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Analytical assessment of *Sukumara Ghrita* using HPTLC and GCMS: Exploring its phytoconstituents and probable mode of action

Research Article

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Abstract

Background: *Sukumara Ghrita* an Ayurvedic ghee-based formulation is renowned for its efficacy in addressing various ailments. Objective: This study employs advanced analytical techniques, specifically High-Performance Thin-Layer Chromatography (HPTLC) and Gas Chromatography-Mass Spectrometry (GC-MS), to comprehensively characterize Sukumara Ghrita and to explore bioactive principles present in Sukumara Ghrita and understand their probable mode of action in treating various ailments. Materials and Methods: The medicine was procured from GMP certified Sitaram Ayurveda Pharma and was subjected to HPTLC and GC–MS analysis after due processing. The methanolic extracts were used for HPTLC and GCMS studies. HPTLC fingerprints for methanolic extracts of *Sukumara Ghrita* was established with good separation and resolution in solvent system Toluene: Ethyl acetate: Hexane (6:3:1). Results: GC-MS analysis of *Sukumara Ghrita* revealed the presence of 11 phytochemical compounds. HPTLC analysis detected the presence of 9 and 14 active biological constituents at 254 nm and 366 nm respectively, which remains to be identified. Conclusion: The probable mode of action of *Sukumara ghrita* can be understood through the action of phytoconstituents screened through HPTLC and GC-MS. The phytoconstituents screened are said to have Anti-oxidant, Anti-hypercholesterolemic, Antihypertriglyceridemic, Anti-inflammatory etc actions.

Keywords: Gas chromatography–mass spectrometry, *Sukumara Ghrita,* HPTLC, Anti-oxidant, Anti-inflammatory.

Introduction

The World Health Organization (WHO) estimates that 70-95 percent of the global population, primarily in emerging economies, relies on traditional and alternative medicines. The rise in natural therapy use has boosted the popularity of herbal medications, prompting efforts to assess their health benefits and establish quality standards (1).

Standardising Ayurvedic herbal formulations is challenging due to various factors and the lack of reference standards. One of the widely used formulation in Ayurveda therapeutics is *Sukumara ghrita*, as there is very minimal data available about its quantitative and qualitative assessment this study aimed to evaluate the same using HPTLC and GCMS methods and to understand its probable mode of action on the basis of the phytoconstituents (2).

The ancient Ayurvedic text Sharangdhar Samhita highlights the importance of Polyherbalism in managing diseases. Polyherbal formulations, contain numerous bioactive compounds from various herbs at low concentrations, target disorders through multiple mechanisms (3).

GC-MS and HPTLC analysis are essential tools in herbal drug research. They enable the precise identification of phytoconstituents even in smaller amounts, which can be challenging to detect using traditional methods of isolation and manual structure elucidation. These techniques provide a comprehensive understanding of the quality and quantity of phytoconstituents in herbal drugs or formulations (4).

Sukumara Ghrita is utilised for various internal and external therapeutic modalities like *Snehapana* (~fat consumption), Basthi(~enema), *picchu* (~oleation), *Yamaka* (~combination of two) type of *Sneha* containing *Ghrita* (ghee) and *Eranda taila* (castor oil) as ingredients. It comprises *Dashamoola* (ten roots) as *Kashaya dravya*, known as the best *Vata shamaka dravya* (*Vata*-normalizing drug). *Eranda taila*, with its sweet, pungent, and astringent taste, along with hot potency, facilitates clearing obstructions, pacifies Vata and Kapha, purifies gametes, and supports the normal movement of Vata dosa (5). To further substantiate the above, the present study was undertaken and aimed at understanding the probable mode of action of *Sukumara Ghrita*. The objectives were to thoroughly characterise *Sukumara Ghrita* and investigate its bioactive components through HPTLC and GCMS analysis.

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Materials and Methods

Sample collection and drug analysis

Sukumara Ghrita was obtained from GMP certified Sitaram Ayurveda Pharma (Batch No-23EGR0568) and Coded As QC/SG/05/23/322.

Physicochemical standardisation

The physicochemical standardisation of the samples was carried out in accordance with the API for the parameters: Organoleptic properties, specific gravity and microbial limits at Quality Control Department, Sitaram Ayurveda Pvt. Ltd (Report No: SAPL/QC/TR/ 2023/248)

Microbial limits

In microbial limit tests, the number of viable aerobic micro-organisms present are estimated. In this study, the presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhimurium* was estimated. The protocol followed for this test was as per the API.

