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Research Article

SIMULTANEOUS QUANTIFICATION OF GALLIC ACID, BETA SITOSTEROL AND OLEANOLIC ACID IN THE EXTRACT OF SYZYGIUM CUMINI PLANT AND ITS FORMULATION USING HPTLC

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ABSTRACT

A simple, precise, accurate and rapid High-Performance Thin Layer Chromatographic method has been developed and validated for the simultaneous estimation of gallic acid, beta sitosterol and oleanolic acid in the plant extract of Syzygium cumini and its formulation. The stationary phase used was precoated silica gel 60F254. The mobile phase used was a mixture of n hexane: Ethyl acetate: Ethanol: Formic acid 7:2:1:0.1 (v/v/v/v). The detection of spots was carried out at 276nm for gallic acid and 540nm for beta sitosterol and oleanolic acid. This HPTLC method was validated statistically and recovery study was performed to confirm the accuracy of the method. It 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, HPTLC, Gallic acid, Beta sitosterol and Oleanolic acid.

1. INTRODUCTION

Traditional systems of medicine have been a integral part of healthcare for centuries all over the world. One of which is use of herbs as medicine, which has been used in all cultures throughout history. By definition, 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'.

Herbal medicines also termed as herbalism is the use of herbs or herbal products for their therapeutic value. Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. There is increasing awareness and general acceptability of the use of herbal drugs in today's medical practice.

Syzygium cumini (Family Myrtaceae) is also known as Syzygium jambolanum and Eugenia cumini. Other common names are Jambul, Black Plum, Java Plum, Indian Blackberry, Jamblang, Jamun etc. Jamun is slow growing tree, medium to large sized up to height of 30mt. The plant is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin. Also they have been reported to be rich in flavonoids, a well-known antioxidant, which accounts for the scavenging of free radicals and protective effect on antioxidant enzymes. The leaves are rich in acylated flavonol glycosides, quercetin, myricetin, myricitin, myricetin 3-O-4-acetyl-L-rhamnopyranoside.

2. Experiment

2.1 Chemical

HPLC grade n hexane, ethyl acetate, ethanol and formic acid were procured from E.Merck, Mumbai, India. Reference standards of β-sitosterol, oleanolic acid and gallic acid were purchased from Sigma-Aldrich Chemie (Aldrich Division; Steinheim, Germany).

2.2 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 TC-1 has been

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.

2.3 Preparation of Stock Solutions

Stock solutions of gallic acid, β-sitosterol and oleanolic acid were prepared in methanol, and by appropriate dilution standard solutions were prepared in the concentration range of 0.1 to 1.0 mg/ml.

2.4 Sample Preparation

5 gm of dried powder of S. cumini was weighed in a round bottom flask. 100 ml of Methanol was added to the flask and the mixture was extracted by Soxhlate extraction after 12 hrs. The extract was then filtered through Whatman filter paper no. 41 (E. Merck, Mumbai, India and filtered through syringe filters of mesh size 0.45µ. The volume was made upto 100ml and used.

2.5 Formulation Sample

For analysis of the formulation sample 1 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, Mumbai, India). The same procedure was performed twice and filtrate obtained was combined together and made up to 100 mL with methanol.

2.6 Chromatography

HPTLC was performed on 200 mm X 100 mm aluminium backed plates pre-coated with 0.2 mm layers of silica gel 60 F254 (Merck, Mumbai, India). A CAMAG TLC system comprising of a Linomat-5 applicator and CAMAG TLC III scanner. Stationary phase used was silica gel G60F254, 20x10 cm TLC plate. The Reference standard gallic acid, beta sitosterol and oleanolic acid was obtained from Sigma-Aldrich Corporation, Bangalore India. Standard solutions of Gallic acid, β-sitosterol and oleanolic acid and sample solutions were applied to the plates as bands 8.0 mm wide, 7.0 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate by use of a CAMAG (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100-ul Hamilton (USA) syringe. Ascending double development to a distance of 80 mm was performed at room temperature (28±2°C), with n hexane : ethyl acetate : ethanol : formic acid 7:2:1:0.1 (v/v) as mobile phase, in a CAMAG glass twin-trough chamber previously saturated with mobile phase vapour for 10 min. After development, the plates were dried in air first and then by keeping on the CAMAG TLC plate heater at 90°C for 5 min. The separated band appeared for gallic acid in uv which was scanned at 276nm with a CAMAG TLC Scanner with winCATS3 software, using the Deuterium lamp. After double development and drying, the plates were derivatized by dipping for 1 sec in 10 % Methanolic sulphuric acid reagent followed by heating on a TLC plate heater at 120°C for 4 min. The separated bands appeared as β-sitosterol and Oleanolic acid violet in colour at different Rf. The plates were then scanned at 540 nm with a CAMAG TLC Scanner with winCATS3 software, using the Tungsten lamp.

