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ORA- Analytical study

Phytochemical Fingerprinting of Polyherbal Oil and Its Topical Derivatives (Gel and Ointment) using LC-MS analysis.

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ABSTRACT:

Background: Polyherbal formulations play a pivotal role in Ayurveda therapeutics; while their complex phytochemical composition demands analytical validation for their quality and safety. Pharmaceutical development of classical Ayurveda formulations to advanced dosage forms is the dire need for enhanced patient compliance. Aim: To develop a polyherbal oil and its topical derivatives (gel and ointment) and comparatively characterize their phytochemical fingerprints using LC–MS analysis. **Methods:** The Polyherbal oil was prepared by following the basic principles of *Sneha Kalpana* (oil and ghee preparations) and its conversion to gel and ointment were done by the contemporary principles and their comparative analytical evaluation is carried out using Liquid Chromatography–Mass Spectrometry (LC-MS) under identical chromatographic conditions. **Results:** LC-MS analysis revealed the presence of flavonoids, terpenoids, phenolic glycosides, sterols, and fatty acids across all formulations. Polyherbal oil demonstrated broader phytochemical diversity with compounds such as α-pinene, harmol, curdione, campesterol, and lupeol, while the ointment and gel retained selective phytoconstituents including glyasperine D, syringing, homoorientin, lupeol derivatives, flavonoids glycosides, and isoflavonoids. Comparative analysis showed partial retention of key phytochemical groups despite formulation modifications. Conclusion: The study demonstrated retention of major phytochemical groups across all formulation dependent variations in LC-MS profiles. LC-MS fingerprinting was found useful for comparative phytochemical evaluation of topical dosage forms.

KEYWORDS: Herbal Gel, Herbal Ointment, LC–MS, Phytochemical Fingerprinting, Polyherbal

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1. INTRODUCTION

Polyherbal formulations constitute an important component of Ayurveda therapeutics owing to their synergetic therapeutic potential. Among the various dosage forms, *sneha kalpana* facilitates the extraction of lipid-soluble phytoconstituents; [1] however; classical oil preparations may present limitations such as greasiness, difficulty in handling, and reduced patient acceptability. To overcome these limitations, pharmaceutical modification of oils into semisolid dosage forms such as gel and ointment has gained increasing importance. Despite such modifications, comparative phytochemical evaluation of classical Ayurvedic oils and their semisolid derivatives remains inadequately explored. The complex phytochemical composition of polyherbal formulations necessitates advanced analytical techniques for comprehensive characterization and quality assessment. In this context, Liquid Chromatography – Mass Spectrometry (LC-MS) [2] provides sensitive and comprehensive phytochemical fingerprinting, and previous studies have demonstrated its utility in the characterization of phytoconstituents in herbal formulations. [3] However, limited information is available regarding the influence of pharmaceutical modification on the phytochemical profile and constituent retention of Ayurvedic oil-based formulations. Therefore, the present study was undertaken to develop a polyherbal oil containing *Datura metel, Vitex negundo, and Strychnos nux-vomica* and to comparatively evaluate its gel and ointment derivatives using LC-MS fingerprinting.

Objectives:

- To prepare polyherbal oil as per classical *Sneha kalpana* and develop its gel and ointment
- To perform LC-MS based phytochemical profiling
- To compare the phytochemical composition of oil, gel and ointment and
- To assess the effect of formulation on phytochemical integrity.

2. MATERIALS AND METHODS:

2.1 Materials: Polyherbal oil was prepared using *Raw tilataila (Sesamum indicum), Manjishta (Rubia cordifolia), Haritaki (Terminalia chebula), Vibhitaki (Terminalia bellirica), Amalaki (Emblica officinalis), Bala (Sida cordifolia), Haridra (Curcuma longa), Musta(Cyperus rotundus), Lodhra (Symploca racemosa), Ketaki (Pandanus odorifer), Vatankura (Ficus benghalensis), Nalika (Phyllanthus acidus), were procured from* Shri Gurukrupa Ayurveda, Mahalakshmipuram Bengaluru *and* were identified and authenticated in the Department of Dravyaguna, Government Ayurvedic Medical College, Bengaluru. *Shuddha Kupilubeeja (strychnos nux-vomica), Nirgundimoola (Vitex negundo)* were procured from KLE Society Ayurved Pharmacy and *Datturapatra (Datura metel),* was procured from HERBO concepts, Vidyapeeta road, Bengaluru and. were authenticated through macroscopic and Organoleptic evaluation at Rajiv Gandhi Education Society's Ayurvedic Medical College and Hospital, Rona, Gadag District, Karnataka, an Ayush approved laboratory. Toxic ingredients such as Datura metel and Strychnos nux-vomica were used after classical purification ensuring detoxification. [4], [5], [6] The preparation of Polyherbal oil was carried out at PG Department of Rasashastra and Bhaishajya kalpana, Ayurvedic Medical College, Bengaluru. All raw drugs used in the study were authenticated through macroscopic and Organoleptic evaluation. Excipients used for gel formulation included Carbopol 934, glycerine , triethanolamine, ethanol, polyethylene glycol-400 (PEG-400), sodium lactate, citric acid, sorbic acid, potassium sorbate, and distilled water of pharmaceutical grade (IP) were used for preparation. Ointment formulation utilized Wool fat, hard paraffin, yellow soft paraffin, cetearyl alcohol, glyceryl monostearate, cinnamon oil, vitamin E, and preservatives Euxyl of pharmaceutical grade (IP) were used for preparation. LC-MS

grade methanol, acetanitrile and formic acid were used for analysis. The excipients procurement and development of gel and ointment and their analytical profiling was carried out at Department of Pharmaceutics, Acharya and B.M. Reddy college of Pharmacy, Bengaluru.

