IJBPAS, September, 2025, 14(9): 4974-4982

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANALYTICAL STUDY OF SHOOLAPRASHAMANA CHOORNA –GCMS STUDY

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Received 15th April 2024; Revised 20th Aug. 2024; Accepted 15th Sept. 2024; Available online 1st Sept. 2025

https://doi.org/10.31032/IJBPAS/2025/14.9.9379

ABSTRACT

Aim: For the purpose of analyzing the active principle in the obtained extracts of the herbs, GC-MS is considered as an important tool. This study includes two techniques where the components is separated by Gas chromatography and the mass spectroscopy does the analysis of the active component. As a part of such an analytical aspect, Shoolaprashaman Churna is subjected to GC-MS to study their bio active compounds.

Methodology – 1grm of sample was extracted with 10 ml of methanol and was filtered through a syringe filter (Nylon 13mm 0.2um) and injected 1ml /min at temp. Of 260°C at GCMS. The components of the oil were identified by comparison of their mass spectra with those of the spectrometer/mass spectral database using NIST Library (NIST-08 SPECTRAL DATA). The identifications were confirmed by comparison of fragmentation pattern and their retention indices with those reported in literature.

Result: Result shows peaks with R.T mins as 11.052, 11.807, 11.942 and the compounds identified are Retrofractamide- A, C20H25O3N

(2E, 4E,8E)-9-(Benzo [D], Dioxol-5-YL), (Piperidin-1-YL)Nona-2,-Trien-1-O,C21H25O3N , Trans-2-Methoxy-.Beta.-Methyl-.Beta.-Nitrostyrene 193 C10H11O3N 6306, Safrole , C10H10O2 respectively .

Discussion-Identified compounds have anti-inflammatory, analgesic activity used in the treatment of pain related diseases and also in the management of acute and chronic inflammatory diseases.

Conclusion-Thus, this GC-MS study indicates the scientific validation of Shoolaprashamana choorna as a potent medicine as claimed by Ayurvedic literature and practice.

Keywords: Shoolaprashamana choorna, GC-MS, NIST Library

INTRODUCTION

Assessment of natural synthetic biologically substance and testing of purity is the major aspect for subjecting the chemical analysis in pharmaceutical preparations **[1]**. For analysis of bio-active compounds present in them Chromatography methods are an excellent tool.

Systematic assessment of the qualitative and quantitative compositions in a compound is done by both planar techniques, as well as high-performance liquid chromatography and gas chromatography. In recent years, in both routine analysis and in research centers chromatographic techniques have become more popular **[2]**. In recent research aspects of Ayurveda, analysis always requires fast, highly efficient and reliable methods. Low efficiency and sensitivity, and nonspecific interactions in the analyzes used in our routine clinical applications cause the reliability of these techniques to decrease. As a result, more specific analyzes should be preferred today than traditional methods **[3]**. GC-MS method used for the analysis of obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetics, drugs, the pharmaceutical or food industry, environmental and forensic applications **[4]**. It combines two analytical techniques into a single method of analyzing mixtures of chemical compounds. This study includes two techniques where the components is separated by Gas chromatography and the mass spectroscopy does the analysis of the active component. As a part of such analytical techniques, Shoolaprashamana Churna is subjected to GC-MS to understand their bioactive compounds

MATERIAL AND METHODS

Shoolaprashamana ***Churna*** **[5]**:

This *Churna* (powder) is mentioned in the Charak samhita sutrasthana under Shadvirechanashatasritiya adhayaya 4th chapter.

Shoolaprashamana ***Churna*** is an ayurvedic formulation used for treatment of *Shoola* (Pain).

The medicinal aspects of each plant constituent of *Shoolaprashamana churna* ingredients

is given in **Table 1, 2**.

