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Full Length Original Research Paper

Preparation and Standardization of Polyherbomineral Formulation

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Abstract:
Standardization of herbal formulation is essential in order to assess the quality of drugs. The present paper reports preparation and standardization of a herbomineral formulation which contains *Zingiber officinalis* (rhizome), *Piper longum* (fruit), *Piper nigrum* (fruit), *Emblica officinalis* (fruit,seed), *Terminalia chebula* (mature fruit), *Terminalia belerica* (pericarp of ripe fruit), *Piper retrofractum* (stem), *Coriander sativum* (fruit), *Cuminum cyminum* (fruit), Mercury, sulphur, loh bhasam, abharak bhasam. This Ayurvedic formulation is used to treat cold and cough. Here we discussed and focused about Morphology, Microscopy, Total ash, Acid insoluble ash, Water soluble and Alcohol soluble extractive value, bulk density, tapped density, Carr's index, Hausner ratio, phytochemical tests, and Thin layer chromatography (TLC) etc. These parameters are required for authentication of any herbal drug and its Herbo-mineral formulation.

Keywords: Standardization, Extractive value, Carr's index, Herbo-mineral.

Introduction

In the present era, market of all commodities has become global. Health has been of utmost importance since ancient times for the mankind. Market of health-related products has been active and these products are manufactured at different parts of the world and sold all over. Standardization is necessary to make sure the availability of a uniform product in all parts of the world (1). Standardization assures a consistently stronger product with guaranteed constituents. WHO collaborates and assists health ministries in establishing mechanisms for the introduction of traditional plant medicines into primary healthcare programs, in assessing safety and efficacy, in ensuring adequate supplies, and in the quality control of raw and processed materials (2). The present paper reports the preparation and standardization of herbomineral formulation based on organoleptic characters, physical characteristics, and physicochemical properties.

Materials and Methods

Plant materials were collected from local market of Mathura, U.P. The authenticity of the species of all the herbs was checked and confirmed from Botany department, BSA, College, Mathura, U.P., India.

Preparation of Herbomineral formulation:

The formulation was prepared as per the procedure of Ayurvedic Sarsangrah (3). All the ingredients were powdered separately, passed through 80# sieve, and then mixed together in specified proportions to get uniformly blended herbomineral formulation.

Marketed Sample

Marketed sample of formulation and the LPH formulation were standardized based on their

organoleptic characters, physical characteristics, and physicochemical properties.

Macroscopy of Herbomineral formulation:

The colour, odour and taste of all herbal drugs and formulation were determined.

Microscopy of Herbomineral formulation:

About 2 g of herbs and lab prepared herbo-mineral (LPH) formulation was taken and thoroughly cleaned with chloral hydrate as a clearing agent and then microscopic study (4,5,6,7,8) was done by staining the powder mixture with phloroglucinol, iodine solution and sudan red etc.

Physicochemical studies:

Physicochemical studies like total ash, acid insoluble ash, water soluble and alcohol soluble extractive values were carried out as per the WHO guidelines (4, 5, 6, 7, 9) for individual herbs, LPH formulation and marketed formulation.

Evaluation of micromeritics characteristics:

Various micromeritics characteristics like bulk density, tapped density, Carr's index and hausner's ratio were carried out as per the standard methods (10,11).

Preliminary Phytochemical screening:

The active phytochemical constituents like carbohydrates, alkaloids, steroids, and saponins were identified in aqueous and alcoholic extract of herbs; LPH and marketed formulation (5,12,13,14,15).

Thin layer chromatography:

Identification of compound of Herbo-mineral formulation by TLC

About 10µl of the sample was spotted on precoated Silica gel-G aluminium plates of uniform thickness of 0.5mm as a stationary phase. Thin layer chromatograms were developed by using a mixture of different solvents as a mobile phase. 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 Dragondroff'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 (4,5,6,7,8,12).

