RESEARCH PAPER

Physicochemical, Phytochemical, and HPTLC Characterisation of an Ayurvedic Polyherbal Formulation used in Chronic Suppurative Otitis Media

Akshata Nara[1], Rajesh Kumar[2] and Manoj Kumar[3]

[1]PhD Scholar, Department of Shalakya Tantra, Faculty of Ayurveda, IMS, BHU, Varanasi, Uttar Pradesh, India
[2]Professor, Department of Otorhinolaryngology, IMS, BHU, Varanasi, Uttar Pradesh, India
[3]Professor, Department of Shalakya Tantra, Faculty of Ayurveda, IMS, BHU, Varanasi, Uttar Pradesh, India
Corresponding Author: Akshata Nara

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT

Aim: This study evaluates the traditional Ayurvedic polyherbal formulation *Karna Dhoopana Yoga* by establishing its analytical fingerprint, including its physicochemical parameters, phytochemical profile, and HPTLC characteristics.

Methods: The formulation was prepared following the classical Ayurvedic method. Physicochemical parameters such as total ash, acid-insoluble ash, loss on drying, and moisture content were determined as per pharmacopeial guidelines. HPTLC fingerprinting was performed to identify the presence of various phytoconstituents. Preliminary phytochemical screening was conducted using standard qualitative chemical tests.

Results: The formulation exhibited a total ash value of 26.08 % and an acid-insoluble ash value of 2.17 %, indicating acceptable purity and minimal contamination. The moisture content 5.572%, and the loss on drying 6.0 % were within pharmacopeial limits, confirming good stability and a low risk of microbial growth. HPTLC analysis revealed eight distinct spots under short UV and eight spots under long UV, denoting the presence of multiple bioactive components. Phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, phenols, and terpenoids, each contributing to the formulation's potential pharmacological activity.

In Ayurvedic pharmaceutics, plant-based fine powder *Churna Kalpana*is regarded as one of the most ancient and widely utilised solid dosage forms. These herbal powders may be prepared from a single medicinal plant or as a combination of multiple ingredients mixed in specific proportions as described in classical texts. The therapeutic efficacy of such formulations is influenced not only by the intrinsic phytochemical composition of the ingredients but also by the methods and conditions employed during their preparation.

This ear-fumigation formulation, Karna Dhoopana[1]It is described in the *Sushruta Samhita*with therapeutic indication of wound management (*Vrana Upachara*). It is a polyherbal preparation composed of seven herbs mixed in equal proportions: *Guggulu* (*Commiphora mukul*), *Agaru* (*Aquilaria agallocha*), *Sarja Rasa* (*Vateria indica*), *Vacha* (*Acorus calamus*), *Shweta Sarshapa* (Brassica juncea), *Saindhava Lavana* (rock salt), *Nimba Patra* (*Azadirachta indica*), along with *Go-ghrita* (clarified butter).

The raw drugs for *Karna Dhoopana Yoga*were procured from an authenticated herbal supplier and underwent botanical verification at the Department of Botany, Faculty of Science, BHU. Further analytical examinations were carried out at the Department of Dravyaguna, Faculty of Ayurveda, IMS–BHU, and at the Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka.

How to cite this article: Nara A, Kumar R, Kumar M. Physicochemical, Phytochemical, and HPTLC Characterisation of an Ayurvedic Polyherbal Formulation used in Chronic Suppurative Otitis Media. Int J Drug Deliv Technol. 2026;16(38s): 929-934. DOI: 10.25258/ijddt.16.38s.99

Source of support: Nil.

Conflict of interest: None

OBJECTIVES

1. To analyse the physicochemical properties of *Karna Dhoopana Yoga*.
2. To evaluate the phytochemical characteristics of *Karna Dhoopana Yoga*.
3. To perform HPTLC fingerprinting for *Karna Dhoopana Yoga* to identify its phytoconstituent profile.

**Author for Correspondence: Akshata Nara*

MATERIALS AND METHODS

Table 1: Ingredients of *KarnaDhoopana Yoga*

S.NO	Drug name	Botanical name	Parts used
1	GUGGULU	*Commiphera mukul*	Resin
2	AGARU	*Aquilaria agallocha*	Skin
3	SARJA RASA	*Vateria indica*	*Resin*
4	VACHA	*Acorus calamus*	Roots
5	SHWETA SARSHAPA	*Brassica juncea*	*Seed*
6	SAIDHAVA LAVANA	Rock salt	-
7	NIMBA PATRA	*Azadiracta indica*	Leaves
8	GO GHRITA	Clarified butter	-

All the drugs were taken in equal quantities by weight.

