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ORA - Analytical Study

Analytical profile of *Swarnaprashana*

[1]S Niveditha, [2*]Reena Kulkarni, [3]P Sivakami Sundari, [4]Suchitra N Prabhu

ABSTRACT:

Background: The Ayurveda classics describes the ancient practice of *Swarnaprashana*, a traditional method of administering gold in the form of emulsion. It has the potential to boost *Medha* (intelligence), *Agni* (digestive capacity), *Bala* (strength), *Ojus* and *Ayu* (longevity). *Swarnabhasma* is administered in combination with *Medhya rasayana* (nootropic) herbs like *Vacha, Kalyanaka ghrita* (ghee), and *Madhu* (honey). Various formulations containing with or without gold have been described in the context of *Lehana*. However, analytical studies on these gold containing lickable are limited. Thus, in this study we explore the analytical profile of *Swarnaprashana Yoga* which was prepared using *Kalyanaka ghrita* (described in *Unmada chikitsa*), *Madhu*, *Vacha* and *Swarnabhasma* and used in the *Unmada chikitsa*. **Objective:** To develop an analytical profile of *Swarnaprashana* prepared with *Kalyanaka ghrita* as base. **Materials and Methods:** Preparation of *Swarnaprashana*: *Swarnabhasma* was purchased from a GMP certified pharmacy and verified with *Bhasma pareeksha for* its quality. Honey, and *Kalyanaka ghrita* was evaluated for purity. To prepare the *Swarnaprashana*, *Swarnabhasma* was triturated until it was homogenous with one part *Kalyanaka ghrita* and one half *Madhu*. Analysis was done on the finished emulsified product. Method of Analytical profiling of *Swarnaprashana*: Analytical profile of *Swarnaprashana* was developed as per standard procedures used for evaluation of organoleptic characters, specific gravity, moisture, and saponification and iodine values along with HPTLC. **Result:** The product passed organoleptic checks and showed Specific gravity (0.571), Moisture content (11.33 %), Saponification value (14.96) and Iodine value (1.586375) and HPTLC (High performance thin layer chromatography). At room temperature the formulation remained stable for 12 months. **Conclusion:** The study validates a reproducible method for preparation and Analytical profiling of *Swarnaprashana*, setting the stage for wider clinical trials.

KEYWORDS: *Analytical study, Ayurveda, HPTLC, Kalyanaka ghrita, Physico-chemical parameters, Swarnaprashana*

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Corresponding Author Email:

drreenakulkarni@gmail.com

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1. INTRODUCTION

Ayurveda, the ancient science of life is gaining global recognition in the present era. Therefore, it is essential to assure the quality assessment and standardization of the formulations. *Swarnaprashana* is used since ages for its multispectral benefits. It is practiced all over the nation in various methods. In spite of its common usage, developing quality standards for formulation of *Swarnaprashana* is necessary for its greater credibility.

Since ancient times, gold has been considered superior among the metals. It is also believed to possess various medicinal properties for preventive and curative action on diseases. In Ayurveda, Gold is indicated for internal use for its *Rasayana guna* (rejuvinative property) which can be attributed to the antioxidants present in it. *Swarnabhasma* is described as having *Madhura Rasa* (sweet taste)*, Snigdha* (unctous) and *Laghu guna* (light), *Sheeta veerya* (cold potency)*,* and *Madhura vipaka* (sweet bio-transformed rasa). It is *Tridosha Shamaka (*pacifies *tridosha), Medhya* (nootropic)*, Smritikara* (enhances memory), *Agnideepana* (enhances agni), *Brimhana* (bulk promoter), and *Ojaskara* (enhances ojus). [1] These are nanoparticles which are directly absorbed into the blood stream. [2] In modern medicine, gold nanoparticles find significant applications in drug delivery as they are capable of encapsulating active drug and targeting. [3] Therefore, it is used in treating wide range of ailments. *Swarnaprashana* is one such formulation, which is widely used in the paediatric population. [4]

