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Pharmacognostical and Preliminary physicochemical evaluation of *Triphaladi* granules – A polyherbal Ayurvedic formulation

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Abstract

Triphaladi Kwatha, a polyherbal Ayurvedic formulation, is recommended by Chakradatta and Yogaratnakara in the management of *Prameha* which has resemblance with type 2 diabetes mellitus. The present study deals with development of pharmacognostical and preliminary pharmaceutical profile of *Triphaladi* granules. The pH (5% aqueous extract) was 6.0, water-soluble extract 48.66% w/w, alcohol-soluble extract 33.91% w/w, ash value 5.97% w/w, and loss on drying at 105°C was 6.53% w/w. High performance thin layer chromatography were carried out after organizing appropriate solvent system in which maximum nine spots were distinguished and few of the R_f values were identical in the alcoholic extract.

Key words: High performance thin layer chromatography, Pharmacognosy, *Prameha*, *Triphaladi* granules

Introduction

Recent years have witnessed a gradual decline of medicinal plant availability as well as the number of tribal traditional healers and their medicinal knowledge. Herbal medicines have a long history for their therapeutic application and are still serving many of the health needs of a global population. However, the quality control and quality assurance still remains a challenge because of the high variability of chemical components involved. Herbal drugs, singularly or in combination, contain numerous active principles in complex matrices in which no single active constituent is responsible for the overall efficacy. This creates a challenge in establishing specific quality control standards of finished products.

Administration of drug in various dosage forms provides an opportunity to the physician to choose better options. Various dosage forms have been described in Ayurvedic texts. One among them is *Kwatha*,[1] which is highly effective when used freshly prepared, but they are often overlooked due to preparation method and palatability. In the present study, *Triphaladi Kwatha*,[2] a known formulation used in *Prameha* (type 2 diabetes mellitus), was converted into granules by *Rasakriya* method to make it palatable, easy to dispense and for dose fixation, etc. As no standard finger print is available for this compound formulation, an attempt has been made to evolve preliminary physico-chemical profile of *Triphaladi* granules (TG).

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Materials and Methods

Collection and authentication of raw drugs

The formulation composition of TG was obtained from Pharmacy of Gujarat Ayurved University, Jamnagar. The API standards were used for pharmacognostical authentication of *Amalaki*,[3] *Asana*,[4] *Bibhitaki*,[5] *Haritaki*,[6] *Kutaja*,[7] *Daruharidra*,[8] and *Musta*[9] based on the morphological features, organoleptic characters and powder microscopy of individual drugs.

Method of preparation of *Triphaladi* granules

Dried raw materials in equal proportions [Table 1] were crushed to prepare coarse powder separately and mixed with 8 parts of water in a stainless steel container. Continuous mild heat was applied until it was reduced to one-fourth of its initial quantity. During heating process, continuous stirring was done to facilitate the evaporation and avoid any deterioration due to burning of materials. After a desirable reduction in volume was achieved, *Kwatha* was filtered through single folded cotton cloth and collected into a separate vessel.

Subsequently, the *Kwatha* was boiled again over slow fire on a gas stove, maintaining the temperature between 90°C and 95°C till a semisolid consistency was obtained. As the water evaporated, the viscosity of the extract increased, resulting in solid mass (*Ghana)*.[10] *Ghana* was mixed with 10% of the fine powder of *Triphaladi Kwatha*.

The solid mass (*Ghana)* was passed through a sieve no. 8 to prepare granules and were then dried at 50°C in a hot air oven for 5 h.[11]

Pharmacognostical evaluation

Pharmacognostical analysis of TG comprises of organoleptic characters [i.e., color, odor, taste, and texture, was recorded] and microscopic studies. Small Quantity of TG was dissolved in distilled water, filtered through filter paper. The precipitate was treated with and without stain to find out the lignified material along with other cellular components. These findings were compared with the characters of individual components of TG. The microphotographs were taken under Carl Zeiss Binocular microscope attached with camera.[12,13]

Physicochemical evaluation

TG were analyzed through relevant physicochemical parameters such as loss on drying, ash value, water-soluble extract, alcohol-soluble extract, and pH value.[14-16] In qualitative analysis, presence of glycosides, tannins, and flavonoids were assessed. High performance thin layer chromatography (HPTLC) is carried out with methanolic extract of TG.[17-18]

High performance thin layer chromatography

Methanolic extract of TG was spotted on pre-coated silica gel GF 60_{254} aluminum plate by means of Camang Linomat V sample applicator fitted with a 100-µL Hamilton syringe. Chloroform: MeOH (9:1) was used as the mobile phase. After development, densitometric scan was performed with a Camag TLC scanner III in reflectance absorbance mode at UV detection as 254 nm and 366 nm under the control of Win CATS Software (V 1.2.1. Camag). Then the plate was sprayed with vanillin sulfuric acid followed by heating and then visualized in daylight.

