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Quantitative estimation of gallic acid and tannic acid in bhuvnesvara vati by RP-HPLC

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ABSTRACT

Bhuvnesvara vati (BV) is an Ayurvedic formulation containing Emblica officinalis, Terminalia belerica, Terminalia chebula, Trichyspermum ammi, Aegle marmelus and two minerals namely rock salt and Soot as main ingredients. The study was aimed to develop finger printing methods for well-known Ayurvedic formulation. Three sample batch of BV were prepared in the laboratory and two different marketed formulations were procured from Ayurvedic medicine shop. A HPLC method was developed for the estimation of gallic acid and tannic acid in laboratory and marketed formulations. The concentration of gallic acid present in raw material is found to be 3.174±0.049% w/w in Emblica officinalis, 8.920±0.173% w/w in Terminalia belerica, 4.092±0.117% w/w in Terminalia chebula, 1.831±0.973% w/w in Aegle marmelus and 0.264±0.365% w/w in Trichyspermum ammi. Gallic acid content in three identical laboratory batch of Bhuvnesvara vati BV-I, BV-II and BV-III, was found to be 2.623±0.746%, 2.589±0.356% and 2.632±0.239% w/w respectively. Two marketed formulation of Bhuvnesvara vati M-I and M-II showed gallic acid concentration to be 2.019±0.872 % and 2.019±0.872 % w/w respectively. The concentration of tannic acid present in raw material was found to be 6.172%±0.365w/w in Emblica officinalis, 8.667%±0.0 319w/w in Terminalia belerica, 13.956%±0.745w/w in Terminalia chebula, 4.789±0.983% w/w in Aegle marmelus and 0.668±1.002% w/w in Trichyspermum ammi respectively and in three identical laboratory batch of Bhuvnesvara vati BV-I, BV-II and BV-III, was found to be 2.623±0.746%, 2.589±0.356%, 2.632±0.239% w/w respectively. In order to obtain precision and accuracy, the recovery study was performed and result obtained with mean value 99.69% for gallic acid and 99.38% for tannic acid, which prove reproducibility of the result. This show significant precision of methods at 95% confidence level. The mean of % RSD value was found to be 0.357 for gallic acid & 0.353 for tannic acid. Results of statistical analysis shows that present HPLC method for determination of gallic acid and tannic acid is simple, precise, accurate and suitable for routine analysis of gallic acid and tannic acid in BV. The developed fingerprints can be used as a standard and gallic acid and tannic acid can be used as a possible marker compound for fingerprinting of BV.

Keywords: Bhuvnesvara Vati (BV), Fingerprints, Gallic acid, Tannic acid, marker, HPLC.

INTRODUCTION

Ayurveda, the health care system indigenous to India, has an impressive evolutionary history that spans a period of many thousands of years [1]. Ayurveda is a science dealing not only with treatment of some diseases but is a complete way of life. This Indian system of medicine has laid down principles and methods of treatment for various diseases including chronic illness where there is no definite treatment, and sympatomatic relief is the only exiting treatment option [2,3]. Chromatographic fingerprint have been suggested to check for authenticity or provide quality control of herbal medicine [4]. Chromatography has the advantage of separating a complicated System into relatively simple sub-systems and then presenting the chemical patterns of herbal medicine in the form of a chromatogram [5,6]. The World Health Organization (WHO) accepts fingerprint chromatography as an

identification and quality evaluation technique for medicinal herbs since 1991 [7]. Fingerprints can be a unique identification utility for herbs and their different species [8,9] and can be used for modeling pharmaceutical activities [10]. Now, chromatographic fingerprint technique plays an important role in controlling the quality of samples and focusing on the identification and assessment of the stability of the components [11]. HPLC analysis for marker compounds may provide additional information in the form of chromatographic fingerprints. The present study is undertaken to develop certain fingerprints for Bhuvnesvara Vati, an Ayurvedic formulation. The Bhuvnesvara Vati is found beneficial in all types of diarrhoea and dysentery.

MATERIALS AND METHODS

Instrumentation

A C-18 LUNA (5 micron 25 cm×4.6 mm) column from Phenomenex a binary gradient high- pressure liquid chromatography (Shimadzu HPLC class VP series) with two LC–10 AT VP pumps, variable wavelength programmable UV/Visible SPD 10 AVP were used. All the chemicals and solvents were used of A.R. Grade.

Procurement of drug

Crude drugs were purchased from local market and identified morphologically and microscopically 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 purchased from local pharmacy.

Sample preparation

The powdered Bhuvnesvara vati (1gm) was refluxed with 60 ml methanol for 90 minute and filtered. The marc was reflux with 40 ml of methanol for another 1hours. Filter and the filtrate were combined. The methanol extract was concentrated under vacuum till the semisolid mass is obtained. The residue was dissolved in 75 ml methanol and filtered 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 methanol.

