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PHARMACO-ANALYTICAL STUDY OF TRAYODASHANGA GUGGULU VATI

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ABSTRACT

Trayodashanga Guggulu is an Ayurvedic herbo-mineral formulation found to be very effective in Vatakapha condtions especially vata vyadhi. It is indicated in nervous disorders and musculoskeltal disorders. The overall aim of drug standardization is to ensure the quality, efficacy and uniformity of the products, in terms of their chemical and biological properties, across the manufactures and it should be started with the identification and authentication of the drug. **Materials and Method:** Pharmacognostical evaluation carried out at Pharmacognosy department, ITRA, Jamnagar, pharmaceutical and HPTLC study were done at Pharmaceutical chemistry department, ITRA, Jamnagar. Microbiological evaluation was carried out at department of Microbiology I.T.R.A, Jamnagar. **Results:** Organoleptic examination: Colour: Blackish, Odour: Ghee odour, Taste: Astringent, bitter, Touch: Hard Pharmaceutical evaluation: Loss on drying: 7.75% Ash Value: 13.25% Weight variation (Average weight):498.1mg, Water solubility: 25.26% Alcohol solubility: 17.6% Hardness of Vati (Average): 2.16kg/cm^{2}pH: 6.5. The HPTLC of TG, 6 major spots were observed at 254nm (short wave) showed mainly 7 major spot and at 366 nm (long wave) showed 5 major spots. No organisms isolated in aerobic and fungal culture, in microbiology evaluation. **Conclusion:** quality control parameters were followed during the collection, identification,

preparation and packing of the sample. HPTLC evaluation confirmed the authenticity of the sample. Absence of contamination is confirmed by the microbiological investigation.

KEYWORDS: Pharmaceutical analysis of Trayodashanga Guggulu, Analysis of Vati, Standardization of Vati, Pharmacognosy of Trayodashanga Guggulu.

INTRODUCTION

Ayurveda belongs to the great ancestry of Vedas in which medicinal herbs are an integral part. Due to the undesirable effect of modernization and industrialization, scarcity of medicinal plants is up-surging and it also makes the genuine resources unavailable. This in turn favouring the threat of adulteration. Different ayurvedic formulations are prepared by many pharmaceutical companies strictly following ayurvedic classical references, still there are some variations observed among same products of different companies. Here arise the need of standardization. The overall aim of drug standardization is to ensure the quality, efficacy and uniformity of the products, in terms of their chemical and biological properties, across the manufactures.[1] But standardization is a difficult thing to execute in Ayurveda as most of the preparations in them are poly herbal combination and complex in manufacturing procedures. So the standardization should start from Identification and authentication of drugs itself.

Trayodashanga Guggulu is an Ayurvedic herbo-mineral formulation mentioned in Vatavyadhi Prakarana of Bhaishajya Ratnavali[2] in which Guggulu is the basic ingredient. This is in Vati/Tablet form found to be very effective in Vatakapha condtions, and is having Anti-inflammatory effect also.TG is indicated in nervous disorders and musculoskeltal disorders. Already some works were carried out regarding the analysis of TG, but they didn't include microbiological investigations moreover values of pharmaceutical evaluations were found different. So for a better understanding and comparison the present study has been done. As per the Ayurvedic pharmacopoeia of India the analysis of Vati include 7 procedures and are as follows[3]:

1. Loss on drying,
2. Ash Value
3. Weight variatio
4. Water solubility
5. Alcohol solubility
6. P^H value

7. Hardness of Vati

MATERIALS AND METHODS

All the drugs of Trayodashanga Guggulu were obtained from Pharmacy, Gujarat Ayurved University, Jamnagar. Then the drugs were powdered separately, and they were subjected to pharmacognostical evaluation at Pharmacognosy laboratory, ITRA Jamnagar, Jamnagar, Gujarat then the vati is prepared at Pharmacy of ITRA, Jamnagar. Once the formulation was ready, it was subjected to Pharmaceutical evaluation and HPTLC at pharmaceutical laboratory, ITRA, Jamnagar, Gujarat. Microbiological investigation was done and will continue for every month at Microbiology Dept ITRA, Jamnagar.

