EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 3
371-379
Year: 2017

A PHARMACOGNOSTICAL AND PHYTO-CHEMICAL ASSAY OF *KANCHANARA GUGGULU* – A PROMISING MEDICINE FOR *BEEJAKOSHA GRANTHI* (OVARIAN CYST)

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Article Received on 17/01/2017 **Article Revised on 07/02/2017** **Article Accepted on 27/02/2017**

ABSTRACT

Introduction: Women have many Diseases in common, Ovarian cysts are at the top of the list. An Ovarian cyst is a fluid-filled sac that forms in the ovary. Ovarian cysts affect fertility if they interfere with normal ovulation or represent a mechanical obstacle for the fertilization process. In Ayurved context to *Granthi*, situated in *Beejakosha* can be correlated with ovarian cyst. *Kanchanara Guggulu* has been selected in present study. Till date there is no scientific evaluation has been observed about the Pharmacognosy and Pharmaceutical study. **Aim:** Authentication of raw drug of *Kanchanara Guggulu* and Phyto-chemical evaluation of finished product. **Material & Methods:** The present study deals with the Pharmacognostical identification of the ingredients of *Kanchanara Guggulu* and its physicochemical analysis. High-Performance Thin Layer chromatography study (HPTLC) was also developed. **Results:** Pharmacognostical results shows Black debris of *Maricha*, Epidermal cells of *Patra*, Group of stone cells of *Maricha* & *Pippali*, Lignified scleroids of *Haritaki*, Lignified stone cells of *Pippal*, Oil globule of *Patra & Twak*, Prismatic crystal of *Kanchanara*, Rhomboidal crystals of *Varuna*, Scleroids of *Amalaki, Haritaki & Bibhitaki*, Silica deposition of *Amalaki*, Starch grains of *Sunthi*, Stone cell of *Kanchanara* & *Haritaki*, Trichome of *Bibhitaki*. Qualitative study shows that pH is 6.5, Ash value is 14.1% w/w, Loss on drying is 9.26% w/w, Water soluble extraction is 27.2% w/w & Alcohol soluble extraction is 9.64% w/w, Acid soluble Ash 6.05%w/w, Uniformity of tablet-Maximum weight 3.120gm, Minimum weight 2.80gm, Average weight 2.961gm,Tablet hardness 4.75kg/cm^2. High-performance thin layer chromatography (HPTLC) revealed maximum 13 spots in short wave ultraviolet (UV) 254 nm and 12 spots were obtained in long wave UV 366 nm. **Conclusion:** Pharmacognostical study revealed genuinity of raw drugs. Physicochemical and HPTLC studies inferred that the formulation meets the minimum quality standards. The inference from this study may be used as reference standard in the further quality control researches.

KEYWORD: *Beejakosha Granthi*, *Kanchanara Guggulu*, Pharmacognostical & Phyto-chemical Analysis.

INTRODUCTION

Women have many diseases in common; ovarian cysts are at the top of the list. In past some years, there is a domestic rise in female related illness which was rarely seen before in history. Ovarian cyst is an emerging problem among the women of reproductive age group.[1] Ovarian cysts are fluid filled sacs inside the ovary.[2] Ovarian cysts affect fertility if they interfere with normal ovulation or represent a mechanical obstacle for the fertilization process. Nearly 2% of the adnexal masses are ovarian carcinomas or border line tumors. Ovarian cyst is becoming a burning problem in current scenario affecting all age group of women. Ovarian cancer is 2nd most common of genital cancers and accounts for 10-15% of all gynecological cancers in developing countries including Iindia.[3]

Acharya Sushruta[4] has given elaborate description of *Granthi* from its etiopathogenesis classification and its management, but not mentioned about neoplastic swelling of female genital organs, though a reference related to *Granthi* of male genital tract is available. *Samanya Chikitsa* includes Conservative treatment which improve immune mechanism and revert the disease process e.g. *Kanchanara Guggulu* (B.P.44/34-44), *Chandraprabha Vati* (S.S.M.K.), *Triphala Guggulu*, *Varunshigru Kwatha* etc. have been mentioned in Ayurveda. The classical formulation of *Kanchanara*

Guggulu (Sha. Mad.7/95-100) was selected in *Beejakosha granthi* (ovarian cyst), because it is prescribed in management of *Gandamala*, *Apachi*, *Arbuda*, *Granthi* etc. It is made up of *Kanchanara Twak*, *Triphala*, *Trikatu*, *Varuna*, *Ela*, *Twak*, *Patra*. Due to its *Vatakaphahara*, *Shothahara*, *Lekhana and Mootrala* effect and these are considered for better result in *Beejakosha Granthi*. This formulation has properties like *Kaphamedohara*, *Lekhana*, *Granthihara*, *Mutrakruchahara*, *Shothahara* and it is considered for better result in *Mootraghata*. *Kanchanara* contains flavonoids and fatty oils. It is diuretic in action, chiefly acting on the glomerulus of the kidneys and the heart, increasing the beats and strength and raising the peripheral blood pressure ultimately.

During the past few decades there has been increasing acceptance of natural products and therapies in the world. Also increase in use of ayurvedic remedies globally. Therefore, quality control for efficacy and safety of herbal products is of main concern.[5, 6] The development of this traditional system of medicine with the perspective of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of the natural products in healthcare. Initial steps in quality standardization of compound formulation are to establish the presence of each ingredient in the finished product, followed by the pharmaceutical analysis. Chromatographic techniques were adopted for the separation of active moieties present in the formulation. Therefore, an attempt has been made to standardize *Kanchanara Guggulu* Pharmacognostical, Physico-chemical and HPTLC fingerprint profile.

