

Pharmacognostical and pharmaceutical analysis of pramehahar guggulu—An ayurvedic polyherbal formulation for clinical trial of diabetic nephropathy

Kanchan Bala¹, Alankruta R Dave², Harisha CR³, VJ Shukla⁴

¹ M.D. Scholar, Department of Kayachikitsa Institute of Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

² Associate Professor, Department of Kayachikitsa, Institute of Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

³ HOD of Pharmacognosy Department and Institute of Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

⁴ HOD of Pharmaceutical Chemistry Laboratory, Institute of Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Abstract

Background: Diabetic nephropathy is a disorder affecting the kidneys, in the form of damage to the glomerular basement membrane, mesangial cell proliferation and glomerulosclerosis. The kidney involvement is heralded by persistent albuminuria (>300 mg/24 hr or 200µg /min) and is the hallmark of diabetic nephropathy. Diabetic Nephropathy is not directly mentioned with a name in the Ayurvedic classical text books. Because of main characteristic features of albuminuria with hyperlipidemia, it may be included under the title of *prameha* (a disease of urinary system with altered composition, frequency and quantity of urine). Albuminuria makes urine concentrated, viscid or dense. These features can be correlated with *sāndrameha* a subtype of *prameha*.

Aim: To analyze the Pharmacognostical and Pharmaceutical properties of *Pramehahar Guggulu*.

Materials and Methods: *Pramehahar Guggulu* was subjected to microscopic evaluation for pharmacognostical study, physico-chemical analysis like hardness, weight variation, loss on drying, ash value, acid insoluble extract, pH value, water soluble extract, alcohol soluble extract and high performance thin layer chromatography (HPTLC).

Result: Pharmacognostical study showed the presence of certain identifying characteristics of all the ingredients of *Pramehahar Guggulu* that is *Punarnva*, *Guggulu*, *Haridra*, *Shirish*, *Varun*, *Shigru*, *Guduchi*, *Amalki*, *Usheer* and *Dhanyak*. In pharmaceutical study, preliminary physico-chemical analysis showed that hardness of *Pramehahar Guggulu* was 6.8kg/cm², ash value 10.39% w/w, loss on drying 10.17% w/w, water soluble extract 32.6% w/w, alcohol soluble extract 19.2% w/w and pH value 6.5. HPTLC analysis showed eleven spots in 254 nm and nine spots in 365nm.

Conclusion-Present work was carried out to standardize the polyherbal formulation *Pramehahar Guggulu* in terms of its identity, quality and purity. Pharmacognostical and physico-chemical observations revealed the specific characters of all the active constituents in the preparation.

Keywords: diabetic nephropathy, pharmaceuticals, pharmacognosy, pramehahar guggulu

Introduction

Diabetic nephropathy is defined clinically by persistent proteinuria if the following additional criteria are fulfilled and: presence of diabetic retinopathy and absence of clinical or laboratory evidence of other kidney or urinary tract diseases. Diabetic nephropathy is the 3rd leading cause of death, among end stage renal disease patients. Kidney disease subjects had greater prevalence rate of micro-albuminuria with retinopathy and overt nephropathy, logistic regression analysis has shown that HbA1C, smoking, duration of diabetes, blood pressure, were associated with micro-albuminuria. Duration of diabetes and systolic B.P were associated with overt nephropathy. The prevalence of micro-albuminuria and overt diabetic nephropathy has been reported to be 26.9 % and 2.2 % respectively in urban and diabetics in Chennai Urban rural epidemiology study [1]. There is no specific treatment available at present in contemporary science. The optimal therapy for this is prevention; as it necessitates dialysis,

which is much cumbersome. Life style modification is an integral part in its management where a moderate protein diet, low sodium and low potassium diet is encouraged. Considering Diabetic nephropathy under *YapyaVyadhi* is precise, where even a slight variation in the diet brings about increase in proteinuria, which is explained in classics with the lines *Pathyahara Vihararyapyo Yapneeyaha* [2]. The diseases involving *Marma* can be counteracted by careful intervention through *Rasayana* drugs or *Shamana Chikitsa*.

