



Standardization of Poly Herbal Cosmetic Formula on Abnormal Pigmentation in Cutaneous Wound Healing – A Preliminary Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author EDTPG designed the study. Author KAJSP performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EDTPG and RDHK managed the analyses, literature searches and finalized the draft. All authors read and approved the final manuscript.

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ABSTRACT

Abnormal pigmentation in cutaneous wound healing has become a major cosmetic issue in the current society. Rising popularity of green labeled cosmetics which are environmentally sustainable with minimum side effects have greater demand in the world cosmetic market. Standardization of herbal formula is essential in order to assess the quality of drugs. Hence, the research study was designed to standardize the selected herbs based on comprehensive literary study on selected Varnaya Dravya (complexion promoting herbs) mentioned in Ayurveda authentic texts and Sri Lankan traditional manuscripts comprising of *Terminalia chebula* Retz, *Terminalia bellerica* (Gaertn). Roxb, *Phyllanthus emblica* Linn, *Rubia cordifolia* Linn, *Curcuma longa* Trim, and *Sesamum indicum* Linn. Authentication of herbal ingredients has been carried out at Bandaranayaka Memorial Ayurveda Research Institute, Nawinna, Sri Lanka. Microscopic identification, Moisture content, pH value, Total ash content and Colouring matter were tested determined through paper chromatography under WHO (2011) guidelines. Microscopic identification revealed that the poly herbal cosmetic formula in powder form contains yellow colour cells, stone

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cells, group of elongated stone cells, transparent cells, pitted vessels, spiral vessels, vessels filled with red, small fibers, oil globules, starch granules, needle shape crystals, hair and Trichoid. Moreover, moisture content of the formula was 6.3%, pH - 5.92 and total ash value - 4.45%. Determination of colouring matter indicated formula does not contain synthetic dyes. In addition, secondary plant metabolites like phenolic, tannins and saponins were present. Pharmacodynamics potential of the formula showed 25% kashaya (astringent) and tikta (bitter) rasa (taste) accordingly. Further, 46% and 27% of formula showed ruksha (dryness) and laghu (lightness) guna (properties) along with 83% and 67% indicated ushna virya. (hot in potency) and madura (sweet) vipaka respectively. Due to presence of secondary metabolites, the poly herbal formula has anti-bacterial, anti-fungal and anti-oxidant potentials which will be favorable for skin complexion protection from bacteria and fungi. Moreover, Lekhana action gives scraping effect which is important for removing the scar. The study suggested that parameters found to be sufficient to evaluate the herbal cosmetic powder and could be used as reference standards and applicability of this formulae should be validated with a preclinical and clinical trials.

Keywords: Poly herbal cosmetic formula; standardization; Varnaya Dravya.

1. INTRODUCTION

Wound healing refers to living organism replacement of damage tissue by newly produce tissue [1]. Negative outcome of the process of wound healing after cutaneous injury on the skin has long been a major issue in current society. It affects for both men and women at any age. Wound healing seems to be the reconstitution of the resulting cellular defects and restoration of the skin which might be leads to dyspigmentation of the skin. Melanin pigment responsible for producing colour in the skin [2] and it is produced by melanocytes at the lower layer of the epidermis. Disruption to normal melanogenesis within the wounded skin will result in discoloration of surrounding skin either hyperpigmentation or hypopigmentation with a range of symptoms including inflammation, erythema, dryness and pruritus. This results in significant psychosocial impact [3] on individuals and their quality of life.

The scar which develop in the final stage of the wound healing named as "Vrana Wasthu" and disappearing scar along with surrounding discoloration is called "vaikritha paham." according to Ayurveda point of view [4]. The complex and lengthy management options are available for dyspigmented areas, but none which completely abolish the problem and it will have increased the burden on health care expenses.

So far, herbal cosmetics play a vital role in solving these issue as it enhances the skin complexion, cost effective with minimal side effects. Currently, the herbal cosmetics are extensively popularized in cosmetic field and it

has become great demand due to increasing awareness and general acceptability. Therefore, it is needed the development of standardization parameters to assure the quality, purity, efficacy and safety of herbal preparation.

Hence, the present study has been focused to standardize poly herbal cosmetic formula of Nisha Tiladi churna in powder form; on abnormally pigmented cutaneous marks. The research is coupled with the previous literary research done on Varnya dravya mentioned in herbal remedies of Ayurveda authentic texts and Sri Lankan Traditional manuscripts for restoration of skin colour of cutaneous marks.

