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RESEARCH ARTICLE

Pharmaceutical Standardization of a Novel Anti Leukemic Ayurvedic Herbomineral Formulation

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ABSTRACT

The aim of this pharmaceutical study was to develop standard manufacturing process of Leukchem 14, a novel herbo-mineral formulation, which was designed for the treatment of Leukemia. The drug consists in specific proportions of dried powders of *Ashwagandha* (Withania somnifera Dunal.) root, *Bilwa* (*Aegle marmelos* Carr.) fruit pulp, *Guduchi* (*Tinospora cordifolia* (Willd) Miers.) stem, *Haridra* (*Curcuma longa* Linn.) rhizome, *Kanchanar* (*Bauhinia variegata* Blume) stem bark and *Triphala* and mineral drugs viz. *Samaguna Kajjali* (black sulphide of purified Mercury) and *Shuddha Manahshila* (purified Realgar). By adopting the principles of *Kharaliya Rasayana*, the homogenous mixture was prepared with these drugs, which was further levigated with fresh cow urine and decoction of *Manjishtha* (*Rubia cordifolia* Linn.) root respectively each three times in three batches. During the procedures of *Shodhana* and *Bhavana*, there were various physicochemical changes were observed. In first step, 317.82, 319.25, and 318.0 g of weight with the *Bhavana* of *Gomutra* was obtained from 306.50 g of basic homogenous mixture of herbal *Churna, Kajjali* and *Shuddha Manahshila* in I, II, and III batches respectively. In second step, 244.79, 241.72, and 243.00 g of Leukchem 14 with the *Bhavana* of *Manjishtha Kwatha* was obtained from 200 g of *Gomutra Bhavita* materials in I, II, and III batches respectively. The percentage increase in weight was observed after levigation with both the media progressively, 3.87 % by cow urine and 21.59% by *Manjishtha Kwatha*. At the end of Pharmaceutical study, dark brown coffee coloured powder was obtained.

Key words: Herbomineral formulation, Kharaliya Rasayana, Leukchem 14, Leukemia

1. INTRODUCTION

Ayurvedic Pharmaceutics is that branch of Ayurveda in which herbal, mineral / metal and animal origin substances are processed either together or individually for the formation of medicament, which are having high therapeutic potency [1]. Various dosage forms of these medicated compounds have been practiced to treat various diseases by adopting multiple treatment strategies since thousands of years back. Ayurvedic doctrine of treatment is based on *Hetu* (etiological factor), *Linga* (symptom / manifestation), and *Aushadh* (medicine) [2] and all these stand for the purpose to understand *Dosha-Dushaya Samurchana* (etiology and pathology of disease) and *Samprapti Vighattan* (infringement of pathology of disease to get healthy state of functions). Ayurvedic practice of medicine aimed the restoration of equilibrium of *Doshik* level of the body through *Vyadhipratinika Chikitsa* and maintains their symmetry for long period via *Rasayana Chikitsa*.

In this study, the novel drug Leukchem 14 was design for the treatment of leukemia, which is a type of *Kharaliya Rasayana* (herbomineral formulation prepared by triturating in Stony mortar and pestle with or without levigation with liquid media either decoction or fresh extracted juices, or animal's by products) **(Table 1)**.

Table 1: Showing compositions of herbomineral formulation Leukchem 14

Name of herbal drug	Botanical name	Part use	Proportion
Ashwagandha	*Withania somnifera* Dunal.	Root	1 part
Bilwa	*Aegle marmelos* Carr.	Fruit Pulp	1 part

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Guduchi	*Tinospora cordifolia* (Willd.) Miers.	Stem	1 part
Haridra	*Curcuma longa* Linn.	Rhizome	1 part
Kanchanar	*Bauhinia variegata* Blume	Stem Bark	1 part
Haritaki	*Terminalia chebula* Retz.	Dried Fruit Pericarp	1/3 part
Bibhitaka	*Terminalia belerica* Roxb.	Dried Fruit Pericarp	1/3 part
Amalaki	*Emblica officinalis* Gaertn.	Dried Fruit Pericarp	1/3 part
Mineral drug	**English name**	***Shodhana Dravya***	**Proportion**
Shuddha Gandhaka	Sulphur (S)	Cow milk and ghee	1/16 part
Shuddha Parada	Mercury (Hg)	Lime powder, Garlic and *Saindava Lavana* (rock salt)	1/16 part
Shuddha Manahshila	Realgar (Arsenic sulphide; As4S4)	*Ardraka* (Ginger juice)	1/100 part
Bhavana Dravya			
Gomutra	Urine of *Bos indicus*- Cow	Urine	Three times
Manjishtha Kwatha	Decoction of *Rubia cordifolia* Linn.	Root	Three times