High performance thin layer chromatography

HPTLC Analysis of Sukumara Ghrita was carried out at CARe Keralam Ltd., Koratty, Thrissur, India (Test report ID: 07E70810040A310F) for standardisation.

The HPTLC analysis for the methanol extract of the *Sukumara Ghrita* was carried out. Normal Phase: At a distance of 12.5 mm each, 2 µL sample was applied in three bands of 8mm each on pre-coated silica gel 60 G 254 aluminium plates (5mm × 10 mm) with Linomat 5 applicator attached CAMAG HPTLC system, having WINCATS software. TLC chambers were pre-saturated with Toluene: Ethyl Acetate: Hexane (6:3:1) as mobile phase for 30 min and then the plates were developed. Developed plates were read using Densitometry TLC scanner 3 at 254 and 366 nm in UV cabinet. Anisaldehyde-Sulphuric Acid Reagent was used for Post Chromatographic derivatisation.

GC–MS Protocol

Instrument:7890 A GC with 5975C with triple axis detector.

Sample Preparation

Methanolic extract of drug was prepared and, filtered through a syringe filter (Nylon 13 mm 0.2 µm) and injected to GCMS.

The GC–MS column consisted of DB 5MS (30 mm × 0.25 mm diameter × 0.25 micro meter thickness). Analysis was performed by injecting 1 µl of the sample with a split ratio 1:10. Helium gas (99.999%) 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°C constant. The compounds are identified by GC–MS Library (NIST and WILEY).

Results

Results of Physico-chemical parameters follows the range of standards laid down by Ayurvedic Pharmacopeia of India for Sukumara Ghrita formulation and microbial limits are also in permissible limits that shows safety and quality of the formulation(Table 1).

HPTLC analysis detected the presence of 9 and 11 active biological constituents at 254 nm and 366 nm respectively, which remains to be identified (Table 2 and Figures 1, 2, 3).

Figure 4 indicates the GC–MS profile details of Sukumara Ghrita. Table 3 represents the GC–MS profile of Sukumara Ghrita along with the possible medicinal role of each molecule in it.

Table 1: Physiochemical analysis of Sukumara Ghrita sample

	Parameters tested	Specification/ range	Result
1	Colour	Green	Complies
2	Odour	Characteristic	Complies
3	Taste	Slightly bitter	Complies
4	Loss on drying @105 degree C	Not more than o.5%	0.40
5	Specific gravity @ 5 degree c	0.910-0.920	0.917
6	Acid Value	Not more than 4	1.64
7	Refractive index	1.460-1.465	1.462
8	Total bacterial count	1	Less than 40
9	Total Yeast And Mould		Absent
10	E.coli	Absent	Absent
11	Salmonella	Absent	Absent
12	S.aureus	Absent	Absent
13	P.aeruginosa	Absent	Absent

Table 2: Showing Rf values obtained from HPTLC fingerprint profile of Sukumara Ghrita sample

Scanning Wavelengths	Rf Values of Major Spots
At 254nm	0.06, 0.08, 0.22, 0.27, 0.43, 0.56, 0.71, 0.83, 0.98
At 366nm	0.07 (red), 0.08 (green), 0.14 (red), 0.16 (blue), 0.25 (blue), 0.28 (blue), 0.41 (blue), 0.42 (light blue), 0.54 (blue), 0.61 (blue), 0.71 (blue), 0.76 (blue), 0.83 (purple), 0.98 (blue).

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Table 3: The retentions values, the types of possible compound, peak height, their Functional group and medicinal roles of each compound of GC–MS profile of Sukumara Ghrita

Sl No	Retention Time	Compound Name	Peak Height	Functional Group	Biological Activity
1	6.061	Propane, 2,2-diethoxy-	1010282	Acetone	Antifungal and anti inflammatory (6)
2	52.933	Tetradecanoic acid, ethyl ester	34626	Fatty acids	Antioxidant, antimicrobial (7), Antifungal, Antioxidant, cancerpreventive, nematicide, hypercholesterolemic (8)
3	57.845	Hexadecanoic acid, ethyl ester	1421967	Fatty Acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavor, hemolytic, 5-alpha reductase inhibitor (7)
4	61.552	Ethyl Oleate	2226440	Fatty acid	Anti-inflammatory, Antiandrogenic Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor (9)
5	62.094	Octadecanoic acid, ethyl ester	647161	Ester	-
6	65.785	(5β)Pregnane-3,20β-diol, 14α,18α-(4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl))-, diacetate	89263	Steroid derivative	-
7	66.473	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	42039	Steroid Derivative	Anti microbial, antitumor (10)
8	70.610	7,8-Epoxylanostan-11-ol, 3-acetoxy-	184755	Alcoholic compound	Antimicrobial anti-inflammatory (11)
9	72.529	Squalene	267006	Terpernes	Anti-hypercholesterolemic, Antihypertriglyceridemic, Anti-oxidant Anti-inflammatory (12)
10	74.292	Ethyl iso-allocholate	141744	Steroid derivative	Antimicrobial, Anti inflammatory, diuretic, antiashmatic (13)
11	74.765	1',1'-Dicarboethoxy-1β,2β-dihydro-17β-propionoxy(3'H)cyhcloprop(1,2)androsta-1,4,6-trien-3-one	210310	Steroid derivative	-