MATERIALS AND METHODS

Collection of Raw Materials

All the raw drug materials required for *Kanchanara Guggulu* were collected from the pharmacy of Gujarat Ayurveda University, Jamnagar. The ingredients and parts used of the drugs are given in Table 1.

Method of Preparation of *Kanchanara Guggulu Vati*

Ingredients enlisted in Table 1 were made into fine powder and sieved in Mesh no.80. The powder was mixed well in mass mixing machine till the homogeneous mixture was obtained. Then the purified *Guggulu* by *Triphala Kwatha* was added. At the last, sufficient quantity of Ghee (approximately 250 ml) was added so that the *Vati* can be formed with proper shape and density. These all materials were mixed and then *Vati* was prepared as per *Guggulu Kalpa* method.[7]

Pharmacognostical Study

Raw drugs were identified and authenticated by the pharmacognosy laboratory, I.P.G.T. & R.A, Jamnagar. The identification was carried out based on Organoleptic characters of *Vati*.[8] Later pharmacognostical evaluation of the *Vati* was carried out. *Vati* was dissolved in a small quantity of distilled water, filtered through filter paper, studied under the Carl Zeiss Trinocular microscope attached with camera, with stain and without stain. The microphotographs were also taken under the microscope.[9,10] Organoleptic evaluation shows various characters such as colour, odor, taste and touch of drugs was observed and recorded.[11]

Pharmaceutical Evaluation

Pharmaceutical Evaluation *Kanchanara Guggulu Vati* was analyzed using qualitative and quantitative parameters at the pharmaceutical laboratory, I.P.G.T. & R.A., Jamnagar. The common parameters mentioned for *Vati* in Ayurvedic Pharmacopeia of India and Central Council for Research in Ayurvedic Sciences guidelines are total Ash value, pH value, Water and Methanol soluble extracts.[12] On these base, the parameters were selected. The presence of more moisture contents in a sample can create preservation problem. Hence, loss on drying was also selected as one of the parameters.[13, 14]

High-performance Thin Layer Chromatography (HPTLC)

HPTLC was performed as per the guideline provided by API. Methanolic extract of drug sample was used for the spotting. HPTLC was performed using toluene + ethyl acetate (9:1 v/v) solvent system. The colour and Rf values of resolved spots were noted.[15]

High-Performance Thin Layer Chromatography study Methanol extract of Sample was spotted on pre-coated silica gel GF254 aluminum plate as 6 mm bands, 5 mm apart and 1 cm from the edge of the plates, by means of a Camang Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Toluene (7ml), Ethyl acetate (2ml), formic acid (0.5ml) was used as the mobile phase. After development, Densitometry 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 (v1.2.1 camag). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 20 mm s-1.

OBSERVATIONS AND RESULTS

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of *Kanchanara Guggulu Vati*. For this, coarse powder of all the ingredients was subjected to Organoleptic and Microscopic evaluation separately to confirm the genuineness of all the raw drugs. Later, after the preparation of *Vati*, pharmacognostical evaluation was carried out.

Pharmacognostical Study

Powder Organoleptic Characters

- Colour -Black, Taste- *Kashaya Tikta*, Odor- *Guggulu* like resin smell, Nature of the Powder – Hard.

Microscopic Characters Identified

Microscopic evaluation was conducted by powdering the *Vati* and dissolving it in the distilled water and studied under a microscope for the presence of characteristics of ingredient drugs. The diagnostic characters are *Kanchanara Guggulu* (Image 1), Black debris of *Maricha* (Image 2), Epidermal cells of *Patra* (Image 3), Fiber of *Twak*, *Varuna*, *Sunthi* (Image 4) (Image 5) (Image 6) Group of stone cells of *Maricha* (Image 7), Group of stone cells of *Pippali* (Image 8), Lignified fibers of *Twak* (Image 9), Lignified scleroids of *Haritaki* (Image 10), Lignified stone cells of *Pippali* (Image 11),Oil globule of *Patra* (Image 12), Oil globules of *Twak* (Image 13), Prismatic crystal of *Kanchanara*(Image 14), Rhomboidal crystals of *Varuna* (Image 15), Scleroids of *Amalaki* (Image 16), Scleroids of *Haritaki* (Image 17), Scleroids of *Bibhitaki* (Image 18), Silica deposition of *Amalaki* (Image 19), Starch grains of *Sunthi* (Image 20), Stone cell of *Kanchanara* (Image 21), Stone cells of *Haritaki (Image 22)*, Tenin contain of *Twak (Image 23)*, Trichome of *Bibhitaki(Image 24).*

Physico -Chemical Study

- **Organoleptic analysis**

The characters of the sample are tabulated in Table no.2.

- **Physico-chemical analysis**

Physicochemical parameters of the *Vati* like Uniformity of the weight, Hardness, Loss on drying, pH, Ash Value, Water soluble extract, Methanol soluble extract, Acid insoluble ash, HPTLC was evaluated. The results are placed at Table no.3.

Qualitative Test of *Kanchanara Guggulu*

The Methanol extract of the sample was analyzed qualitatively for different functional groups. Details are placed at Table no.4.

HPTLC Detection: 1- Short (254nm) and long (366nm) wave UV radiation.

