

Original Article

Physicochemical evaluation of Triphala churna

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ARTICLE INFO

Received 25 Oct 2014

Revised 15 Nov 2014

Accepted 01 Dec 2014

Keywords:

- HPTLC
- Standardization
- Physical evaluation

ABSTRACT

Standardization of herbal formulation is essential in order to assess the quality of drugs for therapeutic value. In the present study the marketed formulation has been standardized in order to assess the quality of marketed formulation based on the physicochemical characters (loss on drying, extractive values, ash values and pH), physical characters viz. (bulk density, tapped density, angle of repose, hausner's ratio and carr's index), phytoconstituents and HPTLC analysis according to pharmacopoeial and standard methods. The set parameters viz. loss on drying (12% w/w), water soluble extractive (46.2% w/w), alcohol soluble extractive (8.6% w/w), total ash (10.2% w/w), pH value (6.2), bulk density (0.4879 gm/ml), tapped density (0.6455 gm/ml), angle of repose (22.3°), hausner's ratio (1.3230) and carr's index (24.415) were found to be sufficient to standardize the Triphala Churna and can be used as reference standards for the quality control/quality assurance study. HPTLC analysis of the formulation showed an R_f value, 0.54 for gallic acid which confirmed the presence of tannins in the formulation. The results obtained may also be considered as tools for assistance to the regulatory authorities, scientific organization and manufacturers for developing standard formulation of great efficacy.

1. INTRODUCTION

In the long struggle to overcome the powerful forces of nature, the human beings have always turned towards plants for food, shelter, clothing, and healing. Even today herbal medicine plays an important role in the management of diseases. Though we are in 21st century where modern technology and scientific discoveries are ushering remarkable changes in our lives, nevertheless, the story of plants as herbal medicines definitely continues to unfold, however, quietly and independently [1]. Standardization is a system to ensure that every packet of medicine that is being sold has the correct substances in the correct amount and will induce its therapeutic effects [2]. It is an essential factor for ayurvedic formulation in order to assess the quality of drugs based on the concentration of their active principle. It is very important to establish a system of standardization for every plant medicine in the market, since the scope of variation in different batches

of medicine is enormous. Triphala' is an age old commonly used Ayurvedic powdered preparation in Indian systems of medicine. This well known formulation is made by combining *Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis*, in equal proportions based on the observation of Ayurvedic Formulary of India (AFI). The formulation is prescribed in the first line treatment of many ailments and is used as laxative, detoxifying agent and rejuvenator [3].

2. MATERIALS AND METHODS

Collection of material

A marketed Triphala Churna formulation was purchased from an authentic vendor of Moradabad. Marketed formulation was identified on the basis of standard organoleptic and microscopic characters in Pharmacognosy Research Lab, Moradabad

Educational Trust Group of Institutions, Faculty of Pharmacy, Moradabad. Formulation was kept in a safe dry place away from direct sunlight till used for further studies.

Chemical and reagents

All the analytical grade reagents and chemicals of Qualigens Fisher Scientific, Central Drug House and Loba Chem, India were employed during the tenure of research work.

Microscopic evaluation

A small quantity of powder was cleared using chloral hydrate solution with little heating in a watch glass. The cleared powder was subjected to staining with microscopy reagents in order to identify diagnostic microscopic characters. Properly stained slides were observed at total magnification of 100X on a digital trinocular microscope (Sciencetech, India) and representative microscopical features were documented using conventional hand drawing method.

Physiochemical evaluation

The physiochemical characters viz. Foreign matter, loss on drying, extractive values (Water and alcohol soluble), ash values (Total ash), pH (1% solution) were determined according to official methods [4, 5, 9].

Fluorescence and powder drug analysis

A small quantity of the powder formulation was spread onto a grease free microscopic slide and placed in a UV-visible light cabinet (Sciencetech India). The visible lamp was switched on and observed for responding color. Thereafter the slide was observed under UV short (254 nm) and UV long (365 nm) light lamps simultaneously. In the second experiment an equal amount of formulation was spread over the microscopic slides and to the each slide a different chemical reagent was added and mixed by gentle tilting. After 2 minutes, the slides prepared were placed into a UV-Visible light cabinet and viewed in day, UV short (254) and UV long (365) radiations. The colors observed after application of different chemical reagents in different radiations were then recorded [6].

Physical evaluation

Physical characters viz. Bulk density, Tapped density, Angle of repose, Hausner's ratio and Carr's index were determined according to standard methods [7].

Preparation of extract

An accurately weighed 50 gm of powder formulation was subjected to hot percolation in a Soxhlet extractor with solvent methanol. The resultant extract was concentrated using Buchi type rotary evaporator (Sciencetech, India) below 40°C and evaporated to dryness over a water bath and stored in a desiccators over indicator silica gel. The amount of extract obtained with solvent was weighed and calculated the percentage extractive yield.

Preliminary phytochemical analysis

Preliminary phytochemical tests were performed on reconstituted methanol extract of Triphla Churna formulation according to standard methods to identify different primary and secondary metabolites viz. carbohydrates, alkaloids, flavonoids, steroids, tannins and phenolic compounds [8-10].

