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Research Article

Physical, Phytochemical and Chromatographic Evaluation of *Triphala Guggul* Tablets

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Abstract

Objective: In the present study, an attempt was made to formulate in-house '*Triphala guggul'* tablets, which were subjected to evaluations of physical properties, Phytochemical and chromatographic evaluation of triphala guggul tablets. Methods: Thin Layer Chromatography (TLC) of the formulations were performed for the confirmation of different phytoconstituents.Further, the study included standardization of these formulations by developing a simple, precise and accurate High Performance Thin Layer Chromatography (HPTLC) method using gallic acid, piperine and guggulsterone E and Z as chemical markers. Result: Chromatographic analysis was performed on silica gel 60 F254 precoated TLC plates using toluene: ethyl acetate: formic acid (4:4.5:1 v/v/v) for gallic acid, benzene: ethyl acetate: diethyl ether (6:3:1 v/v/v) for piperine and petroleum ether: ethyl acetate (6:2 v/v) for guggulsterone E and Z as mobile phase. The R_f values of gallic acid, piperine and guggulsterone E and Z were the basis of confirmation of these markers. The total peak areas of standard markers and corresponding formulations were compared and their contents were estimated in all the formulations. The results demonstrated that, the formulation prepared by company B (MF 2), showed less quantity of gallic acid and guggulsterone E and Z, while piperine content in all the marketed formulations was higher than the in-house formulations (LF1, LF2 and LF3). Conclusion:The analytical method developed herein for standardization of *'Triphala guggul'* preparation including its tablets, will be helpful in obtaining a quality control profile of this formulation.

1. INTRODUCTION

In developed as well as developing countries, herbal medicines have been used as a potential source of home remedies. In recent years, there is great increase in demand of herbal medicines in pharmaceutical industries which covers a substantial proportion of the global herbal drug market over the counter drug products. However, herbal drugs are prone to contamination and deterioration, which leads to variation in composition of constituents and may end up in little or no therapeutic efficacy[1]**.** To minimize the amount of adulteration and misrepresentation of herbal drugs, care should be taken beginning with proper identification of plants, seasons, area of collection as well as their extraction and purification process in order to obtain quality oriented herbal formulations that will act as a reference standard for that particular crude drug[2]**.**

'*Triphala guggul'* is a traditional Ayurvedic herbal formulation that consists of dried fruits of three medicinal plants *Terminalia chebula* (Combretaceae), *Terminalia belerica* (Combretaceae) and *Emblica officinalis* (Euphorbiaceae) which are also combined with *Commiphora mukul* (Burseraceae) and *Piper longum* (Piperaceae). The formulation has been implemented in treatment of sinusitis, allergies, boils, constipation, piles, high cholesterol, mal-absorption and as a purgative. Current research substantiates its benefit for the treatment of elevated blood lipids and coronary and arterial plaque known as atherosclerosis. As a result, today standardized guggul extracts are being approved for lowering elevated serum cholesterol and triglyceride levels in India[3]**.** Therefore it was envisaged to incorporate three phytoconstituents of significant importance i.e. Gallic acid, Piperine, Guggulsterone E and Z, contributing as the major components of *Triphala Guggul* for the purpose of standardizing this formulation in any of its form including tablets. Gallic acid is a strong antioxidant that possesses wide range of applications such as antimutagenic, anti-inflammatory, and anticarcinogenic activities[4]**.** The interaction of piperine with drug-metabolizing enzymes is responsible for oxidation, hydroxylation and glucuronidation and is also reported to be a potential bioenhancers for many Allopathic, Ayurvedic and Unani drugs[5]**.** Guggulsterone E and Z, the main constituents of the plant *Commiphora mukul* inhibits angiogenesis *in vitro* and *in vivo*[6]**.** Considering the pharmaceutical application of these markers, an attempt was made to standardize the '*Triphala guggul'* marketed and in-house formulation by estimation of piperine, gallic acid and guggulsterone E and Z using High Performance Thin Layer Chromatography. Thus, the present study will help in developing a quality profile about the different formulations of these crude drugs being currently used in the market.