2.2 Preparation of Polyherbal Oil: This is an exploratory polyherbal oil formulated using the drugs as mentioned in Table 1 which are *Dattura (Dattura metel), Nirgundi (vitex negundo) and Kupilu (strychnos nux-vomica)* as the major ingredient having *murchita tila taila* as the base and also developed further to its ointment and gel. Although a direct classical reference for the formulation is unavailable, the formulation was developed as an exploratory pharmaceutical preparation intended for standardization and comparative phytochemical profiling through LC-MS.

For preparation of Polyherbal oil the 'general method of preparation [7] i.e., 1 part of *kalkadravya* (paste of drugs) to 4 parts of *tilataila* (sesame oil) to 16 parts of *Dravadravya* (liquid media) is adopted.

Preliminary procedures: ***Tila taila murchana:*** *Taila murchana* is the preliminary step in preparation of any *tailas* which intended to remove the *ama dosha* and *durgandha nashana* present in the raw *tila taila*. [8]

Ingredients:

Table 1: Ingredients for the preparation of Polyherbal oil

Key ingredient	Name of the drug	Botanical name	Voucher specimen number/reference number	Quantity
Kalkadravya	*Dhatturapatra*	*Datura metel*	DTLRC/24/231	375 gm *(125 gm each)*
	Nirgundimoola	*Vitex negundo*	DTLRC/24/230	
	Shodhita Kupilubeeja	*Strychnos nux-vomica*	DTLRC/24/229	
Snehadravya	*Murchita tilataila*	*Sesamum indicum*		1500 ml
Dravadravya	*Dhatturapatra swarasa*	*Datura metel*		6000 ml
	Nirgundimoola Kashaya	*Vitex negundo*		
	Kupilu Kashaya	*Strychnos nux-vomica*		

Table 2: Ingredients for *Taila murchana*

Key ingredients	Drug	Botanical name	Voucher specimen number/reference number	Quantity used
Kalka dravya	*Manjishta*	*Rubia cordifolia*	DG/GAMC/2025/001	125 gm
	Haritaki	*Terminalia chebula*	DG/GAMC/2025/002	31.2 gm
	Vibhitaki	*Terminalia bellirica*	DG/GAMC/2025/003	31.2 gm
	Amalaki	*Emblica officinalis*	DG/GAMC/2025/004	31.2 gm
	Bala	*Sida cordifolia*	DG/GAMC/2025/005	31.2 gm
	Haridra	*Curcuma longa*	DG/GAMC/2025/006	31.2 gm
	Musta	*Cyperus rotundus*	DG/GAMC/2025/007	31.2 gm
	Lodhra	*Symploca racemosa*	DG/GAMC/2025/008	31.2 gm
	Ketaki	*Pandanus odorifer*	DG/GAMC/2025/009	31.2 gm
	Vatankura	*Ficus benghalensis*	DG/GAMC/2025/010	31.2 gm
	Nalika	*Phyllanthus acidus*	DG/GAMC/2025/011	31.2 gm
Sneha dravya	*Tila taila*	*Sesamum indicum*	DG/GAMC/2025/012	2000 ml
Drava dravya	Water	-	_	8000 ml

Procedure: 2000 ml of raw *tilataila* was taken in a stainless steel vessel and placed over mild flame. *Taila* was heated until froth starts to appear. Soon the fire was lit off and waited for *Nishphenabhava* (devoid of froth) and for *shaityabhava* (self-cool) of *taila*. After self-cool *taila* was again placed over mild flame and the *kalka dravyas* as mentioned in Table 2 are added along with 8000 ml of water is added and the mixture was heated on *mandagni* with constant stirring to avoid adhering of *kalka* to the bottom of the vessel. And the procedure was continued till *sneha siddhi lakshanas*. After confirmation of completion of procedure the *murchita tila taila* was stored in an airtight container for further pharmaceutical use.

***Kupilubeeja shodhana*:** 250 gm of *Ashuddha kupilu beeja* was subjected to *swedana* in *dolayantra* containing *godugdha* for 3 hours. [9] The *pottali* was opened and looked for the changes like swollen seeds and their soft outer covering was ready to peel off indicating the shodhana process was completed. The outer coating was peeled off, testa and embryo were removed, and cotolydon is cut into small pieces and dried for further use. *Shuddha kupilubeeja* obtained –191 gm

***Kupilu kashaya* preparation:** 500gm coarse powder (Sieve no. 10) of *shuddha kupilubeeja* is added with 8000ml of water in a stainless steel vessel and boiled on *mandagni* till it reduced to 2000ml. [10] then it is filtered and used for further pharmaceutical procedures.