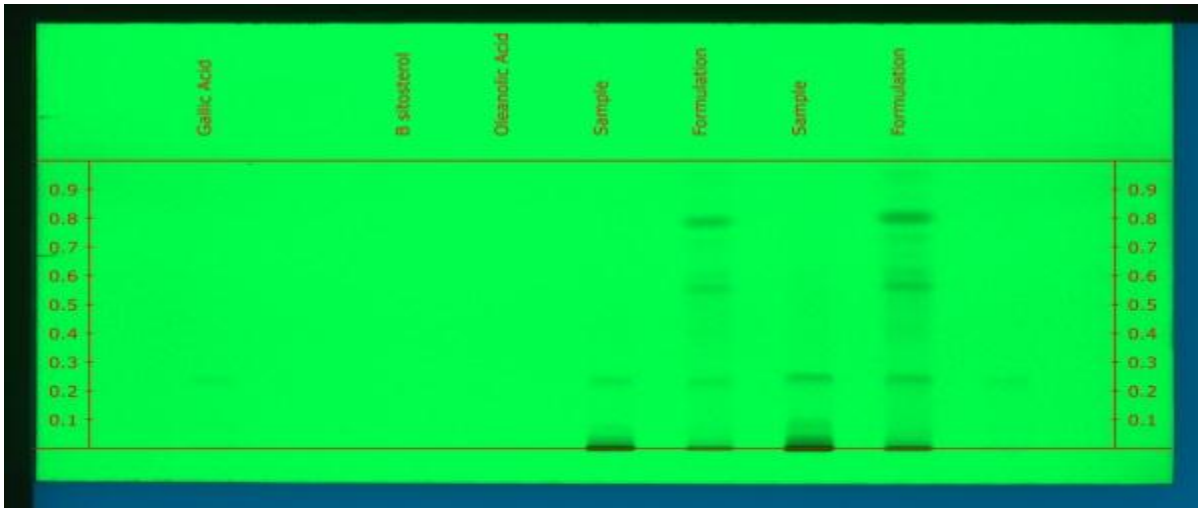
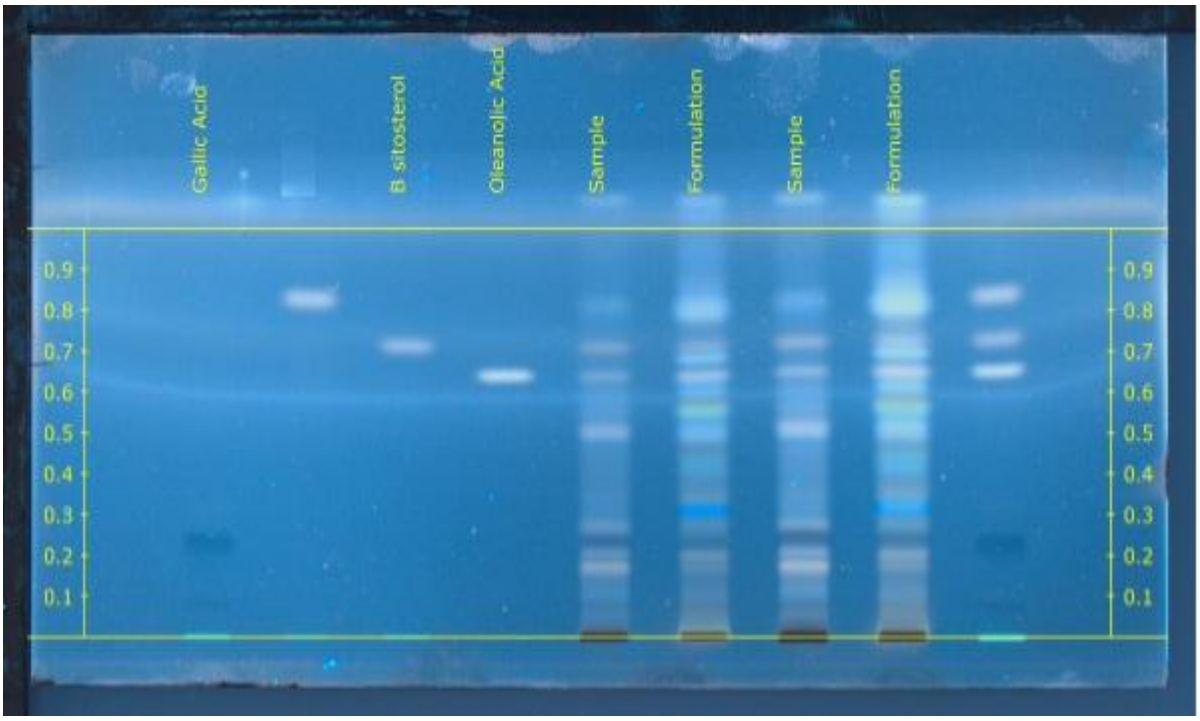


