Pharmaceutico-analytical study of Nagakesaradi Dhoopana Yoga

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Abstract-
Introduction: Nagakesaradi Dhoopana Yoga is explained in Kriyakaumudi, Malayalam Visha Chikitsa textbook written by V.M. Kuttikrishna Menon in the context of Jaladhi Sudhikarana. The ingredients of this Yoga are Nagakesara, Daruharidra, Ela, Twak, Kushta, Priyangu, Laksha, Ativisha, Musta, Nirgundi and is indicated for Dhoopana in Vishavayu. Materials and methods: All the drugs were taken in equal quantity and Nagakesaradi Dhoopana Yoga was prepared as per the general method of Choorna preparation and analytical study was conducted as per the guidelines in Ayurvedic Pharmacopoeia of India. Physicochemical parameters such as Organoleptic characters, Total ash, Acid insoluble ash, Water soluble ash, Loss on drying, Volatile oil content, Water soluble extractive value, Alcohol soluble extractive value, pH, Phytochemical test, Powder microscopy, HPTLC were conducted.
Observations and results: The phytochemical analytical study showed the presence of Alkaloids, Tannins, Saponins, Coumarins, Carboxylic acid which are having both anti-bacterial and anti-fungal property, as well as the presence of Eugenol in HPTLC is a very strong evidence for stating the anti-microbial property of the Yoga. Conclusion: This study will help to standardize Nagakesaradi Dhoopana Yoga and to analyse its probable mode of action as fumigant.

***Keywords- Nagakesaradi Dhoopana Yoga*, Standardization, Pharmaceutico-Analytical study**

I. INTRODUCTION

"Standardization" expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It is also means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations. Herbal products studies cannot be considered scientifically valid if the product tested has not been authenticated and characterized in order to ensure reproducibility in the manufacturing of the product in question. Standardized herbal products of consistent quality and containing well-defined constituents are required for reliable clinical trials and to provide consistent beneficial therapeutic effects. Pharmacological properties of a herbal formulation depend on phytochemical constituents present therein [1] .
Dhoopana karma is a *Bahya parimarjana Chikitsa* which is followed for both preventive and curative purpose. It is the classical method for air purification. Dry drugs are either made in to *Choorna* or *Varti* and *Dhoopana* is done by adding *Choorna* in to burning charcoal with *Ghrita* as fuel or simply igniting the *Varti*. *Dhoopana karma* has wide range of indications; such as *Jwara, Karna roga, Yoni roga, Unmada, Vrana, Graha, Visha* etc. and is even mentioned for *Kumaragara, Suthikagara, Vranithagara, Bheshajagara* etc. for the purpose of disinfection.
Kriyakaumudi is a Malayalam *Visha Chikitsa* Textbook written by V.M. Kuttikrishna Menon; which is comprehensive textbook of *Visha Chikitsa*. *Nagakesaradi Dhoopana Yoga* is explained in the Chapter of *Sthavara Visha Prakarana* in the context of *Jaladhi shudhikarana* [2]. This *Yoga* is indicated for *Vishadushita Vayu* and against microorganisms. *Nagakesara, Daruharidra, Ela, Twak, Kushta, Priyangu, Laksha, Ativisha, Musta* and *Nirgundi* are the drugs explained in this *Yoga*. Among these *Nagakesara, Daruharidra, Twak, Kushta, Priyangu* and *Ativisha* are having *Vishaghna Karma*; *Twak, Laksha, Ativisha, Musta* and *Nirgundi* are having *Krimighna Karma* [3].

II. MATERIALS AND METHODS

Plant materials and Preparation: All the drugs were collected in equal quantity, authenticated and Choorna (Average Coarse Powder) were prepared as per the general method from G.M.P. certified S.D.M. Ayurveda Pharmacy, Kuthpady, Udupi, Karnataka, India. Ingredients of Nagakesaradi Dhoopana Yoga are tabulated in Table no.1 and pictures are depicted from Fig.1 to Fig.11.