Quantity of *Kupilubeeja kashaya* obtained – 2000 ml

***Datturapatra swarasa* preparation:** 2.5 kg of freshly collected *Datturapatra* was washed in running water, cut into small pieces and subjected to grinding. Later it is strained to obtained *swarasa.* [11]

Quantity of *Dattura swarasa* obtained – 2000 ml

***Nirgundimoola kashaya* preparation:** 500 gms coarse powder (Sieve no. 10) of *Nirgundimoola* is added with 8000 ml of water in a stainless steel vessel and boiled on *mandagni* till it reduced to 2000 ml then filtered and used for further pharmaceutical procedures. [10]

Quantity of *Nirgundimoola kashaya* obtained – 2000 ml

Preparation of Polyherbal oil: 1500 ml of *Murchita tilataila* was taken in a wide mouthed vessel and heated over a mild fire. *Taila* was heated until foam starts to appear. Soon the fire was lit off and waited for *Nisphenabhava* (devoid of froth) and *Saityabhava* (selfcool) of *taila*. *Taila* was again placed over mild fire and 6000 ml of *dravadravya* was added as shown in Figure 2 When the mixture started boiling, the *kalka* of 375 gm was added into vessel as shown in Figure 1 This mixture was heated on *mandagni (mild flame)* with frequent stirring to avoid adhering of *Kalka* to the bottom of the vessel as shown in Figure 3.Temperature was monitored. After 1 hour of continuous heating, heat was discontinued and allowed to cool. On the second day the heating process was continued with constant stirring. Temperature was monitored. *Taila* was dark brown in color. After 6 hours of continuous heating, heating was discontinued and allowed to cool. On third day the heating process was continued until all the *Sneha siddhi lakshana (test of completion)* appeared and the *taila* part only remained. Temperature was monitored. The mixture is stirred often to prevent adhering of *Kalkadravya* on the bottom of the vessel. After attaining *Sneha siddhi lakshana* as shown in Figure 4, the stove was lit off. [12] The *taila* was filtered through a kora cloth. The filtered *taila* was allowed to cool and stored in air tight container as shown in Figure 5.

2.3 Preparation of Gel and Ointment

Formulation of Gel: 17.53 ml of Distilled water and 0.2 ml of glycerin were mixed, followed by dissolution of 0.05 mg sorbic acid, 0.15 ml of potassium sorbate, and 0.1 ml of citric acid; 0.12 mg of Carbopol 934 was then sprinkled gradually under stirring and allowed to hydrate fully. Separately, the 1 ml of active ingredient i.e., polyherbal oil was dispersed in 0.4 ml of PEG-400 and 0.4 ml of ethanol, and this phase was slowly

added to the hydrated Carbopol dispersion with continuous stirring. The pH was adjusted to ~6–7 using 0.04ml of sodium lactate and 0.01 ml of TEA for gelation, after which the gel was deaerated, filled into containers as shown in Figure 6, and stored at ambient temperature protected from light. [13]

Formulation of ointment: The weighed solid and semi-solid excipients (1.2 gm of wool fat, 1.2 gm of glyceryl monostearate, 1.2 gm of hard paraffin, 0.5 gm of cetearyl alcohol, 15.0 gm of yellow soft paraffin) were melted together in a water bath at approximately 70°C and mixed until a uniform molten mass was obtained. The mixture was removed from heat and allowed to cool below 40°C, after which 0.2 mL of Euxyl preservative and 0.3 mL of cinnamon oil were incorporated with gentle stirring. The active ingredient (herbal oil of 1.0 mL) was added last at low temperature to prevent thermal degradation and supports uniform distribution. The formulation was stirred continuously during cooling to room temperature to obtain a smooth, homogeneous ointment, which was then transferred to labeled containers and stored protected from light and heat. [14]

Figure 1. Adding *kalka Dravya*

Figure 2. Adding *drava dravya*

Figure 3. *Taila paka*

Figure 4. *Varti Pareeksha*

Figure 5. Polyherbal oil

Figure 6. Gel

Figure 7. Ointment

2.4 Analytical study:

2.4.1. Polyherbal oil: The basic analytical tests like organoleptic characteristics, Acid value, total fatty acid, free fatty acid, iodine value, peroxide value, rancidity test (kries test), refractive index, saponification value, specific gravity, and unsaponifiable matter, LC-MS were carried out for polyherbal oil as per the standard reference of Ayurveda Pharmacopoeia of India. [15]

2.4.2. Ointment and Gel: The basic analytical tests like organoleptic characteristics, P^H, spreadability, Extrudability, Homogeneity, washability, Greasiness, and LC-MS were carried out for polyherbal oil as per the standard reference of Ayurveda Pharmacopoeia of India. [16]

2.4.3Liquid Chromatography – Mass Spectrometry (LC-MS) Analysis

LC-MS Instrument specification: LC-MS analysis was conducted using an acquity UPLC system coupled with a

waters SQ mass spectrometer equipped with electrospray ionization (ESI) source operating in positive ion mode. Chromatographic separation was achieved using an Acquity BEH column (50 x 2.1 mm, 1.7 µm). The mobile phase consisted of 0.1% formic acid in water (Mobile phase A) and 0.1% formic acid in acetonitrile (Mobile phase B) as shown in Table 3. The chromatographic conditions were maintained at a flow rate of 0.5 ml/min with an injection volume of 2 µL and total run time of 6 minutes.

