



**PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF
TRAYODASHANGA GUGGULU VATI: AN AYURVEDIC POLYHERBAL
FORMULATION**

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ABSTRACT

Introduction: *Trayodashanga Guggulu Vati*, is *Guggulu* based hearbal formulation .It provides strength to the nerves, bones, joints, muscles and ligaments. It is effective in all types of diseases related to nervous system and musculoskeletal system. Though *Trayodashanga Guggulu Vati* is *Vatasamaka Rasayana*, *Balya*, *Amapachana*, Rejuvenating property but till date, its pharmacognostical and pharmaceutical study has not been carried out. **Aim:** Authentication of raw drug of *Trayodashanga Guggulu Vati* and phytochemical evaluation of finished product. **Materials and Methods:** The present study deals with the pharmacognostical identification of the ingredients of *Trayodashanga Guggulu Vati* and its physicochemical analysis. **Results:** Powder microscopy revealed the presence of annular vessels of *Shatavari*, border pitted vessel of *Guduchi*, stone cells of *Babbula*, stone cells of *Vraddadaru*, starch grains of *Shunti*, etc., Physicochemical parameters such as total ash value (16.1%), water soluble extract (24.3%), methanol soluble extract (16.8%) were assessed in preliminary physicochemical scanning. High-performance thin layer chromatography (HPTLC) revealed maximum 9 spots in short wave ultraviolet (UV) 254 nm. And four 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 as per quality standards.

KEY WORDS: HPTLC, pharmacognosy, physicochemical analysis, *Trayodashanga Guggulu Vati*.

INTRODUCTION

‘Ayurveda’ is a natural healing system of medicine to maintain health of a healthy person and curing the ailments of an ailing person. Curious scholars of different system of medicine have been working to find out better remedy for *Asthi Sandhigata Vata* (Musculoskeletal Disorder) since ancient period. In modern medicine, various treatment options are available like conservative treatment, epidural steroid injection, per radicular infiltration and surgical methods. But all these modalities have their own limitations and complications. In *Ayurveda* mainly two methods of treatment i.e. *Shodhan chikitsa* and *Shaman chikitsa* are employed. Different tools of *Shodhan chikitsa* are *Snehana*, *Upanaha*, *Agni karma*, *Raktamokshana*, *Pancakarma* etc are described. Various formulations used for *Shaman chikitsa* are *Kwath*, *Vati*, *Guggulu*, *Taila*, *Ghrita* etc are described.^[1,2] Among those, *Trayodashanga Guggulu* have its own heritage as therapeutic value for the treatment of musculoskeletal

disorders. *Trayodashanga Guggulu* mentioned in *Bhaishajya Ratnavali Vata Vyadhi Chikitsa*. [Table 1]

To control the degenerative process *Rasayana*, *Balya*, *Vatasamak Amapachana* drugs are suitable. To solve this problem *Trayodashanga Guggulu* is selected for internal use to pacify the vitiated *Vata Kapha Dohsa*. Majority of *Dravyas* of *Trayodashanga Guggulu* contains *Vatashamaka*, *Rasayana*, *Balya*, *Amapachana* property^[3] which Supports healthy neck, shoulder and joint functions. Supplement for those who spend long hours seated at work or extendedly in front of a computer or table work. Helps to support healthy ligaments of wrist and other parts of the body which happens due to overuse of computer & work overload, maintain flexibility of joint, detoxify the body, promote healthy digestion. Helps in proper circulation of nutrients, strengthen the muscles & provide nutrition to nerves.^[4]

The initial step in quality standardization of the compound formulation is to establish the presence of

each ingredient in the finished product, [5] followed by the pharmaceutical analysis. In the present study, *Trayodashanga Guggulu Vati* was subjected to pharmacognostical (powder microscopy), high performance thin layer chromatography (HPTLC), densitogram, and pharmaceutical evaluation for various physicochemical parameters to prepare a preliminary profile of formulation for future.

MATERIALS AND METHODS

All the raw drug materials were collected from the pharmacy. The ingredients and parts used of the drugs are given in Table 1.