Maximum 13 spots were obtained under short wave ultra violet light (254 nm) and Maximum 12 spots were obtained under long wave ultra violet light (366 nm). Rf values of the spots obtained were at a comparable level which indicates the presence of some definite constituents in the sample. Chromatogram shows 13 prominent spots at hRf 0.03,0.20,0.27,0.37,0.40,0.45,0.51,0.56,0.66,0.72,0.79,0.87,0.95 in short wave uv 254 nm and 12 prominent spots at hRf 0.03,0.20,0.27,0.37,0.45,0.50,0.56,0.62,0.72,0.79,0.90,0.95 in long wave uv 356 nm. 4 spots at hRf 0.03, 0.20, 0.27, 0.37 are common in both UV light. Though it was not possible to identify particular chemical constituent from the spot obtained, the pattern may be used as a reference standard for further quality control researches. (Images: 25 - 29).

TABLE NO. 1: INGREDIENTS OF *KANCHANARA GUGGULU VATI*[16]

Sr.no	Drug	Botanical name	Part use	Ratio
1	*Kanchanara*	*Bauhinia Variegate* Linn.	*Twak*	480 gm(10 *Pala*)
2	*Triphala*	*Haritaki-Terminalia chebula* Retz	*Phala*	
		Bibhitaki-Terminalia Bellerica Roxb	*Phala*	288 gm(6 *Pala*)
		Aamalaki-Emblica Officinalis Gaert	*Phala*	
3	*Trikatu*	*Sunthi-Zingiber Officinale* Rosc	*Kanda*	
		Maricha-Piper Nigrum Linn	*Phala*	144gm (3 *Pala*)
		Pippali-Piper Longum Linn	*Phala*	
4	*Varuna*	*Crateva nurvala* Buch ham	*Twak*	48 gm (1 *Pala*)
5	*Ela*	*Elettaria cardamomum* Maton	*Beeja*	
6	*Twak*	*Cinnamomnm zeylanicum* Breyn	*Twak*	48 gm (1 *Karsha*)
7	*Patra*	*Cinnamnm tamala* Nees & Eberm	*Patra*	
8	*Guggulu*	*Commiphora mukul* Hook Exstocks	*Niryas*	As equal as *Choorna*

TABLE 2. ORGANOLEPTIC CHARACTORS OF *KANCHANARA GUGGULU*

Physical Properties	*Kanchanara Guggulu*
Odour	*Guggulu* like resin
Taste	*Kashaya Tikta*
Colour	Black
Touch	Tablet hard

TABLE NO.3 PHYSICO-CHEMICAL PARAMETERS

No.	Name of the Test	Value
1.	Loss of drying (at 110^{o}c)	9.26 % w/w
2.	Ash Value	14.1 % w/w
3.	Acid insoluble Ash	6.05 % w/w
4.	Water soluble extraction	27.2% w/w

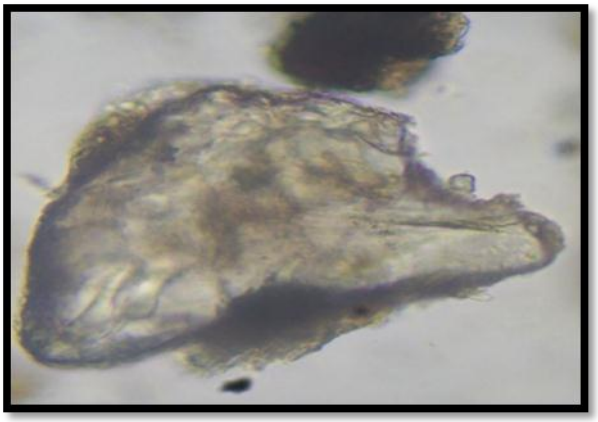
5.	Alcohol soluble extraction	9.64% w/w
6.	pH value by pH paper	6.5
7.	Uniformity of tablet Maximum weight	3.120 gm
	Minimum weight	2.803 gm
	Average weight	2.961 gm
8.	Tablet hardness	4.75 kg/cm^2

TABLE NO.4 CHROMATOGRAPHIC FINGERPRINTING OF *KANCHANARA GUGGULU*

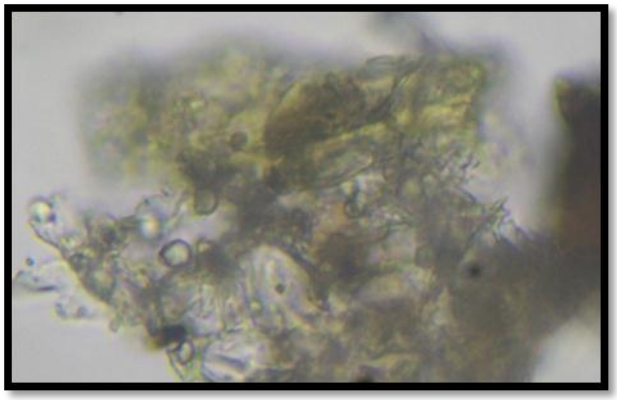
SOLVENT SYSTEM	SHORT UV RADIATION 254 NM		LONG UV RADIATION 366 NM	
	No. of spot separated	Retention factor Rf	No. of spot separated	Retention factor Rf
	13	0.03,0.20,0.27,0.37,0.40,0.45,0.51,0.56,0.66,0.72,0.79,0.87,0.95	12	0.03,0.20,0.27,0.37,0.45,0.50,0.56,0.62,0.72,0.79,0.90,0.95