Result and Discussion

LPH formulation was evaluated as per the WHO guidelines. Pharmacognostical parameters revealed that the LPH formulation was blackish brown in color with Fragrant, Aromatic, and taste was spicy, slightly saline (table 1). Microscopical studies showed the presence of sclereids, rosettes calcium oxalate, stone cells, starch grains, fixed oil glands (table 2a,2b). It also showed the presence of stone cells with brownish matter, calcium oxalate crystals, starch grain and aleurone grains (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 32.93% w/w. The amount of acid-insoluble siliceous matter present in the plant was 1.1% 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 passable at carrr' index was found to be 24.4898±0.81, which was further confirmed by high value of Hausner ratio. The results obtained from phytochemical screening reveals that phytoconstituents like carbohydrate, alkaloids,

proteins, Steroids, and sapposins 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 preparation and standardization of polyherbo-mineral formulation. We had done pharmacognostic study of polyherbo-mineral formulation. We had studied various morphology, microscopy, total ash, acid insoluble ash, ater soluble and alcohol soluble extractive value, bulk density, tapped density, carr's index, hausner ratio, phytochemical tests and TLC. In Thin layer chromatographic studies, Rf value of herbs are more close to Rf value LPH formulation, marketed formulation and standards. This suggested that a precoated TLC plate gives perfect and close results which can be repeated in next future.

These parameters are required for authentication of any herbal drug and its Herbo-mineral formulation and also helpful in standardization and development of the quality control protocol of Herbomineral formulation.

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

Ingredients	Colour	Odour	Taste
Zingiber officinalis (Ginger)	Buff	Agreeable, Aromatic	Agreeable, Aromatic
Piper longum (Pipali)	Greenish black –Black	Aromatic	Pungent
Piper nigrum (Kali mirch)	Greyish black – Black	Aromatic	Pungent
Emblica officinalis (Amla)	Grey – Black	-	Sour ,Astringent
Terminalia chebula (Hare)	Yellowish brown	-	Astringent
Terminalia belerica (Bahera)	Whitish shine,Grey –greyish brown	-	Astringent
Piper retrofractum (Chavya)	Greyish brown	Peppery	Acrid
Coriander sativum (Coriander)	Fawn –Brown	Aromatic	Spicy, Characteristic
Cuminum cyminum (Jeera)	Greenish brown	Aromatic, Characteristic	Spicy
Mercury	Silver	-	-
Sulphur	Greenish yellow	-	-
Loh Bhasma	Dark Brown	-	-
Abharak Bhasma	Brown	-	-
LPH Formulation	Blackish brown	Fragrant , Aromatic	Spicy, Slightly saline
Marketed Formulation	Blackish Brown	Fragrant , Aromatic	Spicy, Slightly saline

Table 2a: Preliminary microscopy of Herbs

Slide description	Observation	Inferences
Powdered drug + Chloral hydrate + Heated and mounted with glycerin	Calcium oxalate crystals	Calcium oxalate crystals
Powdered drug + Chloral hydrate + Phloroglucinol mounted with glycerin	Brownish Structure	Stone cells
Powdered drug + Chloral hydrate + Iodine solution mounted with glycerin	Violet colour Grains	Starch grains
Powdered drug + Chloral hydrate + Phloroglucinol + Picric acid 1%	Greenish yellow grains	Aleurone grains

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

Herbs	Standard	Observed
Zingiber officinalis (Ginger)	Flattened Starch grains(Oblong to Sub rectangular to oval), Fibers associated with vessels	Starch Grains (Flattened, Oval to Sub rectangular)
Piper longum (Pipali)	Starch grains (3-8 µm), Stone cells (Oval to Elongated)	Starch grains (3-8 µm), Stone cells (Oval to Elongated)
Piper nigrum (Kali mirch)	Slightly Elongated Stone cells , Starch grains	Slightly Elongated Stone cells , Starch grains
Emblica officinalis (Amla)	Irregular Silica crystals , Stone cells	Irregular Silica crystal Aggregates, Stone cells with Starch grains
Terminalia chebula (Hare)	Group of Sclereids, Fibers , Vessels	Sclereids
Terminalia belerica (Bahera)	Rosettes Shaped Calcium oxalate crystals , Stone cells in Parancymatous cells	Rosettes Calcium oxalate, Stone cell
Piper retrofractum (Chavya)	Round to Oval Starch grains, Vessels , Fibers	Round to Oval Starch grains
Coriander sativum (Coriander)	Prismatic Calcium oxalate crystals, Fixed oil glands	Prismatic Calcium oxalate crystals, Fixed oil glands
Cuminum cyminum (Jeera)	Fixed oil glands, Short , Bristle Hairs	Fixed oil glands
LPH Formulation	-	Sclereids, Rosettes Calcium oxalate, Stone cells, Starch grains, Fixed oil glands
Marketed formulation	-	Rosettes Calcium oxalate, Stone cells, Starch grains, Fixed oil glands, Fibers, vessels