1.1 Objectives of the Study

1.1.1 General objective

To standardize Nisha Thiladi churna; a poly herbal cosmetic formula

1.1.2 Specific objectives

- i. To identify microscopic characteristic features of poly herbal cosmetic formula
- ii. To analyze physical parameters, colouring matters and phytochemicals of poly herbal cosmetic formula
- iii. To study pharmacodynamics properties of each ingredient in poly herbal cosmetic formula

2. METHODOLOGY

2.1 Data Collection

Data has been collected from Authentic texts, Sri Lankan traditional manuscripts, Journals and

other texts from library of Institute of Indigenous Medicine and Published related research articles via Internet.

2.2 Identification and Authentication of Poly Herbal Formula (Nisha Thiladi churna)

All the ingredients of Nisha Thiladi churna were separately identified and authenticated from Bandaranayaka Memorial Ayurvedic Research Institute, Nawinna, Maharagama in Sri Lanka.

2.3 Collection of Raw Materials and Method of Preparation of Poly Herbal Formula (Nisha Thiladi churna)

Raw materials were collected from local market in Colombo, Sri Lanka and herbal formula was

prepared according to the churna paribhasha mentioned in Ayurveda authentic text [5] in the pharmacy at department of Dravyaguna Vignana, Institute of indigenous medicine, university of Colombo, Rajagiriya, Sri Lanka. The physical impurities were removed from the ingredients, and properly cleaned and dry under the shade for 2 months. Shade dried endocaps, seeds, roots and rhizomes of the ingredients of Nisha Thiladi Churna were powdered separately and filter through strainer sieve no 80 and made into a fine powder. Finally, mixed 20 g from each fine powder to make a uniformly blended churna to use for the pharmacognostical study. Nisha Thiladi churna (Table 1) was prepared as per the classical references [5]. A physicochemical analysis of the final product was carried out at the Industrial Technology Institute, Malabe, Sri Lanka.

2.4 Ingredients of Poly Herbal Formula (Nisha Thiladi churna)

Table 1. Ingredients of poly herbal formula [6] (Nisha Thiladi churna) [Fig. 1-6]

	Sanskrit name	Botanical name	Used part
1.	Haritaki	<i>Terminalia chebula</i> Retz.	Endocap
2.	Vibhitaka	<i>Terminalia bellerica</i> (Gaertn).Roxb	Endocap
3.	Amalaki	<i>Phyllanthus emblica</i> Linn.	Endocap
4.	Manjistha	<i>Rubia cordifolia</i> Linn.	Stem
5.	Vacha	<i>Curcuma longa</i> Trim.	Rhizome
6.	Thila	<i>Sesamum indicum</i> Linn.	Seeds



Fig. 1. Aralu -*Terminalia chebula* Retz.



Fig. 2. Bulu-*Terminalia bellerica*



Fig. 3. Nelli -*Phyllanthus emblica* Linn. (Gaertn).Roxb



Fig. 4. Welmadata -*Rubia cordifolia*



Fig. 5. Kaha -*Curcuma longa* Trim.



Fig. 6. Thala -*Sesamum indicum* Linn.

Figs. 1-6. Ingredients of poly herbal formula (Nisha Thiladi churna)



Fig. 7. *Terminalia chebula* Retz.



Fig. 8. *Phyllanthus emblica* Linn.



Fig. 9. *Terminalia bellerica* (Gaertn.) Roxb



Fig. 10. *Rubia cordifolia* Linn.



Fig. 11. *Curcuma longa* Trim.



Fig. 12. *Sesamum indicum* Linn.

Figs. 7-12. Fine powder of ingredients of poly herbal formula (Nisha Thiladi churna)



Fig. 13. Final product of Nisha Thiladi Churna

2.5 Evaluation Parameters of Herbal Drugs

2.5.1 Microscopic identification of poly herbal formula (Nisha Thiladi churna) [Fig. 7-12]

Small quantity of fine powdered formula was kept on a glass slide and added few drops of chloral hydrate and covered with slip. Then examined under 10X of compound microscope.

2.5.2 Organoleptic evaluation of poly herbal formula [Fig. 13]

The formulation was yellowish brown in color, odour of mixture of all herbs, solid consistency in texture and pungent taste.

