ORIGINAL ARTICLE

Pharmacognostical and phytochemical evaluation of *Tryoshnadi Guggulu Vati*: An ayurvedic polyherbal formulation

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Introduction: *Tryoshnadi Guggulu Vati*, a polyherbal formulation is recommended for the management of diseases due to *Medodushti* (abnormal fat metabolism) like obesity, diabetes mellitus, dyslipidemia, coronary artery diseases, etc. Though *Tryoshnadi Guggulu Vati* is widely used for the treatment of diseases due to abnormal metabolism, but till date, its pharmacognostical and pharmaceutical study has not been carried out. **Aim:** Authentication of raw drug of *Tryoshnadi Guggulu Vati* and phytochemical evaluation of finished product. **Materials and Methods:** The present study deals with the pharmacognostical identification of the ingredients of *Tryoshnadi Guggulu Vati* and its physicochemical analysis. **Results:** Powder microscopy revealed the presence of annular vessels of *Musta*, starch grains of *Vacha,* stone cells of *Pippali*, stone cells of *Chitraka,* oleoresins of *Shunthi,* starch grains of *Musta,* etc., Physicochemical parameters such as total ash value (15.91%), water soluble extract (13.5%), methanol soluble extract (17.2%) were assessed in preliminary physicochemical scanning. High-performance thin layer chromatography (HPTLC) revealed maximum 10 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 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.

Key words: High-performance thin layer chromatography, pharmacognosy, physicochemical analysis, *Tryoshnadi Guggulu Vati*

INTRODUCTION

Since ancient times, humanity has depended on the mixture of the plant resources for food clothing, protection, and traditional medicine to cure a number of ailments. Ayurveda is an Indian system of medicine with vast numbers of compound formulations for various disease entities. *Tryoshnadi Guggulu Vati* is a formulation of nine ingredients [Table 1] mentioned in *Bhavprakash Samhita*, which is meant for curing *Medodushti* (abnormal fat metabolism).[1] Contents of *Tryoshnadi Guggulu Vati* have shown its applicability in diverse spectra of diseases manifesting with dyslipidemia as the main pathological condition because it contains proven hypolipidemic, cardio-protective, antianginal, hypoglycemic, anti-inflammatory, and anti-diabetic properties.[2] According to National Commission on Macroeconomics on Health, there would be around 62 million patients with coronary artery disease (CAD) by 2015 in India and of these, 23 million would be patients younger than 40 years of age.[3] CAD is usually due to atherosclerosis of large and medium-sized arteries, and dyslipidemia has been found to be one of the most important contributing factors.

During the past decades, there has been increasing acceptance as well as public interest in natural therapies in both developing and developed countries. At this juncture, we cannot assure drug industries insulation from adulterations and quality decrement. Thus, quality control for efficacy and safety of herbal products is of prime concern.[4,5] Maintaining the quality standards of the formulation is a challenge. 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.[6,7] The initial step in quality standardization of the compound formulation is

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to establish the presence of each ingredient in the finished product,[8] followed by the pharmaceutical analysis. In the present study, *Tryoshnadi 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*,[9] 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.[10,11]

Method of Preparation of *Tryoshnadi 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* (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.[12]

Pharmaceutical Evaluation

Tryoshnadi 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.[13] 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.[14,15]

High-Performance Thin Layer Chromatography

Methanol extract of *Tryoshnadi Guggulu Vati* was spotted on precoated silica gel GF CO_{254} 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 µL. 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 6.00 mm × 0.45 mm, and the scanning speed was 20 mm/s.[16]

OBSERVATIONS AND RESULTS

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of *Tryoshnadi 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 *Tryoshnadi 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 annular vessels of *Musta* [Figure 1], starch grains of *Musta* [Figure 2], fibers of *Musta* [Figure 3], starch grains of *Vacha* [Figure 4], oleoresin of *Vacha* [Figure 5], stone cells of *Pippali* [Figure 6], tannin of *Pippali* [Figure 7], stone cells of *Chitraka* [Figure 8], oleoresins of *Shunthi* [Figure 9], fibers of *Shunthi* [Figure 10], fibers

