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Original Research Article

Pharmacognostical and pharmaceutical analysis of *Punarnava guggulu* an ayurvedic polyherbal formulation

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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 turpenthum* 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 *(Embelia ribes* Durm.f.) and *Suvrna Makshika Bhasma. Punarnava Guggulu* is mainly indicated for the treatment of Amavata and Vatarakta in a classical text of Ayurveda like Bhaishjya Ratnavali.[1] It is also indicated for the treatment of *Vriddhi*, *Gridhrasi*, *Jangha*, *Uru*, *Prishtha*, *Trika*, and *Bastigata Vyadhi*. 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 Pharmacogonosy 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 *Shunthi* with 8 part of water. It was boiled until water was reduced up to 4th 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 ware added. Then *Vati* of 300 mg was prepared and stored in bottles under hygienic condition.[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.[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-zeisstrinocular microscope.[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.[5,6]

High performance thin layer chromatography[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 camage TLC scanner III reflectance absorbance mode at 254 nm and 366 nm under control of win cats software (V 1.2.1 manufactured by camage 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.

Table 1: Ingredient of *Punarnava guggulu*.

Drug	Botnical 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	*Operculina 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	*Embelia ribes* Durm.f.	Fruit	1
Suvarna makshika Bhasma	*Ferri sulphuratum*	*Bhasma*	0.25
Punarnava	*Boerhavia diffusa* Linn	Root	1

Table 2: Organoleptic characters of *Punarnava guggulu.*

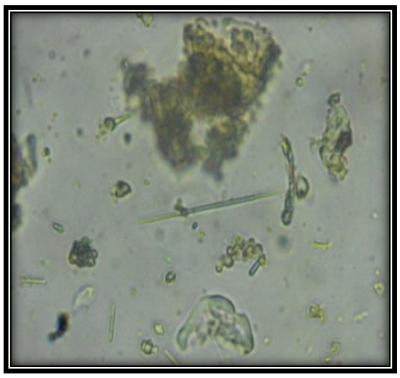
Drug	Colour	Odour	Taste	Consistency
Punarnava Guggulu	Muddy brown	Oily smell	*Lavana-Kashaya*	Hard, *Vati*

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

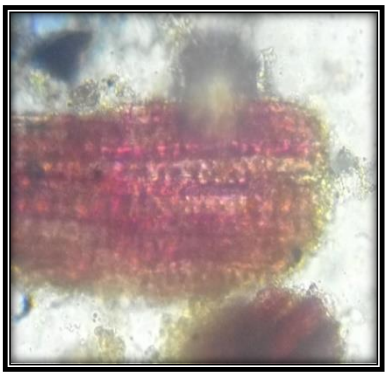
Name of the Analysis	Value of *Punarnava guggulu*
Loss on drying percentage	1.6% w/w
Acid insoluble Ash	1.56%w/w
Ash value percentage	12.84% w/w
pH value (5% aqueous)	6.5
Water soluble extract percentage	35.93% w/w
Alcohol soluble extract percentage	22.14% w/w
Weight variation of *Guggulu*	Average wt.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

Table 4: HPTLC results for methanolic extract of *Punarnava guguulu.*

	254 nm		366nm	
	No. of Spots	**R_f Value**	**No. of Spots**	**R_f Value**
HPTLC	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.

