



# I J A P C

INTERNATIONAL JOURNAL OF AYURVEDA AND  
PHARMACEUTICAL CHEMISTRY

[www.ijapc.com](http://www.ijapc.com)

E ISSN - 2350-0204

**VOLUME 9**

**ISSUE 1**

**10TH JULY**

**2018**

Greentree Group Publishers



## A Novel Approach for Preparation and Evaluation of *Dhoopana* Formulation

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### ABSTRACT

Ayurveda, the ancient science of life, has always focused on the nurture and maintenance of good health in an individual. Ayurveda believes in striking a balance between an individual (body, mind, soul, spirit). Ayurvedic fumigation, *Dhoopana* is an example of drug delivery through the inhalation route having several advantages including ease of drug administration, higher bioavailability and high potential to penetrate the blood brain barrier. Dhoop formulation made in this work is with an aim to minimize the usage of chemicals or disinfectants to cleanse the environment as well as an eco-friendly treatment against cough and cold. Efforts were made to devise an herbal Dhoop using cow dung, cow ghee, camphor, Ajwain seeds (*Trachyspermum ammi*) and Dill (*Anethum graveolens L.*) Having an appreciable fragrance. The current work focuses on preparation and bioanalytical evaluation of natural and herbal Dhoop formulation for cleansing the environment and an eco-friendly treatment against cough and cold. The antimicrobial activity of the prepared Dhoop was checked and it was found that it can be a potential source against respiratory pathogens namely, *Staphylococcus aureus* and *Klebsiella pneumoniae*.

### KEYWORDS

*Ayurveda, Dhoopana, Ajwain, Dhoop, Herbal, Cough and Cold*



**Greentree Group Publishers**

Received 14/06/18 Accepted 09/07/18 Published 10/07/18



## INTRODUCTION

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practised in India. Ayurveda, the ancient science of life, has always focused on the nurture and maintenance of good health in an individual. The health of an individual is the end result of the total sum of the processes inside his body as well as his vicinity. The seers of Ayurveda realised this dynamic equation and emphasised on the importance of man and his environment. The concepts of *Din Charya*, *Ratri-Charya* and *Ritu Charya* present in the classical treatise provide an insight as to the influence of environment upon the health of an individual<sup>7</sup>.

Dhoopana is a method by which drugs of herbal, herbomineral or animal origin are used for fumigation. Since Vedic period sterilization of house & environment around it by Dhoopana, has been going on

traditionally. Dhoopana has been mentioned in *Atharva Veda*. Dhoopana has also been mentioned for its antimicrobial and growth promoting activities for the healthy production of plants. Ample references of Dhoopana are found in *Brihatrayi* proving their vital role in disinfection and sterilisation. It consists of a variety of *Dhoopana Kalpanas* aimed at deriving health, combating diseases and even purifying environment. These formulations have been used widely since long. The *Dhoopana Dravyas* mentioned in the *Brihatrayi* have a lot of potential to manage different diseases and for sterilising in an economical and eco-friendly way without developing any drug-resistant microorganisms. It is necessary to undergo detailed study to identify the bioactive chemical moieties of the formulations and establish their safety and efficacy profiles. Standardising, characterising and marketing these *Dhoopana* formulations is the need of the hour to bring a natural eco-friendly and cheap tool to combat benefits of Dhoopana formulation.

Common side effects of cough medication includes dizziness, drowsiness, diarrhea, headaches, nausea and constipation. Some medications advised for cough may contain ingredients that are habit-forming. Another disadvantage of the habitual use



of cough syrups includes development of drug resistance, toxicity and hypersensitivity reactions which is commonly observed.

Therefore, *Dhoopana* can play a major role as a disinfectant and Ayurvedic therapeutic technique in today's era. This eco-friendly and economical technique is a viable option for treating respiratory diseases in developing countries like India.

## MATERIALS & METHODS

Plant materials viz. Ajwain seeds (*Trachyspermum ammi*) and Dill (*Anethum graveolens* L.) which are recognised for their antimicrobial potentials were procured from local market and used for the preparation after the quality assessment<sup>3</sup>. Dried Cow dung was acquired from a local dairy milk supplier from thane district. The cow dung was then pulverized in a domestic grinder and sieved to obtain the fine powder. Cow's ghee was also procured from local market after checking its quality<sup>5</sup>.

### Preparation of Dhoop Sticks:

All the above mentioned components are taken in the ratio of "10: 1:5: 2.5", respectively and then macerated finely after adding adequate distilled water to get thick paste. Cow's ghee was added in the proportion of 1% of the total mixture and

then macerated using clean, dry mortar and pestle. Dhoop sticks weremade using clean dry cork borer and glass rod. These Dhoop sticks were dried for 2 days in a hot air oven at 40<sup>0</sup>C and then stored in an air tight container<sup>5</sup>.