The contents of Trayodashanga guggulu along with Latin name, parts used and ratio are as mentioned below:-

Table 1:

Drug	***Scientific name***	***Part used***	***proportion***
Aabha (babbul)	*Acasia Arabica(L)Delile*	*Stem bark*	*1 part*
Ashwagandha	*Withania somnifera(L) Dunal*	*Roots*	*1 part*
Hapusha	*Juniperus communis(L)*	*Fruit*	*1 part*
Guduci	*Tinospora cordifolia(Willd)*	*Stem bark*	*1 part*
Shatavari	*Asparagus racemosus (Willd)*	*Tuber*	*1 part*
Gokshur	*Tribulus terrestris(L)*	*panchamool*	*1 part*
Vridha daru	*Argyria speciosa (L)*	*Root*	*1 part*
Rasna	*Pluchea lanceolata(C B Clarke)*	*Leaves*	*1 part*
Shata pushpa	*Foeniculum valgare (Mill.)*	*Seed*	*1 part*
Karchur	*Hedychium spicatum Ham. Ex.Smith*	*Fruit*	*1 part*
Yavani	*Trachispermum ammi(L)*	*seed*	*1 part*
Shunti	*Zingiber officinale(Rosc)*	*Rhizome*	*1 part*
Guggulu	*Commiphora mukul(Engl)*	*Resin*	*12part*
Ghee			*Q.S*

Preparation of trayodashanga guggulu

After proper identification and authentification, the ingredients of Trayodashanga Guggulu were powdered separately; and then sieved and weighed all the 12 drugs. Purified Guggulu was weighed and then crushed after that other ingredients in powder form were added to it and pounded well. Ghritha was added in small quantity at regular intervals and the process continued till attaining a semi-solid uniformly mixed mass was obtained. Then the mixture was expelled through a vati machine with a suitable die, and the vati were cut into desired weight. Rolled the vati on flat surface to round them by circular motion of gloved palm and

smeared with ghee. Dried the vati in a tray – drayer at a temperature not exceeding 60^0 C for 8-10 hours. After proper drying it was stored in a properly labelled air-tight container.

OBSERVATIONS

Pharmacognostic analysis

Organoleptic examination

This is the evaluation done using the sense organs ie, characters like colour, odour, taste and touch.

Table 2:

1	Colour	Blackish
2	Odour	Ghee odour
3	Taste	Astringent, bitter
4	Touch	Hard

Each tablet weighs 500mg

Powder microscopy

Here Powdered drugs were studied microscopically and the characteristic features were noted. Powdered sample was dissolved in water and then it is evaluated for its microscopic features, without adding any stain. Evaluation was done again after staining with Phloroglucinol + HCl. Microphotographs of the sample were also taken under Corl-zeiss trinocular microscope.

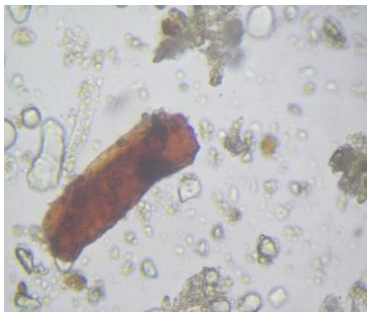
Table 3:

SL. No	Name of drug	Observations
1	*Acasia Arabica(L)Delile*	Prismatic crystal Tannin/Brown content Stone cells
2	*Withania somnifera(L) Dunal*	starch grains pitted vessels
3	*Juniperus communis(L)*	stone cells crystal fibre Parenchyma cells Trichome
4	*Tinospora cordifolia(Willd)*	starch grains cork cells Border pitted vessels
5	*Asparagus racemosus (Willd)*	Fibres stone cells acicular crystal cluster crystal

		scalariform vessels
6	*Tribulus terrestris(L)*	Fibres Stone cells
7	*Argyria speciosa (L)*	Fibres Trichome cluster crystal Tannin/Brown content
8	*Pluchea lanceolata(C B Clarke)*	pitted vessel vesicular cells rosette cells starch grains
9	*Foeniculum valgare (Mill.)*	fibres with oil globule mesocarp cells
10	*Curcuma zedoaria(Roscoe)*	starch grains scalariform vessels Trichome
11	*Trachispermum ammi(L)*	mesocarp cells oil globule starch grains
12	*Zingiber officinale(Rosc)*	starch grains