2.5.3 Physical evaluation [7]

Determination of moisture content: 2.0000g [W] of sample and dried in hot air oven at 105°C

for 4 hours to a constant weight[W₁]. Then placed in a desiccator and allowed to cool and again heated for 1 hour and taken the weight. The moisture content of drug was taken by the using of following formula

$$\frac{(W - W_1)}{W} \times 100\% = \text{Moisture content \% by weight}$$

W = Weight of the sample taken (g)

W₁ = Constant weight of the sample after heating (g)

Determination of total ash content [Fig. 13]:

The residue remaining after incineration is the ash content of drugs, which simply represents inorganic salts, naturally occurring in drugs or adhering added to it as form adulteration. It uses to detect the contamination and adulteration like sand or earth, unwanted part mixed in crude drug. Accurately weighed 2g of powder (W) and placed in a previously ignited crucible. Ignited the sample in Muffle furnace for 4 hours under

5000°C – 6000°C. Allowed to cool in a desiccator and weight was taken (W1). Measured the weight of empty crucible (W2).

Calculation

$$\text{Total ash \% by weight} = [(W_2 - W_1) / W] \times 100$$

- W1 = Weight of the empty crucible
- W2 = Weight of the crucible with ash
- W = Weight of the sample taken

$$\begin{aligned} \text{Total ash content} &= [(W_1 - W_2) / W] \times 100 \\ &= [(34.463 - 34.374) / 2] \times 100 \\ &= 4.45\% \end{aligned}$$

Determination of pH value of poly herbal formula (Nisha Thiladi churna) [Fig. 13]:

Determine pH value at room temperature using pH meter. At first the powder was macerated with dil.H₂O for 2 hours and filtered it. Then pH meter was calibrated from the standard pH buffer available at the laboratory. Then the solution pH was measured. Triplicates were taken.

2.5.4 Colouring matter of poly herbal formula (Nisha Thiladi churna) [Fig. 13]

Method – paper chromatography: 1g of powder sprinkled with few drops of dil.H₂O followed by extracting with methanol. Paper chromatography was run by using following solvent by the proportionate of Butanol: Acetic acid: Water into 20: 5: 12. Paper chromatography pattern was compared with chromatographic with permitted synthetic dyes.

Table 2. Phytochemical analysis of formula (Nisha Tiladi Churna) [8,9]

Phytochemicals	Method
1. Phenolics	To a few drops of extract, a few drops of Lead acetate were added.
2. Tannin	Add 2ml of 10% FeCl ₃ to the 2ml of the extract
3. Saponins	To 0.5 ml filtrate, added 5ml of water. Shaked vigorously.

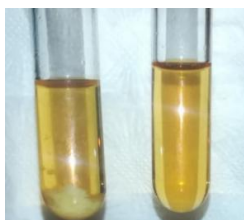


Fig. 14. Test for phenolics



Fig. 15. Test for tannins



Fig. 16. Test for saponins

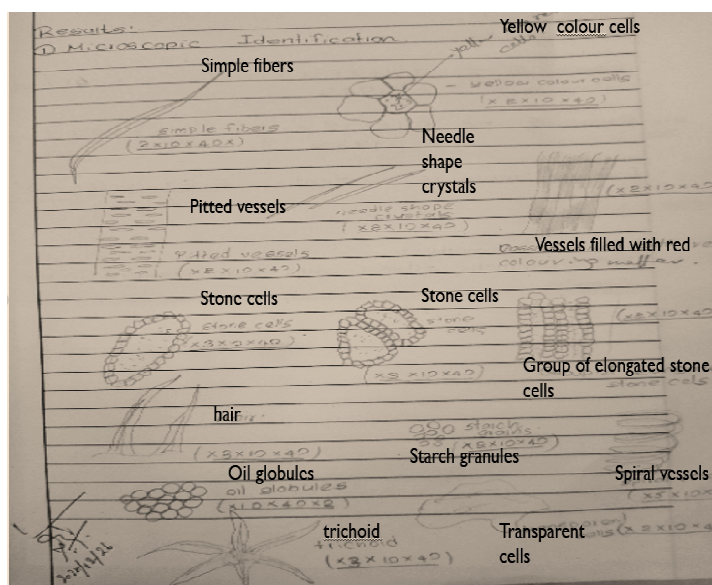


Fig. 17. Microscopic evaluation of poly herbal formula

3. OBSERVATION AND RESULTS

3.1 Microscopic Evaluation of Poly Herbal Formula

Microscopically, yellow colour stone cells, group of elongated stone cells, vessels filled with red, spiral vessels, pitted vessels, needle shape crystals, small fibers, starch granules, oil globules were observed in the formula.