Physio-Chemical Analysis of *Karna Dhoopana Yoga*(Ayurvedic polyherbal formulation)

Determination of Total Ash

About 2 g of the powdered drug was accurately weighed and placed in a pre-weighed silica crucible. It was incinerated gradually at a temperature not exceeding 450 °C until carbon-free ash was obtained. The final weight was recorded to calculate the total ash content.

Determination of Acid-Insoluble Ash

The total ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited to constant weight. The residue obtained represented the acid-insoluble fraction of the total ash.

Determination of Water-Soluble Ash

The ash was boiled with 25 ml of distilled water for 5 minutes. The mixture was filtered through ashless filter paper, and the residue was washed with hot water. This residue was ignited at a temperature not exceeding 450 °C for about 15 minutes. The weight of the insoluble residue was subtracted from the weight of the total ash, and the difference was taken as the water-soluble ash content.

Determination of pH

Dissolved 2 grams of the sample in 20 ml of distilled water (pH = 7) to prepare a stock solution. From this stock solution, two different dilutions were prepared for *Karna Dhoopana Yoga,*with a concentration of 1 mg/ml and another with 10 mg/ml. Measured the pH of these diluted solutions using a pH700-EUTECH meter (S/N 2882510).

Table 1: Physico-Chemical Analysis of *Karna Dhoopana Yoga*

S.No.	Test	Unit	Min.	Max	Test result
1.	Description		0.0	0.000	Fine brownish yellow powder with a saline and slightly bitter taste
2.	Moisture content	%w/w	5.0	10.0	5.572%
3.	Loss on drying[2]	% w/w	0.0	10.0	6.00 %
4.	Ash[2]	% w/w	6.0	16.0	8.06 %
5.	Acid-insoluble ash	% w/w	0.0	2.0	2.17 %
6.	Water-soluble ash	% w/w	2.0	3.0	22.62 %
7.	Water-insoluble ash	% w/w	0.89	13.7	6.02%
8.	Ph	1mg/ml			5.2

Heavy Metal Analysis of *Karna Dhoopana Yoga*

Nitric-Hydrochloric Acid Digestion (1:3)

1. To the samples, 10 mL of a freshly prepared acid mixture of a 1:3 mixture of 65 % HNO_3 (SD Fine-Catno-39335L05) and 37 % HCl (SD Fine-Catno-20940L05) was added (2.5 mL nitric acid and 7.5 mL hydrochloric acid), respectively.
2. The mixture was boiled gently over a water bath (95 °C) for 4–5 h (or until the sample had completely dissolved.

Instrument: AAS (Atomic Absorption Spectrophotometer- Analyst 400 Perkin Elmer)

Table 2: Heavy Metal Analysis of*Karna Dhoopana Yoga*

Sr. No	Compound	Unit	Result	Reference Method
1	Arsenic	Ppm	BDL (DL- 0.05)	ARAS/CH/WI/55
2	Lead	Ppm	BDL (DL- 0.5)	ARAS/CH/WI/55
3	Mercury	Ppm	BDL (DL- 0.05)	ARAS/CH/WI/55
4	Cadmium	Ppm	BDL (DL- 0.5)	ARAS/CH/WI/55

DL- Detection Limit, BDL-Below Detection Limit

Phytochemical Analysis of *Karna Dhoopana Yoga*

Phytochemical analysis of *Karna Dhoopana Yoga*was carried out to detect the presence of various compounds,

including alkaloids, flavonoids, tannins, steroids, glycosides, terpenoids, phenols, saponins, and quinones. For phytochemical screening, 1ml of *Karna Dhoopana Yoga* extract (5mg/mL) was used to obtain a filtrate, which served as the test solution.

