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Research Article

# PHYSICO CHEMICAL EVALUATION OF NEELIBHRINGADI KERA TAILAM MANUFACTURED BY AYURVEDIC COMPANIES IN KERALA

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**ABSTRACT**

The quality control of herbal medicines has become a major concern to health authorities, health care providers and the manufacturing industries at every stage of production. The standardization of raw drugs and formulations with the help of modern analytical tools increase their scope, acceptance and scientific validity. The safety and efficacy of herbal medicines largely depend on their quality. Neelibhringadi keratailam is an excellent hair growth promoting oil widely prescribed and marketed in Kerala. All the ingredients in the formulation are best hair growth promoters. The knowledge of hair products, their mode of action, efficacy and ingredients has become more relevant today. Neelibhringadi keratailam manufactured by GMP certified Ayurvedic companies in Kerala was assessed by evaluating and comparing the physico chemical parameters of market samples of Neelibhringadi keratailam with that of prepared Neelibhringadi keratailam. Physico chemical parameters such as Acid value (AV), Iodine value (IV), Refractive index (RI) Saponification value (SV), Specific gravity (SG), Weight per milliliter, HPTLC, ICP-MS and microbial contamination of market samples were evaluated and compared with those of prepared sample. Neelibhringadi keram was prepared and its physicochemical analysis was done and compared with market samples. Analysis of the market samples showed significant variation in physicochemical analytical parameters, colour and consistency from the prepared samples.

**Keywords**: Neelibhringadi keratailam, Physico chemical analysis, GMP, ICP-MS, HPTLC

## INTRODUCTION

Hair is a complex structure made up of many components that work together to protect the scalp and provide physical attractiveness to the self-perception of beauty. Haircare has become increasingly important in people's lives these days.[1]. A number of hair growth promoting oils are available in commercial market today. Herbal medicines contain phytoconstituents in complex matrices, of which no single active constituent is responsible for the overall effectiveness. Quality of a medicine depends on the genuinity of raw drugs used for the preparation. This creates a problem in establishing quality control standards and standardizing herbal medicines. In order to ensure quality of raw drug, care should be taken from the identification and authentication of raw drugs to the verification process of final product.[2] Therefore, to provide quality control measures and safety and efficacy of Ayurvedic formulations standardization is an essential tool[3]. Neelibhringadi Tailam is one of the most famous Ayurvedic preparations that is renowned for abundant growth of long, dark and dense hair. This tailam is mentioned in Chikitsa Manjari Siro roga Chikitsa[4] and Sahasrayogam Taila prakaranam[5]. As per classical reference, for anjana, galena (lead sulphide) is used. But as per Ayurvedic Pharmacopoeia of India (API)[6] Daruharidra dried stem extract is taken instead of anjana (Lead Sulphide). All the ingredients in the formulation are excellent hair growth promoters which are capable to reduce the rate of hair loss. The medium of preparation is either Tila tailam or Kera tailam. There are 12 ingredients in the formulation. Neeli, Bhrngaraja, Satakratulata, Dhatri Phala, Aja Kshira, Nalikera Kshira, Mahishi Kshira, Go Kshira, Kera Tailam (Coconut oil), Yasti Madhu, Gunja root, Anjana (Galena).

Neelibhringadi tailam is widely prescribed and marketed in Kerala. Yet no previous studies have been published. Therefore, the present study, which will be useful to the pharmaceutical industry and Ayurvedic practitioners, is being conducted to authenticate commercially available samples of Neelibhringadi tailam.

