Asian Journal of Chemistry Vol. 21, No. 9 (2009), 7111-7116

Simulataneous Quantitation of Gallic Acid from Fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz.

AMBIKA SHARMA and SUNITA SHAILAJAN*
Herbal Researh Laboratory, Ramnarain Ruia College, Matunga (East), Mumbai-400 019, India
E-mail: sunitashailajan@yahoo.co.in

Fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. are commonly used as herbal raw materials in many Ayurvedic and herbal formulations. These fruits are either used individually or in combination known as 'Triphala'. Fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. have been reported to contain gallic acid. Gallic acid is a widely occurring phenolic compound of plant origin. Gallic acid is selected as a bioactive marker due to its easy availability, common presence in these fruits and as antiobesity property. Recently, the concept of marker-based standardization of herbal drugs is gaining momentum. A simple, sensitive and reliable high performance thin layer chromatographic method has been established for simultaneous quantification of gallic acid from fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. Gallic acid separation was achieved from *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. of different geographical regions of India and Nepal in a common mobile phase toluene:ethyl acetate:formic acid, 2:7:1 (v/v). After development, detection and quantitation of plates were performed by densitometry at 275 nm. The response to gallic acid in all the three fruits extracts was a linear function of concentration over the range 20 to 100 μg mL^{-1}. There was significant variation in gallic acid content of fruit collected from different regions. Gallic acid was maximum in *Phyllanthus emblica* Linn. collected from Dehradun (0.721 %), in *Terminalia bellirica* (Gaertn.) Roxb. collected from Maharastra (Karjat) (0.561 %) and *Terminalia chebula* Retz. collected from Madhya Pradesh (0.905 %).

Key Words: *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb., *Terminalia chebula* Retz., Gallic acid, antiobesity, HPTLC.

INTRODUCTION

Fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. the three widely used plant drugs in various Ayurvedic and herbal formulations. Triphala churanam is an herbal formulation prepared from equal parts of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. used extensively in Ayurveda and Siddha system of Indian

traditional medicine. Since it contains enormous amount of tannins such as ellagic acid and gallic acid, it is extensively used as an astringent. In Siddha system of medicines it is used for treating wounds and local ulcers[1]. Because of such wide use of these plants it is necessary to analyse the raw material for quality control before they can be added in the formulations. Fruits of *Phyllanthus emblica* Linn. commonly known as Amla has been reported to contain gallic acid, phyllemblic acid, lipids and emblicol[2], lupeol, tannins, polyphenolic compounds; 1,2,3,6-trigalloylglucose, terchubin, alkaloids, phyllatidine and phyllatine[3]. Fruits of *Terminalia bellirica* (Gaertn.) Roxb. commonly known as Baheda contain 20 to 30 % tannins; gallic acid, ellagic acid, chebulagic acid, bellaricanin, phyllemblin; termilignan, thaninlignan, 7-hydroxyl-3',4'-(methylenedioxy)flavan and anolignan B[4], β-sitosterol[5]. Fruits of *Terminalia chebula* Retz. commonly called as Harad has been reported to contain chemical constituents such as gallic acid, chebulic acid, ellagic acid, a tannin terchebin, syringic acid, an ellagitannin terchebulin[6]. In the present research work gallic acid was selected as a marker standard due to its common presence in *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz., antiobesity property[7] and easy availability.

Thus, established HPTLC method was successfully used to compare and evaluate the gallic acid (Fig. 1) content from the fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. collected from different parts of India like Dehradun, Madhya Pradesh, Maharastra (Karjat and Malvan) and from Nepal[8]. Nepal being the neighbouring country and rich in biodiversity like India, herbal raw material is also imported from there to be used in herbal formulations. The method was validated for linearity, precision, accuracy, robustness and can be used for routine identification of these fruit powders to be used in the herbal formulations.

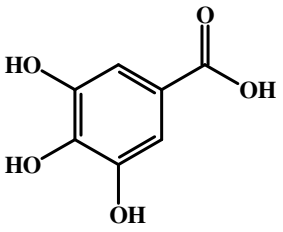

Fig. 1. Structure of gallic acid (GA)

EXPERIMENTAL

Fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. were collected from different regions like Dehradun (Uttarakhand), Madhya Pradesh, Maharastra (Karjat and Malvan) and Nepal. The fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. were collected, washed to remove the adhered contaminants and dried. These dried fruits were then individually packed in self-sealing bags. A

card with details of the plant like common name, botanical name, place of collection, name of the collector and the characteristics of the plant is attached to the sample. Standard gallic acid (99 % purity) was procured from Sigma-Aldrich Chemie Gmbh (Aldrich Division, Steinheim, Fedreal Republic of Germany). The solvents toluene, ethyl acetate and glacial acetic acid were of analytical grade and were purchased from Qualigens Fine Chemicals, Mumbai, India was used in the analysis.