Observations and Results

Pharmacognostical analysis

Detailed pharmacognostical evaluation was carried out for all the ingredients of TG. Organoleptic characters of TG are placed in Table 2 powder microscopy of TG showed striking characters of all individual components of TG. Microscopy of *Terminalia chebula* Retz. showed features like brownish content [Figure 1a], fibers and sclereids [Figure 1b], pitted fibers [Figure 1c], rosette crystals [Figure 1d], sclereids and stone cells [Figure 1e], starch grains [Figure 1f], tannin contents, fibers, etc., *Emblica officinalis* Gaertn. showed group of fibers [Figure 1g], pitted stone cells [Figure 1h], prismatic crystals of calcium oxalate [Figure 1i], spiral vessels [Figure 1j], and yellowish colored parenchyma cells in surface view. Whereas *Terminalia bellerica* Roxb. presented with epidermal cells in surface view [Figure 2a], pitted sclereids [Figure 2b], starch grains [Figure 2c], pitted vessel and group of fibers [Figure 2d], rosette crystals and brownish colored matter [Figure 2e], stone cells and sclereids [Figure 2f], etc., *Berberis aristata* D.C. consisted of cork cells in surface view, group of fibers [Figure 2g], pitted sclereids and parenchymas [Figure 2h], pitted stone cells [Figure 2i], pitted vessel [Figure 2j], prismatic crystals [Figure 2k], starch grains, and yellowish and brownish colored content [Figure 2l]. *Pterocarpus marsupium* Roxb. Showed pitted vessel [Figure 2m], group of fibres [Figure 2n], parenchyma cells having brownish deposition [Figure 2o], and prismatic crystal [Figure 3a]. *Hollarrhena antidysenterica* Wall. consisted of cork cells in surface view, parenchyma cells embedded with starch, pitted sclereids and stone cells [Figure 3b], pitted vessel and stone cells [Figure 3c], prismatic crystals and brownish colored content [Figure 3d], and starch grains [Figure 3e]. Whereas *Cyperus rotundus* Linn. presented with brownish content, cork cells in surface view [Figure 3f], group of fibers [Figure 3g], parenchyma cells having brownish content [Figure 3h], reticulate vessel [Figure 3i], and starch grains [Figure 3j]. The diagnostic features obtained by powder microscopy were found to be complying with the standards mentioned at respective volumes of API.

Table 1: Ingredients of TG

Drug	Latin name	Part used	Proportion
Haritaki	*Terminalia chebula* Retz.	Pericarp	1 Part
Bibhitaki	*Terminalia bellerica* Roxb.	Pericarp	1 Part
Amalaki	*Emblica officinalis* Gaertn.	Pericarp	1 Part
Asana	*Pterocarpus marsupium* Roxb.	Heart wood	1 Part
Kalinga	*Hollarrhena antidysenterica* Wall.	Bark	1 Part
Musta	*Cyperus rotundus* Linn.	Rhizome	1 Part
Daruharidra	*Berberis aristata* D.C.	Stem	1 Part

TG:Triphaladi granules

Table 2: Organoleptic characters of TG

Parameters	Observation
Color	Brown
Odor	Characteristic
Taste	Astringent–bitter (*Kashaya–Tikta*)
Consistency	Solid

TG:Triphaladi granules

Physicochemical analysis

TG was analyzed using relevant physicochemical parameters at the pharmaceutical chemistry lab. The observations are presented in Table 3.

Preliminary phytochemical analysis and high performance thin layer chromatography

Qualitative tests

Presence of glycosides, flavonoids, and tannins was confirmed through the suitable tests while alkaloids could not be detected in TG [Table 4].

