



Research Article

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ANALYTICAL CHARACTERIZATION OF SWARNAMRITHAPRASHANA

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ABSTRACT

In present era of increasing popularity of traditional medicines, steps towards standardization of Ayurvedic formulations are need of the hour. Swarnamrithaprashana is a popular formulation being administered to a significant diaspora of pediatric population. Present study was carried out as a part of project entitled "Efficacy of Swarnamrithaprashana on Promotion of Immunity in Children – A Randomized Double-Blind Trial" sponsored by Rajiv Gandhi University of Health Sciences, Karnataka, Bengaluru, India. The study aimed at assessing the efficacy of Swarnamrithaprashana (modified form of Swarnaprashana) in school going children, with an aim of analytical characterization of Swarnamrithaprashana as a preliminary step towards standardization of the formulation. Microscopic examination and Physico-chemical studies like Refractive index, Specific gravity, disintegration time, Unsaponifiable matter and HPTLC were carried out as per the WHO and Ayurvedic Pharmacopoeia guidelines. The observed values can be considered as reference data for quality assessment and future studies on Swarnamrithaprashana.

Keywords: Swarnamrithaprashana, HPTLC, Swarnaprashana

INTRODUCTION

In recent times, the proportion of traditional medicines in health care sector is in its upsurge.¹ As global inclination towards Ayurveda and other traditional medicines increasing, quality assessment and standardization of formulations also become a matter of paramount importance.² Swarnaprashana is a method of administration of gold to children in order to enhance their immunity, intellect and growth. Swarnaprashana is being widely practiced throughout India in different forms.³ Though Swarnaprashana is well accepted by general public, it is requirement of the hour to develop quality standards of formulations for its wider acceptance and propagation.⁴ Need for drug standardization at different levels of pharmaceutical procedures (raw material, process and finished product) have been highlighted in Ayurvedic treatises along with its principles.⁵ Analytical characterization including High-performance thin-layer chromatography (HPTLC)⁶ is an important step towards standardization of a formulation.⁷

Present study was carried out as a part of project entitled "Efficacy of Swarnamrithaprashana on Promotion of Immunity in Children – A Randomized Double-Blind Trial" sponsored by Rajiv Gandhi University of Health Sciences, Karnataka, Bengaluru, India. The study aimed at assessing the efficacy of Swarnamrithaprashana (modified form of Swarnaprashana) in school going children. Swarnamrithaprashana is a formulation prepared with mixture of Swarnabhasma, Amrithadighritha and Honey.

Amrithadighritha is prepared by processing 50 parts of cows ghee with 200 parts of decoction prepared from dry stem of Guduchi (*Tinospora cordifolia* (Wild) Meirs) and paste of dry root of Yastimadhu (*Glycyrrhiza glabra* Linn.), whole plant of Shankhapushpi (*Convolvulus pluricaulis* Choisy), rhizome of Vacha (*Acorus calamus* Linn.), root of Jatamamsi (*Nardostachys jatamansi* DC.), root of Ashwagandha (*Withania somnifera* Dunal.), dried fruits Pippali (*Piper longum* Linn.), whole plant of Brahmi (*Bacopa monnieri* (Linn.) Wettst.) Above ingredients were mixed homogenously so that each ml of Swarnamrithaprashana consists of 0.5 ml of Amrithadighritha, 0.5 ml of Honey and 5 mg of Swarnabhasma. This mixture was filled into gelatinous capsules each containing 0.4 ml of mixture in it. Soft gel encapsulation was done by following international standards in ISO certified pharmaceutical company (CARE KERALAM, Thrissur and Kerala, India).

MATERIAL AND METHODS

Microscopic examination and Physico-chemical studies like Refractive index, Specific gravity, disintegration time, Unsaponifiable matter and HPTLC were carried out as per the WHO⁸ and Ayurvedic Pharmacopoeia guidelines.⁹

Microscopy

A drop of Swarnamrithaprasha was mounted on a slide and coverslip was placed upon it. Slide was observed under microscope for pollen grains of different size and shape. Photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.¹⁰

Refractive index

Abbe's Refractometer is used for the purpose of determination of refractive index. Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the centre; noted the reading. Distilled water has a refractive index of 1.3325 at 25°C. The difference between the reading and 1.3325 gives the error of the instrument. If the reading is less than 1.3325, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index of the test samples was measured at 28°C.

Specific gravity

Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Sample solution was cooled to room temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper, removed the surplus liquid and noted the weight. Same procedure was repeated using distilled water in place of sample solution.