2. MATERIALS AND METHODS

2.1 Drugs and chemicals used

The extracts of *Terminalia chebula* (Hirda), *Terminalia belerica* (Baheda), *Emblica officinalis* (Amla), *Piper longum* (Pimpli) and *Commiphora mukul* (Guggul) were procured from Natural Remedies, Bangalore, India. The three different batches of marketed formulations (tablets) of 'Triphala guggul' of two different manufacturers in India were procured for this study from the market in Nagpur, Maharashtra, India. i.e. Formulation 1 (Batch 329 [TN1], 356 [TN2], 357 [TN3]) of company A (MF1) and Formulation 2 (Batch 030035 [TP1], 051052 [TP2], 050302 [TP3]) of Company B (MF 2). Standard piperine and gallic acid were procured from Sigma-Aldrich, St. Louis, MO, USA while guggulsterone E and Z were purchased from Yucca enterprises, Mumbai, India. All the solvents used in the present study were of analytical grade. Further, preparation of in-house '*Triphala guggul*' tablets were carried out using different plant extracts. (**Table 1)** using standard monograph given in Indian Pharmacopoeia[7]**.**

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Table 1: Formula for preparation of Triphala Guggul tablets

Ingredients	mg/tab		
	LF1	LF2	LF3
Terminalia chebula	27.5	27.5	27.5
Terminalia belerica	27.5	27.5	27.5
Emblica officinalis	27.5	27.5	-
Piper longum	27.5	-	27.5
Commiphora mukul	138.4	138.4	138.4
Pulverised sugar	45.6	73.1	73.1
Talc	6.0	6.0	6.0

2.2 Evaluation of physical properties

All the formulations i.e. marketed as well as in-house preparations of *'Triphala guggul'* tablets were first subjected to evaluation of physical parameters such as hardness, friability, disintegration and weight variation tests. (**Table 2)** The tests were performed as per the monographs mentioned in the Indian Pharmacopoeia[7]**.**

Table 2: Physical properties of *Triphala Guggul* tablets

Test	MF1	MF2	LF1	LF2	LF3
Hardness (Kg/cm²)	2-3	2-3	3-4	3-4	3-4
Friability (%)	2.24	0.37	0.43	0.49	0.38
Disintegration Time (min)	60	48	60	60	55
Weight variation test	Pass	Pass	pass	pass	Pass

2.3 Preliminary phytochemical screening

Preliminary phytochemical screening of different '*Triphala guggul*' tablets (marketed and in-house prepared) was carried out using standard procedures for the presence of various phytoconstituents[8]**.**

2.4 Thin layer chromatography (TLC)

The presence of markers i.e. gallic acid, piperine and guggulsterone E and Z was confirmed using TLC, where pre - coated silica gel 60 F254 TLC plates (Merck, Germany) were used as stationary phase. The mobile phase used for gallic acid was toluene: ethyl acetate: formic acid (4:4.5:1 v/v/v), for berberine mobile phase was benzene: ethyl acetate: diethyl ether (6:3:1 v/v/v) and in case of guggulsterone E and Z, the plate was developed using petroleum ether: ethyl acetate (6:2) as mobile phase[9]**.**

2.5 High performance thin layer chromatography (HPTLC) study

All the formulations were further standardized simultaneously using gallic acid, piperine and guggulsterone E and Z as chemical markers after confirmation of their presence using high performance thin layer chromatography (Figure1 and figure 2). A stock solution of formulations and standards in methanol was prepared in concentration of 5 mg/mL and 0.2 mg/mL respectively. The mobile phase for developing the chromatogram was same as used in TLC analysis. The study was carried out using Camag-HPTLC instrumentation equipped with Linomat V sample applicator, Camag TLC scanner 3, Camag TLC visualizer and WINCATS 4 software, for data interpretation. The R_f values were recorded and the developed plates were screened and photo-documented at ultra violet range with wavelength (λ_{max}) of 254 nm (gallic acid), 365 nm (berberine) and 254 nm (guggulsterone E and Z) respectively. The calibration curve of standards were prepared by plotting concentration verses area under curve on the basis of which percentage of respective markers were estimated in marketed and in-house; formulations **(Table 3).**

Table 3: Quantification (in % w/w) of phytoconstituents in *Triphala Guggul* tablet

Batch	Gallic acid	Piperine	Guggulsterone E	Guggulsterone Z
MF1	0.49	0.155	17.02	16.21
MF2	0.09	0.137	6.31	7.38
LF1	0.47	0.042	13.30	19.90
LF2	0.49	-	15.34	23.16
LF3	0.47	0.042	14.00	21.10

