Original Article

The Standardization of *Daruharidradi Ghanavati*

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Abstract

Background: Many effective formulations are available in Ayurveda for various diseases. These formulations are lagging in standardization due to the absence of reference standards, whereas maintaining quality standards of given medicines is the need of an hour. *Daruharidradi Ghanvati* is one such combination of six herbal drugs containing *Daruharidra, Meshshrungi, Vijaysar, Mamajjak, Jambubija,* and *Methikabij*. Each drug is described in various *Ayurvedic* antidiabetic formulations**. Aim:** The present study was aimed at setting a standard pharmacognostical and pharmaceutical profile of *Daruharidradi Ghanavati*. **Materials and Methods:** The study included the preparation of *Daruharidradi Ghanavati* using raw drugs. Later, *Daruharidradi Ghanavati* was subjected to pharmacognostical, physiochemical, and thin-layer chromatography analysis as per standard protocols. **Results:** The final observations were recorded. Pharmacognostical findings matched with that of individual raw drugs with no major change in the microscopic structure of the raw drugs during the preparation of *Ghanavati*. **Conclusion:** The quality of *Daruharidradi Ghanavati* tablet can be tested by pharmacognostical, physiochemical screening for the observations of the present study.

Keywords: *Daruharidradi Ghanavati, diabetes mellitus, high-performance liquid chromatography, thin-layer chromatography*

Introduction

To increase the global acceptability of herbal drugs and to prove their clinical efficacy, it is very important to standardize Ayurvedic herbal formulations. World Health Organization has also considered phytotherapy in its health programs and has been promoting traditional medicines for the last few decades. It has suggested basic guidelines and procedures for the validation of Ayurvedic drugs.[1] Standardization is necessary to ensure the authenticity, quality, strength, and purity of Ayurvedic formulations. Thus, to standardize these medications as safe drugs, establishing various parameters using modern techniques for analysis is very important. Ayurvedic formulations are available in the form of *Panchvidha Kashay Kalpna* such as *Swaras*, *Kvath*, *Vati, Ghana, Churna, Arishta, Avlehas,* and *Ghritas*. In the present scenario, *Ghana* is widely acceptable dosage form due to its advantages such as easy administration, shelf life, and palatability. Considering this concept, *Daruharidradi Ghanavati* is prepared.

The global burden due to diabetes mellitus (DM) is mostly contributed by type 2 diabetes which constitutes 80%–90% of the total diabetic population. The estimates by the International Diabetes Federation show that 285 million adults (20–79 years) are affected by the disorder in 2010[2] and that without proper control and prevention, its prevalence will increase further to 438 million in 2030. This accounts for a global increase by 54%, its prevalence of 6.6%–7.8% in 20 years. Nearly, 70% of people with diabetes live in developing countries; the largest numbers are in the Indian subcontinent and China.[2]

A lifestyle measure such as increased physical activity and dietary modifications are an important component in the management of DM. Our ancient physicians *Charaka* and *Sushruta* stressed on the role of exercise and dietary management in the treatment of DM.[3] Oral hypoglycemic agents (OHAs) have been used for more than five decades in the management of type 2 DM. There are so many groups of OHAs available in the market for the management of type 2 DM. Every OHA has some side effects such as cardiovascular disease and other side effects.