Disintegration time

The tank of the digital tablet disintegration apparatus microprocessor based was filled with distilled water up to the mark. 750 ml of distilled water in each of the 1000 ml beaker was taken. The timer of the instrument was set for 60 minutes. The temperature of water in beakers to 37°C and that of water in the main tank to 37.5°C was maintained. One capsule was introduced into each tube and added a disk to each tube. The assembly was suspended in the beaker containing water and the apparatus was operated. The time duration at which the capsule disintegrated was noted.

Determination of Unsaponifiable matter

Weighed 5 g of the Swarnamrithaprasha into the flask; added 50 ml alcoholic KOH into the sample; boiled gently but steadily under reflux condenser for one hour. The condenser was washed with 10 ml 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. Altogether, 50 ml of water was added to the separating funnel followed by an addition of 50 ml petroleum ether. The stopper was inserted and shaken vigorously for 1 minute and allowed it 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 times more using 50 ml 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 25 ml of aqueous alcohol and shake vigorously and drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 25 ml 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 dryness on a water bath; placed the flask 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 dryness on a water bath. Cooled in a desiccator to remove last traces of moisture and then weighed.

Sample preparation for HPTLC

Sample obtained from the 'Unsaponifiable matter' procedure was dissolved in 10 ml of chloroform and chloroform soluble portion was used for HPTLC.

HPTLC

3, 6, 9 µl of the chloroform fraction of above sample was applied on a pre-coated silica gel F₂₅₄ on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator using wincats software. The plate was developed in Toluene – Ethyl acetate (9:1) and the developed plates were visualized in CAMAG visualization chamber under short UV, long UV and after derivatisation in vanillin-sulphuric acid spray reagent and scanned in CAMAG TLC scanner 4 at UV 254 nm, 366 nm and 620 nm (Following derivatisation). R_f, colour of the spots and densitometric scan were recorded.

RESULTS

Microscopy

In microscopic analysis, pollens of various size (ranging from 5 micron to 20 microns) and shapes (circular, oblong, ovate and polygonal) were observed. Multiple needle shaped crystals are observed in scattered and clustered form. (Figure 1)

Physico-chemical parameters

Various physico-chemical parameters such as refractive index, specific gravity, disintegration time (min) and unsaponifiable matters (%W/W) were analyzed and the observed values are tabulated (Table 1)

HPTLC

Chloroform fraction of Swarnamrithaprashana was subjected to HPTLC as described in methodology, and the developed plates were observed under short UV (254 nm) and long UV (366 nm). After derivatisation in vanillin-sulphuric acid spray reagent the developed plates were observed under 620 nm. The observations were recorded in form of densitograms and R_f values were tabulated. (Figure 2, Figure 3a, Figure 3b, Figure 3c and Table 2)

DISCUSSION

In microscopic analysis of the Swarnamrithaprashana sample, pollens of various size and shapes were observed which is suggestive of multiple floral source indicating good quality of the honey. Results of different physico-chemical parameters such as refractive index (1.33229), specific gravity (1.1762), disintegration time (8 min) and unsaponifiable matters (4.17 %W/W) were observed and can be considered as reference values for Swarnamrithaprashana formulation. The observations of HPTLC were recorded in form of densitograms (Figure 3) and following R_f values were observed under 366 nm showed bands at 0.49 (F. blue), 0.59 (F. blue), 0.71 (F. blue), 0.83 (F. blue), 0.87 (F. blue) and after derivatisation showed bands at 0.07 (Purple), 0.36 (Purple), 0.45 (Purple), 0.53 (Purple), 0.85 (Purple) which can be the reference HPTLC fingerprint for assessing the quality of Swarnamrithaprashana.