Table 1: Showing the Ingredients of Shoolaprashamana *Churna*

S. No.	*Sanskrit name*	*Scientific name*	*Parts used*	*Quantity*
1	*Pippali*	*Piper Longum*	Fruit	1Part
2	*Pippali moola*	*Root of piper longum*	Root	1Part
3	*Chavya*	*Piper retrofractrum*	Root	1Part
4	*Chitraka*	*Plumbago zeylanica*	Root	1Part
5	*Nagara*	*Zingiber officinalia*	Rhizome	1Part
6	*Maricha*	*Piper nigrum*	Fruit	1Part
7	*Ajamoda*	*Carum carvi*	Fruit	1Part
8	*Ajaji*	*Cymium cumini*	Fruit	1Part
9	*Ajagandha*	*Cleome viscosa*	Whole plant	1Part
10	**Gandeer*	*Coleus forskohili*	Root	1Part

***As Gandeer is contraversial drug is not used in formulation**

Table 2: Showing the medicinal aspects of each plant constituent of Shoolaprashamana churna

S. No.	*Sanskrit name*	*Scientific name*	*Action*
1	*Pippali*	*Piper Longum*	Anti-inflammatory [16]
2	*Pippali moola*	*Root of piper longum*	neuroprotective, anti-inflammatory, analgesic, antibacterial, antidiabetic, antiulcer [17]
3	*Chavya*	*Piper retrofractrum*	*Anti-helminthic,anti-inflammatory* [18]
4	*Chitraka*	*Plumbago zeylanica*	*Anti-helminthic,anti-inflammatory,anti-diabetic,anti-toxic* [19]
5	*Nagara*	*Zingiber officinalia*	*Anti-inflammatory* [16]
6	*Maricha*	*Piper nigrum*	antioxidant, antipyretic,antifungal, anti-obesity, antibacterial, hepatoprotective [12]
7	*Ajamoda*	*Carum carvi*	*Anti-inflammatory* [11]
8	*Ajaji*	*Cymium cumini*	*Anti-helminthic* [13]
9	*Ajagandha*	*Cleome viscosa*	Anti-inflammatory activity of stems of *Gynandropsis pentaphylla* Linn [14, 15]
10	**Gandeer*	*Coleus forskohili*	-

GCMS Introduction

This present GC-MS study is to see the Biomolecules present in Shoolaprashamana choorna and tries to see and understand the medicinal efficacy of the drug. During the process of this formulation the chemical constituent that contributed must have interacted to produce the Biomolecules observed by GC-MS analysis.

Plant material

All the ingredients of Shoolaprashamana choorna were purchased from authorized Ayurveda shop at Vijayapur and was certified

(letter dated 21/05/2022). All the ingredients were dried in shade, coarsely powdered, and stored for future reference.

Preparation of Extracts

1g of sample was extracted with 10mL of methanol and was filtered through a syringe filter (Nylon 13 mm 0.2um) and injected to GCMS.

GC-MS analysis

GC-MS analysis was performed on the Instrument Model Auto system XL with Turbo mass Gas

Chromatograph/Mass Selective Detector (GC/MSD) with the Triple-Axis High Energy Diode (HED) Electron Multiplier (EM) Detector. Column - DB 5MS 30 m x 0.250mm Diameter x 0.25 Micro Meter

Thickness. Analysis was performed by injecting 1 µL of the sample with a split ratio of 100:1. Helium gas (99.9995%) was used as the carrier gas at a flow rate of 1 mL/min. The analysis was performed in the EI (electron impact) mode with 70 eV of ionization energy. The injector temperature was maintained at 260°C (constant). The column oven temperature program is 75^0C hold for 5minutes Rate 10ºC per minute upto 280ºC hold for 10 minutes.

Table 3

Oven	Rate ºC/min	Value °C/min	Hold time
Initial		**75**	**2**
Ramp 1	**10**	**220**	**5**
Ramp 2	**30**	**280**	**30**

The components of the oil were identified by comparison of their mass spectra with those of the spectrometer using NIST library (NIST - 08 SPECTRAL DATA). The identifications were confirmed by comparison of the fragmentation pattern and their retention indices with those reported in the literature .

Result of GCMS analysis in Shoolaprashamana Churna

Result shows three peaks with R.T mins as11.052,11.807,11.942 and the compounds identified are RETROFRACTAMIDE-A , C20H25O3N (2E,4E,8E)-9-(BENZO[D],DIOXOL-5-YL),(PIPERIDIN-1-YL)NONA-2,-TRIEN-1-O,C21H25O3N , TRANS-2-METHOXY-.BETA.-METHYL-.BETA.-NITROSTYRENE 193 C10H11O3N 6306, SAFROLE , C10H10O2 respectively . The details of each component are given in **Table 4**.