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(%)
Zingiber officinalis (Ginger)	2.49	0.3	19.6	12
Piper longum (Pipali)	3.15	0.8	11.2	18
Piper nigrum (Kali mirch)	2.58	0.9	24.7	22
Emblica officinalis (Amla)	4.06	0.5	57.9	59
Terminalia chebula (Hare)	3.47	0.9	73.0	63
Terminalia belerica (Bahera)	4.64	0.9	60.7	15.67
Piper retrofractum (Chavya)	8.05	0.8	18.88	9.3
Coriander sativum (Coriander)	4.34	0.9	33.56	19.0
Cuminum cyminum (Jeera)	5.62	0.2	39.12	18.56
LPH Formulation	32.93	1.1	36.7	21.3
Marketed Formulation	34.18	1.5	30.2	18.9

Table 4: Micromeritics properties of Herbomineral formulation

Formulation	Bulk Density# (g/ml)	Tapped Density# (g/ml)	Hausner Ratio#	Carr's Index#
LPH Formulation	0.625±0.0018	0.827±0.007	1.324324±0.014	24.4898±0.81

#N=3±S.D.

Table 5: Chemical tests of Herbs

Herbs	Tests Reagent	Observations
Zingiber officinalis (Ginger)	Boil with 5% KOH	Pungency Destroyed
Piper longum (Pipali)	10% NaOH	Brown Colour
Piper nigrum (Kali mirch)	5% Iodine	Black Colour
Emblica officinalis (Amla)	Ferric chloride	Bluish Black Colour
Terminalia chebula (Hare)	Ferric chloride	Bluish Black Colour
Terminalia belerica (Bahera)	Ferric chloride 50%HNO_3	Brownish Green Brick Red Colour
Piper retrofractum (Chavya)	5% Iodine	Black Colour
Coriander sativum (Coriander)	5% $FeCl_3$	No Change in Colour
Cuminum cyminum (Jeera)	Alcoholic Solution of Sudan III	Red Colour

Table 6: Phytochemical test of LPH formulation and Marketed formulation

Test	Reagents	Observation	Sign (LPH Formulation)	Sign (Marketed Formulation)
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(keton)	+	+
c. Barfoed's tests	Barfoed reagents	Red colour (monosaccharide) after 10 min.colour form(disaccharide)	+	+
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 Proteins				
a. Ninhydrine tests	Ninhydrin solution	Violet colour	+	+
b. Millon's tests	Millon reagents	White precipitate	+	+
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 Saponin				
a. Foam test	Water	Foam persists for 10 min	+	+