The same procedure was performed for each batch of Bhuvnesvara vati, two marketed formulation M-I and M-II and separately powdered *Emblica officinalis*, *Terminalia belerica*, *Terminalia chebula*, *Aegle marmelus* and *Trichyspermum ammi*, and solution (100 ml) of their extract were prepared.

Preparation of standard solution

The stock solution of gallic acid and tannic acid was prepared by dissolving 10.0 mg of each in 100.0 mL methanol, creating a 100 µg/mL solution. This solution was diluted with the solvent as needed to prepare different standard solutions (2, 4, 6, 8, 10, 12, 14, 16, 20µg/mL).

Chromatographic conditions

The chromatographic runs were performed at a flow rate was 1.2 ml/min. The wavelength of detection was 264nm.The column temperature was ambient and the injection volume was 10 µl.

Validation parameters

Linearity

Standard solutions (2, 4, 6, 8, 10, 12, 14, 16, 18, 20µg/mL), each in three replicates, were injected into the system. The method of linear regression was used for data evaluation. Peak area ratios of standard compounds were plotted against theoretical concentrations of standards. Linearity was expressed as a correlation coefficient (figure 1 and 2).

Precision

The precision of the method was tested by injecting a standard solution of gallic acid and tannic acid (20µg/mL and 2µg/mL) three times. Peak areas were determined and compared. Precision was expressed as percentage relative standard deviation (R.S.D.) (Table 1).

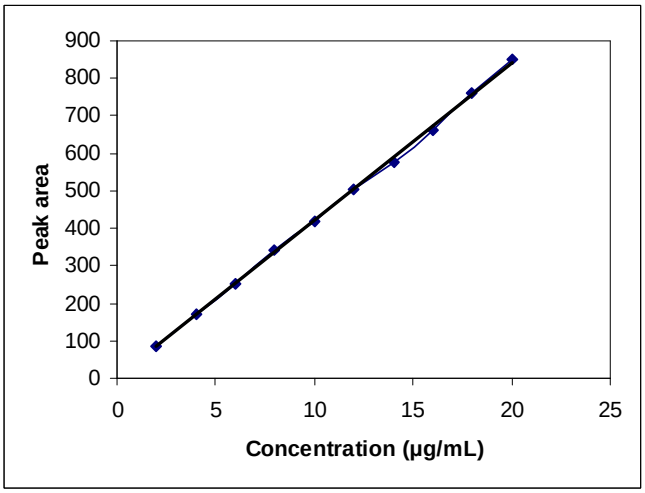

Figure 1: Standard curve of tannic acid

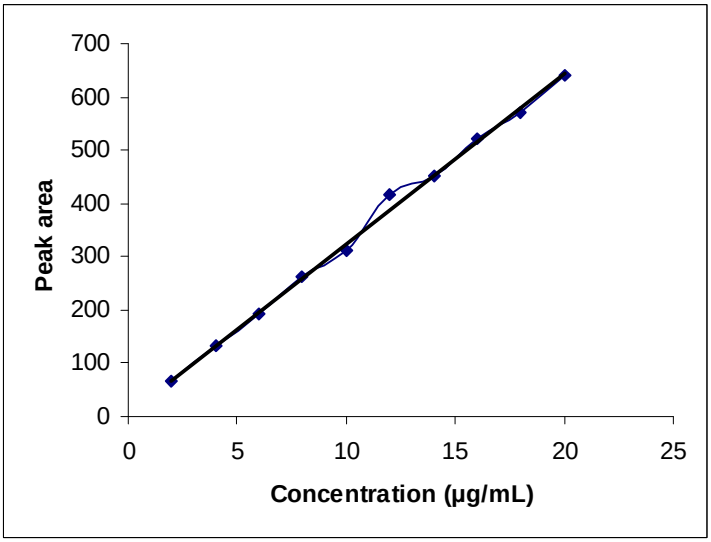

Figure 2: Standard curve of gallic acid

Table 1: Validation Parameter of gallic acid and tannic acid

S.No.	Parameter	Gallic acid	Tannic acid
1	Retention time	3.410min	25.976 min
2	Beer's Law limit	2-20µg/ml	2-20µg/ml
3	Regression equation (y= bx+a)	y= 32.033x + 4.14	y=41.98x+0.53
4	Intercept (a)	4.14	0.53
5	Slope (b)	32.03	41.68
6	Correlation coefficients (r^2)	r^2 = 0.9967	r^2 = 0.9991
7	Precision (n=3 % RSD)	0.357	0.353
8	Accuracy (%)	99.69	99.38
9	Limit of quantification(LOQ)	1.456µg/ml	0.754µg/ml
10	Limit of detection(LOD)	0.478µg/ml	0.249µg/ml

Repeatability

Inter and intra-day variation was performed by injecting the standard solutions (2, 4, 6, 8, 10,12, 14, 16, 18, 20µg/mL), each in three replicates, twice on the same day, and once on the next day and Peak areas were determined and compared (Table 2 and table 3).