Phytochemical Analysis of *Karna Dhoopana Yoga*

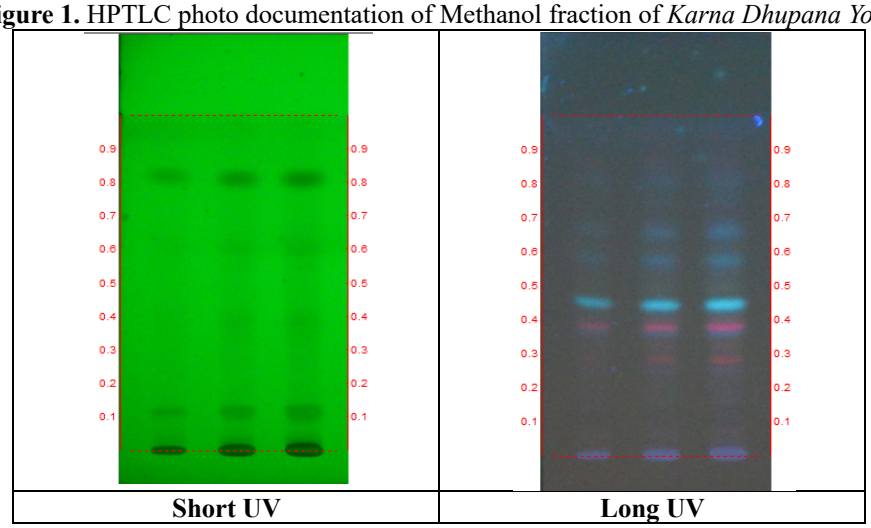
Phytochemical screening of the formulation revealed the presence of several major bioactive groups. Flavonoids, glycosides, saponins, terpenoids, tannins, phenols, carbohydrates, starch, volatile oils, and fats and oils tested positive, indicating a diverse range of phytoconstituents with potential therapeutic relevance. In contrast, alkaloids, steroids, and proteins were absent, suggesting that the formulation's activity is predominantly attributed to its non-alkaloidal, non-steroidal, and non-proteinaceous components.

HPTLC

1 gram of *Karna Dhoopana Yoga*was weighed and dissolved in 10 ml of methanol. 3, 6 and 9µl of the above was applied on a pre-coated silica gel F_{254} on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl Acetate: Glacial acetic acid (1.0: 0.5: 0.1). The developed plates were visualized in short UV, long UV and scanned under UV 254nm, 366nm. Rf, color of the spots and densitometric scan were recorded

Part C: Results

Figure 1. HPTLC photo documentation of Methanol fraction of *Karna Dhupana Yoga*



track 1 - *Karna Dhoopana Yoga* – 3µl

track 2 – *Karna Dhoopana Yoga* – 6µl

Track 3 - *Karna Dhoopana Yoga* – 9µl

Solvent system – Toluene: Ethyl Acetate: Glacial acetic acid (1.0: 0.5: 0.1)

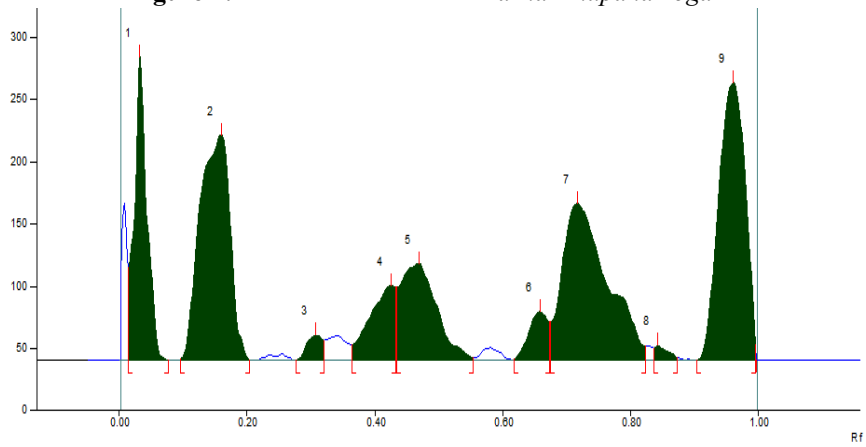
Rf - 0.65 (Gallic acid)

Table 1: R_f values of the sample of *Karna Dhoopana Yoga*

Short UV	Long UV
0.12 (Green)	-
-	0.28 (F. red)
0.39 (Green)	0.38 (F. red)
-	0.45 (F. blue)
-	0.58 (F. blue)
0.60 (Green)	-
-	0.66 (F. blue)
0.81 (Green)	-

***F – Fluorescent; L –Light; D – Dark**

Figure 2: Densitometric scan of *Karna Dhupana Yoga*



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area (AU)	Area %
1	0.01 Rf	74.2 AU	0.03 Rf	244.0 AU	24.80 %	0.08 Rf	0.0 AU	3437.2 AU	11.87 %
2	0.10 Rf	0.6 AU	0.16 Rf	181.3 AU	18.42 %	0.20 Rf	0.3 AU	6181.7 AU	21.35 %
3	0.28 Rf	0.4 AU	0.31 Rf	20.2 AU	2.05%	0.32 Rf	16.0 AU	353.4 AU	1.22%
4	0.36 Rf	12.2 AU	0.43 Rf	60.4 AU	6.14%	0.43 Rf	58.9 AU	1718.2 AU	5.93%
5	0.44 Rf	58.9 AU	0.47 Rf	77.8 AU	7.90%	0.55 Rf	1.8 AU	3130.4 AU	10.81 %
6	0.62 Rf	0.5 AU	0.66 Rf	39.1 AU	3.97%	0.67 Rf	30.5 AU	834.9 AU	2.88%
7	0.68 Rf	30.6 AU	0.72 Rf	126.2 AU	12.83 %	0.82 Rf	11.0 AU	6410.7 AU	22.14 %
8	0.84 Rf	9.7 AU	0.84 Rf	11.9 AU	1.21%	0.87 Rf	1.7 AU	183.8 AU	0.63%
9	0.90 Rf	0.2 AU	0.96 Rf	223.2 AU	22.68 %	1.00 Rf	12.6 AU	6704.7 AU	23.16 %

