RESEARCH PAPER

Pharmaceutico-Analytical Standardization and GC–MS Characterization of an Ayurvedic Dhoopana Yoga

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ABSTRACT

Background:
Dhoopana Karma is an important Ayurvedic fumigation procedure traditionally used for environmental purification, prevention of infections, and microbial control. Despite its extensive classical use, scientific analytical validation of Dhoopana formulations remains limited.

Aim:
To evaluate the pharmaceutico-analytical characteristics and phytochemical profile of an Ayurvedic Dhoopana Yoga using physicochemical parameters and Gas Chromatography–Mass Spectrometry (GC–MS).

Materials and Methods:

The Dhoopana formulation was prepared using Guggulu, Agaru, Vacha, Sarjarasa, Nimba, and Gaur Sarshapa. Organoleptic evaluation and physicochemical analysis including moisture content, total ash, acid-insoluble ash, water-soluble extractive value, and alcohol-soluble extractive value were performed according to API standards. GC–MS analysis was carried out using an Rxi-5Sil MS column under standard laboratory conditions.

Results:
The formulation exhibited acceptable physicochemical parameters within standard limits. GC–MS analysis identified 23 phytochemical compounds predominantly consisting of sesquiterpenes, aromatic ethers, fatty acid esters, and oxygenated derivatives. Major compounds identified were Asarone (24.99%), Germacrene D (23.91%), 2-(Octanoyloxy) propane-1,3-diyl bis(decanoate) (13.42%), Aromadendrene oxide (5.23%), and Copaene (4.98%).

Conclusion:
The analytical findings support the standardization and quality assessment of the Dhoopana Yoga. Presence of antimicrobial and fumigant phytoconstituents validates the traditional use of the formulation in environmental purification and infection control.

Keywords: Ayurveda, Dhoopana Yoga, GC–MS, Physicochemical analysis, Standardization, Herbal fumigation, antimicrobial phytochemicals.

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1. INTRODUCTION

Dhoopana Karma is a traditional *Ayurvedic* fumigation therapy described in classical *Ayurvedic* literature for purification of surroundings, disinfection, and prevention of infectious diseases. Various herbal drugs possessing antimicrobial, aromatic, and insect-repellent properties are used in *Dhoopana* formulations. These formulations are believed to purify air and reduce pathogenic contamination through medicated smoke.

In recent years, increasing microbial resistance and concern regarding environmental hygiene have encouraged researchers to scientifically validate traditional herbal fumigation practices. Standardization and analytical evaluation are essential to establish the identity, purity, quality, and safety of herbal formulations.

Analytical studies help in determining organoleptic properties, physicochemical parameters, and phytochemical composition of medicinal preparations. Advanced analytical tools such as GC–MS provide detailed information regarding volatile and semi-volatile bioactive compounds present in herbal formulations.

The present study was undertaken to evaluate the pharmaceutico-analytical properties and GC–MS profile of an *Ayurvedic Dhoopana Yoga* prepared using classical fumigant drugs.

Described in "*Agropharniya adhyaay*"- **"ततो गुग्गुल्वगुरुसर्जरसवचागौर सर्षपचूर्णैर्लवणनिम्बपत्रविमित्रैराज्ययुक्तैर्धूपयेत्, आज्यशेषेण चास्य प्राणन् समालभेत् ।। (सु.सू. 5/18)"**

