

# Qualitative Analysis of Gallic Acid by HPLC Method In Different Extracts of *Terminalia Bellerica Roxb.* Fruit

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**Qualitative Analysis of Gallic Acid by HPLC Method In Different Extracts of *Terminalia Bellerica Roxb.* Fruit**

## SUMMARY

The aim of this work is to evaluate the qualitative HPLC-analysis of Gallic acid in chloroform, methanolic and ethanolic extracts of *Terminalia bellerica* plant fruit. The chromatographic conditions used for the qualitatively examination of Gallic acid in chloroform, methanolic and ethanolic plant fruit extracts were performed on a Shimadzu LC-20AT, SPD-20A HPLC instrument. The autosampler injector and Spinchrom LC Solution software, Phenomenex Luna C18 (4.6 x 250mm, 5µm particle size) was used as stationary phase. Rheodyne manual injector with 20µL capacity loop and mobile phase water: Acetonitrile (80: 20 % v/v) and pH- 3.00 by O-phosphoric acid was chosen with detection wavelength 272 nm. The injection volume 20 µL was loaded for qualitative analysis of standard and different extracts of plant samples. The peak areas corresponding to different samples extracts integrated by comparison with standard of Gallic acid peak area. The HPLC analysis resulted in a sharp peak of Gallic acid standard at the retention time of 5.343min and authentic sharp peaks of ethanolic extract and methanolic extracts at the retention time of 5.346 min and 5.093 min respectively. But chloroform extract of fruit plant has been absent for Gallic acid peaks.

**Key Words:** Gallic acid, terminalia bellerica, HPLC, qualitatively analysis, polyphenol, extract

***Terminalia Bellerica Roxb.* Meyvalarının Farklı Ekstrelerdeki Gallik Asidin HPLC Yöntemi ile Kalitatif Analizleri\***

## ÖZ

Bu çalışmanın amacı, *Terminalia bellerica* bitkisinin meyvelerinin metanolik ve kloroform ekstraktlarındaki gallik asidin kalitatif HPLC analizini değerlendirmektir. Etanolik, metanolik ve kloroform bitki meyve ekstraktlarında gallik asidin kalitatif olarak incelenmesi için bir otomatik örnekleyici enjektörü ve Spinchrom LC Çözelti yazılımı ve 20µL kapasiteli looplü Rheodyne manuel enjektör ile donatılmış bir Shimadzu LC-20AT, SPD-20A HPLC aletinde gerçekleştirilmiştir. Sabit faz olarak Phenomenex Luna C18 (4 x 250mm, 5µ partikül büyüklüğü) ve hareketli faz olarak O-fosforik asit ile pH-3.00 değerine ayarlanmış su : asetonitril (% 80: 20 v / v) çözeltisi kullanılmıştır ve algılama dalga boyu 272 nm olarak seçilmiştir. Standart ve farklı bitki numunelerinin ekstraktlarının kalitatif analizinde için enjeksiyon hacmi olarak 20 µL kullanılmıştır. Farklı örneklerle karşılık gelen pik alanları, standart kalibrasyon eğrileri ile karşılaştırılarak entegre edilmiştir. HPLC analiziyle, standart gallik asidin 5.343 dakikadaki alıkonmazamanında keskin bir pik şeklinde ve etanolik ve metanolik ekstraktlarda sırasıyla 5.346 dak ve 5.093 dakikalarda gallik asidin otantik keskin piklerinin gözlenmesi ile sonuçlanmıştır. Ancak, bitki meyvesinin kloroform ekstresinde, gallik asit pikleri mevcut değildir.

**Anahtar Kelimeler:** Gallik asit, terminalia bellerica, HPLC, niteliksel analiz, polifenol, ekstre

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## INTRODUCTION

Gallic acid (3, 4, 5-trihydroxybenzoic acid) (Figure 1) is a naturally occurring polyphenolic compound found in processed beverages such as red wines and green teas. It occurs in plants in the form of free acids, esters, catechin derivatives and hydrolysable tannins (Tang et al., 2003 a; Tang et al., 2003 b). The interest in these compounds is due to its pharmacological activity as radical scavengers. It has been proved to have potential preventive and therapeutic effects in many diseases, where the oxidative stress has been implicated, including cardiovascular diseases, cancer, neurodegenerative disorders and in aging (Karamaa et al., 2005; Kaur et al., 2005; Nikolic 2006). Gallic acid has been reported to occur in a number of plants, some of them are *Allan blackia floribunda*, *Garcinia densivenia*, *Bridelia micrantha*, *Caesalpinia sappan*, *Dillenia indica*, *Diospyros cinnabarina*, *Paratecoma peroba*, *Terminalia bellerica*, etc. Many Gallic acid derivatives occur naturally in plant, these include from *Frankenia laevis* and *Tamarix amplexicaulis*, 3-methyl-4-O-[3,4-dihydroxy-5-methoxybenzoyl-6-β-D-glucopyranoside], *Rhus glabra*, 3-ethyl ether from *Phyllanthus emblica*, and 4-ethyl ether from *Eucalyptus gunnii*, *Terminalia chebula* and *Elephantorrhiza elephantina* (Shahriar et al., 2010).