Table 1: Ingredients of *Tryoshnadi Guggulu Vati*

Drug name	Latin name	Part used	Proportion
Pippali	*Piper longum* (Linn.)	Fruit	1 part
Maricha	*Piper nigrum* (Linn.)	Fruit	1 part
Nagar	*Zingiber officinale* (Rosc.)	Rhizome	1 part
Vidanga	*Embelia ribes* (Burm.)	Fruit	1 part
Vacha	*Acorus calamus* (Linn.)	Rhizome	1 part
Chitraka	*Plumbago zeylanica* (Linn.)	Root	1 part
Musta	*Cyperus rotundus* (Linn.)	Rhizome	1 part
Guggulu	*Commiphora mukul* (Linn.)	Gum resin	7 part
Cow Ghee	-	-	7 part

Bhavamishra. Mededosha chikitsa. In– Mishra PB, editor. Bhavaprakasha Samhita. 11th ed. Varanasi– Chaukhambha Sanskrita Sansthana; 2005. p. 225.

Table 2: Organoleptic characters of *Tryoshnadi Guggulu Vati*

Parameter	Results
Color	Brown
Odor	Like cow urine
Test	Pungent
Consistency	Hard

of *Maricha* [Figure 11], black debris of *Maricha* [Figure 12], coloring matter of *Vidanga* [Figure 13], scleroids of *Vidanga* [Figure 14], stone cells of *Vidanga* [Figure 15], which are placed with microphotographs 1–15.

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 17.2% and 13.5%, respectively. Details are shown in Table 3.

High-performance Thin Layer Chromatography

Densitometry scanning of the HPTLC pattern showed 10 spots at corresponding R_f values 0.02, 0.10, 0.16, 0.22, 0.36, 0.46, 0.54, 0.56, 0.70, 0.80, in short wave ultraviolet (UV) 254 nm [Figures 16-18]. And four spots at corresponding R_f values 0.01, 0.46, 0.58, 0.88 obtained in long wave UV 366 nm [Figures 18-20] and [Table 4].

Table 3: Physicochemical analysis of *Tryoshnadi Guggulu Vati*

Parameter	Value
Loss on drying at 110°C	5.58%
Ash value	15.91%
Acid insoluble ash	0.077%
Water soluble extract	13.5%
Methanol soluble extract	17.2%
pH (By pH indicator paper)	4.5
Average weight of *Vati*	501 mg
Highest weight	540 mg
Lowest weight	460 mg
Hardness	1.3 kg/cm^2

Table 4: R_f values of *Tryoshnadi Guggulu Vati*

	R_f values at 254 (nm)	R_f values at 366 (nm)
HPTLC	0.02, 0.10, 0.16, 0.22, 0.36, 0.46, 0.54, 0.56, 0.70, 0.80	0.01, 0.46, 0.58, 0.88