Swarnprashana is a unique formulation mentioned in Ayurveda which acts as an excellent medicine for enhancing immunity. Various formulations containing gold have been described for the same purpose. [5] It is practiced all over the nation in various methods like drops, capsules, lickable and dispersible tablets. It can be given right from birth and is a potion which is the nectar for a child. Acharya Kashyapa has coined the term *Swarnaprashana* for the process of administration of *Lehana* in his *Lehanadhyaya*. It is said to have a wide spectrum of benefits. It is *Medhya*, which promotes intellect, kindles the digestive power, enhances the metabolism, improves physical strength and boosts immunity. [6]

Madhu, having *Madhura* and *Kashaya rasa* (bitter taste)*, Laghu, Ruksha* (dry)*, Yogavahi guna* (bioenhancer), *Sheeta veerya*, and *Madhura vipaka*, is *Tridosha shamaka*. [7] Thus, it enhances the absorption and action of the other ingredients like *Swarnabhasma* and *Kalyanaka ghrita*, while also making the formulation palatable for children. *Madhu* is sourced from plants, carried by the honey bees, it contains pollens. When this is introduced in the body, it triggers the antigen and antibody reaction thereby stimulating the immune system.

Kalyanaka ghrita is well-documented in classics for its efficacy in treating *Manovikara* (disorders of *Manas*), *Smriti Nasha* (disorders of memory), and *Unmada* (psychological disorders). [8] It consists of *Haritaki* (*Terminalia chebula* Retz.), *Vibhitaki* (*Terminalia bellerica* Roxb.), *Amalaki* (*Phyllanthus emblica* Gaertn.), *Vishala* (*Citrullus colocynthis* Linn.), *Bhadraila* (*Amomum subulatum* Roxb.), *Devadaru*(*Cedrus deodar* Roxb.), *Elavaluka* (*Prunus cerasus* Linn.), *Shweta Sariva*

(*Hemidesmus indicus* Linn.), *Krishna Sariva* (*Cryptolepis buchanani* Roem & Schult.), *Haridra* (*Curcuma longa* Linn.), *Daruharidra* (*Berberis aristata* DC.), *Shalaparni* (*Desmodium gangeticum* DC.), Prushnaparni (*Uraria picta* DC.), *Phalini* (*Callicarpa macrophyla* Vahl.), *Natha* (*Valeriana wallichii* Roxb.), *Bruhathi* (*Solanum indicum* Linn.), *Kushta* (*Saussurea lappa* Falc.), *Manjishta* (*Rubia cordifolia* Linn.), *Nagakeshra* (*Mesua ferrea* Linn.), *Dadima* (*Punica granatum* Linn.), *Vella* (*Embelia ribes* Burm.f.), *Talisapatra* (*Abies webbiana* Lindl.), *Ela* (*Elettaria carddamomum.), Malati (Jasminum sambac* Linn.), *Utpala* (*Nymphaea stellate* Willd.), *Danti* (*Balliospermum montanum* Muell-Arg.), *Padmaka* (*Prunus cerasoides* D.Don.), *Hima* (*Pterocarpus santalinsus* Linn.) and *ghrita* as the base. By the virtue of its ingredients, this acts as *Medhya, Rasayana, Agnideepana*, and *Tridoshahara*. The lipophilic action of *Ghrita* facilitates transportation to a target organ and final delivery into the cell as the cell membranes contain lipid. It does *Dhatu poshana*, balances *Pitta dosha*, and enhances the bioavailability of the active constituents. Thus, *Kalyanaka ghrita* enhances intellect, emotional stability and overall health in children. [8]

Kalyanaka ghrita and *Vacha churna* (fine powder) were purchased from GMP (Good Manufacturing Practice) certified SDM Pharmacy with GMP certification number AUS 783, prepared as per the AFI (Ayurveda formulary of India). *Swarnabhasma* was procured from Virgo UAP Pharma with Batch Number – BHL150 and GMP certification number GA/1696. Honey was taken from AGMARK certified Siri Gramodyoga company.

