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RESEARCH ARTICLE

Pharmacognostical Study and Analytical Standardization of *Bhringaraja Taila*: An *Ayurvedic* Oleaginous Medicine

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Abstract

Background: *Bhringaraja Taila* is an *Ayurvedic* classical formulation. The formulation is indicated as *keshaya*. The present study is structured around validating the fact of preparing the formulation with and without the addition of mineral (*Gairika*). **Aim:** The objectives of this study were to prepare and analyze *Bhringaraja Taila.* **Method:** After performing the *murchanna samsakara*, a specified amount of *Drava, Kalka and Sneha dravyas* were taken and subjected to moderate heating till the watery portion evaporated *Bhringraja Taila* prepared with and without *Gairika*. **Results:** The refractive index of *Bhringraja Taila* with and without the addition of *Gairika* was found to be decreased in both samples. The specific gravity and saponification values of both samples were found to be within the limit but slightly increased in the sample prepared with the addition of *Gairika*. The iodine value of both samples was found to be less than the limit. A decreased iodine value signifies less tendency toward rancidity. The acid value of both samples was found to be within the limit but more in the sample prepared with the addition of *Gairika*. The peroxide value of the sample prepared with the addition of *Gairika* was higher than the sample prepared without *Gairika*. Higher peroxide value indication of rancidity. The pH of the sample prepared with the addition of *Gairika* was found to be acidic and the sample prepared without the addition of *Gairika* was found to be basic. **Conclusion:** Thus considering the above fact it can be concluded that the formulation was prepared without mineral (*Gairika*) can be more stable but the therapeutic efficacy between the two formulation cannot be confirmed until and unless there is clinical validation.

Keywords: Murchanna*, Sneha Kalpana,* Sneha Paka, Standardization

1. Introduction

Sneha Kalpa is an oleaginous medicine that uses a variety of ingredients, including decoction, paste, and liquids[1-3]. Lipid-soluble substances easily penetrate the body's bio-membranes, which is why the *sneha kaplas* can produce a much better beneficial effect in compression to other forms of dosage[4-6]. Hair loss has been an issue that affects people of all races and genders to varying degrees. Premature hair loss has been a tragedy for men and women of all nations and ethnicities for thousands of years[7]. The plant *Bhringraj* (*Eclipta alba*) recognized for its ability to stimulate hair

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growth, is used as the main ingredient of *Bhringraj Taila*, an *Ayurvedic* herbal oil[8]. It is said that the oil can strengthen and nourish hair, stop hair loss, and encourage the development of new, healthy hair[8]. To assess improved quality and therapeutic efficacy, an attempt is made in the current study to make *Bhringaraj Taila* with and without the addition of *Garika*. To eliminate the *Ama* and *Gandha Doshas* of *Taila*, *Taila Murchana Samskara* was done before *Taila Paka*[9].

2. Materials and Methods

The process was carried out in two steps i.e. pharmaceutical study and analytical study.

2.1 Pharmaceutical Study

2.1.1 Collection and Authentication of Raw Materials

The herbal drugs, *Garika* and *Tila taila* were used for the preparation of *Bhringraja taila* purchased from the local market Jalandhar, Punjab on dated 5/10/2012. Authentication of the herbal drug was done from Guru Nanak Dev University, Amritsar, Punjab on dated 12/10/2012 with ref. no. 1065. *Gairika* and *Tila Taila* were authenticated from Ayurlab Herbal Pvt. Ltd, Vadodara, Gujarat on dated 15/02/2013 with sample code-AHPL/13/03, AHPL/13/04.

2.2 Analytical Study

2.2.1 Determination of Foreign Matter

After random sampling, a 500 g sample was obtained and spread out onto a tray. With a magnifying lens, remove any undesired material and determine the proportion of foreign materials[10-20].

2.2.2 Loss on Drying (at 105°C)

Approximately 10 g air dried sample kept in hot air oven (at 105°C for 5 hours) and take weight. The process repeated till the difference between the two progressive readings was not less than 0.25 % then finally computed the percentage of LOD[10-20].

2.2.3 Determination of Total Ash

Weighed 2-3 g of drug sample incinerate at 450°C in a muffle furnace until it is carbon-free. Now calculate the total ash percentage[10-20].

