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Standardization of Triphala Churna: Spectrophotometric Approach

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A widely used Ayruvedic preparation, 'Triphala Churna' prepared using dried fruits of amla, baheda and harda, was estimated spectrophotometrically for its tannic acid content. Three-laboratory batch of Triphala Churna and powdered amla, bahera, harda, were estimated for their tannic acid contents against standard tannic acid solution on double beam UV-Visible Spectrophotometer at λ_{max} 276 nm. The tannic acid content of all the three batches is found to be in close proximities with each other and recovery studies are indicated of reproducibility of method. Hence the present method is simple, sensitive, precise and accurate and can be adopted for routine quality control of Triphala Churna.

Key Words: Triphala churna, Spectrophotometry, Tannic acid.

INTRODUCTION

Triphala is among the most common formulations used in Ayurvedic medicine, comprised of the fruits of three medicinally important plants, Indian gooseberry (Amalaki, *Emblica officinalis*), Belleric myrobalan (Vibhitaka, *Terminalia balerica*) and Chebulic myrobalan (Haritaki, *Terminalia chebula*). Triphala is mentioned throughout the ancient literature of Ayurvedic medicine as a tonifying blood cleanser and gentle laxative, highly prized for its ability to regulate the process of digestion and elimination. Used by itself or in formulation, Triphala plays an essential role in the treatment of a wide variety of ailments[1].

Now-a-days most of the Ayurvedic formulation are lacking in their defined quality control parameters and method and therefore, they are not being accepted worldwide[2]. Hence World Health Organization (WHO) Assembly, in its resolution WHA 31.33 (1978), WHA 40.33 (1987), WHA 42.43 (1989) has emphasized the need to ensure the quality of medicinal plant products by using modern controlled technique and applying suitable standards[3].

In this connection an attempt has been made to develop the method of estimation of tannic acid, which is chemically hydrolyzable tannin

corresponding to a complexity of pentadigalloyl glucose[4] $C_{76}H_{52}O_{46}$ an important content in triphala churna. Presently tannis are estimated on the basis of visible-spectrophotometric method[5] and tannic acids are estimated on the basis of isolation technique. Present study is based on UV spectrophotometric analysis, which is a simple, precise and accurate method that can be considered as one of the quality control method for routine analysis.

EXPERIMENTAL

Plants: Dried fruits of amla, bahera and harda was purchased from local market Raipur, India and identified on the basis of morphological and microscopical characters and compared with standard Pharmacopeial Monograph[6-9]. All the chemicals and solvents were used of A.R. Grade.

Preparation of triphala churna: Three batches of triphala churna named TP-I, TP-II, TP-III, were prepared in laboratory using method described in Ayurvedic formulary[10]. The individual fruit of amla, bahera and harda was also powdered. These three batches of triphala churna and powdered amla, bahera, harda, were estimated for their tannic acid contents against standard tannic acid solution on UV-visible spectrophotometer (Shimadzu, UV-1700, Pharmaspec).

Preparation of tannic acid extract of triphala churna: Extract the powdered triphala churna (1 g) with 6 volume of denatured spirit on a shaker for 2 h. Filter the extract and re extract the mass left with 4 volumes of denatured spirit for another 1 h. Filter and combine the filtrate. Concentrate the denatured spirit extract under vacuum till the semisolid mass is obtained. Dilute with distilled water (1 : 50) and keep it overnight at 5ºC. Now filter the extract and discard the flocculent precipitate. Extract the filtrate with equal volume of ethyl acetate thrice. Concentrate the ethyl acetate extract till the semisolid mass is obtained. Dissolve the residue in 75 mL 0.1 N hydrochloric acid and filter through sintered glass funnel (G-2) by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 min, the supernatant was collected in 100 mL volumetric flask and volume was made with 0.1 N hydrochloric acid.

The same procedure was performed for each batch of triphala churna and separately powdered amla, bahera, harda and solution (100 mL) of their tannic acid extract were prepared.

Preparation of standard solution of Tannic Acid: As tannic acid show good solubility in 0.1 N hydrochloric acid, an accurately weighed amount of tannic acid (100 mg), from Himedia, A.R. Grade, was dissolved in 0.1 N hydrochloric acid and volume was made up to 100 mL with 0.1 N hydrochloric acid in volumetric flask. 2 mL of this solution was diluted with 0.1 N hydrochloric acid up to 100 mL in volumetric flask to give 20 μg/mL tannic acid solution.

Calibration curve from standard solution of tannic acid was prepared and with the help of this curve the tannic acid content of triphala churna estimated. The method was validated for precision and accuracy.

Calibration curve of tannic acid: A series of calibrated 10 mL volumetric flask were taken and appropriate aliquots of the working standard solution of tannic acid were withdrawn and diluted up to 10 mL with 0.1 N hydrochloric acid. The absorbance was measured at absorption maxima 276 nm, against the blank prepared in similar manner without the tannic acid. The absorption maxima and Beer's law limit were recorded and data that prove the linearity and obey Beer's law limit were noted.

