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A COMPARATIVE PHARMACEUTICO ANALYTICAL STUDY OF BHAGANDHARA-HARA LEPA WITH ITS MODIFIED FORM AS A HYDROGEL

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ABSTRACT

Objective of the study: To the develop standard operative procedures of *Bhagandharahara lepa* and change the form of *Bhagandharahara lepa* into the form of the Hydrogel. To comparatively analysis *Bhagandharahara lepa* and its pharmaceutically modified form of Hydrogel with classical and advanced analytical techniques. **Methods: Pharmaceutical study:** *Bhagandharahara lepa* and its modified form as a Hydrogel is prepared with standard household parameters. **Analytical study:** Comparative analysis of both *Bhagandharahara lepa* and its modified form as a Hydrogel will be comparatively analysed with suitable physicochemical parameters and advanced instrumental methods of analysis. **Results:** In Stability evaluation of BHL, classically prepared *lepa* got decomposed next day with foul smell and become darker mustard field in colour, so *lepa* (wet form) stability remains within 24 hrs. and here physical and chemical stability of dried form of *lepa churna* is good throughout 3 months of period and in Stability evaluation of BHG prepared hydrogel Light Ochre in colour with easily spreading and physicochemical parameters also like homogeneity and extrudability, viscosity, pH, irritancy test, melting point within limit there is no any major changes throughout the stability period 3 months. So *Bhagandharahara* Hydrogel is almost remained

stable till 3 months. C**onclusions:** As *lepa* is having shorter shelf life, it cannot be preserved to longer time, so in this study its modification to Hydrogel form facilitates a longer shelf life. Due to some special features of Hydrogel like extrudability, spreadability, viscosity, pH, homogeneity it is easy to apply to the target site.

Keywords: Bhagandhara, Lepa Kalpana, Hydrogel.

INTRODUCTION

Ayurveda with its specified branch of *Shalya Tantra* has found success in treating the *Bhagandhara* with various treatment like application of *kshara, varti, taila* etc.[1]

The present study of *Bhagandharahara lepa*[2,3] is selected. As application of *lepa* in the cases of *Bhagandhara* many a times may not be convenient especially in case of long track fistulae or multiple track fistulae, an effort is planned to modify the *lepa* into a Hydrogel form to make it easier and convenient for the surgeon to apply the gel into the communicating tract of fistula in ano with ease.

Herbal drugs were not used precisely in modern pharmacy due to their lengthy process and various procedure, recently a trend of modifying such herbal formulation to pharmaceutically modified form such as cream, gel, ointment etc. have been started.

These pharmaceutically modified forms of herbal formulations have increased their market value and ease in application also enhanced their therapeutic efficacy, shelf life and acceptability. Considering the above fact, the present study is framed.

Aim and Objectives

1. To develop a standard method of preparation of *Bhagandharahara lepa.*
2. To convert the *Bhagandharhara lepa* into a Hydrogel form.
3. To comparatively analyse both *Bhagandharhara lepa* and its pharmaceutically modified form of a Hydrogel with classical and advanced analytical techniques.

Materials and Method

The reference of *Bhagandharahara lepa* is from *Sahasrayogam*. It is poly herbal formulation in the form of *lepa*, here *Haridra, Haritaki, Nimba patra, Arka moola, Saindhava lavana* and *Takra* 6 ingredients are the described. All the 5 drugs (*Haridra*[4], *Haritaki*[5], *Nimbapatra*[6], *Saindhava lavana*[7], *Arkaroola*[8]) 1 part each and *takra*[9] 10 parts (q.s) were used for *lepa* preparation.

Method of preparation of *lepa*

All the drugs mentioned in classical reference is collected. The drugs are individually powdered and sieved to get a micro fine powder and *mardana* is done with *Takra* and *lepa* is obtained. This prepared *lepa* is divided into two parts, one part of *lepa* is stored in a glass air tight container and another part of prepared *lepa* is placed in a steel tray for dry procedure with the help of vacuum oven drier finally dried form of *lepa* is obtained and this dried form of *lepa* is collected from steel tray and triturated well with the help of Mortar and pestle finally *lepa churna* is obtained micro fine form and stored in a glass air tight container.

