Research Article

Simultaneous quantification of gallic acid, myricetin and quercetin in the extract of *Syzygium cumini* plant and its formulation using HPLC

Vijaykumar Chavan[1]*, Tanmay K. Chache[1], Vikas Yadav[2]

[1]*Ramnarain Ruia Autonomous College, L. N. Road, Matunga (East), Mumbai 400 019 India*

[2]*Department of Chemistry, Ramnarain Ruia College, Mumbai, Maharashtra, India*

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Abstract

Objective: A precise, accurate and reproducible HPLC method is validated for simultaneous quantification of three bioactive markers Gallic acid, myricetin and quercetin in the plant of *Syzygium cumini* and its marketed formulation. **Materials and methods:** The mobile phase was a gradient mixture of 0.1% OPA and methanol 65:35 (v/v). The column used for the chromatographic technique was Phenomex, C-18 having dimensions of 250 mm x 4.6 mm, 5 µm. The detection of chromatograms was carried out at 220nm. This HPLC method was validated statistically and recovery study was performed to confirm the accuracy of the method. **Results and conclusion:** A precise method for simultaneous quantification of Gallic acid, myricetin and quercetin was developed by using High performance Liquid Chromatography. The analytical standards were compared that to methanolic extract of plant and formulation with their respective Retention time. The method can be used for routine quality control of herbal raw materials as well as formulations containing any or all of these compounds.

***Keywords*:** Simultaneous quantification, HPLC, gallic acid, myricetin and quercetin

Introduction

Syzygium cumini is a large evergreen and densely foliaceous tree with greyish-brown thick bark, exfoliating in woody scales. The leaves are leathery, oblong-ovate to elliptic or obovate-elliptic with 6 to 12 centimeters long. Flowers are scented, greenish-white, in clusters of just a few or 10 to 40 and are round or oblong in shape and found in dichotomous paniculate cymes (Muniappan and Pandurangan, 2012). *Syzygium cumini* is known to possess wide range of medicinal properties, which have been attributed to the presence of bioactive compounds in different parts of the plant. It is known to have anti-diabetic, anti-oxidant, anti-inflammatory, anti-microbial activities (Ruan et al., 2008; Amutha and Aishwarya, 2010; Ecker et al., 2015). Also phytoconstituents such as gallic acid, quercetin, β-sitosterol, oleanolic acid, kaempferol etc are known to be present which aid in for different medicinal properties in the plant (Jadhav et al., 2009; Syama et al., 2017).

As in the pharmaceutical industry, HPLC could be regarded as the central standardization workhorse in many herbal analytical or manufacturing laboratories (Damle and Dalavi, 2015). Nowadays, HPLC equipment is very sophisticated and reliable, and it comes with different types of detector and auto-samplers as well as computers that control operation, processing, data storage, and retrieval. The present work thus was carried out to simultaneously quantitate three different phytoconstituents Gallic acid, myricetin and querectin in the plant of *Syzygium cumini* and its formulation (Patel et al., 2009; Patil et al., 2017).

Address for Corresponding Author:
Dr. Vijaykumar Chavan
Ramnarain Ruia Autonomous College, L. N. Road, Matunga (East), Mumbai 400 019 India
Email**:** Vijay.chavvan@gmail.com

Materials and Methods

Chemical

Reference standards of Gallic acid, myricetin and quercetin were purchased from Sigma-Aldrich Chemie (Aldrich Division; Steinheim, Germany). All chemicals and solvents used were of analytical grade and purchased from Merck (Darmstadt, Germany).

Plant Material

Plant of S. cumini was collected in Mumbai. Herbarium samples of S. cumini were prepared in duplicate and authenticated by Botanical Survey of India (BSI), Pune, India. A voucher specimen numbered TKC-01 has been

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retained in the herbarium section of BSI, Pune for future reference. The plant were washed with water to remove any dust particles, dried in shade, powdered and then sieved through BSS mesh size 85 and stored at 25°C in an airtight container.