Table no.1; Ingredients of *Nagakesaradi Dhoopana Yoga* [4- 6]

Sl. No.	Drugs	Botanical Name	Part Used	Quantity Used
1.	***Nagakesara***	*Mesua ferrea* Linn.	Stamens	200gms
2.	***Daruharidra***	*Berberis aristata* DC.	Root	200gms
3.	***Ela***	*Elettaria cardamomoum* (Linn.) Maton	Fruits and seeds	200gms

4.	***Twak***	*Cinnamomum zeylanicum* Blume.	Stem Bark	200gms
5.	***Kushta***	*Saussurea lappa* C.B. Clarke	Root	200gms
6.	***Priyangu***	*Callicarpa macrophylla* Vahl.	Seeds	200gms
7.	***Laksha***	*Laccifer lacca* (Kerr).	Resin	200gms
8.	***Ativisha***	*Aconitum heterophyllum* Wall. ex Royle	Tuberous root	200gms
9.	***Musta***	*Cyperus rotundus* Linn.	Tubers	200gms
10.	***Nirgundi***	*Vitex negundo* Linn.	Leaves	200gms

Fig. 1; *Nagakesara*

Fig. 2; *Daruharidra*

Fig. 3; *Ela*

Fig. 4; *Twak*

Fig. 5; *Kushta*

Fig. 6; *Priyangu*

Fig. 7; *Laksha*

Fig. 8; *Musta*

Fig. 9; *Ativisha*

Fig. 10; ***Nirgundi***

Fig. 11; ***Nagakesaradi Dhoopana Yoga***

Instrumentation and techniques:
All the tests including HPTLC (High Performance Thin Layer Chromatography) were conducted from S.D.M. Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India, as a part of the thesis study undergoing in the Department of Agadatantra in S.D.M. college of Ayurveda and Hospital,. Kuthpady, Udupi, Karnataka under Rajiv Gandhi University of Health Sciences.
Physicochemical parameters, Powder microscopy and HPTLC were conducted as per the Standard guidelines

Physicochemical parameters [7-11]
Physicochemical analysis was conducted to find out the following parameters

a. Organoleptic Characters [7]
b. Loss on drying at 105ºC [8]
c. Total Ash [8]
d. Acid insoluble Ash [8]
e. Water soluble ash [8]
f. Alcohol soluble extractive [8]
g. Water soluble extractive [8]
h. Determination of pH [9]
i. Volatile oil estimation [10]
j. Preliminary Phyto- chemical Analysis [11]
1. Tests for alkaloids
2. Tests for carbohydrates
3. Test for saponins
4. Test for tannins
5. Test for flavonoids
6. Test for phenol
7. Test for coumarins
8. Test for triterpenoids
9. Test for carboxylic acid
10. Test for resin
11. Test for quinone

Powder microscopy [12]
Powder microscopy was conducted to find out the specific microscopic features of the sample.

HPTLC [13-14]
1.0g of *Nagakesaradi Dhoopana Yoga* powder was suspended in 10.0ml ethanol filtered after 24hrs. 3, 6 and 9µl of each of the above extract were applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7mm using Linomat 5 TLC applicator. The plate was developed under Toluene: Ethyl Acetate (9.3: 0.7). The developed plates were visualized in short UV, long UV, White light and then scanned at 254nm, 366nm, derivatised with Anisaldehyde sulphuric acid reagent subsequently scanned at 620nm. R_f, colour of the spots, densitometric scan and 3-D chromatograms were recorded.

III. RESULTS AND DISCUSSION

Organoleptic characters:
Characters such as Colour, Odour and Taste were properly tested and tabulated in table no.2

Table no. 2; Organoleptic characters of *Nagakesaradi Dhoopana Yoga*

Parameters	Results
Colour	Straw yellow
Odour	Aromatic, pleasant
Taste	Astringent

The *Choorna* is characterized by an aromatic, pleasant odour, as many of its contents such as *Nagakesara, Ela, Twak, Nirgundi, Laksha, Priyangu* and *Musta* contain essential oil. Whereas *Ela, Twak, Kushta* and *Nirgundi* possess particular aromatic odour. Caryophyllene oxide is the aromatic compound present in *Nirgundi* and *Kushta*; β-Caryophyllene is the aromatic compound exclusively present in *Nirgundi*, which showed very good antimicrobial activity towards Gram-positive bacteria in a study [15]

Physico-chemical parameters

Physico-chemical parameters such as Loss on drying, Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive value, Water soluble extractive value, pH, Volatile oil has been tabulated in Table no.3.