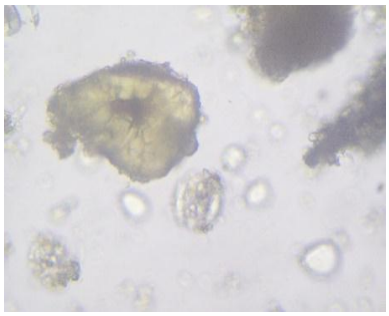
Micro-photographs of each ingredients

1. ***Aabha (babbul)***



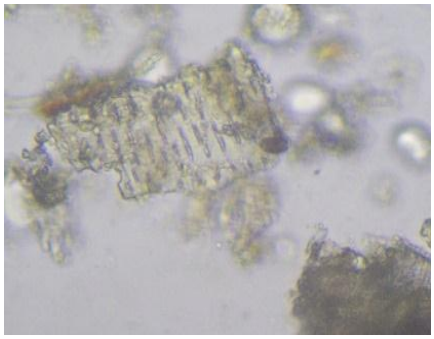
1.1 *Brown content*

1.2 *Crystal fibre*

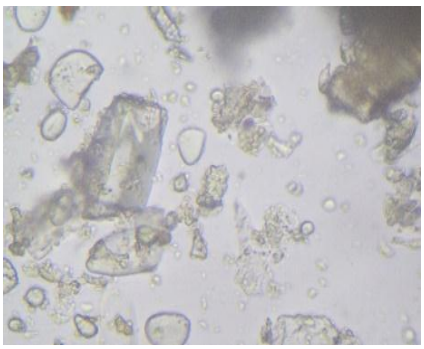


1.3 *Stone cells*

2. ***Ashwagandha***

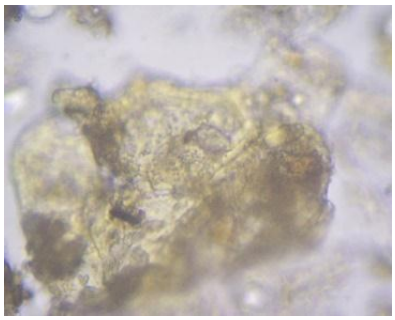


2.1 Starch grain

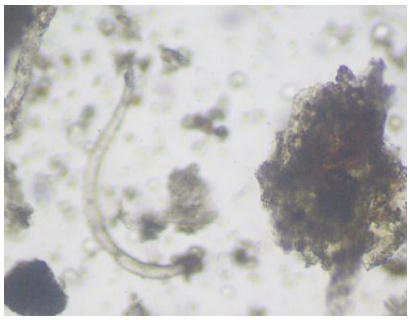


2.2 Pitted vessel

3. ***Hapusha***

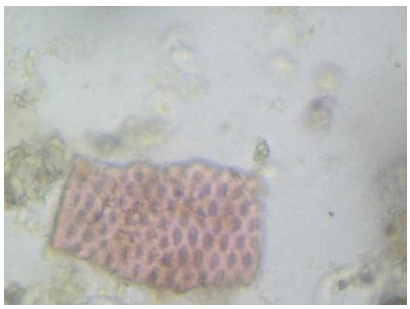


3.1 Parenchyme cells

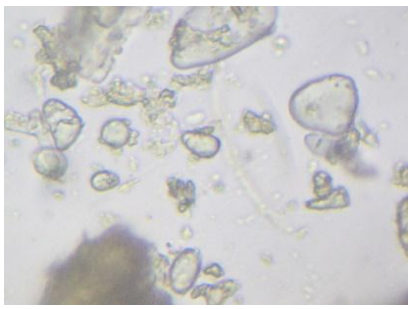


3.2 Trichome

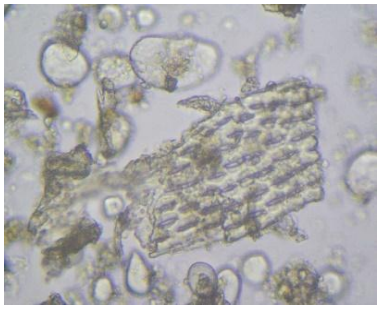
4. Guduci



4.1 *Border pittted vessels*

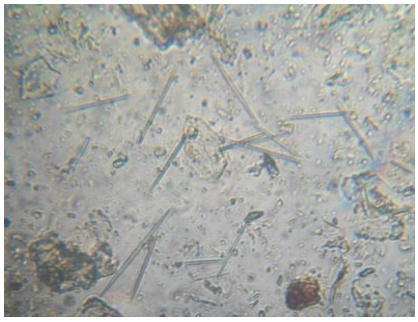


4.2 *Starch grain*

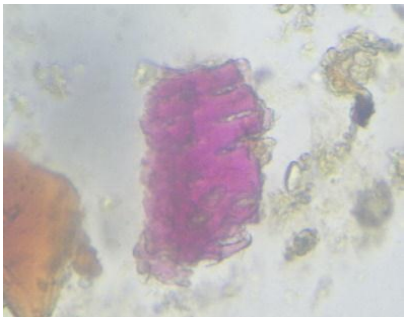