For the present study, *Pramehahar guggulu* (*Anubhut yog*), a polyherbal formulation contains different amount of ten herbal drugs [Table no.1] that is *Punarnva* (*Boerhavia diffusa* Linn.), *Guggulu* (*Commiphora mukul* Hook ex Stocks.), *Haridra* (*Curcuma longa* Linn.), *Shirisha* (*Albizia lebeck* Linn.), *Varuna* (*Crataeva nurvala* Linn.), *Shigru* (*Moringa oleifera* Lam.), *Guduchi* (*Tinospora cordifolia* Linn.), *Amalaki* (*Phyllanthus emblica* Linn.), *Ushira* (*Chrysopogon spinosus* Linn.), *Dhanyak* (*Coriandrum*

sativum Linn.) In *Pramehahara Guggulu* drugs *Punarnava*, *Dhanyaka*, *Varuna*, *Shigru*, *Ushira* etc. having the Mutral properties and has significant effect on *Mutravasrotas*. Drugs like *Haridra*, *Amla*, *Guduchi* are having Anti-diabetic effect as well as *Rasayana* effects as main line of management include to treat the main disease.

Aims and Objectives

1. To evaluate raw drugs of *Pramehahar Guggulu* for authenticity through various pharmacognostical procedures.
2. To develop the pharmacognostical and phyto-chemical profile *Pramehahar Guggulu*.

Materials and Methods

Collection, Identification and Authentication of raw drugs

The raw materials were collected from the pharmacy of Gujarat Ayurved University, Jamnagar. All the raw drugs were identified and authenticated in the Pharmacognosy Laboratory, Institute of Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar.

Preparation of Drug

The *kwatha* prepared out of 32 parts of *Punarnava* and 6 times of *Jala*, according to *Kwathanirman Vidhi*, 8 Parts of *Shodhit Guggulu* was mixed with the *Kwatha* and subjected to *Agni* till the appearance of *Gudapakalakshanas*. Afterwards, mix 8 Parts of other ingredients (one Part each ingredient) be mixed well and *Vati* prepared of the size of one *Shana matra* Weight of each *Vati*, about 500mg. Shape: Round shape Size: About 0.5 cm, greenish greyish in colour with characteristic odour, Hard to touch. Storage: kept in well closed polythene bags. Ingredients of *Pramehahar Guggulu* are as shown in (Table no. 1) This *Vati* was analyzed using various standard physicochemical parameters such as, Loss on drying, PH, water soluble extract, and methanol soluble extract at the pharmaceutical chemistry lab, ITRA, Jamnagar.

Pharmacognostical Study

The Pharmacognostical study comprises of organoleptic study and microscopic study of finished product.

Organoleptic Study

The Organoleptic characters of polyherbal drugs are very important and give the general idea regarding the genuinity of the sample. Organoleptic parameters i.e. taste, colour, odour and touch of *Pramehahar guggulu* were scientifically studied as per the standard references.

Microscopic Study

Pramehahar guggulu was powdered and dissolved with water and microscopy of the sample was done without stain and after staining with phloroglucinol + HCL. Microphotographs of *Pramehahar guggulu* were also taken under Corl-zeisstrinocular microscope [3].

Physico-Chemical Analysis

Pramehahar guggulu as analyzed using various standard physico-chemical parameters. The common parameters mentioned for compressed tablets in Ayurvedic Pharmacopeia of India [4], and CCRAS [5], guidelines are loss on drying, total ash value, acid insoluble ash, pH value, water soluble extract, methanol soluble extra total ash, and water and alcohol soluble extractives.

High Performance Thin Layer Chromatography (HPTLC)

Methanol extract of *Pramehahar guggulu vati* were spotted on pre coated silica gel GF 60254 aluminium plate as 5mm bands, 5mm apart and 1 cm from the edge of the plates, by means of a Camag Linomate V sample applicator fitted with a 100 μ L Hamilton syringe. Toluene (7 ml), Ethyl acetate (2 ml), Acetic acid (1 ml) was used as mobile phase. After Development, Densitometric scanning was performed with a Camag TLC scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of win CATS software (V 1.2.1 Camag). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 20 mm [6, 7].

Results and Discussion

Organoleptic characters of *Pramehahar Guggulu* -

Organoleptic characters contents of *Pramehahar guggulu* like colour, taste, touch, Odor were recorded. The color of *Pramehahar guggulu* was Greenish Grey. *Pramehahar guggulu* contain its characteristic smell and Tikta taste which is shown in Table -2.

