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ORIGINAL ARTICLE

Preparation and Phytochemical Analysis of Lasuna Ksheerapaka with Special Reference to G.C.M.S

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ABSTRACT
Ayurvedic medicines have been valued since ancient times for their therapeutic efficacy, which primarily depends on the diverse phytoconstituents present in the formulations. Lasuna Ksheerapaka is a classical Ayurvedic preparation composed of Lasuna (Allium sativum), milk, and water, prepared according to traditional textual references. The formulation is prepared by adding one part of coarsely powdered Lasuna to eight parts of milk and thirty-two parts of water, followed by boiling and reduction until the final volume equals that of milk, and subsequently filtered. The formulation was subjected to comprehensive standardization parameters, including organoleptic evaluation, physicochemical analysis, phytochemical screening, High-Performance Thin Layer Chromatography (HPTLC) fingerprinting, and Gas Chromatography–Mass Spectrometry (GC–MS) analysis. Physicochemical parameters revealed that the pH was 6.01, loss on drying (LOD) was 82.15%, acid-insoluble ash was 0.001%, total ash was 0.89%, alcohol-soluble extractive value was 19.11%, and water-soluble extractive value was 26.89%. Preliminary phytochemical screening confirmed the presence of alkaloids, glycosides, flavonoids, saponins, triterpenoids, carbohydrates, as well as essential and fatty oils. Elemental analysis indicated the presence of important minerals such as calcium, magnesium, potassium, sodium, iron, phosphate, and chloride. HPTLC fingerprinting demonstrated the presence of multiple phytoconstituents, while GC–MS analysis provided a detailed chemical profile of the formulation. The obtained analytical values comply with the standards prescribed in the Ayurvedic Pharmacopoeia of India (API), thereby authenticating the classical preparation. The identified phytoconstituents and chemical components collectively contribute to the pharmacological potential of Lasuna Ksheerapaka.
Keywords: *Lasuna Ksheerapaka, physicochemical analysis, phytoconstituents, HPTLC fingerprinting, GC–MS.*

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INTRODUCTION

Ayurveda is known as the science of life lying close to nature. Herbal and herbo-mineral preparations are included in Ayurvedic medicines. Ayurvedic formulations are basically classified into Swaras a (juice extracts of the drug), kalka (paste of the drugs), kwatha (decoctions), Hima (cold infusions) [1]. There are several other formulations which are modifications or derivatives of these formulations. Ksheerapaka is an ayurvedic formulation which comes under modification of Kashaya kalpana. In Kashaya the drugs are processed in water and hence the water extractive of the drug is obtained. But in ksheerapaka the drug is processed with water and milk and hence the water soluble and the fat-soluble component of the drug is extracted. The formulation becomes more palatable and milk adds the nutritive value and reduces the tikshnata of the formulation. The use of ksheerapaka can be traced from the samhitha period in Ayurveda. Cow's milk is used in ksheerapaka. For the preparation of ksheerapaka the drugs with astringent, pungent, spicy are mainly used. In ayurvedic classics for the preparation of ksheerapaka various methods are explained. They are the ratio of drug: milk: water is in the ratio 1:8:32 [2],1:15:15 [3], preparation of Kashaya and then adding milk to the Kashaya and formulating it to ksheerapaka[4]. In this study the general method of preparation of ksheerapaka as per Sharangadharacharya is taken into consideration. In this study one part of Lashuna(garlic) is added with 8 times of milk and 32 times of water and is reduced to

the quantity of milk. Lasuna is indicated for cardiac disorders[5]. As per classics Lasuna Ksheerapaka is indicated for the same.
The proper identification and authentication are mandatory for preparing standard formulation. There are various standardization parameters that has to be followed in every steps. Physico-chemical parameters, phyto-chemical evaluations, HPTLC and GCMS are evaluated here. The analysis of the phytoconstituents is very important and is the main part of the study.

MATERIAL AND METHODS

Collection and authentication

The drugs of Lasuna Ksheerapaka as per API were obtained from local market, Kerala. Cow's milk is purchased from farm. The drugs were powdered separately, and they were subjected to Pharmacognostical evaluation at Care Keralam, Kerala.