4.3 *Pitted vessels*

5. ***Shatavari***

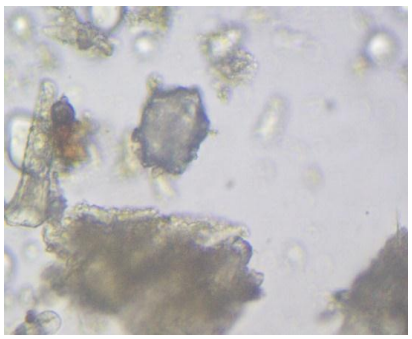


5.1 Acicular cells

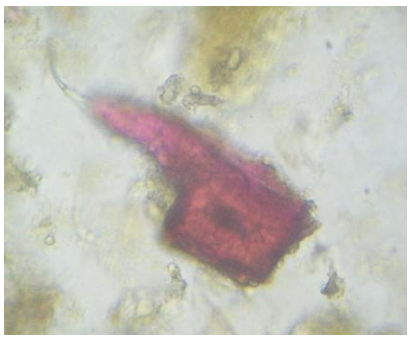


5.2 Scalariform cells

6. Gokshura

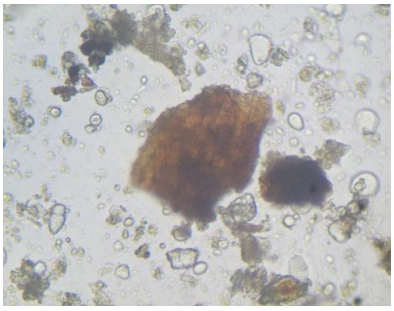


***6.1* Cluster crystal**

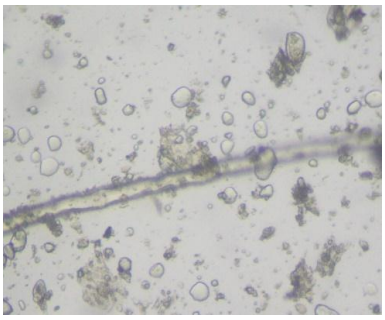


***6.2* Stone cells**

7. ***Vridhadaru***

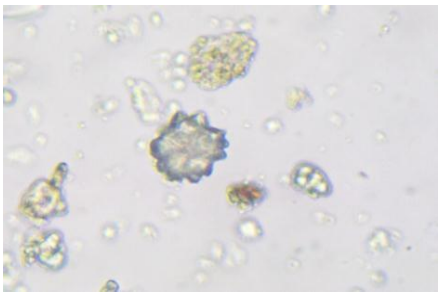


7.1 **Brown content**

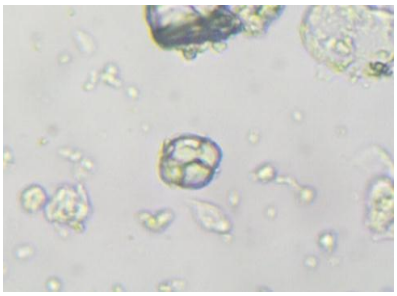


7.2 **Fibres**

8. ***Rasna***

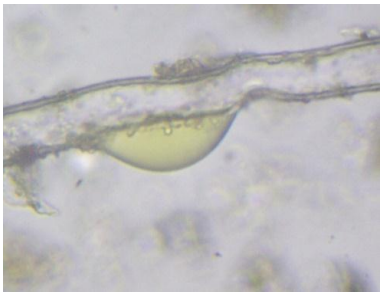


8.1 **Rosette crystal**

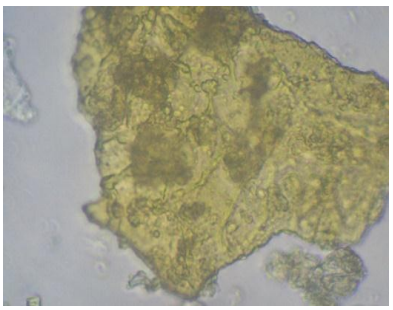


8.2 **Starch grain**

9. ***Shatapushpa***

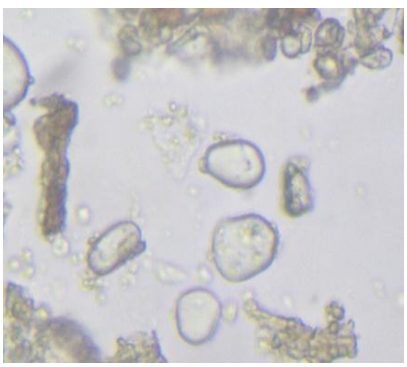


9.1 **Fibre with oil globule**