Pharmacognostical Study

Raw drugs were identified and authenticated by the pharmacognosy laboratory. The identification was carried out based on organoleptic characters of *Vati*. [6] 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. [7,8]

Method of Preparation of *Trayodashanga 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 Kwath* was added. At the last, sufficient quantity of *Ghee* 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. [9]

Pharmaceutical Evaluation

Trayodashanga Guggulu Vati was analyzed using qualitative and quantitative parameters at the pharmaceutical laboratory. 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. [10] On its 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. [11,12]

High-Performance Thin Layer Chromatography

Methanol extract of *Trayodashanga Guggulu Vati* was spotted on precoated silica gel GF CO₂₅₄ aluminum plate as 5 mm bands, 5 mm apart and 1 cm from the edge of the plates, by means of Camag Linomat V sample applicator fitted with a 100 μ L 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 the control of win CATS Software (V 1.2.1, manufactured by Camage Switzerland). The slit dimensions were 6.0mm \times 0.45 mm, and the scanning speed was 20mm/s. [14]

OBSERVATIONS AND RESULTS

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of *Trayodashanga 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.

Organoleptic Evaluation

Organoleptic features such as color, odor, and taste of the *Trayodashanga Guggulu Vati* were recorded and are placed in Table 2.

Microscopic Evaluation

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 acicular crystal of *Shatavari* [Fig. 1], annular vessel of *Shatavari* [Fig.2], annular vessel of *shati*[Fig.3], border pitted vessel of *Guduchi*[Fig.4], collenchyma cells of *Guduchi*[Fig.5], stone cells of *Babbula*[Fig.6], fibres of *Vruddadaru*[Fig.7], prismatic crystal of *Babbula*[Fig.8], Rhomboidal crystal of *Vruddadaru*[Fig.9], stratified fibres of *Ajmoda*[Fig.10], warty trichome of *Gokshura*[Fig.11], road shaped crystal of *Rasna*[Fig.12], silica deposition of *Shati* [Fig.13], group of stone cells of *Vruddadaru*[Fig.14], oil globules of *Ajmoda*[Fig.15], oil globules of *Shatapushpa*[Fig. 16], simple starch grain of *Shunthi*[Fig.17], fibres of *Hapusha* [Fig.18], which are placed with microphotographs 1–18. Plate1 (1-18)

Physicochemical Parameters

Physicochemical parameters of the *Vati* like uniformity of the weight, hardness, loss on drying, pH values were found within the normal range. Methanol and water soluble extractive values were found to be 16.8% and 24.3%, respectively. Details are shown in Table 3.

High-performance Thin Layer Chromatography

Densitometry scanning of the HPTLC pattern showed 9 spots at corresponding R_f values 0.02, 0.33, 0.43, 0.62, 0.70, 0.76, 0.88, 0.94, 0.98, in short wave ultraviolet (UV) 254 nm [Fig. 19-20-23]. And four spots at corresponding R_f values 0.02, 0.33, 0.62, 0.73 obtained in long wave UV 366 nm [Fig.21-22] and [Table 4].

Table 1: Ingredients of Tryodashanga Guggulu Vati (Ref-13)

No	Ingredients	Latin Name	Part Used	Part
1	Abha	<i>Acacia arbica</i> Linn.	Stem bark	1 Part
2	Aswagandha	<i>Withania somnifera</i> Dunal	Root	1 Part
3	Hribera	<i>Juniperus comunis</i> Linn.	Fruit	1 Part
4	Guduci	<i>Tinospora cordifolia</i> Willd.	Stem	1 Part
5	Satavari	<i>Asparagus racemosus</i> Willd.	Root	1 Part
6	Gokshura	<i>Tribulus terrestris</i> Linn.	Root	1 Part
7	Vrudhadaru	<i>Argyrea spinosa</i> Linn.	Root	1 Part
8	Rasna	<i>Pluchea lanceolata</i> Oliver & Hiem	Leaf	1 Part
9	Satahwa	<i>Foeniculum vulgare</i> Roxb.	Fruit	1 Part
10	Sati	<i>Hedychium spicatum</i> Ham. Ex Smith	Rhizome	1 Part
11	Yavani	<i>Trachysprum ammi</i> Linn.	Fruit	1 Part
12	Sunthi	<i>Zingiber officinale</i> Roxb.	Rhizome	1 Part
13	Guggulu	<i>Commiphora mukul</i> Anr.	Oleo gum resine	13 Part
14	Ghrita	Clarified Butter		½ Part