HPTLC – High-performance thin layer chromatography

Figure 1: Annular vessels of *Musta*

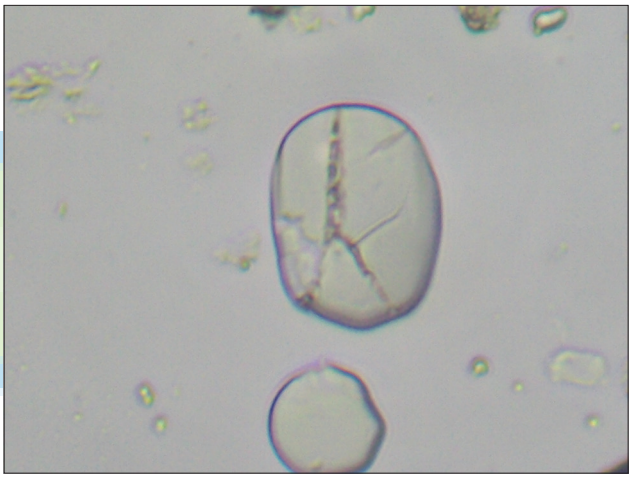

Figure 2: Starch grains of *Musta*

Figure 3: Fibers of *Musta*

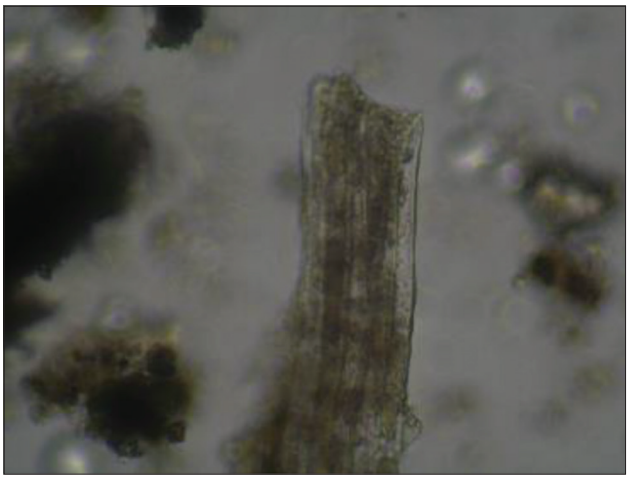

Figure 4: Starch grains of *Vacha*

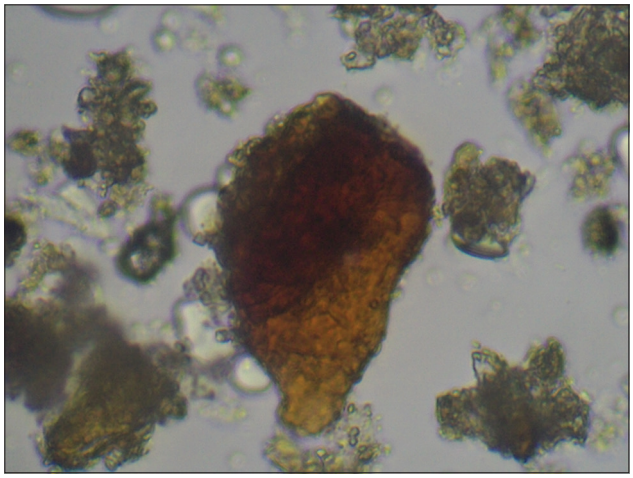

Figure 5: Oleoresin of *Vacha*

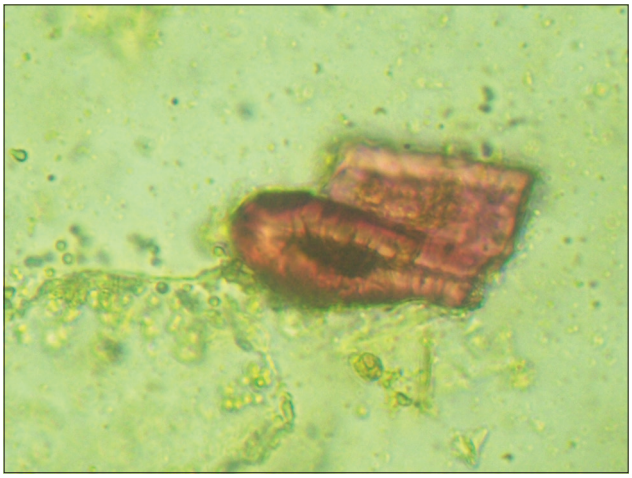

Figure 6: Stone cells of *Pippali*

