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PHARMACOGNOSTICAL AND PRELIMINARY PHYSICO CHEMICAL ASSAY OF *RASAYANA* GRANULES – A PILOT STUDY

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ABSTRACT

In *Ayurveda* context *Acharya Sushruta* has mentioned to take *Shita, Madhura, Drava Ahara* for *Garbhini*. *Rasayana* drugs also having these qualities and no side effects. Drug that nourishes, energizes & vitalizes are *Rasayana* drugs. The drugs are seasonal and pregnant women have taste variations along with morning sickness. As the powder is not palatable, its form has been converted in to granules form. Till date there is no scientific evaluation has been observed. An attempt is made to evaluate its Pharmacognostical and physicochemical profile. Pharmacognostically authenticated *Rasayana* drugs were used for the preparation of granules and it was analyzed through qualitative and quantitative analysis of physicochemical parameters. High-Performance Thin Layer chromatography study (HPTLC) was also developed. Pharmacognostical results shows that Stone cells of *Jeevanti*, Brown content of *Draksha*, Silica deposition of *Amalaki*, Chollenchyma cells of *Guduchi* & Acicular crystal of *Shatavari* etc is found. Qualitative study shows that ph is 6.5, ash value is 6.55% w/w, loss on drying is 1.945% w/w, water soluble extract is 78% w/w & alcohol soluble extract is 34.84% w/w. HPTLC study shows that Maximum 7 spots were obtained when viewed under short wave ultra violet light (254 nm) and 4 spots viewed under long wave ultra violet light (366 nm).

KEYWORD: *Bala, Shatavari, Guduchi,* Phytochemical Analysis, Chromatography.

INTRODUCTION

In the description of *Shodasakala Chikitsa*[1] (Sixteen Pre-requisite Qualities), Ayurvedic classics have given special importance to the physician's ability to understand the rationale

behind the utility of natural components in modifying the disease conditions and in re-establishing the equilibrium of *Doshas* (Humour), *Dhatus* (Tissues) and *Mala's* (Waste Products) or in other words, maintaining the health. *Acharya Charaka*, explained *Guduchi* under *Vayasthapan, Dahaprashmana, Trishnanigrahana, Stanyashodhan, Triptighna Gana and Acharya Sushruta* included this drug under *Guduchyadi, Patoladi, Aragvadhi, Kakolyadi, Vallipanchamula. Acharya Charaka*, explained *Amalaki* under *Vayasthapana, Virechanopaga gana and Acharya Sushruta* included this drug under *Parushakadi, Triphala Gana.*[2][3][4][5][6] *Acharya Charaka*, explained *Jeevanti* under *Jivaniya, Madhura Skandh and Acharya Sushruta* included this drug under *Kakolyadi Gana.*[7] *Acharya Charaka*, explained *Shatavari* under *Balya, Vayasthapan, Madhurskandh Gana and Acharya Sushruta* included this drug under *Vidarigandhadi, Kantakpanchmula,Pittaprashmana Gana.*[8][9] *Acharya Charaka*, explained *Bala* under *Balya, Brihaniya, Prajasthapan, Madhurskandh Gana and Acharya Sushruta* included this drug under *Vatasanshamana Gana.*[10] *Acharya Charaka*, explained *Vatashunga* under *Mutrasangrahaniya, Kashayaskandh Gana and Acharya Sushruta* included this drug under *Nyogrodhadi Gana.*[11][12][13][14][15][16][17][18][19][20] *Acharya Charaka*, explained *Draksha* under *Snehopaga, Virechanopaga, Kasahara, Jwarahara Gana and Acharya Sushruta* included this drug under *Kakolyadi, Parushakadi Gana.*[21][22][23]

Literature survey reveals that *Guduchi* is used in *Chhardi, Aruchi, Agnimandhya, Amlapitta* & has antiemetic, digestive, carminative, appetizer & haematinic effect. Amalaki has *Dahaprashamana, Balya, Rochana, Deepana, Anulomana, Stambhana, Hridya, Shonitasthapana, Garbhasthapana, Rasayana, Rasa, Rakta, vardhana property* & has antimutagenic, antimicrobial, antioxidant, immunomodulatory effect.[2][3][4][5][6] Jeevanti is used in uterine haemorrhages & habitual / threatened abortions.[7] Shatavari has antiabortifacient (shatavarin I), antioxytoxic (shatavarin IV) & antispasmodic activity.[8][9] Bala has *Prajastapaka, Raktapittashamaka, Balya, Brihana, Ojovardhaka property and use as Garbhaposhnartha in Garbhashaya Daurbalya.*[10] *Vatashung has Raktapittahara, Raktarodhaka, Stambhaka & Garbhastapaka property.*[11][12][13][14][15][16][17][18][19][20] *Draksha has* Soumanasyajanana, Garbhasthapaka, Jeevaniya, Balya, Brumhana property.[21][22][23]