2. MATERIALS AND METHODS:

Analytical parameters consisting of Organoleptic Characters, Specific gravity, Moisture content, Saponification value, Iodine value and High Performance Thin Layer Chromatography (HPTLC) were conducted as per the Indian Pharmacopeia, Ayurveda Pharmacopeia and WHO guidelines.

a. Organoleptic character

Assessment of organoleptic character was carried out with the help of sense organs. It was Light brown, semisolid mass with pleasant odour having Buttery taste. [9]

b. Specific gravity

Accurate weight of an empty specific gravity bottle was taken. The bottle was filled completely with the sample and weighed accurately. Then the bottle is cleaned and filled completely with distilled water and weighed accurately noting the temperature. [10]

c. Moisture content

To determine the moisture content and loss on drying, petridish is cleaned and dried and the empty weight is noted. The sample substance is added and again the weight of petridish is noted. Petridish is placed in tray dryer and is weighed at every 5 minutes and allowed to dry until getting the constant dry weight. The percentage of loss on drying and percentage of moisture content was calculated. [11]

d. Saponification value

Saponification value was calculated using the 0.5N HCl and 0.5N alcoholic KOH solution and Reflux condenser as per the standard procedure. [12]

e. Iodine value

Iodine value is a measured using Iodine flask, Hanu's iodine solution, Bromine solution, 15% KI solution, Standard 0.1N sodium thiosulphate solution, 1% starch indicator and chloroform as per the standard procedure. [13]

f. HPTLC (High performance thin layer chromatography)

Determination of Unsaponifiable matter

5g of the *Swarnaprashana* was weighed into the flask which was added with 50ml alcoholic KOH. This is subjected to gentle but steady boiling under reflux condenser for one hour. The condenser was washed with 10ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. 50ml of water was added to the separating funnel followed by an addition of 50ml petroleum ether. The stopper was inserted and shaken vigorously for 1 minute, allowed to settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six more times using 50ml of petroleum ether for each extraction. All the extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 25ml of aqueous alcohol and shaken vigorously and drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 25ml of water until the water no longer turns pink on addition of a few drops of Phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dry on a water bath. The flask is placed in an air oven at 85°c for about 1 hour to remove the last traces of ether. A few ml of acetone was added and evaporated to dry on a water bath, which is cooled in a desiccator to remove last traces of moisture and then weighed.

Sample preparation for HPTLC

Unsaponifiable matter obtained from the above procedure is dissolved in 10 ml of chloroform. Chloroform soluble portion was used for TLC.

HPTLC

3, 6, 9ul of the chloroform fraction of samples of *Swarnaprashana* was applied on a pre-coated

Silica gel Fzs4 on aluminium plates to a band width of 8mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9.0: 1.0) and the developed plates were visualized under short UV, long UV and after derivatisation in Vanillin sulphuric acid spray reagent and Scanned under UV 254nm, 366nm, 620nm. Rf, colour of the spots and densitometric scan were recorded.