Pharmaceutical Study

The Lasuna Ksheerapaka is prepared at Department of Rasasastra and Bhaishajya Kalpana Laboratory of Santhigiri Ayurveda Medical College, Palakkad.

The Lasuna was collected. Its outer covering was removed and was ponded. The preparation of Lasuna ksheerapaka was carried out as per the opinion of Sharangadara Acharya. One part of the coarsely powdered drug is added with 8 parts of milk and 32 times of water and is boiled to the quantity of milk. This is then filtered and the filtrate is ksheerapaka. Here cleaned, pounded Lasuna(20g) is added with 8 times of milk(160ml) and 32 times of water(640ml) and is boiled in mild fire and is reduced to the quantity of milk. Then it is filtered and the filtrate is used.

Analytical Study

The Analytical studies, Phytochemical studies ,HPTLC and GCMS of milk and Lasuna ksheerapaka was conducted at CARE KERALAM LTD(CKL), Koratty, Kerala.

The study includes organoleptic characters – Color, odour, consistency. In Physico-chemical analysis, wet analysis includes foreign matter, P^H, Loss on drying, Total ash, Alcohol soluble extractive, Water soluble extractive, Acid insoluble ash and Loss on drying carried out as per API [4, 5]. The phytochemical screening for alkaloids, glycosides, flavonoids, saponins, triterpenoids, tannins, proteins, steroids, carbohydrates, essential oil, fatty oil and carbonates. HPTLC and GCMS profiling were also done.
HPTLC Profile attached, done as per CKL/ANL/HPTLC-001 method.

High Performance Thin Layer Chromatography

HPTLC is the technique that is used in separation of compounds from a mixture. This is the major technique that is used for analysis of herbal medicine. A finger print of the formulation is obtained **[6, 7]**.

Procedure

Develop the plate using the solvent system in twin trough chamber previously saturated with the solvent for 30 min, wash the syringe twice with the methanol. Dry the plate and place it in the scanner. Open a file and enter all parameters for scanning, integration and spectrum. For absorption reflection mode scan the plate in the uv 254 nm and 366nm using Deuterium, Tungsten and Mercury lamp respectively. Scan all the tracks and then scan the UV spectrum of each scanning. Take the finger print of each track. UV spectra spots can be compared in the spectrum display.

Table - 1 Equipment and material specification for HPTLC

Instrument	CAMAG Linomat V Automatic Sample Spotter (Camag Muttenz, Switzerland); the syringe, 100μL (from Hamilton)
Development mode	Ascending
Chamber type	CAMAG glass twin trough chamber (5x10 cm)
Absorbant	Silica gel 60 F254 TLC plates(E.Merk) 0.2mm thickness
Solvent System	Isopropanol: Dichlormethane: Water(5:2.5:1)
Scanning Wave length	366nm
Lamp used	Deuterium,Tungsten, Mercury

A 3 g sample was extracted with methanol and applied onto a silica gel TLC plate. The plate was developed in a suitable solvent system and air-dried.
The developed plate was first observed under UV light at 366 nm, and the Rf values and colors of the resolved bands were recorded.
Subsequently, the plate was sprayed with anisaldehyde–sulphuric acid reagent and heated at 105°C until colored bands appeared. The Rf values and colors of the bands after derivatization were again recorded for

comparison.
This procedure helps in the identification and characterization of phytoconstituents based on their Rf values and color reactions.

Fatty Acid Profile – GCMS

Two drops of sample was mixed with 1ml hexane, shaken for 10 seconds. 200µL of 2N methanolic NaOH was added vortexed, then 200µL of 2N methanolic HCl was added and vortexed. Supernatant solution was taken through a syringe filter (Nylon 13mm 0.2um) and injected to GCMS.