Table 3: Phase wise Gradient time for the three samples

Gradient: Time (min)	Mobile phase-A	Mobile phase-B
0.0	95	5
3.0	5	95
4.0	5	95
5.0	95	5
6.0	95	5

The mass spectrometer is operated in electrospray ionization mode (ESI) with parameters: Capillary voltage 2.8 kV, source temperature 150 °C, desolvation temperature 350 °C, gas flow 600 L/hr. The scan range is 50–1200 m/z. Data acquisition and processing were carried out using MassLynx software. The chromatograms were monitored at 254 nm.

Sample preparation:

Sample of Polyherbal oil (sample 1), ointment (sample 2) and gel (sample 3) were separately extracted using methanol. The mixtures were sonicated for 20 minutes and centrifuged at 5000 rpm for 10 minutes. The supernatant was filtered through a 0.22 um membrane filter and subjected to LC-MS analysis.

Running sample:

Prepared samples of polyherbal oil, ointment and gel were injected individually into the LC-MS system under identical chromatographic and mass spectrometric conditions. The acquired chromatograms and mass spectra were analyzed for identification of phytoconstituents based on retention time, molecular ion peaks, and spectral library matching.

3. RESULTS:

The observations and results during the preparation and analysis of the Polyherbal oil, Ointment and Gel were recorded as below

3.1 Assessment of Raw *tila taila* & *Murchita tila taila*:

Table 4: Basic comparative physico chemical results of Raw *tila taila* & *Murchita tila taila:*

Test name	Raw *tila taila*	*Murchita tila taila*
Refractive index	1.46550 (Brix – 71.75%)	1.46677 (Brix – 72.27%)
Specific gravity	0.9671	0.9648
Acid value	14.9877	30.097
Saponification value	194.64	172.02
Iodine value	391.37	371.56

3.2 Observations during *Taila murchana* & Polyherbal Oil preparation:

Table 5: showing Observations during *Tila taila murchana*

Day	Time	Temp.	Color	Odor	Observation
Day	10:40 am	35^0C	Dark brown	*Tila taila* smell	Heating of oil
1	11:40 am	95^0C	Dark brown	Odor of *taila* and *kalka*	Heterogenous mixture was seen
	1:43 pm	96.2^0C	Dark brown	Same odor	Same
Day	10:50 am	32.10C	Dark brown	Same odor	Same
2	1:15 am	96.8^0C	Dark brown	Odor of *tila taila* & *manjishta* was appreciated	Same
Day	9:15 am	33.8^0C	Dark brown	Same odor	Same
3	10:15 am	92.7^0C	Dark reddish brown	Characteristic odor of *kalka dravya*	Slight froth started
	11:10 am	94.7^0C	***Kalka*-** 1. Traces of water was present (+); 2. Produced crackling sound when placed on fire; 3. *Kalka* was very sticky		

			in nature and rolling varti was difficult when pressed in between two fingers ***Taila****- 1.* Traces of water were absent (-); 2. Not Producing crackling sound when put on fire		
	11:20 am	80^0C	Dark reddish brown	Same odor	1. *Kalka* was not sticky and formation of *varti* when rolled between fingers 2. Appearance of froth

Total duration of *paka*: 07 hours and 33 minutes; Quantity of *murchita tila taila* obtained: 1810 ml; Yield percentage of *Murchita tila taila* obtained: 90.50%

Table 6: Observations during the preparation of Polyherbal oil

Day	Time	Temp.	Color	Odor	Observation
Day	3:50 PM	48^0C	Dark brown	*Characteristic odor of Murchita tila taila*	Heating of oil
1	4:20 PM	56^0C	Dark brown	*Characteristic odor taila & kalka*	Same
	4:50 PM	60^0 C	Dark brown	*Characteristic odor taila & kalka*	Heterogenous mixture was seen
Day	9:20 AM –12:20 PM	36^0C – 89.4^0C	Dark brown	*Characteristic odor taila & kalka*	Heterogenous mixture was seen
2	12:50 PM	90^0 C	Dark brown	Strong odor of *taila & kalka*	Boiling of mixture
	1:20 PM – 3:50 PM	90^0 C - 97^0 C	Dark brown	Same odor	Slight separation of *kalka*
Day	10:30AM	48^0C	Dark brown	Characteristic odor of *kalkadravya*	-
3	11:00AM	65^0C	Dark brown	Same odor	Slight sticking of *kalkadravya*
	11:30AM	79^0C	Dark brown	Characteristic odor of *kalkadravya*	Slight froth started
	12:00PM	80^0C	Same color	Same odor	Same
	12:10 PM	84.7^0C	***Kalka –*** 1) Traces of water was present (+); 2) Produced crackling sound when placed on fire; 3) *Kalka* was very sticky in nature and rolling *varti*(wick like)was difficult when pressed in between two fingers ***Taila****-1)* Traces of water was absent (-); 2) Not Producing crackling sound when put on fire		
	12:40 PM	90^0 C	Dark reddish brown	Same odor	*Kalka* was not sticky and formation of *varti* when rolled between fingers; Appearance of froth

Total duration of *paka*: 10 hours and 40 minutes; Final weight of *taila* obtained*:* 1050ml; Yield percentage of *taila* obtained: 70%