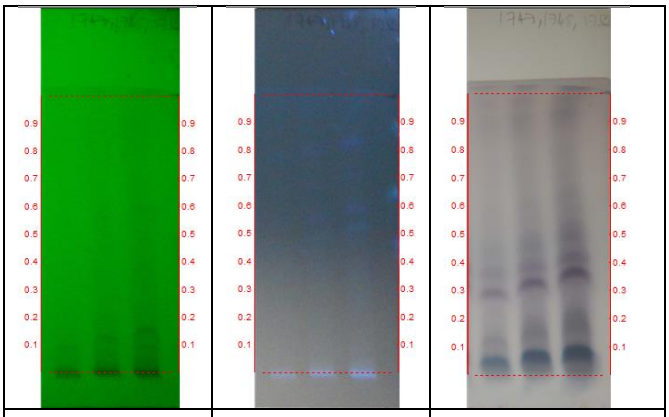


Figure 1. HPTLC photo documentation of Chloroform fraction of *Swarnaprashana*

Track 1 – ***Swarnaprashana*** – 3µl

TRACK 2 – ***Swarnaprashana*** – 6µl

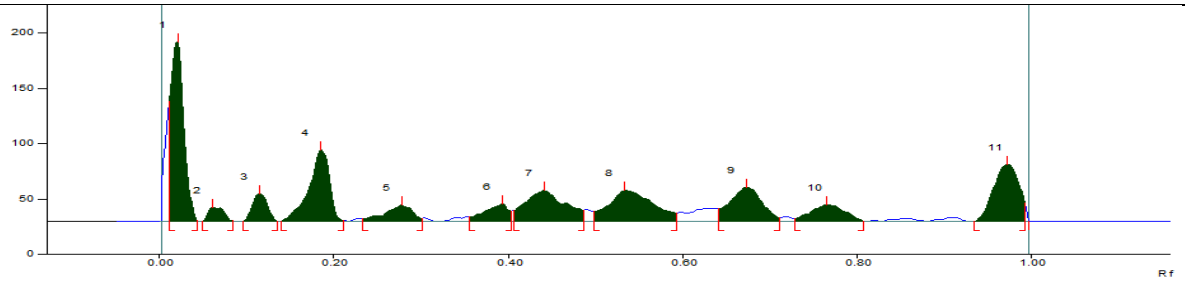
Track 3 – ***Swarnaprashana*** – 9µl

Solvent system – Toluene: Ethyl acetate (9.0: 1.0)

Table 1: R_f values of sample of *Swarnaprashana*

Short UV	Long UV	Post derivatization
0.10 (Green)	-	0.09 (Purple)
0.15 (Green)	-	0.15 (Purple)
-	-	0.33 (Purple)
-	-	0.39 (Purple)
-	-	0.50 (Purple)
-	0.52 (F. blue)	-
-	0.59 (F. blue)	0.59 (Purple)
-	-	0.64 (Purple)
-	0.83 (F. blue)	-
-	-	0.93 (Purple)

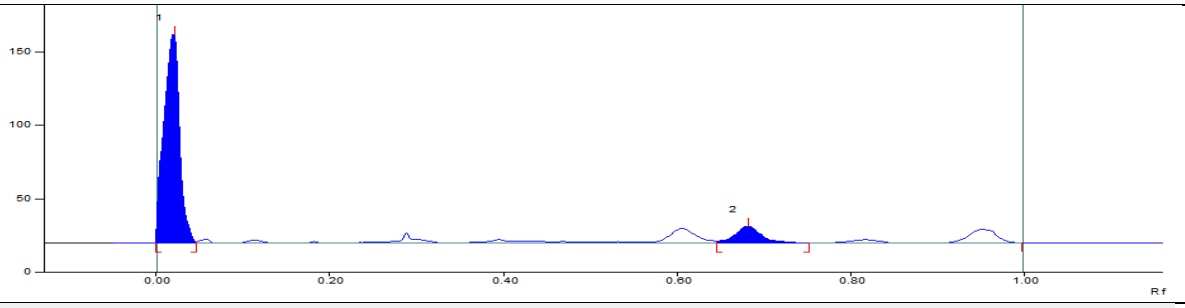
***F – Fluorescent; L –Light; D – Dark**