These drugs are described in the Ayurvedic Pharmacopoeia of India (API) and used as one of ingredient in various formulations with different dosage form described in the Ayurvedic formulary of India (AFI) [3, 4]. Most of the drugs have *Rasayana* property. Section 3 (*h*) of Drug & Cosmetic (D & C) Act 1940 [5], under the clause of I (B) of Rule 158-B of D & C Rule 1945 [6], allow us to develop a new formulation with the ingredient (s) mentioned in the formulae described in the authoritative books of the Ayurveda specified in the first schedule of D & C Act 1940. All the ingredients used in the preparation of Leukchem 14 have been selected based on their proof of effectiveness in the treatment of various types of cancer specially in leukemia and associated symptoms in vitro, in vivo, and clinical trials [7, 8, 9]. In preparation of any dosage form, a great many varieties of factors are there related to raw drug selection, processing, formulation-preparation, use of purification processes, and use of adjuvant have been observed to have strong influence on the expression of pharmacological activity thus possibly on therapeutic effect. Thus, it will maintain safety, efficacy, and quality, which not only help to preserve this system heritage but also to rationalize the use of natural products in healthcare. With this prime aim of this pharmaceutical study was planned to develop standard manufacturing process of Leukchem 14, a novel herbo-mineral formulation.

2. MATERIALS AND METHODS

2.1. Procurement and authentication of Raw materials:

Major ingredients:

Raw mineral drugs *Parada, Gandhaka* and *Manahshila*, were procured from genuine supplier of Varanasi and characterized and authenticated as per their accepted characters (*Grahya Lakshan)* specified in the authoritative texts of Rasa Shastra. Raw herbal drugs *Ashwagandha*, *Bilwa*, *Guduchi*, *Haridra*, *Kanchanar*, ingredients of *Triphala* (*Amalaki*, *Haritaki*, and *Vibhataki*), and *Manjishtha* with their respective used part were collected from the local Gola Deena Nath market of Varanasi and authenticated by expert of the department of *Dravya Guna*, faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University. *Gomutra* was freshly collected from the Cowshed under the Institute of Agriculture Science of Banaras Hindu University.

Associate ingredients:

The drugs, which were considered as associate drugs, used as *Shodhana* media. *Sudha Raja* (powder of limestone), *Lashuna* (garlic bulb), and *Saindava Lavana* (rock salt), were used for the *Shodhana* of *Parada*, *Ksheera* (Cow's milk) and ghee (clarified butter) for the *Shodhana* of *Gandaka* and *Ardraka* (ginger) for the *Shodhana* of *Manahshila*, were also collected from the local market of Varanasi.

2.2. Pharmaceutical processing:

The steps involved in the pharmaceutical processing of Leukchem 14 were as follows:

Table 2: Showing brief of pharmaceutical processing of Leukchem 14

S No	Steps	Reference	Method	Equipments & accessories
1	*Shodhana* of *Parada*[10]	Rasa Tarangini (R.T.) 5/27-30	*Mardana* with suitable media (s) & *Prakshalana*	*Khalva Yantra*, cloth, stainless steel (SS) vessels, spatula
2	*Shodhana* of *Gandhaka*[11]	Rasamrita 2/3	*Dhalana* and *Prakshalana*	*Khalva Yantra*, SS Vessels, Plate, Cloth, weighing machine
3	Preparation of *Kajjali*[12]	R.T. 2/27	*Mardana*	*Khalva Yantra*, Plate, weighing machine, spatula
4	*Shodhana* of *Manahshila*[13]	Rasa Ratna Samucchya (R.R.S.) 3/93	*Bhavana*	*Khalva Yantra*, Plate, weighing machine, spatula
5	Powder preparation of herbal drugs[14]	API part II	*Churna Nirmana Vidhi*	*Khalva Yantra*, weighing machine, Plates, Sieve size No. 85
6	Preparation of *Manjishtha Kwatha*	R.T. 2/51	*Kwatha Nirmana* for *Bhavana*	*Khalwa Yantra*, measuring flask 1000

				ml capacity, S.S. vessels, Heating device, spoons, beaker
7	Preparation of Leukchem 14	API part II	*Bhavana*	*Khalva Yantra*, SS Vessels, Measuring flask, Plate, Heating device, weighing machine, spatula

2.2.1. Pharmaceutical process of *Parada Shodhana*:

Ashuddha Parada 300 g, *Sudha Raja* 300 g, *Nistusha Lashuna* 278 g and *Saindhava Lavana* 139 g were taken as ingredients during this practical study.