2.2.4 Determination of Acid Insoluble Ash

Boil the obtained total ash with 25 ml of 6N hydrochloric acid for five minutes on a hot plate. Filter and collect the insoluble ash on ash-less filter paper, and wash it 3-4 times with hot water to make it chlorine-free. Incinerate it at 450°C till constant weight is achieved. Calculate the percentage with reference to the air-dried drug[10-20].

2.2.5 Determination of Alcohol-soluble Extractive

About 5 g of coarsely ground drug is macerated with 100 ml of alcohol for 24 hours in a closed flask with frequent shaking for 6 hours and allows the mixture to stand for 18 hours. Filter carefully using Whattman filter paper. To the filtrate, transfer 25 ml of the filtrate in a tarred evaporating dish and evaporate on a water bath till dry. Weighed it and kept it in hot air oven at 60-70 °C for 3 hours to dry it. Weigh the filtrate. Determine the percentage of alcohol-soluble extractive in relation to the air-dried drug[10-20].

2.2.6 Determination of Water-soluble Extractive

The process was the same as substituting alcohol with water in an extractive that was soluble in alcohol[10-20].

2.2.7 pH

pH can be defined as the logarithm of the reciprocal of the concentration of hydrogen ions, expressed in grams per liter. The pH meter must be calibrated before each measurement using two or three buffer solutions or buffer tablets with known pH values mainly pH 4, pH 7, and pH 9.2. Potentiometric method consists use of a glass electrode, a reference electrode, and a pH meter[10-20].

2.2.8 Refractive Index

The relationship between the sine of the angle of incident light and the angle of refraction of a light emission entering a substance from the air is known as the refractive index of drugs relative to air. The wavelength of the light used to measure it affects it[10-20].

2.2.9 Determination of Iodine Value

Take a 20 ml sample in an iodine flask and add carbon tetrachloride (10 ml), and iodine monochloride (20 ml). Keep the prepared mixture in a dark place for

30 minutes now add potassium iodine (15 ml) and water (100 ml). Titrate it against sodium thiosulphate (0.1 N) by taking starch solution as an indicator. Note down the burette reading in terms of the number of ml consumed as (a) repeat the same procedure by omitting the sample for blank reading (b)[21,22].

2.2.10 Determination of Peroxides Value

In a conical flask (250 ml) take a sample (5 g) and add a 30 ml mixture of glacial acetic (3 part) acid and chloroform (2 part). Now add saturated potassium iodide (0.5 ml) and water (30 ml) then titrate it against sodium thiosulphate (0.01M) till the disappearance of yellow colour. Now add indicator 0.5 ml (starch solution) and titrate again till the disappearance of blue colour as reading (a), repeat the same procedure by omitting the sample for blank reading (b)[21,22].

2.2.11 Determination of Saponification Value

In a conical flask (250 ml) take a sample (2 g) and add potassium hydroxide (25 ml) alcoholic solution. Boil the mixture in a water bath by using a reflux condenser for 1 hour. Add 1 ml phenolphthalein indicator (1 ml) after colling and titrate the mixture against hydrochloric acid (0.5 N). Note down the burette reading in terms of the number of ml consumed as (a) repeat the same procedure by omitting the sample for blank reading (b)[21,22].

2.2.12 Determination of Acid Value

In a conical flask (250 ml) take a sample (10 g) and add 50 ml mixture prepared by using alcohol (25 ml) and ether (25 ml). Add phenolphthalein (1 ml) to neutralize the mixture. Now titrate the mixture against potassium hydroxide (0.1 N) till the appearance of pink colour persists for at least 15 seconds. Note down the burette reading in terms of the number of ml consumed for completing the reaction[21,22].

2.2.13 Determination of Specific Gravity

The liquid's sample weight at room temperature is compared to the weight of an equivalent amount of water at the same temperature[10-20].

2.2.14 Microbial Load

The Ayurvedic Pharmacopoeia of India's technique was followed to determine the existence of microbial burden[10-20].