Analysis

Instrument model – 7890 A GC with 5975C with triple axis detector
Column – DB 5MS 30mx0.250mm diameter x 0.25 mm thickness
Analysis was performed by injecting 1µL of the sample with a split ratio of 100:1. Helium gas (99.995%) was used as the carrier gas at a flow rate of 1ml/min. The analysis was performed in the EI (electron impact) mode with 70 EV of ionization energy. The injector temperature was maintained at 280^{o}C (constant).

Table – 2: Thermal analysis

Oven	Rate ^{o}C/min	Value ^{o}C/min	Hold time
Initial		80	5
R amp 1	4	230	5

RESULTS

Pharmaceutical part:

Lasuna ksheerapaka weighing 20g were taken and 160ml of milk was added and to this 640ml of water was added and reduced to quantity of milk . Final product obtained was 160ml.

Analytical Part:

The results of the analytical studies are enlisted in the tables 03 to 10. The results of the HPTLC and GCMS are also enlisted.

Table – 3 .Observations during the preparation of Lasuna Ksheerapaka

Amount of pounded Lasunsa(g)	Quantity of milk (ml)	Quantity of water (ml)	Max temp Degree Celsius	Total yield ml
20g	160ml	640ml	90-95^{o}c	160ml

Table – 4 Organoleptic characters

Parameters	Result
Colour	White
Odour	Garlic smell
Consistency	Liquid

Result: The sample was white in colour with a characteristic garlic-like odour and exhibited a liquid consistency. These organoleptic properties indicate the presence of volatile sulphur-containing compounds and suggest a uniform liquid formulation.

Table – 5 Physico-chemical analysis- wet analysis

Parameters	Result	Test Method
Foreign matter	Nil	
Total ash	0.89%	IP 2018
Alcohol soluble extractive	19.11%	IP 2018
Water soluble extractive	26.89%	IP 2018
Acid insoluble ash	0.01%	IP 2018
P^{H}	6.01%	API Part 1, Vol 1
Loss on drying	82.15%	API Part 1, Vol 1

Interpretation: The physico-chemical evaluation indicates that the sample is free from foreign matter, confirming its purity. The low total ash (0.89%) and very low acid-insoluble ash (0.01%) suggest minimal inorganic and siliceous contamination.
The higher water-soluble extractive value (26.89%) compared to alcohol-soluble extractive (19.11%) indicates the predominance of polar constituents. The pH of 6.01 shows the sample is slightly acidic, which

is generally acceptable for herbal formulations.
The high loss on drying (82.15%) reflects a significant moisture or volatile content, consistent with its liquid nature. Overall, the results support the quality and acceptable physicochemical characteristics of the formulation.
the analytical profile suggests that Lashuna ksheerapaka sample is satisfactory showing low organic and extraneous matter contamination (low total ash and acid insoluble ash). Alcohol soluble and acid soluble extractive values gives the evidence for presence of phytoconstituents, volatile oil, Sulphur compounds, polar compounds, proteins.

Table – 6 : Phyto chemical screening

Parameters	Result	Test Method
Alkaloids	Present	Dragendroff"s reagent test
Glycosides	Present	Picric acid test
Flavanoids	Present	Shinoda test
Saponins	Absent	Foam test
Triterpenoids	Present	Salkowski reaction test
Tannins	Absent	Ferric chloride test
Proteins	Absent	Biurette test
Steroids	Absent	Salkowski reaction test
Carbohydrates	Present	Benedict solution test
Essential oil	Absent	Solubility test
Fatty oil	Present	Solubility test
Carbonates	Absent	Experimental chemistry

Result: The phytochemical screening of screening of Lasuna ksheerapka showed the presence of alkaloids, glycosides, flavonoids, triterpenoids, carbohydrates and fatty oil where as the tests for saponins, tannins, proteins, steroids, fatty oils and carbonates were absent.