Procedure:

Parada was triturated with *Sudha Raja* and then it was filtered through a double folded cloth. The remaining mixture of *Sudha Raja* was washed with hot water till *Parada* was remained. Total 36 hours were taken in this step. Then procured *Sudha Raja Shodhita Parada* was triturated with the paste of *Nistusha Lashuna Kalka* (paste of garlic) and *Saindhava Lavana* until *Kalka* became black for next 36 hours. Finally, it was washed with hot water until complete removal of *Kalka* and then filtered through a double folded cotton cloth. *Shuddha Parada* was weighed and packed in a glass bottle.

Observations & Results

After trituration with *Sudha Raja*, complete *Parada* was mixed with it and colour of the mixture was changed from white to grey. In the second step of *Parada Shodhana*, yellowish light green *Lashuna Kalka* was changed to black and minute globules of mercury were easily visible in the paste of *Lashuna* and *Saindava*. Total 267g of yield with 33 g, and 11% of weight loss of *Parada* were found during the practical study of *Parada Shodhana*.

2.2.2. Pharmaceutical process of *Gandhaka Shodhana*:

The *Gandhaka Shodhana* was carried out as per the reference of Rasamrita 2/3.

Gandhaka 400 g, *Godugdha* (Cow milk) 1600 ml x 7 times = 11,200 ml (Gravimetrically four times quantity to the *Gandhaka* each time), ghee 35g x 7 times = 245 g were taken as ingredient during practical study.

Procedure:

Gandhaka was made into powder in a clean *Khalwa Yantra*. Then, powdered *Gandhaka* was heated with ghee over *Mandagni* (mild heat) in a stainless steel pan. After melting, it was poured (*Dhalana*) into *Godugdha*, which was placed in a steel vessel, through the ghee-smeared cloth. Granular form with some solid mass of *Gandhaka* was taken out, washed with hot water, and dried well. After complete drying, the procedure was repeated for next six times. After completion of procedure, *Gandhaka* was collected, washed with hot water, and dried well. After drying, it was powdered, weighed, and kept in a sealed polyethylene packet.

Observations & Results

It was observed that, average time taken to melt the *Gandhaka* was 6 minutes. Total 35 g ghee was smeared over vessel and cloth for melting and pouring of *Gandhaka*. Crystalline dark yellow *Gandhaka* turned into granular and dull yellow after *Shodhana*. Total 378 g of yield with 22 g, and 5.5 % of weight loss of *Gandhaka* were found after practical study of *Gandhaka Shodhana*.

2.2.3. Preparation of *Kajjali*:

225 g of *Shuddha Parada* and 225 g of *Shuddha Gandhaka* was taken in this practical study.

Procedure:

Shuddha Parada and *Shuddha Gandhaka* were taken in equal proportion, mixed together, and triturated in *Khalwa Yantra* until colour of mixture was completely turned into black coloured powder. After completion of this practical *Kajjali* was weighed, and preserved into a glass bottle.

Observation:

After 20 minutes of trituration yellow powder of *Shuddha Gandhaka* was started to turn into black coloured precipitate. Completion test was confirmed by the test of *Nishchandratva* (lusterless while examined under bright Sun light), *Varitara* (floating of particles on stable surface of water), followed by *Unam Pariksha* (ability of particles to float on water, even after a grain was put over film formed by *Kajjali* particles over water), *Rekhapurnatwa* (particle were inserted in furrows of finger).

Results:

Total 447 g of final yield was obtained with 3 g of loss may be due to adherence with and spill out the sample from *Khalwa*.

2.2.4. Pharmaceutical process of *Manahshila Shodhana*:

Shodhana of *Manahshila* was carried out as per the reference of R.R.S. 3/93.

Procedure:
Shodhana of *Manahshila* was carried out by seven *Bhavana* (levigations) with *Zingiber officinalis* Rosc. (Ginger) rhizome juice. Raw *Manahshila* was taken into a dried clean *Khalwa* and powdered by using pestle. Powdered *Manahshila* was levigated with fresh ginger juice. After completion of one *Bhavana* same procedure was repeated for next six times. Prepared dried *Shodhita Manahshila* was packed in to polyethylene packet.