3. Result

The master formula of *Bhringraja Taila is* mentioned in (Table 1)**.** During *Murchana Samsakara* 11.5% loss of *Taila* was seen due to the evaporation of watery content and absorption of *Taila* by *Kalka*. Table 2 describe the *taila paka* observations of days 1, 2, and 3

Table 1. Master formula of *Bhringraja Taila*

Sr. No.	Raw Drug	Quantity	Sr. No.	Raw Drug	Quantity
1	*Bhringraj* (*Pachang*)	3.0721 g	8	*Haridra* (Rhizome)	48 g
2	*Manjistha* (Root)	48 g	9	*Daruharidr* (Stem)	48 g
3	*Padmaka* (Heart Wood)	48 g	10	*Nagkesar* (Stamen)	48 g
4	*Lodhra* (Stem)	48 g	11	*Priyangu* (Flower)	48 g
5	*Chandan* (Heart Wood)	48 g	12	*Mulethi* (Root)	48 g
6	*Bala* (Root)	48 g	13	*Prapau ndarika* (Root)	48 g
7	*Shudh Gairika*	48 g	14	*Svet Sariva* (Root)	48 g
15	*Tila Taila*	768 ml			

Table 2. Observation during the preparation of *Bhringraja taila*

Sr. No.	Day	Heating Time	Status of Oil	Observation	Comments
1	1st	3 hrs	Brownish oil with water	Oil bubbles were floating on the surface of *dravya*	*Bhringaraja taila* was prepared with and without *Gairika* which resulted in the formation of oil bubbles, Varti of kalka was formed on 2nd day and the bubble was produced without any sound
2	2nd	3 hrs	Oil boils with sound	*Varti* of *kalka dravya* was formed	
3	3rd	3 hrs	Oil boils without sound	Oil test +ve and *Kalka* test +ve mentioned for *sidha sneha paka*	

including the status of oil. Preliminary phytochemicals analysis of prepared *Bhringraja taila* was carried out and observations are shown in Table 3. Colour brownish, odour characteristics, consistency of oily and texture of oily liquid were observed in prepared *Bhringraja taila* (Table 4). Physicochemical properties such as Loss on Drying (LOD), Total Ash (TA), Acid Insoluble Ash (AIA), and Water-Soluble Extractive (WSE) and Alcohol Soluble Extractive (ASE) of herbal drugs are carried out, and compression is done with the values mentioned in API. (Table 5a- Table 5e) physico-chemical properties such as Refractive Index (RI), specific gravity (Sp.gr.), Iodine Value (IV), and Acid Value (AV) of *Tila taila* are mentioned in (Table 6). Physico-chemical results of *Bhringraja taila* prepared with and without *Gairika* include parameters such as refractive index, specific gravity, iodine value and acid value as per *Ayurvedic* protocols (Table 7). Microbial analysis of total bacterial count, *E. coli, Staphylococcus* spp., *S. aureus,* and *P. aeruginosa* were observed and found absent in prepared samples (Table 8).

4. Conclusion

Sneha Kalpana is the preparation in which both oil-soluble and water-soluble active principles are extracted. The refractive index of both prepared formulations was found to be decreased and the specific gravity of both prepared samples lies within the limit. Not much significant changes were observed in the saponification value of both prepared samples but it was significantly increased in the sample prepared with the addition of *Gairika*. The iodine value of both samples was found to be less than the standard limit. The acid value of both samples was found to be within the limit. The peroxide value of *Bhringraja Taila* with *Gairika* was found to be above the limit and *Bhringraja taila* without *Gairika* was found within the limit. A high peroxide value indicates a rancid fat. pH of sample prepared with addition of *Gairika* found to be acidic while in sample prepared without addition of *Gairika* found to be basic in nature.

Table 3. Primarily phytochemical screening

Sr. No.	Test Name	Constituents	Finding
1	Ferric chloride test	Tannins	+
	Lead acetate test		+
	Bromine water		+
2	Borntrager's test	Glycoside	-
	Liebermann's Test		-
	Keller-Kiliani Test		-
	Salkowski's Test		-
3	Foam test	Saponin	+
4	Millon's test	Protein	+
	Biuret Reagent test		+
	Ninhydrin Test		+
5	Benedict's solution test	Carbohydrates	+
	Fehling's test		+
	Molisch's test		+
6	Mayer's reagent	Alkaloids	+
	Dragendroff reagent		+
	Wagner's reagent		+
	Hager's reagent		+
7	Liebermann Burchard's reaction	Steroids	+
	Salkowski test		+
8	Ferric Chloride Test	Phenols	+
	Liebermann's nitroso reaction		+
	Lead Acetate test		+
	Gelatin test		+
9	Alkaline reagent test	Flavonoids	+
	Zinc hydrochloride test		+