Fig 2a. At 254nm, R_f - 0.65 (Gallic acid)

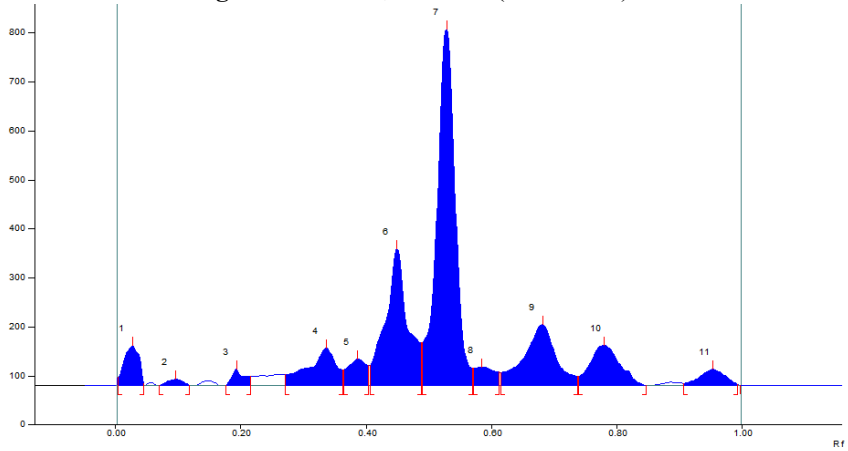


Fig 2b. At 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area (AU)	Area %
1	0.00 Rf	17.5 AU	0.03 Rf	81.0 AU	5.26%	0.05 Rf	3.1 AU	1382.9 AU	3.73%
2	0.07 Rf	0.1 AU	0.10 Rf	12.4 AU	0.81%	0.12 Rf	0.5 AU	222.9 AU	0.60%
3	0.18 Rf	0.6 AU	0.19 Rf	33.4 AU	2.17%	0.22 Rf	18.1 AU	477.3 AU	1.29%

4	0.27 Rf	22.6 AU	0.34 Rf	76.9 AU	4.99%	0.36 Rf	32.2 AU	2445.3 AU	6.60%
5	0.36 Rf	32.4 AU	0.39 Rf	53.5 AU	3.48%	0.40 Rf	41.0 AU	1159.0 AU	3.13%
6	0.41 Rf	41.3 AU	0.45 Rf	278.2 AU	18.07%	0.49 Rf	86.6 AU	7097.5 AU	19.15%
7	0.49 Rf	87.5 AU	0.53 Rf	727.1 AU	47.22%	0.57 Rf	34.9 AU	15134.7 AU	40.83%
8	0.57 Rf	35.0 AU	0.59 Rf	37.6 AU	2.44%	0.61 Rf	27.1 AU	875.7 AU	2.36%
9	0.62 Rf	26.9 AU	0.68 Rf	123.7 AU	8.04%	0.74 Rf	18.6 AU	4513.7 AU	12.18%
10	0.74 Rf	18.7 AU	0.78 Rf	82.6 AU	5.37%	0.85 Rf	0.1 AU	2792.8 AU	7.53%
11	0.91 Rf	4.0 AU	0.95 Rf	33.1 AU	2.15%	0.99 Rf	2.8 AU	962.9 AU	2.60%

DISCUSSION

The present analytical investigation on *Karna Dhoopana Yoga* was undertaken to characterise its physicochemical, phytochemical, and chromatographic profiles, thereby generating primary reference parameters that define the formulation's intrinsic properties and analysis. The formulation, being polyherbal, integrates multiple classical ingredients such as *Guggulu (Commiphora mukul)*, *Agaru (Aquilaria agallocha)*, *Sarja rasa (Vateria indica)*, *Vacha (Acorus calamus)*, *Shweta Sarshapa (Brassica juncea)*, *Saidhava Lavana (Rock salt)*, *Nimba Patra (Azadirachta indica)*, and *Go Ghrita (Cow ghee),*each possessing distinct therapeutic properties mentioned in Ayurvedic texts for their antimicrobial, anti-inflammatory, and wound-healing potential.