### Analysis of the Dhoop Sticks:

Proximate analysis of Dhoop sticks was carried out as per the standard protocols of Ayurvedic Pharmacopoeia if India. The dhoop stick was also subjected to TLC analysis for preliminary detection of Thymol. Toluene: Ethyl Acetate (90:10 v/v) was used as a mobile phase and thymol was detected by derivatising the plate by vanillin sulphuric acid reagent followed by heating of the plate at 110<sup>0</sup>C.

Further GC-MS analysis was carried out in order to identify the phytochemical constituents that are present in the formulation. Details of GC-MS analysis are as given below:

**Sample Preparation:** Ethanol was found to be suitable for polar and non-polar compounds. 1 gram Dhoop sample was extracted with 10ml ethanol overnight and filtered using syringe filter of 0.45 micron. 2 µl of prepared sample was injected into the GC-MS instrument (Shimadzu Gc-2010 Plus).

**Parameters for GC-MS analysis were maintained as stated below:**



Carrier gas-1ml per min, Split Ratio-10:1, Detector-Mass detector Shimadzu, Sample injected-2 $\mu$ l, Column-Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25mm x 0.25 $\mu$ m df, Oven temperature Programme-110 $^{\circ}$  C with 2 min hold, Up to 200 $^{\circ}$  C at the rate of 10 $^{\circ}$  C/min without hold, Up to 280 $^{\circ}$  C at the rate of 5 $^{\circ}$  C / min with 9 min hold, Injector temperature-250 $^{\circ}$  C, Total GC running time-36 min.

MS Shimadzu Analytical Parameters: Inlet line temperature -200 $^{\circ}$ C, Source temperature-200 $^{\circ}$ C, Electron energy-70 eV, Mass scan (m/z):45-450, Solvent Delay:0-2 min, Total MS running time-36 min.

In the MS Programme, NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained<sup>4</sup>.

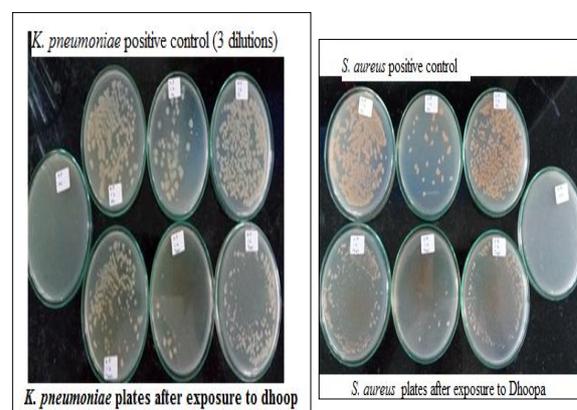
### Microbiological evaluation:

Microbiological evaluation was carried out to assess the efficacy of the formulation with respect to its antagonistic effect against respiratory pathogens. Replica

plating technique was used to observe the inhibitory effect of Dhoopana on the count of the organism. To achieve this the cultures of *S.aureus* & *K. pneumoniae* were grown on a plate using spread plate method. After 24 hours the colonies were observed. Using replica plate technique two plates were replicated out of which one was exposed to fragrant fumes of burning dhoop stick (Figure 1) the unexposed plate worked as a positive control for microbial growth. Next day the colony count was recorded to observe the effect of dhoopana on above mentioned microbes (Fig 1).



**Fig 1** In house Dhoop sticks prepared by using macerated mixture



**Fig 2** Results before and after *Dhoopana* (1:2, 1:4, 1:6 dilutions)



Survey consisting of various questions based on in house Dhoopana formulation was carried out. A questionnaire was constructed to understand the opinion regarding the in-house formulation after burning it. Outcomes of the survey are recorded and percentage was calculated for concluding the survey.

Following questions were included in questionnaire:

1. Do you suffer more often with cough and cold throughout the year?
2. Do you have baby in your home?
3. Do you know “Dhoopana” practice/protocol from Ayurveda?
4. Do you have allergy with Dhoop which is available in market?
5. Would you like an herbal Dhoop stick to relieve you from cough?
6. Would you like to use or accept Dhoop as medicine?
7. Do you like the appearance of Dhoop?
8. Do you like the smell of Dhoop?
9. Can you inhale these fumes? Does it irritate you?
10. Does smoke irritate your eyes?
11. Does it feel pleasant in surrounding?
12. Are you suffering from cold? If yes, please comment on the effectiveness of Dhoopana