Table no. 3; Results of standardization parameters of *Nagakesaradi Dhoopana Yoga*

Parameter	Results n = 3 (%w/w) (Avg ± SD)
Loss on drying	89.39±0.02
Total Ash	7.08±0.00
Acid Insoluble Ash	2.57±0.02
Water soluble Ash	1.79±0.01
Alcohol soluble extractive value	1.26±0.02
Water soluble extractive value	19.03±0.00
pH	4.82
Volatile oil (%)	0.17

Optimum moisture content of the sample is maintained. The pH of the sample is 4.82, i.e., acidic due to the presence of various fatty acids and other acid compounds in the *Choorna* and even the phytochemical test reveals the presence of carboxylic acid. Fumigation with Formaldehyde has long been an accepted sterilization method for areas where microbiological cleanliness is required. Formaldehyde fumigation is the recognized and most commonly used method because of its cost effective procedure [16]. The pH of formaldehyde solution is 2.8 – 4.0 [17], here the pH of sample is 4.82, stating that the *Yoga* is having similar pH as that of the standard drug used in the fumigation, for the disinfection of air.

Powder microscopy:

Powder microscopy revealed the presence of starch grains as the sample contain *Ela, Ativisha, Musta, Nirgundi* and *Twak*; Perisperm due to *Ela*; Pollen due to *Nagakesara*; Tracheids due to *Kushta, Nirgundi*; Parenchyma due to E*la, Twak, Kushta, Priyangu, Ativisha, Musta* and *Nirgundi*; Cork due to *Kushta, Ativisha* and *Nirgundi*; Fibre due to *Nagakesara, Ela, Twak, Kushta, Priyangu, Ativisha, Musta* and *Nirgundi*; Trichome due to *Nirgundi*; Pigments due to *Laksha* and *Musta*; Sclerenchyma due to *Ela, Twak* and *Kushta*. Results of powder microscopy are illustrated in Fig.12(12a to 12).