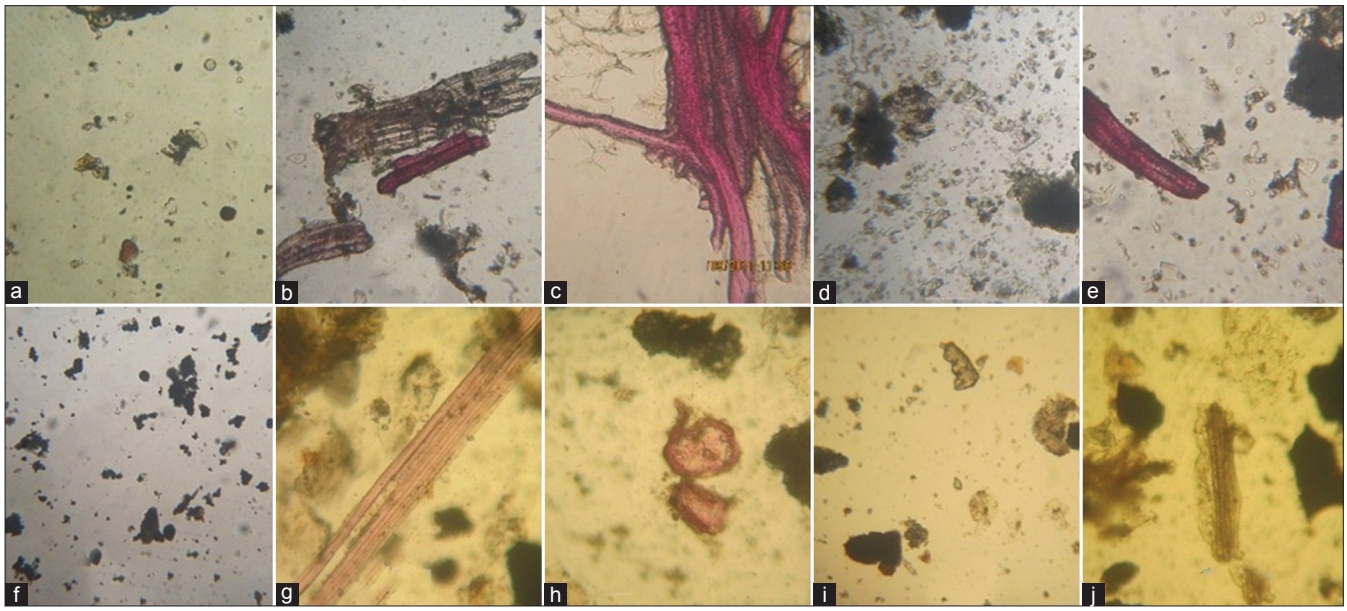

Figure 1: Microscopic characters: (a) brownish content; (b) fibers and sclereids; (c) pitted fibers; (d) rosette crystals; (e) sclereids and stone cells; (f) starch grains; (g) group of fibers; (h) pitted stone cells; (i) prismatic crystal of calcium oxalate; (j) spiral vessels