Table 2: System repeatability of gallic acid

Concentration (µg/mL)	Day 1 peak area	Day 1 peak area	Day 2 peak area
2	65.01±0.231	65.21±0.981	65.89±0.456
4	132.16±0.569	132.19±0.369	132.72±0.652
6	192.14±0.634	192.16±0.258	193.12±0.832
8	263.43±0.432	263.33±0.147	264.22±0.624
10	312.12±0.526	312.08±0.546	312.96±0.496
12	416.23±0.236	416.21±0.236	417.02±0.238
14	450.27±0.864	450.65±0.985	451.23±0.234
16	521.43±0.651	521.22±0.924	522.03±0.941
18	570.12±0.196	570.36±0.846	571.09±0.743
20	642.12±0.298	642.13±0.754	643.02±0.725

Mean ± S.D. (n = 3).

Table 3: System repeatability of tannic acid

Concentration (µg/mL)	Day 1 peak area	Day 1 peak area	Day 2 peak area
2	85.89±0.654	85.94±0.827	86.02±1.020
4	171.12±0.236	171.22±0.854	171.53±0.981
6	251.03±0.453	251.16±0.946	251.92±0.479
8	342.01±0.821	342.09±1.231	342.97±0.673
10	420.02±0.763	420.13±0.238	421.07±0.612
12	505.2±0.367	505.29±0.734	505.83±0.825
14	574.2±0.964	574.24±0.439	574.97±0.946
16	660.25±0.239	660.31±0.824	661.29±0.256
18	761.02±0.546	761.14±0.559	761.73±0.652
20	852.03±0.547	852.18±0.629	853.13±0.987

Mean ± S.D. (n = 3).

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 quantitated 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 were assayed (Table 1).

Estimation of gallic acid and tannic acid

The appropriate aliquots from extract of each batch of Bhuvnesvara vati, its two marketed formulations and separately *Emblica officinalis*, *Terminalia belerica*, *Terminalia chebula*, *Aegle marmelus* and *Trichyspermum ammi* were withdrawn in 10 ml volumetric flask separately. The corresponding concentration of gallic acid and tannic acid against respective peak areas value was determined using the gallic acid and tannic acid calibration curve respectively (Table 4).

Table 4: HPLC Estimation of gallic acid and tannic acid

S.no.	Name		Gallic acid content %w/w	Tannic acid content %w/w
1	*Emblica officinalis*		3.174 ± 0.49	6.172±0.365
2	*Terminalia chebula*		4.092± 0.117	13.956±0.745
3	*Terminalia belerica*		8.920± 0.173	8.667±0.032
4	*Aegle marmelus*		1.831±0.973	4.789±0.983
5	*Trichyspermum ammi*		0.264±0.365	0.668±1.002
6	Bhuvnesvara vati	BV-I	2.623±0.746	4.914±0. 782
		BV-II	2.589±0.356	4.789±0.636
		BV-III	2.632±0.239	4.854±0.698
		M-I	2.019±0.872	4.251±0.993
		M-II	2.019±0.872	3.987±0.368

Mean ± S.D. (n = 3).

Recovery Studies

The recovery studies performed at three levels by adding known amount of gallic acid and tannic acid to extract of Bhuvnesvara Vati, of which the gallic acid and tannic acid content have been estimated previously. The data were obtained and recovery was calculated (Table 1).