Fig. 12; Powder microscopy of *Nagakesaradi Dhoopana Yoga*

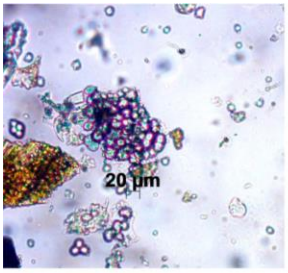


Fig.12a. Starch grains in bundle

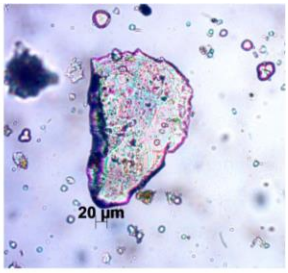


Fig.12b. Perisperm

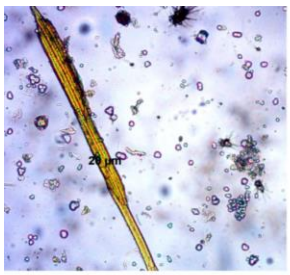


Fig.12g. Fibre

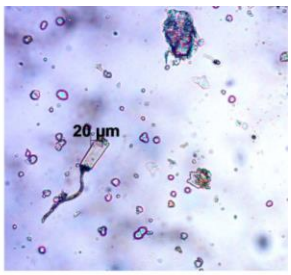


Fig.12h. Trichome

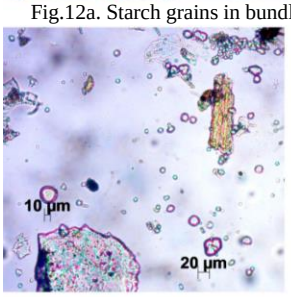


Fig.12c. Pollens

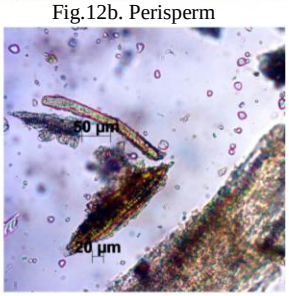


Fig.12d. Tracheid

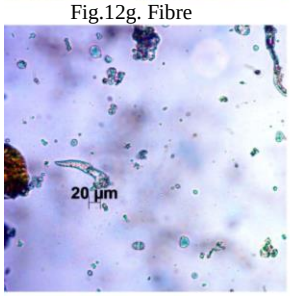


Fig.12i. Covering trichome

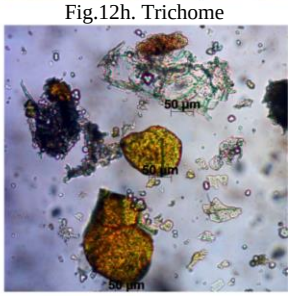


Fig.12j. Pigment layer

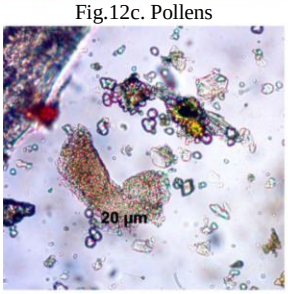


Fig.12e. Parenchyma

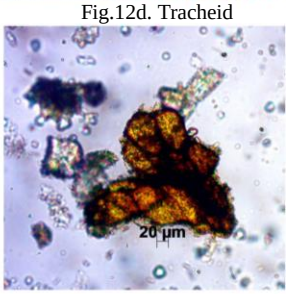


Fig.12f. Cork cells

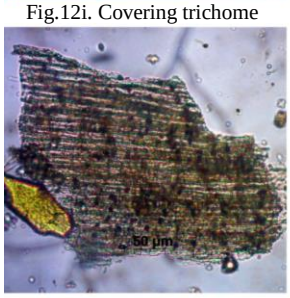


Fig.12k. Phloem fibres

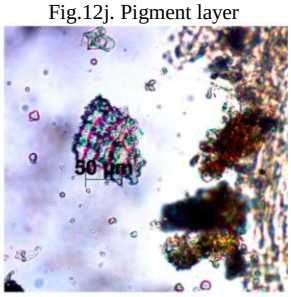


Fig.12l. Sclereids

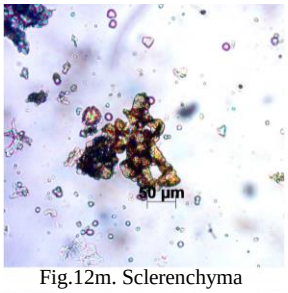


Fig.12m. Sclerenchyma

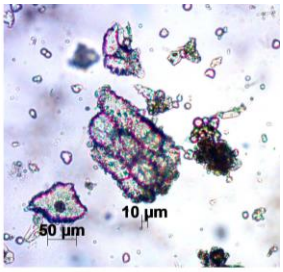


Fig.12n. Sclereids

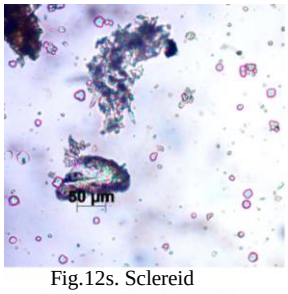


Fig.12s. Sclereid


Fig.12t. Trichome

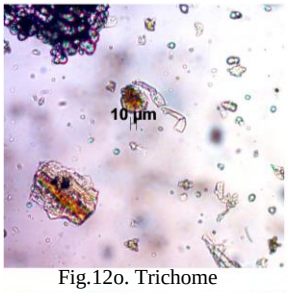


Fig.12o. Trichome

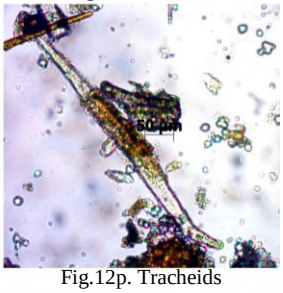


Fig.12p. Tracheids

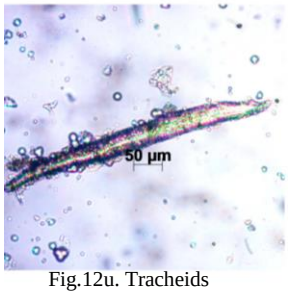


Fig.12u. Tracheids

Fig.12v. Tracheids

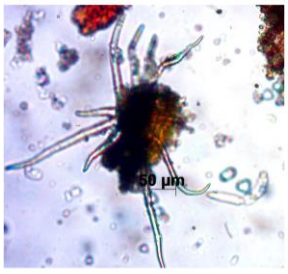


Fig.12q. Trichomes

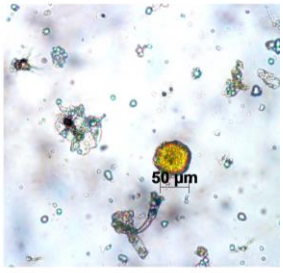


Fig.12r. Trichome

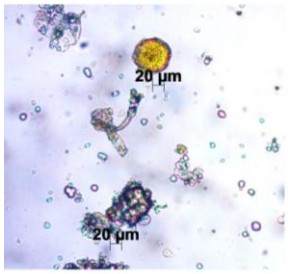


Fig.12w. Trichome and starch

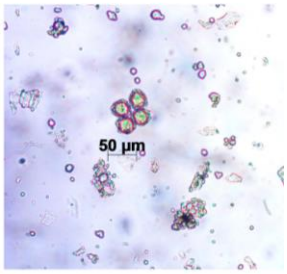


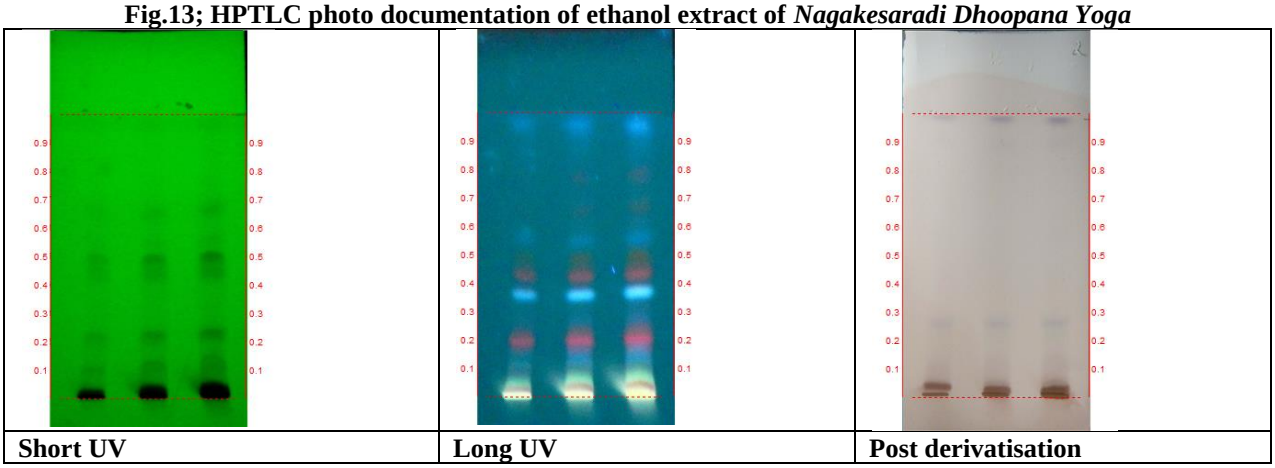
Fig.12 x. Bunch of Pollen

High Performance Thin Layer Chromatography (HPTLC):