## MATERIALS AND METHODS

The study comprised of 4 stages
1.Online survey
2.Preparation of Neelibhringadi keratailam
3.Selection of market samples of Neelibhringadi keratailam
4.Physico chemical analysis of prepared and markets samples of Neelibhringadi keratailam

**Table 1: Ingredients of Neelibhringadi Kera Tailam**

| Ingredients | Botanical Name | Family | Parts used |
|---|---|---|---|
| Neeli | *Indigofera tinctoria* Linn | Fabaceae | Leaf juice |
| Bhrngaraja | *Eclipta alba* Hassk | Asteraceae | Plant juice |
| Satakratulata | *Cardiospermum halicacabum* Linn | Sapindaceae | Plant juice |
| Dhatri Phala | *Emblica officinalis* Gaertn | Euphorbiaceae | Pericarp juice |
| Aja Kshira | Goat milk | | |
| Nalikera Kshira | *Cocos nucifera* Linn | Arecaceae | Milk from Endosperm |
| Mahishi Kshira | Buffalo milk | | |
| Go Kshira | Cow's milk | | |
| Kera Tailam | *Cocos nucifera* Linn | | |
| Yasti Madhu | *Glycyrrhiza glabra* Linn | Fabaceae | Root |
| Gunja | *Abrus precatorius* Linn | Fabaceae | Root |
| Anjana (Galena) | Lead Sulphide | | |

**Online Survey**

In the present study a preliminary online survey was conducted among GMP certified pharmacies in Kerala regarding manufacturing methods adopted and ingredients used in production of Neelibhringadi. In Ayurvedic classics even though Neelibhringadi tilatailam is mentioned, the market availability of Neelibhringadi tilatailam is inappreciable. So, for an information about the annual production of both Neelibhringadi keratailam and Neelibhringadi tilatailam a survey was conducted. There is also lack of uniformity in the ingredients used for formulation preparation by various GMP (Good manufacturing practice) certified Ayurvedic pharmacies. Detailed questionnaire was prepared on consultation with subject experts. Google form link of questionnaire attached with covering letter from head of department was sent to the production heads of the pharmacies manufacturing Neelibhringadi and responses were collected. As per survey responses received majority pharmacies are having more production of Neelibhringadi keratailam using Anjana (Galena or Lead sulphide) compared to Neelibhringadi tilatailam using Anjana (Galena or Lead sulphide). So, samples of Neelibhringadi keratailam with Anjana from GMP certified Ayurvedic companies in Kerala were selected for physico chemical analysis in comparison with prepared Neelibhringadi keratailam.

**Preparation of Neelibhringadi keratailam**

The pharmaceutical work in the present study includes –

Collection of raw drugs, processing of raw drugs and preparation of formulation.

The raw drugs, Yastimadhu and Anjana were collected from local drug store in Thiruvananthapuram and its morphological identity was authenticated by subject experts. All the other ingredients Neeli, Bhrngaraja, Satakratulata, Dhatri phala, Gunja mula, milks of cow, goat and buffalo were freshly and directly collected from sources.

The freshly collected Neeli leaves, whole plant of Bhrngaraja and Satakratulata were thoroughly cleaned and washed in running water to remove dirt and other extraneous matter. It was then crushed individually in khalwayantra and after pounding it was transferred to a mixer grinder for proper crushing. The crushed mass was put into a clean double layered cotton cloth, wrapped and squeezed tightly to obtain juices of Neeli, Bhrngaraja and Satakratulata. 350 ml of Neeli swarasa, 340 ml of Satakratulata swarasa and 200 ml of Bhrngaraja swarasa were extracted from 244 gm of Neeli leaves, 220 gm of Sakralatha and 160 gm of Bhrngaraja plant respectively. 882 gm of Dhatri phala were collected, seeds were discarded after proper washing. The pericarp portion was chopped into small pieces and juice was collected as before to obtain 560 ml of Dhatri swarasa. From the above four juices 130 ml each were measured and kept aside for preparation of tailam.

The raw drugs Yastimadhu and Gunja mula were cleaned, washed, dried and were powdered separately using a pulverizer. The powdered drugs were then sieved individually through sieve number 85 and stored in an airtight container. In the same manner Anjana (lead sulphide) was also finely powdered and sieved through sieve number 85 and stored in an airtight container. 50.5 gm of Yastimadhu fine powder, 32 gm of Gunja mula fine powder and 107 gm of Anjana (Galena) fine powder were obtained from 100 gm of Yastimadhu, 57 gm of Gunja mula and 109.5 gm of Anjana (Galena) respectively. From these 5.4 gm each was weighed and kept aside for preparation of formulation. 5.4 gm, each of fine powders of Yastimadhu and Gunja mula were ground with sufficient quantity of water in khalwayantra to form a fine paste or kalka.