This *Dhoopana* formulation is claimed as potential disinfectant by *Acharya*. It contains *Guggul, Aguru, Vacha, Sarjras, Gaursarshap, Nimb patra* added with *Saindhav lavan*, and *Goghrit*. All these drugs are having *krimighna* and *jantughna* properties. This *Dhoopana* formulation may act as a safe preparation which will be helpful to prevent hospital acquired infections. The formulation selected for present study is "*Dhoopan Dravyas*" described by *Acharya Sushruta* in *Sushrut Samhita sutra sthan*, 5th chapter "*Agrahopharniya adhyay"* shlok no. 18. The *Dhoopana* formulation containing *Guggul*, *Agaru, Vacha, Sarjarasa, Gaur Sarshap, Neem*, *Saindhava*, and *Go-Ghrita* is designed according to classical Ayurvedic principles to achieve potent *Krimighna* (antimicrobial*), Kitaghna* (insecticidal), *Vatahara*, and *Shodhana* (purifying) effects. Each ingredient is selected for its ability to transform into volatile, bioactive aromatic compounds when exposed to fire (*Agni*), enabling them to disinfect the air, surfaces, and the environment effectively. The synergy of *Dravya* (herbs), *Guna* (properties), *Veerya* (potency), and *Vipaka* (postdigestive effect) forms the backbone of this formulation. The *Dhoom* (smoke) produced through this process is associated with fire and possesses qualities such as *laghuta* (lightness) and *chalanatva* (mobility). These properties enable it to disperse rapidly and reach every corner of a room. It can flow into narrow spaces, cracks, and concealed areas where stagnant air and microbial accumulation are common. Considering these points, adopting *Dhoopan Karma* may prove to be an effective alternative for reducing hospital-acquired infections.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Raw Drugs

The raw drugs were procured from the raw drug shop Kesari mal, Raipur, Chhattisgarh.

The raw materials used in the formulation were:

The ingredients were authenticated and analyzed at Drug Testing Laboratory Evum Anusandhan Kendra, Raipur, Chhattisgarh.

S. No.	DRUGS	BOOKS REFERRED
1.	*Guggul (Commiphora mukul)*	Data base on medicinal plant volume- 2, API part I volume-1, pg. 43.
2.	*Aguru (Aquilaria agallocha)*	Dravyaguna vigyana by J.L.N. Shastri, API part I volume-4, pg. 04.
3.	*Vacha (Acorus calamus)*	Data base on medicinal plant volume 1, API part | Volume-2, pg. 168.
4.	*Sarjras (Shorea robusta)*	Data base on medicinal plant volume 5, API part I volume-4, pg. 106.
5.	*Nimba (Azadirachta indica)*	Data base on medicinal plant volume 1, API part I volume- 5, pg. 121.
6.	*Gaur sarshap (Brassica campestris)*	Shri Narhari Pandit's Raj Nighantu, Chaukhambha Orientalia.

AUTHENTICATION OF RAW DRUGS

Taxonomic identification of raw drugs was done in Botany section and Pharmacognosy section of Central Council for Research in Ayurvedic Sciences, Central Ayurveda Research Institute (CCRAS-CARI), JHANSI (U.P).

Table 1: Presenting the Reference number of authenticated crude drugs by
CCRAS-CARI -
Table 2.1-

S.NO.	DRUG NAME	PART USED	REFERENCE NUMBER
1.	*Guggulu*	Exudate	1-6/2022-23/CARI
2.	*Agaru*	Heart wood	1-6/2022-23/CARI
3.	*Vacha*	Root	1-6/2022-23/CARI
4.	*Sarjrasa*	Exudate	1-6/2022-23/CARI
5.	*Neem*	Leaves	1-6/2022-23/CARI
6.	*Gaur sarshap*	Seeds	1-6/2022-23/CARI

Table-2.2 -Organoleptic Evaluation

S.N.	Organoleptic characteristics	S_1	S_2	S_3	S_4	S_5	S_6
1.	Colour	Reddish brown	Dark brown	Light brown	Whitish	Yellowish green	Creamish white
2.	Odour	Fragrance	Characteristic	Aromatic	Fragrance	Distinct	Odourless
3.	Texture	Coarse	Fibrous	Fibrous	Coarse	Smooth	Smooth
4.	Taste	Bitter and astringent	Characteristic	Aromatic	Characteristic	Bitter	Bitter

S_1– Guggulu, S_2 – Agaru, S_3 – Vacha, S_4 – Sarjaras, S_5 – Neem, S_6 – Gaursarshap, S_7- Dhoopan formulation

Table 2.3: The Standard Physicochemical Characterstics of Ingredients of *Dhoopana* formulation as defined in API