Figure 1: HPTLC of Sukumara Ghrita at 254 nm

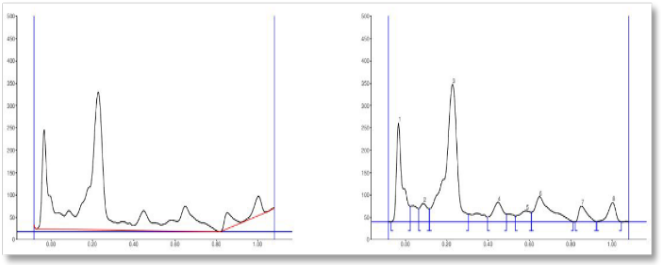


Figure 2: HPTLC of Sukumara Ghrita at 366 nm

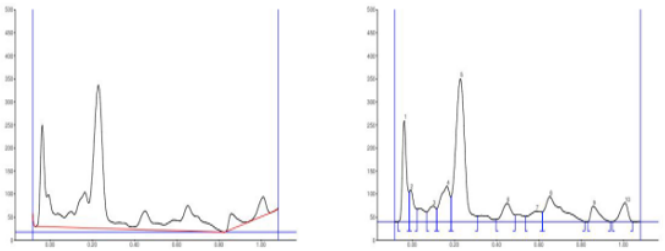


Figure 3: Image information of HPTLC of Sukumara Ghrita at 254nm and 366 nm.

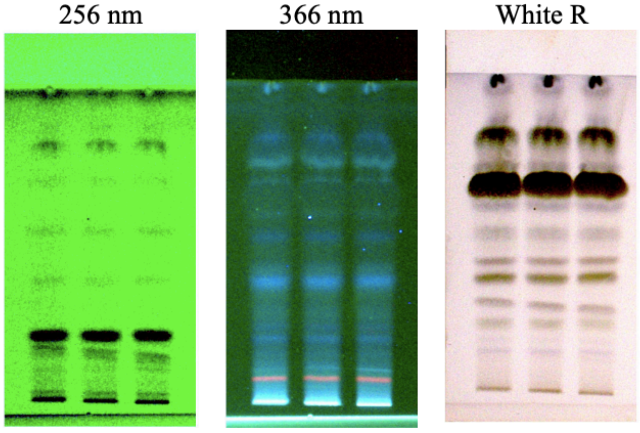
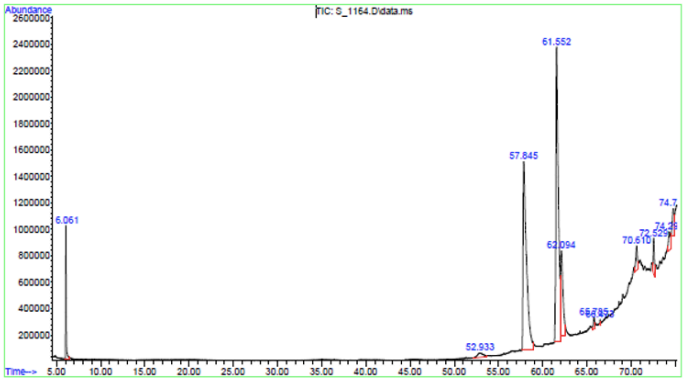


Figure 4: Represents the GC–MS profile of Sukumara Ghrita



Discussion

HPTLC analysis

A combination of flavonoids, phenolic chemicals, or terpenoids may be indicated by the presence of bands at both 254 and 366 nm. Certain chemicals, such as steroids and glycosides, may become more visible after derivatisation, suggesting potential medicinal benefits. Components were successfully separated by the solvent system (Toluene: Ethyl Acetate: Hexane, 6:3:1), demonstrating the chemical diversity of the extract**.**

HPTLC Analysis at 256 nm (UV Light)

Band Pattern & Consistency

Triplicate sample application is indicated by three identical lanes. Reproducibility in sample preparation and chromatographic settings is confirmed by the banding pattern's apparent consistency throughout all three lanes. Multiple compounds may be present, as shown by a number of discrete bands at various Rf values.