3.3 Results of Polyherbal oil, ointment & gel:

A. Organoleptic Characters:

Table 7: Showing Organoleptic Characteristics of Polyherbal oil, Ointment and Gel:

Physical test	Polyherbal Oil	Ointment	Gel
Color	Dark brown	Light brown	Pale white
Odor	Characteristic odor	Characteristic odor	Characteristic odor
Taste	Slightly Bitter	Bitter	Bitter
Texture	Viscous liquid	Semisolid	Semisolid

B. Physico-Chemical Parameters of Polyherbal oil:

Table 8: Showing Physico-Chemical results of Polyherbal oil

Sl no	Test	Results
1	Acid value	20.05 mgKOH/g
2	Free fatty acid	10.03g/100g
3	Iodine value	105.74
4	Peroxide value	7.57 meq/Kg
5	Rancidity test (kries test)	Absent
6	Refractive index	1.4705
7	Saponification value	190.25 mgKOH/g
8	Specific gravity	0.9226
9	Unsaponifiable matter	0.625g/100g

C. Results of Ointment and Gel:

Table 9: Showing Analytical results of Ointment & Gel

Test	Ointment	Gel
P^H	6.0	6.7
Viscosity @ 10 rpm	52,620 cP	27,350 cP
Spreadability	4.1 cm	6.9 cm
Extrudability	0.41 ± 0.02 g/10s	0.82 ± 0.04 g/10s
Washability	64% ± 3.4 removal efficiency	92% ± 2.1 removal efficiency

D. Accelerated Stability study observations:

Samples of the polyherbal based gel and ointment were stored under the following ICH recommended conditions and monitored for the parameters like Ph and Organoleptic characters as shown in Table 10.

Condition: Accelerated

Temperature: 40±2^{O}C / 75% RH

Purpose: To predict shelf life

Table 10: Accelerated Stability study of Gel and Ointment

Sample	Parameters	Day 1	Day 15	Day 30	Day 45
Gel	pH	6.7	6.7	6.72	6.72
	Organoleptic characteristics	Good	Good	Good	Good
Ointment	pH	6.0	6.02	6.04	6.04
	Organoleptic characteristics	Good	Good	Good	Good

E. Liquid Chromatography – Mass Spectrometry Analysis

LC-MS analysis demonstrated the presence of diverse phytochemical groups including flavonoids, terpenoids, sterols and fatty acids. The polyherbal oil produced a wider phytochemical range compared to its gel and ointment. Principle compounds such as lupeol and glyaspetin D were consistently detected across all samples.

LC-MS of Polyherbal oil: LC–MS analysis revealed the presence of diverse classes of phytoconstituents including flavonoids, terpenoids, sterols and fatty acids. The major phytochemicals tentatively identified are listed in the Table 11 and as shown in Figure 8.

LC-MS of Ointment: The major phytochemicals tentatively identified in LC-MS of Ointment are listed in the Table 12 and as shown in Figure 9.

LC-MS of Gel: LC–MS analysis tentatively identified the major phytochemicals and is listed in the Table 13 and as shown in Figure 10.

Comparative LC-MS Evaluation:

The Chromatographic profiles of Polyherbal oil, its gel and ointment were compared based on the factors like retention time, m/z values and peak patterns. The presence or absence of phytoconstituents and fluctuations in peak intensity were analyzed to quantify the impact of formulation on phytochemical composition.