Table – 7 Inorganic chemical constituents

Parameters	Results	Test Method
Calcium	0.017%	AOAC 21stEdition
Magnesium	0.02%	CKL/ANL/FP-019
Potassium	0.133%	AOAC21st Edition 2019
Sodium	0.129%	AOAC21stEdition 2019
Iron	0.003%	AOAC21st Edition 2019
Sulphate	0.022%	IS:3025 Part 24
Phosphate	0.0003%	IS:1797, Page 7
Chloride	0.28%	IS:1797,Page 7
Nitrate	Not detected	IS:3025 Part 34

Results: The inorganic chemical analysis ruled out the presence of Calcium, Magnesium, Potassium, Sodium, Iron, Sulphate, Phosphate and Chloride, Nitrate is not detected.
HPTLC of Lasuna Ksheerapaka

winCATS Planar Chromatography Manager

Integration

Properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	5.0 mm
Track end position	75.0 mm
Display scaling	Automatic

All tracks at WavelengthSc4

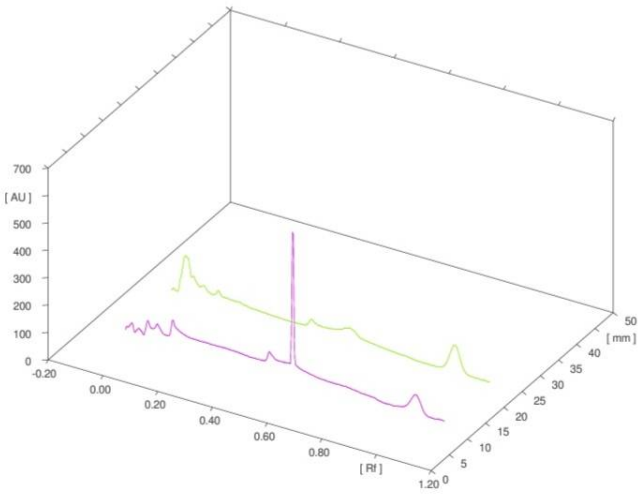

Fig 1: HPTLC of Lasuna Ksheerapaka

Interpretation: In the quality control analysis of the sample, there are many small active compounds to be correlated with tabular peak list below

Table – 8**:** Tabular Peak list data of Lasuna Ksheerapaka

winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.08	11.8	-0.06	30.9	3.30	-0.05	2.0	291.0	2.03	unknown *
2	-0.05	5.2	-0.04	17.8	1.90	-0.02	0.0	186.2	1.30	unknown *
3	-0.02	0.6	-0.00	55.8	5.97	0.01	31.5	673.6	4.70	unknown *
4	0.02	31.9	0.03	53.6	5.72	0.06	22.0	1039.1	7.24	unknown *
5	0.07	22.0	0.09	83.7	8.95	0.14	38.7	2300.0	16.04	unknown *
6	0.41	30.9	0.44	70.1	7.49	0.47	47.2	1787.1	12.46	unknown *
7	0.50	47.5	0.53	531.6	56.79	0.59	31.2	4957.6	34.56	unknown *
8	0.71	23.7	0.72	24.9	2.66	0.80	15.3	1031.0	7.19	unknown *
9	0.89	4.3	0.98	67.6	7.23	1.03	0.5	2077.8	14.49	unknown *

Interpretation: The densitometric scanning of the sample revealed a total of 9 resolved peaks, indicating the presence of nine distinct chemical constituents. Among these, Peak 7 was found to be the dominant component, contributing the highest intensity/area to the chromatogram.

The secondary prominent constituents were identified as Peaks 5, 9, and 2, which also showed comparatively higher responses than the remaining peaks.

GCMS

GCMS of milk and Lasuna Ksheerapaka was carried out.The compounds were identified after comparing the spectral configrations obtained with that of available mass spectral data base(NIST-08 SPECTRAL DATA.