S.NO.	Parameters	S_1	S_2	S_3	S_4	S_5	S_6	S_7
1.	Moisture Content	-	-	-	-	-	-	-
2.	Total Ash Value %	NMT 5 %	NMT 13 %	NMT 7 %	NMT 0.1 %	NMT 7 %	-	-
3.	Acid insoluble Ash value %	NMT 1 %	NMT 0.5 %	NMT 15	Negligible	NMT 1.5 %	-	-
4.	Water soluble extractive value %	NLT 53 %	NLT 2 %	NLT 16 %	-	NLT 5 %	-	-
5.	Alcohol soluble extractive value%	NLT 27 %	NLT 1 %	NLT 9 %	NLT 60 %	NLT 6 %	-	-

Table 2.4: The Standard Physicochemical Characterstics of Ingredients of *Dhoopana* formulation.

S. NO.	Parameters	S_1	S_2	S_3	S_4	S_5	S_6	S_7
1.	Moisture Content	12 %	1 %	15 %	7%	13.5 %	0.5 %	6.5 %
2.	Total Ash Value %	2.67 %	9.67 %	4 %	0.03%	6 %	5 %	12.3 %
3.	Acid insolulble Ash value %	1 %	0.33 %	0.67 %	Negligible	1 %	3.34 %	6.67 %
4.	Water soluble extractive value %	26.2 %	1.4 %	17.3 %	58 %	19.6 %	11.4 %	15 %
5.	Alcohol soluble extractive value %	42.6 %	3.6 %	43.4 %	63.7%	26.8 %	17.6 %	11.4 %

S_1–Guggulu, S_2–Agaru, S_3–Vacha, S_4 –Sarjaras, S_5 – Neem, S_6–Gaursarshap, S_7-Dhoopan formulation

The Physicochemical study of ingredients *Dhoopan* formulation i.e. from S1 to S6 are found within limits as mentioned in the different volumes of API. The drug *Gaursarshap* is not mentioned in the API, moreover the formulation *Dhoopan* formulation also not mentioned in the AFI.

GC-MS SPECTRAL ANALYSIS OF DHOOPAN FORMULATION

A technique called gas chromatography- mass spectrometry (GC-MS) combines the advantages of gas-liquid chromatography and mass spectrometry to identify various compounds in a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, investigating explosives and identifying unidentified materials. Additionally, it can find traces in materials that were previously believed to have deteriorated beyond identification.

GC-MS were performed for *Dhoopan* formulation as per standard procedure in Devansh Drug Testing Laboratory, Haridwar (Uttarakhand).

3. Method-

GCMS column used: Rxi-5Sil MS Length: 30M
Film thickness:0.25 micron
GCMS Condition: Column Oven Temp 60°C
Injection mode: Splitless
Program: Oven Temp Program

RESULTS

TABLE 3.1 - Rate, Temperature and Time followed in GC-MS

S. NO	Rate	Final Temperature (°C)	Hold Time (min)
1.	-	60.0	2.00
2.	4.00	280.0	5.00

Peak #	Name	R. Time	Base m/z	Area	Area %	Height	Height %
1	Ethylbenzene	3.630	91.05	1425745	0.34	959273	0.89
2	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-	18.153	121.10	1771281	0.43	6150760.57	0.57