PLATE NO.1 PHOTOMICROGRAPHS OF *KANCHANARA GUGGULU*

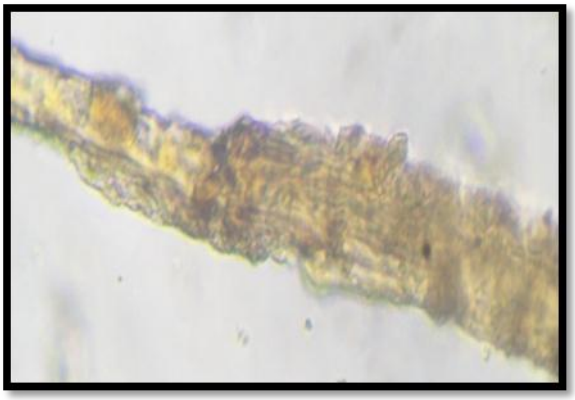
1. *Kanchanara Guggulu*



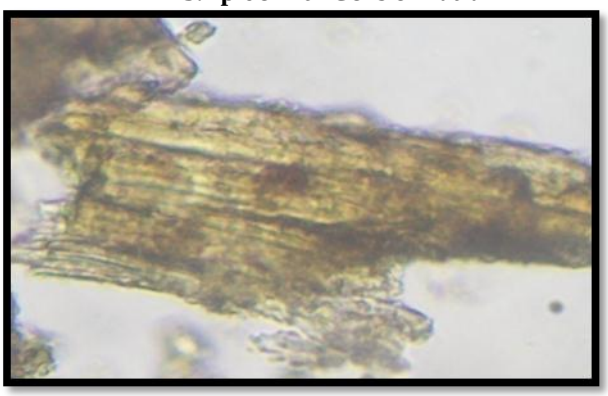
2. Black Debris of *Maricha*



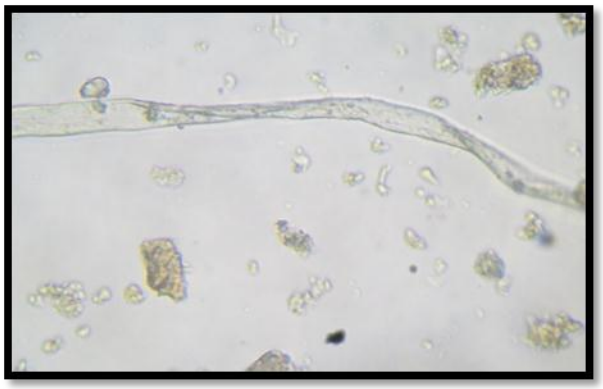
3.Epidermal Cells of *Patra*



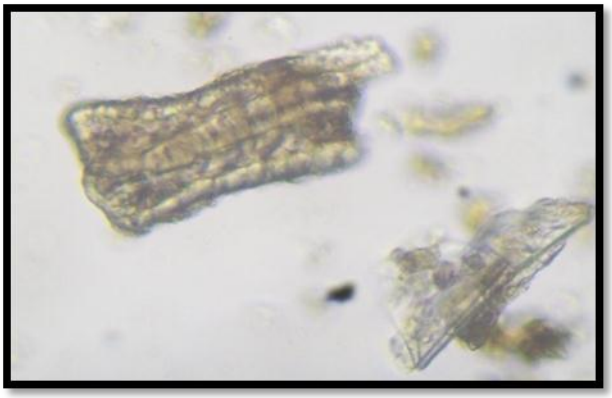
4. Fiber of *Twak*



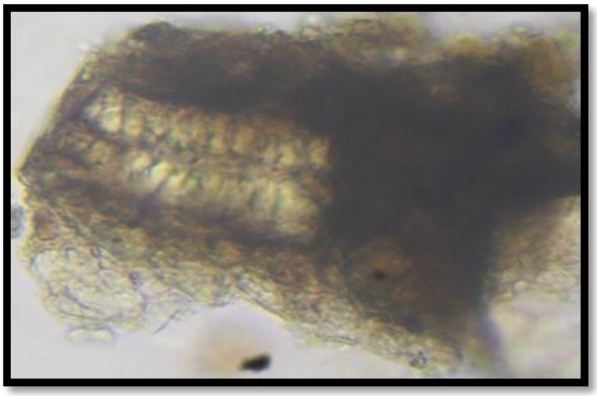
5.Fiber of *Varuna*



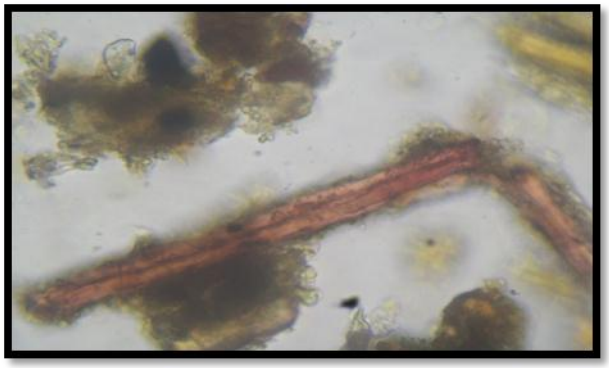
6. Fibers of *Sunthi*



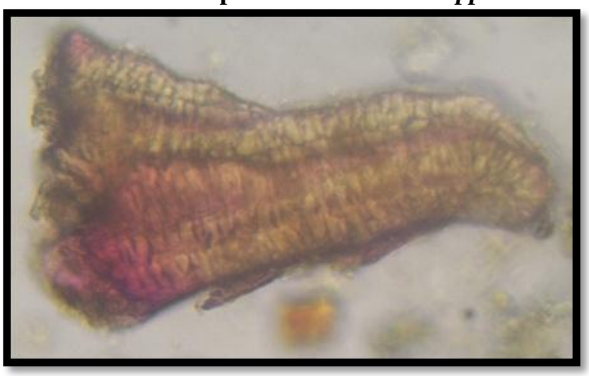