Track 9, ID: Swarnaprahana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	108.5 AU	0.02 Rf	161.9 AU	36.19 %	0.04 Rf	0.5 AU	1745.2 AU	21.24 %
2	0.05 Rf	0.1 AU	0.06 Rf	12.8 AU	2.86 %	0.08 Rf	0.4 AU	170.6 AU	2.08 %
3	0.10 Rf	0.2 AU	0.11 Rf	24.7 AU	5.51 %	0.14 Rf	0.2 AU	307.0 AU	3.74 %
4	0.14 Rf	0.3 AU	0.19 Rf	64.3 AU	14.38 %	0.21 Rf	1.0 AU	1084.6 AU	13.20 %
5	0.23 Rf	2.7 AU	0.28 Rf	15.0 AU	3.34 %	0.30 Rf	2.7 AU	337.8 AU	4.11 %
6	0.36 Rf	3.7 AU	0.39 Rf	16.0 AU	3.57 %	0.40 Rf	9.5 AU	310.4 AU	3.78 %
7	0.41 Rf	9.7 AU	0.44 Rf	27.8 AU	6.22 %	0.49 Rf	10.2 AU	922.1 AU	11.22 %
8	0.50 Rf	9.3 AU	0.53 Rf	27.8 AU	6.22 %	0.59 Rf	7.4 AU	1028.5 AU	12.52 %
9	0.64 Rf	11.5 AU	0.67 Rf	30.8 AU	6.89 %	0.71 Rf	3.4 AU	818.7 AU	9.96 %
10	0.73 Rf	2.6 AU	0.77 Rf	14.9 AU	3.34 %	0.81 Rf	0.2 AU	427.7 AU	5.20 %
11	0.94 Rf	0.0 AU	0.97 Rf	51.3 AU	11.47 %	0.99 Rf	16.5 AU	1065.4 AU	12.96 %

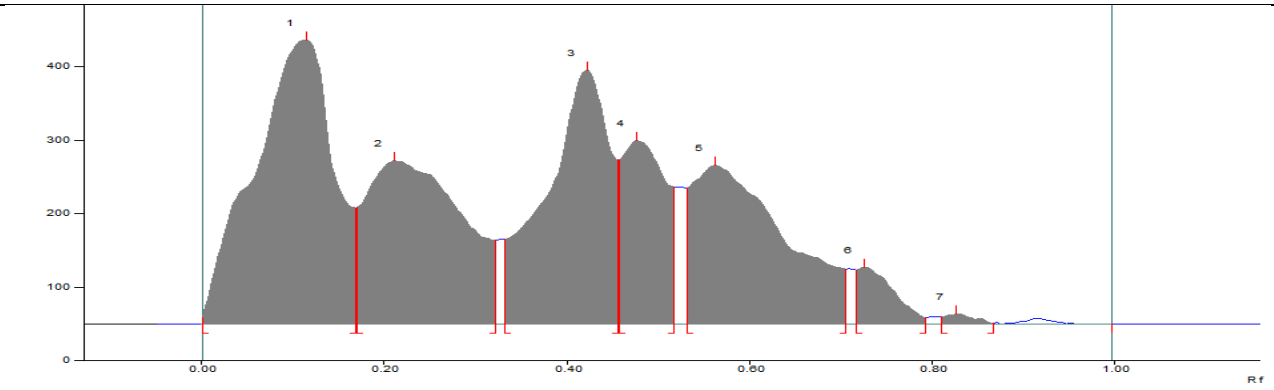
Fig 2a. At 254nm



Track 9, ID: Swarnaprahana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	141.5 AU	92.60 %	0.05 Rf	0.9 AU	1798.4 AU	87.92 %
2	0.65 Rf	1.5 AU	0.68 Rf	11.3 AU	7.40 %	0.75 Rf	0.0 AU	247.1 AU	12.08 %

Fig 2b. At 366nm



Track 9, ID: Swarnaprahana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	8.1 AU	0.11 Rf	385.7 AU	25.57 %	0.17 Rf	58.1 AU	24191.7 AU	28.34 %
2	0.17 Rf	158.4 AU	0.21 Rf	221.9 AU	14.71 %	0.32 Rf	13.6 AU	16895.2 AU	19.79 %
3	0.33 Rf	115.5 AU	0.42 Rf	345.0 AU	22.87 %	0.46 Rf	22.5 AU	17179.0 AU	20.12 %
4	0.46 Rf	222.9 AU	0.48 Rf	249.5 AU	16.53 %	0.52 Rf	86.2 AU	8550.1 AU	10.01 %
5	0.53 Rf	184.5 AU	0.56 Rf	215.7 AU	14.30 %	0.71 Rf	74.7 AU	15963.0 AU	18.70 %
6	0.72 Rf	73.6 AU	0.73 Rf	77.4 AU	5.13 %	0.79 Rf	8.9 AU	2268.9 AU	2.66 %
7	0.81 Rf	9.6 AU	0.83 Rf	13.5 AU	0.89 %	0.87 Rf	1.6 AU	326.5 AU	0.38 %

