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Research Article

Estimation of Gallic Acid in the Seed Extracts of *Syzygium cumini*

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ABSTRACT

The aim of this work was to estimate the amount of gallic acid present in raw seed powder, and in the aqueous and alcoholic extracts of *Syzygium cumini* seed by using reverse phase High pressure liquid chromatography. The method was carried out on a Phenomenex kromosil C18 column (250 × 4.6 mm, 5µ) using mobile phase of 2ml orthophosphoric acid in 1000ml water and methanol with a gradient elution at the flow rate of 1 ml/min, with the detection wavelength at 272 nm. The linear regression analysis data for the calibration showed a good linear relationship with correlation co-efficient of 0.999. The developed method has been validated as per ICH guidelines and the method has the advantage of being both rapid and easy and hence it can be applied for routine quality control analysis of herbal preparations containing *Syzygium cumini* constituents.

Key-words: Syzygium cumini , Gallic acid, High Performance liquid chromatography (HPLC).

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INTRODUCTION

The *Syzygium cumini* (or *Eugenia jambolana*) tree belongs to the Myrtaceae family. Other common names of *Eugenia jambolana* are Jamun, Jambul, Jambol in India and Malaya. All parts of this tree are utilized for its medicinal properties. The extract of the barks, leaves and seeds have been reported to possess anti-bacterial [1] antiulcerogenic, [2] antimalarial, [3] and radioprotective [4] activities. High Pressure Liquid Chromatography (HPLC) currently represents the most popular and reliable technique for analysis of phenolic compounds. Various supports and mobile phases are available for the analysis of phenolics including anthocyanins, proanthocyanidins, hydrolysable tannins, flavonols, flavan-3-ols, flavanones, flavones, and phenolic acids in different plant extract and food samples [5]. The objective of the present investigation was to estimate the amount of gallic acid in aqueous and hydro-ethanolic extract of *Syzygium cumini* and in the raw seed powder using the fast and sensitive HPLC method.

MATERIAL AND METHODS

Chemicals

Reference compound Gallic acid was obtained from Sigma-Aldrich, Bangalore, India. Ortho Phosphoric acid, Ethanol and Methanol used were of analytical grade obtained from S D Fine chemicals, Mumbai India.

HPLC Analysis

Chromatography analysis for the quantification of the chemical marker gallic acid in *Syzygium cumini* seed kernel extracts was conducted on a Shimadzu®HPLC controlled by LC Software and with quarternary pumps (LC-10AD), an on-line degasser (DGU-14A), and an injection valve (Rheodyne) with a 20 µl loop. The equipment included a UV–Vis detector. The column used was a Phenomenex kromosil C18 (250 mm × 4.6 mm, 5µ). The chromatographic separation was carried out using a mobile phase with 2ml of ortho phosphoric acid in 1000ml of purified water as solvent A and methanol as solvent B at a flow rate of 1 ml/min and elute with the detection wavelength at 272 nm using gradient program explained in Table 1

Time(mins)	SOLVENT A (%)	SOLVENT B (%)
0.01	90	10
10	70	30
13	40	60
18	60	40
21	80	20
24	90	10
30	90	10

TABLE 1: GRADIENT PROGRAM

Standard preparation

Weighed accurately 25.2mg of gallic acid and transferred to 50ml volumetric flask. Added about 30ml of 50% ethanol and sonicated to dissolve the content and made up to mark with diluent to obtain a stock solution having concentration of 500mcg/ml. Pipetted out 5ml of this solution and made upto to 25ml with diluent and the concentration of 100mcg/ml was obtained and used for analysis.

Sample preparation

Preparation of plant extract

The *Syzygium cumini* fruits were first washed well with water and the pulp removed from the seeds. The outer membrane of the seeds was removed and the inner kernel were taken and dried under shade. Then the kernel was powdered and stored in airtight container. 100gm of the powder was taken for extraction along with 500ml of distilled water to prepare the aqueous extract and preparation of hydroalcoholic extract was done with ethanol and water in the ratio 70:30 v/v. The extraction was carried out by using Soxhlet's apparatus for 48 hours. The aqueous and hydroethanolic extract yields were obtained as 18.3%w/v and 9.1%w/v respectively.

The raw seed powder of *Syzygium cumini* (500.1mg) was dissolved in 100ml of diluent. 10ml of the resulting solution was taken and made up to 25ml by adding more diluent. The Ethanol extract (126.7mg) was taken and dissolved in 100ml of diluent while the aqueous extract (134.2mg) was dissolved in 100ml of the diluent, filtered and the filtrates were used for the studies. The above three sample solutions were injected respectively under the chromatographic conditions and the scans were recorded as shown in the figure 2-5. The amount of drug present in various extracts was calculated by comparing the mean peak ratio from the standard.