Table 4: Showing the values in GC-MS analysis

SHOOLAPRASHAMANA CHOORNA **160224PARULAYURUNI-3**

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	793	537	4-PENTADECYNE, 15-CHLORO-	242	C15H27Cl	56554-70-2
2	790	503	9,12-OCTADECADIENOIC ACID, (2-PHENYL-1,3-DIOXOLAN-4-YL)METHYL ESTER, CI	442	C28H42O4	56599-47-4
3	789	519	9-OCTADECYNENITRILE	261	C18H31N	56599-96-3
4	753	519	(3R,3AR,4AR,8AS,9AR)-3,8A-DIMETHYL-5-METHYLENEDECAHYDRONAPHTHO[2,3-B]	234	C15H22O2	72523-74-1
5	746	468	9,12-OCTADECADIENOIC ACID, (2-PHENYL-1,3-DIOXOLAN-4-YL)METHYL ESTER, TR	442	C28H42O4	56599-48-5
6	745	426	4-VINYLBICYCLO[3.3.1]NONANE-2,7-DIONE	178	C11H14O2	900210-93-4
7	730	630	10-12-PENTACOSADIYNOIC ACID	374	C25H42O2	66990-32-7
8	721	409	Z,Z,Z-8,9-EPOXYEICOSA-5,11,14-TRIENOIC ACID, METHYL ESTER	334	C21H34O3	900368-68-2
9	720	381	Z-2-ACETOXY-12-TETRADECENITRILE	279	C17H29O2N	900130-78-6
10	718	452	9,12-OCTADECADIENOIC ACID, 2-PHENYL-1,3-DIOXAN-5-YL ESTER, CIS-	442	C28H42O4	56687-50-4
11	718	360	BENZENEACETIC ACID, .ALPHA.,.ALPHA.,4-TRIMETHYL-, ETHYL ESTER	206	C13H18O2	32454-24-3
12	714	406	FUMARIC ACID, HEXYL TETRADEC-3-ENYL ESTER	394	C24H42O4	900348-83-7
13	708	426	NAPHTH[2,3-B]OXIRENE, DECAHYDRO-	152	C10H16O	21399-51-9
14	707	452	CYMEN-7-YL ANGELATE, P-	232	C15H20O2	900383-66-9
15	707	587	10,12-TRICOSADIYNOIC ACID	346	C23H38O2	66990-30-5
16	703	614	METHYL 10,12-PENTACOSADIYNOATE	388	C26H44O2	900342-58-7
17	702	564	2-PROPEN-1-OL, 1-(6,6-DIMETHYLBICYCLO[3.1.1]HEPT-2-YL)-2-PHENYL-	256	C18H24O	900163-97-1
18	701	602	SUCCINIC ACID, DODEC-2-EN-1-YL 3-PHENYLPROP-2-EN-1-YL ESTER	400	C25H36O4	900391-04-9
19	697	335	PHENACYL 11-OCTADECENOATE	400	C26H40O3	900336-79-7
20	697	543	8A(2H)-PHENANTHRENOL, 7-ETHENYLDODECAHYDRO-1,1,4A,7-TETRAMETHYL-, A	332	C22H36O2	41756-14-3

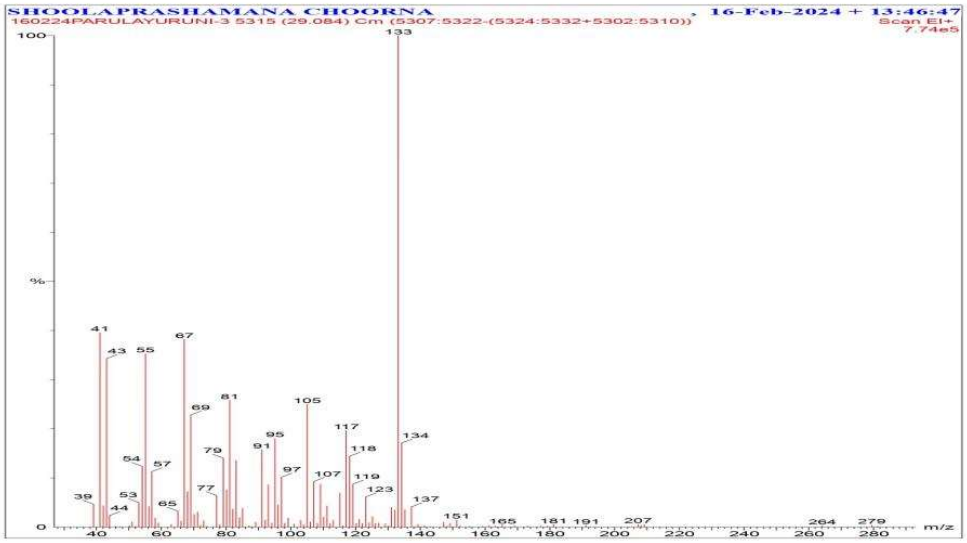
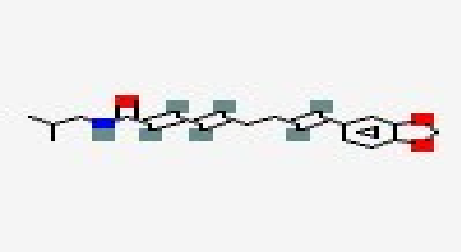