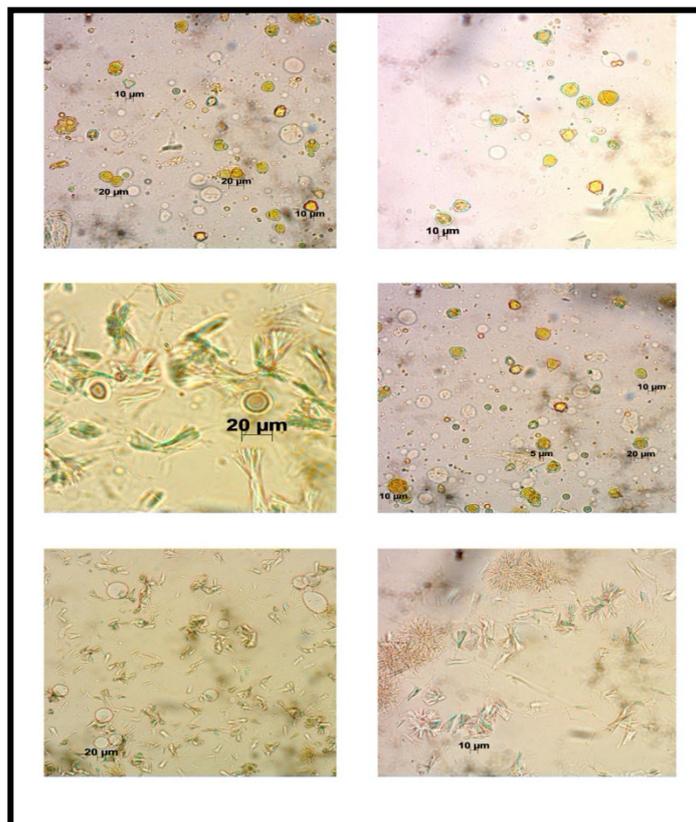
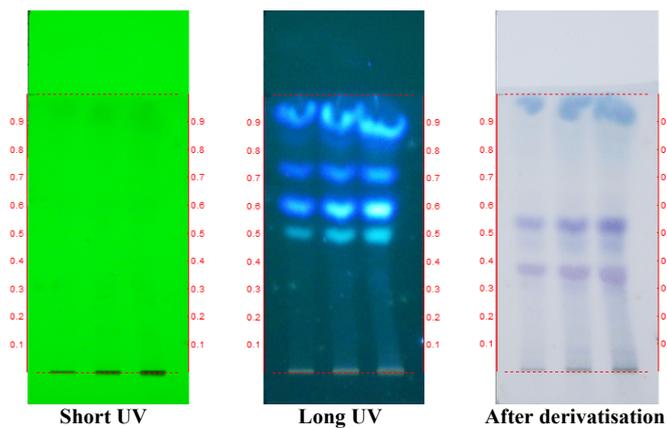


Figure 1: Microscopy of Swarnamrithaprashana – Pollens of different size, clusters of needle shaped crystals

Table 1: Results of standardization parameters

Parameter	Results n = 3 %w/w	
	Swarnamrithaprasha	Swarnamritha capsule
Refractive index	1.33229	-
Specific gravity	1.1762	-
Disintegration time (min)	-	8 min
Unsaponifiable matter (%W/W)	4.17	



Solvent system - Toluene: Ethyl Acetate (9.0: 1.0)

Track 1 – Swarnamrithaprasha– 3 μl
 Track 2 – Swarnamrithaprasha– 6 μl
 Track 3 – Swarnamrithaprasha– 9 μl

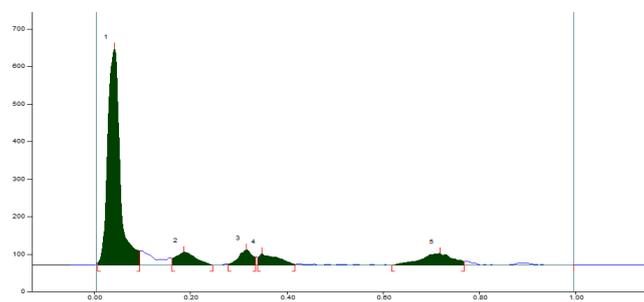
Figure 2: HPTLC photo documentation of Chloroform fraction of Swarnamrithaprashana

Table 2: R_f values of Swarnamrithaprasha

Swarnamrithaprasha		
Short UV	Long UV	Post derivatisation
-	-	0.07 (Purple)
-	-	0.36 (Purple)
-	-	0.45 (Purple)
-	0.49 (F. blue)	-
-	-	0.53 (Purple)
-	0.59 (F. blue)	-
-	0.71 (F. blue)	-
-	0.83 (F. blue)	-
-	-	0.85 (Purple)
-	0.87 (F. blue)	-

*D – dark; L – light; F – fluorescent

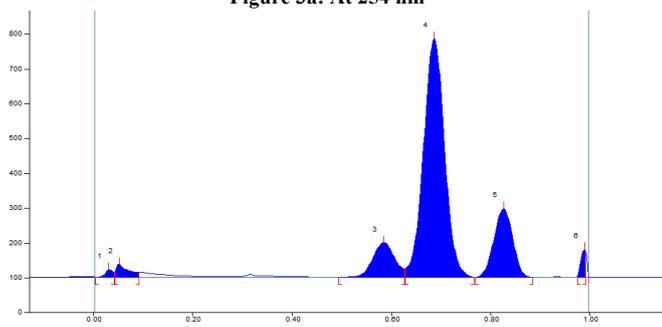
Figure 3a: Densitometric scan of Swarnamrithaprasha