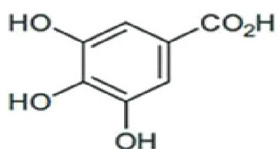


Figure 1. Structure of Gallic acid

*Terminalia bellerica* Roxb. (Combretaceae) is one of the ingredients of Ayurvedic purgative medicament of 'Triphala' available in the Indian market for the treatment of dyspepsia, diarrhea, and dysentery, inflammation of the small intestine biliousness, flatulence, liver disease and leprosy (Sharma et al., 2005). Chemically, the presence of β-sitosterol, Gallic acid, ellagic acid, galactose, ethyl gallate, chebulagic acid, mannitol, glucose, galactose, fructose and rhamnose in the fruit of *Terminalia bellerica* have also been reported (Row et al., 1970). The aim of this work is to evaluate the qualitative HPLC-analysis of Gallic acid in chloroform, methanolic and ethanolic extracts of *Terminalia bellerica* plant fruit and only the qualitative examination of crude extracts of fruit sample in Gallic acid presence or not. Because the most of the studies have been shown to it responsible to therapeutic activity of plant.

## MATERIALS AND METHODS

### Collection and authentication of *Terminalia bellerica*

The fruits of *Terminalia bellerica* were purchased from local market of Mathura. The plant fruit was authenticated by the Dr. Sunita Garg (Emeritus Scientist Raw Materials Herbarium & Museum), NISCAIR, New Delhi. Voucher specimen No. NISCAIR/RHMD/Consult/2018/3138-87, Dated 16/01/2018 was preserved for further references. The fruits washed 2-3 times from distilled water then dried in shade and grinded into fine powder, stored in closed container separately with proper labeling for further use.

### Reagents

Gallic acid was procured from Sigma Chemicals (St Louis, Mo, USA). All other chemicals used were of analytical grade and were purchased from S.D. Fine Chemicals Ltd, Mumbai, India.

### Successive solvent extraction and preparation of sample

Extract about 25 g of the air dried powdered plant material successively with the following sequence of solvent in a soxhlet assembly

(Chloroform → Methanol → Ethanol).

In every time before extracting with the next solvent, the marc of drug dried in oven below 50 °C. The solvent was expelled under reduced pressure below 45 °C to get the dried extract. Pure and dried all three extracts of the plant fruit thus prepared were stored at 4-5 °C until used. Further completion of extract procedure, they have been used for HPLC analysis (Anis 1994).

The each extracts were re-dissolved in 50 ml of methanol and passed through a Samprep RP<sub>18</sub> column, previously conditioned with 5 ml of methanol and 5 ml of water. The loaded cartridge was eluted with 4.5 ml of 30% aqueous methanol to remove the phenolics.

### Conditions of HPLC analysis

The Gallic acid was analyzed qualitatively by HPLC method. The suspensions from different extraction methods were filtered through filter paper followed by centrifugation at 14,000×g (Eppendorff, Switzerland) for 10 min and aliquots of supernatant were transferred to glass vials. The chromatography was performed on a Shimadzu LC-20AT, SPD-20A HPLC instrument equipped with an autosampler injector and Spinchrom LC Solution software. Phenomenex Luna C18 (4.6 x 250mm, 5µm particle size) was used as stationary phase. Rheodyne manual injector with 20µL capacity loop was used. Based

on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard and sample solutions were injected into column using different mobile phases. From the various mobile phases, Water: Acetonitrile (80: 20 %v/v) and pH- 3.00 by O-phosphoric acid was chosen with detection wavelength 272 nm. The injection volume 20 µL was loaded for qualitative analysis of standard and different extracts of plant fruit samples.

The peak areas corresponding to different samples extracts integrated by comparison with standards. The analytical operation can be completed in 10 min (Fulzele et al., 2005 a; Fulzele et al., 2005 b; kiran et al., 2012).