Preparation of Stock Solutions

Stock solutions of standards were prepared in methanol immediately before use. 10 mg each of Gallic acid, Quercetin and Myricetin were dissolved in 10 ml of methanol to make a concentration of 1000 µg/ml. All three standards were further diluted using methanol to make a solution of appropriate concentrations.

Sample Preparation

The plant powder of *Syzygium cumini* was being carried out by Soxhlet extraction. Five grams of plant powder was weighed and packed in a Whatman paper thimble. It was then extracted with 100 ml methanol for 12 hours using Soxhlet extractor. . Extracts were filtered through a syringe filter of pore size 0.45 µm (Merck, India) before further analysis.

Formulation Sample

For the analysis of formulation sample, 5 gm was accurately weighed into a round bottom flask. 30 mL of methanol was added to the flask and the mixture was refluxed on a boiling water bath for about 30 min. The extract was then filtered through Whatman filter paper no. 41 (E. Merck, India). The same procedure was performed twice and filtrate obtained was combined together and made up to 100 mL with methanol.

Chromatographic conditions

High Performance Liquid Chromatography was performed using Shimadzu instrument comprising of LC – 20 AD Binary pump, SIL-20 AC autosampler CTO-20 AC column oven at 25°C and SPD – M 20 A Photo Diode Array detector. An phenomex C-18 column (250 mm x 4.6 mm i.d. x 5 µm) was employed. Separations were done in the gradient mode, using 0.1% orthophosphoric acid and methanol at a flow rate of 0.7ml/min from 0-7.51 mins and 0.5ml/min from 7.52 to 15 mins; with an injection volume of 10 µl; PDA detection was at 220 nm.

Validation of the Method

ICH harmonized tripartite guidelines were followed for the validation of the developed analytical method. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

System Suitability and Specificity

Suitability of the system was checked by injecting six replicates of each standard. The solution was injected under the optimized conditions. Parameters like retention time and peak area were evaluated for the system suitability. Specificity test was carried out by injecting 10 µl of the diluent, mobile phase and individual standards.

Precision

The variability of the method was studied by carrying out repeatability, inter-day and intra-day precision. Repeatability was carried out in same laboratory, on same day as well as on two consecutive days, by analyzing standard solutions of Gallic acid, myricetin and quercetin using optimized chromatographic conditions.

Linearity

The Linearity of a method is the measure of how well a calibration plot of detector response against concentration approximates to a straight line. The concentration range of 1.0 µg/mL to 600.0 µg/mL for Gallic acid, 2.0 µg/mL to 500 µg/mL for myricetin, 0.5µg/mL to 600.0µg/mL for quercetin was selected for linearity.

Limit of Detection and Limit of Quantification

ICH defines the limit of detection (LOD) is the lowest concentration of an analyte that can be detected under the operational conditions of the method but not necessarily quantitated as an exact value. The limit of quantification (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy, under the operational conditions of the method.

Assay

Ten microliters of sample solution *Syzygium cumini* plant extract and of formulation extract were injected. Also different concentrations of Gallic acid, myricetin and quercetin were injected under optimized chromatographic conditions. The peak corresponding to Gallic acid, myricetin and quercetin in the sample solution was identified by comparing the Retention time values of the sample, with that of standards. The amount of Gallic acid, myricetin and quercetin present in sample solution was determined from the calibration curve by using the peak area of standards generated by the chromatogram.

Recovery

The recovery experiment was carried out to check if there is any interference of other constituents with the peaks of Gallic acid, myricetin and quercetin present in extract of *Syzygium cumini* and formulation. Accuracy of the method was established by carrying out recovery experiment at three different levels, using standard addition method. Each

sample was analyzed in seven replicates and the amounts of Gallic acid, myricetin and quercetin recovered for each level, were determined. The value of percentage recovery for the three components was then calculated.

Ruggedness

Ruggedness of the method was studied by determining the effects of small variations, of mobile phase composition (±2%), oven temperature (±5%).