Nalikera was scraped, ground and squeezed through double layered cotton cloth to obtain Nalikera Kshira. The other ingredients -milks of cow, goat and buffalo were freshly and directly collected from sources.130 ml each from the four milks was measured and kept aside.

Before preparation of oil, Kera tailam samples from three different manufacturing units were collected and were subjected for analytical test to detect for the mentioned qualities as per AGMARK[7] standards. The keratailam sample which matched more with AGMARK standards was chosen for Neelibhringadi keram preparation.

The formulation was prepared in a bronze vessel. The vessel was cleaned thoroughly and wiped dry. It was slightly warmed to remove any moisture content that may have been present. Into this vessel 130 ml of kera tailam was added. The fine paste of kalka was mixed with 520 ml of four swarasa. The mixture was slowly added to the keratailam contained in the vessel and were properly mixed with the help of clean and dried steel ladle. The paka was done under mandagni. The mixture was stirred continuously until mridu paka was attained. On attaining mridu paka 520 ml of four milks were added. Heating was again continued under mandagni with frequent stirring and kalka was constantly checked for paka lakshanas. The paka was continued till khara paka that was when kalka became brittle, when rolled in between fingers. Then the stove was turned off and the tailam was filtered through a clean cotton cloth into a dried and cleaned vessel containing 5.4 gm of powdered anjana (Galena). It was then stirred well. On cooling it was stored in an airtight glass bottle.

Total three samples of Neelibhringadi keram were prepared. The prepared samples were coded as $P_1$, $P_2$ and $P_3$.

Three samples of Neelibhringadi keram with Galena were subjected to analytical study to ensure reproducibility of Neelibhringadi in which each were prepared following same manufacturing method and same ingredients. The prepared Neelibhringadi keratailam was analyzed for various physico-chemical parameters such as Acid value (AV), Iodine value (IV), Refractive index (RI) Saponification value (SV) and Specific gravity (SG). Rancidity test and HPTLC were also done.

**Table 2: Results obtained after preparation in ml***

| Observations | P1 | P2 | P3 |
|---|---|---|---|
| Initial quantity of keratailam | 130 | 130 | 130 |
| Final product obtained | 126 | 120 | 120 |

*ml – Milliliter

**Method of Market Sample Collection of Neelibhringadi keratailam and its Analysis**

As per survey response, Neelibhringadi keratailam with Anjana (galena) as ingredient is manufactured more by GMP certified pharmacies and is widely available in Kerala market. Hence six samples with above combination were randomly selected from list of manufacturers preparing Neelibhringadi keratailam using lottery method, for physicochemical analysis and comparison. The selected market samples were coded as M1, M2, M3, M4, M5 and M6. All the nine samples were analyzed for the physico chemical parameters such as Weight per milliliter (Wt/ml) Acid value (AV), Iodine value (IV), Refractive index (RI) Saponification value (SV), Specific gravity (SG) and Rancidity test, and the result obtained was compared with that of prepared sample.

**Physicochemical Evaluation- Neelibhringadi keratailam**

The following tests were done to evaluate the physico chemical parameters as per the methods described in The Ayurvedic Pharmacopoeia of India, Part1 Volume 6 (Department of Ayurveda, Yoga-Naturopathy, Unani, Siddha and Homoeopathy. Ministry of Health and Family Welfare, Govt. of India: 2008)[8].

1. Weight per milliliter
2. Determination of specific gravity
3. Determination of refractive index
4. Determination of saponification value
5. Determination of acid value
6. Determination of iodine value
7. Rancidity test (Kreis test)

**OBSERVATION AND RESULT**

**Table 3: Analysis of Raw Keratailam**

| Parameters | Value | AGMARK |
|---|---|---|
| Specific gravity | 0.921 | 0.915-0.920 |
| Moisture content | 0.094% | - |
| Refractive index | 1.4620 | 1.4481-1.4491 |
| Saponification value | 270 | Not less than 250 |
| Iodine value | 9 | 7.5-10 |
| Acid value | 0.911 | 0.5 |
| Wt/ml | 0.845 | - |
| Rancidity | Not rancid | - |

