Pharmacognostical and pharmaceutical analysis of Punarnava guggulu an ayurvedic polyherbal formulation

Chaitali Kakadiya1*, Mandip Goyal1, C. R. Harisha2, V. J. Shukla3

1Department of Kayachikitsa, 2Department of Pharmacognosy Laboratory, 3Department of Pharmacutics Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

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*Correspondence:
Dr. Chaitali Kakadiya,
E-mail: Chaitali.kakadiya@yahoo.com

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ABSTRACT

Background: Punarnava Guggulu is a polyherbal formulation mentioned in Bhaishajya Ratnavali containing various Ayurvedic medicinal drugs and specially indicated for the treatment of Amavata and Vatarakta. For assurance of quality of herbal compounds pharmacognostical and pharmaceutical analysis should be done.

Methods: Punarnava Guggulu was subjected to microscopic evaluation for Pharmacognostical study, physiochemical analysis like hardness, weight variation, loss on drying, ash value, acid insoluble extract, pH value, water soluble extract, alcohol soluble extract, high performance thin layer chromatography (HPTLC).

Results: Pharmacognostical study showed the presence of certain identifying characters of all of the ingredients of Punarnava Guggulu that is Punarnava, Erandamula, Shunthi, Guggulu, Eranda Tail, Trivruta, Danti, Guduchi, Haritaki, Bibhitaki, Amalaki, Maricha, Pippali, Chitraka, Bhallataka and Vidanga. In pharmaceutical study, preliminary physiochemical analysis showed that hardness of the Vati was 4.05 Kg/cm², ash value 12.84% w/w, acid insoluble ash value 1.56% w/w, loss on drying 1.6% w/w, water soluble extract 35.93% w/w, alcohol soluble extract 22.14% w/w and HPTLC showed 13 spots in 254nm and 8 spots in 366nm.

Conclusions: Present work was carried out to standardize the polyherbal formulation Punarnava Guggulu in terms of its identity, quality and purity. Pharmacognostical and physico-chemical observations revealed the specific characters of all active constituents in the preparation were present in it.

Keywords: Punarnava guggulu, Pharmacognocy, Pharmaceutics

INTRODUCTION

Punarnava Guggulu, a polyherbal formulation contains various herbal drugs (Table 1) that is Punarnava (Boerhavie diffusa Linn), Eranda Mula and Tail (Ricinus communis Linn), Shunthi (Zingiber officinale Roxb) Guggulu (Commiphora myrrha (Ness) Engl.), Trivruta (Operculina turpethum N (L) Salve Manse), Danti (Baliospermum montanum Muell), Guduchi (Tinospora cordifolia Willd. Miers. Ex Hook.), Haritaki (Terminalia chebula Retz.), Bibhitaki (Terminalia bellirica Roxb.), Amalaki (Emblica officinalis Gaertn.), Maricha (Piper nigrum Linn), Pippali (Piper longum Linn.), Chitraka (Piper retrofractum Vahl), Saindhava Lavana, Bhallataka (Semecarpus anacardium Linn), Vidanga (Embella ribes Durm.f.) and Sivrrna Makshika Bhasma. Punarnava Guggulu is mainly indicated for the treatment of Amavata and Vatarakta in a classical text of Ayurveda like Bhaishya Ratnavali.1 It is also indicated for the treatment of Vridhh, Gridhrusi, Jangha, Uru, Prishtha, Trika, and Bastigata Vyaadhi. Ingredients of Punarnava Guggulu are having Katu, Tikta, Kashaya Rasa, Laghu, Ushna and
Ruksha Guna, Ushna Virya and Katu Vipaka. Thus, Punarnava Guggulu mainly pacify Kapha and Vata Dosha. In the case of internal administration of hrebomineral drug, it should be safe, effective and free from adulteration, with appropriate quantity and ingredients. It is difficult to identify herbal drug in dry or powdered form.

This condition leads to increase in adulteration. So, it is a need of time to set proper parameters for standardization of herbal drugs. Pharmacognostical studies reveals plant identification and sets parameters for standardization which can be done in the case of herbal traditional medicine. Generally, physiochemical analytical study of drugs help to interpret the pharmacokinetics and pharmacodynamics involved. With the help of physiochemical analytical studies, it is possible to standardize the drug and differentiate the adulterants.

High performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) are the conventional methods used in the analysis of secondary metabolites originating from plants. It is necessity of time in the field of Ayurveda to go for quality control of the raw drugs as well as final products using modern parameters which provides credibility to Ayurvedic medicines and also help in the globalization of Ayurveda.