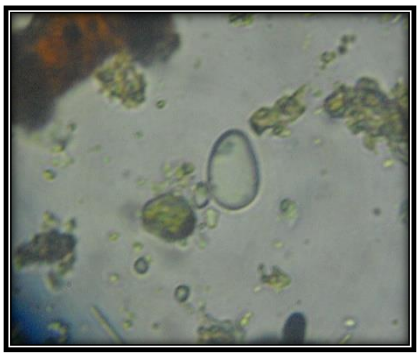


1: Acicular crystal of *Punarnava*

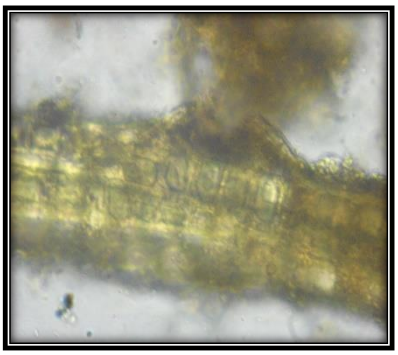


2: Pitted vessels of *Punarnava*

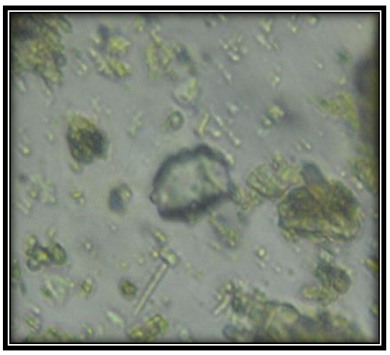
3: Fibers of *Erandmoola*



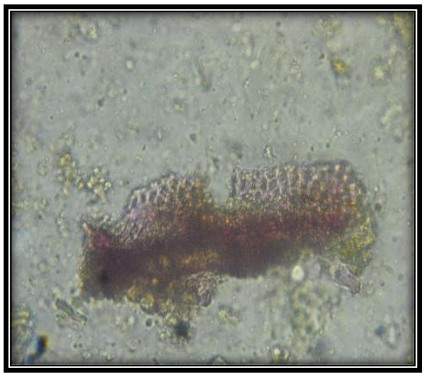
4: Starch grains of *Shunthi*



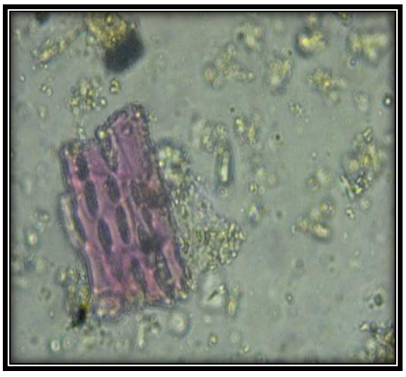
5: Crystal fiber of *Trivruta*