Microscopic Study of *Pramehahar Guggulu*-

Identifying characters of ingredients of *Pramehahar guggulu* under the microscope were stone cells of *Varuna*, stone cells of *Shirisha*, stratified fibres of *Ushir*, Rhomboidal crystal of *Shirisha*, Tannin content of *Shirisha*, pitted border of *Guduchi*, starch grains of *Haridra*, annular grain of *Dhanyak*, cork cells of *Guduchi*, Parenchyma cells of *Haridra*, Oil globules of *Ushira*, tannin content of *Varuna*, Collenchyma cells of *Guduchi*, starch grains of *Shigru*, stone cells of *Punarnava* [Plate 1(1 to -15)].

Physico-chemical analysis of *Pramehahar Guggulu*-

Physico-chemical analysis of *Pramehahar Guggulu* revealed the value as hardness 6.8 Kg/cm², ash value 10.39 %w/w, loss on drying 10.17 %w/w, water soluble extract 32.6%w/w, alcohol soluble extract 19.2%w/w and pH value was 6.5. [Table No-3.]

High performance thin layer chromatography of *Pramehahar Guggulu*-

On performing HPTLC, visual observation under UV light showed few spots but on analyzing under densitometer much more was observed and at 254nm, the chromatogram showed 9 peaks with R_f values 0.26, 0.46, 0.57, 0.61, 0.74, 0.82, 0.87, 0.95, 0.99 While at 366nm the chromatogram showed 6 peaks with R_f values 0.26, 0.35, 0.46, 0.65 0.79, 0.99 [Table No-4.]

Table 1: Ingredients of *Pramehahar Guggulu* (*Anubhuta*)

S.NO	Drug name	Botanical name	Part used	Proportion
1	<i>Punarnava</i>	<i>Boerhavia diffusa</i> Linn.	Root	32 part
2	<i>Guggulu</i>	<i>Commiphora mukul</i> Hook ex Stocks.	Gum resin	8 part

3	<i>Haridra</i>	<i>Curcuma longa</i> Linn.	Rhizome	1 part
4	<i>Shirisha</i>	<i>Albizia lebbek</i> Linn.	Bark	1 part
5	<i>Varuna</i>	<i>Crataeva nurvala</i> Linn.	Root	1 part
6	<i>Shigru</i>	<i>Moringa oleifera</i> Lam.	Root	1 part
7	<i>Guduchi</i>	<i>Tinospora cordifolia</i> Linn.	Stem	1 part
8	<i>Amalaki</i>	<i>Phyllanthus emblica</i> Linn.	Fruit	1 part
9	<i>Ushira</i>	<i>Chrysopogon spinosus</i> Linn.	Root	1 part.
10.	<i>Dhanyak</i>	<i>Coriandrum sativum</i> Linn.	Whole plant	1part