Results and discussions

A precise, accurate and reproducible HPLC method is validated for simultaneous

quantification of three bioactive markers Gallic acid, myricetin and quercetin. The method was validated for specificity, linearity, LOD, LOQ, intra- day and inter-day precision, recovery, ruggedness and stock solution stability. The method was found to be linear from 1.0 µg/mL to 600 µg/mL for Gallic acid, 2.0 µg/mL to 500.0 µg/mL, myricetin and 0.5 µg/mL to 600.0 µg/mL for quercetin respectively. The correlation coefficient was found to be ≥ 0.995 for all the three components. The relative standard deviation for inter-day and intra-day precision was <2%. This indicates the method is precise. The recovery values of the standards were within acceptable limits. The values of recovery of Gallic acid in plant extract and formulation were 94.02% and 93.41%, recovery of myricetin in plant extract and for formulation were 92.98% and 92.65%, recovery of quercetin in plant extract and formulation were 90.57% and 93.96% respectively. The assay value for samples of *Syzygium cumini* plant powder was found to be 0.48%, 0.19 % and 0.24% for Gallic acid, myricetin and quercetin respectively, while for the formulation it was found to be 0.43 %, 0.18% and 0.23% for Gallic acid, myricetin and quercetin respectively. The method is specific for all the three components because it resolved all standards well in the presence of other phytochemicals in *Syzygium cumini*.

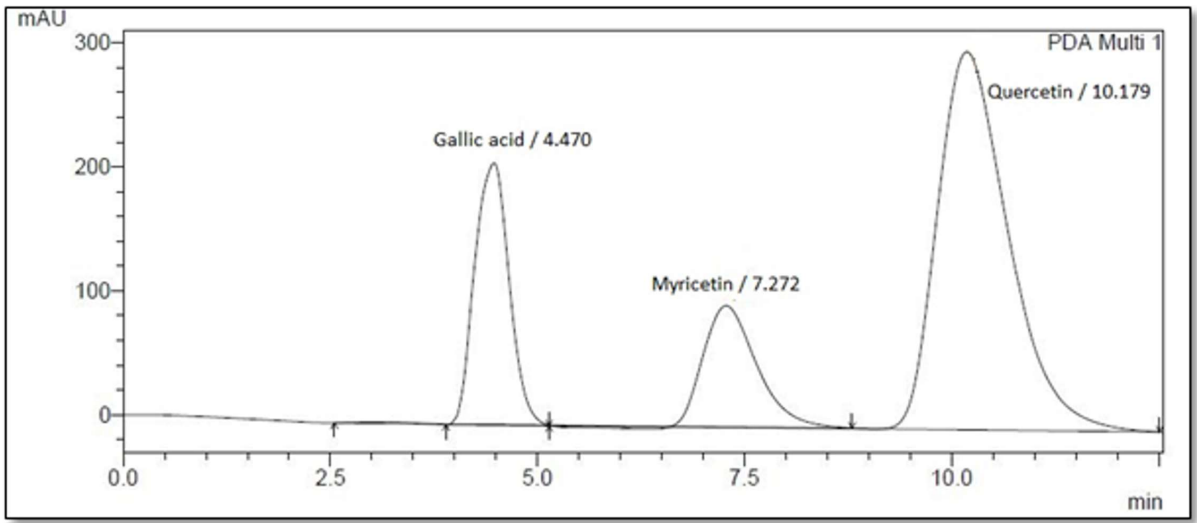

Figure 1. A typical chromatogram of standard gallic acid, myricetin and quercetin