Table 2: Organoleptic characters of Tryodashanga Guggulu Vati

Parameters	Result
Color	Black
Odor	Characteristic
Test	Pungent
Touch	Hard

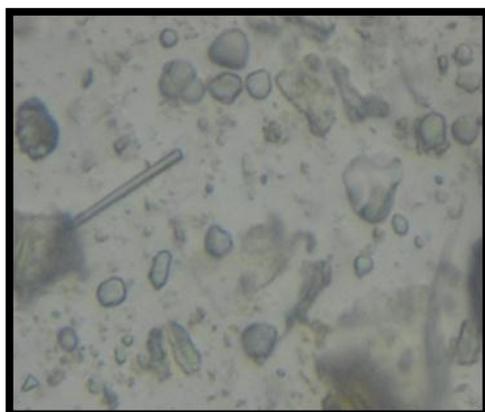
Table 3: Physicochemical analysis of Trayodashanga Guggulu Vati

Parameters	Value
Loss on drying at 110°C	12.7%
Ash value	16.1%
Acid in soluble ash	0.077%
Water soluble extract	24.3%
Methanol soluble extract	16.8%
p ^H	6.0
Average weight of Vati	509.5 mg
Highest weight	578 mg
Lowest weight	425 mg
Hardness	2.8kg/ cm ²

Table 4: R_f values of Trayodashanga Guggulu Vati (Plate 2)

	R _f values at 254 (nm)	R _f values at 366 (nm)
HPTLC	0.02, 0.33, 0.43, 0.62, 0.70, 0.76, 0.88, 0.94, 0.98	0.02, 0.33, 0.62, 0.73

