STANDARDIZATION OF ASTANGAVALEHA: A SPECTROPHOTOMETRIC APPROACH

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Abstract: Selective and efficient analytical methods are required not only for quality assurance but also for authentication of herbal formulations. The main objective of the present study is to develop a simple, rapid and validated UV fingerprint method for estimation of gallic acid in Astangavaleha and selected crude drugs. Astangavaleha is official in Ayurvedic formulary of India and it is the most common formulation used for respiratory disorders like asthma, cough, COPD etc. in ayurvedic medicine. It comprises of the medicinal important plants Myrica esculenta (Kaiphal), Inula racemosa (Pohakar), Pistacia integerrima (Karkatasrangi), Trachyspermum ammi (Ajwain), Carum carvi (Jira), Zingiber officinale (Adraka), Piper nigrum (Kalimirch), Piper longum (Lindi Pippal), Madhu & Ardrak Svarasa. The estimation was carried out with three laboratory batches and one marketed formulation by spectrophotometric approach at 270 nm.

Keywords: Astangavaleha, Ayurvedic, Gallic acid, UV.

Introduction: Spectrophotometric is emerging as a versatile, high throughput & cost-effective technology that is uniquely suited to assessing the identity and quality of botanical materials. The World Health Organization (WHO) has emphasized the need to ensure the quality of herbal / Ayurvedic formulations by using suitable standards and technique. Astangavaleha is official in Ayurvedic formulary of India and it is the most common formulation used for respiratory disorders in Ayurvedic medicinal preparation. It composition of the medicinal important plants *Myrica esculenta (*Kaiphal*), Inula racemosa (*Pohakar*), Pistacia integerrima (*Karkatasrangi*), Trachyspermum ammi (*Ajwain*), Carum carvi (*Jira*), Zingiber officinale (*Adraka*), Piper nigrum (*Kalimirch*), Piper longum (*Lindi Pippal*),* Madhu & Ardrak Svarasa. Spectrophotometric determination gallic acid used as an internal standard. Polyphenols constitute one of the most numerous and ubiquitous groups of plant metabolites and are an integral part of both human and animal diets. Gallic acid (GA), a plant polyphenol, has been described as having antioxidant activities. The main objective of the present study is to quantification of one of the active constituent of total polyphenols as gallic acid in Astangavaleha and selected crude drugs (*Myrica esculenta, Pistacia integerrima* & *Trachyspermum ammi*). spectrophotometric analysis, which is a simple, precise and accurate method that can be considered as one of the quality control method for routine analysis. [1-7]

Materials and Methods:

Plant material: Dried crude drugs of *Myrica esculenta (*Kaiphal*), Inula racemosa (*Pohakar*), Pistacia integerrima (*Karkatasrangi*), Trachyspermum ammi (*Ajwain*), Carum carvi (*Jira*), Zingiber officinale (*Adraka*), Piper nigrum (*Kalimirch*)* and *Piper longum (*Lindi Pippal*)* were purchased from local market of Ujjain (M.P.) 456010, INDIA. The sample of crude drug was also authenticated by Dept. of Botany, Govt. Madhav Science college, Ujjain (M.P.), India 456010.

Preparation of the formulation: Astangavaleha was prepared in laboratory, as per the method described in Ayurvedic Formulary of India. The composition of Astangavaleha with their botanical identities and parts used were given in table 1. The prescribed weight of all the raw materials were taken in the form of powder than boiled with ardrak svarasa & mixed together well than madhu was added when the preparation was cool & mixed well. Three laboratory formulation batch of Astangavaleha were prepared using above mentioned methods and were named as AS-I, AS-II & AS-III. One Marketed formulations named ASU-I was purchased from local pharmacy store Ujjain. These formulation samples were stored at identical conditions of temperature, light and moisture. [1]

Method development of UV [4 -11]:

Chemicals: All the chemicals and solvents were used of A.R. Grade. Standard gallic acid was procured from Himedia laboratories Pvt. Limited Bombay, INDIA

Instrument: Astangavaleha was estimated for their gallic acid contents against standard gallic acid solution on UV-Visible Spectrophotometer (Shimadzu, UV-1700, Pharmaspec).