[Here as we know wet form of *lepa* is having short shelf life, this prepared *lepa* were divided into 2 parts for further subjected analysis purpose.]

Method of preparation of Hydrogel

It consists of 3 steps.

Step 1- Preparation of *Lepa churna* (To dried form)

Step 2-Preparation of Drug extract water

Step 3- Preparation of Hydrogel[10]

STEP 1: Preparation of *Bhagandharahara Lepa churna*

All the drug mentioned in the preparation of *bhagandharahara lepa* are individually powdered and mixed well with buttermilk & vacuum dried.

STEP 2: Preparation of Drug extract water

The dried form of *Bhagandharahara lepa churna* is macerated with the distilled water in the ratio of 1:10 in a closed conical flask for twenty-four hours, shaking frequently during six hours and kept stagnant for eighteen hours. After eighteen hours the liquid is filtered using the filter paper and drug extract water is obtained.

STEP 3: Preparation of Hydrogel

Quantity sufficient Drug extract water is taken in a beaker and at the same time, weighed quantity of Sodium benzoate[11] 0.5% ($C_7H_5O_2Na$) is added and mixed with drug extract water.

Weighed quantity of Carbomer[12]940 5% w/v is dissolved with drug extract mixture & kept for 24 hours.

After 24 hours weighed quantity of PEG 400 2% (Polyethylene glycol[13]) was taken in another beaker and added to the above mixture, mixed well by continuous stirring (with mortar & pestle).

Quantity sufficient of Triethanolamine[14] solution was taken in another beaker and added to the above obtained mixture drop by drop for the pH adjustment and mixed well with the help of mortar & pestle and hand blender, Finally *Bhanghandharahara* Hydrogel is obtained and stored in a glass air tight container.

Results

1. Raw Material Standardization:

Table 1: Showing Physico-chemical evaluation of raw materials

Drug name	Total ash	Water soluble extractive	Alcohol soluble Extractive	Acid insoluble ash	Loss on drying	pH
Nisa (Haridra)	6.95%	12.96%	9.20%	0.95%	6.82%	-
Pathya (Haritaki)	4.05%	82.64%	41.74%	0.70%	4.14%	-
Nimba patra	9.95%	27.00%	25.36%	3.90%	4.98%	-
Arka moola	3.60%	7.84%	4.56%	1.00%	10.34%	-
Saindhava lavana	0.5%	-	-	1%	0.1%	8.90
Takra	-	-	-	-	-	4.32

Table 2: Showing Phytochemical evaluation of raw materials in water extract and methanol extract

Drug name	Alkaloids		Tannins		Amino acids		Sugars		Glycosides		Phenolic compound		Flavonoids		Proteins		Saponins	
	Water extract	methanol extract	water extract	methanol extract	water extract	methanol extract	water extract	methanol-extract	water extract	methanol extract	water extract	methanol extract	water extract	methanol extract	water extract	methanol extract	water extract	methanol extract
Nisa (Harid)	+	+	+	+	-	-	+	+	+	+	-	+	-	+	+	+	-	-
Pathya (Haritak)	-	-	+	+	-	-	+	+	+	+	+	+	-	-	+	+	-	-
Nimba patra	-	-	+	+	-	-	+	-	+	-	+	+	-	-	+	+	+	+
Arka moola	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	-

2. Finished Product Standardization: (A) *Bhagandharahara Lepa*:

Table 3: Organoleptic evaluation of *Bhagandharahara lepa* (wet form)

BATCH	Time (day)	Evaluation parameters			
		Colour	Odour	Texture	Consistency
BHL	0	Mustard field	Smell of *Nimba*	Smooth	Paste
	1	Dark Mustard field	Foul Smell	Smooth	Paste

Table 4: Showing Organoleptic evaluation of *Bhagandharahara lepa churna* (dried form)

BATCH	Time (day)	Evaluation parameters			
		Colour	Odour	Texture	Consistency
BHL	0	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	15	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	30	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	45	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	60	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	75	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	90	Mustard field	Smell of *Nimba*	Smooth	Fine powder