HPTLC – High-performance thin layer chromatography

**Fig.1: Acicular crystal of Shatavari****Fig.2: Annular vessel of Shatavari****Fig.3: Stone cells of Gokshura**

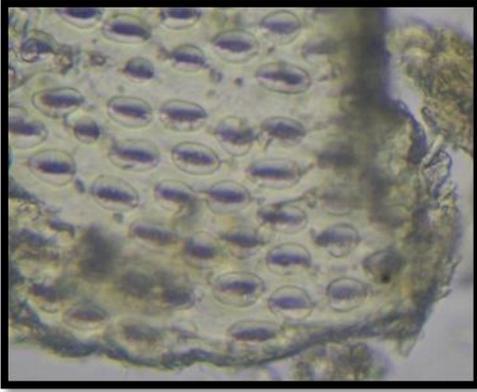


Fig. 4: Border pitted vessel of *Guduchi*



Fig. 5: Collenchyma cells of *Guduchi*

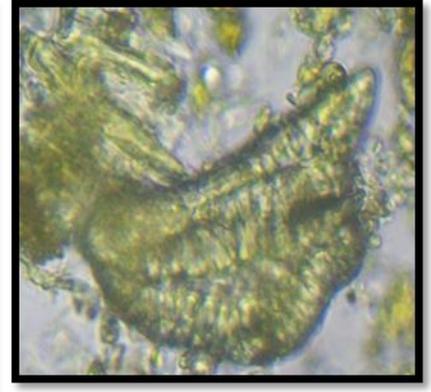


Fig.6: Stone cells of *Babbula*

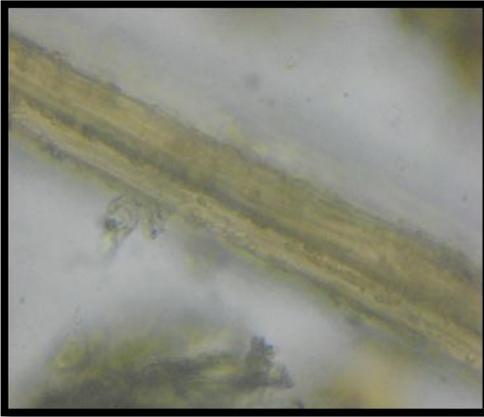


Fig.7:Fibres of *Vruddadaru*

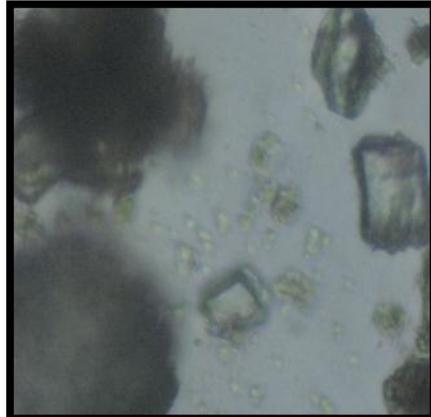


Fig.8:Prismatic Crystal of *Babbula*

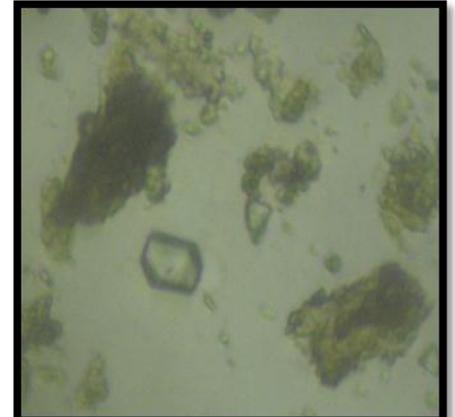


Fig.9:Rhomboidal crystal of *Vruddadaru*

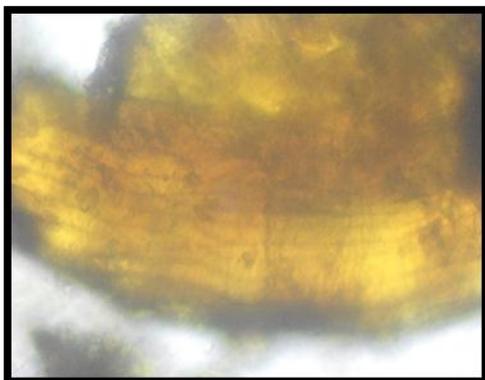


Fig.10: Stratified fibre of *Ajmoda*



Fig.11:Warty trichome of *Gokshura*

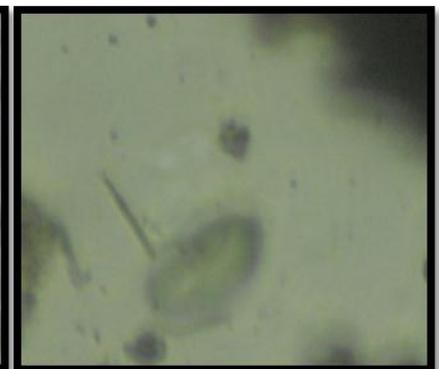


Fig.12:Road shaped crystal of *Rasna*