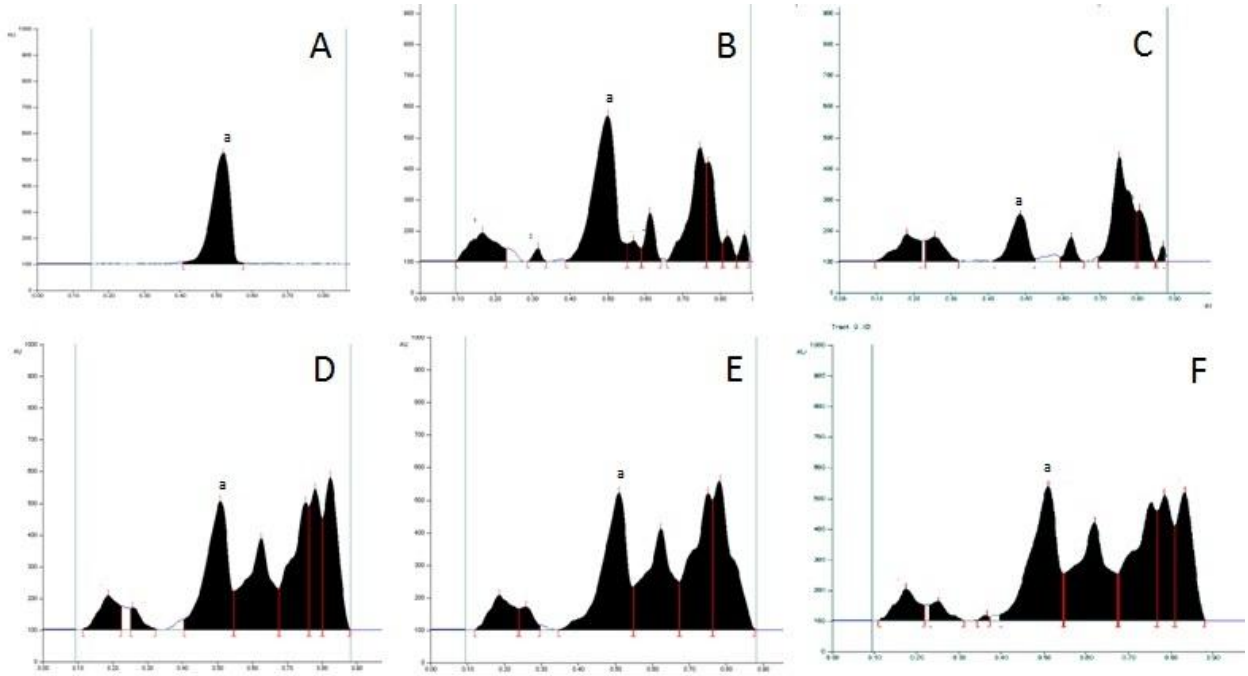

Figure 1: HPTLC chromatogram of gallic acid (a) in different formulations of Triphala guggul tablets. In figure A: Standard peak of gallic acid, B: Peak of gallic acid present in MF 1, C: Peak of gallic acid present in MF 2, D: Peak of gallic acid present in LF 1, E: Peak of gallic acid present in LF 2 and F: Peak of gallic acid present in LF 3.

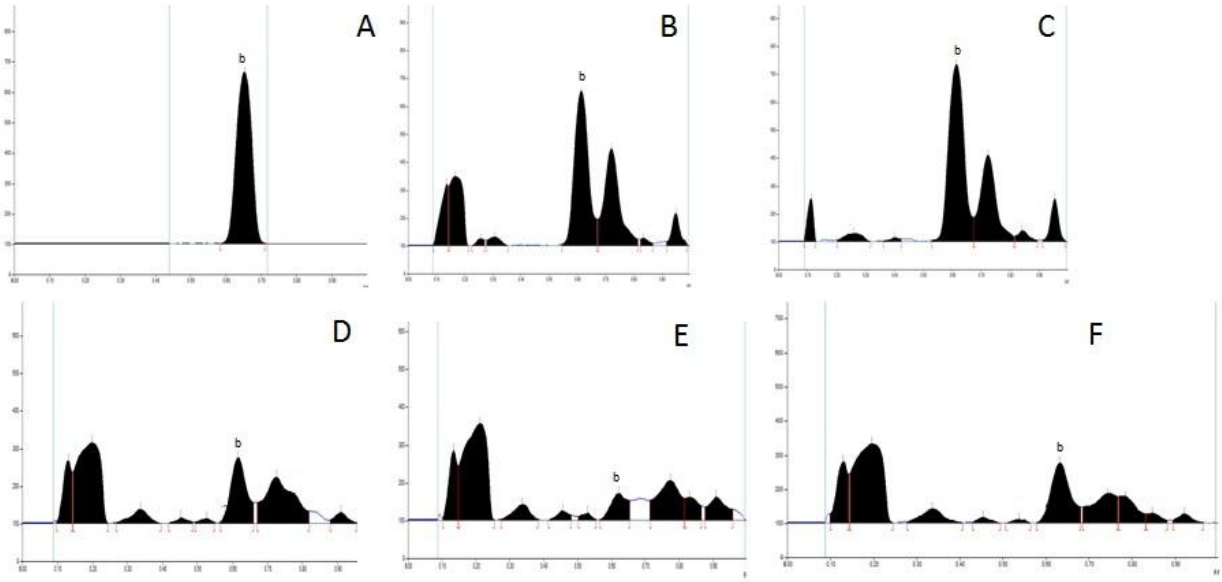

Figure 2: HPTLC chromatogram of piperine (b) in different formulations of *Triphala guggul* tablets.