HPTLC fingerprint of ethanol extract of *Nagakesaradi Dhoopana Yoga* was developed. HPTLC photo documentation of ethanol extract of *Nagakesaradi Dhoopana Yoga* with Short UV, Long UV and Post derivatisation are depicted in Fig.13 and Densiometric Scan at UV 254, 366, 620nm are depicted in Fig.14. The R_f values are presented in Table no.4. Ethanol extract of *Nagakesaradi Dhoopana Yoga* at 254 nm showed 6 spot (0.12 Green, 0.22 Green, 0.45 Green, 0.51 Green, 0.57 Green, and 0.67 Green), at 366nm it showed 6 spots (0.13 Blue, 0.20 Red, 0.28 Blue, 0.36 Blue, 0, 42 Red, 0.56 Blue) and at 620nm (post derivatisation) showed 2 spots (0.26 Purple, 0.89 Purple) using Toluene: Ethyl Acetate: (9.3: 0.7) as solvent system. R_f value 0.57 (Green) in short UV and 0.56 (F. blue) in long UV (highlighted with red) indicates the presence of Eugenol.

Eugenol is an allyl chain-substituted guaiacol, which is a member of the allylbenzene class of chemical compounds. [18] Eugenol is used as a pesticide and fumigant in agricultural applications to protect foods from microorganisms such as *Listeria monocytogenes* and *Lactobacillus* during storage. The assessment of the effects of eugenol on the growth of some species of Gram-positive (*Bacillus cereus; Bacillus subtilis; Staphylococcus aureus*) and Gram-negative (*Escherichia coli; Salmonella typhi; Pseudomonas aeruginosa*) bacteria were done by the agar well diffusion method. At the concentration of 1000 µg/mL, Eugenol has shown an inhibitory effect on the growth of the *P. aeruginosa*. At 2000 µg/mL the complete inhibitory effect against such bacteria was shown and similar effects of eugenol also have confirmed against various pathogens such as *S. aureus, E. coli, B. cereus, Helicobacter pylori, Streptococcus pneumoniae*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*. It was also found that production of *Staphylococcal* enterotoxin has been reduced significantly depending on the concentration of eugenol [19].