**Preparation of standard stock solution:**

An accurately weighed quantity of Gallic acid (10 mg) was transferred to 10 ml volumetric flask, dissolved and divided to the mark with Water: Methanol (9: 1 %v/v) to obtain standard stock solution of 1000µg/ ml.

**Preparation of Calibration curve**

A calibration curve established with six dilutions of standard, at concentrations ranging from 10 to 100 µg/ml. Aliquots of (0.1, 0.2, 0.4, 0.6, 0.8 and 10 ml) standard stock solution (1000 µg/ml) was transferred to 10 ml of volumetric flask and made up to the mark with Water: Methanol (9: 1 %v/v) to get concentration of (10, 20, 40, 60, 80 and 100 µg/ml). Each concentration was measured in triplicate.

**Method validation for HPLC**

For qualitative purposes, the method was evaluated by taking into account the precision in the

retention time and selectivity of marker compound eluted. A high repeatability in the retention time was obtained both for standards and extracts even at high concentration. For quantitative purpose LOD (Limit of Detection) and LOQ (Limit of Quantification) were evaluated. LOD and LOQ were calculated by using equations,

$LOD = 3.3x \sigma / Slope$  and  $LOQ = 10x \sigma / Slope$ , (where  $\sigma$  = Standard deviation; Slope= Slope of the calibration curve). The calibration curve was drawn for concentration v/s AUC for Gallic acid (Figure 2).

**Statistical analysis**

Statistical calculations were carried out with the Microsoft Excel 2007 for Window Software package.

**RESULTS AND DISCUSSION**

The qualitative HPLC assay for chromatogram for ethanolic, methanolic and chloroform extracts of *Terminalia bellerica* fruit were examined. The peaks, retention time (min), area (mAU\*s), percentage area, suitable baseline and heights (mAU) were documented in (Table 1). The HPLC analysis resulted in a peak of Gallic acid standard at the retention time of **5.343 min** shown in (Figure 3) and authentic sharp peaks of ethanolic extract and methanolic extracts at the retention time of **5.346 min** and **5.093 min** respectively shown in (Figure 4 and 5). The chloroform extract at the retention time of **1.386** peaks has been not shown any standard peak of Gallic acid in (Figure 6). The LOD and LOQ values were found to be 0.65 µg/ml and 2.5 µg/ml respectively (Table 2). The HPLC studies confirmed the Gallic acid active phytoconstituent present in the ethanolic and methanolic fruit extract of samples.

**Table 1.** HPLC analysis of standard Gallic acid and different extracts of *Terminalia bellerica* fruit

S.No	Parameters	Gallic Acid (Standard)	Ethanolic Extract	Methanolic Extract	Chloroform Extract
1	Retention Time (min.)	5.343	5.346	5.093	1.386
2	Area (mAU*s)	7573.30	486.98	229.49	219.27
3	Height (mAU)	355.68	18.36	2.99	23.98
4	Area %	100	69.92	12.72	39.38

**Table 2.** Limit of Detection (LOD) and Limit of Quantification (LOQ) of the proposed HPLC method

Compound	Conc. (µg/ml)	LOD	LOQ	Regression equation	R <sup>2</sup>
Gallic acid	10-100	0.65	2.5	Y= 27132x+14337	0.997