Observations and Results:
Brick red *Manahshila* turned into orange red colour after *Shodhana*.
A total of 590 ml ginger juice was digested in 300 g *Manahshila* by manual trituration for 34 hours and 46 min. A total 36 g, 12% weight was increased after *Shodhana* of *Manahshila*.

2.2.5. Preparation of *Churna*:

Procedure: Herbal ingredients were pulverized to powder separately and then sieved through No. 85. To obtain maximum yield, remaining portion of these drugs were again powdered by manual grinding in to *Khalwa Yantra* and sieved again. They were packed separately in labeled polyethylene bags.

Observations and results:
The yield was about 92% - 96% from each herbal drug except *Bilwa Phala Majja*. *Bilwa Phala Majja* (pulp) yield was only 84%, it contains mucilaginous, and fatty oil matters may be the reason of fewer yields.

2.2.6. Pharmaceutical process of Leukchem 14:
The Pharmaceutical study of Leukchem 14 was carried out in three batches to fix the standard. *Bhavana* process was completed in two steps, *Bhavana* with one *Dravya* (levigation liquids) was considered as one-step. In first step of *Bhavana*, in each batch *Kajjali* 6 g, *Shuddha Manahshila* 500 mg, and powders of herbal drugs each 50 g X 6 = 300 g (as per their proportional ratio) were taken in to a clean *Khalwa Yantra* and prepared the mixture of these ingredients homogeneously. Then prepared mixture of ingredients was levigated (*Bhavana*) with fresh collected *Gomutra* (cow urine). Total three levigations were performed with cow urine. *Gomutra Bhavita* material was collected and weighed in Electronic balance. In the next step of *Bhavana*, *Gomutra Bhavita* material 200 g was again levigated with freshly prepared *Manjishtha Kwatha* (MK) for three times. MK was prepared by taking equal quantity of *Manjishtha* (proportion taken by weight of *Gomutra Bhavita* material), crushed well, and soaked overnight with sufficient quantity of water. Next day, *Kwatha* was prepared by adding eight times of water, and reduced to $1/8^{th}$ on mild heat. *Kwatha* was filtered and used for *Bhavana* purpose. Final prepared drug was collected and preserved in labeled polyethylene bags by mentioning manufacturing date etc.

Observation:
The colour of *Gomutra Bahvita* sample was found to be converting from green to dark green in each successive *Bhavana*. While the colour of *Manjishtha Kwatha Bhavita* sample was turned completely into dark brown coffee colour. Total weight of mixture was increased after completion of *Bhavana*. While weight of Cow urine *Bhavita* material was significantly less increased, in comparison of *Manjishtha Bhavita* materials. It may be due to presence of more solid content (phyto-constituents) in *Manjishtha Kwatha*.

Precaution:
Fresh cow urine and freshly prepared *Manjishtha Kwatha* were used for each *Bhavana* of Leukchem 14.

Table 3: Summary of *Bhavana* procedure of step I of Leukchem 14

Step I. *Bhavana* with *Gomutra*								
Batch	**No. of *Bhavana***	**Weight before *Bhavana* (g)**	**Quantity of *Bhavana Dravya* (ml)**	**Weight after *Bhavana* (g)**	**Duration (hours : minutes)**	**Colour**	**Weight gain (g)**	**Average weight gain (g)**
I	1	306.50	330	311.42	5 : 12	Light Green	4.92	11.86
	2	311.42	300	314.50	4: 40	Green	3.08	
	3	314.50	300	317.82	5 :10	More dark than previous	3.32	
		Total *Bhavana Dravya* (ml)	930	**Total duration (hr:min)**	**15 : 02**	**Total weight gain (g)**	11.32	
II	1	306.50	345	312.50	5 : 24	Green	6	
	2	312.50	300	315.50	5 : 05	Green	3	
	3	315.50	300	319.25	5 : 12	More dark than previous	3.75	
		Total *Bhavana Dravya* (ml)	945	**Total duration (hr:min)**	**15 : 41**	**Total weight gain (g)**	12.75	
III	1	306.50	340	312.00	5 : 20	Green	5.50	
	2	312.00	300	315.12	5 : 02	Green	3.12	
	3	315.12	300	318.00	4 : 42	More dark than previous	2.88	
		Total *Bhavana*	940	**Total duration**	**15 : 04**	**Total weight gain (g)**	11.50	