***Positive (+) Negative (-)**

Table 4. Organoleptic property of *Bhringraja taila*

Sr. No.	Property	With *Gairika*	Without *Gairika*	Comments
1	Colour	Brownish	Greenish	*Bhringaraja taila* prepared without *Gairika* showed greenish colour but the *taila* prepared with *Gairika* showed brownish colour. Apart for that all other organoleptic property are same.
2	Odour	Characteristics	Characteristics	
3	Appearance	Oily	Oily	
4	Consistency	Oily Liquid	Oily Liquid	

Table 5a. Observations of herbal drug physicochemical properties

Sr. No.	Parameter	Standard (API)	*Bhringraja* (Whole Plant)	Standard (API)	*Haridra* (rhizome)	Standard (API)	*Lodhra* (st. bk)	Standard (API)	*Svet sariva* (root)
1	FM	NMT 2%	1.03%w/w	NMT 2%	1.05%w/w	Nil	0.45%w/w	NMT 2%	1.22%w/w
2	LOD (105°C)	Not mentioned	6.6% w/w	Not mentioned	7.2% w/w	Not mentioned	5.5% w/w	Not mentioned	8% w/v
3	TA	NMT 22%	11.3%w/w	NMT 9%	4.5%w/w	NMT 12%	1.3%w/w	NMT 4%	2% w/w
4	AIA	NMT 11%	1.5% w/w	NMT 1%	0.5% w/w	NMT 1%	0.5% w/w	NMT0.5%	0.5% w/w
5	ASE	NLT 5%	13.8%w/v	NLT 8%	11.7%w/v	NLT 9%	15.2%w/v	NLT 15%	23.7%w/v
6	WSE	NLT 15%	12.8%w/v	NLT 12%	18.9%w/v	NLT 15%	24% w/v	NLT 13%	16.2%w/v

*FM- Foreign Matter, TA – Total Ash, LOD – Loss on Drying, AIA – Acid Insoluble Ash, ASE – Alcohol Soluble Extractive, WSE – Water Soluble Ash, NMT – Not More Then, NLT – Not Less Then

Table 5b. Observations of herbal drug physicochemical properties

Sr. No.	Parameter	Standard (API)	*Daruharidra* (stem)	Standard (API)	*Nagkesar* (stamen)	Standard (API)	*Manjistha* (stem)	Standard (API)	*Padmakh* (heart wood)
1	FM	NMT 2%	0.9% w/w	NMT 2%	0.8% w/w	NMT 2%	1.2% w/w	NMT 1%	0.9% w/w
2	LOD (105°C)	Not mentioned	9.2% w/w	Not mentioned	12.8%w/w	Not mentioned	10.2%w/w	Not mentioned	3.3% w/w
3	TA	NMT 14%	2.8% w/w	NMT 6%	2.2% w/w	NMT 12%	3.3% w/w	NMT 1%	0.5% w/w
4	AIA	NMT 5%	1.5% w/w	NMT 3%	1% w/w	NMT 0.5%	0.5% w/w	NMT 0.5%	0.0% w/w
5	ASE	NLT 6%	12.8%w/v	NLT 15%	18.1% w/v	NLT 3%	11.3% w/v	NLT 3%	10.6%w/v
6	WSE	NLT 8%	10.6%w/v	NLT 12%	18.7% w/v	NLT 17%	24.8% w/v	NLT 1%	12% w/v