Preparation of standard solution of gallic acid: An accurately weighed gallic acid (100 mg) was dissolved in methanol and volume was made up to 100 ml with methanol in volumetric flask. Two ml of this solution was diluted with methanol up to 100 ml in volumetric flask to give 20 µg/ml gallic acid solution.

Preparation of sample of Astangavaleha & selected crude drugs: The sample of selected components of AS (*Myrica esculenta, Pistacia integerrima* & *Trachyspermum ammi*), prepared laboratory batches (AS-I, AS-II & AS-III) & marketed preparation (ASU-I) were prepared separately by

weigh sample accurately 100mg and extracted in methanol by heating and make up to 100 ml volume with methanol. The prepared samples separately were used for UV analysis.

Table 1 Composition of Astangavaleha

S. No	Sanskrit Name	Hindi / comman name	Botanical Name	Family	#Part Used	Qt.
1	Katphala	Kaiphal	*Myrica esculenta*	Myricaceae	Fr.	1 part
2	Pauskara	Pohakar	*Inula racemosa*	Asteraceae	Rt	1 part
3	Srngi	Karkatasrangi	*Pistacia integerrima*	Anacardiaceae	Gi	1 part
4	Yamani	Ajwain	*Trachyspermum ammi*	Umbelliferae	Fr.	1 part
5	Karavi	Jira	*Carum carvi*	Umbelliferae	Fr.	1 part
6	Sunthi	Adraka	*Zingiber officinale*	*Zingiberaceae*	Rz.	1 part
7	Marica	Kalimirch	*Piper nigrum*	Piperaceae	Fr.	1 part
8	Pippali	Lindi Pippal	*Piper longum*	Piperaceae	Fr.	1 part
9	Madhu	Sahad	-	-	-	Q. S.
10	Ardrak Svarasa	Adraka	*Zingiber officinale*	Zingiberaceae	Rz.	Q. S.

Fr.- fruit; Rt.-root; Rz.- rhizome; Gi.- Gall;

Calibration curve of gallic acid: A series of calibrated 10 ml volumetric flask were taken and appropriate aliquots of the working standard solution of gallic acid were withdrawn and diluted up to 10 ml with methanol. The absorbance was measured at absorption maxima 270 nm, against the reagent blank prepared in similar manner without the gallic 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 concentrations (X-axis) and absorbance (Y-axis) were graphically presented and 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. The results were shown in Figure 1.

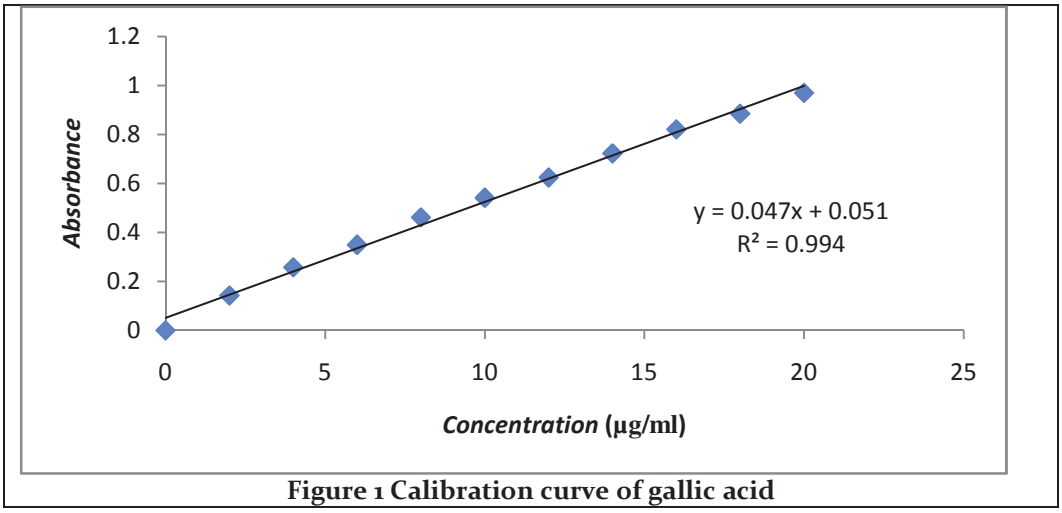

Figure 1 Calibration curve of gallic acid

Method validation: The method was validated for precision, accuracy, limit of detection (LOD), limit of quantification (LOQ). The results were illustrated in table 3.

