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Research Article

Quantitative Analysis of Tannic Acid in Crude Drug and its Ayurvedic Formulation by UV Spectrophotometry

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ABSTRACT

A simple and reproducible UV- spectrophotometric method for the quantitative determination of Tannic acid in Bhuvnesvara vati (BV) were developed and validated in the present work. The parameters, precision, accuracy, Limit of Detection and Limit of Quantitation were studied. In this present study a new, simple, rapid, sensitive, precise and economic spectrophotometric method in ultraviolet region has been developed for the determination of Tannic acid in laboratory ayurvedic formulation of Bhuvnesvara vati (BV). Each ingredient was purchased from the local market and identified morphologically and microscopically and compared with standard pharmacopoeial monograph. The concentration of tannic acid present in raw material is found to be 6.1%±0.27w/w in *Emblica officinalis*, 8.7%±0.31w/w in *Terminalia belerica*, 14.05%±0.29w/w in *Terminalia chebula*, 4.8%±0.94 in *Aegle marmelus* and 0.67±0.49 in *Trichyspermum ammi* and in three identical laboratory batch of Bhuvneshavara vati BV-I, BV-II and BV-III 4.90%±0.42, 4.79%±0.86, 4.85%±0.75w/w respectively with mean value 4.85%±0.53 w/w. Tannic acid has the maximum wavelength at 276 nm and hence the UV spectrophotometric method was performed at 276 nm. Tannic acid was found to follow beer lambert's law in concentration range 2-20µg/ml. The mean of %RSD value was found to be 0.399 with the mean standard error were 0.275.The content of Tannic acid in ayurvedic formulation was determined. The results of analysis have been validated statistically and confirmed the accuracy of the proposed method. Hence the proposed method can be used for the reliable quantification of Tannic acid in herbal formulations.

Key words: Bhuvnesvara vati, finger printing, Tannic acid, UV Spectrophotometer.

INTRODUCTION

Herbal drugs have been used since ancient times as medicines for treatment of a wide range of diseases. Medicinal plants have played a key role in world health. An increasing number of research papers and reviews clearly indicate that medicinal plants exhibit a variety of therapeutic properties[1-3]. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds[4-5]. Ayurveda is a unique holistic system, based on the interaction of body, mind and spirit[6].

Herbal medicines are in great demand in the developed as well as in developing countries for primary health care because of their wide biological activities, higher safety margins and lesser costs[7]. Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration, variation in composition and level of active constituents due to variation in climatic conditions. Also variation in the chemical profile of the herbal formulations is due to the factors like growing, harvesting, storage and drying processes. Therefore quality control of herbal medicines offers a host of problems. It is very important that a system of standardization is established for every plant medicine available in the market because the scope for variation in different batches of medicine is enormous[8-11].

A comprehensive and quantifiable identification method for fingerprint development; is able to reveal chemical information of herbal medicines with spectrogram and other analytical techniques[12]. The identification of herbal medicines from various sources is crucial in order to ensure authenticity, quality, safety and efficacy. Most of the herbal formulations are lacking in their defined quality control parameters. Therefore, they are not well accepted in global market. Hence, WHO has emphasized the need to ensure the quality of medicinal plant products by using modern analytical technique and applying suitable standards[13-14].

The formulation of Bhuvnesvara vati (BV) is well known ayurvedic formulation, is official in Ayurvedic Formulary of India[15] and cited in standard traditional literature of ayurveda i.e. Bhasaijyaratnavali, used for diarrhoea in ayurvedic medicine[16] .Though it is very popular medicine, no establishment of quality control for this drug studies have been performed yet. This paper includes estimation of Tannic acid for different batch sample of Bhuvnesvara vati (BV) by UV spectroscopic methods. The present study is an attempt to develop the fingerprinting method for

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Table 1: Validation Parameter of tannic acid

S.No.	Parameter	Value
1	Absorption Maxima	276 nm
2	Beer's Law limit	2-20µg/ml
3	Regression equation (y= bx+a)	y= 0.0417x + 0.0128
4	Intercept (a)	0.0128
5	Slope (b)	0.0417
6	Correlation coefficients (r2)	r2 = 0.9995
7	Precision (n=6, % RSD)	0.399
8	Accuracy (%)	99.71
9	LOQ	0.362µg/mL
10	LOD	0.129µg/mL

Table 2: Estimation of tannic acid content in Bhuvneshavara vati

S.no.	Name		Tannic acid content %w/w	Confidence level (95%)
1	*Emblica officinalis*		6.1% ± 0.27	±0.216
2	*Terminalia belerica*		14.05%± 0.29	±0.248
3	*Terminalia chebula*		8.7% ± 0.31	±0.232
4	*Aegle marmelus*		4.8%±0.94	±0.796
5	*Trichyspermum ammi*		0.67%±0.49	±0.412
6	Bhuvneshavara Vati	BV-I	4.90%±0.42	±0.402
		BV-II	4.79%±0.86	±0.722
		BV-III	4.85%±0.75	±0.644
		M-I	4.25±0.69	±0.684
		M-II	3.98±0.98	±0.972

Mean ï SD of six determinations,

Bhuvnesvara vati (BV) by spectrophotometric determination using as tannic acid as a standard, which is an important and major content in formulation. The developed spectroscopic fingerprints can be used as a standard tannic acid and can be used as a possible marker compound for fingerprinting of Bhuvnesvara vati (BV).