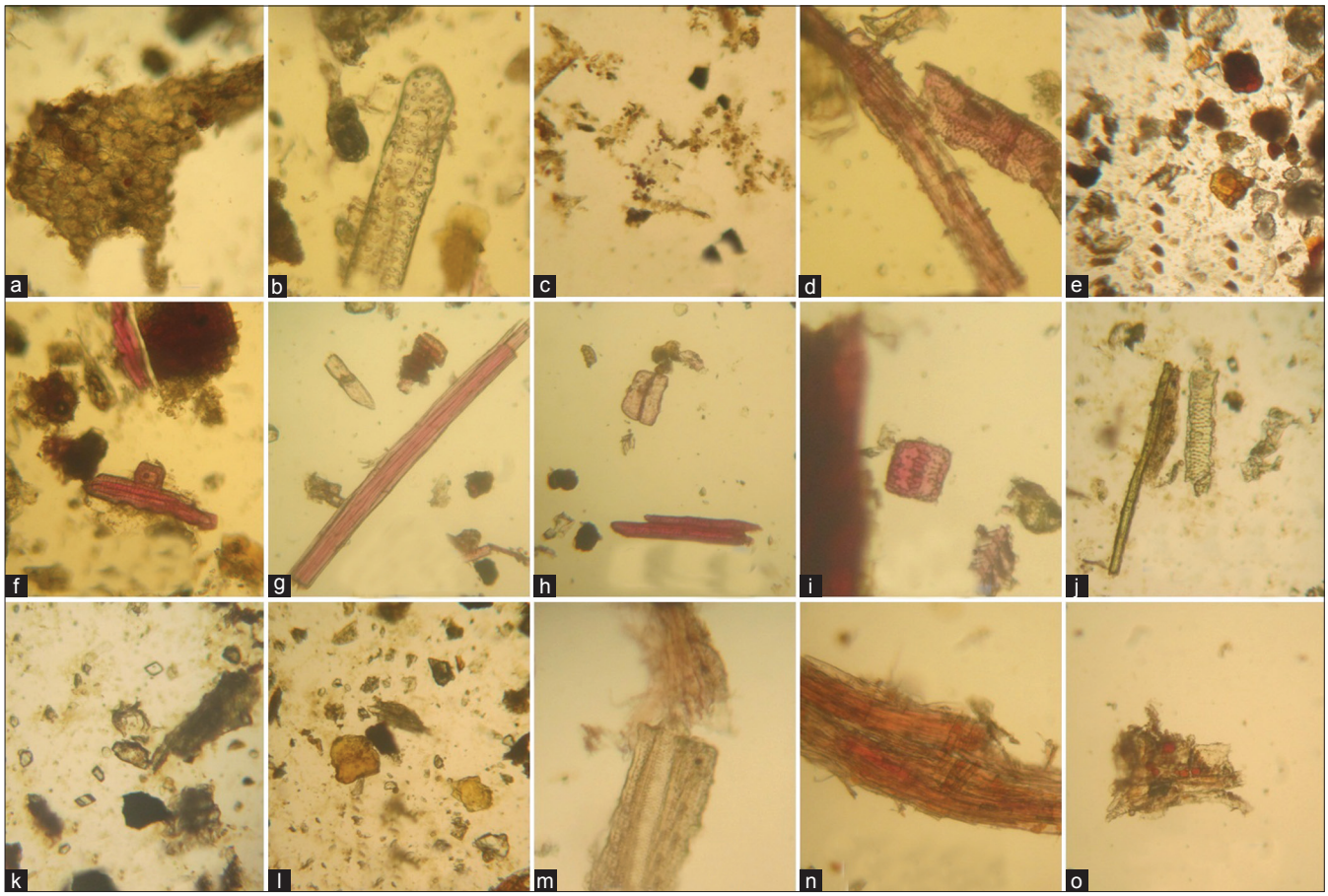

Figure 2: Microscopic characters: (a) epidermal cells in surface view; (b) pitted sclereids; (c) starch grains; (d) pitted vessel and group of fibers; (e) rosette crystals and brownish colored matter; (f) stone cells and sclereids; (g) group of fibers; (h) pitted sclereids and parenchymas; (i) pitted stone cells; (j) pitted vessel; (k) prismatic crystals; (l) yellowish and brownish colored content; (m) bordered pitted vessel; (n) group of fibers; (o) parenchyma cells having brownish deposition