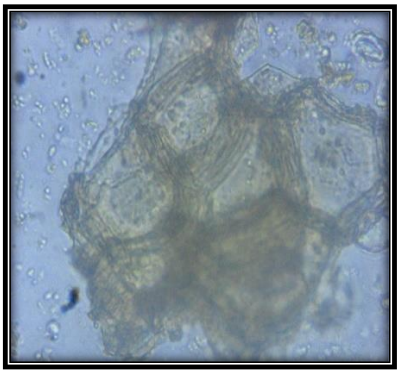
6: Rhomboidal crystal of *Trivruta*



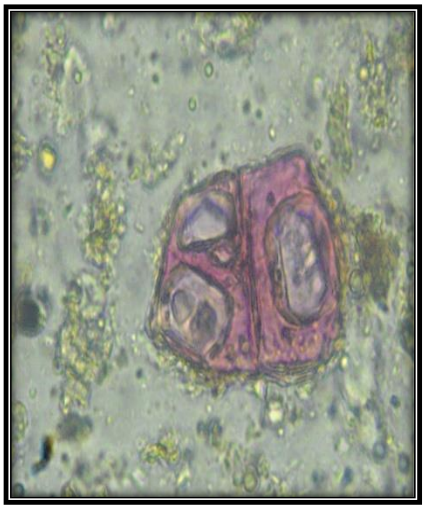
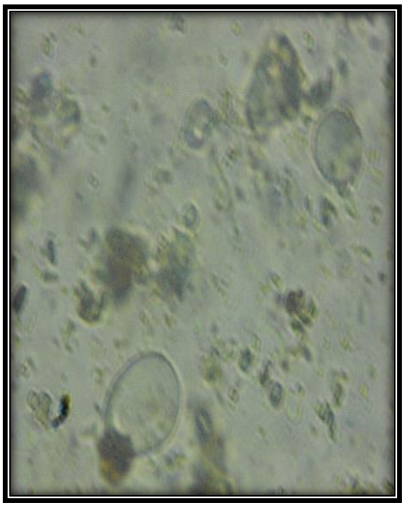
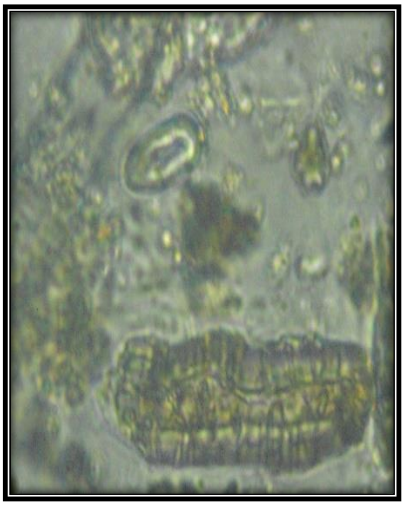
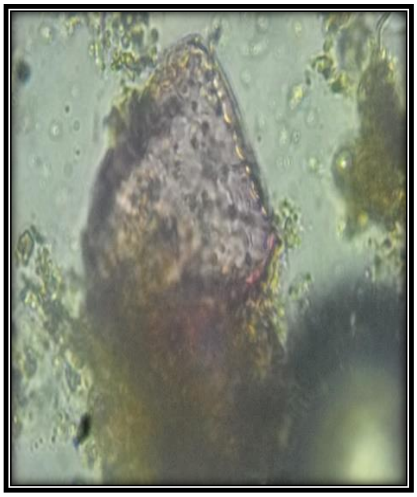
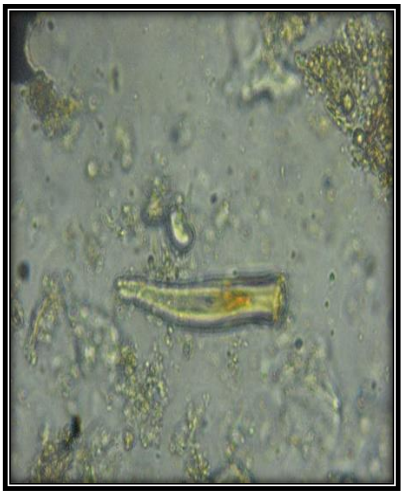
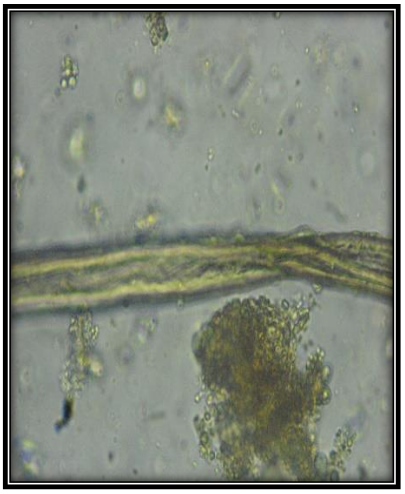
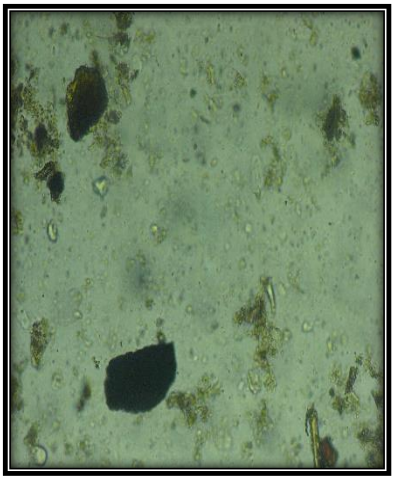
7: Pitted vessels of *Dhanti*