Figure 1: Showing the GCMS analysis

Structure of Active Compounds

1. RETROFRACTAMIDE-A [10]

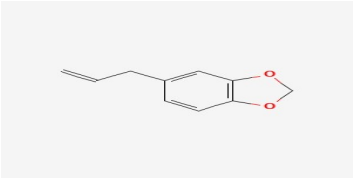
Structure



Ten alkaloids, namely chabamide (1), pellitorine (2), retrofractamide A (3), pyrroperine (4), isopiperolein B (5), piperamide C9:1 (8E) (6), 6,7-dehydrobrachyamide B (7), 4,5-dihydropiperine (8), dehydropipernonaline (9), and piperine (10), were isolated from the fruits of Piper nigrum. Among these,

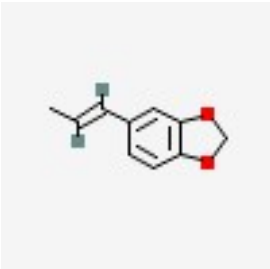
chabamide (1), pellitorine (2), retrofractamide A (3), isopiperolein B (5), and 6,7-dehydrobrachyamide B (7) exhibited significant inhibitory activity on lipopolysaccharide-induced nitric oxide (NO) production in RAW264.7 cells, with IC_{50} values of 6.8, 14.5, 30.2, 23.7, and 38.5 μM, respectively. Furthermore, compound 1 inhibited lipopolysaccharide-induced NO production in bone marrow-derived macrophages with IC_{50} value of 9.5 μM. Consistent with NO inhibition, treatment of RAW264.7 cells with chabamide (1), pellitorine (2), and 6,7-dehydrobrachyamide B (7) suppressed expression of inducible NO synthase and cyclooxygenase-2. Chabamide (1), pellitorine (2), and 6,7-dehydrobrachyamide B (7) induced heme-oxygenase-1 expression at the transcriptional level. In addition, compound 1 induced the nuclear translocation of nuclear factor-E2-related factor 2 (Nrf2) and upregulated the expression of Nrf2 target genes, NAD(P)H:quinone oxidoreductase 1 and γ-glutamyl cysteine synthetase catalytic subunit, in a concentration-dependent manner in RAW264.7 cells. These findings suggest that chabamide (1) from P. nigrum exert antiinflammatory effects via the activation of the Nrf2/heme-oxygenase-1 pathway; hence, it might be a promising candidate for the treatment of inflammatory diseases **[11]**.

2. Safrole



Four new aryl-sulfonamide derivatives (3a, 4a, 5a-b), having methylenedioxy group attached to phenyl ring, were prepared from natural safrole and evaluated as anti-inflammatory agents. The N-methylsulfonamide 3a and corresponding retrosulfonamide derivative 5a were more active than standards indomethacin and nimesulide, at the same molar concentration, in carrageenan-induced pleurisy assay **[12]**.

3.1,3-BENZODIOXOLE, 5-(1-PROPENYL) **[13]**



CONCLUSION

GC-MS analysis **[19]** of the present study is shown by the retention time, peak values of the possible compounds, name of the compounds and their reported medicinal roles. These identified compounds with medicinal properties will contribute the formulation

effectively. Therefore the study indicates the scientific validation of Shoolaprashamana choorna as a potent medicine claimed by Ayurvedic literature.

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