Table 5: Showing the table physico-chemical evaluation of *Bhagandharahara Lepa* (wet form)

Parameter	BHL	
Time (Day)	0	1
P^H (10% aqueous extract)	4.68	4.85

Table 6: Showing Physico-chemical evaluation of *Bhagandharahara lepa churna* (dried form)

Parameter	BHL						
Time (Day)	0	15	30	45	60	75	90
Loss on drying (%)	4.7	4.58	4.67	4.91	4.85	4.88	4.83
Total ash (%)	17.35	17.49	16.75	17.70	17.25	16.85	17.05
Acid insoluble ash (%)	2.14	2.06	2.18	2.06	2.06	2.04	2.26
Alcohol-soluble extractive (%)	29.00	28.90	30.05	29.88	29.58	29.06	29.33
Water soluble extractive (%)	`64.88	65.4	64.70	64.59	64.80	64.58	64.70
P^H (10% aqueous extract)	4.62	4.64	4.65	4.60	4.68	4.50	4.83
Particle size	Micro fine	Micro fine	Micro fine	Micro fine	Micro fine	Micro fine	Micro fine

Table 7: Showing Phyto chemical evaluation of *Bhagandharahara lepa churna* in water soluble extractive and Methanol soluble Extractive

Parameter	BHL water soluble extractive							BHL Methanol soluble Extractive						
Time (Day)	0	15	30	45	60	75	90	0	15	30	45	60	75	90
Alkaloids (Mayer's test)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+	+	+	+	+	+	+
Sugars (Fehling's solution test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides (Keller kiliyani test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenolic compound	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids (Shinoda test)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Nin-Hydrin test (Amino acids)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
proteins (Biuret test)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+	+	+	+	+	+	+
Tannins (Ferric chloride test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

HPTLC Determination

1) **Standard curcumin**: Rf 0.24 AUC: 40490.1
2) **BHL**: **Rf** 0.26 AUC: 13127.8

Table 8: Showing HPTLC determination of *Bhagandharahara lepa churna* Quantification of *Curcumin* in sample

Sample	Percentage content of Curcumin
BHL	6.28 %

Stability Test: (Physical and Chemical Stability)
The stability study was performed as per ICH guidelines and formulation were stored under room temperature stability conditions (Temperature 25°C ± 2°C, Humidity 65% ± 5% RH) were carried for a period of 3 months. The physical and chemical analysis was assessed periodically at 15 days interval for a period of 3 months.

Table 9: Showing Physico Organoleptic Stability evaluation of *Bhagandharahara lepa* (wet form)

BATCH	Time (day)	Evaluation parameters			
		Colour	Odour	Texture	Consistency
BHL	0	Mustard field	Smell of Nimba	Smooth	Paste
	1	Dark Mustard field	Foul Smell	Smooth	Paste

Table 10: Showing Physico-chemical stability evaluation of *Bhagandharahara Lepa* (wet form)

Parameter	BHL	
Time (Day)	0	1
P^H (10% aqueous extract)	4.68	4.85

Table 11: Showing Physico-organoleptic Stability evaluation of *Bhagandharahara lepa churna* (dried form)

BATCH	Time (day)	Evaluation parameters			
		Colour	Odour	Texture	Consistency
BHL	0	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	15	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	30	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	45	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	60	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	75	Mustard field	Smell of *Nimba*	Smooth	Fine powder
	90	Mustard field	Smell of *Nimba*	Smooth	Fine powder

Table 12: Showing Physico-chemical Stability evaluation of *Bhagandharahara lepa churna* (dried form)

Parameter	BHL						
Time (Day)	0	15	30	45	60	75	90
Loss on drying (%)	4.7	4.58	4.67	4.91	4.85	4.88	4.83
Total ash (%)	17.35	17.39	18.16	17.88	17.41	18.18	18.11
Acid insoluble ash (%)	2.04	2.06	2.18	2.14	2.56	2.04	2.26
Alcohol-soluble extractive (%)	29	28.90	30.05	29.88	29.58	29.06	29.33
Water soluble extractive (%)	64.88	65.4	64.70	64.59	64.80	64.58	64.70
pH (10% aqueous extract)	4.62	4.64	4.65	4.60	4.68	4.50	4.83

