PHARMACOPEIAL STANDARDIZATION OF MRDVIKADI LEHYA: A AYURVEDIC FORMULATION

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Abstract

Standardization of herbal formulation is essential in order to assess the quality of drugs for therapeutic value. Mrdvikadi Lehya is official in Ayurvedic formulary of India and prepared as prescribed in the official book of Ayurvedic Formulary of India. It is most common formula used for Cough in Ayurvedic medicine. It comprised of the fruits of two medicinal important plants, *Vitisvinifera* (Draksha), *Piper longum* (Pippali) and Sarkara (sugar) and madhu (Honey). It was standardized in order to assess the quality of drugs, based on the concentration of their active principles according to world health organization guidelines. The various parameters performed included organoleptic and physicochemical characteristics. The set parameters were found to be sufficient to standardize the Mrdvikadi Lehya and can be used as reference standards for the quality control/ quality assurance study mostly on plant drugs for their primary health care needs. The results obtained may be considered as tools for assistance to the regulatory authorities, scientific organization and manufacturers for developing standard formulation of great efficacy.

Keywords : *Standardization, Ayurvedic, Quality Control, Mrdvikadi Lehya.*

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1.Introduction

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing andagreeing upon technical standards, standardization is a tool in the quality control process.[1]WHO has also issued Guidelines for quality control methods for medicinal plant material in 1992 with a clear objective to provide general test methods for correct botanical evaluation and identification of medicinal plants widely used in traditional and home remedies. [2]Mrdvikadi Lehya, which are official in Ayurvedic formulary of India and cited in standard traditional literature of ayurveda i.e. Sarangdharasamhita and Bhasaijya Ratnavali. It is most common formula used for Cough in Ayurvedic medicine. It comprised of the fruits of two medicinal important plants, *Vitisvinifera* (Draksha), *Piper longum* (Pippali) and Sarkara (sugar) and madhu (Honey). Three batches of Mrdvikadi Lehya designated as ML- I, ML - II, ML - III were prepared in laboratory. One marketed formulations named MML - I was purchased from local pharmacy store Ujjain. The preparation of Mrdvikadi Lehya is based on traditional methods in accordancewith the procedures given in classical texts.This may not have the desired quality and batch to batch

consistency. Hence this formulation required standardizationaccording to guidelines given by WHO. [3]

2. Materials and Methods

2.1 Authentication of raw material

Dried crude drugs of *Vitisvinifera* and *Piper longum* were purchased from local market of Ujjain (M.P.) 456010, INDIA and identified morphologically and microscopically and compared with standard Pharmacopoeial monograph. The sample of crude drug was also authenticated by Dept. of Botany, Vikram University, Ujjain (M.P.) 456010, INDIA.

2.2 Preparation of MrdvikadiLehya

Mrdvikadi Lehya is prepaed in laboratory, as per the method described in Ayurvedic Formulary of India. The authenticated crude drugs of these formulations were crushed in to fine powder separately. Finally, the prescribed weights of all the raw materials was mixed together in stone mortar and pestle and were grinded with honey and kept the resulting Lehya in a tight closed container. [4] The component of crude drugs used in Mrdvikadi Lehya with their botanical identities and parts used are given in table 1.

Table 1. Raw ingredients of MrdvikadiLehya

S. no	Sanskrit Name	Hindi /comman name	Botanical Name	Family	Part Used	Quantity
1	Mrdvika	Draksha	*Vitis vinifera*	Vitaceae	Dr. fr.	50 in number
2	Pippali	Lady pippal	*Piper longum*	Piperaceae	Fr.	30 in number
3	Sarkara	Sugar				48gm
4	Madhu	Honey				QS

2.3 Coding of formulation

Three sample batch of MrdvikadiLehya were prepared using above mentioned methods and were named as ML-I, ML-II, ML-III. One Marketed formulations named MML-I was purchased from local pharmacy store, Ujjain. These samples were stored at identical conditions of temperature, light and moisture.