		***Dravya* (ml)**		**(hr:min)**				

Table 4: Summary of *Bhavana* procedure in step II of Leukchem 14

Step II. *Bhavana* with *Manjishtha Kwatha*								
Batch	**No. of *Bhavna***	**Weight before *Bhavana* (g)**	**Quantity of *Bhavana Dravya* (ml)**	**Weight after *Bhavana* (g)**	**Duration (hours : minutes)**	**Colour**	**Weight gain (g)**	**Average weight gain (g)**
I	1	200.00	200	214.66	4: 24	Brown reddish green	14.66	43.17
	2	214.66	200	228.92	4 : 40	Brown	14.26	
	3	228.92	200	244.79	4 : 52	Brownish colour	15.87	
		Total *Bhavana Dravya* (ml)	600	**Total duration (hr:min)**	**13 : 56**	**Total weight gain (g)**	44.79	
II	1	200.00	200	213.50	5 : 12	Brown reddish green	13.50	
	2	213.50	180	227.50	4 : 50	Brown	14.00	
	3	227.50	180	241.72	4 : 25	Brown coffee colour	14.22	
		Total *Bhavana Dravya* (ml)	560	**Total duration (hr:min)**	**14 : 27**	**Total weight gain (g)**	41.72	
III	1	200.00	200	213.00	5 : 00	Brown reddish green	13.00	
	2	213.00	180	226.50	4 : 30	Brown	13.50	
	3	226.50	180	242.00	4: 42	Brown coffee colour	16.50	
		Total *Bhavana Dravya* (ml)	560	**Total duration (hr:min)**	**14 : 12**	**Total weight gain (g)**	43.00	

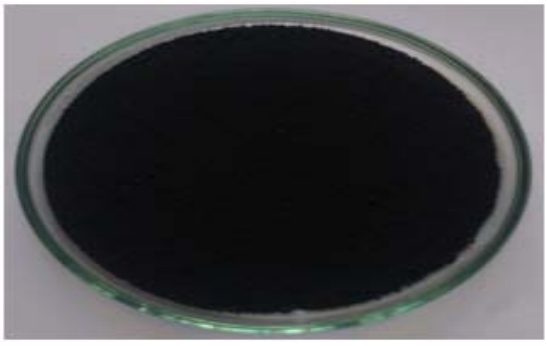
Table 5: Organoleptic characters of Leukchem 14

Parameters	***Gomutra Bhavita* (step I)**	***Manjishtha Kwatha Bhavita* (step II)**
Colour	Dark green	Coffee brown
Touch	Smooth +	Smooth ++
Odour	*Gomutra*	Characteristic
Taste	Pungent	Sweet, slight Pungent