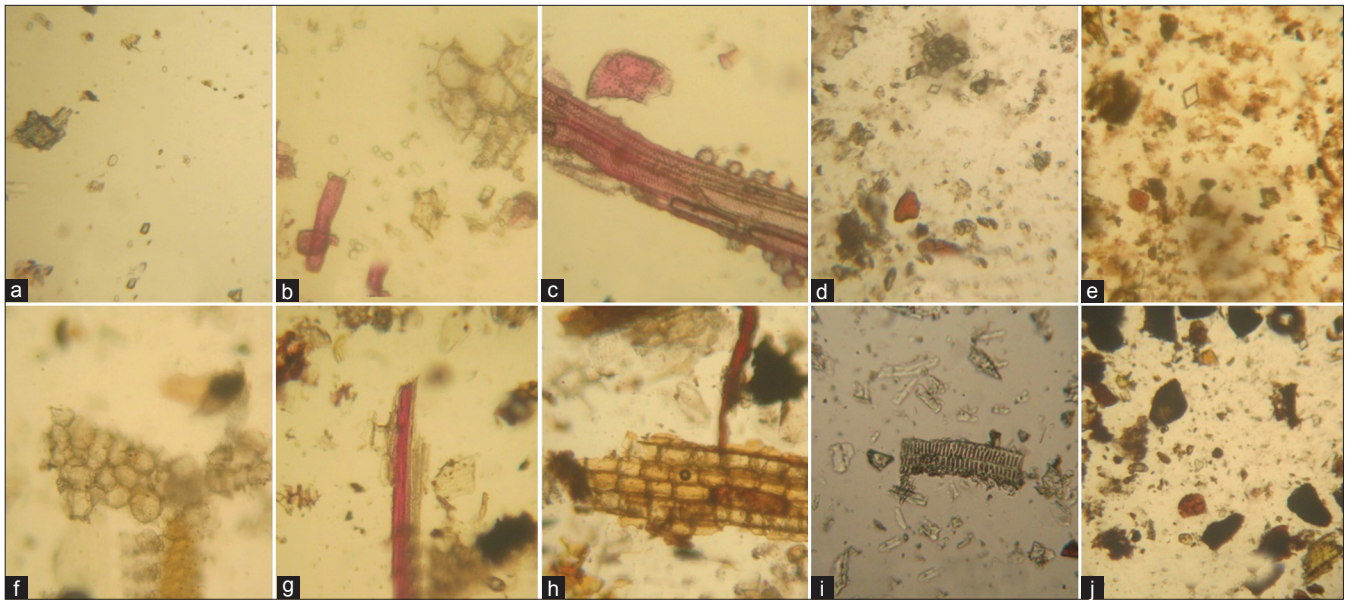

Figure 3: Microscopic characters: (a) prismatic crystal; (b) pitted sclereids and stone cells; (c) pitted vessel and stone cells; (d) prismatic crystals and brownish colored content; (e) starch grains; (f) cork cells in surface view; (g) group of fibers; (h) parenchyma cells having brownish content parenchyma cells; (i) reticulate vessel; (j) starch grains

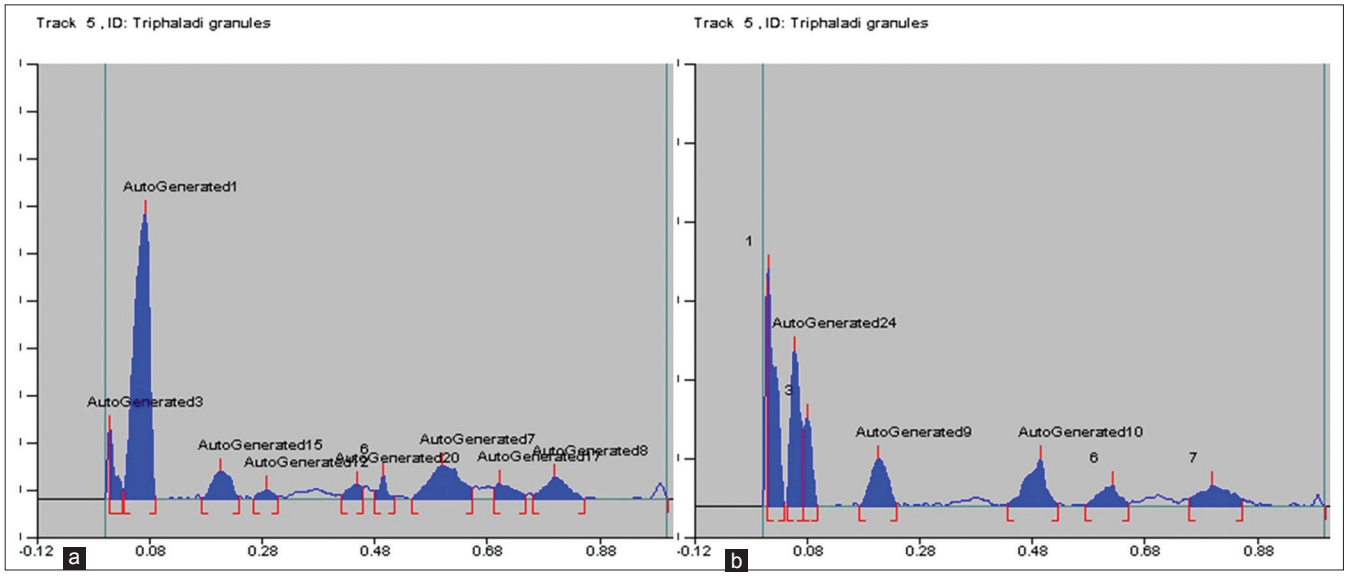

Figure 4: (a) Densitogram curve of methanol extract of *Triphaladi* granules at 254 nm; (b) densitogram curve of methanol extract of *Triphaladi* granules at 366 nm

Table 3: Physicochemical parameters

Test	Result
Aqueous extract	48.66% w/w
Alcohol extract	33.91% w/w
pH	6.0
Ash value	5.974% w/w
Loss on drying	6.53% w/w

TG: Triphaladi granules

HPTLC

On analyzing under densitometer at 254 nm, the chromatogram showed nine peaks. While at 366 nm, the chromatogram showed seven peaks. When the plate was sprayed with vanillin sulphuric acid followed by heating and then visualized in daylight showed eight spots [Figures 4a and b, 5a-c, and Table 5].