7.Group of Stone Cells of *Maricha*



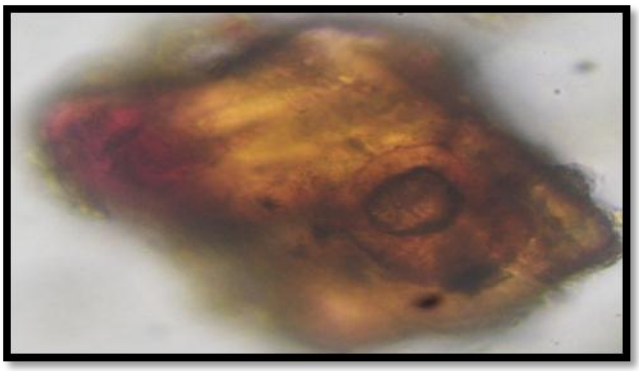
8.Group of Stone Cells of *Pippali*



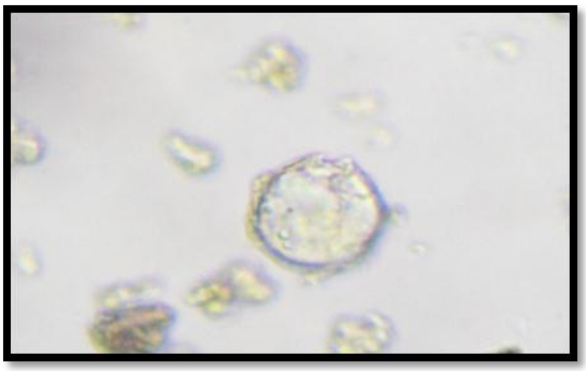
9.Lignified Fibers of *Twak*



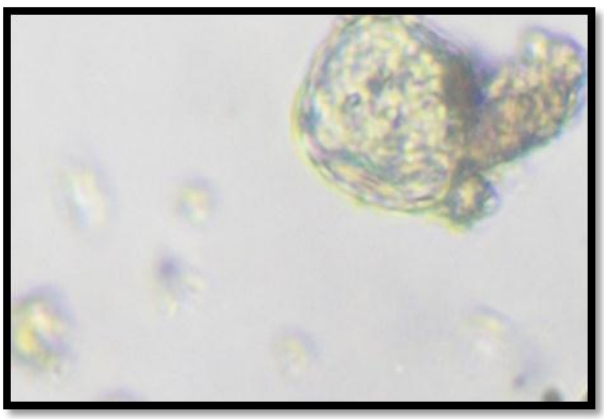
10.Lignified Scleroids of *Haritaki*



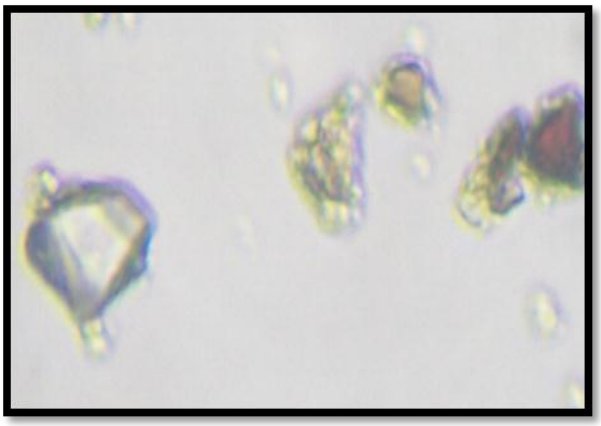
11. Lignified Stone Cells of *Pippali*



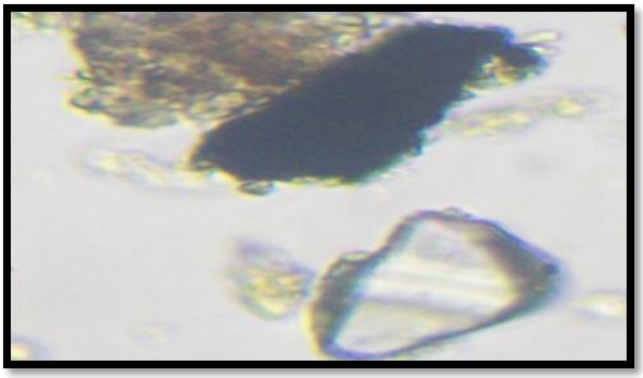
12. Oil Globule of *Patra*



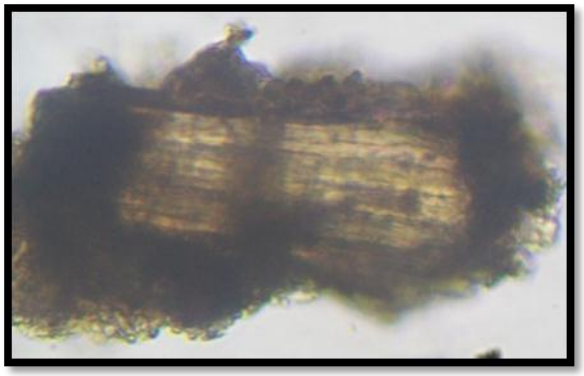
13. Oil Globules of *Twak*