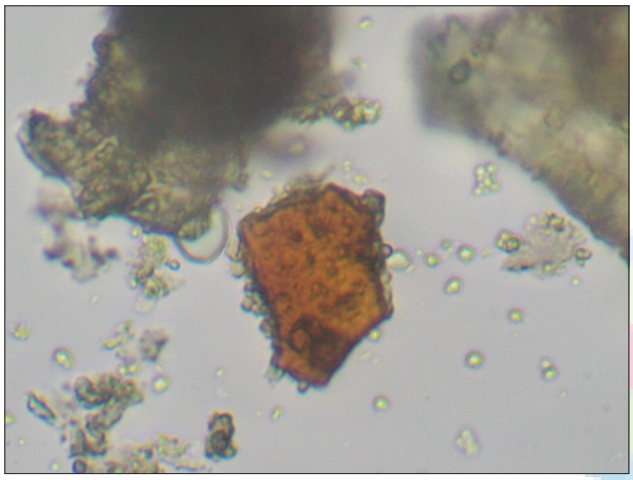

Figure 7: Tannin of *Pippali*

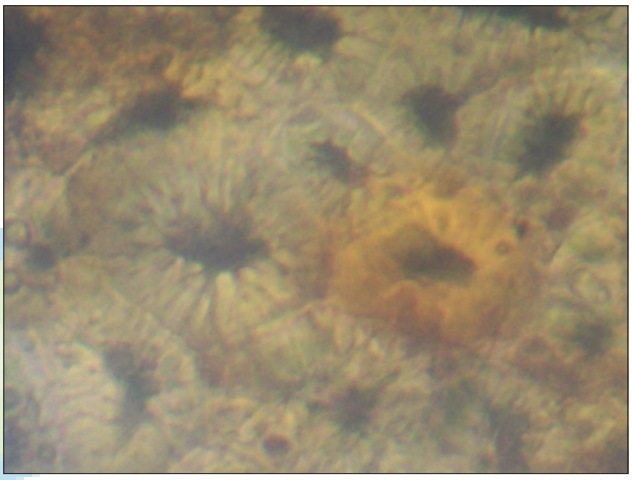

Figure 8: Stone cells of *Chitraka*

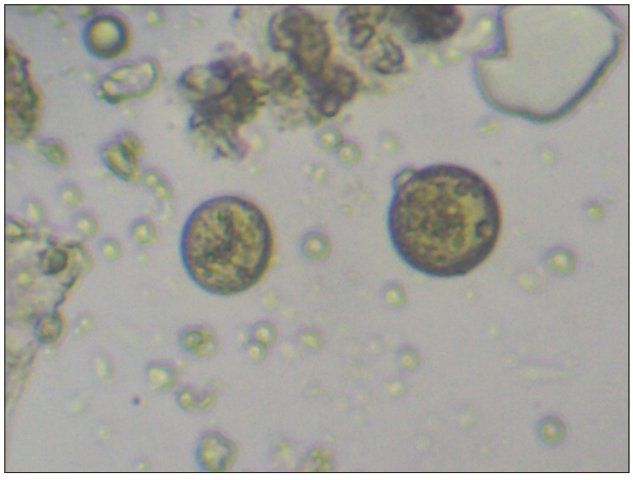

Figure 9: Oleoresins of *Shunthi*

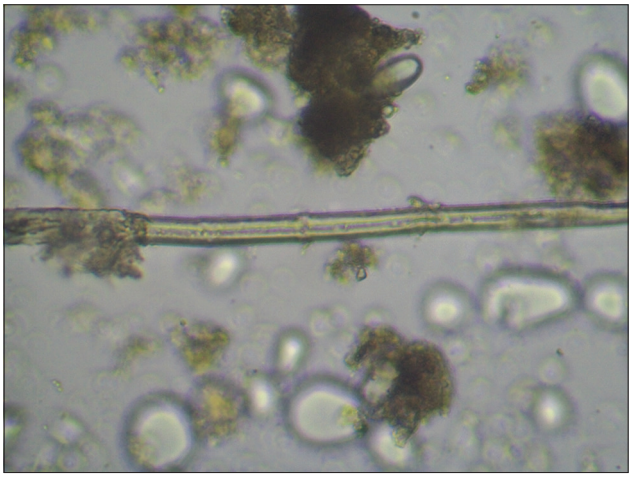

Figure 10: Fibers of *Shunthi*

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 *Troshnadi Guggulu Vati* revealed the diagnostic characters like annular vessels of *Musta,*