MATERIAL AND METHOD

Procurement of crude drug: The crude drugs were procured from local market and identification was confirmed by macroscopic and microscopic features and compared with standard pharmacopoeial monograph.

Preparation of formulations: Three sample batches of Bhuvnesvara Vati were prepared as per the method described in Ayurvedic Formulary of India and were named as BV-I, BV-II, BV-III. The same procedure was performed for each batch of Bhuvnesvara vati. Two Marketed formulations named M-I and M-II were procured from local pharmacy.

Chemicals: All the chemicals and solvents were used of A.R. Grade.

Instrument: Bhuvnesvara vati, 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 Bhuvnesvara vati : Extract the powdered Bhuvnesvara vati (1gm) with 6 volume of denatured spirit on a shaker for 2 hours. Filter the extract and re extract the marc left with 4 volumes of denatured spirit for another 1hours. 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 0.1N hydrochloric acid and filter through sintered glass funnel (G-2) by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 minutes, the supernatant was collected in 100 ml volumetric flask and volume was made with 0.1N hydrochloric acid. The same procedure was performed for each batch of Bhuvnesvara vati (BV-I, BV-II and BV-III), marketed formulations (M-I and M-II) and separately powdered *Emblica officinalis*, *Terminalia belerica*, *Terminalia chebula, Trichyspermum ammi* and *Aegle marmelus* and solution (100 ml) of their tannic acid extract were prepared.

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

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 reagent blank prepared in similar manner without the tannic acid. The absorption maxima and Beer's law limit were recorded and data that prove the

Table 3: Recovery study

S.No	Amount of Tannic Acid (µg/ml)			RSD%	SE	Recovery%
	sample	Added	Estimated			
1	100	50	149.06±0.72	0.483	0.294	99.37±0.63
2	100	100	200.10±0.63	0.314	0. 257	100.05±0.12
Mean				0.399	0. 275	99.71

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

linearity and obey Beer's law limit were noted.

The slope (b), intercept (a), and correlation coefficient (r^2) were calculated out for linear equation (Y= bx + a) by regression analysis using the method of the least square (Table 1).

Estimation of tannic acid: The appropriate aliquots from tannic acid extract of each batch of Bhuvnesvara vati and *Emblica officinalis, Terminalia belerica, Terminalia chebula, Aegle marmelus* and *Trichyspermum ammi* separately were withdrawn in 10 ml volumetric flask. Absorbance for aliquots of each 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 is also performed (Table 2)

Determination of limit of quantitation and limit of detection: The limit of detection (LOD) is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. The limit of quantitation (LOQ) is the lowest amount of analyte which can be quantitatively determined with suitable precision. The LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution and the lowest concentrations assayed (Table 1).

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 extract of Bhuvnesvara vati, of which the tannic acid content have been estimated previously. The data were obtained and recovery was calculated (Table 3).

RESULTS

The UV spectroscopy fingerprinting method was developed via estimation of tannic acid for each batch of Bhuvnesvara vati, its two marketed formulations and separately its raw material *Emblica officinalis, Terminalia belerica, Terminalia chebula, Aegle marmelus* and *Trichyspermum ammi* which are important contents in Bhuvnesvara vati. Tannic acid was found to follow Beer Lambert's law in concentration range 2-20µg/ml at λ_{max} 276 nm. The correlation coefficient (r^2) was calculated where the r^2 value 0.9995 indicates the good linearity between the concentration and absorbance. The concentration of tannic acid present in raw material is found to be 6.1%±0.27w/w in *Emblica officinalis*, 8.7%±0.31w/w in *Terminalia belerica*, 14.05%±0.29w/w in *Terminalia chebula*, 4.8%±0.94 in *Aegle marmelus* and 0.67±0.49 in *Trichyspermum ammi* and in three identical laboratory batch of Bhuvneshavara vati BV-I, BV-II and BV-III 4.90%±0.42, 4.79%±0.86, 4.85%±0.75w/w respectively with mean value 4.85%±0.53 w/w.

In order to obtain precision and accuracy, the recovery study was performed at two levels by adding known amount of tannic acid with pre -analyzed sample of tannic acid in Bhuvnesvara vati. The experiment was repeated six times at both level and result shows 99.37%±0.63 and 100.05%±0.12 recovery of tannic acid at both the level with mean value 99.71%±0.37 which prove reproducibility of the result. This shows significant precision of methods with 95% confidence level. The %relative standard deviation (% RSD) value was found to be 0.483 and 0.314 with mean 0.399 at both the level while the standard error was 0.294 and 0.257 with mean 0.275 respectively. From the data's it was observed that the present method of spectrophotometric determination of tannic acid is simple, precise, accurate and suitable for routine analysis of tannic acid in Bhuvnesvara vati.

As Bhuvnesvara vati 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 Bhuvnesvara vati.

CONCLUSION

The developed method was found to be accurate, simple, precise and rapid. It can therefore be applied for routine analysis of tannic acid in ayurvedic formulation Bhuvnesvara vati.

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