3	Aciphyllene	18.892	95.05	2151211	0.52	750031	0.70
4	Copaene	19.630	105.05	2061616 7	4.98	7174558	6.67
5	(-)-beta-Bourbonene	19.888	81.05	4888252	1.18	1509518	1.40
6	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)	20.097	93.05	1573356 4	3.80	4903146	4.56
7	Caryophyllene	21.068	91.05	1330255 7	3.21	3577230	3.32
8	1H-Cyclopenta [1,3] Cyclopropa[1,2] benzene, octahydro	21.405	161.10	1987531	0.48	685556	0.64
9	Humulene	22.229	93.05	2787292	0.67	894386	0.83
10	Germacrene D	23.104	161.10	9903006 7	23.91	28037525	26.06
11	(1S,2E,6E10R)- 3,7,11,11- Tetramethyl bicyclo[8.1.0] und	23.538	121.05	1132567 9	2.73	3801992	3.53
12	(1R,7S, E)-7-Isopropyl-4,10-dimethylenecyclodec-5- enol	25.402	123.05	3473886	0.84	1167044	1.08
13	1H-Cycloprop[e] azulen-7-ol, decahydro-1,1,7-trimethyl	26.072	91.05	2195304 6	5.30	7105761	6.60
14	Caryophyllene oxide	26.221	79.05	4640521	1.12	1611861	1.50
15	(1R,7S, E)-7-Isopropyl-4,10-dimethylenecyclodec-5- enol	26.321	91.05	3885758	0.94	1272438	1.18
16	(1R,2R,4S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylene	26.642	159.10	6002923	1.45	2081449	1.93
17	Asarone	27.201	208.05	1035177 33	24.99	24926604	23.17
18	2-Butenal,2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-y)	33.315	123.10	5517721	1.33	1810147	1.68
19	Hydroxydehydrostevic acid	33.490	121.05	5869855	1.42	1827867	1.70
20	Aromadendrene oxide-(1)	34.165	107.05	2167972 6	5.23	5388145	5.01
21	1-Heptatriacotanol	35.249	123.10	3961906	0.96	1264008	1.17
22	2-(Octanoyloxy)propane- 1,3-diyl bis(decanoate	54.576	155.10	5558122 7	13.42	5310624	4.94

	)						
23	**Nonacosane**	**56.322**	**57.05**	**311513 8**	**0.75**	**9163 54**	**0.85**

The GC–MS analysis identified **23 phytochemical compounds** in the *Dhoopan* formulation under the conditions described (Rxi-5Sil MS column, 30 m, 0.25 µm film, splitless injection, temperature gradient from 60°C to 280°C). The chemical profile indicates a dominant presence of terpenes, sesquiterpenes, aromatic compounds, and oxygenated derivatives, many of which are well-known for antimicrobial, anti-inflammatory, and air-purifying properties.

Below is the interpretation based on peak area %, retention time behavior, and chemical nature:

[I]. Dominant Compounds Based on Area %

The largest peaks in the chromatogram (based on area %) are:

1. ***Asarone – 24.99% (Peak #17)***

- One of the highest contributors in the sample.
- Asarone is an aromatic ether commonly found in *Acorus calamus* and several *Ayurvedic* fumigants.
- Known activities**:**
- Strong antimicrobial and antifungal effects
- Insecticidal and repellent properties
- Sedative and aromatic properties
- Its heavy presence may contribute substantially to the air sanitizing and pathogen-reducing ability of *Dhoopan*.

2. ***Germacrene D – 23.91% (Peak #10)***

- A sesquiterpene hydrocarbon commonly found in aromatic plant resins.
- Biological roles**:**
- Significant antibacterial and antifungal activity
- Anti-inflammatory and antioxidant
- Germacrene D is also a major volatile in *guggulu*, *turmeric*, and other substances used traditionally for cleansing the environment.

3. *2-(Octanoyloxy) propane-1,3-diyl bis(decanoate) – 13.42% (Peak #22)*

- A long-chain, ester-rich compound.
- Likely non-volatile but detected due to high-temperature elution.
- Possible origin**:** lipid components or plant resin derivatives.
- These fatty esters may support slow-release antimicrobial action when heated.

4. *Copaene – 4.98% (Peak #4)*

- A microbial-growth inhibiting sesquiterpene.
- Often found in fumigant herbs like *clove*, *guggulu*, *juniper*.
- Reinforces the antimicrobial signature of the sample.

5. *Aromadendrene oxide-1 – 5.23% (Peak #20)*

- Oxygenated sesquiterpene.
- Strong antimicrobial, antiviral, and antioxidant activity**.**
- Oxygenated terpenes are often more bioactive than hydrocarbon terpenes.