Precision and accuracy: The method was validated for precision and accuracy, by performing the recovery studies at three levels by adding known amount of gallic acid extract of Astangavaleha, of which the gallic acid content have been estimated previously. The data were obtained and recovery was calculated.

Limit of detection (LOD) and limit of quantification (LOQ): In order to estimate the limit of detection (LOD) and limit of quantification (LOQ),

blank methanol was spotted six times. The signal to noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting known concentrations of gallic acid until the average responses were approximately three or ten times the standard deviation of the responses for six replicate determinations.

Table 2 Recovery study

S. no.	Amount of gallic acid (µg/ml)			RSD%	SE	Recovery%
	Sample	Added	Estimated			
1	100	50	148.62±0.293	0.197	0.120	99.08
2	100	100	198.20±0.482	0.243	0.197	99.10
3	100	150	247.92±0.651	0.263	0.266	99.16
Mean				**0.234**	**0.194**	**99.11**

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

Table 3 Validation parameters of gallic acid

S. No	Parameters	Observations
1	Absorption maxima	270 nm
2	Beer's law limit (µg/ml)	0-20
3	Correlation coefficient (r^2)	0.994
4	Regression equation (y) Slope (a)Intercept (b)	y=0.047x+0.051 0.047 0.051
5	LOD(µg/ml)	0.536
6	LOQ (µg/ml)	1.769
7	Precision (% R.S.D.) (n=6) Repeatability Intraday precision Interday precision	0.473 0.586 1.144
8	Recovery Studies Accuracy(%RSD) SE Recovery%	0.234 0.194 99.11

Table 4 UV estimation of gallic acid

S. No	Crude drugs & formulations		Gallic acid Content (%w/w)	Standard error
1	*Myrica esculenta*		3.272 ± 0.473	0.193
2	*Pistacia integerrima*		2.784 ± 0.691	0.282
3	*Trachyspermum ammi*		1.102 ± 0.214	0.087
4	Astangavaleha	AS-I	0.125 ± 0.365	0.149
		AS –II	0.130 ± 0.412	0.168
		AS–III	0.132 ± 0.357	0.146
		ASU-I	0.124 ± 0.564	0.230

(Mean ± SD of 6 determinations)

Results And Discussion: In order to obtain precision and accuracy, the recovery study was performed at three levels by adding known amount of gallic acid with pre -analyzed sample of gallic acid in Astangavaleha. The experiment was repeated six times at both level and result shows 99.08 %, 99.10 % and 99.16 % recovery of

gallic acid at all the level with mean value 99.11 % which prove reproducibility of the result. The %relative standard deviation (% RSD) value was found to be 0.197, 0.243 and 0.263 with mean 0.234 at all the level while the standard error was 0.120, 0.197 and 0.266 with mean 0.194 respectively. From the data it was observed that the present method of spectrophotometric determination of gallic acid was simple, precise, accurate and suitable for routine analysis of gallic acid in Astangavaleha (Table 2 & 3).

Estimation of gallic acid in Astangavaleha & crude drugs: The appropriate aliquots from gallic acid extract of each batch of Astangavaleha (AS-I, AS-II and AS-III), marketed formulations (ASU-I) and selected crude drugs *Myrica esculenta, Pistacia integerrima* & *Trachyspermum ammi* separately were withdrawn in 10 ml volumetric flask. Absorbance for aliquots of each was noted at 270 nm. The corresponding concentration of gallic acid against respective absorbance value was determined using the gallic acid calibration curve. The concentration of gallic acid present in crude drugs was found to be 3.272 ± 0.473 % w/w in *Myrica esculenta*, 2.784 ± 0.691 % w/w in *Pistacia integerrima* and 1.102± 0.214 % w/w in *Curcuma zedoria* and in three identical laboratory batches of Astangavaleha (AS-I, AS-II & AS-III) and one marketed preparations (ASU-1) 0.125± 0.365 % w/w, 0.130± 0.412 % w/w, 0.132± 0.357 % w/w and 0.124± 0.564 % w/w were found to be respectively (Table 4).

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