**Table 4: Organoleptic characters of Neelibhringadi Keratailam**

| Sample | Colour | Odour | Texture |
|---|---|---|---|
| P1 | Dark grape colour | Strong aromatic smell | Smooth |
| P2 | Dark grape colour | Strong aromatic smell | Smooth |
| P3 | Dark grape colour | Strong aromatic smell | Smooth |
| M1 | Light green | Aromatic smell | Smooth |
| M2 | Light green | Aromatic smell | Smooth |
| M3 | Light green | Aromatic smell | Smooth |
| M4 | Light green | Characteristic smell | Smooth |
| M5 | Light green | Aromatic smell | Smooth |
| M6 | Pale green | Characteristic smell | Smooth |

**Table 5: Comparison of physico chemical characters of prepared and market samples of Neelibhringadi keratailam**

| Sample name | Acid value | Iodine value | Saponification value | Refractive index | Specific gravity | Weight/ml | Rancidity |
|---|---|---|---|---|---|---|---|
| P1 | 5 | 11.02 | 252.59 | 1.460 | 0.920 | 0.904 | Not rancid |
| P2 | 6.9 | 11.63 | 253.72 | 1.460 | 0.918 | 0.902 | Not rancid |
| P3 | 6.7 | 7.65 | 258.77 | 1.470 | 0.922 | 0.906 | Not rancid |
| M1 | 9.97 | 11.63 | 266.53 | 1.454 | 0.924 | 0.905 | Not rancid |
| M2 | 14.27 | 17.5 | 283.38 | 1.455 | 0.925 | 0.903 | Rancid |
| M3 | 13 | 11.16 | 279.58 | 1.453 | 0.924 | 0.905 | Not rancid |
| M4 | 12.67 | 12.2 | 273.80 | 1.456 | 0.921 | 0.902 | Not rancid |
| M5 | 13.20 | 10.47 | 297.85 | 1.456 | 0.921 | 0.903 | Not rancid |
| M6 | 15.87 | 11.78 | 282.55 | 1.454 | 0.923 | 0.904 | Rancid |

**Lead content in samples by Inductively coupled plasma mass spectrometry (ICP-MS)**

ICP-MS is a technique employed for the detection of heavy metals in the samples.

As Neelibhringadi formulation contains anjana (lead sulphide or galena), ICP-MS was done for analyzing the amount of lead content in samples following the test method CKL/ANL/AY-008. The prepared sample P2 and market samples M2, M3, M4 were randomly selected for analysis. The lead concentration in market samples were within permissible limits as in WHO quality control standards for herbal materials but was found higher in prepared sample. From that it can be inferred that market samples may not be adding galena in formulation in required amount as in reference.

The results obtained were as follows.

**Table 6: Lead percentage in prepared and market samples**

| Sample | Result (mg/kg) | Specification | Detection limit |
|---|---|---|---|
| M2 | 1.36 | NMT 10.0 mg/kg | 0.05 |
| M3 | 3.45 | NMT 10.0 mg/kg | 0.05 |
| M4 | 0.29 | NMT 10.0 mg/kg | 0.05 |
| P2 | 15.07 | NMT 10.0 mg/kg | 0.05 |

**HPTLC of Neelibhringadi samples**

The HPTLC (High-performance thin layer chromatography) of randomly selected one prepared sample (P1) and six market samples were performed in solvent system Toluene: Ethyl acetate: hexane (6:3:1). Samples are in M1, M2, M3, M4, M5, M6 and P1 order in photodocumentation.

