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Preliminary Pharmacognostical and Physicochemical Analysis: A Poly Herbomineral Formulation

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Abstract:
Standardization of Ayurvedic herbal formulation is essential in order to assess the quality, therapeutics efficacy and safety. Ayurvedic herbomineral formulation consists of *Myristica fragrans* (fruit), *Myristica fragrans* (flowering top), *Datura metel* (seed), *Pueraria tuberosa* (root), *Asparagus racemosus* (root), *Grewia hirsuta* (Bark), *Barringtonia acutangula* (seed), *Tribulus terrestirs* (Fruit), *Abutilon indicum* (root) Mercury, Sulphur, Abharak Bhasma and Camphor, and specifically used to treat cold and cough. This study was aimed at standardization of Ayurvedic herbal formulation with respect to study of powder Morphology, Microscopy, Total ash, Acid insoluble ash, Water soluble and Alcohol soluble extractive value, Bulk density, Tapped density, Carr's index, Hausner ratio, Phytochemical test, and Thin layer chromatography (TLC) parameters. These result of parameters obtained may be serve as diagnostic tools for assistance to the regulatory authorities, scientific organizations and manufacturers for authentication and developing standard herbomineral formulation of great efficacy .

Keywords: Herbomineral, Standardization, Carr's index, Thin layer chromatography

Introduction

Now a day, eighty percent population of the word utilizes the herbal medicines in developing countries for primary healthcare. Health has been of utmost importance since ancient times for the mankind. So concern has been drawn to quality, safety, efficacy and standards of the Ayurvedic formulations as per the increasing demand for safer drugs (1,2). Variation in chemical constituents of plant material due to bulk utility, and differ in therapeutics efficacy because of different batches of collection like collection from different site and season with different geographical location or climate (3). The market of health-related products has been active and manufactured these products at different parts of the world and sold. Standardization is essential to confirm the availability of a uniform product in all parts of the world (4). Standardization assures a consistently stronger product with guaranteed constituents. World Health Organization (WHO) collaborates and assists health ministries in establishing policies for the introduction of traditional plant medicines for public health care in developing nation into primary healthcare programs, in assessing safety and efficacy, in ensuring adequate supplies, and in the quality control of raw and processed materials (5). The manuscript presents the preliminary pharmacognostical and physicochemical analysis of a poly herbomineral formulation based on organoleptic characters, physical characteristics, and physicochemical properties for cough and cold.

Materials and Methods

All the plant materials were collected from local market of Mathura, U.P. The species of all the herbs was checked, confirmed and authenticated from Botany department, BSA, College, Mathura, U.P., India. All the chemicals used in the experiment were of analytical grade.

Preparation of Herbomineral formulation:

According to the procedure of Ayurvedic Sarsangrah (6), the formulation was prepared. All the ingredients were powdered separately, passed through sieve number 80#; weighing separately each powder ingredient and mix together in specified proportions to get homogeneous blend of herbomineral formulation.

Marketed Sample

The marketed sample and LPH formulation were standardized based on their organoleptic characters, physical characteristics, and physicochemical properties.

Macroscopy of Herbomineral formulation:

The organoleptic characteristics like colour, odour and taste etc of all herbal drugs and formulation were determined.

Microscopy of Herbomineral formulation:

Microscopy study of herbs and lab prepared herbo-mineral (LPH) formulation was made with dried powder samples (2g) and thoroughly cleaned with chloral hydrate as a clearing agent and then stain the powder mixture with phloroglucinol, iodine solution and sudan red etc (7,8,9,10,11).

Physicochemical studies:

Total ash, acid insoluble ash, water soluble and alcohol soluble extractive values were carried out as per the WHO guidelines (7,8,9,10,12) for individual herbs, LPH formulation and marketed formulation.

Evaluation of micromeritics characteristics:

Various micromeritics characteristics include bulk density, tapped density, Carr's index as well as hausner's ratio were carried out as per the standard methods (13,14).

Preliminary Phytochemical Analysis:

The presence of active phytochemical constituents like alkaloids, carbohydrates, saponins, steroids, and proteins were identified in aqueous and alcoholic extract of herbs; LPH and marketed formulation (8,15,16,17,18,19,20).