***Bhagandharahara* Hydrogel:**

Table 13: Showing Organoleptic evaluation of *Bhagandharahara* Hydrogel

Sample	Time (Day)	Colour	Odour	Texture	Consistency	Spreadability
BHG	0	Light Ochre (colour code 7926)	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	15	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	30	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	45	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	60	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	75	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	90	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading

Table 14: Showing Homogeneity evaluation of *Bhagandharahara* Hydrogel

Parameter	BHG						
Time (Day)	0	15	30	45	60	75	90
Homogeneity test	++	+	+	+	+	+	+

++= very good, += good

Table 15: Showing the pH evaluation of *Bhagandharahara* Hydrogel.

Parameter	BHG						
Time (Day)	0	15	30	45	60	75	90
P^H (10% aqueous extract	4.90	4.63	4.46	4.41	4.41	4.29	4.29

Table 16: Showing Viscosity evaluation of *Bhagandharahara* Hydrogel

Parameter	BHG						
Time (Day)	0	15	30	45	60	75	90
Viscosity (cp)	>30, 650	>30, 650	>30, 650	>30, 650	>30, 650	>30, 650	>30, 650

Table 17: Showing Spreadability evaluation of *Bhagandharahara* Hydrogel

Parameter	BHG						
Time (Day)	0	15	30	45	60	75	90
Spreadability (g.cm/sec)	5.00	4.62	4.10	3.40	3.40	3.40	2.88

Table 18: Showing Extrudability evaluation of *Bhagandharahara* Hydrogel

Parameter	BHG						
Time (Day)	0	15	30	45	60	75	90
Extrudability (g.cm/sec)	16.66	16.66	16.66	20.00	20.00	20.66	20.66

Table 19: Showing Irritancy test evaluation of *Bhagandharahara* Hydrogel

Parameter	BHG						
Time (Day)	0	15	30	45	60	75	90
Irritancy test	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant

Table 20: Showing solubility test evaluation of *Bhagandharahara* Hydrogel

Solubilizing agent	BHG
Methanol	Poorly soluble
Ethanol	Poorly soluble
Toluene	Not soluble
Water	Less soluble
Hexane	Not soluble
Chloroform	Not soluble

Sterility Test

1. Total Aerobic Microbial Count

(a) Plate count:

For bacteria.

Table 21: showing the table of total aerobic microbial count plate count for bacteria

Sample	No. of colonies in stock solution	No. of colonies in 1st dilution
BHG	5	1

For bacteria.

Table 22: showing the table of total aerobic microbial count plate count for fungi

Sample	No. of colonies in stock solution	No. of colonies in 1st dilution
BHG	5	1

(b) Multiple-tube or serial dilution method:

Turbidity or growth in the samples (BHG) test tubes was not observed after incubation period. The positive control showed growth and negative control did not show any growth.

Samples BHG passed the test having less number of organism than the cut-off value set by pharmacopoeia.

2. Tests for Specified Micro-Organisms

Table 23: showing the table of tests for specified micro-organisms

Bacteria	Agar medium	Sample No.	Observation
E. coli	MacConkey	BHG	No colour change was observed and there were no colonies
P. aeruginosa	Cetrimide	BHG	No greenish colonies were observed.
S. aureus	Mannitol salt	BHG	No change in colour

Samples BHG did not show the presence of *E. coli, P. aerouginosa* and *S. aureus*.

Melting Point Test

Table 24: Showing Melting Point test evaluation of *Bhagandharahara* Hydrogel

Parameter	BHG						
Time (Day)	0	15	30	45	60	75	90
Melting Point (^{O}C)	108	108	107	107	108	109	110

HPTLC Determination:

Result:

1) **Standard curcumin: Rf** 0.36 **AUC**: 49777.0
2) **BHG: Rf** 0.00 AUC: 00

Table 25: Showing HPTLC determination of *Bhagandharahara* hydrogel Quantification of *Curcumin* in sample

Sample	Percentage content of *Curcumin*
BHG	0 %

Stability Test[15]: (Physical and Chemical Stability)

The stability study was performed as per ICH guidelines and formulation were stored under room temperature stability conditions (Temperature 25°C ± 2°C, Humidity 65% ± 5% RH) were carried for a period of 3 months. The physical and chemical analysis was assessed periodically at 15 days interval for a period of 3 months.