2.4 Development of Organoleptic evaluation

The entire laboratory batch of Mrdvikadi Lehya ML-I, ML-II, ML-III, marketed formulation MML-I and separately powdered *Vitis vinifera and Piper longum* were subjected to Organoleptic examination.Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste etc.[5]

Table 2. Organoleptic evaluation of MrdvikadiLehya and its Raw Material

S.	Name		Colour	Odour	Taste
1	*Vitisvinifera*		Dark brown	Sweetish and pleasant	Bitter
2	*Piper longum*		Dark Brown	Characteristic	Pungent
3	MrdvikadiLehya	ML-I	Light Brown	Characteristic	Sweet
		ML-II	Light Brown	Characteristic	Sweet
		ML-III	Light Brown	Characteristic	Sweet
		MML-I	Light Brown	Characteristic	Sweet

2.5 *Development of physical characteristics*

The Raw materials of Mrdvikadi Lehya (*Vitis vinifera and Piper longum)* were subjected to evaluation of physical properties which were determined by means of tap density, bulk density, angle of repose, hausner ratio and carr's index.[6,7]

2.6 Bulk density

Bulk density, $\rho_b$is defined as the mass of a powder divided by the bulk volume. A sample of about 50 cm^3 of powder that has previously

been passed through a U.S. Standard no. 20 sieve is carefully introduced into a 100 ml graduated cylinder. The cylinder is dropped at 2-sec intervals on a hard wooden surface three times from a height of 1 inch. The bulk density is then obtained by dividing the weight of the sample in gm by the final volume in cm3 of the sample contained in the cylinder.

2.7 Tap density

Tap density, $\rho_T$is defined as the mass of a powder divided by the Tapped volume. A sample of about 50 cm^3 of powder that has previously been passed through a U. S. Standard no. 20 sieve is carefully introduced into a 100 ml graduated cylinder. The cylinder is dropped at 2-sec intervals on a hard wooden surface hundred times from a height of 1 inch until no further decrease in the volume of powder takes place. The tap density is then obtained by dividing the weight of the sample in gm by the final volume in cm3 of the sample contained in the cylinder.

2.8 Angle of repose

A glass funnel is held in place with a clamp on ring support over a glass plate. The glass plate is placed on a micro-lab jack. Approximately 100 g of powder is transferred in to the funnel (that has previously been passed through a number 10 mesh size), keeping the orifice of funnel blocked by the thumb. As the thumb is removed, the lab –jack is adjusted so as to lower the plate and maintain about 6.4 mm gap between the bottom of funnel stem and top of the powder pile. When the powder is emptied from the funnel, the angle of the heap to the horizontal plane is measured with a protector. Measure the height of the pile (h) and the radius of the base(r) with the ruler. The angle of repose is thus estimated by following formula.

$$\theta = \tan^{-1}(h/r)$$

2.9 Hausner ratio

The Hausner ratio is calculated by the formula given below, where ρB is the freely settled bulk density of the powder, and ρT is the tapped density of the powder.

$$H = \frac{\rho_T}{\rho_B}$$

2.10 Carr's index

The Carr index is an indication of the compressibility of a powder. It is calculated by the following formula, where V_B is the freely settled volume of a given mass of powder, and V_T is the tapped volume of the same mass of powder.

$$C = 100\frac{V_B - V_T}{V_B}$$

It can also be expressed as follows, where ρ_B is the freely settled bulk density of the powder, and ρ_T is the tapped density of the powder.

$$C = 100 \times (1 - \frac{\rho_B}{\rho_T})$$

Table3. Physical characteristics of raw material of MrdvikadiLehya

S.	Name	Tap density	Bulk density	Angle of repose	Hausner ratio	*Carr's index*
1	*Vitis vinifera*	0.9644	0.7172	49.77	1.34	34.421
2	*Piper longum*	0.4798	0.3901	28.34	1.23	18.6953

2.11 Determination of Loss on Drying

An excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Limits for water content should therefore be set for every given plant material. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. The presence of excess amount of moisture in crude drugs may affect their quality. Therefore, the percent loss on drying was determined for each batch of MrdvikadiLehya, one marketed formulations and separately powdered raw material*Vitis vinifera*and *Piper longum*.(Table.4)

2.12 Determination of foreign matter

Medicinal plant materials should be entirely free from visible signs of contamination by moulds or insects, and other animal contamination, including animal excreta. No abnormal odour, discoloration, slime or signs of deterioration should be detected.