Thin layer chromatography:

Identification of compound of Herbomineral formulation by TLC

For TLC about 10µl of the sample was spotted on precoated Silica gel-G aluminium plates of uniform thickness (0.5mm) as a stationary phase. Mixtures of different solvent systems were adopted as a mobile phase to developed thin layer chromatograms. The development was stopped when the solvent front had advanced about 7.5 cm. After drying plates in air, for some time, Iodine chamber and Dragendroff's reagent was used as a detecting agent for the detection of compound. Compound present in the LPH formulation were identified by comparison with the spot of the reference standard (7,8,9,10,11,15).

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Table 1: Macroscopy of Herbs, LPH formulation and Marketed formulation

Ingredients	Colour	Odour	Taste
Jayphal	Greenish -brown	Pungent, Aromatic	Strong, Aromatic
Javitri	Yellowish ,Orange	Aromatic	Aromatic, Strong
Datura	Light brown	-	Bitter, Acrid
Vidari	Reddish brown	-	Sweetish
Shatavari	Whitish -Brown	-	-
Mercury	Silver	-	-
Sulphur	Greenish yellow	-	-
Abharak Bhasma	Brown	-	-
Camphor	Pearl White	Aromatic	-
LPH Formulation	Blackish Brown	Pleasant	Slightly Bitter
Marketed Formulation	Blackish Brown	Pleasant	Slightly Bitter

Table 2: Microscopy of Herbs, LPH formulation and Marketed formulation

Herbs	Standard	Observed
Jayphal	Occasional Tannin iodoblasts, Less regular Aleurone grains	Irregular Aleurone grains
Javitri	Aleurone grains , Stone cells	Aleurone grains , Stone cells
Datura	Glandular , Nonglandular Tricomes	Nonglandular Tricomes
Vidari	Prismatic Calcium oxalate crystals , Starch grains , Xylem vessels	Starch grains
Shatavari	Stone cells (Rectangular) , Raphides , Lignified thick wall cells	Lignified thick walled cells
LPH Formulation	-	Aleurone grains, Stone cells, Tricomes , Fibers, Lignified thick walled cells
Marketed formulation	-	Irregular Aleurone grains, Stone cells

Table 3: Total ash, Acid insoluble ash, Water soluble extractive value and Alcohol soluble extractive value of different herbs, LPH formulation and Marketed formulation.

Ingredients	Total Ash (%)	Acid Insoluble Ash (%)	Water Soluble Extractive Value(%)	Alcohol Soluble Extractive Value(%)
Jayphal	3.96	0.1	9.2	12.2
Javitri	5.52	0.1	33	29
Datura	13.2	1.1	29.2	13
Vidari	10.95	1.2	49	11.2
Shatavari	2.04	1.0	23	28
LPH Formulation	32.2	1.2	34.2	18.6
Marketed formulation	23.23	0.8	29.4	20.98

Table 4: Micromeritics properties of Herbomineral formulation

Formulation	Bulk Density# (g/ml)	Tapped Density# (g/ml)	Hausner Ratio#	Carr's Index#
LPH Formulation	0.624±0.0040	0.825±0.011	1.322992±0.016	24.41373±0.95

#N=3±S.D.

Table 5: Chemical tests of Herbs

Herbs	Tests Reagent	Observations
Jayphal	5% $FeCl_3$	No Change in Colour
Javitri	5% $FeCl_3$	No Change in Colour
Datura	Vitali Morin Test	Violet Colour
Vidari	5% $FeCl_3$	No Change in Colour
Shatavari	80% H_2SO_4	Deep Yellow Colour
LPH Formulation	5% $FeCl_3$	No Change in Colour
Marketed Formulation	5% $FeCl_3$	No Change in Colour

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Table 6: Phytochemical test of LPH formulation and Marketed formulation