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

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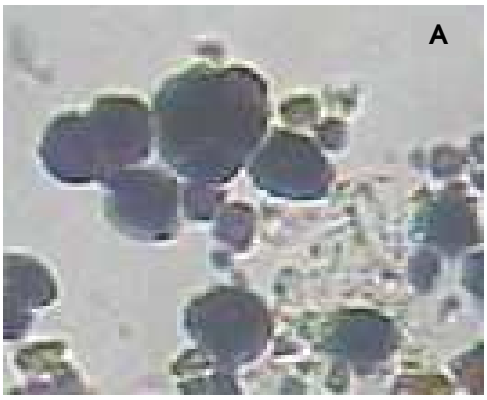
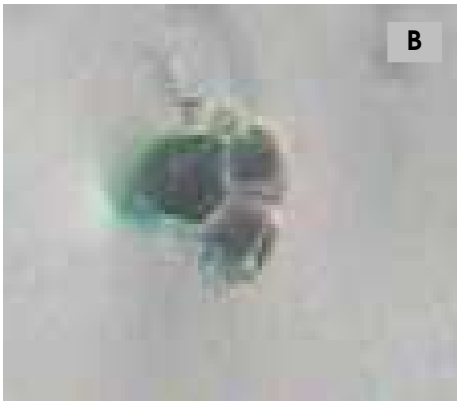
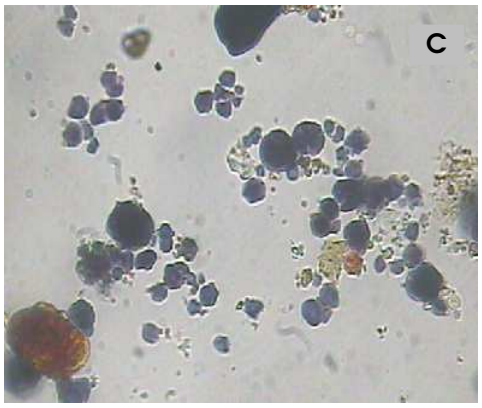
Herbs/Compound	Mobile Phase	R_f of Ingredients	R_f of LPH Formulation	R_f of Marketed Formulation
Zingiber officinalis (Ginger)	Hexane: Diethyl ether (2:3)	0.59	0.58	0.61
Piper longum (Pipali)	Toluene: Ethyl acetate (9:1)	0.35	0.32	0.34
Piper nigrum (Kali Mirch)	Toluene: Ethyl acetate (7:3)	0.48	0.48	0.47
Emblica officinalis(Amla)	Toluene: Ethyl acetate (93:7)	0.39	0.42	0.40
Terminalia chebula (Hare)	n-propanol: Ethyl acetate: Water: Glacial acetic acid (40:40:20:1)	0.63	0.64	0.63
Terminalia belerica(Bahera)	n-propanol: Ethyl acetate: Water: Glacial acetic acid (40:40:20:1)	0.78	0.79	0.79
Piper retrofractum (Chavya)	Pet. Ether: Benzene (2:11)	0.82	0.8	0.81
Coriander sativum (Coriander)	Toluene: Ethyl acetate (9.3:0.7)	0.27	0.29	0.32
Cuminum cyminum (Jeera)	Toluene: Ethyl acetate (9.3:0.7)	0.29	0.3	0.28







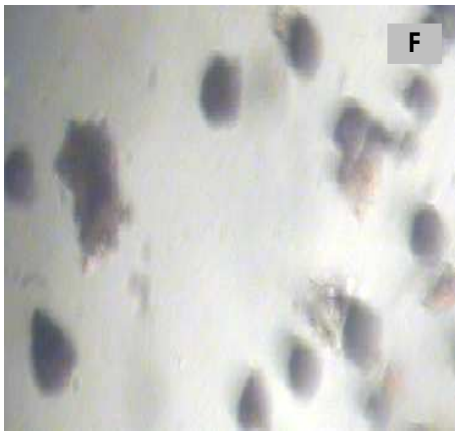


Figure 1: A-Oval (Flattened) starch grains of *Zingiber officinalis;* B-Subrectangular starch grains of *Zingiber officinalis*; C- Starch grains (Oval) & Stone cells of *Piper longum;* D- Starch grains (Elongated) & Stone cells of *Piper nigrum;* E- Group of sclereids of *Terminalia chebula;* F- Round to Oval starch grains of *Piper retrofractum;* G- Irregular Silica crystal aggregates *of Emblica officinalis;* H- Stone cells with Starch grains *of Emblica officinalis;* I- Rosettes Calcium oxalate of *Terminalia belerica;* J- Stone cell *of Terminalia belerica;* K- Prism shape Calcium oxalate crystals of *Coriander sativum;* L- Oil glands *of Coriander sativum;* M- Oil glands of *Cuminum cyminum.*