Table 5c. Observations of herbal drug physicochemical properties

Sr. No.	Para meter	Standard (API)	*Rakatchandan (Heart Wood)*	Standard (API)	*Priyangu* (fruit)	Standard (API)	*Parpundrika* (root)	Standard (API)	*Bala (root)*
1	FM	NMT 2%	0.9%w/w	NMT 2%	0.9% w/w	Not mentioned	3.3% w/w	NMT 2%	1.2% w/w
2	LOD (105°C)	Not mentioned	9.5%w/w	Not mentioned	9.3% w/w	Not mentioned	8.4% w/w	Not mentioned	6.2% w/w
3	TA	NMT 2%	1.5%w/w	NMT 6.5%	3.5% w/w	NMT 10%	2% w/w	NMT 8%	2% w/w
4	AIA	NMT 0.3%	0% w/w	NMT 1%	1% w/w	NMT 2.5%	1% w/w	NMT 3%	1% w/w
5	ASE	NLT 1%	9.5%w/v	NLT 3%	12.6%w/v	NLT 10%	16.8%w/v	NLT 3%	9.1% w/v
6	WSE	NLT 1%	13% w/v	23.2%w/v	22.4%w/v	NLT 20%	26.9%w/v	NLT 9%	17.8%w/v

Table 5d. Observations of herbal drug physicochemical properties

Sr. No.	Para meter	Standard (API)	*Amalaki (Fruit Pulp)*	Standard (API)	*Ketaki (root)*	Standard (API)	*Nayagrodha (st.bk)*	Standard (API)	*Haritaki (Fruit Pulp)*
1	FM	NMT 3%	1.5% w/w	NMT 2%	1.3% w/w	NMT 2%	1.1% w/w	NMT 1%	0.5%w/w
2	LOD (105°C)	Not mentioned	8.2% w/w	Not mentioned	5.3% w/w	Not mentioned	3.3% w/w	Not mentioned	6.6% w/w
3	TA	NMT 7%	3.3% w/w	NMT 11%	3.6% w/w	NMT 8%	3.1% w/w	NMT 5%	1.3% w/w
4	AIA	NMT 2%	1% w/w	NMT 2%	1.5% w/w	NMT 3%	1.3% w/w	NMT 5%	1% w/w
5	ASE	NLT 40%	44.4%w/v	NLT 9%	12.8%w/v	NLT 6%	12% w/v	NLT 40%	47.2%w/v
6	WSE	NLT 50%	56.8%w/v	NLT 16%	22.3%w/v	NLT 8%	20.7%w/v	NLT 60%	65.1%w/v

Table 5e. Observations of herbal drug physicochemical properties

S. No.	Parameter	Standard (API)	*Bhibhtaki (Pulp)*	Standard (API)	*Musta (rhizome)*	Standard (API)	*Tvak (st.bk)*
1	FM	NMT 2%	0.8% w/w	NMT 2%	1.3% w/w	NMT 2%	1% w/w
2	LOD (105°C)	Not mentioned	6% w/w	Not mentioned	3.4% w/w	Not mentioned	2.4% w/w
3	TA	NMT 7%	1.3% w/w	NMT 8%	2.3% w/w	NMT 3%	1.5% w/w
4	AIA	NMT 1%	0.8% w/w	NMT 4%	1.3% w/w	NMT 2%	0.5% w/w
5	ASE	NLT 8%	12.2%w/v	NLT 5%	16.8%w/v	NLT 2%	10.8%w/v
6	WSE	NLT35%	41% w/v	NLT 11%	23.3%w/v	NLT 3%	15% w/v

Table 6. Observations of *Tila taila* physico-chemical properties

S. No.	Parameters	Result
1	RI	1.4652
2	Sp.gr.	0.9169
3	Sap. V	196.3
4	IV	81.21
5	AV	1.4
6	PV	9
7	pH	4.25

Table 7. Physico-chemical result of *Bhringraja taila* prepared with and without *Gairika*

Sr. No.	Para meters	Standard Value (API)	With *Gairika*	Without *Gairika*
1	RI	1.451 to 1.464	1.301	1.312
2	Sp.gr.	0.910 to 0.932	0.926	0.924
3	Sap. V	188 to 194	193	189
4	IV	90 to 100	76.14	65.98
5	AV	3 to 6	4.768	3.927
6	PV	NMT 6	5.5	5
7	pH	Not mentioned	4.37	9.18

Table 8. Microbial load

Microbial Analysis	Total Bacterial Count	Total Yeast and Mould	*Escherichia coli*	*Staphylococcus* spp.	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Limit mentioned in API	NMT 10^5 CFU/ml	NMT 10^3 CFU/ml	-	-	-	-
Observed values	49000 CFU/ml	350 CFU/ml	-	-	-	-

Absent(-)

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