High performance thin layer chromatographic analysis

Preparation of sample

1 g of Triphla churna was extracted with 20 ml, 2M Hydrochloric Acid. Extract was filtered on a vacuum pump and the filtrate obtained was treated with diethyl ether in a separating funnel. The ethereal part was collected and the solvent was allowed to evaporate to isolate gallic acid. The gallic acid thus obtained was dissolved in sufficient distilled water to produce 50 ml solution.

Chromatographic conditions

The experiment was performed on a pre-coated silica gel 60 F₂₅₄ (0.2 mm thickness, Merck) HPTLC plates (20×10 cm) without prewashing. Samples were applied to the plates as 8mm bands, 8 mm apart and 10 mm from the edges of the plate, with a Camag Linomat V automatic sample applicator. The plates were developed by the ascending technique, to a distance of 80 mm, at 25±5°C, relative humidity 50–60%, in a Camag twin trough glass chamber with a stainless steel lid, using the mobile phase ethyl acetate: toluene: acetone (4.5:4:1) for gallic acid. The chamber was saturated with solvent system vapors for 20 minutes. After development, plates were dried using a hot-hair dryer, viewed in a Camag UV cabinet, and then scanned with a Camag TLC Scanner, using winCATS software (version 1.4.2), in absorbance mode, with slit dimensions 6.00 x 0.45 mm, Micro. Detection was carried out at 254 nm wavelength. The R_f value was found to be 0.54±0.01 for gallic acid.

3. RESULTS AND DISCUSSION

In the present study the marketed powder formulation of Triphla was evaluated in order to establish its quality control parameters. In the case of powder herbal formulations the presence of moisture content is always unwanted because it may lead to deterioration of formulation and must be controlled in order to ensure the stability of product. The results of physiochemical analysis are shown in Table 1 and Fig. 1.

Table 1. Results of physiochemical evaluation

S.No.	Parameter	obtained Value
1.	Foreign matter	NIL
2.	Loss on drying	12% w/w
3.	Extractive values	
	Water soluble extractive	46.2% w/w
	Alcohol soluble extractive	8.6% w/w
4.	Ash value	
	Total ash value	10.2 %
5.	pH (1% w/v Soln.)	6.2

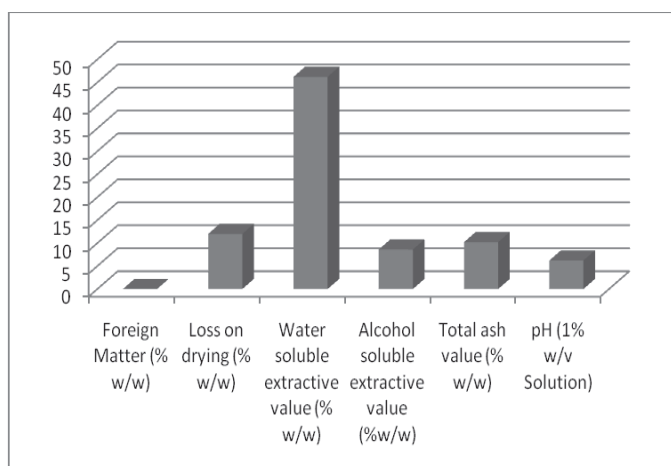


Fig. 1. Results of physicochemical evaluation

The marketed formulation on analysis for moisture content showed 12% w/w (Not more than 15% w/w) loss on drying which is a good indication for its stability. The total ash value is an indicative of total amount of inorganic material after complete incineration, which might have arisen due to improper washing of individual crude drug involved in formulation manufacturing. The marketed formulation on analysis for total ash yield 10.2% w/w ash which was found to be very high and could be due to the presence of adulteration or improper washing.

Table 2. Results of fluorescence and powdered drug analysis

Material	Visible Light	UV-Short (254nm)	UV-Long (365nm)
*PF alone	Light yellow	Brown	Black
PF + Methanol	Dark yellow	Spinach green	Black
PF + Acetic Acid	Dark yellow	Dark green	Black
PF + Petroleum ether	Light yellow	Light green	Black
PF + 1N NaOH	Reddish Brown	Dark green	Black
PF + Con. HNO ₃	Reddish brown	Greyish green	Black
PF + Con. HCl	Brown	Dark green	Black

*PF = Powder formulation

Results of fluorescence and powder drug analysis are mentioned in Table 2. Extractive value is another parameter to establish the amount of chemical constituent present in the formulation or to know herb extract ratio. From the Figure 2, given below it is clear that the formulation yield maximum extractive with water, which indicates that water is a potent extracting solvent for the powder formulation. Alcohol also gave appreciable extractive in comparison to water, which indicates that formulation also contains alcohol soluble components. Determination of pH of 1% w/v solution of formulation using a calibrated pH meter showed a 6.2 pH value, which indicates that the plant material yields the acidic solution after extraction with water.