Figure 1 to 10: Showing Ingredients and processing of Leukchem 14

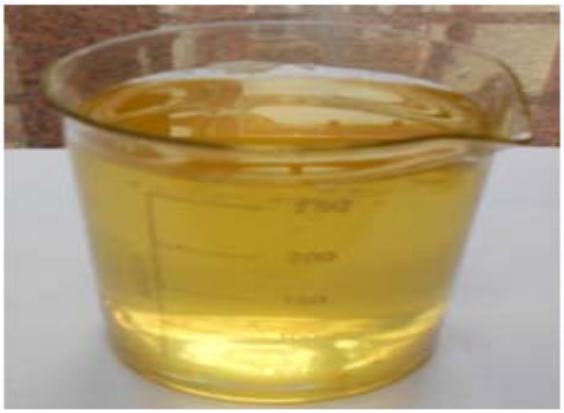
Shuddha Parada

Shuddha Gandhaka



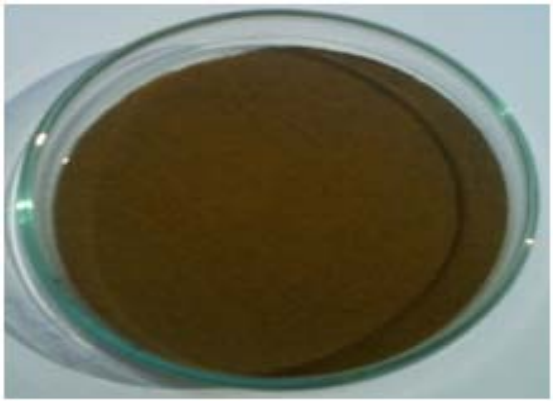
Kajjali

Suddha Manahshila



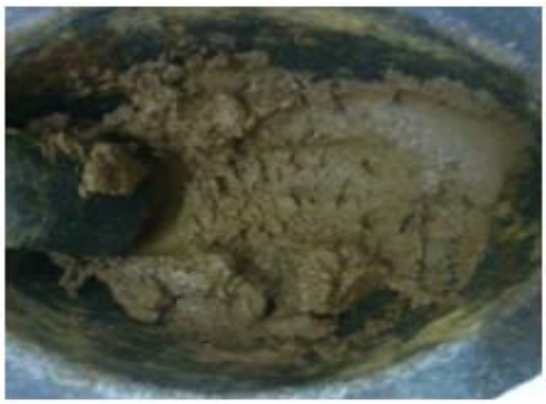
Gomutra

Gomutra Bhavana

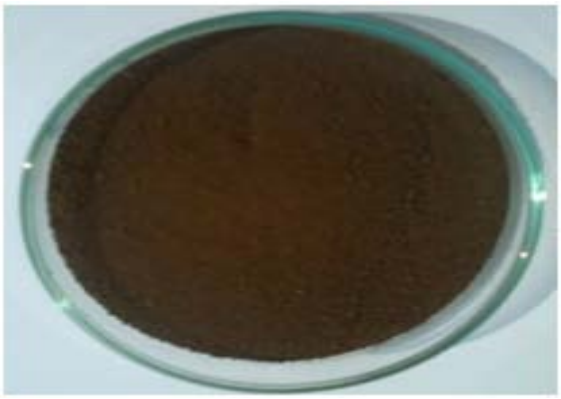


Gomutra Bhavita Material

Manjishtha Kwatha



Manjishtha Kwatha Bhavana



Leukkhem 14

DISCUSSION

Selection of the ingredients

Among all types of Cancer, hematological malignancies are specially considered as fast spreading disease and offered the path of metastasis to other body part [15]. Chemotherapy of these malignancies has known for their excessive cost and intensive side effects [16]. The strengths and weaknesses of the current diagnosis and management of cancer are often not understood well by many patients; therefore, their quest for complementary and alternative medicine (CAM) approaches for cancer care continues globally [17]. Over the last few decades, several anticancer agents, which are being investigated or used, belong to the plant world. This raises hopes for novel approaches used by CAM systems [18]. In particular, the search for a potent anti cancerous and having Rasayana property, Ayurvedic herbo-mineral formulations are the most potent dosage form of Ayurveda. Previous study of some herbo-mineral compounds *Kamdudha Rasa*, *Keharuba Pisti* and *Navjeevana Rasa* in combination have shown complete remission on acute promyelocytic leukemia (APL or specific AML-M3) and acute myeloid leukemia (specific AML-M0 type) successfully [19, 20]. So, after searching the literatures in Ayurvedic classics, this drug particularly prepared containing *Samaguna Kajjali*, *Shuddha Manahshila*, *Ashwagandha*, *Bilwa*, *Guduchi*, *Haridra*, *Kanchanar*, and *Triphala Churna* with the *Bhavana* of *Gomutra* and *Manjishtha Kwatha* each three times respectively (**Table 1)**. The prepared drug was named as Leukchem 14, which was a type of *Kharaliya Rasayana* (herbomineral formulation prepared by triturating with or without liquid media). The herbals and mineral drugs used in the preparation of herbo-mineral formulation were selected on the basis on their proof of effectiveness in various type of cancer, leukemia and cancer related symptoms. Thus, the present study was aimed to standardize the pharmaceutical method of herbo-mineral formulation, design especially for the treatment of Leukemia having anti cancerous property as well as a rich *Rasayana* property.

Rationality of proportion of mixing the ingredients

In API, the therapeutic recommended doses of herbal drugs are ranging from 03 to 06 g twice a daily [14]. Similarly, clinical recommended dose for *Kajjali* is mentioned from half to one *Ratti* [21] (60 to 120 mg) and for *Shuddha Manahshila* is about 05 – 10 mg [22] twice daily. Thus, by considering all these references, each unit dose of Leukchem 14 may consist herbal drug each 500 mg (500 mg X 6= total 3g), *Kajjali* 60 mg and *Shuddha Manahshila* 5 mg with additional weight of solid constituents of *Gomutra* and *Manjishtha*

Kwatha increased by three levigations of each *Bhavana Dravyas*. Thus, solid components of these *Bhavana Dravyas* are adjunct with the mixture of herbal and mineral drug of the pharmaceutical compound too.