Plate Scanned at 276nm before Derivatization

A: Gallic Acid Standard
B: Beta sitosterol Standard
C: Oleanolic acid Standard
D, F: Methanolic Extract of Syzygium cumini
E,G: Methanolic Extract of Formulation containing Syzygium cumini



A B C D E F G

Plate Scanned at 540nm after Derivatization

A: Gallic Acid Standard
B: Beta sitosterol Standard
C: Oleanolic acid Standard
D, F: Methanolic Extract of Syzygium cumini
E,G: Methanolic Extract of Formulation containing Syzygium cumini

3. Validation of the Method

ICH harmonized tripartite guidelines were followed for the validation of the developed analytical method

3.1 Specificity

The specificity of the proposed HPTLC method was ascertained by analyzing standard compounds and samples. The bands from sample solutions were confirmed by comparing the Rf and colour of the bands to those of the standards.

3.2 Linearity

Working standard solutions of Gallic acid (100µg/mL), β-sitosterol (1000µg/mL) and oleanolic acid (1000µg/mL) were applied, in triplicate, to three different plates and developed and scanned using the optimized conditions described above. The densitograms were then acquired and the peak areas were recorded for each concentration of standard. RSD of standard peak areas for each linearity level were less than 5%.

3.3 Precision

3.3.1 Repeatability

Repeatability of sample applications and measurement of peak area was carried out using the six replicates of same spot 10 µl//spot. Repeatability is also termed Intra-assay precision.

3.3.2 Inter-Day and Intra-Day Precision

Variability of the method was studied by analyzing quality control samples of Gallic acid (100 µg/mL), β-sitosterol (1000 µg/mL) and oleanolic acid (1000 µg/mL) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as ≤ 5% RSD.

3.3.3 Recovery Studies

Recovery tests were carried out to further investigate the accuracy of the method by adding three concentration levels (80,100 and 120%) of the mixed standard solutions to known amounts of S.cumini samples. The resultant samples were then extracted and analyzed with the described method. The average percentage recoveries were evaluated by calculating the ratio of detected amount versus added amount.

3.3.4 Robustness

The robustness of method was performed by small but deliberate change in two parameters, i.e. injection volume(± 2%) and mobile phase composition of one of the solvent (±2%) and its impact on area and Rf values were recorded.

3.4 Summary

The method was validated for linearity, precision, specificity, recovery, robustness and stability. The method was found to be linear from 0.1-0.9 µg/µl for gallic acid and 1-9 µg/µl for β-sitosterol and oleanolic acid. The correlation coefficient was found to be ≥0.99 for all the three components. The precision (%RSD) of the method was found to be ≤ 5%, indicating that the proposed method is precise. The recovery values for all the three components were within acceptable limits (85.0 to 105.0%). Solution stability were evaluated by monitoring the peak area response. Standard solutions were analysed right after its preparation and after 72 hrs. There was no significant change (% RSD ≤ 2%) in the Rf and area values of standard peak.

Table 1: Summary of the validation parameters

Parameter	Gallic acid	β-sitosterol	Oleanolic acid
Specificity	Specific	Specific	Specific
Linearity(µg/µl)	0.1-0.9	1-9	1-9
Correlation coeff	0.994	0.997	0.997
LOD (µg/µL)	0.1	1	1
LOQ (µg/µL)	0.3	3	3
Precision (RSD)	≤ 5%	≤ 5%	≤ 5%
Assay (Plant)	0.11%	0.14%	0.21%
Assay (Formulation)	0.06%	1.02%	0.43%
Stock soln. stability (2-8°C)	72 hours	72 hours	72 hours
Robustness	Robust	Robust	Robust

4. RESULT AND DISCUSSION

A normal phase high performance thin layer chromatographic (HPTLC) method for the simultaneous quantification of Gallic acid, β-sitosterol and oleanolic acid from seed powder of S.cumini was developed in the present research work.

5. CONCLUSION

The proposed method is simple, rapid, precise and accurate. The method was found to be suitable for qualitative and simultaneous quantitative analysis of Gallic acid, β-sitosterol and oleanolic acid in the methanolic extract of S.cumini. The method established in this work can therefore be used as quality-control method for other market formulations or dietary supplements containing seed powder of S.cumini.

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