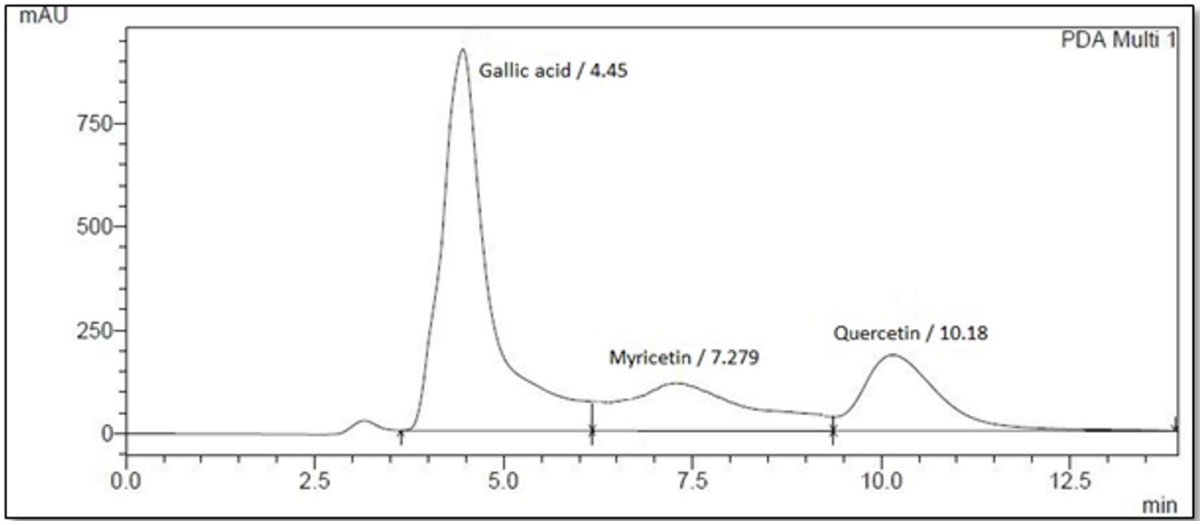

Figure 2. HPLC chromatogram of plant extract of *Syzygium cumini*

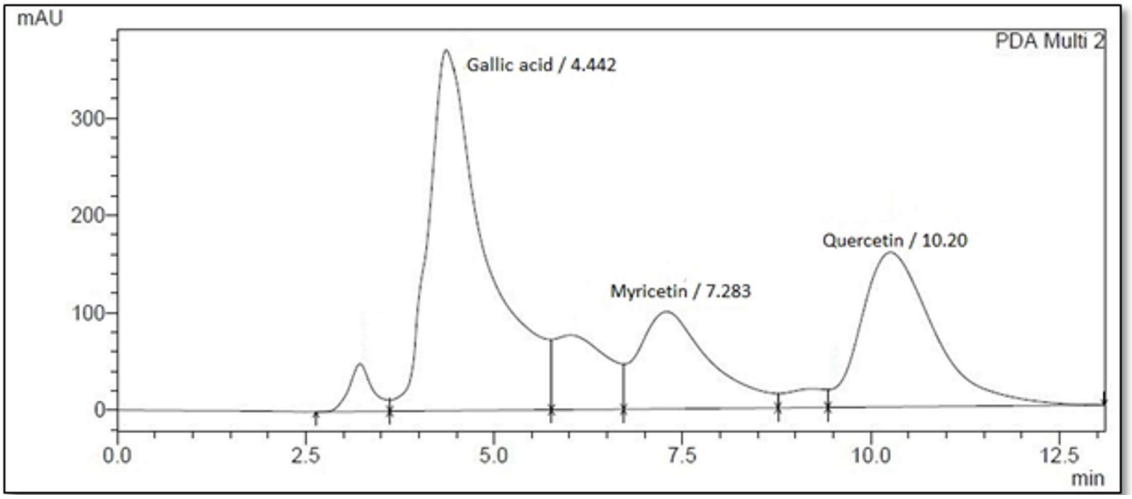

Figure 3. HPLC chromatogram of marketed formulation

Conclusion

The developed method in this research work is precise, accurate and reproducible. It is suitable for qualitative and quantitative analysis of Gallic acid, myricetin and quercetin in the methanolic extracts of plant of *Syzygium cumini*. Also it can be used as a quality control method for other market formulations or dietary supplements containing powder extract of *Syzygium cumini.*

Conflict of interest

The authors whose names are listed certify that they have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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