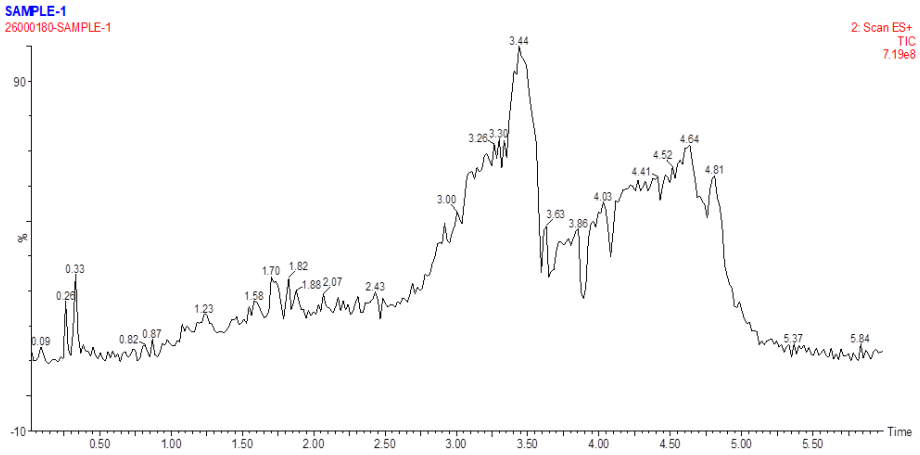

Figure 8: Liquid Chromatography of Polyherbal oil @ 254 nm

Table 11: Tentatively identified Major phytochemicals in Polyherbal oil

Sl no	Phytochemical	Chemical Class	Formula	m/z	Retention time (Min)
1.	Datiscin	Flavonoid-3-O-glycosides	$C_{27}H_{30}O_{15}$	595.4	0.330
2.	Glyasperin D	7-O-methylated isoflavonoids	$C_{22}H_{26}O_5$	371.19	0.955
3.	Demethyleneberberine	Protoberberine alkaloids and derivatives	$C_{19}H_{18}NO_4$	347.106	1.094
4.	Tetrahydropalmatine	Protoberberine alkaloids and derivatives	$C_{21}H_{25}NO_4$	356.18	1.181
5.	Campesterol	Ergosterols and derivatives	$C_{28}H_{48}O$	383.40	1.355
6.	α-Pinene	Bicyclic monoterpenoid	$C_{10}H_{16}$	137.15	1.632
7.	Curdione	Germacrane sesquiterpenoids	$C_{15}H_{24}O_2$	237.17	1.719
8.	Harmol hydrochloride	Harmala alkaloids	$C_{12}H_{10}N_2O$	221.06	2.727
9.	Homoorientin	Flavonoid C-glycosides	$C_{21}H_{20}O_{11}$	449.40	3.213
10.	Lupeol	Triterpenoids	$C_{30}H_{50}O$	427.39	3.248
11.	Palmitoleic acid	Long-chain fatty acids	$C_{16}H_{30}O_2$	254.46	3.282
12.	Atropine sulphate	Beta hydroxy acids and derivatives	$C_{34}H_{48}N_2O_{10}S$	694.34	1.719

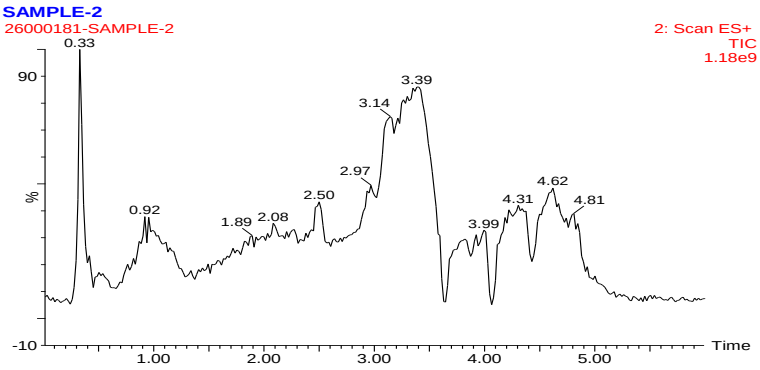

Figure 9: Liquid Chromatography of Ointment @ 254 nm

Table 12. Tentatively identified Major phytochemicals in Ointment

Sl no	Phytochemical	Chemical Class	Formula	m/z	Retention time (Min)
1.	Silybin (Silibinin)	Flavonolignans	$C_{25}H_{22}O_{10}$	500.16	0.313
2.	Biochanin A	4'-O-methylisoflavones	$C_{16}H_{12}O_5$	285.17	0.33
3.	Glyasperin D	Isoflavonoids	$C_{22}H_{26}O_5$	371.19	0.33
4.	Apigenin	Flavones	$C_{15}H_{10}O_5$	271.26	0.347
5.	Daidzein	Isoflavones	$C_{15}H_{10}O_4$	292.97	0.347
6.	Baicalein	Flavones	$C_{15}H_{10}O_5$	271.19	1.059
7.	Luteolin	Flavonoid 8-C-glycosides	$C_{26}H_{28}O_{15}$	581.20	3.473
8.	Eriocitrin	Flavonoid-7-O-glycosides	$C_{27}H_{32}O_{15}$	635.13	1.146
9.	Lupeol	Triterpenoids	$C_{30}H_{50}O$	427.39	1.268
10.	Kirenol	Diterpenoids	$C_{20}H_{34}O_4$	339.24	1.303
11.	Syringin	Phenolic glycosides	$C_{17}H_{24}O_9$	390.166	0.955
12.	Theaflavin	Catechins	$C_{29}H_{24}O_{12}$	587.10	1.528