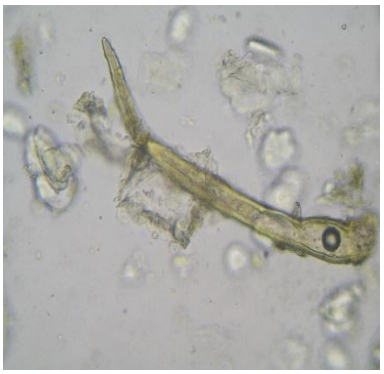


9.2 **Mesocarp cells**

10. ***Karchur***

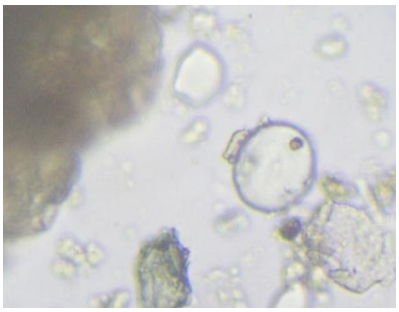


10.1 Starch grain

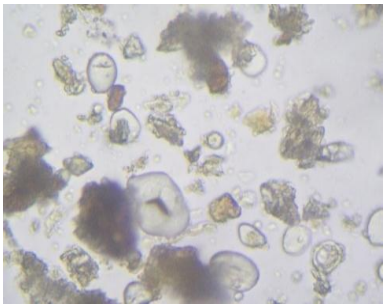


10.2 Trichome

11. Yavani

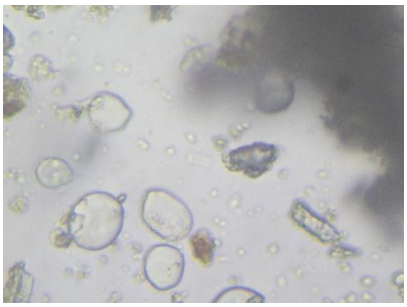


11.1 **Oil globules**



11.2 **Starch grain**

12. Shunti



12.1 **Starch grains**

Pharmaceutical evaluation

Physico-chemical Parameters of Trayodashanga Guggulu were analysed and measurements noted at Pharmaceutical Laboratory, ITRA, Jamnagar. The Analytical criteria for vati as per the Ayurvedic pharmacopoeia of India are Loss on drying, Ash Value, Weight variation, Water solubility, Alcohol solubility, pH value, Hardness of vati.