Standard Operating Procedure (SOP) of Pharmaceutical process of Leukchem 14 (Table 2 & Figures 1 to 10)

Standardization is a process coming under Quality Control (QC), which refers the process of *Samskara*. The role of *Samskara* is very much important behind drug formulation. The mineral drugs *Parada*, *Gandhaka* and *Manahshila* were used in formulation after proper *Shodhana* process. *Shodhana* is a process of purification and detoxification by which physical and chemical blemishes, and toxic materials are eliminated and substances are subjected for further processing [1]. *Parada Sodhana* was done as per reference mentioned in the AFI part-I (quoting the reference of Rasa Tarangini). This classical reference for *Parada Shodhana* portrayed that by trituration with *Sudha*, *Lashuna* and *Saindhava*, *Parada* became free from *Parada- Dosha* completely and can be used in preparation of medicine. It was observed that when *Parada* was triturated with *Sudha*, it was converted into grey coloured powder form. It is difficult to procure whole amount of *Parada* by double folded cloth process as mentioned in classics. Therefore, there was need of *Prakshalana* with hot water to get maximum yield of *Parada*. In second step, *Parada* was triturated with *Nistusha Lashuna Kalka* and *Saindhava*. After trituration when black coloured mixture was obtained, pure *Parada* was collected through *Prakshalana* again with the use of hot water. The total 11% loss was observed in this process. However, maximum loss was observed in trituration with *Sudha Raja* (22 g in weight and 7.6 %).

Gandhaka Sodhana was done as per reference mentioned in the AFI part-I (quoting the reference of Rasamritam). Considering this, sufficient amount of *Godugdha* (cow milk- 1600 ml) was needed for 400 g of *Gandhaka* for each *Dhalana*. Ghee was used to smear over vessel in which *Gandhaka* was melted and the cloth, through which molted *Gandhaka* was poured in to milk. Total 35 g ghee was found sufficient to smear (30 g + 5 g) for each *Dhalana*. After each *Dhalana*, *Gandhaka* was thoroughly washed with hot water to remove fat contents of milk and Ghee. Otherwise, these materials may appear in form of black precipitates after burning. Each time, fresh milk was taken to facilitate detoxification of *Gandhaka*. For the preparation of *Kajjali*, manual trituration in stony mortar with a pestle was done. It was continued till the powder became black in color and very fine like *Kajala* [12] (collyrium) and it fulfilled all the criteria of *Kajjali Pariksha*. *Varitaratva* (floating on stagnant water), *Rekhapurnatva* (filling the furrows when rubbed between two fingers), and *Nischandratva* (lusterless) were found after 78hours of *Mardana* (trituration). *Kajjali* (Black sulphide of mercury – HgS) is a *Sagandha Niragni Murchchana* of *Parada*.

Kajjali (black sulphide of mercury; HgS) and *Shuddha Manahshila* (realgar as As_4S_4) have been used in traditional Indian medicines for thousands of years and are still in practice for treatment of different disease conditions available in various dosage forms[3, 4]. However, both mercury (Hg) and arsenic (As) are highly toxic substances [23, 24], and their safety and risk are of concern. However, *Kajjali* and *Manahshila* are not generally taken directly. Usually, *Kajjali* and or *Manahshila* are combined with other herbal ingredients, subjected to additional *Bhavana* and prepared a complex Rasa formulations (*Rasaushadhies*). *Kajjali* forms the base as one of the ingredient of many *Rasaushadhies*, and it helps further to potentiate their action. The sulphur content of this mixture is essential in the context to allow the full therapeutic effect of the mercury to interact with the body tissues. *Kajjali* have the properties of *Yogavahi* [25] (which potentiating the action of the drug as well as caries the drug to its action site) and *Rasayana* (counteracting the effects of age) [26].

For *Shodhana* of *Manahshila*, *Bhavana* (levigation) process was selected as per reference mentioned in AFI that was carried out by seven levigations with the fresh extracted juice of *Zingeber officinalis* Roscoe. It was observed during *Shodhana* of *Manahshila*, that one fifth part (60 ml) of *Ardraka* juice was sufficient to levigate the *Manahshila*. But, by studying some previous studies on *Manahshila Shodhana* [27, 28], it was decided to take 110 ml of *Ardraka* juice in first *Bhavana*. Simultaneously, amount of *Ardraka* juice was taken 80 ml in each successive levigation process, which may be considered as 85 ml approximately in each levigation. There are several positive factors associated with ginger in the mode of *Shodhana* of *Manahshila* by *Ardraka Swarasa.* Ginger contains two important sulphur-based amino acids called cysteine and methionine,

which can act as phytochelatins and may render *Manahshila* nontoxic. Phytochelatins are heavy metal-binding peptides that play an important role in detoxification of heavy metals by chelation [27]. In one of the study, *Ardraka Shodhita Manahshila* was also found to be nontoxic in experimental animals [29].