Fig.13; HPTLC photo documentation of ethanol extract of *Nagakesaradi Dhoopana Yoga*



Short UV	Long UV	Post derivatisation

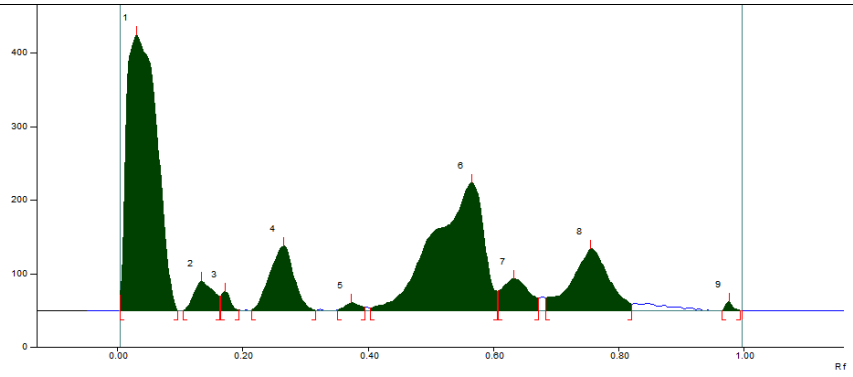
Track 1 - **Ethanol extract of *Nagakesaradi Dhoopana Yoga*** – 3µl
Track 2 - **Ethanol extract of *Nagakesaradi Dhoopana Yoga*** – 6µl
Track 3 - **Ethanol extract of *Nagakesaradi Dhoopana Yoga*** – 9µl
Solvent system – Toluene: Ethyl Acetate: (9.3: 0.7)

Table no. 4; R_f values of sample of *Nagakesaradi Dhoopana Yoga*

Short UV	Long UV	Post derivatisation
0.12 (Green)	**0.13 (F. blue)**	-
-	**0.20 (F. red)**	-
0.22 (Green)	-	-
-	-	0.26 (Purple)
-	**0.28 (F. blue)**	-
-	**0.36 (F. blue)**	-
0.45 (Green)	**0.42 (F. red)**	-
0.51 **(Green)**	-	-
0.57 (Green)	**0.56 (F. blue)**	-
0.67 **(Green)**	-	-
-	-	0.89 (Purple)

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

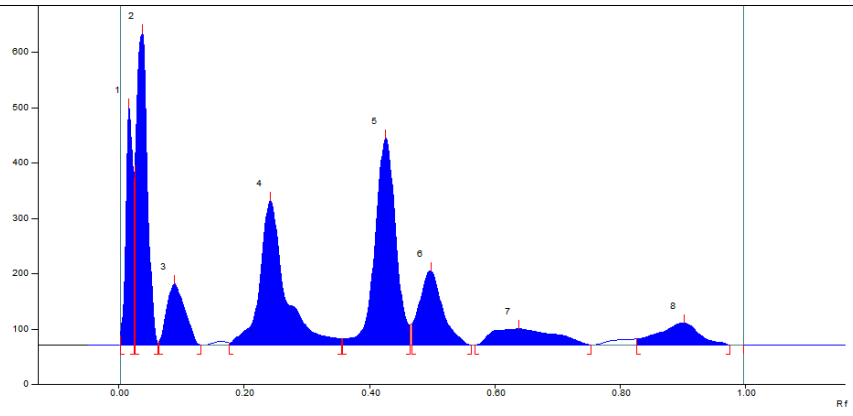
Fig.14; Densitometric scan of *Nagakesaradi Dhoopana Yoga*