Objectives of this studies are to evaluate the authenticity of Punarnava Guggulu through various pharmacognostical procedures and to develop the pharmacognostical and phyto-chemical profile of Punarnava Guggulu.

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 for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India.

Preparation of drug

All raw drugs were purchased from Pharmacy of Gujarat Ayurved University. Decoction was prepared from coarse powder of Punarnavamula, Erandamula and Shuddhi with 8 part of water. It was boiled until water was reduced up to 4\textsuperscript{th} part. Then decoction was filtered. Shuddha Guggulu was added to filtrate decoction and was boiled.

When Guggulu was about to have Asanna Pakva phase, Eranda Taila and other drugs mentioned in table 1 were added. Then Vati of 300 mg was prepared and stored in bottles under hygienic condition.\textsuperscript{2}

Pharmacognostical study

The pharmacognostical study was divided in to organoleptic study and microscopic study of the finished product.

Organoleptic study

The genuinity of the polyherbal formulation can be fined with organoleptic characters of the given sample. Organoleptic parameters comprise taste, colour, odour and touch of Punarnava Guggulu which was scientifically studied as per the standard references.\textsuperscript{3}

Microscopic study

Punarnava Guggulu was powdered and dissolved with water and microscopy of the sample was done without stain and after staining with Phloroglucinol + HCl. Microphotographs of Punarnava Guggulu were also taken under Corl-zeissinocular microscope.\textsuperscript{4}

Physico-chemical analysis

With the help of various standard physico-chemical parameters, Punarnava Guggulu was analyzed. The common parameters mentioned for Guggulu Kaplana in Ayurvedic Pharmacopia of India, and CCRAS, guidelines are loss on drying, hardness, total ash value, acid insoluble ash, pH value, water soluble extract, methanol soluble extra total ash and water and alcohol soluble extractives.\textsuperscript{5,6}

High performance thin layer chromatography\textsuperscript{7}

High Performance Thin Layer Chromatography (HPTLC) is a powerful analytical method suitable for the separation and quantitative determination of a considerable number of compounds even from complicated matrix. HPTLC is used for identification of active constituents, identification and determination of impurities and quantitative analysis of active constituents. Principle of HPTLC remains the same as of TLC i.e. adsorption. One or more compounds can be spotted in a thin layer of adsorbent coated on a chromatographic figure. The mobile phase solvent flows through because of capillary action against gravitational force. The component with more affinity towards stationary phase travels faster. Thus, the components are separated on a thin layer chromatographic figure based on the affinity of the components towards the stationary phase.

Steps involved in HPTLC were as follows:

- Sample and standard preparation
- Selection of chromatographic layer
- Layer pre-washing
- Layer pre-conditioning
- Application of sample
• Chromatographic development
• Detection of spots
• Scanning and documentation.

Methanol extract of *Punarnava Guggulu* were spotted on pre-coated silica gel GF CO254 aluminum figure as 5 mm bands, 5 mm apart and 1 cm from the edge of the figures, by means of camag, linomate V sample applicator fitted with a 100 µL. Hamilton syringe was used as the mobile phase. After development, densitometry scanning was performed with a camag TLC scanner III reflectance absorbance mode at 254 nm and 366 nm under control of win cats software (V 1.2.1 manufactured by camag Switzerland). The slit dimensions were 6.00 x 0.45 mm and the scanning speed was 20 mm per second.\(^8\)

**RESULTS**

**Organoleptic characters of Punarnava Guggulu**

Organoleptic characters contents of *Punarnava Guggulu* like colour, taste, touch, odor were recorded. The Color of *Punarnava Guggulu* was muddy brown. *Punarnava Guggulu* had oily smell, taste was *Lavana-Kashaya* and felt hard on touch which is shown in Table 2.