UV 256 nm Analysis

Compounds that absorb UV light at 256 nm are usually conjugated or aromatic (e.g., flavonoids, alkaloids, phenols, terpenoids). Strong UV-absorbing chemicals are shown by the dark bands. A diverse phytochemical profile is suggested by the presence of several bands.

Band Intensity & Position

The bands in the lower region (close to the baseline) point to non-polar substances such fatty acids, terpenoids, and sterols. Flavonoids, alkaloids, and glycosides are examples of moderately and highly polar chemicals that correlate to the middle and upper area bands. Consistent phytoconstituent concentration across samples is shown by uniform band intensity.

Inference

Several UV-active phytochemicals, such as flavonoids, phenolic compounds, alkaloids, or terpenoids, are present in the methanol extract of Sukumara ghrita. Sample dependability is ensured by the uniform banding pattern throughout the triple application.

HPTLC Analysis at 366 nm (UV Light)

The presence of chemicals that naturally fluoresce or react under UV light is indicated by fluorescent bands visible in HPTLC images taken at 366 nm UV light.

Band Pattern & Fluorescence

Consistent banding in the triplicate lanes verifies sample application homogeneity. A wide variety of phytochemicals are highlighted by the presence of several fluorescent bands at various Rf values.

UV 366 nm Analysis

Flavonoids, coumarins, or phenolic chemicals are probably indicated by greenish and blue fluorescent bands. Under UV light, some substances naturally glow. Lower region reddish or pink bands may be a sign of terpenoids, glycosides, or anthraquinones. Highly polar chemicals that travelled farther with the mobile phase are indicated by bright bands at the top

Comparison with 256 nm UV Analysis

Certain bands that are visible at 256 nm might not be as noticeable at 366 nm, indicating the presence of non-fluorescent UV-absorbing substances (such as tannins and alkaloids). Certain substances that show fluorescence but are less apparent at 256 nm are indicated by the existence of extra fluorescent bands at 366 nm.

Inference

Several kinds of phytochemicals, such as flavonoids, phenolic compounds, and coumarins (because of their blue/green fluorescence), are present in the methanol extract of *Sukumara ghrita*, glycosides or anthraquinones (because of the pink or red bands at the bottom) and perhaps terpenoids that emerge following derivatisation. A chemically diversified extract is confirmed by the presence of both UV-absorbing (256 nm) and fluorescent (366 nm) components.

HPTLC Analysis After Derivatization (Visible Light) - Interpretation

The picture shows the post-chromatographic derivatisation of Sukumara Ghrita's methanol extract, with the use of anisaldehyde-sulfuric acid reagent, which produces colour changes by reacting with particular classes of phytochemicals.

Banding Pattern & Consistency

The three lanes show a consistent banding pattern, indicating that the sample application and separation procedure are repeatable. The presence of several phytoconstituents is indicated by many bands at various Rf values.

Post-Derivatization Interpretation

Steroids, terpenoids, or essential oils reacting with the reagent are usually linked to dark brown to black bands (top region). Flavonoids, phenolics, or glycosides may be indicated by light brown/yellow bands in the middle and lower regions. Alkaloids or tannins may be indicated by faint greenish bands.

Comparison with UV Analysis (256 nm & 366 nm)

Following derivatisation, several bands that were fluorescent at 366 nm UV became coloured, indicating their chemical makeup. Other constituents are now visible due to the appearance of non-UV active chemicals, which were not visible at 256 nm or 366 nm. The upper region's strong black bands indicate the presence of highly concentrated bioactive ingredients.

Inference

A wide variety of phytochemicals, such as flavonoids, terpenoids, phenolics, and maybe steroids and glycosides, are found in *Sukumara Ghrita*. The presence of chemicals that were either weakly UV-active or required a chemical reaction for visualisation is confirmed by the derivatisation stage. The consistency of the banding pattern among the triplicate samples attests to the extract's composition.

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Probable mode of action of *Sukumara ghrita*

It can be understood by the action of phytoconstituents detected through GC-MS. This would also add on to evidence-based practice and also would prove the scientific basis of Ayurvedic treatment.

Acetone

Acetone helps to maintain the pH buffering capacity and acts as a provision for fuel synthesis in peripheral tissues during stressful situations like starvation and diabetic ketosis (14).