In the description of *Chatuspada* (Four Limbs of Treatment), Drugs have the second most importance in treating the diseases and also the drug should possess the good qualities in it.[24] So, proper identification and standardization of the drug is essential. Each and every

drug has its own physical and chemical characteristics that help for separating it from other closely related drugs. Hence physicochemical studies of a particular drug by making use of various parameters help in standardizing the drug and validate it. Chromatographic techniques were adopted for the separation of active moieties present in the formulation. Therefore, an attempt has been made to standardize *Rasayana* granules, an Ayurvedic medicine based on their. Pharmacognostical Physico-chemical and HPTLC fingerprint profile.

Method of preparation of the *Rasayana* Granules

Khadi Shakar was made into powder form and was boiled with water till it lost about 10% of water content, powders of *Guduchi, Amalaki, Jeevanti, Shatavari, Bala, Vatashung* were added and mixed properly. The mixture was again heated. Pulp of *Draksha* was added and mixed properly.

Mixture was given heat till it became homogeneous and consistent i.e. *Darvipralepatva* was achieved. Heating was stopped. Prepared drug was placed in the dryer till it became granular. Bigger granules were broken in to required size. No preservatives were added.

MATERIALS AND METHODS

Plant Material: All *Rasayana* drugs powder is collected from the Pharmacy, Gujarat Ayurved University, Jamnagar. The powder was subjected to powder microscopy.

Organoleptic characters

Organoleptic characters of test drug such as odour, taste, texture and colour were observed and recorded.[25]

Powder microscopy

For examining characters of the test powder, pinch of powder was taken on glass slide and observed as such to see their cell contents and then stained with phloroglucinol and hydrochloric acid to observe the lignifications of the cell wall.[26] The sample was observed under compound microscope and photographs were taken.

Physicochemical parameters

Physico-chemical Parameters like Loss on drying, alcohol soluble extractive and water-soluble extractive values and pH were determined as per the API guidelines for the test sample.[27]

High-Performance Thin Layer Chromatography study

Methanol extract of Sample was spotted on pre coated silica gel GF254 aluminium 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, 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 (v1.2.1 camag). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 20 mm s^{-1}. All HPTLC plates were scanned with filter faction Savitsky-goloy 7, minimum spot-5, minimum height 10AU, minimum area 50AU, maximum height 990 AU with absorption unit.

RESULTS AND DISCUSSION

Pharmacognostical Study

Organoleptic characters: The powder was Light brown in colour, has aromatic odor and *Madhur, Tikta* & *Kashaya* in taste.

Powder Microscopy

The dried powder was mounted in the distilled water to detect the Prismatic crystal of *Jeevanti*, Stone cells of *Jeevanti*, Brown content of *Draksha* Acicular crystal of *Shatavari*, Chollenchyma cells of *Guduchi*, Silica deposition of *Amalaki*, Pitted vessels of *Jeevanti*, Acicular crystal of *Draksha*, Simple starch grain of *Guduchi*, Simple trichome of *Bala*, Septate fibres of *Bala*, Border pitted vessels of *Guduchi*, Stomata of *Vatashung*, Stone cells of *Jeevanti*, Rhomboidal crystal of *Jeevanti*, Lignified fibre of *Jeevanti*, Simple trichome of *Vatashung* Starch grain with hilum of *Jeevanti* & Stellate trichome of *Bala* were observed.(Plate no. 1 .1-21).

Physico - chemical Study

Organoleptic Characters: The characters of the sample are tabulated in table no.2.

Physico-chemical parameters: The granules were evaluated for physico chemical parameters like Total Ash Value, loss on drying, pH value, Sugar estimation (Total sugar, Reducing Sugar, Non - Reducing Sugar), Acid soluble and water-soluble extractive values. The results were placed at table no.3.