| Drug          | Botanical name                  | Part used | Proportion |
|---------------|---------------------------------|-----------|------------|
| Punarnava     | *Boerhavia diffusa* Linn.       | Root      | 100        |
| Eranda        | *Ricinus communis* Linn.        | Root      | 100        |
| Shunthi       | *Zingiber officinale* Roxb.     | Root      | 16         |
| Guggulu       | *Commiphora myrrha* (Ness) Engl.| Gum       | 8          |
| Erand Taila   | *Ricinus communis* Linn.        | Oil       | 4          |
| Trivruta      | *Opercunila turpethum* N(L) Salve Manse. | Root  | 5          |
| Danti         | *Baliospermum montanum* Muell.  | Root      | 1          |
| Guduchi       | *Tinospora cordifolia* Willd.   | Stem      | 2          |
| Haritaki      | *Terminalia chebula* Retz.      | Fruit     | 0.5        |
| Bibhitaki     | *Terminalia bellirica* Roxb.    | Fruit     | 0.5        |
| Amalaki       | *Emblica officinalis* Gaertn.   | Fruit     | 0.5        |
| Shunthi       | *Zingiber officinale* Roxb.     | Root      | 0.5        |
| Maricha       | *Piper nigrum* Linn.            | Fruit     | 0.5        |
| Pippali       | *Piper longum* Linn             | Fruit     | 0.5        |
| Chitraka      | *Piper retrofractum* Vahl.      | Root      | 0.5        |
| Saindhava     | *Sodi chloridium*               |           | 1          |
| Bhallataka    | *Semecarpus anacardium* Linn.f. | Fruit     | 1          |
| Vidanga       | *Emblica ribes* Durm.f.         | Fruit     | 1          |
| Suvarna makshi Bhasma | *Ferri sulphuratum* | Bhasma | 0.25 |
| Punarnava     | *Boerhavia diffusa* Linn.       | Root      | 1          |

**Table 2: Organoleptic characters of Punarnava guggulu.**

| Drug          | Colour         | Odour         | Taste          | Consistency   |
|---------------|----------------|---------------|----------------|---------------|
| Punarnava Guggulu | Muddy brown    | Oily smell    | Lavana-Kashaya | Hard, Vati    |

**Table 3: Physico-chemical parameters of Punarnava guggulu.**

| Name of the Analysis | Value of Punarnava guggulu |
|----------------------|----------------------------|
| Loss on drying percentage | 1.6% w/w                   |
| Acid insoluble Ash    | 1.56% w/w                  |
| Ash value percentage  | 12.84% w/w                 |
| pH value (5% aqueous) | 6.5                        |
| Water soluble extract percentage | 35.93% w/w  |
| Alcohol soluble extract percentage | 22.14% w/w   |
| Weight variation of Guggulu | Average wt. 0.342gm            |
|                        | Highest wt. 0.380gm        |
|                        | Lowest wt. 0.290gm         |
Table 4: HPTLC results for methanolic extract of *Punarnava guguulu*.

| HPTLC | 254 nm | 366 nm |
|-------|--------|--------|
| No. of Spots | R<sub>f</sub> Value | No. of Spots | R<sub>f</sub> Value |
| 13 | 0.07, 0.14, 0.23, 0.29, 0.32, 0.38, 0.43, 0.58, 0.66, 0.68, 0.78, 0.81 and 0.88 | 8 | 0.07, 0.13, 0.22, 0.34, 0.45, 0.54, 0.58 and 0.66. |

1: Acicular crystal of *Punarnava*  
2: Pitted vessels of *Punarnava*  
3: Fibers of *Erandmoola*  
4: Starch grains of *Shunthi*  
5: Crystal fiber of *Trivruta*  
6: Rhomboidal crystal of *Trivruta*  
7: Pitted vessels of *Dhanti*  
8: Border pitted vessels of *Guduchi*  
9: Cork cells of *Guduchi*
10: Lignified collenchyma cells of *Guduchi*

11: Starch grains of *Guduchi*

12: Scleroid of *Haritaki*

13: Stone cell of *Bibhitaki*

14: Trichome of *Bibhitaki*

15: Fiber of *Amalaki*

16: Scleroid of *Amalaki*

17: Silica deposition of *Amalaki*

18: Black debris of *Maricha*
19: Stone cells of *Maricha*  
20: Black debris of *Pippali*  
21: Lignified stone cells of *Pippali*  

22: Fiber of *Chitraka*  
23: Stone cells of *Chitraka*  
24: Tannin content of *Chitraka*  

25: Fixed oil and oleoresine content of *Bhallataka*  
26: Oleoresin content of *Vidanga*  
27: Stone cells of *Vidanga*  

**Figure 1:** Microphotograph of *Punarnava guggulu.*
Figure 2: Densitogram of Punarnava guggulu at 254nm and 366nm (A): Peak display at 254 nm (B): Peak display at 366nm.

Figure 3: Three dimensional HPTLC (3D) densitogram of Punarnava guggulu (A): 254nm (B): 366nm.