Track 3, ID: Nagakesaradi churna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	20.9 AU	0.03 Rf	374.0 AU	43.90 %	0.10 Rf	0.5 AU	12861.7 AU	40.93 %
2	0.10 Rf	0.3 AU	0.13 Rf	40.3 AU	4.74 %	0.16 Rf	19.5 AU	890.4 AU	2.83 %
3	0.16 Rf	19.9 AU	0.17 Rf	26.0 AU	3.05 %	0.19 Rf	1.3 AU	271.0 AU	0.86 %
4	0.21 Rf	1.4 AU	0.27 Rf	87.4 AU	10.26 %	0.32 Rf	0.3 AU	2411.6 AU	7.68 %
5	0.35 Rf	1.4 AU	0.37 Rf	10.7 AU	1.26 %	0.40 Rf	5.0 AU	187.9 AU	0.60 %
6	0.40 Rf	3.7 AU	0.57 Rf	173.8 AU	20.40 %	0.61 Rf	26.4 AU	9834.9 AU	31.30 %
7	0.61 Rf	26.7 AU	0.63 Rf	43.2 AU	5.07 %	0.67 Rf	16.8 AU	1317.2 AU	4.19 %
8	0.68 Rf	17.8 AU	0.76 Rf	84.3 AU	9.89 %	0.82 Rf	9.1 AU	3550.0 AU	11.30 %
9	0.97 Rf	0.4 AU	0.98 Rf	12.2 AU	1.43 %	1.00 Rf	0.4 AU	96.6 AU	0.31 %

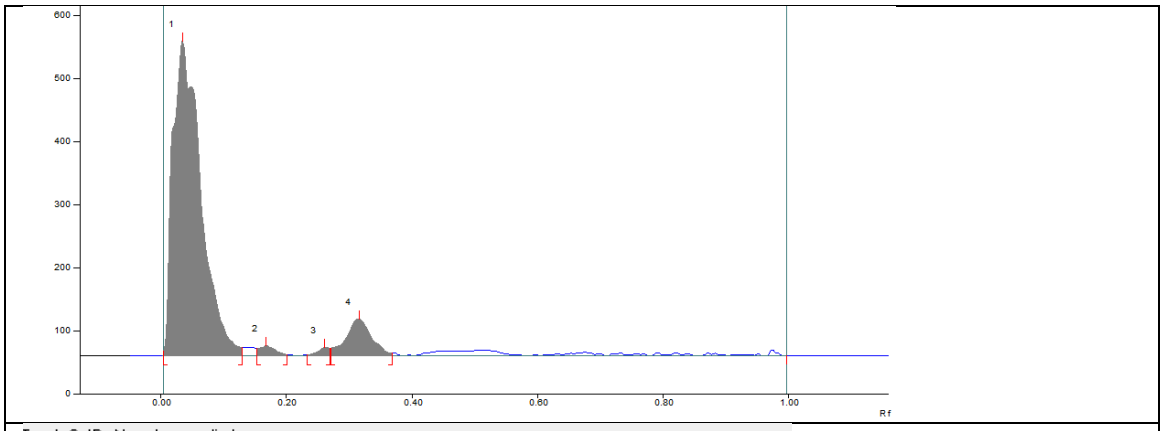
Fig. 14a. At 254nm



Track 3, ID: Nagakesaradi churna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	15.9 AU	0.02 Rf	429.4 AU	22.12 %	0.02 Rf	01.4 AU	3436.0 AU	9.36 %
2	0.03 Rf	324.1 AU	0.04 Rf	563.8 AU	29.04 %	0.06 Rf	3.8 AU	6987.8 AU	19.04 %
3	0.06 Rf	4.7 AU	0.09 Rf	110.5 AU	5.69 %	0.13 Rf	0.0 AU	2276.2 AU	6.20 %
4	0.18 Rf	3.9 AU	0.24 Rf	260.7 AU	13.43 %	0.36 Rf	11.5 AU	7606.4 AU	20.73 %
5	0.36 Rf	11.5 AU	0.43 Rf	373.2 AU	19.22 %	0.47 Rf	37.0 AU	8860.6 AU	24.15 %
6	0.47 Rf	37.8 AU	0.50 Rf	134.1 AU	6.91 %	0.56 Rf	0.1 AU	3536.4 AU	9.64 %
7	0.57 Rf	0.0 AU	0.64 Rf	29.6 AU	1.52 %	0.75 Rf	0.1 AU	2137.7 AU	5.83 %
8	0.83 Rf	10.9 AU	0.90 Rf	40.0 AU	2.06 %	0.98 Rf	0.1 AU	1854.9 AU	5.05 %