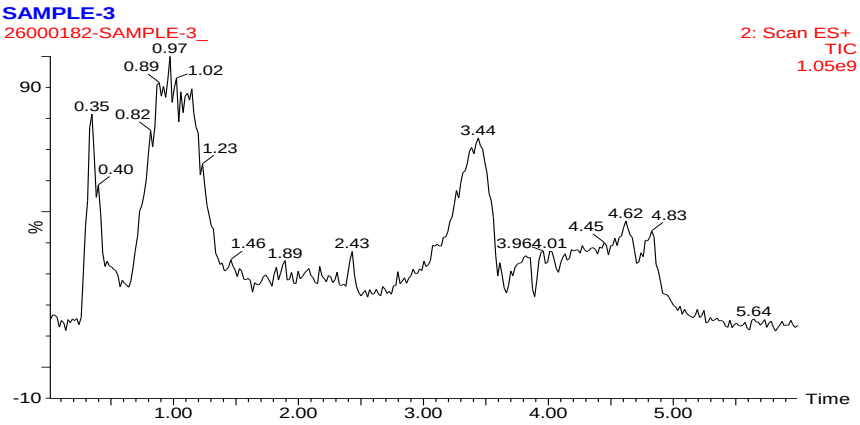

Figure 10: Liquid Chromatography of Gel @ 254 nm

Table 13: Tentatively identified Major phytochemicals in Gel

SI NO	Phytochemical	Chemical Class	Formula	m/z	Retention time (Min)
1.	Glyasperin D	Isoflavonoids	$C_{22}H_{26}O_5$	371.1	0.886
2.	Eriodictyol	Flavanones	$C_{15}H_{12}O_6$	311.050	1.025
3.	Robinin	Flavanones	$C_{15}H_{12}O_6$	311.05	0.347
4.	Syringin	Phenolic glycosides	$C_{17}H_{24}O_9$	390.16	0.903
5.	Homoorientin	Flavonoid C-glycosides	$C_{21}H_{20}O_{11}$	487.05	1.025
6.	Mulberroside F	2-arylbenzofuran flavonoids	$C_{26}H_{30}O_{14}$	589.144	1.077
7.	3-Epilupeol	Triterpenoids	$C_{30}H_{50}O$	449.373	0.886
8.	Isotectorigenin	7-O-methylisoflavones	$C_{18}H_{16}O_6$	329.1651	0.903
9.	Gaultherin	Phenolic glycosides	$C_{19}H_{26}O_{12}$	175.03751	0.99
10.	Vincanidine	Strychnos alkaloids	$C_{19}H_{20}N_2O_2$	309.15317	1.025
11.	Condylocarpine N-oxide	Strychnos alkaloids	$C_{20}H_{22}N_2O_3$	339.17108	4.741

3. DISCUSSION:

The Organoleptic evaluation showed distinct variation among the polyherbal oil, ointment, and gel formulations in terms of color, texture, and consistency, while retaining a characteristic odor and bitter taste. The oil exhibited a viscous liquid nature, whereas the ointment and gel showed semisolid consistency as mentioned in Table 7 which is suitable for topical application.

The physicochemical analysis of the polyherbal oil demonstrated acceptable quality parameters. The acid value and free fatty acid content reflected acceptable lipid characteristics of the formulation, while the peroxide value and rancidity assessment suggested minimal oxidative deterioration. The iodine value suggested the presence of unsaturated constituents, and the saponification value reflected the fatty acid composition of the base oil. Refractive index, specific gravity, and unsaponifiable matter were found within acceptable limits as mentioned in Table 8, indicating consistency and purity of the formulation.

The ointment and gel formulations exhibited pH values near the physiological skin range, suggesting suitability for dermal application. The ointment showed higher viscosity, whereas the gel demonstrated better spreadability, Extrudability, and washability, indicating improved ease of application and patient acceptability as mentioned in Table 9. Accelerated stability studies revealed no significant changes in Ph,

organoleptic properties or physical characteristics over 45 days, ensuring good formulation stability as mentioned in Table 10.

LC-MS based phytochemical profiling of the polyherbal oil (sample 1), ointment (sample 2), and gel (sample 3) demonstrated the presence of multiple phytochemical classes including flavonoids, isoflavonoids, terpenoids, triterpenoids, phytosterols, phenolic glycosides, and polyphenolic compounds. Phytochemical assignments were tentatively made based on LC-MS spectral matching and available literature comparison typically attributed to Polyherbal medicine.

Comparative LC-MS analysis of the polyherbal oil, ointment, gel formulations demonstrated formulation dependent variations in phytochemical composition. The oil and ointment showed similarity in terpenoids and flavonoids constituents including Glyasperin D and Lupeol. The oil formulation demonstrated the presence of α-Pinene, Curdione, Campesterol, Datiscin, Glyasperin D, Homoorientin, and other terpenoids derivatives suggestive of retention of lipophilic phytoconstituents within the lipid matrix.

The ointment formulation demonstrated the presence of flavonoids and terpenoids derivatives including Silybin, Biochanin A, Apigenin, Baiicalein, Eriocitrin, Lupeol, Kirenol, Syringin, and Theaflavin. The semisolid liquid matrix of the ointment may contribute to retention of moderately lipophilic phytoconstituents during pharmaceutical modification.

The gel formulation exhibited comparatively greater representation of polar phytoconstituents including Eriodictyol, Robinin, Homoorientin, Mulberroside F, Isotectorigenin, Syringin, and Gaultherin. The hydrophilic polymeric gel matrix may influence selective retention of polar and glycosidic phytoconstituents

The oil and gel shared flavonoid glycosides, phenolic glycosides, terpenoids derivatives despite contrasting polarity. Homoorientin in the oil and flavonoids-7-O-glycosides in the gel suggest flavonoids stability across lipophilic and hydrophilic systems. Also α-pinene in the oil and terpene lactones in the gel indicates retention of aromatic terpenoids. Both retained alkaloidal metabolites. The oil demonstrated the presence of atropine sulfate, a characteristic tropane alkaloid associated with Datura metel, whereas the gel showed tentatively annotations of Vincanidine and condylocarpine N-oxide, which falls under the Strychnos alkaloid ontology. The ointment and gel demonstrated the greatest similarity in phytochemical composition. Both showed abundant flavonoids glycosides, glycosidic phytoconstituents, phenolic compounds. The ointment contained diterpene glycosides and lignans, while the gel showed syringing, terpene lactones, and repeated Strychnos alkaloid annotations, suggesting semisolid system favor preservation of phytochemicals.