Figure 11: Fibers of *Maricha*

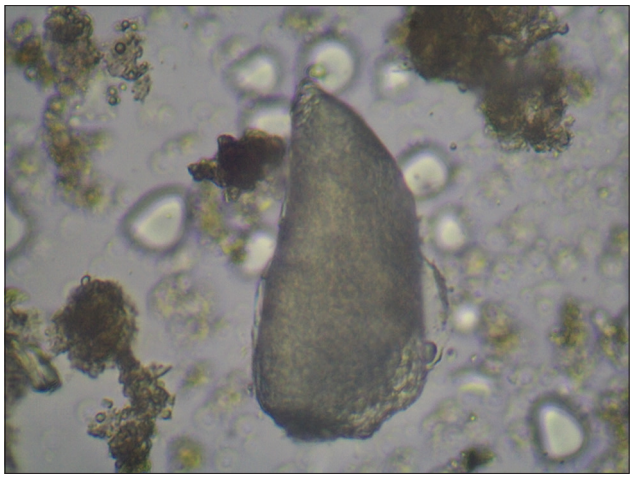

Figure 12: Black debris of *Maricha*

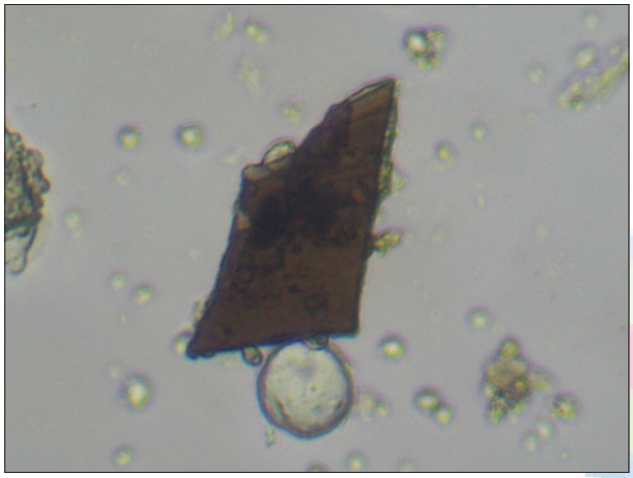

Figure 13: Coloring matter of *Vidanga*

Figure 14: Scleroids of *Vidanga*

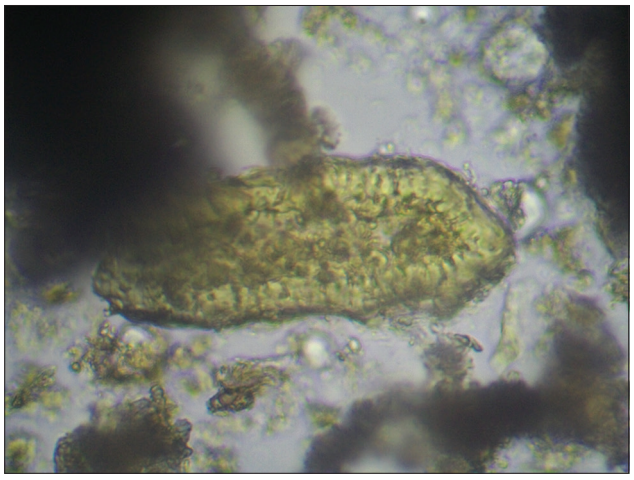

Figure 15: Stone cells of *Vidanga*

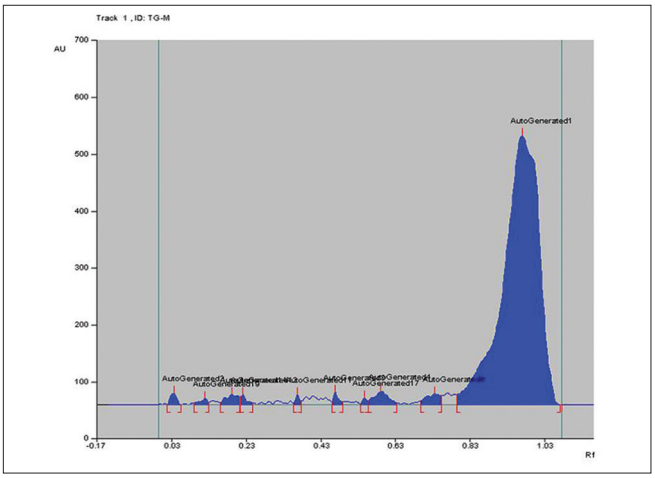

Figure 16: Densitogram of methanolic extract of *Tryoshnadi Guggulu Vati* at 254 nm

starch grains of *Musta*, fibers of *Musta*, starch grains of *Vacha*, oleoresin of *Vacha*, stone cells of *Pippali*, tannin of *Pippali*, stone cells of *Chitraka*, oleoresins of *Shunthi*, fibers of *Shunthi*, fibers of *Maricha*, black debris of *Maricha*, coloring matter of *Vidanga*, scleroids of *Vidanga*, and stone cells of *Vidanga* which authenticate genuineness of the raw drugs of *Tryoshnadi Guggulu Vati*.

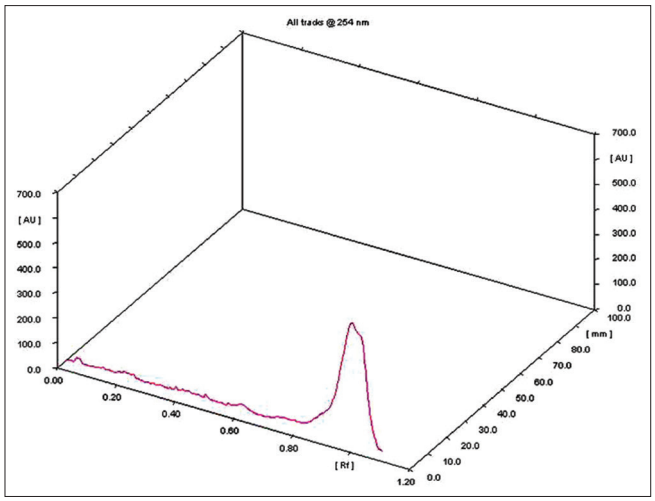

Figure 17: Three-dimensional densitogram of methanolic extract of *Tryoshnadi Guggulu Vati* at 254 nm

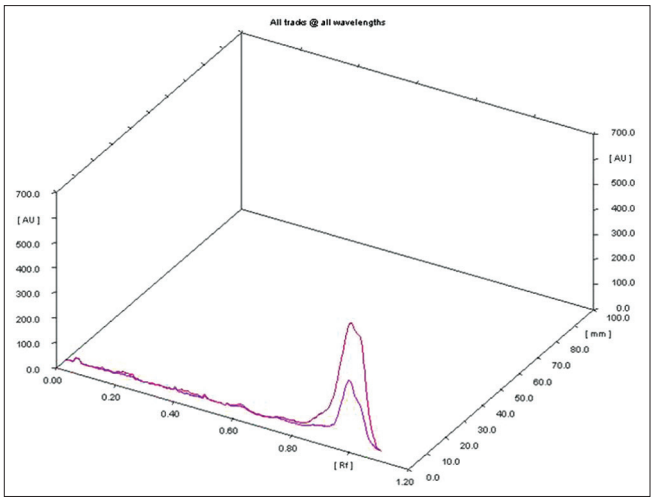

Figure 18: Three-dimensional milled wood lignin of methanolic extract of *Tryoshnadi Guggulu Vati*