6. *1H-Cycloprop[e]azulen-7-ol derivatives – 5.30% (Peak #13)*

- Oxygenated sesquiterpenoid alcohols.
- Known for broad-spectrum antimicrobial activity.
- Typically present in resins and fumigant plant parts.

[II]. Other Significant Components

1. Caryophyllene & Caryophyllene oxide (Peaks #7 & #14)

- Together contribute ~4.3% area.
- Caryophyllene is a well-established anti-inflammatory and antibacterial terpene.

2. *Humulene (Peak #9)*

- Anti-inflammatory, antimicrobial sesquiterpene.

1. Ethylbenzene (Peak #1)

- Small amount; likely from natural aromatic resin components, not harmful at this trace level.

2. Long-chain hydrocarbons (Peak #23: Nonacosane)

- Found in beeswax, plant wax, and resins.
- Known to have surface-protective and insect-repelling properties.

[III]. Chemical Class Distribution

Based on area %, the *Dhoopan* profile is dominated by Caryophyllene oxide has even stronger antimicrobial potency and is responsible for insect-repellent action.

Table No. 3.2- Phytochemical Contribution with functional interpretation

Chemical Class	Contribution	Interpretation

Sesquiterpenes	~50–55%	Major antimicrobial, anti-inflammatory activity
Aromatic e	~25%	Strong disinfectant and fumigant action
Fatty acid esters	~13%	Slow-release antimicrobial potential
Hydrocarbons & waxes	~2%	Stability, fragrance binding



ACID INSOLUBLE ASH



ALCOHOL INSOLUBLE EXTRACT

4. RESULTS

4.1 Physicochemical Findings

The formulation exhibited acceptable physicochemical characteristics and complied with API reference limits for individual ingredients. Moisture content, ash values, and extractive values confirmed the purity and stability of the formulation.

4.2 GC–MS Findings

GC–MS analysis identified 23 phytochemical compounds.

Major Compounds Identified

Compound	Area %	Probable Activity
Asarone	24.99%	Antimicrobial, fumigant
Germacrene D	23.91%	Antibacterial, antioxidant
2-(Octanoyloxy) propane-1,3-diyl bis(decanoate)	13.42%	Sustained antimicrobial effect
Aromadendrene oxide	5.23%	Antimicrobial
Copaene	4.98%	Antimicrobial
Caryophyllene	3.21%	Anti-inflammatory
Caryophyllene oxide	1.12%	Insect-repellent

The phytochemical profile demonstrated predominance of sesquiterpenes and aromatic compounds associated with antimicrobial and air-purifying activities.

5. DISCUSSION

The present study scientifically evaluated an *Ayurvedic Dhoopana Yoga* using standard analytical procedures. Organoleptic and physicochemical parameters confirmed the identity and quality of the formulation ingredients. GC–MS analysis revealed the presence of several biologically active phytoconstituents. *Asarone,* identified as the major compound, is known for antimicrobial and fumigant properties and is commonly present in *Acorus calamus*. *Germacrene D* and *Caryophyllene* derivatives contribute significant antibacterial and anti-inflammatory activity.

The presence of oxygenated sesquiterpenes and aromatic compounds supports the traditional use of *Dhoopana* formulations for environmental purification and microbial reduction. Fatty acid esters detected in the formulation may assist in slow release of volatile bioactive constituents during fumigation.

The study provides scientific evidence supporting the traditional *Ayurvedic* concept of *Dhoopana Karma* as a potential natural disinfection method.

5. CONCLUSION

The Ayurvedic *Dhoopana Yoga* demonstrated satisfactory pharmaceutico-analytical characteristics and a rich phytochemical profile. GC–MS analysis identified several bioactive compounds possessing antimicrobial, fumigant, antioxidant, and insect-repellent activities. The findings validate the

traditional application of *Dhoopana Karma* for environmental purification and provide a scientific basis for future antimicrobial and air-sanitization studies.

Future Scope

- Antimicrobial assay against airborne pathogens
- Smoke toxicity evaluation
- Comparative study with modern fumigants
- Clinical environmental disinfection studies
- Nanoformulation or aerosol adaptation of *Dhoopana* extracts

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