Bhavana is a process in which powdered drug is triturated with certain liquid media until the contents are completely dried. The quantity of liquid medium should be sufficient to ensure that the powder is completely soaked with the liquid media so that, while trituration each minute particles of the material remain in contact with the liquid media. The impregnation of properties of the media to the material leads to unique and suitable physio-chemical changes [1] i.e., induction of trace element from cow urine and herbal juices or decoction to potentiate the biological efficacy of the material and to facilitate the absorption. Cow urine and *Manjishtha Kwatha* were selected respectively for *Bhavana* with three times each **(Table 3 & 4)**. *Manjishtha Kwatha*[30] was prepared freshly for each levigation as per classical reference with ratio of 1:8 (one part of drug and eight parts water) at 100ºC and reduced to 1/8 quantity in 3 hours used for *Bhavana*. In review of the preparation of *Rasaushadhies* of *Kharaliya* types, we find that one to seven *Bhavanas* are common[31]. So, after the expert opinion in between range of three *Bhavana* were selected in the pharmaceutical study of Leukchem 14. In the process of *Bhavana*, *Mardana* (grinding) is involved which was carried out manually by trituration in stony mortar and pestle. It serves two purposes i.e. produces new surfaces for reaction and provides specified size[1]. The speed of trituration was maintained at moderate rate (approximately 34 - 36 frequencies forward and backward movement per minute). The trituration was continued until the homogenous mixture was achieved. It was continued till complete drying.

Difference in duration of *Bhavana* and quantity used of *Bhavita* material was noticed in pharmaceutical processing of Leukchem 14. The quantity of Cow urine was absorbed more in first cycle of *Bhavana* compare to successive *Bhavana*. However, in progressive stage of *Bhavana* less quantity (300 ml) and slight more duration was observed in process of *Bhavana* with cow urine. However, *Bhavana* with *Manjistha Kwatha* found equal proportion of *Kwatha* (200 ml) was needed for first *Bhavana*, while comparatively less quantity of MK (180 ml) was needed in progressive cycles of *Bhavana*. However, approximately similar duration of *Bhavana* was observed. More water soluble solid extractives (22.80%) and accordingly lesser amount of water proportion in *Manjishtha Kwatha*[32, 33] and less solid content (urea 2.5%, minerals, salts, hormones, and enzymes- 2.5%) and more water amount (95%) in Cow urine may be reason of these observations [9].

It was observed that 317.82, 319.25, and 318.0 g of Leukchem 14 in step I with the *Bhavana* of *Gomutra* was obtained from 306.50 g of basic homogenous mixture of herbal *Churna, Kajjali* and *Shuddha Manahshila* in three batches **(Table 3)**. While 244.79, 241.72, and 243.00 g of Leukchem 14 was obtained with the *Bhavana* of *Manjishtha Kwatha* from 200 g of *Gomutra Bhavita* materials in three batches **(Table 4)**. Increase in weight of *Bhavita* materials was observed in both steps. However, ratio of increase is approximately four times more in *Manjishtha Kwatha Bhavita* material than the cow urine *Bhavita* material. It may be presence of less solid portion in cow urine than the *Manjistha Kwatha*. It was observed that Cow urine and *Manjishtha Kwatha* (if prepared by 1:8 ratio) content 2.85±0.09 and 7.28±0.42 percentage of solids respectively. This percentage of solids may contribute in increasing of weight after each levigation of Cow urine and *Manjishtha Kwatha.*

Particular media for each process of levigation indicates some basic relation between the particular media and material too. Colour indicates the formation of specific compound. The color of levigated mass became light green when *Bhavana* was done by *Gomutra* **(Table 5)**. *Green* color of *Bhavaita* material was increased in each successive *Bhavana*, this observation may be due to absorbing the solid inorganic and organic components of Cow urine in to levigated materials[9]. The levigation with *Manjishtha Kwatha* was turned into greenish reddish brown colour in first *Bhavana*. The color of levigated mass became more brown and coffee colour in progressive *Bhavana* with *Manjishtha Kwatha*.

CONCLUSION

Changes in the raw materials ultimately attribute to the medicinal efficacy and safety of the final compound. The increase in weight was observed after levigation with both the media progressively, 11.86 g & 3.87 % by cow urine and 43.17 g & 21.59% by *Manjishtha Kwatha*. The present study

standards can be set as standards for herbo-mineral formulation Leukchem 14 in large scale and here by used for future considerations.

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