RESULTS AND DISCUSSION

The optimization was achieved by using Phenomenex kromosil C18 (250 mm × 4.6 mm, 5µ) column using 2ml of ortho phosphoric acid in 1000ml of purified water as solvent A and methanol as solvent B. A gradient program of 1ml/min with the detection wavelength at 272 nm with 20µl injection volume. A calibration curve was made and concentration examined within the detection range 25-150µg/ml and correlation values was found to be 0.999 for gallic acid. The precision was determined by repeated analysis and the values are presented in Table 2 and the recovery experiment was performed by spiking method and the values are presented in Table 2.The linearity graph of gallic acid obtained is given in the Figure.1.The amount of gallic acid present in the *S.cumini* seed extracts was found to be 5.85mg/gm, 20.66mg/gm and 48.73mg/gm in the raw seed powder, aqueous extract and hydroethanolic extract respectively and the chromatograms for standard and for extracts depicted in the figure 2-5 and the values are presented in Table 3.

Fig 1: LINEARITY OF GALLIC ACID BY HPLC METHOD

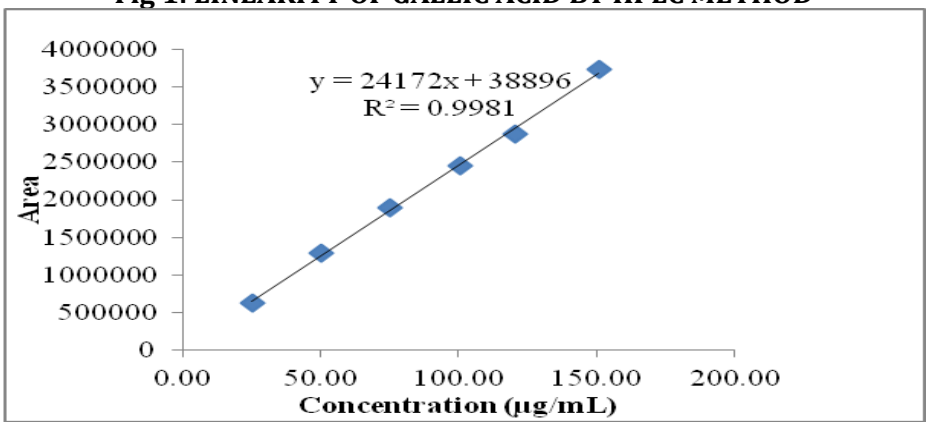


TABLE 2: OPTICAL CHARACTERISTICS OF GALLIC ACID

S.NO	PARAMETERS	RESULTS
1.	Linearity range	25-150µg/ml
2.	Correlation Co-efficient(r)	0.999
3.	Slope	24171.77
4.	Intercept	38896.24
7.	Tailing factor	1.3
8.	Retention Time	5.8
9.	Theoretical Plates	15354.165
10.	%R.S.D	0.564
11.	% Accuracy	99.84

TABLE 3: AMOUNT OF GALLIC ACID PRESENT IN VARIOUS EXTRACTS OF *Syzygium cumini*

S.No	Sample taken	RetentionTime	Mean peak area	Amount of Gallic acid (mg/gm)
1.	Raw seed powder	5.885	674317	5.848
2.	Aqueous extract	5.887	1597993	20.66
3.	Ethanol extract	5.862	3559107	48.73
4.	Standard	5.903	5723025	-

Fig: 2 CHROMATOGRAM OF *Syzygium cumini* RAW SEED POWDER

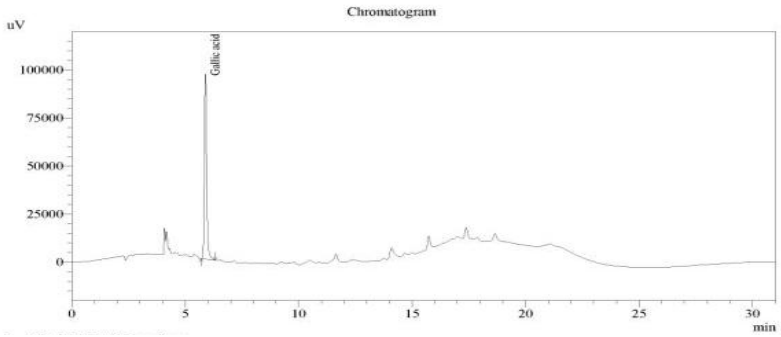


Fig: 3 CHROMATOGRAM OF *Syzygium cumini* SEED EXTRACT (Aqueous)