Table – 9 Identified compounds of Lasuna ksheerapaka- GCMS

Parameters	Unit	Milk	Lasuna Ksheerapaka	Test Method
Caprylic Acid(C8:0)	%	0.79	0.80	CKL/ANL/GC-003
Capric Acid(C10:0)	%	1.96	1.99	CKL/ANL/GC-003
Lauric Acid(C12:0)	%	3.11	3.20	CKL/ANL/GC-003
Myristic Acid(C14:0)	%	11.89	11.65	CKL/ANL/GC-003
Palmitic Acid(C16:0)	%	36.59	39.55	CKL/ANL/GC-003
Stearic Acid(C18:0)	%	3.96	41.06	CKL/ANL/GC-003
Oleic Acid(C18:1)	%	36.59	1.17	CKL/ANL/GC-003
Linoleic acid(C18:2)	%	1.21	0.58	CKL/ANL/GC-003

GCMS-Milk Chromatogram Graph- 2

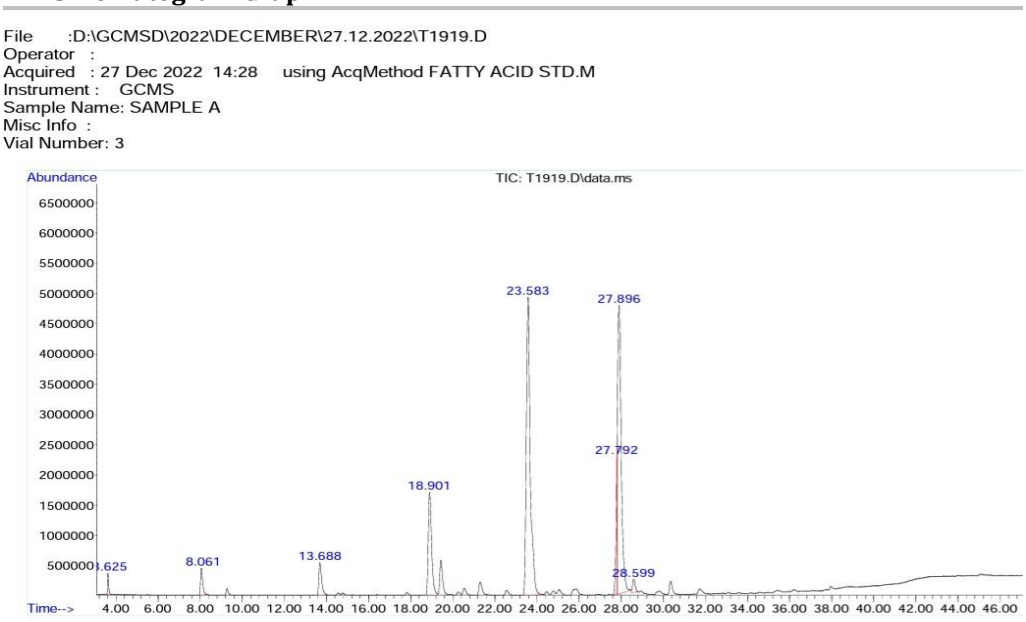

Fig 2: GCMS-Milk Chromatogram

Interpretation: This figure illustrates the total chromatogram obtained from GCMS of milk to find its fatty acid composition.The chemical profile reveals two dominant peaks. The first one eluted RT of 23.583 minutes and second one of RT 27.816 minutes representing volatile fatty acid methyl esters. Minor lipid fractions were also detected indicating the presence of lower molecular weight or shorter chain fatty acids.

Table-10 – GCMS – MILK%

Area Percent Report

Data Path : D:\GCMSD\2022\DECEMBER\27.12.2022\
Data File : T1919.D
Acq On : 27 Dec 2022 14:28
Sample : SAMPLE A

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.625	55	63	79	BB 2	347031	12429522	1.95%	0.789%
2	8.061	565	578	610	BB 2	441836	30940977	4.85%	1.964%
3	13.688	1214	1232	1273	BB 2	532641	48981735	7.68%	3.108%
4	18.901	1816	1838	1883	BV 4	1697760	187329257	29.36%	11.888%
5	23.583	2352	2383	2463	BB 2	4922740	638002176	100.00%	40.489%
6	27.792	2852	2872	2873	M3	2261056	62408295	9.78%	3.961%
7	27.896	2873	2884	2946	M5	4773969	576556704	90.37%	36.590%
8	28.599	2952	2966	2993	VB 7	213623	19089505	2.99%	1.211%

Sum of corrected areas: 1575738172

Interpretations: The table lists 8 distinct peaks representing different chemical compounds detectected in ther sample. Peaks 5 and 7 are the major components making over77% of the sample composition. Peak 5 is the absolute peak. Shorter chain fatty acids appear at peaks 1,2,and 3.