Table 26: Showing Physico Organoleptic Stability evaluation of *Bhagandharahara* hydrogel

Sample	Time (Day)	Colour	Odour	Texture	Consistency	Spread ability
BHG	0	Light Ochre (colour code 7926)	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	15	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	30	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	45	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	60	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	75	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading
	90	brownish yellow	Characteristics pungent odour	Smooth	Porosity and soft	Easily spreading

* The colour of the hydrogel gradually changed over a period of stability studies from Light Ochre to dark brownish yellow *

Table 27: Showing Physico-chemical stability evaluation of *Bhagandharahara* Hydrogel

Parameter	BHG						
Time (Day)	0	15	30	45	60	75	90
Homogeneity test	++	+	+	+	+	+	+
P^H (10% aqueous extract)	4.90	4.63	4.46	4.41	4.41	4.29	4.29
Viscosity test (cp)	>30, 650	>30, 650	>30, 650	>30, 650	>30, 650	>30, 650	>30, 650
Spreadability (g.cm/sec)	5.00	4.62	4.10	3.40	3.40	3.40	2.88
Extrudability (g/cm^2)	16.66	16.66	16.66	20.00	20.00	20.66	20.66
Irritability	Non irritant	Non irritant	Non irritant	Non irritant	Non irritant	Non irritant	Non irritant
Melting point (°C)	108	108	107	107	108	109	110

Fig 1: *Bhagandharahara Lepa* Ingredients

Fig 2: *Bhagandharahara* Hydrogel Ingredients

Fig 3: Final product of *Bhagandharahara Lepa* and *Bhagandharahara* Hydrogel

DISCUSSION

Advances in ayurvedic pharmaceutics is just an old *ghrita* in new bottle the basic principle is same as advice by acharya in *Ayurveda* classical books, we can change the only way of presentation by using modern technologies.

In *ayurvedic* clinical practice, practitioner faces difficulties during prescribe *Ayurveda* dosage form due to its appearance, non-palatable, non-portability. Because of these demerits it's a great challenge in front of *Ayurveda* pharmaceutics to improve Ayurveda dosage form which will be easily palatable, long self-life simplify to dispense easily portable and good appearance with increasing therapeutic, utility, potency and increase market value. Hence an effort was made to present study is aimed to prepare *Bhagandharahara lepa* with its modified form as a Hydrogel with pharmaceutical and analytical approach.

In HPTLC Analysis of *Bhagandharahara* Hydrogel sample, *curcumin* was not detected. It was found that *lepa churna* samples is having good content of *curcumin* and *Bhagandharahara* Hydrogel having no curcumin content, it may be because *Haridra* is not dissolved in aqueous extract. Outcome of HPTLC here indicate that hydrogel doesn't contain curcumin due to its insolubility in water but is seen in *lepa* as the whole drug is to be used.

CONCLUSION

The drugs used were reviewed and it was found that having several pharmacology actions as anti-inflammatory, Antiallergic, Antifungal, wound healing activity, excellent cleansing property and helpful in treating *Bhagandhara* disease.

As *lepa* is having shorter shelf life, it cannot be preserved to longer time, so on this study its modification to hydrogel form facilitates a longer shelf life.

Due to some special features of prepared *Bhagandharahara* Hydrogel like extrudability, spreadability, viscosity it is easy to apply to the target site.

With regards to the pharmaceutical and dermatological character which includes better stability and skin friendly nature. It looks that modification of *lepa* into Hydrogel form is a better choice. If preservative is used self-life further can be extended, but pharmacological and clinical intervention are essential to establish the efficacy of Hydrogel as it doesn't contain curcumin.

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