The linear correlation between these concentrations (X-axis) and absorbance (Y-axis) was graphically presented and the slope (b), intercept (a), and correlation coefficient (r^2) were calculated for linear equation (Y = bx + a) by regression analysis using the method of the least square, Table-1 and Fig. 1.

TABLE-1
OPTICAL CHARACTERISTICS, STATISTICAL REGRESSION DATA AND VALIDATION PARAMETER OF TANNIC ACID

S.No.	Parameter	Value
1	Absorption maxima	276 nm
2	Beer's law limit	2-20 μg/mL
3	Regression equation (y = bx + a)	y = 0.0417x + 0.0128
4	Intercept (a)	0.0128
5	Slope (b)	0.041682
6	Correlation coefficients (r^2)	r^2 = 0.9995
7	Precision (n = 6, % RSD)	0.251
8	Accuracy (%)	99.30

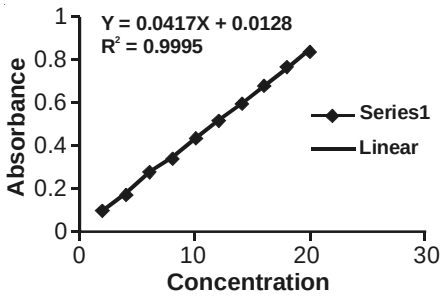

Fig.1 Calibration curve of tannic acid

Estimation of tannic acid: The appropriate aliquots from tannic acid extract of each batch of triphala churna and separately powdered amla, bahera and harda were withdrawn in 10 mL volumetric flask separately and absorbance of each aliquots was noted at 276 nm. The corresponding concentration of tannic acid against respective absorbance value was

determined using the tannic acid calibration curve. The statistical analysis for checking uniformity in batches also performed (Table-2).

TABLE-2
ESTIMATION OF TANNIC ACID CONTENTS IN TRIPHALA CHURNA

S.No.	Name		Tannic acid content (%) w/w	Confidence level (95%)
1	Amla		6.10 ± 0.27 %	± 0.216
2	Harda		14.05 ± 0.31 %	± 0.248
3	Bahera		8.70 ± 0.29 %	± 0.232
4		TP-I	9.02 ± 0.39 %	± 0.312
5	Triphala churna	TP-II	9.83 ± 0.42 %	± 0.336
6		TP-III	9.24 ± 0.43 %	± 0.344

*Mean ±SD of six determinations, TP-I: Triphala churna Batch I,
TP-II: Triphala churna Batch II, TP-III: Triphala churna Batch III,

Precision and accuracy: The method was validated for precision and accuracy, by performing the recovery studies at two levels by adding known amount of tannic acid to extract of triphala churna, of which the tannic acid content have been estimated previously. The data were obtained and recovery was calculated (Table-3).

TABLE-3
COMPILATION OF DATA OF RECOVERY STUDY

S.No.	Amount of tannic acid (μg/mL)			RSD (%)	SE	Recovery (%)
	In sample	Added	Estimated			
1	100	50	149.23 ± 0.39	0.261	0.159	99.48 ± 0.26
2	100	100	198.25 ± 0.48	0.242	0.196	99.13 ± 0.24
Mean				0.251	0.177	99.30

Mean ± SD of six determinations, RSD = Relative Standard Deviation
SE = Standard Error

RESULTS AND DISCUSSION

Tannic acid obeys Beer Lambert's law in concentration range 2-20 μg/mL at λ_{max} 276 nm. The correlation coefficient (r^2) was calculated where the r^2 value 0.9995 indicates good linearity between the concentration and absorbance.

The estimation of tannic acid content of triphala churna (three identical laboratory batch) and powdered amla, bahera and harda was carried out separately. The concentration of tannic acid present in raw material was found to be 6.10% ± 0.27 w/w in amla, 8.7% ± 0.31 w/w in bahera and 14.05% ± 0.29 w/w in harda, respectively and in three identical laboratory batch of triphala name TP-I, TP-II, TP-III, was 9.02% ± 0.39, 9.83% ± 0.42, 9.24% ± 0.43 w/w (Table-2), respectively with mean value 9.36% ± 0.41 w/w.

In order to obtain precision and accuracy recovery study was performed at two levels by adding known amount of tannic acid to pre-analyzed sample of triphala churna. The experiment was repeated six time at both level (Table-3) and result shows 99.48% ± 0.26 and 99.13% ± 0.24 recovery of tannic acid at both the level with mean value 99.30% ± 0.25 which prove reproducibility of the result. This shows significant precision of methods with 95% confidence level. The percentage relative standard deviation (% RSD) value was found to be 0.261 and 0.242 with mean 0.251 at both the level while the Standard Error was 0.159 and 0.196 with mean 0.177, respectively. From the data it is obvious that the present method of Spectrophotometric determination of tannic acid is simple, precise, accurate and suitable for routine analysis of tannic acid in triphala churna. As triphala churna is a good source of tannic acid, these findings can be taken as one of the parameter, along with other parameters, for quality control of triphala churna.

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