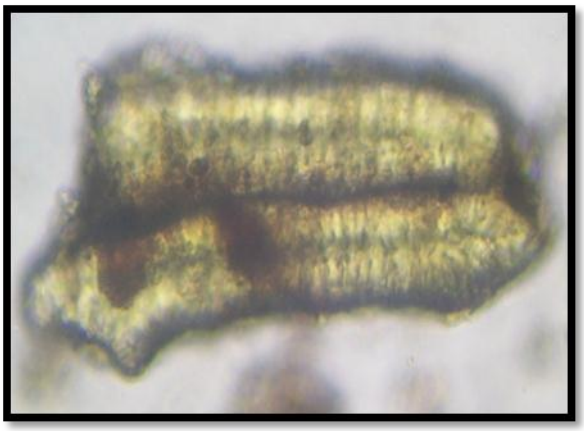
14.Prismatic Crystal of *Kanchanara*



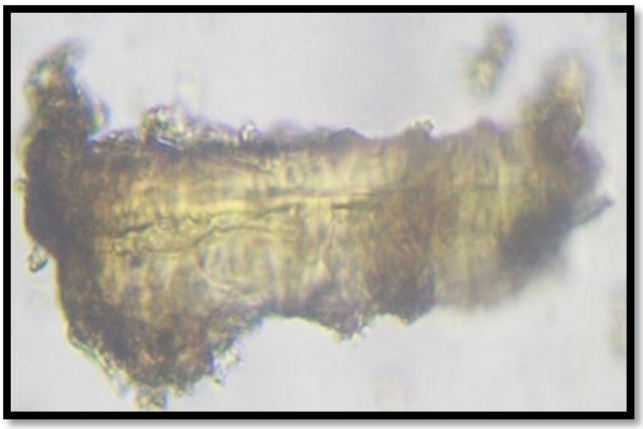
15.Rhomboidal Crystals of *Varuna*



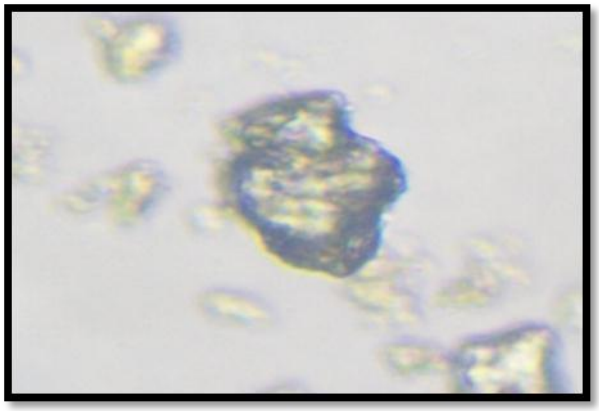
16.Scleroids of *Amalaki*



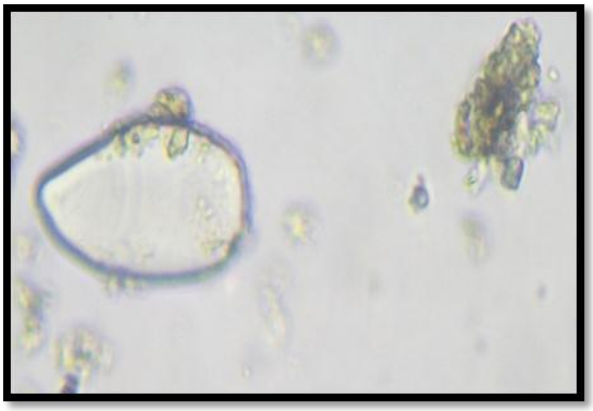
17.Scleroids of *Haritaki*



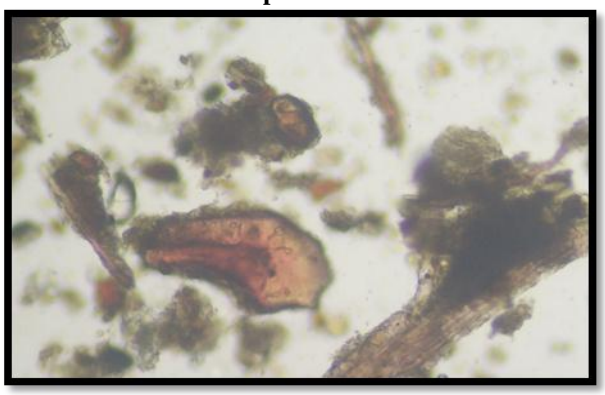
18.Scleroids of *Bibhitaki*



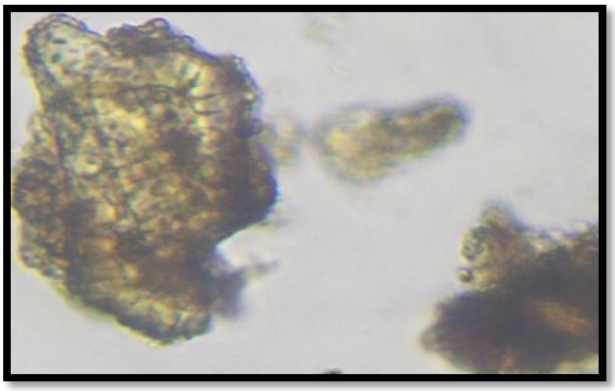
19.Silica Deposion of *Amalaki*



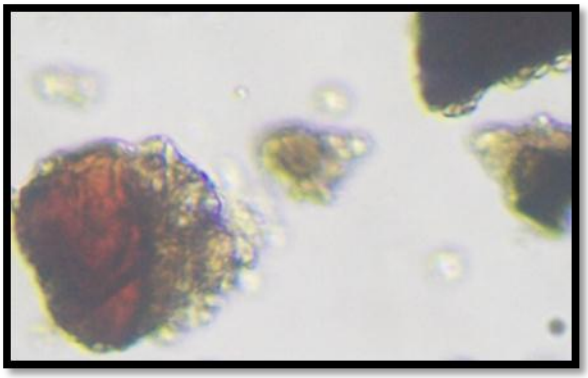