GCMS- Lasuna Ksheerapaka chromatogram

File :D:\GCMSD\2022\DECEMBER\27.12.2022\T1921.D
Operator :
Acquired : 27 Dec 2022 17:02 using AcqMethod FATTY ACID STD.M
Instrument : GCMS
Sample Name: SAMPLE C
Misc Info :
Vial Number: 5

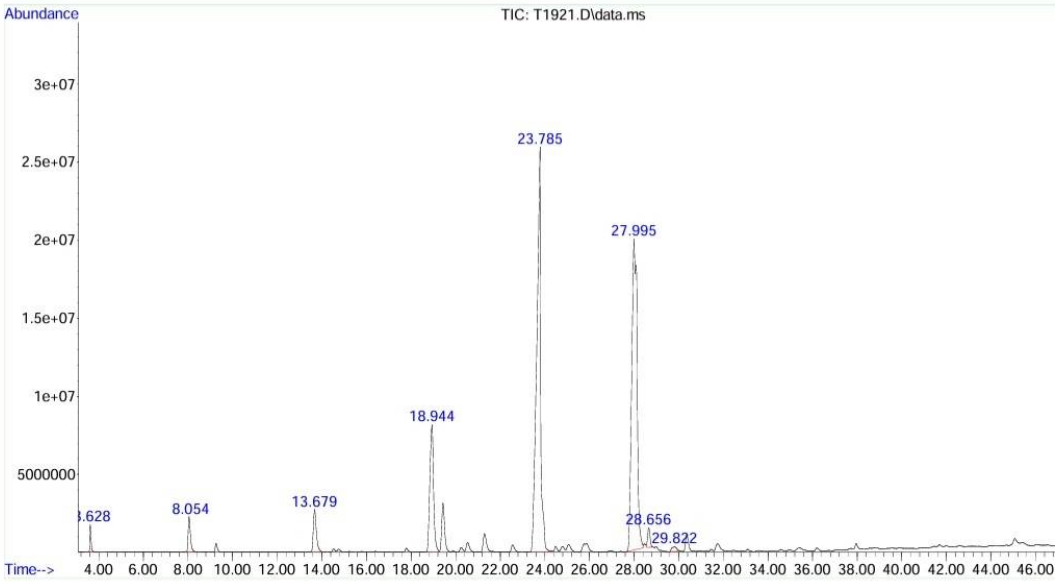

Fig 3: GCMS- Lasuna Ksheerapaka chromatogram

Interpretation: The system was calibrated using a standard fatty acid protocol, ensuring accurate identification of components based on retention time. The chromatogram revealed that the dominant compound appeared at a retention time of 23.785 min, representing the highest peak, which indicates the presence of a major bioactive fatty acid successfully extracted into the milk medium.

The second most abundant compound was observed at a retention time of 27.995 min, suggesting another significant fatty acid constituent. In addition, several minor peaks were detected, representing fatty acids present in lower concentrations.

The major peak at 23.785 min likely corresponds to standard fatty acids such as stearic acid, as supported by comparison with standard data (Table 9). Overall, the chromatographic profile confirms the presence of multiple fatty acids, with one predominant compound and several minor constituents, indicating effective extraction and a well-defined fatty acid composition.

Table – 10 GCMS- Lasuna Ksheerapaka %

Area Percent Report

Data Path : D:\GCMSD\2022\DECEMBER\27.12.2022\
Data File : T1921.D
Acq On : 27 Dec 2022 17:02
Sample : SAMPLE C

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.628	53	63	82	BB	1758454	64716789	1.95%	0.799%
2	8.054	559	577	611	BB	2261488	160969246	4.84%	1.987%
3	13.679	1210	1231	1276	BB	2717736	259510620	7.80%	3.203%
4	18.944	1811	1843	1881	BV 3	8138387	944244259	28.38%	11.654%
5	23.785	2350	2406	2473	BB	25816676	3204283766	96.32%	39.548%
6	27.995	2848	2896	2945	M9	19935966	3326647270	100.00%	41.058%
7	28.656	2960	2972	2989	M5	1245623	94573358	2.84%	1.167%
8	29.822	3075	3108	3138	M5	294607	47276450	1.42%	0.583%