Fig.13:Silica deposition of *Shati*



Fig.14:Group of stone cells of *Vruddhadaru*

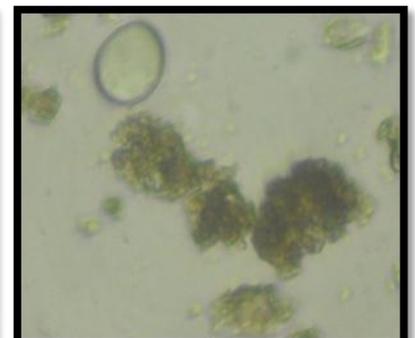


Fig.15:Oil globules of *Ajmoda*

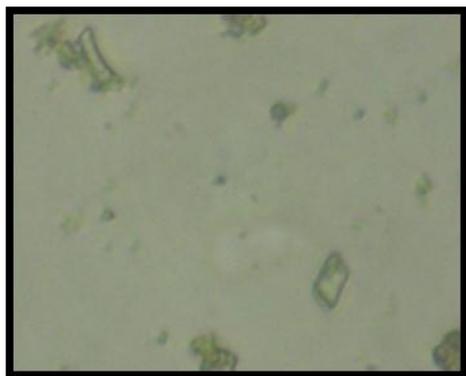


Fig.16:Microcrystal of Gokshura

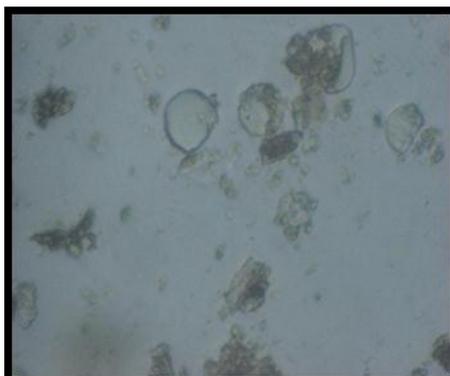


Fig.17:Simple starch grain of Shunthi



Fig.18:Fibres of Hapusha

Plate1: Microphotographs of *Tryodashanga Guggulu Vati*

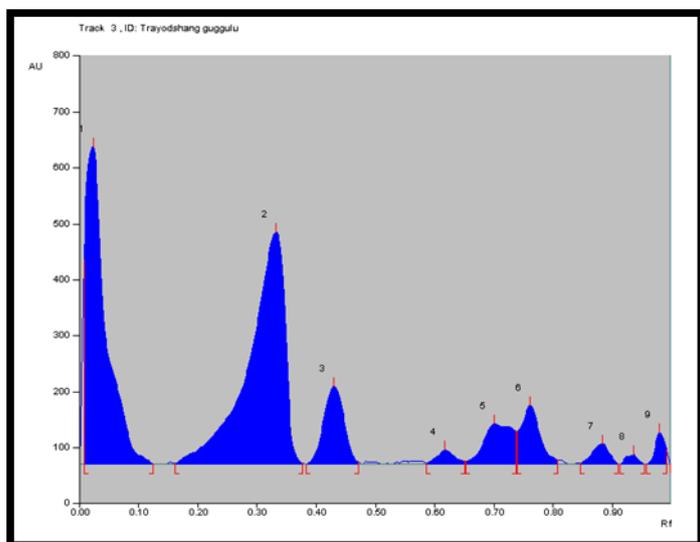


Fig.19:Densitogram of methanolic extract of *Trayodashanga Guggulu Vati* at 254 nm

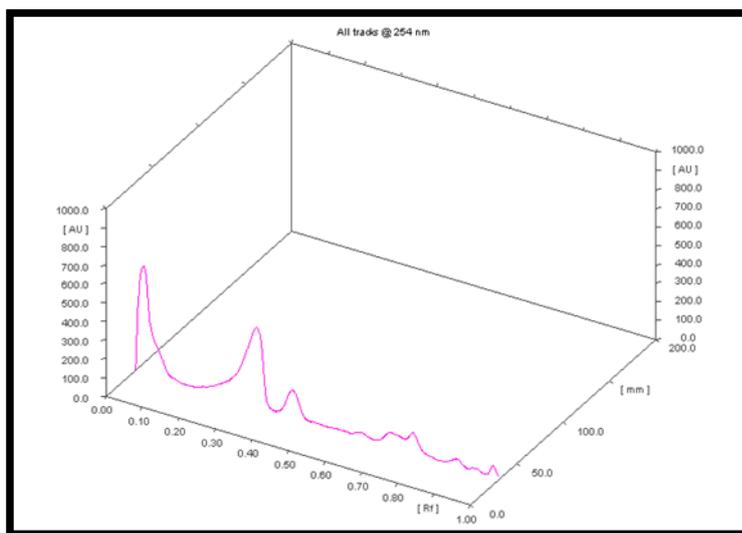


Fig.20:Three dimensional densitogram of methanolic extract of *Trayodashanga Guggulu Vati* at 254 nm

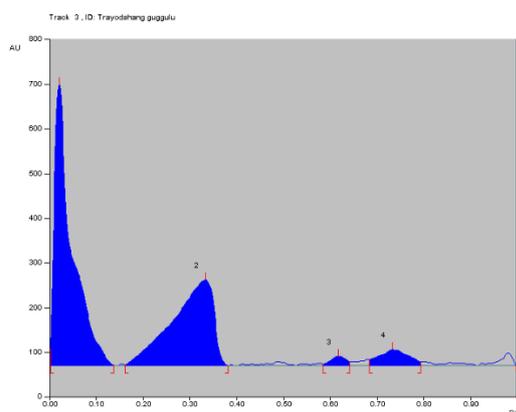


Fig.21: Densitogram of methanolic extract of *Trayodashanga Guggulu Vati* at 366 nm