Track 3, ID: Swarnamritha prasha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	5.2 AU	0.04 Rf	577.2 AU	80.57 %	0.10 Rf	38.4 AU	10650.5 AU	71.58 %
2	0.16 Rf	18.3 AU	0.19 Rf	34.5 AU	4.81 %	0.25 Rf	0.6 AU	1029.5 AU	6.92 %
3	0.28 Rf	3.0 AU	0.32 Rf	41.9 AU	5.85 %	0.34 Rf	21.8 AU	833.5 AU	5.60 %
4	0.34 Rf	20.5 AU	0.35 Rf	31.0 AU	4.33 %	0.42 Rf	2.3 AU	867.3 AU	5.83 %
5	0.62 Rf	0.1 AU	0.72 Rf	31.8 AU	4.43 %	0.77 Rf	10.0 AU	1497.4 AU	10.06 %

Figure 3a: At 254 nm



Track 3, ID: Swarnamritha prasha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.03 Rf	21.9 AU	1.95 %	0.04 Rf	14.9 AU	246.2 AU	0.80 %
2	0.04 Rf	16.6 AU	0.05 Rf	37.8 AU	3.37 %	0.09 Rf	13.8 AU	685.0 AU	2.22 %
3	0.50 Rf	0.1 AU	0.59 Rf	99.4 AU	8.86 %	0.63 Rf	24.9 AU	3149.2 AU	10.21 %
4	0.63 Rf	25.3 AU	0.69 Rf	686.2 AU	61.13 %	0.77 Rf	0.1 AU	20872.6 AU	67.67 %
5	0.77 Rf	0.1 AU	0.83 Rf	197.6 AU	17.60 %	0.89 Rf	0.1 AU	5355.5 AU	17.36 %
6	0.98 Rf	3.5 AU	0.99 Rf	79.5 AU	7.09 %	0.99 Rf	77.0 AU	534.3 AU	1.73 %

Figure 3b: At 366 nm

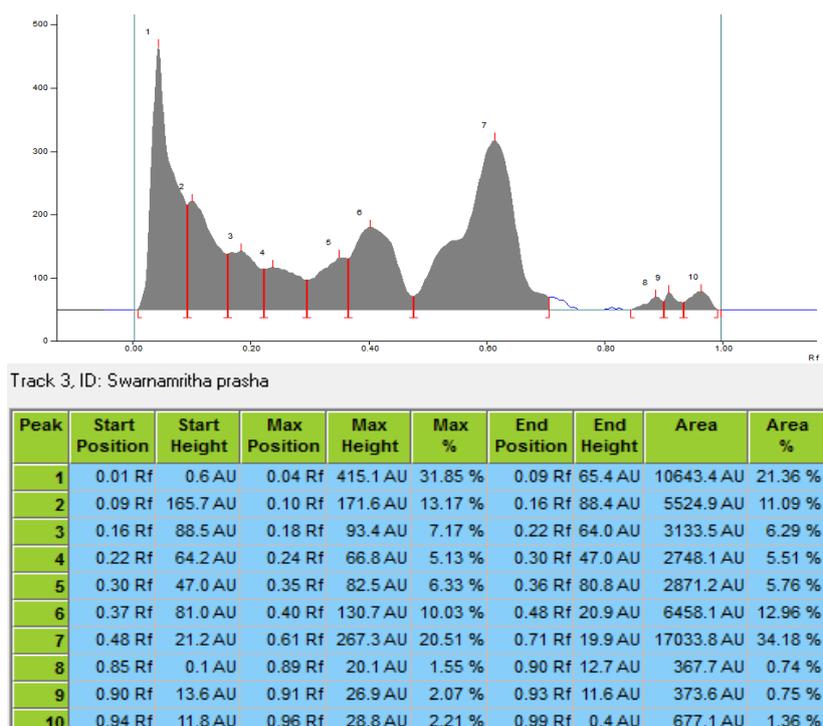


Figure 3c: At 620 nm (Post derivatization)

CONCLUSION

This study being first of its kind on Swarnamrithaprashana, above observations can be considered as the reference for similar studies in future.

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