Fig 2c. At 620nm

Figure 2: Densitometric scan of *Swarnaprashana*

3. RESULT:

Parameters such as organoleptic characters, Specific gravity, Moisture content, Saponification value and Iodine value were analysed using various analytical tests. The observed values are presented in the tabular form. (Table 2)

Table 2: Analytical values of sample of *Swarnaprashana*

Analytical Parameter	Obtained Value	Reference range
Specific gravity	0.571	0.571(IP,vol 2,p –105-106)
Moisture content	11.33 %	11.33
Saponification value	14.96	180 - 210 (API, vol 1,p - 76)
Iodine value	1.586375	33 - 45 (API, vol 1,p - 76)

The chloroform fraction of the *Swarnaprashana* was analysed with HPTLC as described above in the methodology. The developed plates were visualized under short UV, long UV and after derivatisation in Vanillin sulphuric acid spray reagent and Scanned under UV 254nm, 366nm, 620nm. Rf colour of the spots and densitometric scan were recorded. (Table 2, Figure 2, 2a, 2b and 2c).

4. DISCUSSION:

Swarnaprashana is a unique traditional practice with wide spectrum application on immunity, intellect, speech, digestion, complexion and overall health. [4] Recently, many clinical and experimental studies with diversified combination of *Swarnaprashana* have been published. However, pharmacological standardization was limited to one or two studies. Thus, this analytical study focuses on the standardization of *Swarnaprashana* based on the Ayurveda Pharmacopeial and Indian Pharmacopeial standards.

Swarnaprashana used in this study was prepared using *Swarnabhasma, Kalyanaka ghrita, Madhu,* and *Vacha churna taken* in the quantity of 90mg, 12ml, 18ml and

50mg respectively. Ingredients were triturated and made into a homogenous emulsion form. This was administered in the dose of 4 drops daily containing 1mg *swarnabhasma* arbitrarily fixed using the Young's formula considering the adult dose of *Swarnabhasma* to be 1/8 – 1 /4 *Ratti*. [14] Each of these ingredients contributes to collective role in promoting physical, mental, and immunological development in children. Various randomised controlled studies have been carried out to evaluate the immunomodulatory activity of *Swarnaprashana* in infants and is been validated for its safety and efficacy. [15] Gold has been proved for its immune-modulatory effects by its antibacterial action against various organisms, but when mixed with *Madhu* and *Ghrita*, it extends its spectrum of action to stimulate immune cells. [16] In-vitro and Clinical studies have revealed that *Swarnaprashana* is free from toxicity as the components used in its preparation are used only after certification. Wide range of studies in humans and animals have proven that the dose of *Swarnabhasma* to be 15mg/dose as non-toxic. [17] In the present study, *Swarnabhasma* was procured from GMP certified Virgo Pharmacy. The acute toxicity study report of the *Swarnabhasma* showed that the dose up-to 2000mg/kg per oral was safe in human. [18]The ability of antioxidant activity of *Swarnabhasma,* has been reported by in-vivo animal studies on mice and on albino rats model and has shown significant increase of Superoxide dismutase (SOD) and catalase activity, that effectively reduces free radical concentrations. [19-21] an experimental study carried on Albino rats for effect of *Swarnaprashana* on Hippocampus concluded that *Swarnaprashana* had good potential in improving neurodegenerative disorders characterized by deterioration in cognition and memory. The *Swarnaprashana* group showed increased cellularity in Dentate gyrus. [22] To enhance the global acceptance, it is important to standardize the formulation by assessment of various physico-chemical parameters. These parameters validate the quality of the formulation. Analytical studies have been carried out previously to standardize the formulation of *Swarnaprashana*. This study reflects the similar Rf values in the HPTLC. [23]