Test	Reagents	Observation	Sign (LPH Formulation)	Sign (Marketed Formulation)
Test for Alkaloids				
a. Mayer's test	Potassium mercuric iodide solution	Cream precipitate	+	+
b. Dragondroff's test	Potassium bismuth iodide solution	Raddish brown precipitate	+	+
c. Wagner's tests	Iodine potassium solution	Brown precipitate	+	+
d. Hager's tests	Saturated solution of picric acid	Yellow colour	+	+
Test for carbohydrates				
a. Molish test	Alcoholic a-naphthol+ Sulphuric acid	Purple to violet colour rings	+	+
b. Selivanoff's tests	Selivanoff's reagents	Rose colour (ketone)	+	+
c. Barfoed's tests	Barfoed reagents	Red colour (monosaccharide) after 10 min. colour form(disaccharide)	+	+
Test for Saponin				
a. Foam test	Water	Foam persists for 10 min	+	+
Test for Steroids				
a. Salkowaski test	Chloroform and Conc. H2SO4	Chloroform layer -Red colour Acid layer - Greenish yellow fluorescence	+	+
b. Lieberman Burchardt tests	Chloroform, acetic anhydride and Conc. H2SO4	reddish ring	+	+
Test for Proteins				
a. Ninhydrine tests	Ninhydrin solution	Violet colour	+	+
b. Millon's tests	Millon reagents	White precipitate	+	+

Table 7: Thin Layer Chromatography of Herbs, LPH Formulation and Marketed formulation and their R_f value

Herbs/Compound	Mobile Phase	R_f of Ingredients	R_f of LPH Formulation	R_f of Marketed Formulation
Jayphal	Ethyl acetate : Glacial Acetic acid (2:3)	0.72	0.72	0.73
Javitri	Ethyl acetate : Glacial Acetic acid (2:3)	0.71	0.7	0.69
Datura	Chloroform: Methanol (80:20)	0.98	0.97	0.98
Vidari	Toluene : Ethyl Acetate : Methanol (80 : 20 : 0.5)	0.45	0.44	0.45
Shatavari	n-Butanol : Acetic Acid : Water (4: 1 :5)	0.68	0.67	0.67

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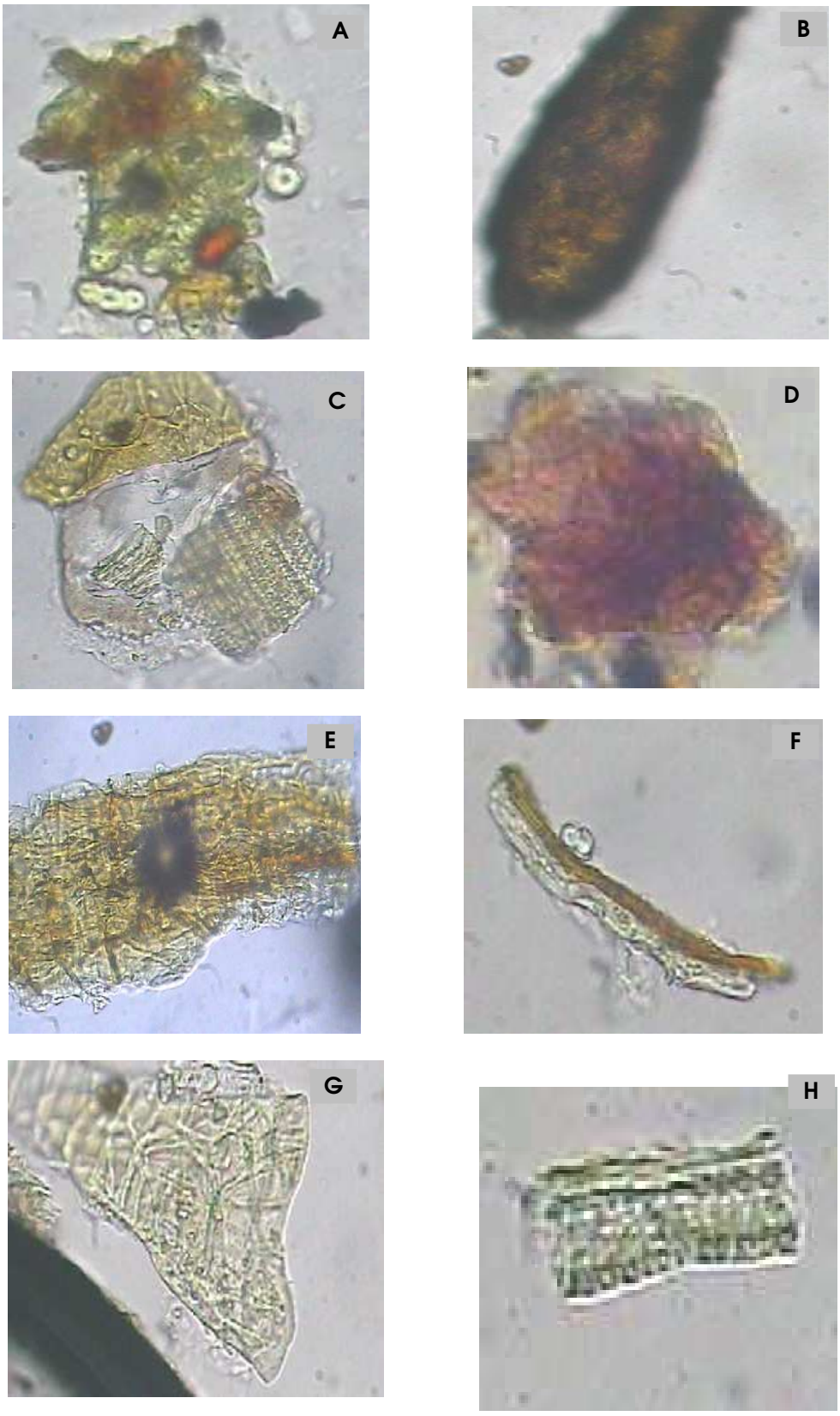