Table 4.Percent of Loss on Drying of MrdvikadiLehyaand raw Material

S.	Name		%LOD*±S.D.(n=6)
1	*Vitis vinifera*		14.118±0.559
2	*Piper longum*		7.212±0.893
3	MrdvikadiLehya	ML-I	18.623±0.336
		ML-II	17.129±0.683
		ML-III	18.259±0.567
		MML-I	14.712±0.491

It is seldom possible to obtain marketed plant materials that are entirely free from some form of innocuous foreign matter. However, no poisonous, dangerous or otherwise harmful foreign matter or residue should be allowed. Accurately weighed (250g) sample of plant materials are spread in a thin layer and foreign matter is sorted by using a magnifying lens (6x or 10x). The remainder of the sample is sifted through a No. 250 sieve; dust is regarded as mineral admixture. The portions of this sorted foreign matter are weighed and the content of each group is calculated in grams per 100g of air-dried sample.The percent foreign matter was determined for raw material of MrdvikadiLehya i.e. *Vitisvinifera* and *Piper longum* and Laboratory formulations of MrdvikadiLehya are prepared after removal of foreign matter was not included in study.

Table 5. Percent of foreign matter of MrdvikadiLehyaand raw Material

S.	Name		*%Foreign matter ±S.D.(n=6)*
1	*Vitis vinifera*		1.601±0.367
2	*Piper longum*		1.201±0.315
3	MrdvikadiLehya	ML-I	NIL
		ML-II	NIL
		ML-III	NIL
		MML-I	NIL

2.13 Determinations of ash values

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. The percent total ash and Acid-insoluble ash was determined for ML and one marketed formulation of each and all raw ingredients of formulation as per the method described below.

2.14 Total ash

About 4g of the ground air-dried material is accurately weighed and Placed in a previously ignited and tarred silica crucible. The material is spread in an even layer and ignited by gradually increasing the heat to a temperature of 500-600°C until it is white, indicating the absence of carbon. The material is Cooled in a desiccators and weighed. The content of total ash is calculated in mg per g of air-dried material.

2.15 Acid-insoluble ash

To the crucible containing the total ash, 25 ml of hydrochloric acid is added, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass is rinsed with 5 ml of hot water and this liquid is added to the crucible. The insoluble matter is collected on an ash less filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter is transferred to the original crucible, dried on a hot-plate and ignited to constant weight. The residue is allowed to cool in suitable desiccators for 30 minutes, and then weighed without delay. The content of acid-insoluble ash is calculated in mg per gm of air-dried material.

The percent total ash and acid-insoluble ash was determined for each batch of MrdvikadiLehya, its marketed formulations MML-I and each of its raw material *Vitis vinifera and Piper longum.*(Table 6)

Table.6 Percent of ash value of MrdvikadiLehya and its raw materials

S.	Name		Total ash (% w/w ± S.D., n=6)	Acid insoluble ash (% w/w ± S.D.,n=6)
1	*Vitisvinifera*		2.759 ± 0.139	0.225±0.614
2	*Piper longum*		4.075 ±0.729	0.689 ± 0.112
3	MrdvikadiLehya	ML-I	1.513±0.349	0.209±0.438
		ML-II	1.518±0.467	0.211±0.287
		ML-III	1.506±0.662	0.210±0.477
		MML-I	1.496±0.473	0.199±0.361

2.16 Determination of extractive values

Accurately weighed 4.0g of coarsely powdered air-dried material is placed in a glass-stoppered conical flask and macerated with 100ml of the solvent for 6 hours, shaking frequently, and then allowed to stand for 18 hours. The mixture is filtered rapidly taking care not to lose any solvent. 25 ml of the filtrate is transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. The residue is dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay.[8]The percent extractive values were determined in various solvents ranging from non polar, semi polar to polar behavior. The extractive values are recorded in Petroleum ether, chloroform, alcohol and water with a view to study the distribution of various constituents of MrdvikadiLehya (ML-I, ML-II, ML-III), Marketed formulation (MML-I) and its raw materials *Vitis vinifera and Piper longum.*

Table .7 Extractive values (%w/w ±S.D. n=6) MrdvikadiLehya and its raw materials

S.	Name		Water soluble	Alcohol soluble	Chloroform soluble	*Petroleum ether soluble*
1	*Vitis vinifera*		71.16±0.446	27.42±0 .349	2.91±0.176	0.742±0.419
2	*Piper longum*		13.98±0.339	10.119±0.681	1.862±0.252	2.699±0.963
3	MrdvikadiLehya	ML-I	65.19±0.467	29.36±0.113	1.89±0.446	0.491±0.224
		ML-II	65.16±0.268	29.37±0.436	1.87±0.086	0.499±0.083
		ML-III	65.06±0.469	29.42±0.086	1.88±0.011	0.498±0.167
		MML-I	64.72±0.286	29.89±0.428	2.01±0.497	0.631±0.342