20.Starch Grains of *Sunthi*



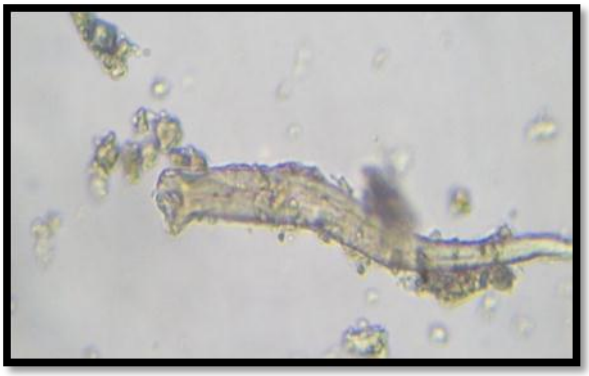
21.Stone Cell of *Kanchanara*



22.Stone Cells of *Haritaki*



23.Tenin Contain of *Twak*



24.Trichome of *Bhibhitaki*

PLATE NO. 2: HPTLC RESULTS

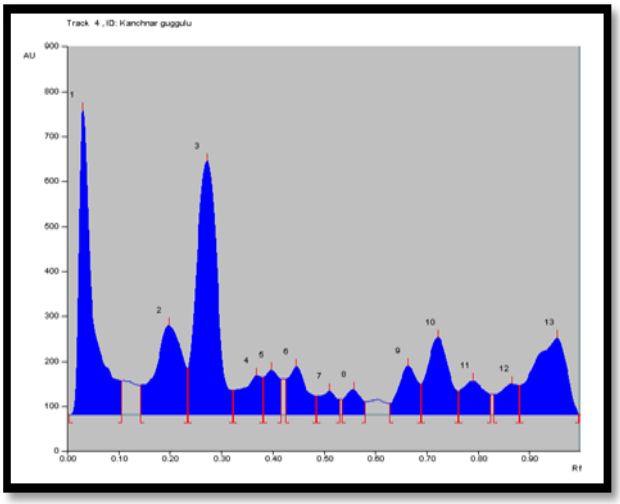


Image 25(254 nm)

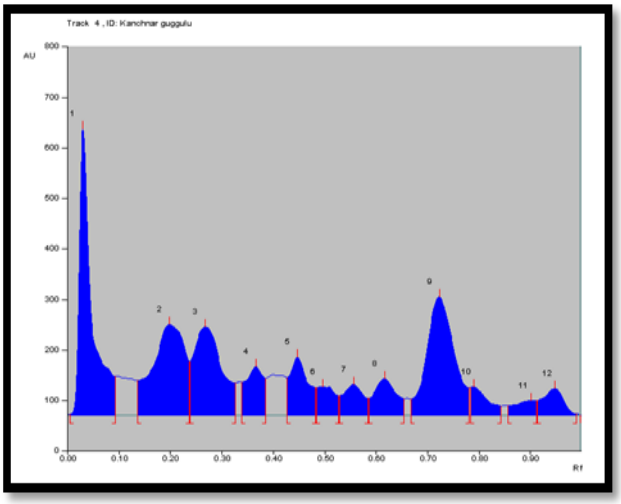


Image 26(356 nm)

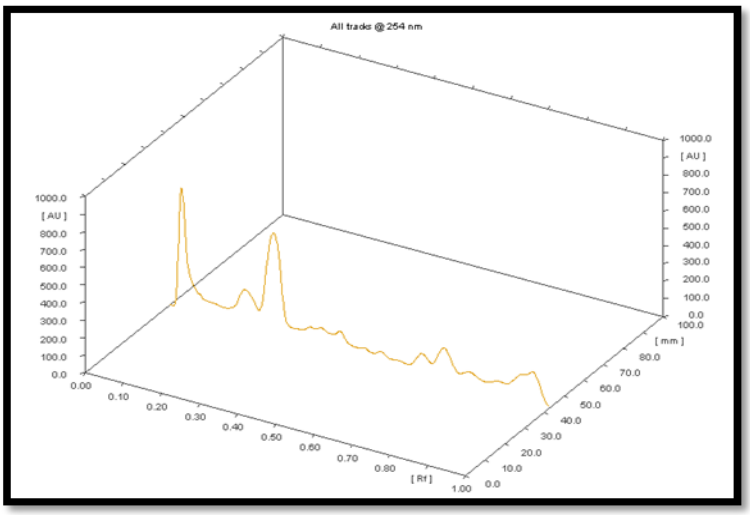


Image 27 Three-dimensional densitogram of methanolic extract of *Kanchanara Guggulu Vati* at 254 nm