Fig. 14b. At 366nm



Track 3, ID: Nagakesaradi churna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	6.9 AU	0.04 Rf	497.9 AU	85.08 %	0.13 Rf	13.4 AU	16068.8 AU	88.00 %
2	0.15 Rf	11.8 AU	0.17 Rf	16.4 AU	2.81 %	0.20 Rf	1.6 AU	301.5 AU	1.65 %
3	0.23 Rf	1.2 AU	0.26 Rf	13.0 AU	2.22 %	0.27 Rf	12.2 AU	176.8 AU	0.97 %
4	0.27 Rf	12.3 AU	0.32 Rf	57.9 AU	9.89 %	0.37 Rf	4.3 AU	1714.0 AU	9.39 %

Fig. 14c. At 620nm

Preliminary phytochemical Analysis

Results of preliminary phytochemical tests reveals the presence of Alkaloids, Carbohydrates, Tannins, Saponins, Coumarins and Carboxylic acid in the given sample and tabulated in Table no:5 and Table no:6.

Table no. 5; Results of Phytochemical screening of *Nagakesaradi Dhoopana Yoga*

Test	**Inference**
	Nagakesaradi Dhoopana Yoga
Alkaloid	+
Steroid	-
Carbohydrate	+
Tannin	+
Flavanoids	-
Saponins	+
Terpenoid	-
Coumarins	+
Phenols	-
Carboxylic acid	+
Amino acids	-
Resin	-
Quinone	-

(+) – present; (-) – negative

Alkaloids are the phytochemicals which have been extensively investigated for their biological activities such as anticancer, antibacterial, antiviral and central nervous depressant activities in both traditional and modern medicine. In recent years, the antibacterial activity of alkaloid's played a significant role in the treatment of many infectious diseases reporting Multiple Drug Resistance phenomenon [20]. Tannins are a group of polyphenols which are commonly occurring in nature. Tannic acid shows various activities against microorganisms (bacteria and viruses). The broad spectrum activity showed by the tannic acid, especially against *Staphylococcus aureus* and *Enterococcus faecalis*, suggests that Gram-positive bacteria were most susceptible to tannic acid than Gram-negative ones. The antibacterial activity of Tannic acid has been proven on Gram-positive and Gram-negative bacteria. Tannic acid inhibits the bacteria attachment to the surfaces, resulting in lack of bacteria adhesion to the surface and bacterial cell death. Moreover, the amino acid and sugar uptake are inhibited by tannic acid what limits the bacterial growth [21] Saponins displays a biological role and medicinal properties such as antibacterial, antifungal, antiviral, anti-inflammatory, insecticidal, and molluscicidal action [22]. An *in-vitro* study reveals that, pure saponin extract has antimicrobial activity against *Staphylococcus aureus, Klebsiella pnuemoniae, Salmonella typhi , Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger*[23] . Coumarins are plants secondary metabolites compounds, whose biological activity varies according to their substitution patterns. A series of 45 coumarin derivatives and the parent coumarin were tested against 4 strains of bacteria: two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) for their antibacterial activity and the results indicated that each compound showed more or less pronounced antibacterial potencies,

affecting both Gram-positive and Gram-negative microorganisms [24]. Studies have reported the importance of the concentration of carboxylic acid exposures in the suppression of the growth of bacteria. Conventional studies on the bacterial resistance, adaptation and membrane permeabilization have demonstrated the dependence of the metabolic responses with the microorganism and acid concentration were evaluated [25]. Nagakesaradi Dhoopana Yoga appreciates the presence of Alkaloids, Tannins, Saponins, Coumarins and Carboxylic acid; these constituents contribute to the anti-microbial activity of the *Yoga*.

IV. CONCLUSION

Kriyakaumudi is a *Keraleeya Visha Chikitsa* textbook written by V. M. Kuttikrishna Menon and *Nagakesaradi Dhoopana Yoga* is explained in the context of *jaladhisudhikarana* for the purification of air and has mentioned particularly against microorganisms. Here Pharmaceutico- analytical study of *Nagakesaradi Dhoopana Yoga* reveals the presence of Alkaloids, Tannin, Saponins, Coumarins, Carboxylic acid, which all have proven anti- bacterial and anti-fungal activity. And the Eugenol presence in the HPTLC indicates the strong antimicrobial activity. pH of the *Nagakesaradi Dhoopana Yoga* is 4.82, which is almost nearer to the pH of Formaldehyde, which is the standard drug for fumigation. Hence this study will help to standardize *Nagakesaradi Dhoopana Yoga* and to analyse its mode of action as fumigant.

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CONFLICT OF INTEREST: None Declared