The physicochemical evaluation serves as a critical measure for assessing the purity, quality, and stability of the drug. The total ash content (26.08%) and acid-insoluble ash (2.17%) indicate the presence of natural inorganic constituents, such as minerals, that may contribute to the formulation's pharmacological action. The acid-insoluble fraction being within acceptable limits suggests minimal contamination by earthy or siliceous matter. The recorded moisture content (5.57%) and loss on drying (6.0%) fall within pharmacopeial norms, confirming that the formulation is adequately dried and thus less prone to microbial degradation. Low moisture values ensure longer shelf life and better preservation of volatile compounds essential for therapeutic action.

The water-soluble ash (22.62%) and water-insoluble ash (6.02%) findings point toward a high proportion of bioavailable minerals and trace elements that may enhance the bio efficacy of the preparation. The pH value (5.2) indicates a mildly acidic nature, which could be beneficial for external application in ear-related pathologies, maintaining the normal acidic milieu of the external auditory canal and preventing pathogenic bacterial overgrowth.

Heavy metal analysis showed that lead, arsenic, mercury, and cadmium levels were below detectable limits (BDL), ensuring the safety of the formulation and compliance with standard regulatory guidelines. This supports the premise that the classical method of preparation and sourcing of authenticated raw materials contributes to the purity of the formulation.

Preliminary phytochemical screening revealed the presence of flavonoids, glycosides, terpenoids, tannins, phenols, carbohydrates, starch, volatile oils, and fats. The absence of alkaloids and steroids was noted. These bioactive constituents play diverse pharmacological roles: flavonoids and phenols act as potent antioxidants and anti-inflammatory agents; tannins contribute to astringent and antimicrobial effects; terpenoids exhibit antimicrobial and anti-inflammatory actions; and volatile oils and fats enhance the formulation's penetration and soothing action during *dhoopana* (fumigation). The synergistic presence of these compounds aligns with the Ayurvedic concept of *yogavahi guna*, where multiple ingredients act in concert to enhance therapeutic efficacy.

HPTLC fingerprinting is an essential modern tool for the standardisation of herbal formulations. The *Karna Dhoopana Yoga* sample displayed eight distinct peaks under short and long UV light, with a characteristic Rf value of 0.65 corresponding to Gallic acid. This phenolic compound is a well-known antioxidant and antimicrobial agent, often found in *Vateria indica* and *Azadirachta indica*, suggesting that these constituents contribute significantly to the observed chromatographic profile. The multiple peaks observed confirm the polychemical nature of the formulation, reflecting the presence of diverse phytoconstituents responsible for its pharmacodynamic effects. These fingerprint profiles can serve as a reference chromatogram for future quality control and comparative analytical studies.

Overall, the study demonstrates that *Karna Dhoopana Yoga* exhibits the essential physicochemical stability, safety profile, and phytochemical richness necessary for an effective Ayurvedic formulation. The results corroborate classical Ayurvedic descriptions that emphasise its role in ear pathologies, particularly in purifying the local environment and reducing the microbial load in otorrhea and chronic suppurative conditions.

CONCLUSION

The analytical evaluation of *Karna Dhoopana Yoga* primarily provides a physicochemical and phytochemical fingerprint for quality assessment and consistency evaluation. Physicochemical parameters confirmed acceptable purity, stability, and absence of heavy metal contamination, supporting its pharmaceutical suitability. Preliminary phytochemical screening showed the presence of flavonoids, glycosides, tannins, terpenoids, and phenolic compounds. HPTLC analysis revealed a characteristic chromatographic fingerprint with an Rf value of 0.65 corresponding to gallic acid, which may serve as a reference marker. This study is limited to preliminary fingerprinting, and compounds not detected cannot be inferred. The findings offer baseline information for future detailed analytical and clinical studies

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Institute of Medical Sciences, Banaras Hindu University, Department of *Shalakya Tantra*, Faculty of Ayurveda, the **Department of Botany, Faculty of Science, BHU**, and to the ***Dravyaguna* Department, Faculty of Ayurveda, Institute of Medical Sciences, BHU**, for their valuable support in the analytical studies.The authors sincerely acknowledge Professor Alka Aggrawal for her valuable suggestionson conducting chemical analysis. The authors also extend heartfelt thanks to the **Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka**, for providing essential facilities and assistance during the pharmaceutical and analytical laboratory work

REFERENCES

1. Sushruta. *Sushruta Samhita*. (Chaukhambha Sanskrit Sansthan, Varanasi, 2015).
2. Govt. of India. *The Ayurvedic Pharmacopoeia of India*. vol. 2 (1999).