established in the present study may serve as a reference for routine quality control and standardization of Pathadi Ghrita. Future investigations should focus on the isolation and characterization of individual bioactive constituents, quantitative estimation of marker compounds and advanced analytical techniques such as LC-MS/MS and GC-MS. Further pharmacological and clinical studies are warranted to correlate the phytochemical profile with the Medhya activity and neuroprotective potential of Pathadi Ghrita, thereby strengthening its scientific validation and therapeutic application.

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