Figure 1: A-Aleurone grains with Less regular crystalloids of *Myristica fragrans ;* B-Non-glandular Tricomes of *Datura metel*; C- Aleurone Grains of *Myristica fragrans (Javitri);* D- Stone cells of *Myristica fragrans (Javitri);* E & F- Lignified thick walled cells of *Asparagus raceomosus;* G- Prismatic Ca. oxalate crystals of *Pueraria tuberosa;* H- *Vessels* of *Pueraria tuberose*

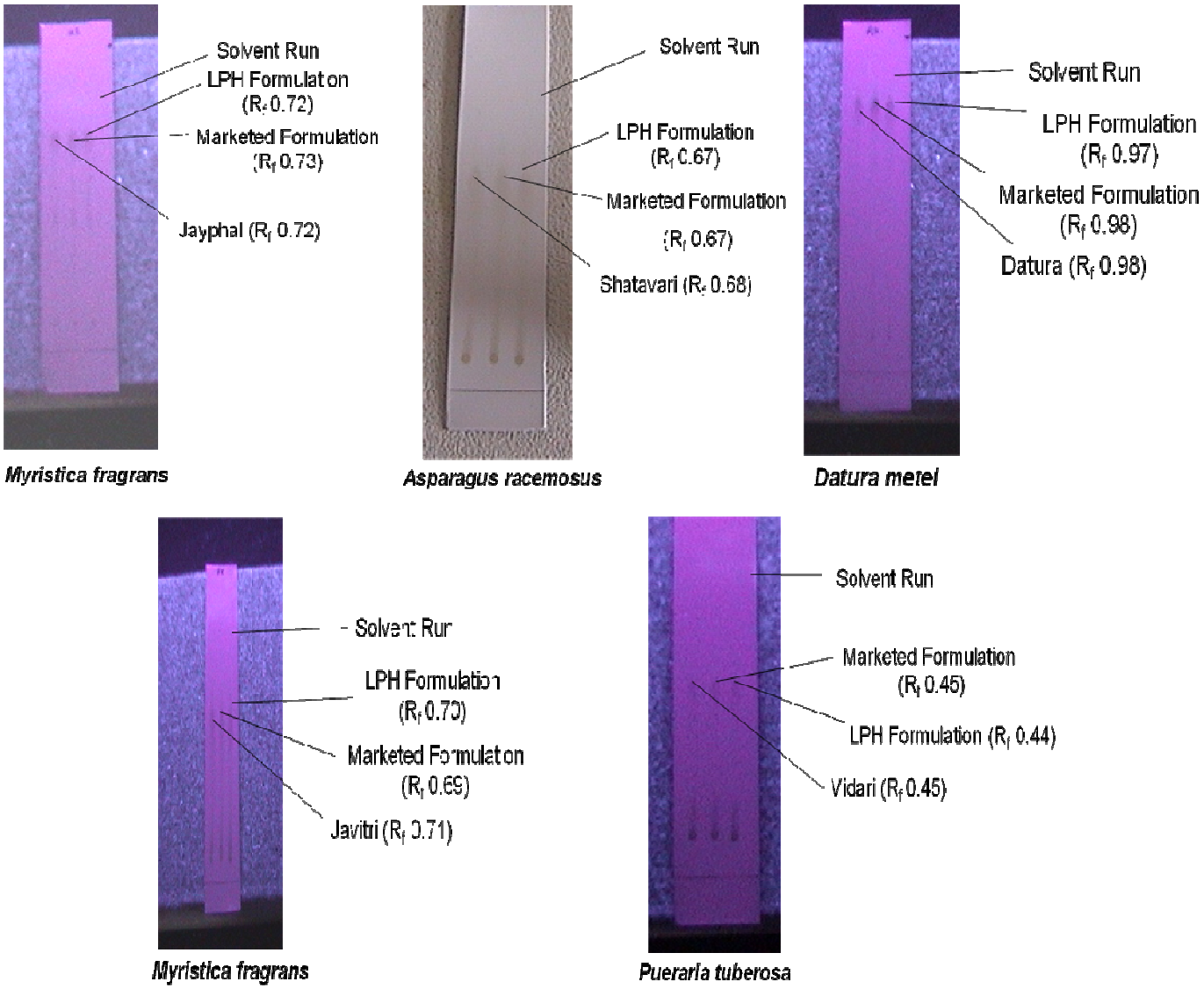

Figure 2: Thin Layer Chromatography of Herbs, LPH formulation and Marketed formulation

RESULT AND DISCUSSION

The LPH formulation was evaluated as per the WHO guidelines. Pharmacognostical parameters revealed that the LPH formulation was blackish brown in color with pleasant odour and taste was slightly bitter (table 1). Microscopical studies showed the presence of Aleurone grains, Stone cells, Tricomes and Fibers (table 2). It also showed the presence of Lignified thick walled cells, stone cells with brownish matter, calcium oxalate crystals and starch grain (Fig. 1). Physico-chemical parameters of LPH formulation were tabulated in table 3. Total Ash value of plant material indicated the amount of minerals, and earthy materials present in the plant material. Analytical results showed the Total Ash value was 23.23% w/w. The amount of acid-insoluble siliceous matter present in the plant was 0.8% w/w. The water-soluble extractive value indicated the presence of sugar, acids, and inorganic compounds. The alcohol soluble extractive values indicated the presence of polar constituents. Micromeritics characteristics of LPH formulation were tabulated in table 4. The flow ability of the LPH formulation was found to be

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passable at carrr' index was found to be 24.41373±0.95, which was further confirmed by high value (1.322992±0.016) of Hausner ratio. The results obtained from phytochemical screening reveals the presence of phytoconstituents like alkaloids, carbohydrate, saponins, proteins and steroids, in LPH formulation (Table 5,6). TLC profile of herbs, LPH formulation and marketed formulation was developed. Thin layer chromatography (Table 7) showed different spots (Fig. 2) that indicate the presence of different herbs in LPH formulation.

Conclusion

The present study involved the pharmacognostical and physicochemical analysis of a poly herbomineral formulation. We had studied various morphology, microscopy, total ash, acid insoluble ash, alcohol soluble and water soluble extractive value, phytochemical tests, bulk density, tapped density, hausner ratio, carr's index and TLC. The physicochemical standards will serve as preliminary test for the standardization of the formulation. In Thin layer chromatographic studies, Rf value of herbs are more close to Rf value LPH formulation, marketed formulation and standards. This manuscript suggested that TLC plate gives perfect and close results which can be repeated in next future.

These parameters are required for authentication of any herbs and herbomineral formulation and also helpful in standardization and development of the quality control of herbs and herbomineral formulation.

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