A TLC scanner with computer system and Cats 3 Version Software were obtained from Camag (Muttenz, Switzerland). The source of radiation was mercury lamp. Camag Linomat IV was used as applicator. Separation was done[9] on silica gel F254 HPTLC precoated plate procured from Merck (Darmstaldt, Germany).

Standard and sample preparation: A stock solution of gallic acid (1000 μg mL^{-1}) was prepared by dissolving 25.0 mg of accurately weighed gallic acid in methanol and diluting to 25.0 mL with methanol. Since gallic acid is light sensitive[8] preparation of standard was carried out in dark. Aliquots (0.2 mL to 1.0 mL) of this stock solution were transferred to 10 mL standard volumetric flasks and the volume of each was adjusted to 10 mL with methanol, to obtain working standard solutions containing 20 to 100 μg mL^{-1}.

Fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. collected from 5 different regions *i.e.* Dehradun, Madhya Pradesh, Maharastra (Karjat and Malvan) and Nepal was individually washed, shade dried, deseeded, powdered, sieved through an 80-mesh (BSS) sieve and stored in an airtight container at 25 °C. 100 mg of the dried fruit powder was accurately weighed and placed in a stoppered tube and 10 mL of methanol was added to each stoppered tubes, the sample was vortexed for 1-2 min and left to stand overnight at room temperature (28 ± 2 °C). The contents of the tube were filtered through Whatmann No. 41 paper (E. Merck, Mumbai, India) and the filtrate was used for experimental work.

Chromatography was performed on silica gel F254 HPTLC pre-coated plate. Samples (10 μL) were applied on the plates as band of 7 mm width with the help of a Camag Linomat IV sample applicator at the distance of 14 mm from the edge of the plates. The mobile phase constituted of toluene:ethyl acetate:formic acid, 2:7:1 (v/v). The plates were developed to a distance of 90 mm in a Camag twin-trough chamber previously equilibrated with mobile phase for 0.5 h. The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. After development of plates, densitometric evaluation of the plates was performed at 275 nm (maximum absorbance) in UV absorbance mode using deuterium lamp with a Camag Scanner II in conjunction with Cats 3 Version software.

Linearity of detector response[10,11]: Each standard solution (10 μL, for gallic acid corresponding to 20, 30, 40, 50, 60, 70, 80, 90, 100 μg mL^{-1}) were prepared in methanol. Each of these solutions (10 μL) was applied to a plate, the plates were developed and the detector response for the different concentrations was measured. A graph was plotted of drug peak area against concentration of gallic acid. The plot

of gallic acid was linear in the range 20 to 100 μg mL^{-1}. The experiment was performed three times and the mean was used for the calculations. The linearity data is given in Table-1.

TABLE-1
LINEARITY DATA

	Gallic acid (GA)
Linearity range (μg mL^{-1})	20 to 100
Slope (m)	27.242
Intercept (c)	6.77
Correlation coefficient (R)	0.9991
LOD (μg mL^{-1})	2
LOQ (μg mL^{-1})	4
Instrument precision RSD % (n = 5)	0.11
Intra-day precision RSD % (n = 3)	0.11
Inter-day precision RSD % (n = 3)	0.09

(y = mx + c, where, y = peak area; m = slope; x = concentration; c = intercept).

Assay procedure: The solution of gallic acid (50 μg mL^{-1}) and 10 μL of plant extract *i.e. Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. of different regions were spotted on HPTLC plate. The amount of gallic acid present in the fruits extract was calculated by comparison of area measured for the sample to that for the standard respectively. The assay procedure described earlier was repeated three times. The results of assay are given on Table-2. The mean assay values of gallic acid in *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. is shown in Tables 2-4, respectively.

RESULTS AND DISCUSSION

Gallic acid standard was detected and quantitated in an accurate manner using silica gel F254 HPTLC pre-coated plates with the mobile phase made of toluene:ethyl acetate:formic acid, 2:7:1 (v/v). The identity of band of gallic acid in the standard in the fruit extracts was confirmed by overlaying the chromatogram obtained from the standards gallic acid and by comparing their R_f (0.54).

The linearity range of gallic acid was observed over a concentration of 20 to 100 μg mL^{-1} with correlation coefficient of 0.999. The concentration of gallic acid in fruits of *Phyllanthus emblica* Linn. was found to be maximum (0.721 μg mg^{-1}) in Dehradun region. where as *Terminalia bellirica* (Gaertn.) Roxb of Maharastra (Karjat) (0.561 μg mg^{-1}) and *Terminalia chebula* Retz. Madhya Pradesh. showed maximum gallic acid content.

Instrument precision, intraday precision, interday precision were measured to evaluate the precision of the method. The % RSD values were found to be less than 2 % indicating that the selected method is precise and reproducible.

TABLE-2
RESULTS OF ASSAY OF GALLIC ACID IN *Phyllanthus emblica* Linn.