Myristic acid

Myristic acid is a long-chain saturated fatty acid that is present in large amounts in milk. Myristic acid consumption is thought to have a beneficial effect on cardiovascular health. Because myristic acid directly affects post-translational protein modifications, it regulates several critical metabolic processes in the human body. When consumed in moderation, it raises plasma phospholipid levels of long-chain omega-3 fatty acids, which may enhance human cardiovascular health indicators. Excessive consumption of myristic acid shown immunomodulatory effects by raising the level of a particular protein that triggers macrophage activation. During pregnancy, saturated fats might also be crucial for promoting the growth of the fetal membrane (15).

Palmitic acid

Palmitic acid (16:0) is a saturated fatty acid which occurs in the diet as well as synthesized endogenously. The infant has 13–15% body fat at birth, with 45–50% palmitic acid, the majority of which is produced by the foetus. Longer fatty acids can be created from palmitic acid, which is the first fatty acid synthesized during lipogenesis, or fatty acid synthesis. It is necessary for lung lecithin production, which is connected to foetal development. The radio chromatogram indicated a significant level of palmitate incorporation by the foetal lung into lecithin (16).

Hexadecanoic acid, ethyl ester

Hexadecanoic acid exhibited strong inhibitory effect in the PLA2 enzyme kinetics research, it might have anti-inflammatory properties. The fatty acid n-hexadecanoic acid is an inhibitor of phospholipase A2, making it an anti-inflammatory molecule, according to structural and kinetic investigations. This supports the Ayurvedic medical system's use of medicinal oils high in hexadecanoic acid to treat inflammatory disorders (17).

Ethyl Oleate

Using an esterase inhibitor, such as ethyl oleate, in heterotopic lipid formulations (emulsions) may help shield ester prodrugs from intestine metabolism and boost their oral bioavailability. Traditionally, ester prodrugs have been created to increase their parent drug's membrane permeability and oral bioavailability. (18), Omega-6, -7, and -9 fatty acids and their esters showed potent antimicrobial action, showing some species-specificity (19).

Octadecanoic acid

Stearic acid (C18:0), as a metabolite that is detected in our diets has a physiological role in mitochondrial morphology and function. Additionally, a study demonstrated that within a few hours of eating, the stearic acid in our food causes our mitochondria to fuse. Stearic acid's ability to lower the risk of cardiovascular disease and cancer may be explained by this. A physiological response for lipid handling, including fatty acid beta-oxidation, is activated when dietary stearic acid signals the intake of lipids to the body, this may be the reason for less fat accumulation in the body. In turn, reducing the cardiovascular risks which justifies its inclusion in our daily diet (20).

Propionic acid

It is a saturated fatty acid with a short chain and has antifungal properties. Propionic acid decreased the levels of tumour necrosis factor-α (TNF-α) and interferon-gamma-induced protein (IP-10) significantly by an approximate of 30%.It is also said to have an inhibitory action on adipose tissue macrophage (ATM) markers, mRNA expression of adipose tissue macrophage markers. It is said to positively increase the expression of lipoprotein lipase (LPL), sterol regulatory-element-binding protein-1c (SREBP-1c) and glucose transporter 4 (GLUT-4), which are associated with lipogenesis and glucose uptake. Mediated by Gi/o protein coupled receptor it produces an anti-inflammatory effect on subcutaneous adipose tissue (21).

7,8-Epoxylanostan-11-ol, 3-acetoxy-

7,8-Epoxylanostan-11-ol, 3-acetoxy- may have an antimicrobial activity (22).

Squalene

An intermediate during the cholesterol biosynthesis pathway is squalene. Animal studies have demonstrated it can effectively inhibit chemically induced skin, colon, and lung tumorigenesis in rodents. This can be further explained by the mechanisms in which there is an inhibition of Ras farnesylation, modulation of carcinogen activation, and antioxidative activities (23).

Ethyl iso-allocholate

Ethyl iso-allocholate may have some anti-bacterial action by inhibitoion of dihydropteroate synthase (24). In zebrafish it has proven to reduce tumour growth, liver metastasis, and angiogenesis (25).

Conclusion

This is first of its kind study on Sukumara ghrita and as per the present study the molecules present in Sukumara Ghrita may have specific mode of actions which can be understood by their anti-oxidant, anti-hypercholesterolemic, antihypertriglyceridemic, anti-

inflammatory activities. It is interesting that there are many molecules in Sukumara Ghrita with no known functions and it would be interesting to work on these molecules which could further throw some light on the therapeutic role of *Sukumara Ghrita*. Hence there is a need to further study *Sukumara Ghrita* with multiple samples from various sources for its characterisation and standardisation.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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