The Common parameters mentioned for *Rasayana drugs* in Ayurvedic Pharmacopoeia of India are total ash, pH Value, water and alcohol soluble extractives. On its basis the parameters like total ash content, water and methanol soluble extractives etc., were selected. Presence of more moisture content in a sample can create preservation problem. Hence loss on drying was also selected as one of parameters. Since, the sample was in the form of granules has the possibility of containing significant quantity of sugar, hence Sugar estimation was considered as another parameter. Total sugar was found to be 50.73% w/w suggesting presence of considerable amount of sugar in the sample.

Qualitative Test of *Rasayana* Granules: 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 7 spots were obtained when viewed under short wave ultra violet light (254 nm) and 4 spots viewed under long wave ultra violet light (366 nm). R_f values of the spots obtained were at a comparable level which indicates the presence of some definite constituents in the sample. Chromatogram shows 7 prominent spots at hR_f 0.01, 0.08, 0.27, 0.43, 0.63, 0.79, 0.89 in short wave uv 254 nm and 4 prominent spots at hR_f 0.01, 0.08, 0.14, 0.28 in long wave uv 356 nm. 2 spots at hR_f 0.01 & 0.08 are common in both UV light. (Plate no. 2).

Table no. 1 *Rasa Panchaka* (Pharmacodynamics) of *Rasayana* Granules

Sr.No.	*Drug*	*Rasa*	*Guna*	*Veerya*	*Vipaka*	*Doshghanta*
1.	*Guduchi*	*Kashaya, Tikta*	*Snigdha, Guru*	*Ushna*	*Madhura*	*Tridoshahar*
2.	*Amalaki*	Amla rasapradhana 5 rasa except Lavana	Guru, Ruksha, Sheeta	*Sheeta*	*Madhura*	Tridoshahara specially Pittahara
3.	*Jeevanti*	*Madhura*	*Snigdha, Laghu*	*Sheeta*	*Madhura*	*Tridoshhar mainly Vaata Pitta Shaamaka*
4.	*Shatavari*	*Madhura, Tikta*	*Snighda,Guru*	*Sheeta*	*Madhura*	*Vaata Pitta Shaamaka*
5.	*Bala*	*Madhura*	*Snigdha, Laghu, Picchil*	*Sheeta*	*Madhura*	*Vaata Pitta Shaamaka*
6.	*Vatashung*	*Kashaya*	*Ruksha,Guru*	*Sheeta*	*Katu*	*Kapha Pitta Shaamaka*
7.	*Kishmish*	*Madhura*	*Snigdha, Guru, Mrudu*	*Sheeta*	*Madhura*	*Vaata Pitta shaamaka*
8.	*Sita*	*Madhura*	*Snigdha*	*Sheeta*	*Madhura*	*Vaata Pitta Shaamaka*

Table 2: Organoleptic Characters of *Rasayana* Granules

	Drug	Touch	Colour	Odour	Taste
1.	*Rasayana* Granules	Rough	Light Brown	Aromatic	Madhura, Tikta, Kashaya

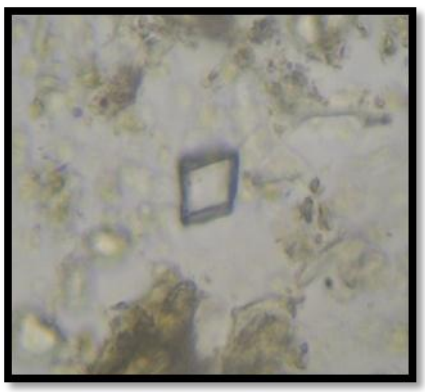
Table No. 3 Physico-chemical parameters

Sr No.	Test name	Drug-*Rasayana granules*
1.	**Loss on drying**	1.945% w/w
2.	**Water soluble extracts**	78% w/w
3.	**Methanol soluble extracts**	34.84% w/w
4.	**Ash value**	2.24
5.	**pH value**	6.5
6.	**Sugar estimation**	
	Total Sugar	50.73%w/w
	Reducing Sugar	11.53%w/w
	Non-ReducingSugar	39.2%w/w