3.2 Organoleptic Evaluation

Organoleptic evaluation of the formula was yellowish brown in color with pleasant odour of mixture of all herbs, solid consistency in texture and pungent taste.

3.3 Physio-chemical Parameters

The pH value of poly herbal cosmetic formula was acidic. Ash value content was 7.45% and moisture contents were 6.3%.

3.4 Phytochemical Study

Phytochemical screening is evaluated in different solvent extraction showed the presences of secondary metabolic like phenol, Tannin and alkaloid which are helpful in predicting their therapeutic properties [Table 2].

3.5 Colouring Matter of Formula- Paper Chromatography

Colouring matter indicates that Nisha Thiladi churna does not contain synthetic dyes.

Table 3. Organoleptic parameters of poly herbal cosmetic formula

Sr. no	Organoleptic parameters	Observation
1.	Appearance	Moderately fine powder
2.	Colour	Yellowish brown colour
3.	Taste	Pungent
4.	Odour	Pleasant

Table 4. Physio-chemical parameters of poly herbal cosmetic formula

Sr no.	Physical parameters	Formula
1.	pH (5% w/v aqueous solution)	5.92
2.	Total Ash value (% w/w)	7.45%
3.	Moisture content	6.3%

Table 5. Phytochemical parameters of poly herbal cosmetic formula

Sr. no	Phytochemical	Observation	Result
1.	Phenolic	Yellow colour precipitate present	Positive
2.	Tannin	Solution of blush green colour	Positive
3.	Saponins	Persistent of 1 cm foam	Positive

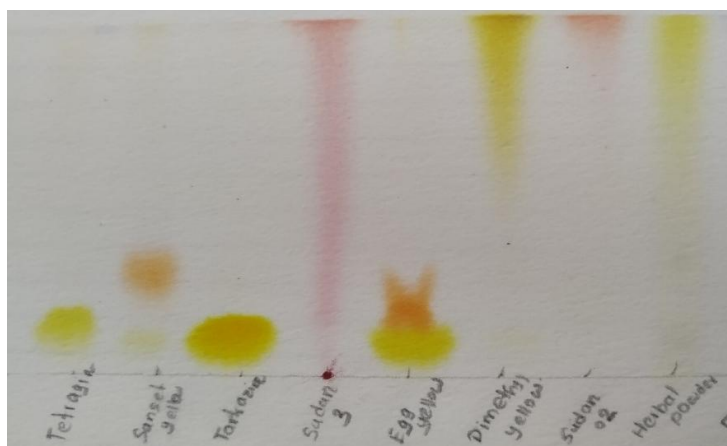


Fig. 18. Paper chromatography pattern of poly herbal formula (Nisha Tiladi churna)

3.6 Pharmacodynamic Actions of the Ingredients of Poly Herbal Formula

Table 6. Pharmacodynamic properties of the ingredients of ploy herbal formula [10,11]

Ingredients of Nisha Tiladi Churna	Rasa					Guna		Virya		Vipaka			
	Madhura	Amla	Katu	Thiktha	Kashaya	Laghu	Ruksha	Guru	Snigdha	Ushna	Shitha	Madhura	Katu
1 <i>Abhya Terminalia chebula</i>	+	+	+	+	+	+	+			+		+	
2 <i>Vibhitaka Terminalia belerica</i>					+	+	+			+		+	
3 <i>Dhatri Phyllanthus emblica</i>	+	+	+	+	+	+	+				+	+	
4 <i>Manjista Rubia cordifolia</i>	+			+	+		+	+		+			+
5 <i>Nisha Curcuma longa</i>			+	+			+			+			+
6 <i>Tila Sesamum indicum</i>	+		+	+	+			+	+	+		+	