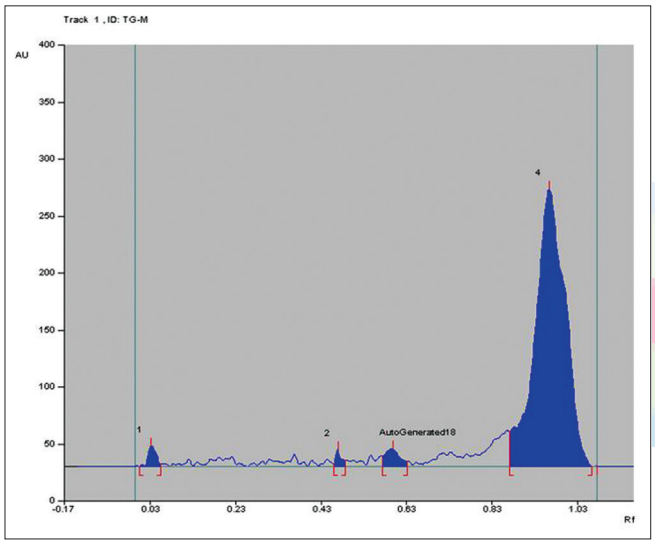

Figure 19: Densitogram of methanolic extract of *Tryoshnadi Guggulu Vati* at 366 nm

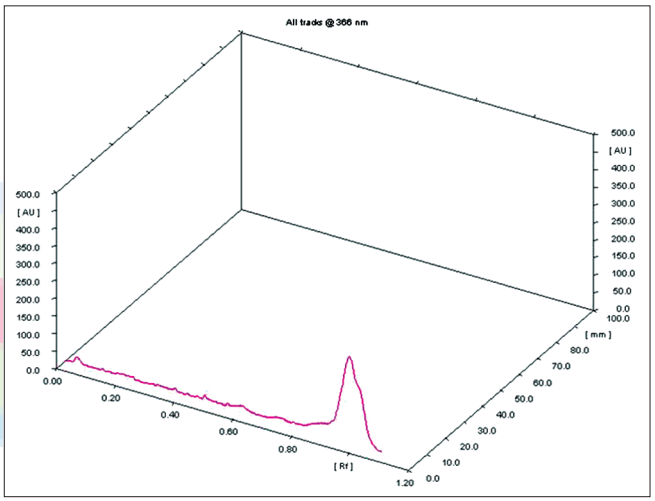

Figure 20: Three-dimensional densitogram of methanolic extract of *Tryoshnadi Guggulu Vati* at 366 nm

Taste of *Tryoshnadi Guggulu Vati* was *Katu* (Pungent) because the majority of contents of *Tryoshnadu Guggulu Vati* having *Katu* (Pungent) taste. *Guggulu* was purified by cow urine which may result in cow urine like odor of formulation.

Tryoshnadi Guggulu Vati was found to have 501 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. *Tryoshnadi Guggulu Vati* was found to have 1.3 Kg/cm^2 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. *Tryoshnadi Guggulu Vati* contains 5.58% 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. *Tryoshnadi Guggulu Vati* contained 15.91% w/w total ash and 0.077% w/w acid insoluble ash. The results revealed that *Tryoshnadi Guggulu Vati* is free from unwanted organic compounds and production site was good enough keeping sample free from dust and other solid matters. About 13.5% w/w of water soluble extractives and 17.2% w/w methanol soluble extractives were present in *Tryoshnadi Guggulu Vati* indicating that the drug has good solubility in water.[17]

In HPTLC study, 10 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 microscopical 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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Nil.

Conflicts of Interest
There are no conflicts of interest.

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