**Microscopic study of Punarnava guggulu**

Identifying characters of ingredients of Punarnava Guggulu under the microscope were acicular crystals (1) and pitted vessels (2) of Punarnava, group of fibers of Eranda Mula (3), starch grain of Shunthi (4), crystal fiber (5) and rhombodial crystal (6) of Trivruta, pitted vessels of Dantimula (7), border pitted vessels (8), cork cells (9), lignified cholenchymal cells (10) and starch grain of Guduchi (11), scleroid of Haritaki (12), stone cells (13) and trichome (14) of Bibhitaki, simple fiber (15), scleroid (16) and silica deposition of Amalaki (17), black debris (18) and stone cells (19) of Maricha, lignified stone cells of Pippali (20), fiber (21), stone cell (22) and tannin content of Chitraka (23), fixed oil (24) and oleoresin content of Bhallataka (25), oleoresin content (26) and stone cell of Vidanga (27).

All these are showed in Figure 1 (1 to 27).

**Physico-chemical analysis of Punarnava guggulu**

Physico-chemical analysis of Punarnava Guggulu revealed the hardness of 4.05 Kg/cm² the ash value was 12.84%w/w, acid insoluble ash value 1.56%w/w, loss on drying 1.6%w/w, water soluble extract 35.93%w/w, alcohol soluble extract 22.14%w/w and pH value was 6.5, (Table 3).
**High performance thin layer chromatography of Punarnava Guggulu**

On performing HPTLC, the chromatogram of **Punarnava Guggulu** showed 13 peaks with maximum Rf values 0.07, 0.14, 0.23, 0.29, 0.32, 0.38, 0.43, 0.58, 0.66, 0.68, 0.78, 0.81 and 0.88 at short wave UV 254nm; while at long wave UV 366 nm, the chromatogram showed 8 spots with maximum Rf values 0.07, 0.13, 0.22, 0.34, 0.45, 0.54, 0.58 and 0.66 (Table 4).

**DISCUSSION**

Pharmacognostical part of the study of **Punarnava Guggulu** was the step towards identification of all raw material present in the finished product. The presence of all contents of raw drugs in the final product showed the genuineness of the final product. Hence **Punarnava Guggulu** is herbomineral drug, identification of mineral parts of **Punarnava Guggulu** cannot be evaluated through pharmacognosy. All the pharmaceutical parameters were done to analyze the values permissible for the **Punarnava Guggulu**. All the parameters tested under the pharmaceutical study are as per the API.\(^9\) The physico-chemical parameters showed that percentage of water soluble extract was more than alcohol soluble extract which indicates the presence of flavonoids, tannins and anthocyanidins in the drug. While alcohol soluble extract value denotes the presence of tannins, resins and alkaloids in the drug. Ash value of the final product is 12.840% w/w shows the presence of inorganic material which cannot be identified through pharmacognosy.

**CONCLUSION**

The pharmacognostical and physico chemical analysis of **Punarnava Guggulu** confirmed the purity and genuineness of the drug. Published information is not available on pharmacognostical and physico-chemical profiles of **Punarnava 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.

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the Institutional Ethics Committee

**REFERENCES**

1. Ambikadattashastri K, Bhaishajyaratnavali of Shri Govinddasasena, ed. Vatarakta Rogadhihara, Chapter-27 Shlola. Varanasi; Chaukhambha Prakashan, 2016: 113.
2. Ambikadattashastri K, Bhaishajyaratnavali of Shri Govinddasasena, ed. Vatarakta Rogadhihara, Chapter-27 Shlola. Varanasi; Chaukhambha Prakashan, 2016: 109-112.
3. Tease and Evans, Pharmacognosy, 15th ed., W. B. Sanders Company Ltd., 1996: 569-570.
4. Wallis TE, Text book of Pharmacognosy, 5th ed., New Delhi; CBS Publishers and Distributors, 2002: 123-132: 210-215.
5. Ayurvedic pharmacopeia of india part 1 volum IX. pdf available at: http://ayush.gov.in/sites/default/files/Ayurvedic%20Pharmacopoeia%20of%20India%20part%201%20volume%20IX.pdf. Accessed 24 April 2020.
6. CCRAS, General guidelines for drug development of ayurvedic formulations, 2009; Available at: http://www.ccras.nic.in/sites/default/files/viewpdf/Publication/CCRAS_Guideline%20of%20Drug%20Development.pdf.
7. Anonymous, Planner Chromatography, Modern Thin layer Chromatography, Switzerland. 1999:2-16.
8. Gupta AK, Introduction to pharmaceutics, Volume (1) 3rd ed, New Delhi: CBS publishers and distributors; 1994: 270.
9. Ayurvedic pharmacopeia of India Part 2 Volume II. Available at: https://naturalingredient.org/wp/wp-content/uploads/API-II-Vol-2.pdf.

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