## RESULTS & DISCUSSION

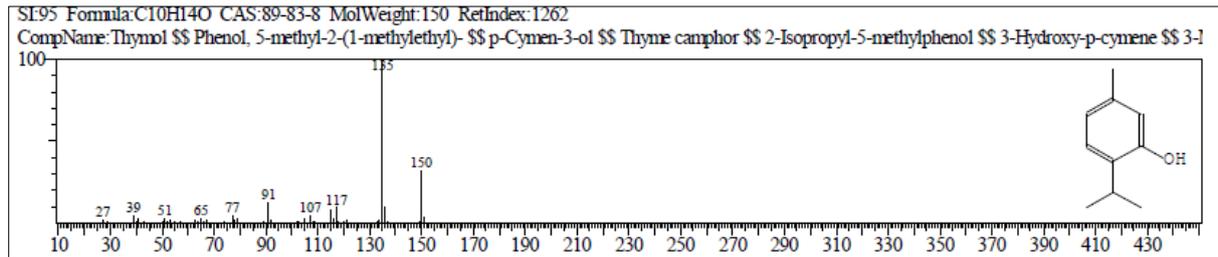
Dhoop stick containing Ajwain seeds (*Trachyspermum ammi*) and Dill (*Anethum graveolens L.*) were prepared using all the natural ingredients. Proximate analysis of the formulation was carried out. This includes Total Ash, Extractive values, LOD, pH etc. The results of the proximate evaluation are reported in Table 1.

**Table 1** Proximate Evaluation of Dhoopa Formulation

Parameter	Result obtained
pH for 1% aqueous solution	6.66
% Loss on Drying	6.8 %.
Water Soluble Extractive	18.4%.
Alcohol Soluble Extractive	2.4%.
Rate of Dhoop stick burning	5.27min/cm

These parameters will not only ensure the quality establishment but also help in maintaining the quality of the formulation. TLC analysis proves that at least 6 components are present in the Dhoop. Presence of Thymol ( $R_f$  0.64) was confirmed by TLC analysis of Dhoop which is the principal active constituent of ajwain of Dhoop, which remains undamaged throughout the process of preparation.

GC-MS analysis (Fig 3) of prepared Dhoop revealed that 51 phytochemical constituents are present in the formulation. The components identified by GC-MS are reported in Table 2. Thymol was found as a major component along with Isoborneol, Borneol, Apiol, and Phytol. The synergy of these plant metabolites consequences



**Fig 3** GC-MS Spectra of Thymol from *Dhoopa* formulation

the potent antimicrobial properties of the formulation. Microbiological evaluation of Dhoop revealed that it is effective against the respiratory pathogens used for current study. As specified in table 3, significant decrease was observed in the microbial

count after the exposure of plates to *Dhoopana*. From the survey it was evident that 50 to 60% people suffer more often with cough and cold throughout the year. More than 70% people are aware of the “Dhoopana” practice from Ayurveda.

**Table 2** Phytoconstituents from Dhoop Formulation Identified by GC-MS

Peak No.	R.Time	Area	Area%	Height	Name
1	1.203	75280860	55.67	11451945	2-Propanol, 1-methoxy-
2	1.315	23188356	17.15	6229915	Ethane, 1,1-diethoxy-
10	3.605	4334808	3.21	1900532	Isoborneol
11	3.691	90657	0.07	37337	Borneol
15	5.033	9536171	7.05	3914383	Thymol
16	5.185	64128	0.05	28456	Phenol, 2-methyl-5-(1-methylethyl)-
19	9.670	3115522	2.30	1664151	Apiol
25	13.758	368025	0.27	150930	Dibutyl phthalate
26	14.032	108144	0.08	45096	Phthalic acid, butyl 2-pentyl ester
27	15.485	37107	0.03	18220	Methyl 11,14-octadecadienoate

**Table 3** Results obtained after Microbiological Evaluation of *Dhoop*

	1:2	1:4	1:6
<i>Staphylococcus aureus</i> positive control	200	50	300
<i>Staphylococcus aureus</i> after exposure to dhoopa	100	20	180
<i>Klebsiella pneumoniae</i> positive control	300	25	320
<i>Klebsiella pneumoniae</i> after exposure to dhoopa	170	10	190

Ratios 1:2, 1:4 & 1:6 indicates the dilution of the master suspension

There is a 74% acceptance for herbal Dhoop as medicine as well for curing the cough and cold. 73% of people like the appearance and smell of Dhoop formulation prepared. 73% of people can inhale the fumes and it irritates them which figuratively shows that active phytochemicals from Dhoop formulation

prepared, dilates the blood vessels in the face region of an individual causing some irritation. 71% of people say that smoke or fumes originating from Dhoop doesn't irritate the eyes and 76% people says that it feels pleasant and positive in surroundings because of Dhoop. 80%



people rated the Dhoop formulation prepared on Eight out of Ten.

## CONCLUSION

Herbal therapies like dhoopan are a part of Indian culture. Such practices which are based on natural products which are easily possible and has great benefits to humans. With changing lifestyle humans are moving away from nature. In cities, it's not possible to practice traditional way of dhoopan which involves burning of wood or coal and subjecting herbs in order to inhale the fumes for therapeutic effectiveness. Therefore, current research work provides user friendly way of dhoopan which avoids almost all the hurdles for practicing dhoopan in cities.

Present work focusses on standardization of Dhoopan using novel approach which involves Proximate, phytochemical, microbiological and sensory evaluation of the prepared Dhoop sticks. The quality control parameters were established for the formulation and was found to be effective against respiratory pathogens namely, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The Dhoop stick formulation is user friendly and effective for cough and cold which is evident from the survey.



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