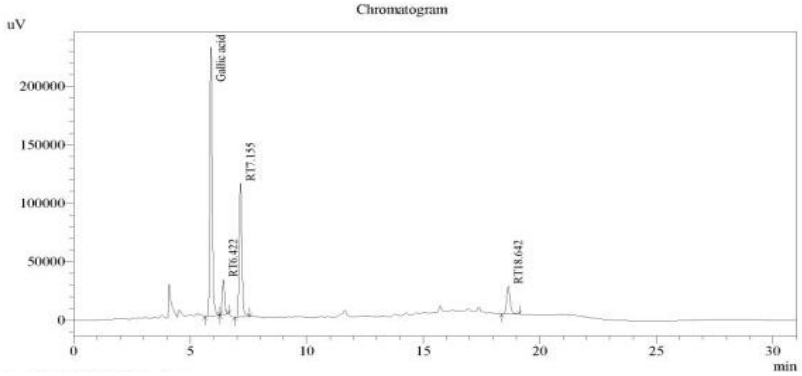


Fig: 4 CHROMATOGRAM OF Syzygium cumini SEED EXTRACT (Hydroethanolic)

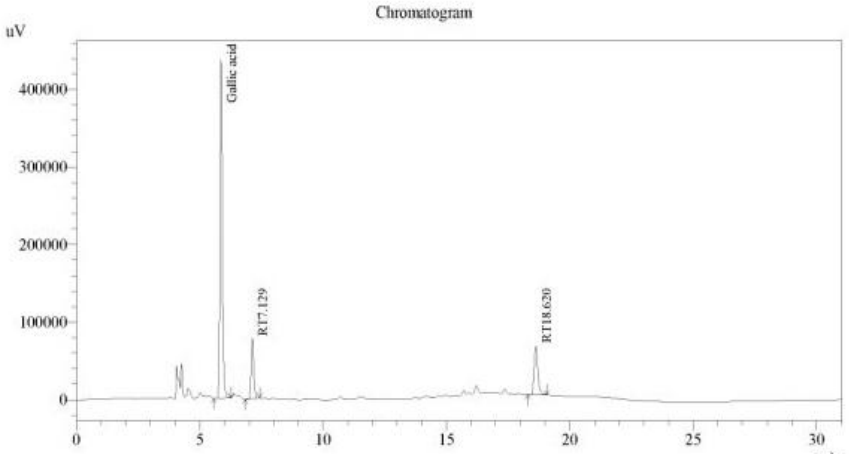
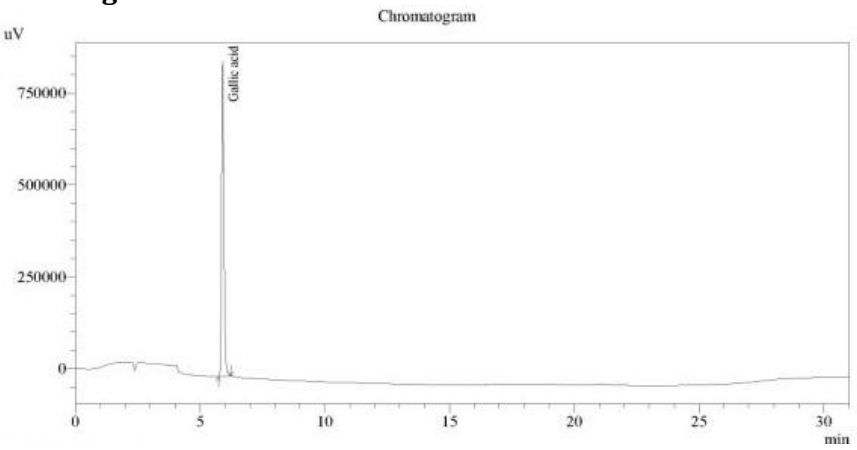


Fig 5: CHROMATOGRAM OF GALLIC ACID STANDARD



CONCLUSION

In this study, a simple, accurate and reliable method was developed to determine the chemical marker of *Syzygium cumini* by using HPLC. The hydro ethanolic extract was found to contain higher amount of gallic acid than that of aqueous extract and raw seed powder. Future studies have to be carried out for estimation of other phenolic compounds.

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