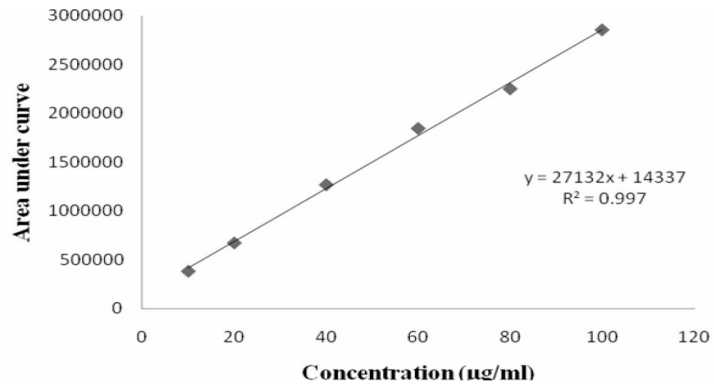


Figure 2. Calibration curve for Gallic acid by HPLC

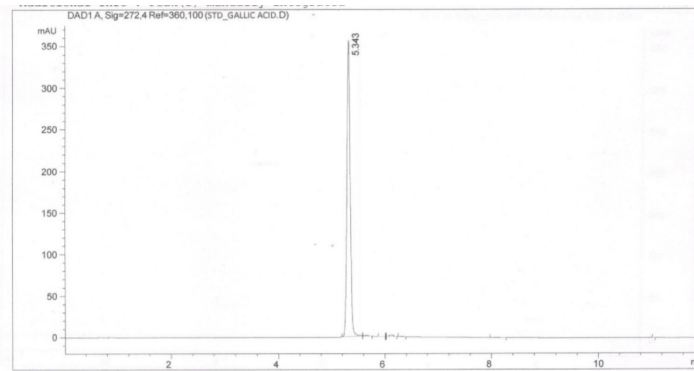


Figure 3. HPLC Chromatogram of standard Gallic acid

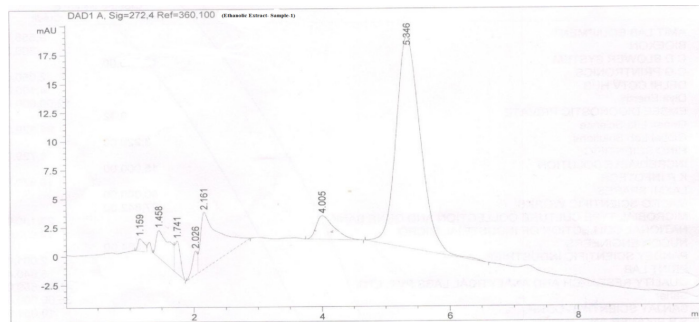


Figure 4. HPLC Chromatogram of ethanolic extract of *Terminalia bellerica* fruit

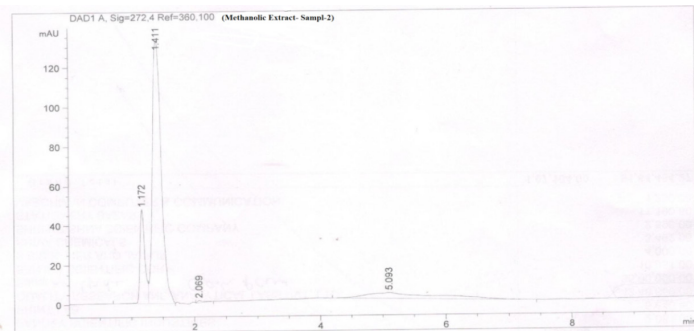
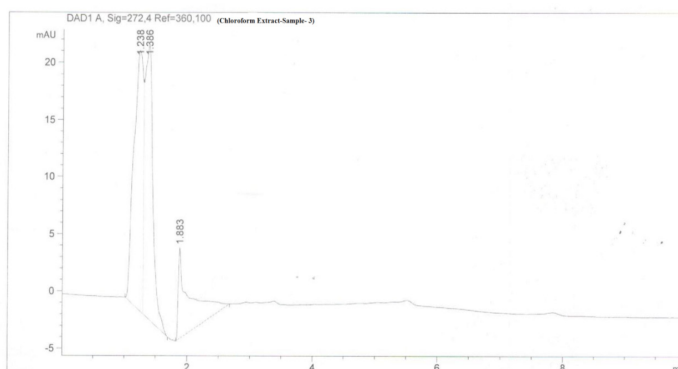


Figure 5. HPLC Chromatogram of methanolic extract of *Terminalia bellerica* fruit



**Figure 6.** HPLC Chromatogram of chloroform extract of *Terminalia bellerica* fruit

## CONCLUSION

The HPLC method estimation of Gallic acid is a simple, rapid and precise for the sample of plant fruit different extracts. As *Terminalia bellerica* fruit is a good source of Gallic acid (Row et al., 1970). After the chromatograms of three extracts were compared with standard Gallic acid HPLC chromatogram, the ethanolic extract was reported to show almost the same time as standard Gallic acid. Gallic acid was also found in methanolic extract when it was compared to standard chromatogram of Gallic acid but Gallic acid peak was not found in the chloroform extract of fruit plant. This study is beneficial for further research of Gallic acid because Gallic acid play a pivotal role in imparting medicinal properties of the plant and therefore it is considered as a promising lead molecule for new drug development. These findings can be used as routine chromatographic fingerprinting method for the standardization of the crude extract of plant fruit samples.

After present investigation it can be concluded that the HPLC study of fruit of *Terminalia bellerica* yielded a set of qualitative parameter or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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#### ABBREVIATIONS AND SYMBOLS USED

- HPLC- High Performance Liquid Chromatography
- v/v- Volume by volume
- μL- micro liter
- % - Percentage
- mg - milli gram
- °C - Degree Centigrade
- ml - milli liter
- mAU- milli absorption
- min - Minute
- LOD- Limit of Detection
- LOQ- Limit of Quantification