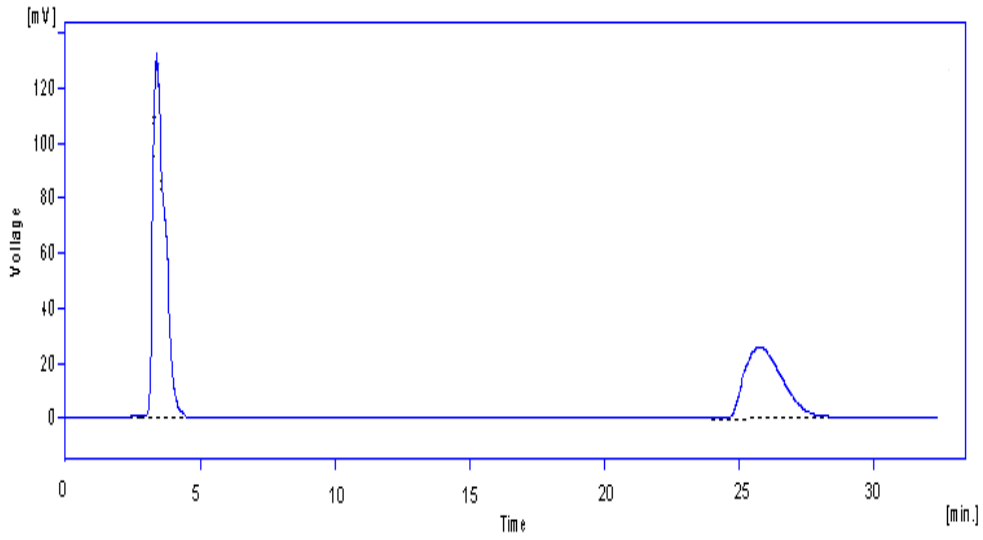

Figure 3: HPLC chromatogram of gallic acid and tannic acid

RESULTS AND DISCUSSION

In the present study, the fingerprint method for quality control of Bhuvnesvara Vati (BV) was developed by simple high-performance liquid chromatography (HPLC) determination using gallic acid and tannic acid as a standard, which are important and major content in formulation. RP- HPLC methods for determination of gallic acid and tannic acid from the fruits of *Emblica officinalis*, *Terminalia belerica*, *Terminalia chebula*, *Aegle marmelus* and *Trichyspermum ammi* and Bhuvnesvara vati have been developed. The chromatogram of gallic acid and tannic acid under experimental condition showed a single peak of the drug at 3.410 min and 25.976 min (Figure 3). The standard curve for gallic acid and tannic acid was linear over the investigated range (2–20 µg/mL) with a percent relative standard deviation (% R.S.D.) of less than 2% based on three successive readings (figure 1 and 2). A correlation coefficient (R^2) is suggested that the developed HPLC method had an excellent linearity over the concentration range of 2-20µg/m of gallic acid and tannic acid. Under the developed HPLC conditions, the limit of quantitation was determined to be 1.456 and 0.754 µg/mL respectively for gallic acid and tannic acid after three successive injections of the sample. Also, the limit of detection was found to be 0.478 and 0.249 µg/mL for gallic acid and tannic acid respectively (Table 1). The concentration of gallic acid present in raw material is found to be 3.174±0.049% w/w in *Emblica officinalis*, 8.920±0.173% w/w in *Terminalia belerica*, 4.092±0.117% w/w in *Terminalia chebula*, 1.831±0.973% w/w in *Aegle marmelus* and 0.264±0.365% w/w in *Trichyspermum ammi*. Gallic acid content in three identical laboratory batch of Bhuvnesvara vati BV-I, BV-II and BV-III, was found to be 2.623±0.746%, 2.589±0.356% and 2.632±0.239% w/w respectively. Two marketed formulation of Bhuvnesvara vati M-I and M-II showed gallic acid concentration to be 2.019±0.872 % and 2.019±0.872 % w/w respectively (Table 4). The concentration of tannic acid present in raw material was found to be 6.172%±0.365w/w in *Emblica officinalis*, 8.667%±0.0319w/w in *Terminalia belerica*, 13.956%±0.745w/w in *Terminalia chebula*, 4.789±0.983% w/w in *Aegle marmelus* and 0.668±1.002% w/w in *Trichyspermum ammi* respectively and in three identical laboratory batch of Bhuvnesvara vati BV-I, BV-II and BV-III, was found to be 2.623±0.746%, 2.589±0.356%, 2.632±0.239% w/w respectively. The results were comparable to marketed formulations (Table 4). The results indicate that the developed method can be used to quantification of gallic acid and tannic acid from Bhuvnesvara vati. In order to obtain precision and accuracy the recovery study was performed and result shows 99.69% and 99.38% for gallic and tannic acid respectively, which prove reproducibility of the result. This shows significant precision of methods at 95% confidence level. The % relative standard deviation (% RSD) value was found to be 0.357 and 0.353 for gallic acid and tannic acid respectively. The recovery of gallic acid and tannic acid from the Bhuvnesvara vati was quantitative (Table 1), and there was no interference from the other compounds present in the formulation when compared to the control.

The HPLC method developed for the simultaneous estimation of gallic acid and tannic acid is a simple, rapid and precise for the routine estimation of Bhuvnesvara vati. The method was validated by statistical analysis and recovery studies. As Bhuvnesvara vati is a good source of gallic acid and tannic acid, these findings can be used as routine chromatographic fingerprinting method for the standardization of the raw materials of the Bhuvnesvara vati as well as finished formulation.

CONCLUSION

The developed high performance liquid chromatographic method for estimation of gallic acid and tannic acid from Bhuvnesvara vati could be used as a valuable analytical tool in the routine analysis, to check the batch to batch variation.

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