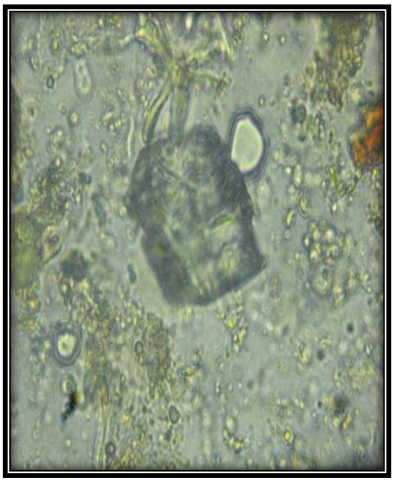
8: Border pitted vessels of *Guduchi*



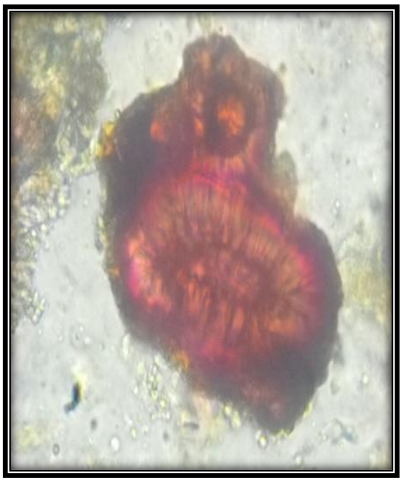
9: Cork cells of *Guduchi*

		
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*

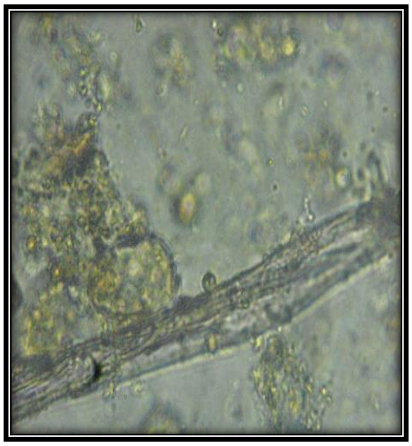
19: Stone cells of *Maricha*



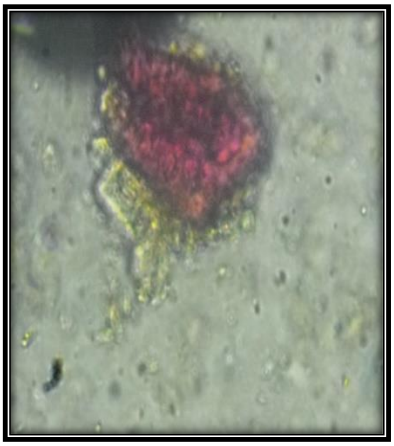
20: Black debris of *Pippali*



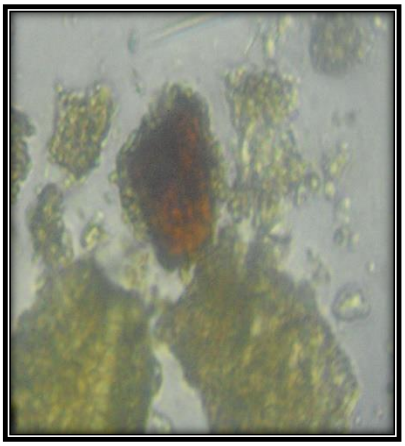
21: Lignified stone cells of *Pippali*



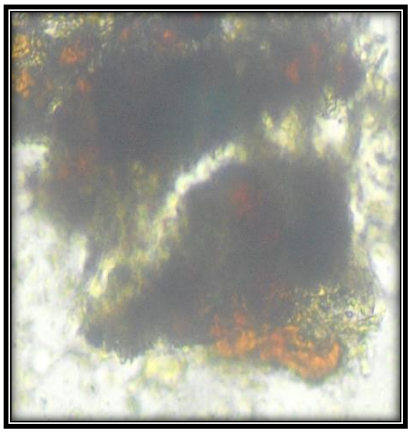
22: Fiber of *Chitraka*



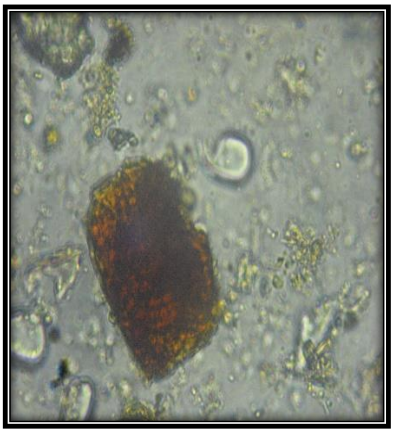
23: Stone cells of *Chitraka*



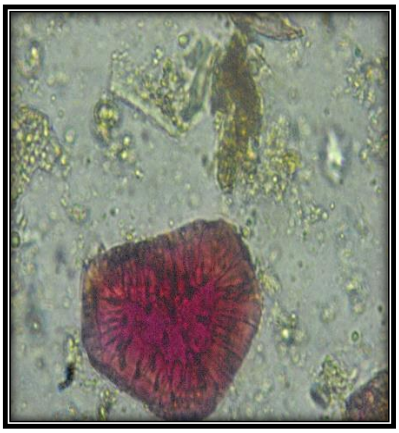
24: Tannin content of *Chitraka*



25: Fixed oil and olioresine contant of *Bhallataka*



26: Oleoresin content of *Vidanga*



27: Stone cells of *Vidanga*