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Daruharidra, Meshshrungi, Vijaysar, Mamajak, Jambubija, and *Methikabij* are few drugs mentioned in *Ayurvedic* texts which are said to have antidiabetic properties individually. *Daruharidra* manages blood sugar levels by increasing the metabolism of glucose and preventing further formation of glucose. It inhibits the formation of fat cells in the body and helps in weight management. It also lowers the bad cholesterol levels in the body. This is mainly due to the active constituent berberine present in *Daruharidra* which has potent antioxidant and anti-inflammatory properties.[4] Extract of *Meshshrungi* was found to bring blood glucose homeostasis, by increasing serum insulin levels. The islets of Langerhans appear to be restored or regulated by the herbal extract. Increased glycoprotein is the major metabolic abnormality in DM which results in nephropathy, retinopathy, and micro- and macroangiopathy, and is brought under control by the administration of the leaf extract.[5] According to one of the studies conducted, several antidiabetic principles (epicatechin, pterosupin, marsupin, and pterostilbene) have been identified in the *Pterocarpus marsupium*. However, it is seen that *Pterocarpus marsupium* extract substantially prevented hypertriglyceridemic levels and hyperinsulinemia. This study suggests the usefulness of *Pterocarpus marsupium* bark (*Vijayasar*) in insulin resistance, the associated disorder of type 2 diabetes; however, future studies are required to certify their efficacy and safety before clinical scenarios. *Mamajjaka* (*Enicostemma littorale*) is a frequently used drug for the treatment of DM. There is no direct reference available for its antihyperglycemic activity in Ayurvedic classics. *Daruharidra, Meshshrungi, Vijaysar, Mamajak, Jambubija,* and *Methikabij* are proven drugs in *Madumeha*. *Bhavmishra* has also suggested *pramehgna* properties of these drugs.[6] The aim of the study is to standardize the combination of these drugs for the research community and evaluate its effect on the basis of further studies.

Materials and Methods

Collection and authentication of raw drugs

Daruharidradi Ghanavati contains *Daruharidra*, *Meshshrungi*, *Vijaysar*, *Mamajjak*, *Jambubija,* and *Methikabij*. *Daruharidradi Ghanavati* was prepared in standard "Good Manufacturing Practices" certified company. Acacia catechu was used as the binding agent while preparing *Daruharidra Ghanavati* [Table 1].[7] Authentication of all the individual drugs was done.

Analytical study

Daruharidradi Ghana was analyzed by employing various analytical parameters. Organoleptic characteristics (color and taste) and physiochemical analysis such as loss on drying at 105°C,[9] ash value,[10] acid-insoluble ash,[11] pH value,[12] water-soluble extractives,[8] and methanol-soluble extractives[13] were carried out. Daruharidradi Ghana was subjected to further analysis such as quantitative estimation for the presence of heavy metals[14] (lead, arsenic, cadmium, and mercury) and tests for microbial contamination.

Table 1: Details of ingredients of *Daruharidradi Ghanvati*[8]

Ingredient's name	Latin name	Family	Proportion
Daruharidra	*Berberis aristata*	*Berberidaceae*	1
Meshashringi	*Gymnema sylvestre*	*Asclepiadaceae*	1
Vijayasara	*Pterocarpus marsupium*	*Leguminaseae*	1
Mamajjaka	*Enicostema littorale*	*Gentianacea*	1
Jambubija	*Syzygium cumini*	*Myrtaceae*	1
Methikabija	*Trigonella foenugreek*	*Leguminaseae*	1

Method of preparation of *Daruharidradi Ghanavati*

The drugs mentioned in Table 1, taken in equal proportions were properly dried and pulverized into a coarse powder. All these drugs were used to prepare decoction as per the classical method. Semisolid consistency was obtained by heating the decoction appropriately. Formed granules of these six drugs were punched into tablets of 500 mg each and the uncoated tablets were packed in airtight packaging. The whole process was carried out in the pharmacy under all the aseptic conditions.

High-performance liquid chromatography

Preparation of sample solution

Accurately weighed powdered pellets equivalent to the content of each capsule in 250 ml were taken in an iodine flask. Thirty ml of methanol was added and refluxed at 60°C for 45 min, then cooled and filtered. The remaining residues were again refluxed with 30 ml methanol for 20 min, the same procedure was repeated for two times. Again, the washing was cooled, filtered, and combined. Then, it was completely dried on water bath. Dry residue was reconstituted with 5 ml of methanol in volumetric flask.