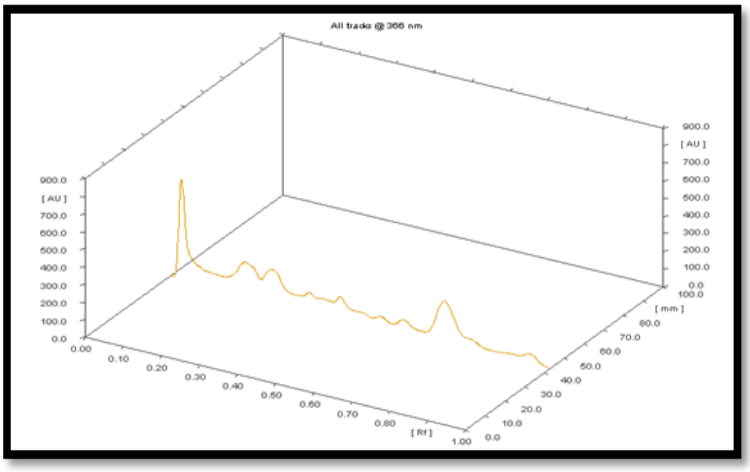


Image 28. Densitogram of methanolic extract of *Kanchanara Guggulu Vati* at 366 nm

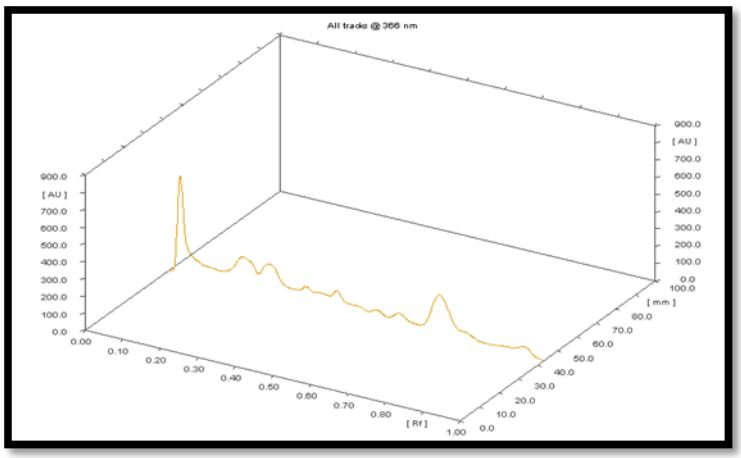


Image 29.Three-dimensional milled wood lignin of methanolic extract of *Kanchanara Guggulu Vati*

DISCUSSION

Powder microscopy of *Kanchanara Guggulu Vati* revealed the diagnostic characters like Black debris of *Maricha*, Epidermal cells of *Patra*, Fiber of *Twak, Varuna*, *Sunthi*, Group of stone cells of *Maricha*, Group of stone cells of *Pippali*, Lignified fibers of *Twak*, Lignified scleroids of *haritaki* , Lignified stone cells of *Pippali*, Oil globule of *Patra*, Oil globules of *Twak*, Prismatic crystal of *Kanchanara*, Rhomboidal crystals of *Varuna*, Scleroids of *Amalaki*, Scleroids of *Haritaki*, Scleroids of *Bibhitaki*, Silica deposion of *Amalaki*, Starch grains of *Sunthi*, Stone cell of *Kanchanara*, Stone cells of *Haritaki*, Tenin contain of *Twak*, Trichome of *Bhibhitaki* which authenticate genuineness of the raw drugs of *Kanchanara Guggulu Vati*. Taste of *Kanchanara Guggulu Vati* was *Kashaya Tikta* (bitter) because the majority of contents of *Kanchanara Guggulu Vati* having *Kashaya Tikta* taste. *Guggulu* was purified by cow urine which may result in cow urine like odor of formulation.

Moisture contents should be minimum to prevent degradation of the product. Excess of water in formulation encourage microbial growth, presence of fungi or insects, and deterioration following hydrolysis. *Kanchanara Guggulu Vati* contains 9.26% w/w moisture, showing that the *Vati* should be protected from the humid atmosphere. Ash values are the criteria to judge the identity and purity of crude drugs where total Ash, Water-soluble and Acid-insoluble ashes are considered. *Kanchanara Guggulu Vati* contained 14.1% w/w total ash and 6.05% w/w Acid insoluble ash. The results revealed that *Kanchanara Guggulu Vati* is free from unwanted organic compounds and production site was good enough keeping sample free from dust and other solid matters. About 27.2% w/w of water soluble extractives and 9.64% w/w methanol soluble extractives were present in *Kanchanara Guggulu Vati* indicating that the drug has good solubility in water.[17] *Kanchanara Guggulu Vati* was found to have 2.961 gm average weight. All the *Vati* were within acceptable range of weight variation as for natural herbal products. Hardness of *Vati* interferes with the bioavailability of drug. *Kanchanara Guggulu Vati* was found to have 4.75 Kg/cm^2 hardness which was noticed in acceptable limit. In HPTLC study, 13 spots at 254 nm and 12 spots at 366 nm were obtained, indicating its possible components of the matrix which may possess its therapeutic effect.

CONCLUSION

The ingredients were identified and authenticated pharmacognostically and were used for the preparation. The formulation was subjected to pharmacognostical study reveals genuineness as that all the ingredient microscopic characters were observed. Physicochemical and HPTLC studies inferred that the formulation meets the minimum quality standards. The inference from this study may be used as reference standard in the further quality control researches.

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