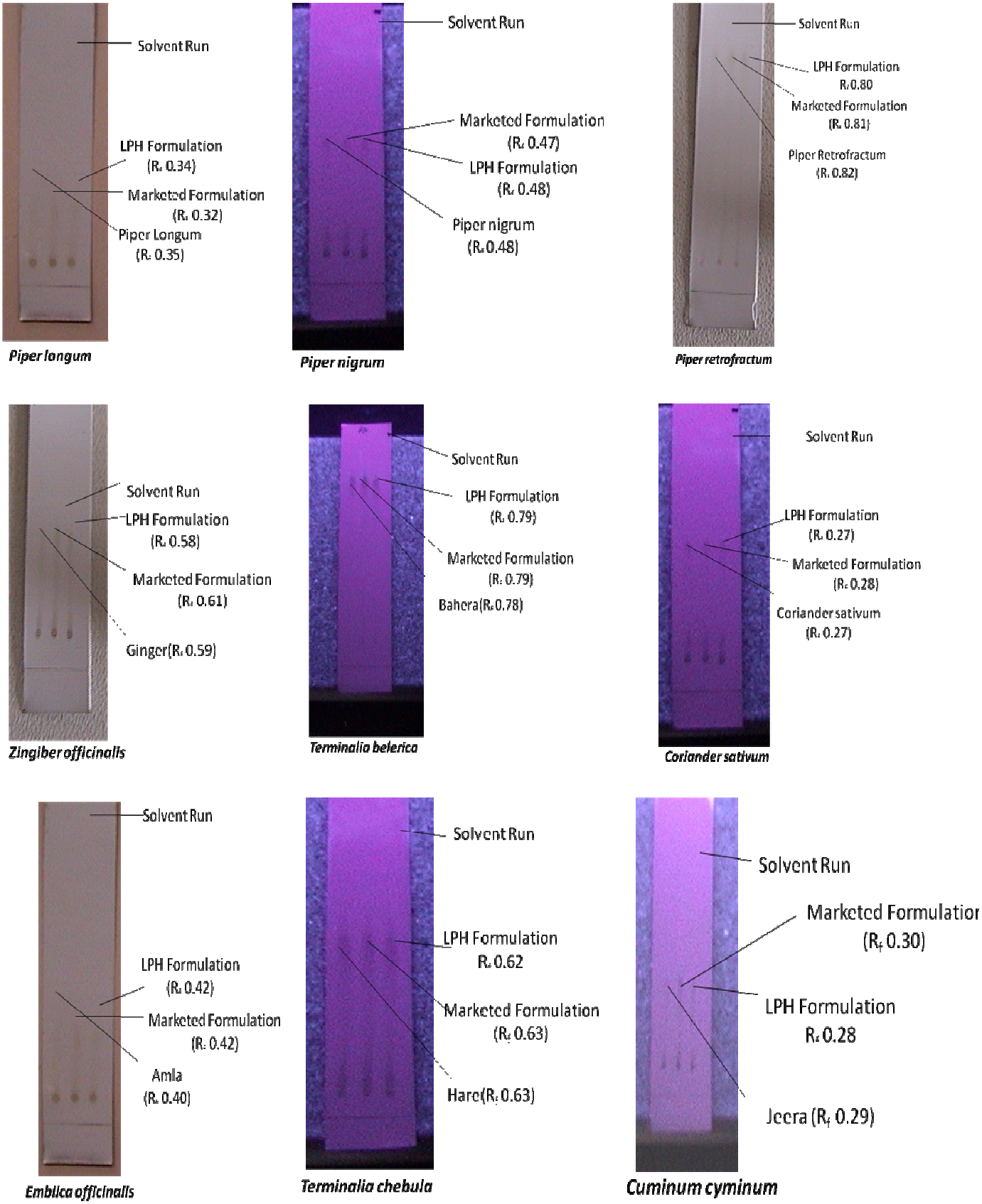


Figure 3: Thin Layer Chromatography of Herbs, LPH formulation and Marketed standard

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