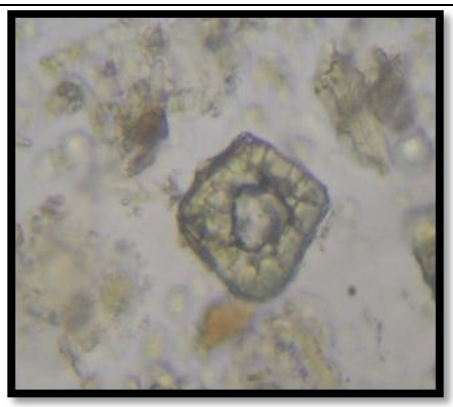
Table No. 4 Chromatographic fingerprinting of *Rasayana* Granules

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)
Toulene:Ethyl Acetate:formic Acid (7:2:0.5 v/v)	**7**	0.01, 0.08, 0.27, 0.43, 0.63, 0.79, 0.89	**4**	0.01,0.08,0.14,0.28

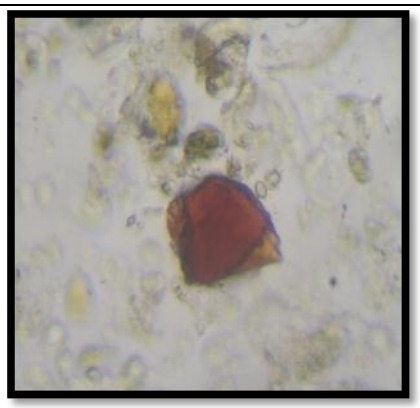
Plate no. 1: Photomicrographs of *Rasayana* Granules



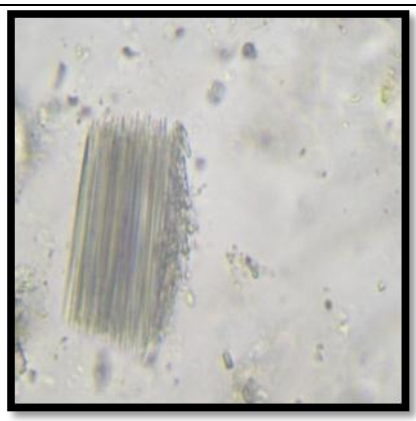