2.17 Qualitative phytochemical studies

MrdvikadiLehya is having *Vitis vinifera*and *Piper longum* as its main constituents. To detect the presence of various phytoconstituents in formulations as well as in raw materials, phytochemical investigation is performed. The tests are performed on alcohol, water, chloroform and petroleum ether extract. To

detect the presence of various phytoconstituents in formulations as well as in raw materials phytochemical investigation is performed. The tests are performed on alcohol, water, chloroform and petroleum ether extract. Qualitative phytochemical analyses were done for the extract of raw materials and of formulations for alkaloids, carbohydrates, tannins and phenols, fixed oils and fats, saponins and gums and mucilage's.(Table.8).[9]

Alkaloids

The extracts were dissolved in diluted sulphuric acid and filtered. The filtrate was treated with Mayer's, Dragendrof, Hager's and Wagner's reagents separately. Appearance of cream, orange brown, yellow and reddish brown precipitates in response to the above reagents respectively indicate the presence of alkaloids.

Carbohydrates

300 gm of aqueous and alcoholic extracts were dissolved in water and filtered. The filtrate was treated with concentrated sulphuric acid and then with Molisch's reagent. Appearance of pink to violet color indicates the presence of carbohydrates.

The filtrate was boiled with Fehling's and with Benedict's solutions. Formation of brick red precipitate in Fehling's and Benedict's solutions is the positive result for reducing sugars and non-reducing sugars respectively.

Tannins and phenols

Small quantity of alcoholic and aqueous extracts was dissolved in water and to that ferric chloride solution (5%) or gelatin solution (1%) or lead acetate solution (10%) was added. Appearance of blue color with ferric chloride or precipitation with other reagents indicates the presence of tannins and phenols.

Phytosterols

The extract is refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The saponification mixture is diluted with distilled water and extracted with ether. The ethereal extract is evaporated and the residue is subjected to Liebermann's test and Liebermann burchard's test.

Gums and mucilage's

About 10 ml of extract was slowly added to 25 ml of absolute alcohol with constant stirring. Precipitation indicates the presence of gums and mucilage's.

Fixed oils and fats

A drop of concentrated extract was pressed in-between two filter papers and kept undisturbed. Oil stains on the paper indicate the presence of oils and fats.

Saponins

About one ml of the alcoholic and aqueous extracts were dissolved separately in 20 ml of water and shaken in a graduated cylinder for 15 minutes. Formation of one cm layer of foam indicates the presence of saponins.

Protein and free amino acid

Small quantities of aqueous or alcoholic extract were dissolved in a few ml of water and the solution is subjected to Millon's, Biuret and Ninhydrin test.

3. Result and Discussion

Evaluations of organoleptic characters of raw materials (*Vitis vinifera and Piper longum)* were performed. Laboratory batches of MrdvikadiLehya (ML-I, ML-II, ML-III) and the one marketed preparation (MML- I) were also evaluated for sensory characters. Our results reveal that the MrdvikadiLehya is light brown in colour with slight sweet taste having characteristic odour. The results for the marketed formulations (MML- I) and laboratory formulation were found comparable. (Table 2.)

Crude powdered drugs possess some physical properties which are determined in form of tap density, bulk density, angle of repose, Hausner ratio and Carr's index. Among the raw material *Piper longum* showed good flow properties while a poor flow was observed in case of *Vitisvinfera.* This is further confirmed by high value of Hausner's ratio and Carr's index. The laboratory formulation was in semi solid preparation so it was not included in study.The Loss on drying or heating to constant weight is determined for crude drugs and finished formulation. The percent moisture content in raw materials was found to be*Vitisvinifera* (14.118±0.559) and *Piper longum* (7.212±0.893). The percent moisture content

for ML-I, ML-II and ML-III were, 18.623±0.336, 17.129±0.683 and18.259±0.567, while it is 14.712±0.491for MML-I.(Table 3 and 4.)