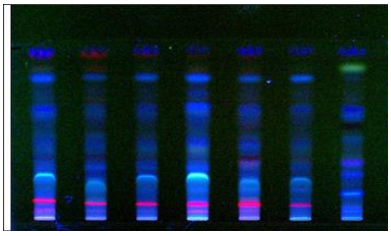

**Fig 1: HPTLC of Neelibhringadi keram samples at 366 nm**

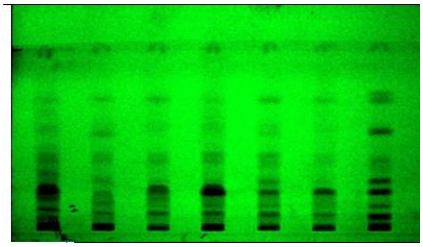

**Fig 2**: **HPTLC of Neelibhringadi keram samples at 254 nm**

At 254 nm sample M5 have maximum number of peaks (11 peaks). Sample M6 was having least number of peaks (6). Samples M1, M2, M3 and M4 showed some unique peaks that were not observed in other samples.

At 366 nm sample M2 have the maximum number of peaks (12 peaks) followedby sample P1. The Rf values of many peaks of market samples were similar compared to prepared samples indicating the presence of same chemical constituents in the market samples. Similar Rf values uniformly presented in all the samples studied indicate the presence of similar Phyto constituents.

Microbial contamination in 6 market samples were analyzed soon after purchase. All the samples were found to be free from contamination.

**DISCUSSION**

Herbal medicines contain Phyto constituents in complex matrices, of which no single active constituent is responsible for the overall effectiveness. This creates a problem in establishing quality control standards and standardizing herbal medicines. Hence the formulations used in Ayurveda must be standardized in order to obtain the optimal concentration of the known active ingredients and to maintain their activities in various physico chemical parameters.

The present study aimed at the physicochemical analysis of Neelibhringadi keratailam manufactured by GMP certified Ayurvedic companies in Kerala. The six market samples were assessed by evaluating and comparing their physicochemical characters with that of prepared Neelibhringadi keratailam. The preliminary parts of study included an online survey, raw drug collection, pharmaceutical work and analytical part. The study was conducted in two parts. Physico-chemical evaluation of prepared Neelibhringadi keratailam, and physico-chemical evaluation of six market samples and the results were compared with that of prepared sample.

The physico-chemical analysis was conducted in Drug Testing Laboratory, Department of Rasasastra and Bhaishajyakalpana, Govt Ayurveda College, Thiruvananthapuram and Drug Standardization Unit, Govt. Ayurveda College, Thiruvananthapuram. HPTLC and ICPMS were conducted at CARe KERALAM Limited, Thrissur.

Market samples of Neelibhringadi keratailam showed variations in their physico chemical parameters when compared with the prepared sample. The higher analytical tests like HPTLC and ICPMS were done for selected few samples only due to non-availability of test facilities in the study setting and high cost for analysis. Rancidity was tested for all the samples by performing Kries test. The market samples M2 and M6 were found to be rancid which indicates that oxidative process has been started in those samples. The mean specific gravityof prepared samples was 0.920. All the market samples were having specific gravity slightly more than the mean value of prepared ranging from 0.921 to 0.925. The wt/ml of the prepared samples were almost similar with market samples. The refractive indices of all the prepared samples were higher than the market samples. The mean refractive index of the prepared sample was 1.463 which was similar to the raw keratailam taken. The mean saponification value of prepared samples was 255.026 which was lower than that of raw oil with saponification value, 270. All the market samples showed an increase in saponification value ranging from

266 to 297. The mean iodine value of prepared samples was 10.1 which was almost similar to raw keratailam taken. The market samples M5(10.47) and M3 (11.16) were more matching to mean iodine value of prepared sample. The market sample M1 had similar iodine value to the prepared sample P2 which was 11.63. The mean acid value of prepared sample was 6.2. All the market samples showed high increase in acid values from the prepared samples, ranging from 9 to 15. On conducting Kries test, two market samples were found to be rancid by forming a pinkish ring in test tube.

In order to compare between prepared sample and market sample, ANOVA test was employed. Significant differences were observed on the statistical analysis between all the parameters of prepared and market samples.

## CONCLUSION

Analysis of the market samples showed significant variation in physicochemical analytical parameters, colour and consistency from the prepared samples. It may be due to difference in ingredients used in the formulation. As Neelibhringadi keratailam is a widely used and prescribed formulation, its proper standardization and quality control is essential to maintain the uniformity and reproducibility in their production and to maintain its efficacy.

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