Sum of corrected areas: 8102221758

FATTY ACID STD.M Wed Dec 28 14:55:06 2022

Interpretation: This analysis was done to identify volatile and lipid soluble constituents. The processing of the sample revealed 8 peaks. Major phytoconstituent (peak 6) resolved at R.T of 27.995 min and secondary major constituent (peak 5) runs at RT of 23.785 minutes. The remain peaks represents trace volatile compounds, intermediate fatty acid esters. The compounds are enlisted in Table 10.

DISCUSSION

Analytical evaluation of *Lasuna Ksheerapaka* revealed the presence of multiple bioactive constituents contributing to its therapeutic potential. The detection of alkaloids suggests possible analgesic, anti-inflammatory, and cardioprotective effects. Glycosides are known to support cardiovascular function and may play a role in neuroprotection, along with exhibiting antimicrobial, anti-inflammatory, and anticancer properties [8].

The presence of flavonoids indicates strong antioxidant activity, along with anti-inflammatory, antimutagenic, and anticancer effects, and their role in reducing the risk of coronary heart disease (CHD). Triterpenoids further contribute to cardioprotection and may help in lowering the incidence of coronary disorders [9, 10].

Essential minerals identified in the formulation also play a crucial physiological role. Calcium is vital for maintaining cardiac muscle contraction, while magnesium regulates neuronal activity, intracardiac conduction, and myocardial function through its influence on ion channels such as calcium and potassium. Potassium is essential for maintaining normal cardiac rhythm and electrical conductivity of the heart. Iron supports oxygen transport, mitochondrial activity, and enzymatic processes. Additionally, phosphate balance is critical for ATP synthesis, and its depletion may result in ventricular arrhythmias and reversible myocardial dysfunction. Chloride contributes to fluid balance and electrolyte regulation, particularly through its role in the renin–angiotensin–aldosterone system [11-14].

GC–MS analysis provided detailed chemoprofiling of the formulation. The presence of caprylic acid indicates active lipid metabolism. Lauric acid, a medium-chain triglyceride, is rapidly metabolized in the liver, supports increased HDL (good cholesterol), and aids in weight management. Oleic acid is known for its protective role against atherosclerosis and its beneficial effects on cardiovascular health and insulin sensitivity [15].

Collectively, these phytoconstituents and nutrients contribute to the cardioprotective and therapeutic efficacy of *Lasuna Ksheerapaka*. The GC–MS analysis enabled identification of these compounds, and interpretation was carried out using the National Institute of Standards and Technology (NIST) database, ensuring reliable characterization of the chemical profile.

CONCLUSION

Lasuna Ksheerapaka was prepared following the classical method described in *Sharangadhara Samhita*. The formulation was subsequently subjected to comprehensive phytochemical and analytical evaluation. Preliminary screening confirmed the presence of alkaloids, glycosides, flavonoids, triterpenoids, and fatty oils, indicating a rich phytoconstituent profile.

In addition, the formulation contains essential inorganic elements, including calcium, magnesium, potassium, sodium, iron, sulphate, phosphate, and chloride, which contribute to its therapeutic potential. Nitrogen was not detected in the analysis.

HPTLC profiling revealed the presence of seven distinct constituents, demonstrating a well-defined chromatographic fingerprint suitable for standardization. Further, fatty acid profiling indicated the presence of caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, and oleic acid in varying proportions.

Overall, these findings confirm that *Lasuna Ksheerapaka* possesses a complex composition of bioactive phytochemicals and essential fatty acids, supporting its traditional use and potential therapeutic applications.

LIMITATIONS AND SCOPE OF STUDY:

This study has analysed the phytoconstituents, responsible for the mode of action of the drug. The formulation can be more logically planned according the condition of the patient.

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