1. Loss on drying

This analysis helps to determine the volatile content of a sample which will help to minimize deterioration of drugs by microbial contamination thereby help to evaluate its shelf life. As per the Ayurvedic pharmacopoeia of India, the value should not be more than 11%.[4]

2. Ash Value (AV)/total ash value

This is the residue remaining after incineration of the sample this will helps to evaluate the percentage of inorganic salts, carbonates, phosphates, silicates etc. naturally occurring in the drug or adhering to it or deliberately added as a form of adulteration. This is a criteria helps to judge the identity of the drug and evaluate its quality and purity. As per the Ayurvedic pharmacopoeia of India, the value should not be more than 15%.[5]

Determination of extractive values

This evaluation gives an idea about the chemical constituents present in the crude drug.

3. Water soluble extract

2.5gm accurately weighed, powdered sample was taken in a conical flask, 50ml of distilled water was added in it, shaken and was kept overnight. Next day it was filtered.50ml of filtrate was taken in a pre-weighed, dried porcelain evaporating dish and was evaporated on a hot water bath. It was dried to constant weight in an oven and weighed at room temperature. From the weight of the residue, the water soluble extractive percentage was calculated.[6]

4. Alcohol soluble extract

The methanol soluble extract was determined by taking accurately weighed 2.5gm of powdered sample in a conical flask to it 50ml of methanol was added, Shaken, closed tightly and was kept overnight. Next day after filtering, of filtrate was taken in a dried, pre-weighed porcelain –evaporating dish and was evaporated on a hot water bath. It was cooled, weighed and from the weight the percentage of methanol soluble extractive was calculated.[7]

5. PH Value

2.5gm of sample was taken and it is mixed with 50ml of distilled water .After thorough mixing PH of the filtration was noted using a filter paper.[8]

6. Weight variation test

20 tablets were selected after random sampling, and their weight were noted individually and then average weight of the tablets were also calculated.

Physicochemical Parameters: Results

Table 4:

No.	Analytical parameter	Trayodashanga Guggulu
1	Loss on drying	7.75%
2	Ash Value	13.25%
3	Weight variation(Average weight)	498.1mg
4	Water solubility	25.26%
5	Alcohol solubility	17.6%
6	Hardness of Vati(Average)	2.16kg/cm^2
7	pH	6.5

HPTLC EVALUATION

High Performance Thin Layer Chromatography is an advanced form of Thin Layer Chromatography and it consists of chromatographic layers of utmost separation efficiency. HPTLC helps to perform standardized methodology based on scientific facts. HPTLC plates provide improved resolution, higher detection sensitivity and enhanced in situ quantification which will be very useful in industrial pharmaceutical densitometric quantitative analysis.

Main principle behind HPTLC is same as that of TLC which is adsorption. Chromatography is a physical process of separation where the conmponents to be separated are distributed between two immiscible substance. Because of the capillary action the mobile phase solvent flows, and they move towards the adsorbent according to their affinity. Those with more affinity towards the stationary phase travels slower and those with lesser affinity moves faster. A fingerprint of the sample was obtained by HPTLC which will help in standardization purpose.

Retardation factor

This is the fundamental parameter used to express the position of a sample zone in a thin – layer chromatogram indicates the ratio of the distance migrated by the sample compared to the distance travelled by the solvent front.[9]

Results: at 254nm (short wave) showed mainly 7 major spot and at 366 nm (long wave) showed 5 major spots.