Preparation of mobile phase

For *Daruharidra* (*Berberis aristata*), ethyl acetate, methanol, and water were taken in the ratio of 8:2:0.5 v/v in a suitable volumetric flask and were mixed well. Similarly, for *Meshashrungi* (*Gymnema sylvestre*), chloroform and methanol were taken in the ratio of 9:1 v/v. For *Vijayasara* (*Pterocarpus marsupium*), toluene, ethyl acetate, and formic acid were taken in the ratio of 4:5:1 v/v. For *Mamajjaka* (*Enicostemma littorale*), toluene, ethyl acetate, and formic acid were taken in the ratio of 8:2:0.1 v/v. For *Jambubija* (*Syzygium cumini*), ethyl acetate, methanol, water, and glacial acetic acid were taken in the ratio of 8:1:1:0.1 v/v. For *Methikabij* (*Trigonella foenum-graecum*), toluene, ethyl acetate, and formic acid were taken in the ratio of 5:4:1 v/v, each in suitable volumetric flask separately and mixed well.

The tank was saturated by pouring sufficient quantity of the mobile phase to form a layer of solvent 5–10 mm deep and was closed and allowed to stand for 1 h at room temperature. Narrow strip of the coating substance was removed about 5 mm wide from the vertical sides of the plate and applied to the plate 5 µl of each solution and allowed to dry in air. The plate was placed in a saturated chamber vertically. The mobile phase was allowed to rise to 10 cm. The plate was dried using an HPTLC Plate Heater. The developed plate was visualized under visible daylight, short ultraviolet (UV) (254 nm), and long UV (366 nm), and after spraying with the vanillin–sulfuric acid, the reagent was again observed in daylight.

Test for microbial limits

Determination of total aerobic count, determination of yeast and molds, determination of *Escherichia coli,* enumeration of *Salmonella,* and enumeration of bile tolerant Gram-negative bacteria tests were carried out as per standard methods to determine the microbial load in *Daruharidradi Ghanvati*.[15]

Other tests

Parameters such as color, taste, uniformity of weight, disintegration time, hardness, and heavy metal presence were checked. Organoleptic characters, average weight, and physicochemical analysis of all the samples were carried out. Quantitative analysis for disintegration time was checked according to the prescribed standard methods in Indian Pharmacopoeia. Heavy metal analysis for lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) were carried out using atomic absorption spectrometry. Thin-layer chromatography (TLC) was used to detect the presence of four important secondary metabolites, namely, steroids, terpenoids, flavonoids, and alkaloids, in the Ayurvedic preparation.

Results

The quantitative parameters of *Daruharidradi Ghanavati* are given in Table 2. In the absence of the marker compound, the HPTLC profile of Daruharidrada, *Meshshrungi*, *Vijaysar*, *Mamajak*, *Jambubija,* and *Methikabij* was attempted. Visual spots and R_f values were recorded [Table 3 and Figure 1]. This particular study can be considered standard for further research work.

In the present study, the level of heavy metals (Pb, As, Hg, and Cd) was checked by the ASS method and was found to be in normal limits set by the Ayurvedic Pharmacopeia of India (API). The level of lead was 10 ppm, that of arsenic was found to be 3 ppm, mercury 1 ppm, and cadmium levels recorded were 0.3 ppm.

The microbial profile of *Daruharidradi Ghanavati* was found satisfactory. The total bacterial plate count was an average of 104 cfu/g, while that of the fungal count was 103 cfu/g and the total viable aerobic count was 105 cfu/g. These recorded values were under the limit set by API. Pathogenic organisms such as *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Salmonella* were found to be absent in the formulation. A test for aflatoxins was also conducted in the study. Aflatoxins B1 was recorded at 2.0 ppb and aflatoxins B1+ B2+ G1+ G2 found was 5.0 ppb. These recorded values were under the limit as per the Laboratory Guide for the Analysis of Ayurveda and Siddha Formulation.[15] These results revealed that the formulation developed was safe for consumption.