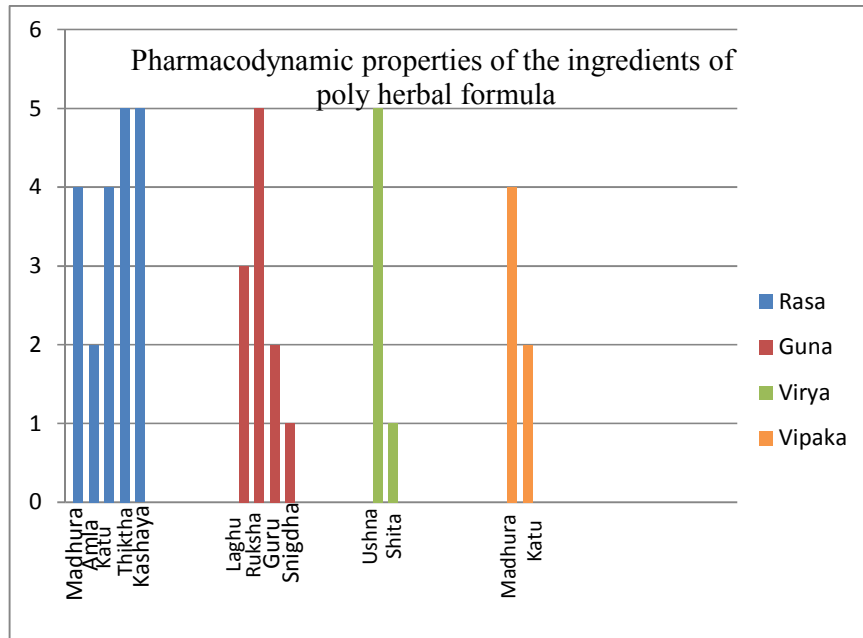


Fig. 19. Pharmacodynamic properties of the ingredients of poly herbal formula

3.7 Pharmacokinetic Actions of the Ingredients of Poly Herbal Formula

Table 7. Pharmacokinetic potential of the ingredients of poly herbal formula [11]

Ingredient	Pharmacodynamic action
<i>Terminalia chebula</i>	Thridoshahara, Anulomana, Rasayana, Prajasthapana, Cakṣuṣya, Hṛdya, Lekhana
<i>Terminalia belerica</i>	Kapha Pittahara, Kesya, Cakṣuṣya, Bhedana
<i>Phyllanthus emblica</i>	Tridosahara, Vayahsthapana, Vrushya, Rasayana
<i>Rubia cordifolia</i>	Kapha Pittahara, Varnya, Vishaghna
<i>Curcuma longa</i>	Kapha Vatahara, Varnya, Lekhana, Vishaghna
<i>Sesamum indicum</i>	Vata shamaka, Twachya, Balya, Kesya

4. DISCUSSION

The average pH of poly herbal formula of Nisha Thiladi churna is 5.92 which supports to regulate the normal function of the skin because the normal skin pH ranged between 4.5 -5.5. Moisture content of the formula is 6.3% and the value also within the normal range of the moisture content of powder; which is less than 12%. Due to minimum percentage of moisture content of the formula is indicated not contaminated by the fungus and other microorganisms. Total ash value was 4.45%.[Table 5] and it involves an oxidation of the components of the formula. Microscopic identification shows yellow colour cells, stone cells, group of elongated stone cells, vessels filled with red, spiral vessels, pitted vessels, needle shape crystals, small fibers, starch granules, oil globules [Fig. 17] and it indicates sample was free from adulterants. Colouring matter indicates that Nisha Thiladi churna does not contain synthetic dyes [Fig. 18]. Further, herbal formula was consisted with phytochemicals such as Phenole, Tannin, Saponin [12] [Table 4]. Hence, the poly herbal formula has anti-bacterial, anti-fungal and anti-oxidant properties which will be beneficial for skin complexion as well as in providing protection from pathogenic bacteria and fungi [12].

According to the Ayurveda perception, selected ingredients mostly contain Tikta (bitter), Kashaya (Astringent), Katu (pungent), Madhura (Sweet) Rasa which pacify Vata, Pitta, Kapha Dosha and nourishes Raktha Dhatu. Madhura Rasa increase all Dhathus (elements) and Ojas. It enhances skin complexion. Furthermore, maximum percentage of ingredients having Ushna virya (Hot in potency) which pacify Vata Dosha and Kapha Dosha but increase Pitta Dosha. Bhrajaka Pitta which is situated in skin, illumines various shades of complexion and supporting and promoting to maintain the healthy skin. All the ingredients in the formula have Thridoshahara, Kapha Pittahara, Kapha Vatahara, Vatashamaka, Varnya, Twachya, Lekhana and Rasayana actions. In addition, Lekhana action gives scraping effect which is important for removing the scar.

5. CONCLUSION

Poly herbal cosmetic formula has been standardized by intervention of modern scientific quality control parameters. Average pH of the formula is safe for the skin and it does not

contain synthetic dyes and adulterants. The formulae exhibit a set of diagnostic characters, which may help in identifying the herbal cosmetic powder and parameters could be used as reference standards for authentication and assessment.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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