Table.8 Qualitative chemical analysis of various extract of MrdvikadiLehya and its raw material

Chemical Test	Extract	ML-I	ML-II	ML-III	MML-I	VV	PL
Alkaloids	Alcohol	+	+	+	+	-	+
	Water	+	+	+	+	-	+
	Chloroform	+	+	+	+	-	+
	Pet. ether	-	-	-	-	-	-
Carbohydrate and Glycoside	Alcohol	+	+	+	+	+	+
	Water	+	+	+	+	+	+
	Chloroform	-	-	-	-	-	-
	Pet. ether	-	-	-	-	-	-
Tannins and phenols	Alcohol	+	+	+	+	+	-
	Water	+	+	+	+	+	-
	Chloroform	-	-	-	-	-	-
	Pet. ether	-	-	-	-	-	-
Gums and Mucilage's	Alcohol	-	-	-	-	-	-
	Water	+	+	+	+	+	+
	Chloroform	-	-	-	-	-	-
	Pet. ether	-	-	-	-	-	-
Fixed oils and fats	Alcohol	-	-	-	-	-	-
	Water	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-
	Pet. ether	+	+	+	+	+	-
Saponins	Alcohol	+	+	+	+	+	-
	Water	+	+	+	+	+	-
	Chloroform	-	-	-	-	-	-
	Pet. ether	-	-	-	-	-	-
Protein and free amino acid	Alcohol	+	+	+	+	+	+
	Water	+	+	+	+	+	+
	Chloroform	-	-	-	-	-	-
	Pet. ether	-	-	-	-	-	-
Volatile oil	By hydro Distillation Method	+	+	+	+	-	+
		+	+	+	+	-	+
		+	+	+	+	-	+
		+	+	+	+	-	+
Phytosterol	Alcohol	-	-	-	-	-	-
	Water	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-
	Pet. ether	-	-	-	-	-	-

(+) Present, (-) Absent

The percent of foreign matter in raw materials was determined macroscopically and through determination by weight as per WHO guidelines. It was found to be: *Vitisvinifera* (1.601±0.367) and *Piper longum* (1.201±0.315). Laboratory formulation of MrdvikadiLehya are

prepared after removal of foreign matter but there is possibility that this may be present in MML-I, because it was obtained in finished form.(Table 5.)

The total ash value indicates both physiological ash, and non-physiological ash. The percent total ash value for *Vitis vinifera* and *Piper longum*was found to be2.759±0.139,4.075±0.729 respectively. The total ash value for ML-I, ML-II and ML-III was 1.513±0.349, 1.518±0.467 and 1.506±0.662% respectively while in MML-I it was 1.496±0.473%. The yield of acid insoluble ash for *Vitis vinifera, Piper longum* was 0.225±0.614%,0.689±0.112% respectively which indicates the amount of silica present, especially as sand and siliceous earth. The percent acid insoluble ash for ML-I, ML-II, ML-III and MML-I was 0.209±0.438, 0.211±0.287, 0.210±0.477% 0.199±0.361%.and respectively.(Table 6.)

Extractive values were determined in various solvents with a view to study the distribution of various constituents of MrdvikadiLehya and its raw material. The extractive values were recorded in Petroleum ether, chloroform, alcohol and water with a view to study the distribution of various constituents of MrdvikadiLehya (ML-I, ML-II and ML-III), Marketed formulation (MML-I) and its raw materials *Vitis vinifera* and *Piper longum.*Highest extractive values were obtained in water and alcohol. This indicates high proportion of tannins and carbohydrates etc. in the formulations as well as raw material.(Table 7.) MrdvikadiLehya is having *Vitis vinifera* and *Piper longum*as its main constituents. To detect the presence of various phytoconstituents in formulations as well as in raw materials phytochemical investigations were performed. The tests were performed on alcohol, water, chloroform and petroleum ether extract. The formulations were found to have phenolics (tannins), carbohydrates, glycosides, phytosterols, fixed oil and fats, saponins, alkaloid, volatile oils and proteins,. Marketed formulation was found to contain the same constituents.(Table 8.)

4. Conclusion

The present research study provides quality and stability parameters of the selected formulation. The physicochemical profile of selected formulation is the mandatory quality standards for the further study. Thus these developed standardization parameters will help to make global acceptability of formulations and support the regulatory requirements.

5. References

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