Table 2: Quantitative parameters of *Daruharidradi Ghanavati*

Test	Specification
Description	Brown colored, circular, compressed flat, uncoated tablets
Diameter	10–11 (mm)
Thickness	4–6 (mm)
Loss on drying (at 105°C)	NMT 2%w/w
Friability	NMT 1%w/w
Hardness	1–4 kg/cm^2
Uniformity of weight	0.475–0.525 (g)
Disintegration time	NMT 30 (min)

NMT: Not more than

Table 3: High-performance liquid chromatography profile of *Daruharidradi Ghanavati*

Drug	TLC plate (nm)	Number of spots	Rf value
Daruharidra	254	5	0.22, 0.38, 0.46, 0.61 and 0.80
Meshshringi	254	2	0.21 and 0.70
Vijayasara	254	4	0.33, 0.50, 0.69 and 0.78
Mamajjaka	254	5	0.23, 0.31, 0.42, 0.61 and 0.78
Jambubija	254	5	0.14, 0.24, 0.40, 0.56 and 0.78
Methikabij	254	4	0.27, 0.42, 0.59–0.77

TLC: Thin-layer chromatography

Discussion

To ensure the quality, strength, purity, and authenticity, standardization and quality control of Ayurvedic formulations is necessary. The present work deals with physiochemical analysis, TLC, HPTLC, microbial profile, and heavy metals analysis of *Daruharidradi Ghanavati*. The organoleptic parameters form the basic criteria to confirm the finished product. This is the primary character to assess the quality of tablets.

The color was buff, the taste was bitter, and the odor was characteristic due to the specific properties of the various ingredients. The results of physicochemical parameters, namely average weight (0.5059 g) and disintegration time (56 min), were found [Table 4]. TLC fingerprint profiles of the formulations were compelling. Heavy metals present in a drug can be carcinogenic and toxic. In the present study, the level of heavy metals (Pb, Cd, As, and Hg) was checked by means of AAS (atomic absorption