Densitogram of *TG*

Image 13

Peak at 254nm

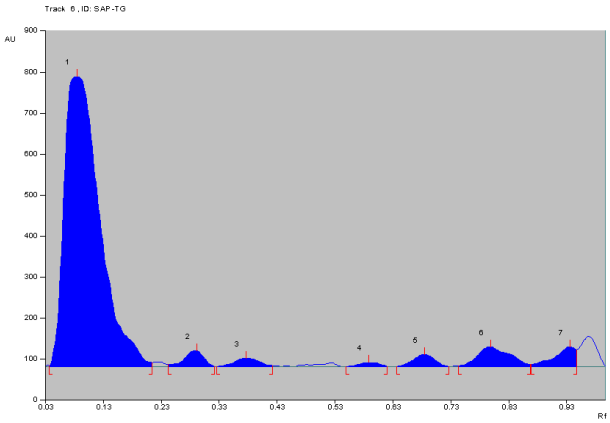
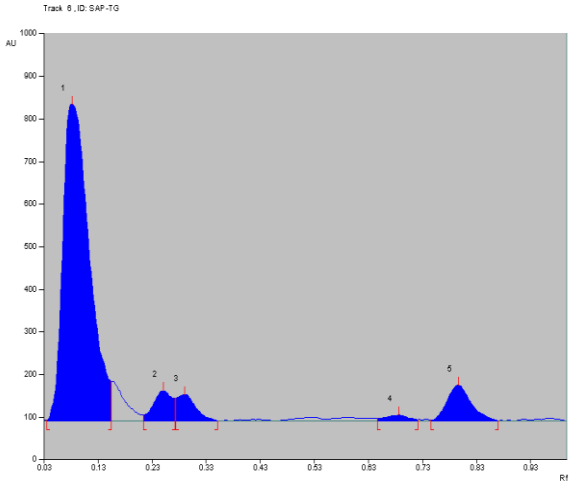


Image 14

Peak at 366nm



Microbiological investigation

Aerobic culture report:

No organisms isolated (After 48 hrs of incubation at 37^{o}C)

Fungal Culture report:

No fungal pathogen isolated (After 7 days of incubation at 37^{o}C)

DISCUSSION

In order to have a good coordination between the quality of raw materials, in process materials and the final products, it is essential to develop a reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental methods of analysis.[10] The overall aim of drug standardization is to ensure the quality, efficacy and uniformity of the products, in terms of their chemical and biological properties, across the manufactures. Ayurveda as a science and drug industry, it is very important to follow stringent and non-compromising quality control parameters to ensure uniformity and standards of the formulations/products across the industry.[11] The organoleptic characters were analysed first, all the vati were hard in touch, black in colour, ghee odour, and astringent and bitter mixed taste. Microscopic examination showed the peculiar features of all 12 drugs in the vati preparation and were already detailed above. Physico chemical parameters were analysed as per Ayurvedic pharmacopoeia of India.

The analysis Loss on Drying helps to determine the moisture content of a drug thereby help to evaluate its shelf life. As per the Ayurvedic pharmacopoeia of India, the value should not be more than 11%, for Trayodashanga Guggulu it is **7.75%.** Ash Value will helps to evaluate

the percentage of inorganic salts, carbonates, phosphates, silicates etc. The higher the inorganic substances present in drugs, more will be the ash value.[12] As per the API, the value should not be more than 15%, here it is 13.25%. Water Soluble Extract (WSE) and Alcohol Soluble Extract (ASE) gives an idea about the chemical constituents present in the sample. As per API, WSE should not be less than 21% here the value for TG is 25.26% and ASE should not be less than 17.5%, here the value for TG is 17.6%.In the previous analytical study of TG, values of pharmaceutical evaluation were not followed the API standards.

The HPTLC of TG, 6 major spots were observed at 254nm (short wave) showed mainly 7 major spot and at 366 nm (long wave) showed 5 major spots indicating its possible compounds in the matrix, which may be responsible for therapeutic activity of the same. Normally medicinal herb preparations carry bacteria and moulds as they originated from soil, here the values were in normal limits, which means proper hygiene is maintained during the preparation and packing of sample.

CONCLUSION

As per the analysis it can be concluded that sufficient quality control parameters were followed during the collection, identification, preparation and packing of the sample. HPTLC evaluation confirmed the authenticity of the sample which can be used as a primary tool for future research works. Absence of contamination is confirmed by the microbiological investigation.

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