Table 2: Organoleptic characters of *Pramehahar Guggulu*

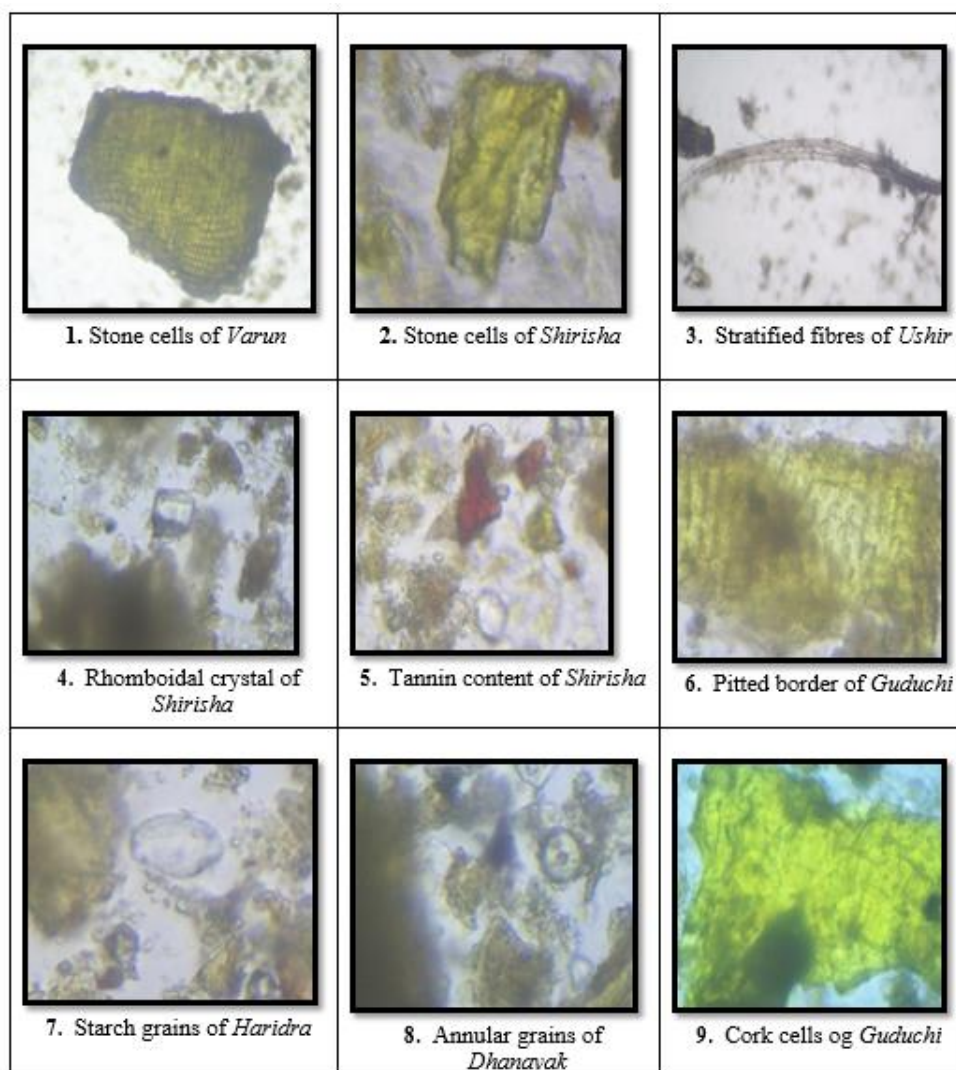
Drug	Color	Odour	Taste	Consistency
<i>Pramehahar Guggulu</i>	Greenish Grey	Charactristic	<i>Tikta</i>	Hard

Table 3: Physico-chemical Parameters of *Pramehahar Guggulu*

Name of the Analysis	Value of <i>Pramehahar Guggulu</i>
Loss on drying percentage	10.17% w/w
Ash value	10.39% w/w
PH value(5% aqueous)	6.5
Water soluble extract percentage	32.6% w/w
Alcohol soluble extract percentage	19.2% w/w
Weight variation	Average wt. 457.55mg Highest wt. 521mg Lowest wt. 388 mg

Table 4: HPTLC results for methanolic extract of *Pramehahar Guggulu* At 254nm at 366nm

No. of spots	Rf Value	No. of spots	Rf Value
9 spots	0.26, 0.46, 0.57, 0.61, 0.74, 0.82, 0.87, 0.95, 0.99	6 spots	0.26, 0.35, 0.46, 0.65 0.79, 0.99