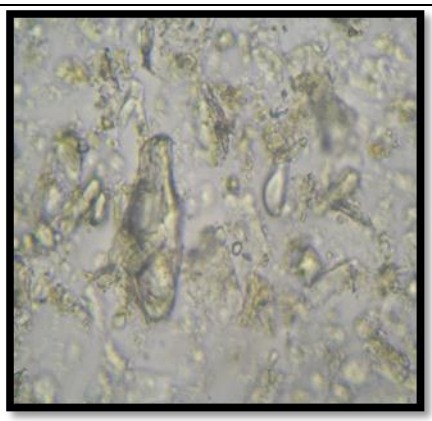
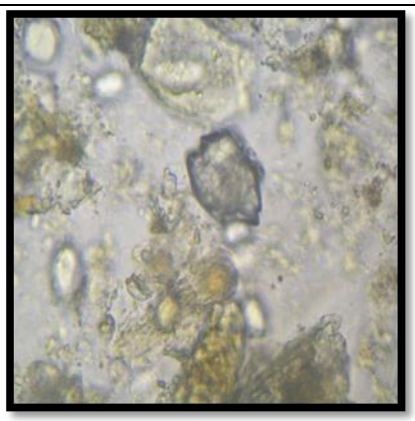
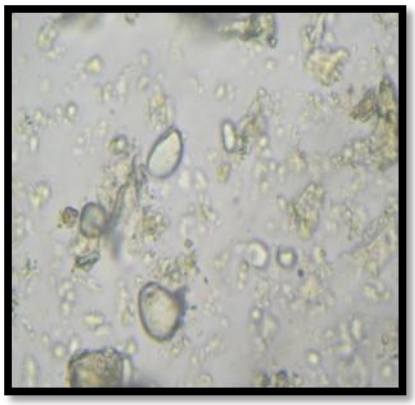
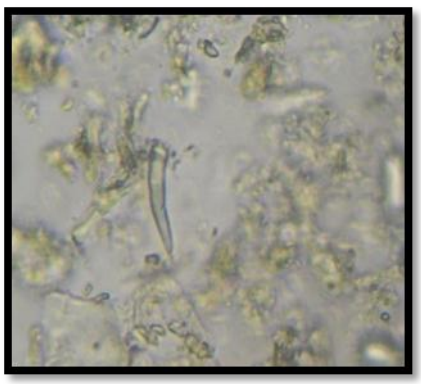
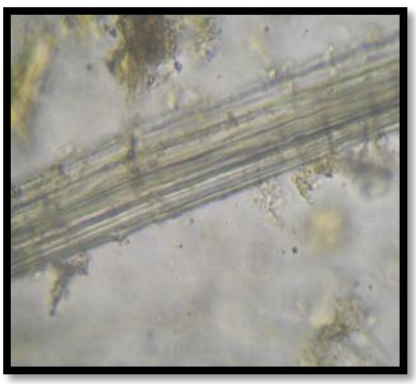
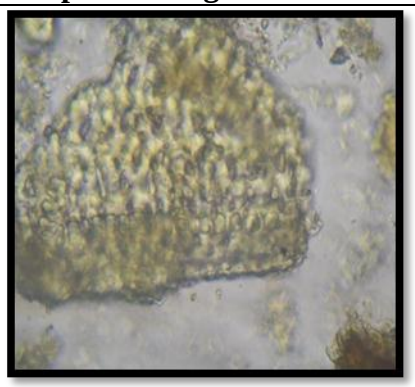
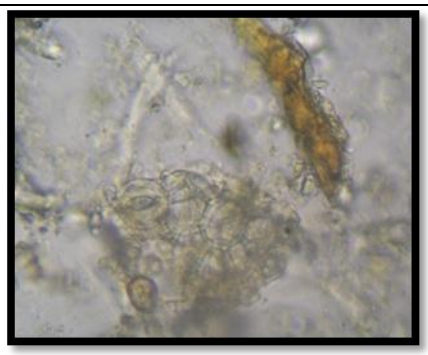
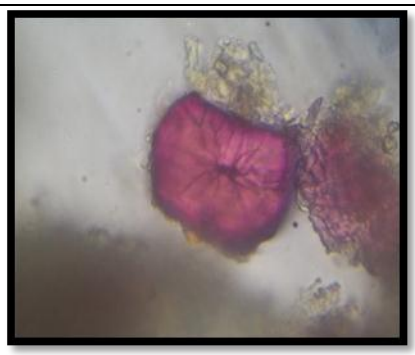
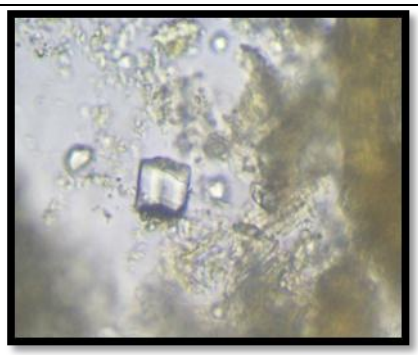
1. Prismatic crystal of Jeevanti