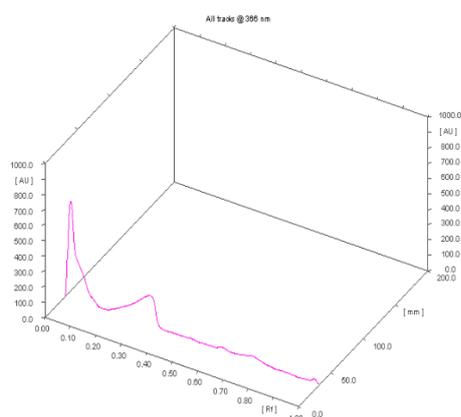


Fig.22:Three dimensional densitogram of methanolic extract of *Trayodashanga Guggulu Vati* at 366 nm

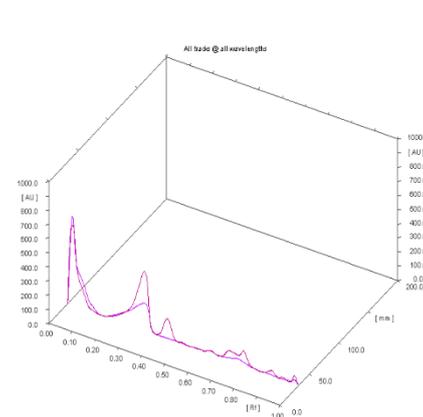


Fig.23:Three dimensional milled wood lignin of methanolic extract of *Trayodashanga Guggulu Vati*

Plate2: Microphotographs of HPTLC results of *Tryodashanga Guggulu Vati*

Though it cannot be possible to identify particular chemical constituent from the spot obtained, the pattern may be used as a reference standard for further quality control researches.

DISCUSSION

Powder microscopy of *Trayodashanga Guggulu Vati* revealed the diagnostic characters like acicular crystal of *shatavari*, annular vessel of *shatavari*, annular vessel of *shati*, border pitted vessel of *Guduchi*, collenchyma cells of *Guduchi*, stone cells of *Babbula*, fibres of *Vruddadaru*, prismatic crystal of *Babbula*, Rhomboidal crystal of *Vruddadaru*, stratified fibres of *Ajmoda*, warty trichome of *Gokshura*, rod shaped crystal of *Rasna*, silica deposition of *Shati*, group of stone cells of *Vruddadaru*, oil globules of *Ajmoda*, oil globules of *Shatapushpa*, simple starch grain of *Shunthi*, fibres of *Hapusha* which authenticate genuineness of the raw drugs of *Trayodashanga Guggulu Vati*. Taste of *Trayodashanga Guggulu Vati* was *Katu* (Pungent) because the majority of contents of *Trayodashanga Guggulu Vati* having *Katu* (Pungent) taste.

Trayodashanga Guggulu Vati was found to have 509.5 mg 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. *Trayodashanga Guggulu Vati* was found to have 2.85 Kg/cm² hardness which was noticed in acceptable limit. 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.

Trayodashanga Guggulu Vati contains 12.07% 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. *Trayodashanga Guggulu Vati* contained 16.1% w/w total ash and 0.077% w/w acid insoluble ash. The results revealed that *Trayodashanga Guggulu Vati* is free from unwanted organic compounds and production site was good enough keeping sample free from dust and other solid matters. About 24.3% w/w of water soluble extractives and 16.8% w/w methanol soluble extractives were present in *Trayodashanga Guggulu Vati* indicating that the drug has good solubility in water.^[15] In HPTLC study, 9 spots at 254 nm and 4 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 microscopically characters were observed. Physicochemical and HPTLC studies inferred that the formulation meets the minimum quality standards as

reported in the American Petroleum Institute at a preliminary level. The inference from this study may be used as reference standard in the further quality control researches.

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