In the current study, the *Swarnaprashana* sample appeared as semisolid, having pleasant odour in light brown colour with buttery taste which indicates proper palatability and preparation. These sensory characteristics of the emulsion is suggestive of the presence of ghee and honey used in the formulation. The findings of the various analytical parameters such as Specific gravity of 0.571 indicates that the emulsion is lighter than water, suggesting the dominance of lipid based components, which is typical for formulations that contain fat-soluble constituents. Moisture content of 11.33 % lies in the acceptable range, preventing the growth of microbes and maintaining the shelf life of the formulation, especially gold-based formulations from hydrolytic degradation. Saponification value of 14.96 implies the presence of long-chain fatty acids, the main characteristic of lipid based preparation. Lower saponification value corresponds to the higher molecular weight of fatty acids present in the *Swarnaprashana*, which is lipid rich in nature. This

parameter also assesses the quality and purity of the ghee/lipid component used in the formulation. Iodine value of 1.586375 is consistent with cow-ghee, which has saturated and monounsaturated fats, reflecting very low unsaturation. [24] Low iodine value establishes stability against oxidative deterioration and rancidity ensuring safety and long-term storage of the formulation. HPTLC photo documentation of Chloroform fraction of *Swarnaprashana* is similar with the standardization of *Kalyanaka ghrita* showing the presence of *Terminalia chebula, Emblica officinalis and* gallic acid. [25] These values can be considered as standard reference values for formulation of *Swarnaprashana*.

The Rf values recorded through HPTLC also coincides with the similar values of the previous study. [23] It revealed 2 spots under Short UV (0.10 green and 0.15 green), 3 spots under long UV (0.52 Fluorescent blue, 0.59 Fluorescent Blue and 0.83 Fluorescent Blue) confirming the presence of various phytochemical constituents present in the formulation. Fluorescent blue bands suggest the presence of aromatic compounds. Multiple purple bands produced by Vanillin-sulfuric acid derivatization shows the presence of triterpenoids, steroids and phenolic compounds in the formulation could be attributed for the wide spectrum of clinical utility of *Swarnaprashana*. The densitometric scans shows 11 peaks in 254nm, 2 peaks in 366nm and 7 peaks in 620nm validating the presence of active principles. Thus, the chromatographic profile obtained in this study serves as a finger print for assuring the quality and can be used as reference standard for future study. In in this study Refractive index, Weight per ml, Acid value, Peroxidase value, Congealing point, Microbial limits and Aflatoxins were not analysed. However, there is scope for these parameters to be studied in the future to solidify the credibility of analytical study of *Swarnaprashana*.

5. CONCLUSION:

The assessments of various analytical parameters are in line with Ayurveda formulations that are gold-based and rich in ghee. Absence of divergent values suggests the purity of the formulation with no adulteration or degradation. The Chromatography profile by HPTLC assigns the reliable identity and purity marker for Quality Control. Overall, the analytical profiling result validates the stability, chemical integrity and authenticity of *Swarnaprashana*.

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Authors Details:

[1]Final year PG Scholar, Department of Kaumarabhritya, Shri Dharmasthala Manjunatheshwara Institute of Ayurveda and Hospital, Anhepalya, Bengaluru – 560074

[2*]Professor and Head, Department of Kaumarabhritya, Shri Dharmasthala Manjunatheshwara Institute of Ayurveda and Hospital, Anhepalya, Bengaluru – 560074

[3]Associate Professor, Department of Pharmacognosy College of Pharmaceutical Sciences

Dayanand Sagar University, Devarakaggalahalli, Harohalli, Kanakapura Road, Bangalore South District - 562112

[4]Research officer, Department of Pharmacognosy and Phytochemistry, Sri Dharmasthala Manjuntheswara centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi – 574118

Authors Contribution:

Conceptualization: NS, RK

Data collection: NS, RK, PSS, SNP

Writing original draft: NS

Reviewing & editing: RK

Approval of final manuscript: All authors

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