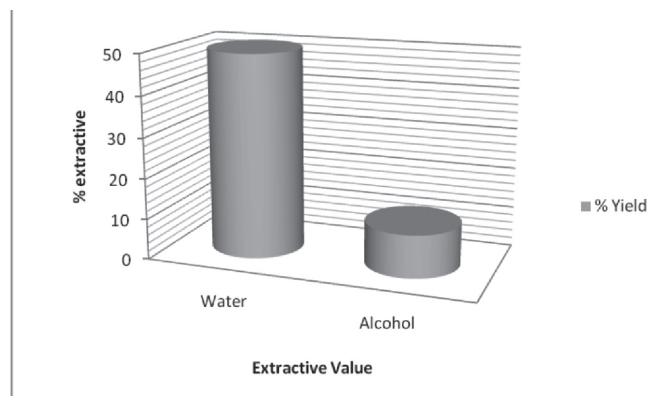


Fig. 2. Graphical comparison of extractive values

Results of physical evaluation are shown in Table 3 and Figure 3. The bulk density of formulation was found to be 0.4879 gm/ml which indicate that a medium size container is a best choice for its packaging. The tapped density of formulation was found to be 0.6455 gm/ml. The angle of repose of formulation was found to be 22.3 which indicate above excellent flowability of formulation thereby it can be easily taken out from container. The Hausner's ratio of formulation was found to be 1.3230 indicating passable flowability. The Carr's Index of formulation was found to be 24.415% indicating passable flowability which could be employed as a useful parameter in granulation process of formulation.

Table 3. Results of physical evaluation

S. No.	Parameter	Obtained Value (Average)
1	Bulk density	0.4879 gm/ml
2	Tapped density	0.6455 gm/ml
3	Angle of repose	22.3°
4	Hausner's ratio	1.3230
5.	Carr's index	24.415

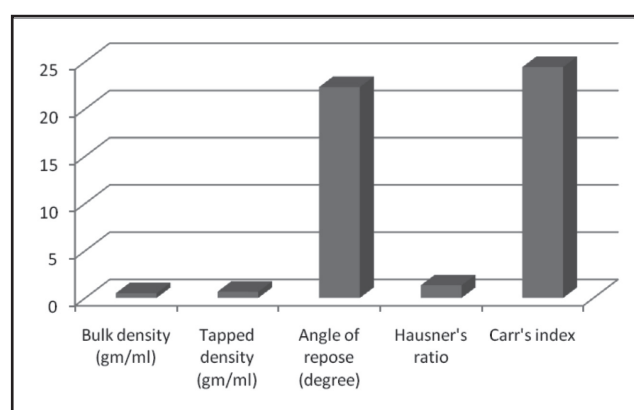


Fig. 3. Results of physical evaluation

Microscopy of powder formulation showed the presence of lignified phloem fibers, pitted stone cells, unicellular and multicellular covering trichomes and lignified epidermal cells characteristics of *Terminalia chebula*, *Terminalia belerica* and *Emblca officinalis*. Results of phytochemical analysis are shown in Table 4. Methanolic extract from the formulation showed

the presence of carbohydrates, phenolics, tannins and acidic compounds while the alkaloids, steroids and saponins were found to be absent.

Table 4. Results of phytochemical analysis

S. No.	Extract	Type of extract	Consistency	Colour	Weight in gm	% Yield (w/w)
1	Methanol	Crude	Semi-Solid	Blackish brown	26.01 gm	52.02

The results of phytochemical analysis confirmed that the raw materials used in formulation was exclusively good in quality and purity. HPTLC analysis (Figure 4) of the formulation showed an R_f value, 0.54 for gallic acid which confirmed the presence of tannins in the formulation.

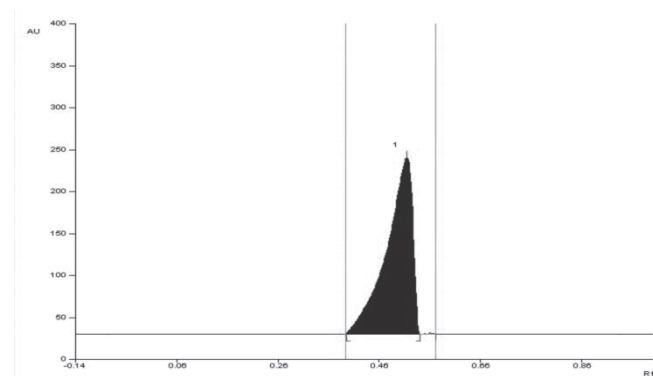


Fig. 4. Results of HPTLC analysis

5. CONCLUSION

The results of present study confirmed that the marketed formulation complies with prescribe standards and on the scale of quality fits to excellent. On the whole the parameters established in the present work would serve as the reference standards for the quality control of the marketed formulation in future. Beside the standards, the present study provide a methodology to researchers on which basis any individual involved in quality control can easily standardized the manufactured formulations.

Acknowledgements

Corresponding author is highly thankful to dignified members of Moradabad Educational Trust, Moradabad, India for providing research facilities.

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