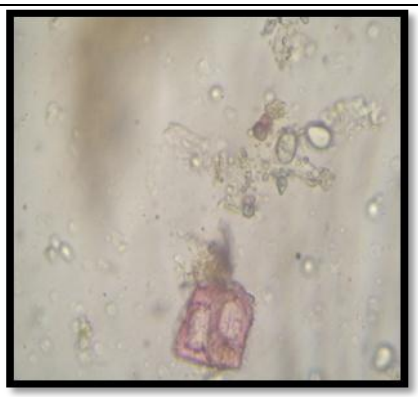
2 Stone cells of Jeevanti



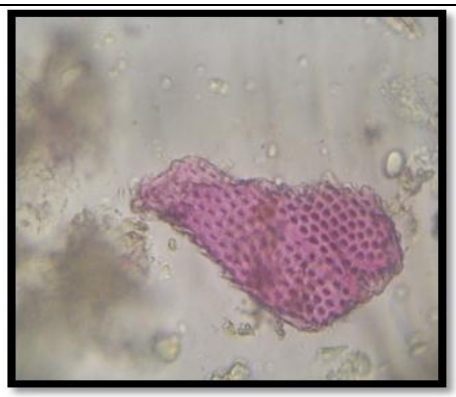
3 Brown content of Draksha

		
4 Acicular crystal of Shatavari	**5 Chollenchyma cells of Guduchi**	**6 Silica deposition of Amalaki**
		
7 Pitted vessels of Jeevanti	**8 Acicular crystal of Draksha**	**9 Simple starch grain of Guduchi**
		
10 Simple trichome of Bala	**11 Septate fibres of Bala**	**12 Border pitted vessels of Guduchi**
		
13 Stomata of Vatashung	**14 Stone cells of Jeevanti**	**15 Rhomboidal crystal of Jeevanti**

16 Lignified fibre of Jeevanti

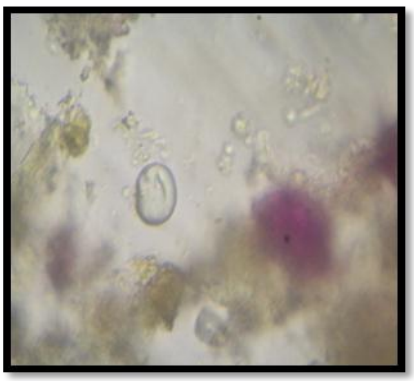


17 Chollenchyma cells of Guduchi

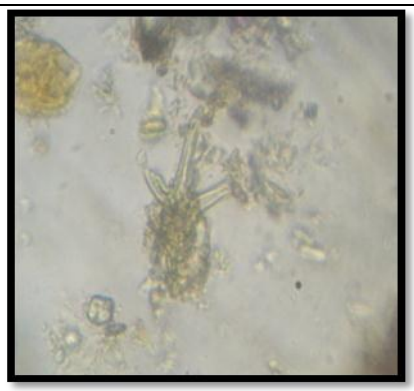


18 Pitted vessels of Jeevanti

19 Simple trichome of Vatashung

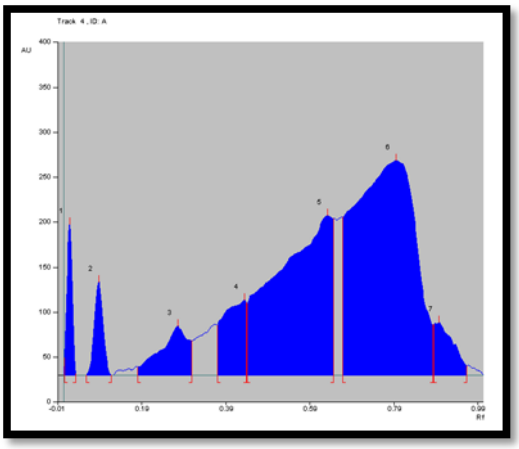


20 Starch grain with hilum of Jeevanti

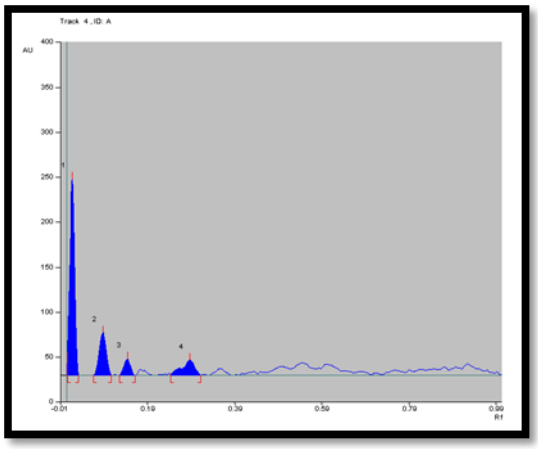


21 Stellate trichome of Bala

Plate no. 2: HPTLC results of *Rasayana* Granules



HPTLC- peaks at 254 nm



HPTLC- peaks at 366 nm

CONCLUSION

The *Rasayana* drugs are used from the ancient time for its medicinal values. In Pregnancy *Rasa* is the main factor responsible for nutrition of mother as well as fetus. *Rasayana* improves the quality of *Rasa* which further cause *Dhatupushti*, thereby leading to proper growth & development of the embryo. In the present study, the granule form was evaluated for pharmaceutical feasibility and for patient palatability. All *Rasayana* drugs were identified

and authenticated phamacognostically and were used as a unique ingredient. The formulation namely, *Rasayana* Granules were subjected to phytochemical, physico-chemical, TLC and HPTLC studies. It is inferred that the formulation meets the minimum qualitative standards as reported in the API at a preliminary level.

On the basis of our observations and experimental results, we created method of preparation of Granules for the first time and are economical in terms of time and machinery. This study may be used as reference standard in the further quality control researches.

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