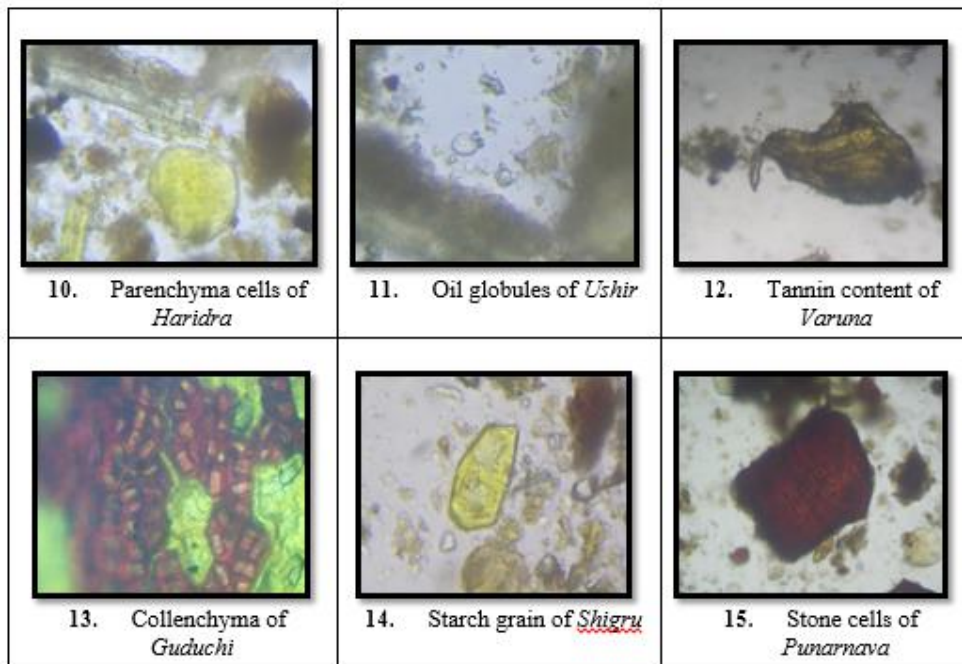


Fig 1

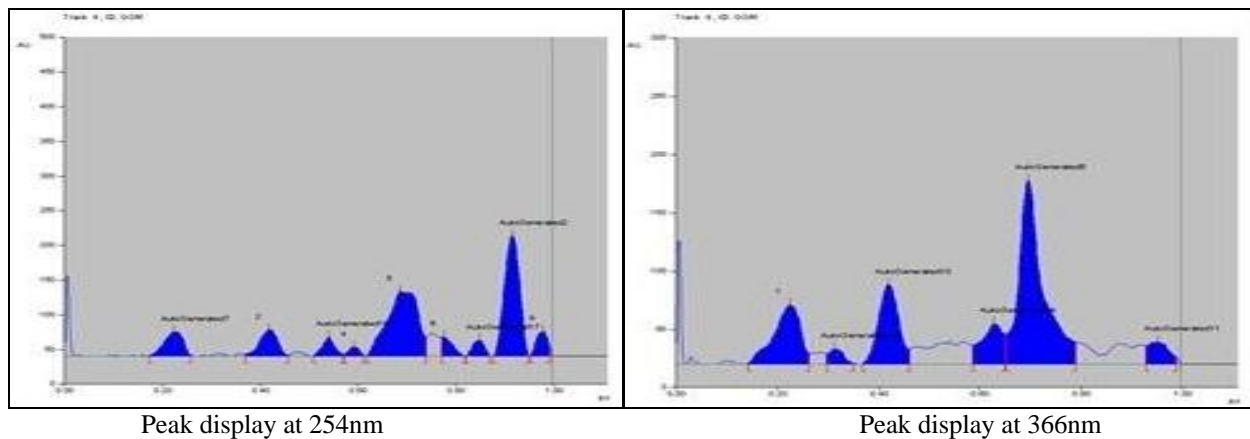


Fig 2: Densitogram of Pramehahar Guggulu at 254nm and 366nm.

Conclusion

The Pharmacognostical and physico chemical analysis of *Pramehahar guggulu* confirmed the purity and genuinity of the drug. A published information is not available on pharmacognostical and physico-chemical profiles of *Pramehahar guggulu*. Information acquired from this study may be beneficial for further research work and can be used as a reference standard for quality control researches.

References

1. API textbook of Medicine VOL 1, 9th edition ISBN 978-93-5025-074-7 page no.529
2. Ibidem Charak Samhita Sutra sthana Maha chatushpada Adhyaya 10/17
3. Trease and Evans, Pharmacognosy, 15th Ed., W. B. Saunders Company Ltd,1996:569:570.
4. Wallis TE, Text book of Pharmacognosy, 5th Ed., New Delhi: CBS Publishers & Distributors,2002:123-132:210-215.
5. Anonymous, Protocol for testing of Ayurveda, Siddha & Unani medicines, Pharmacopoeial laboratory for Indian medicines, Ghaziabad, Ministry of AYUSH, Government of India.
6. Stahl E; Thin layer chromatography, 2nd Ed. Springer-Verlag New York, Inc. 175 5th Ave. New York, NY, 1969, 125-133.
7. Reich E, Schibii A; High Performance Thin Layer Chromatography for the analysis of medicinal plnts, Germany: Thieme medical publishers. Inc, 2007:129-160(206-210):224-240.