Figure 1: Microphotograph of *Punarnava guggulu.*

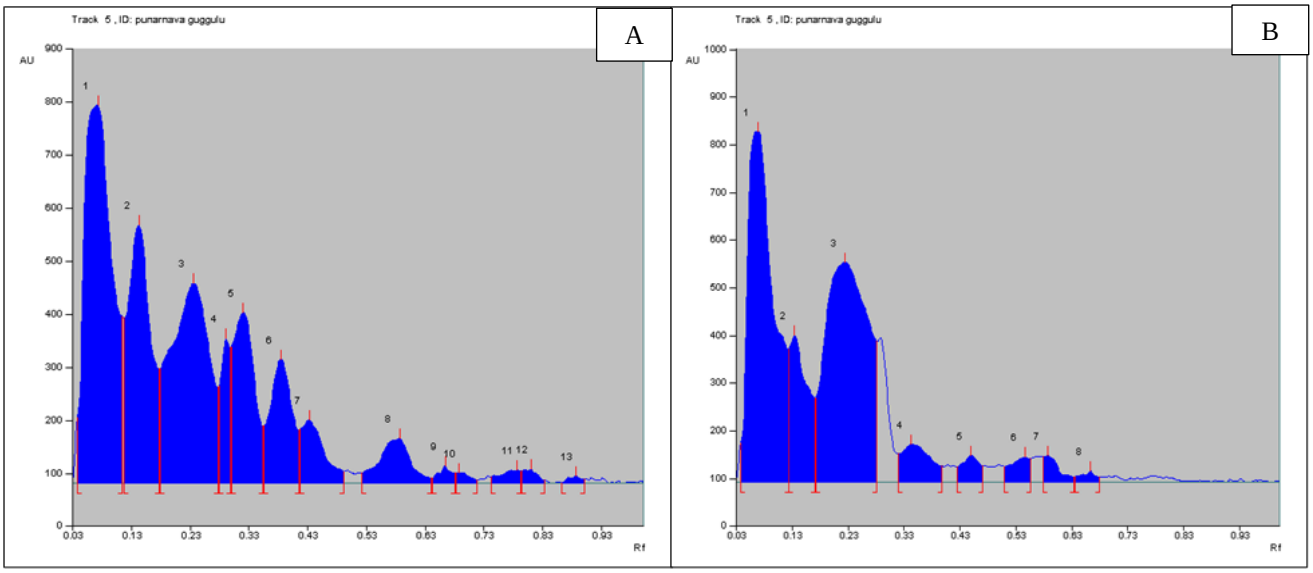


Figure 2: Densitogram of *Punarnava guggulu* at 254nm and 366nm (A): Peak disply at 254 nm (B): Peak display at 366nm.

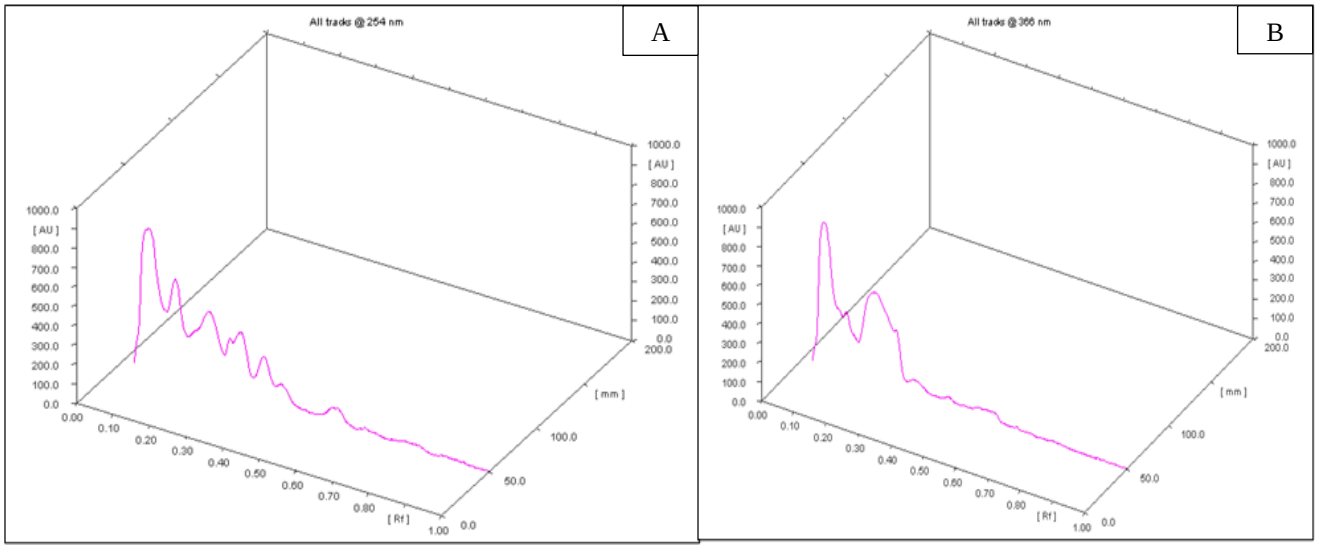


Figure 3: Three dimensional HPTLC (3D) densitogram of *Punarnava guggulu* (A): 254nm (B): 366nm.

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), olioresin 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/cm^2 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 R_f 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 R_f 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 genuinity 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 genuinety 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

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