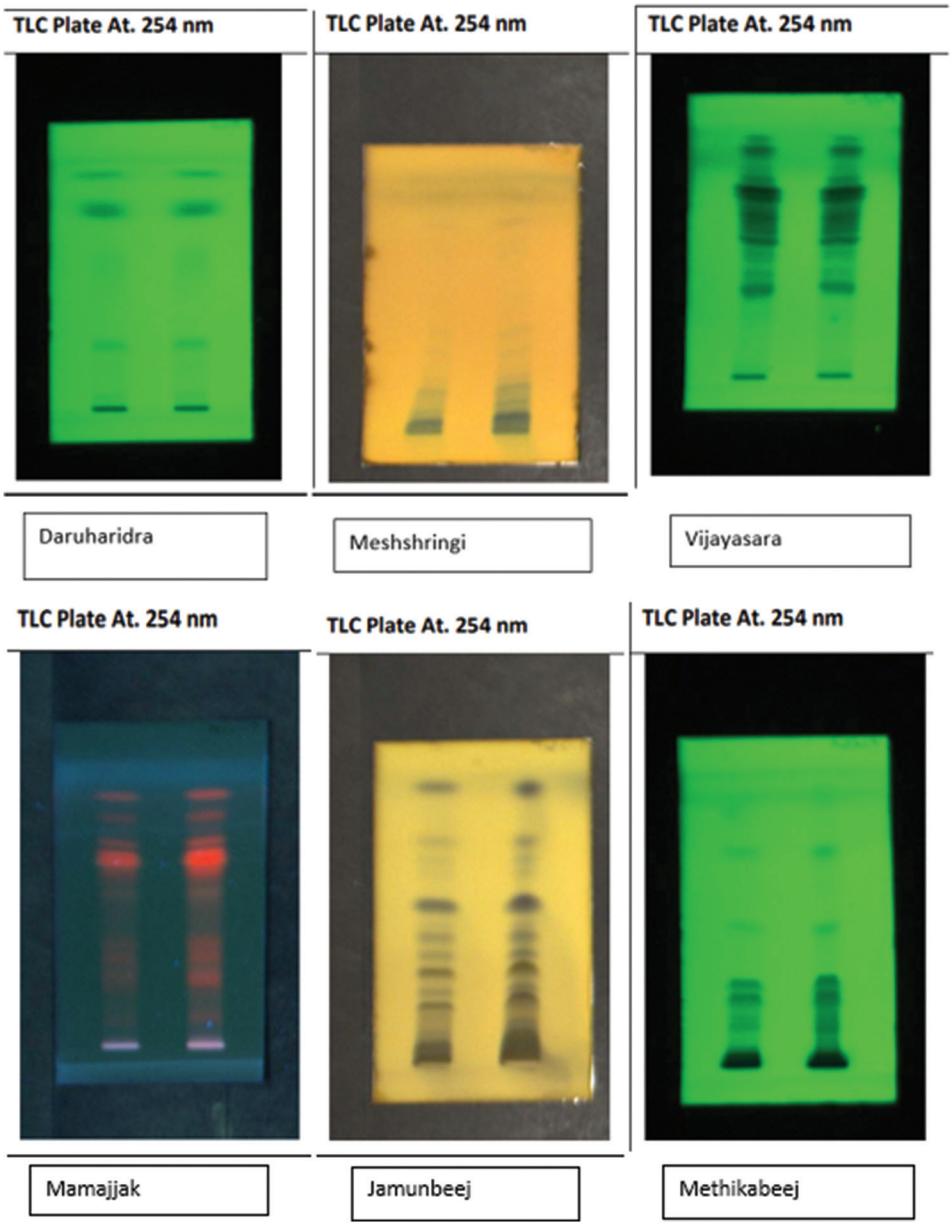

Figure 1: Thin-layer chromatography (TLC) monographs of the components of *Daruharidradi Ghanavati.* TLC precoated plate with Silica gel 60 F254 of 0.2 mm thickness; visualization under 254 nm of ultraviolet light showing major spots at different Rf values. TLC: Thin-layer chromatography

Table 4: Physiochemical data of *Daruharidradi Ghanavati*

Parameters	Specification
Loss on drying (on 105°C)	NMT 2%w/w
pH value (10% aqueous solution)	4–5
Total ash	NMT 18%w/w
Acid-insoluble ash	NMT 8%w/w
Alcohol-soluble extractive	NLT 25%w/w
Water-soluble extractive	NLT 25%w/w
Total sugar	NMT 25%w/w
Reducing sugar	NMT 2%w/w
Nonreducing sugar	NMT 23%w/w

NLT: Not less than; NMT: Not more than

spectrophotometry) and found to be within the limit set by the API. The microbial profile of the *Daruharidradi Ghanavati* was found satisfactory. The total bacterial plate count was an average of 80cfu/g while the yeast and molds counts showed an average of <10 cfu/g. These recorded levels are less than the limit set by API. Pathogenic bacteria, i.e., *E. coli, Salmonella,* and bile-tolerant Gram-negative bacteria were found to be absent in the formulation.

These results thus showed that the formulation of *Daruharidradi Ghanavati* developed was safe for consumption. Thus, it indicates that during the preparation and packaging of the formulation, proper hygiene norms were followed.

Conclusion

Each drug in the formulation of *Daruharidradi Ghanavati* is widely used as a traditional herb in the present era for its antidiabetic effect. The pharmaceutical standardization of such formulation is in need of an hour for its global acceptance. Physicochemical data as well as TLC, heavy metal profile, and microbial overload are essential parameters followed to develop SOP. The data generated together can be used for quality evaluation and the standardization of compound formulations. This particular work can be taken as standard for the preparation of *Daruharidradi Ghanavati* at a laboratory scale.

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Ethical statement

The study was approved by the institutional Ethics Committee of Govt. Ayurved College and Hospital, Nagpur (GACN/Ph.D.S/22/2018 Date: 23/02/2018).

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Conflicts of interest

There are no conflicts of interest.

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