Discussion

Powder microscopy of TG showed striking characters of all individual seven drugs of TG. This confirms the ingredients present in the finished product and there is no major change in the microscopic structure of the raw drugs during the pharmaceutical processes of preparation of granules. By preliminary qualitative analysis, presence of phytochemicals such as glycosides, flavonoids,[19] and tannins[20] was found.

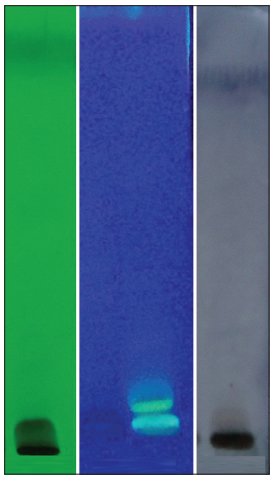

Figure 5: HPTLC: (a) 254 nm; (b) 366 nm; (c) after spray

Table 4: Functional groups of TG

Material	Functional Group	Result
Alcoholic extract of TG	Glycosides	Present
	Flavonoids	Present
	Tannins	Present
	Alkaloids	Absent

TG: Triphaladi granules

Table 5: HPTLC

HPTLC	Spots	Rf values at 254 nm
	9	0.01, 0.07, 0.21, 0.29, 0.45, 0.49, 0.60, 0.70, 0.80
		Rf values at 366 nm
	7	0.01, 0.06, 0.08, 0.21, 0.49, 0.62, 0.80
		Daylight after spray with vanillin sulphuric acid
	8	0.05, 0.11, 0.15, 0.25, 0.40, 0.45, 0.58, 0.67

HPTLC: High performance thin layer chromatography

Conclusion

Preliminary organoleptic features and results of powder microscopy reveal the presence of tannin contents, starch grains, pitted vessel, group of fibers, sclereids, stone cells. etc., This shows TG consist of all the ingredients. In preliminary physico-chemical analysis, water-soluble and alcohol-soluble extract, pH, ash value, and loss on drying were assessed. Preliminary qualitative analysis proves the presence of glycosides, flavonoids, and tannins. As no published information is available on physic-chemical profile of TG, this preliminary information can be used as standard in future.

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हिन्दी सारांश

त्रिफला ग्रेनूल्स का फार्मकोग्नोस्टिकल एवं फायटोकेमीकल अध्ययन

अंकुश गुंजाल, एच. एम. चंडोला, हरिषा सी. आर., वि. जे. शुक्ला, मनदीप गोयल, प्रिती पंड्या

चक्रदत्त एवं योगरत्नाकर मे प्रमेह चिकित्सा के लिये वर्णित त्रिफला क्वाथ एक मिश्रित आयुर्वेदिक फ़ोर्मुलेशन है । आधुनिक काल मे प्रमेह टाईप २ डायबिटीस से समानता रखत है । वर्तमान अध्ययन मे त्रिफला ग्रेनूल्स का फार्मकोग्नोस्टिकल एवं फायटोकेमीकल स्तरों पर मानिकीकरण किया गया है, जैसे कि pH 6.0 (5% conc.), जल मे घुलीत तत्व 48.66% w/w, ऐश मात्रा 5.97% w/w, अल्कोहल मे घुलीत तत्व 33.91% w/w तथा सुखाने पर हानि (105°C) 6.53% w/w थी । एक निश्चित सोल्वेन्ट प्रणाली से टी.एल.सी. और एच. पी. टी. एल. सी. करने पर ९ बिन्दु अलग मिले, जिससे योग मे कुछ तत्वो की उपस्थिती निश्चित की है । इस प्रकार फार्मकोग्नोस्टिकल एवं फायटोकेमीकल अध्ययन से त्रिफला ग्रेनूल्स की गुणवत्ता एवं प्रामाणिकता का परिक्षण किया गया है ।