In figure A: Standard peak of gallic acid, B: Peak of piperine present in MF 1, C: Peak of piperine present in MF 2, D: Peak of piperine present in LF 1, E: Peak of piperine present in LF 2 and F: Peak of piperine present in LF 3.

3. RESULTS AND DISCUSSION

In the present study, an attempt was made to prepare in-house formulations (tablets) of '*Triphala guggul',* which were further compared with some marketed formulations with respect to their physical and phytochemical characteristics. In the present investigation, *Triphala guggul* tablets were prepared by direct compression method. The advantages of direct compression include uniformity of blend, few manufacturing steps, prime particle dissociation, and physical stability. Manufacturing of tablets should be followed by its quality control tests such as the weight variation, friability, hardness and disintegration. Weight variation in tablets is an important factor that is mainly affected by factors such as tooling of the compression machine, head pressure, machine speed and flow properties of the powder, powder or granulate density and particle size. This friability test helps in determining the physical strength of the tablets which is attributed to the tablet breaking force. The disintegration test is a measure of the time required under a given set of conditions for a group of tablets to disintegrate into particles. Factors affecting the disintegration of tablet dosage[1] are physicochemical properties of drug (solubility, particle size and polymorphism), formulation factors (effect of excipients), test apparatus (pH and surface tension of the medium, temperature of medium, and its viscosity), and the tablet-manufacturing process (method of granulation and compression). From the results it was observed that, in-house tablets showed more hardness compared to marketed preparations, while friability was higher in case of MF1. The disintegration time for most of the tablets was 60 min, while for MF 2, it was lowest at 48 min. All the formulations were found to pass the weight variation test as per the monographs of the Indian Pharmacopoeia (Table 2). All the results obtained in the present study were found to be within the permitted range of the monographs as described in Indian Pharmacopoeia. Preliminary phytochemical screening of a formulation gives an idea about the chemical nature of the active constituents present in that formulation[7]**.** The results demonstrated the presence of mainly phenols, flavonoids, tannins, alkaloids, carbohydrates, proteins and amino acids. Phenols and flavonoids have been reported to be effective in quenching of oxygen-derived free radicals and it neutralizes them by donating hydrogen atom or an electron to the free radicals[10]**.** In addition to antioxidant and anti-inflammatory activities (by increasing the capillary permeability), flavonoids have also been associated in treatment of various cardiovascular diseases[11,12]**.** Tannins have a strong astringent property and have also been shown to have potent anti-bacterial, anti-inflammatory, anti-viral and anti-oxidant activities[1, 13,14]. Medicinal value of alkaloids in treatment of cancer, malaria, pain, inflammation, Parkinsonism, hypertension and number of central nervous system disorders is well known[15].

The markers i.e. gallic acid, piperine and guggulsterone E and Z were finalized to standardize these formulations and were then confirmed through Thin Layer Chromatography analysis, where R_f values was the basis of confirmation (gallic acid: 0.48, piperine: 0.60, guggulsterone E: 0.22 and guggulsterone Z: 0.29). From the Thin Layer Chromatography analysis, it was observed that, the marketed formulations showed lesser R_f value as compared to in-house formulations which is mostly likely due to the impact of manufacturing practices. Further, the confirmed markers were quantified in all the formulations by HPTLC and are represented in table 3. The HPTLC analysis depicted well resolved peaks of all the formulations showing the presence of respective markers. The spots of the entire chromatogram were visualized under UV and their percentage w/w was reported on the basis of regression equation. Our observations from the above analysis showed that, the formulation prepared by company B (MF2) showed the lowest concentration of gallic acid, guggulsterone E and Z, while all the marketed formulations showed higher quantity of piperine as compared to in-house formulations (LF1, LF2 and LF3). Thus, through this study we have optimised the analytical method for standardization of marketed as well as in-house preparations of '*Triphala guggul'* tablets. The method will help in obtaining a quality control profile of the various formulations prepared using these crude drugs and it will act as a source of referential information for researchers having interest in the relevant field. However, further studies are underway to obtain a biological standardization profiling of optimized formulation with respect to their pharmacological potential such as anti-inflammatory, antioxidant and hepatoprotective activities.

4. ACKNOWLEDGEMENT

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