Samples of *Phyllanthus emblica* Linn. of different regions	Weight of sample (mg)	Amount of gallic acid in sample (μg mg^{-1})	Average (%) content of *Phyllanthus emblica* Linn.	RSD (%) (n = 3)
Dehradun (Uttarakhand)	100	0.721	0.721	0.08
Madhya Pradesh (Hoshangabad)	100	0.41	0.410	0.14
Karjat (Maharashtra)	100	0.362	0.362	0.15
Malvan (Maharashtra)	100	0.403	0.403	0.37
Nepal	100	0.257	0.258	0.44

TABLE-3
RESULTS OF ASSAY OF GALLIC ACID IN *Terminalia bellirica* (Gaertn.) Roxb.

Samples of *Terminalia bellirica* (Gaertn.) Roxb. of different regions	Weight of sample (mg)	Amount of gallic acid in sample (μg mg^{-1})	Average (%) content of *Terminalia bellirica* (Gaertn.) Roxb.	RSD (%) (n = 3)
Dehradun (Uttarakhand)	100	0.262	0.262	0.76
Madhya Pradesh (Hoshangabad)	100	0.382	0.382	0.30
Karjat (Maharashtra)	100	0.561	0.561	0.27
Malvan (Maharashtra)	100	0.473	0.473	2.68
Nepal	100	0.104	0.104	0.55

TABLE-4
RESULTS OF ASSAY OF GALLIC ACID IN *Terminalia chebula* Retz.

Samples of *Terminalia chebula* Retz. of different regions	Weight of sample (mg)	Amount of gallic acid in sample (μg mg^{-1})	Average (%) content of *Terminalia chebula* Retz.	RSD (%) (n = 3)
Dehradun (Uttarakhand)	100	0.684	0.684	0.16
Madhya Pradesh (Hoshangabad)	100	0.905	0.905	0.22
Karjat (Maharashtra)	100	0.300	0.300	0.76
Malvan (Maharashtra)	100	0.113	0.113	1.54
Nepal	100	0.247	0.247	1.65

The robustness of the method was studied, during method development, by determining the effects of small variation, of mobile phase composition (± 2 %), chamber saturation period, development distance and scanning time (10 % variation of each). No significant change of R_f response to gallic acid was observed, indicating the robustness of the method.

Conclusion

One of the best methods of standardizing herbs and herbal formulations based on the modern scientific tools is chromatography. The developed HPTLC method helps in identification of plant material with respest to gallic acid content. Fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia*

chebula Retz. collected from different geographical regions show significant variation in gallic acid content. Therefore, the fruit materials of *Phyllanthus emblica* Linn. from Dehradun, *Terminalia bellirica* (Gaertn.) Roxb. from Maharastra and *Terminalia chebula* Retz. from Madhya Pradesh may be recommended to be used in anti-obesity formulation as gallic acid plays an important role in anti-obesity. The work needs to be further consolidated by using larger samples covering all agroclimatic zones of India.

REFERENCES

1. V.P. Kamboj, *Curr. Sci.*, **78**, (2000).
2. C.K. Kokate, A.P. Purohit and S.B. Gohale, Drugs Containing Tannins in Text Book of Pharmacognosy, Published by Nirali Prakshan, Pune, India, Ch. 9, edn. 5, pp. 318-323 (1997).
3. P.C. Sharma, M.B. Yelna and T.J. Dennis, Database on Medicinal Plants Used in Ayurveda, Central Council for Research in Ayurveda and Siddha, New Delhi, Vol. 3, pp. 11-16, 158-162, 282-284 (2001).
4. J.B. Harborne, Phytochemical Methods-A Guide to Modern Technique of Plant Analysis, Chapman and Hall London UK, Third Indian Reprint (2007).
5. Quality Standards of Indian Medicinal Plants, Indian Council of Medicinal Research, New Delhi, India, Vol. 1, p. 199 (2005).
6. Quality Standards of Indian Medicinal Plants, Indian Council of Medicinal Research, New Delhi, India, Vol. 1, p. 207 (2005).
7. C.-L. Hsu and G.-C. Yen, *Br. J. Nutr.*, **98**, 727 (2007).
8. E. Stahl, Thin Layer Chromatography-A Laboratory Handbook, CBS, India (1969).
9. J. Sherma and B. Fired, Handbook of Thin Layer Chromatography, Chromatographic Science Series, Marcel Dekkar, New York, ISBN 0-8493-2660-J (1995).
10. ICH, Q2A, Validation of Analytical Procedure: Methodology. In: Proc. Int. Con. Harmonization, Geneva (1994).
11. ICH, Q2A, Validation of Analytical Procedure: Methodology. In: Proc. Int. Con. Harmonization, Geneva (1996).

(*Received*: 24 December 2008; *Accepted*: 10 August 2009) AJC-7738