Overall, the oil retained lipophilic terpenoids and tropane alkaloids, while the ointment and gel preserved a broad spectrum of flavonoids, glycosides, terpenoids, and alkaloidal constituents. Atropine sulphate identified in the oil corresponds to the characteristic tropane alkaloida profile of *Datura metel*, whereas α-pinene and lupeol are phytoconstituents reported in *Vitex negundo*. In the gel, Vincanidine and Condylocarpine N-oxide were tentatively annotated and classified under the Strychnos alkaloid ontology. Collectively, the three formulations retained a common phytochemical backbone despite pharmaceutical modification. Despite these similarities, marked dissimilarities were evident between the samples. The oil was characterized by stronger retention of lipophilic constituents, particularly volatile terpenoids and tropane alkaloids, including atropine sulfate, indicating a phytochemical profile closely associated with *Datura metel.* The ointment exhibited a comparatively balanced profile containing glycosides, lignans, diterpene compounds, and moderate alkaloidal content, reflecting preservation of both lipophilic and semipolar constituents

within the semisolid lipid base. In contrast, the gel formulation showed the richest flavonoid and glycosidic composition, together with tentative annotations of Vincanidine and Condylocarpine N-oxide, both classified under the Strychnos alkaloid ontology. These findings indicate comparatively greater representation of polar phytochemicals and alkaloidal constituents in the gel formulation under the present analytical conditions. The LC-MS analysis suggests that certain constituents detected in the ointment and gel are likely attributable not to the herbal ingredients, but to the excipients and base materials used during preparation. In the ointment, the presence of long chain hydrocarbons, fatty acid esters, lipid derivatives can be because of hard paraffin, soft paraffin, wool fat, glyceryl monostearate, and cetearyl alcohol. Certain volatile aromatic constituents may also be influenced by excipient additives such as cinnamon oil. In the gel small polar peaks and polymer associated fragments are likely contributed by agents like carbopol 934, polyethylene glycol (PEG-400), triethanolamine, glycerine, sodium lactate, citric acid, sorbic acid, and potassium sorbate. These may generate glycol derived fragments, acidic adducts, preservative related ions, and polymeric degradation products during ionization. Therefore, while the major flavonoids, terpenoids, glycosides, and alkaloids represent retained herbal phytoconstituents, some low molecular weight lipid, polymeric, and preservative associated compounds observed in the ointment and gel profiles are more appropriately interpreted as formulation derived constituents originating from the dosage form excipients rather than the herbal drugs themselves.

The base polyherbal oil exhibited a wider chemical diversity, while the gel and ointment reflected selective retention of key constituents. Such diversity can be because of formulation processes (*samskara*) like boiling process, excipients – drug interaction during formulation. In spite of these variations, principle constituents were consistently retained across all three dosage forms, despite differences in composition. The consistently detected phytoconstituents are flavonoids glycosides, flavonoids C- and O-glycosides, phenolic glycosides, terpene derivatives, terpene glycosides. Representative constituents identified across the formulations included Glyasperin D, Homoorientin, Syringin, flavonoids glycosides, terpenoids derivatives, and phenolic glycosides, indicating partial retention of common phytochemical classes across the formulations.

Interestingly, the gel and ointment formulations showed the presence of several flavonoids and flavanoid glycoside constituents in the LC-MS analysis. In gel formulation (sample-3), the specifically identified flavonoids related constituents included flavonoids-7-O-glycosides, flavonoids C-glycosides, eriodictyol and other phenolic flavonoids derivatives. The gel also showed syringing and multiple glycosidic polyphenolic constituents. In the ointment formulation (sample-2), the flavonoids included isoflavonoid O-glycosides, flavonoid glycosides, and lignin-associated phenolic constituents. Both formulations retained substantial flavonoids chemistry, with the gel demonstrated comparatively greater representation of polar flavonoids glycosides. Difference in phytochemical profiles may be influenced by excipients interactions, polarity differences, formulation processing, and differential solubility characteristics.

Overall the outcomes highlights that pharmaceutical development can alter phytochemical expression without impairing essential constituents. LC-MS based fingerprinting served as a useful preliminary analytical approach for comparative phytochemical evaluation of the formulation.

Analytical limitations of the study:

The present study was limited to qualitative LC-MS based phytochemical fingerprinting. Quantitative estimation of individual phytoconstituents, validation parameters such as LOD, LOQ, precision, repeatability, and residual alkaloidal

analysis were not performed. Identification of phytoconstituents was based primarily on spectral library matching and requires further confirmation using reference standards.

5. CONCLUSION:

The present work successfully demonstrates the pharmaceutical development of classical polyherbal oil to its gel and ointment with contemporary pharmaceutical principles.LC-MS fingerprinting supports the presence and retention of vital bioactive phytoconstituents such as flavonoids glycosides, flavonoids C- and O-glycosides, phenolic glycosides, terpene derivatives, terpene glycosides, flavonoids 7-O-glycosides, isoflavonoid glycosides, terpene lactones, diterpene glycosides across all three formulations with not much compositional integrity. Though minor variations were observed might be because of formulation processes. Overall the study establishes LC-MS as a useful preliminary analytical approach for comparative phytochemical evaluation. Further quantitative and validated analytical studies are required to establish comprehensive standardization and safety assessment.

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