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Research Article

Quantitative Analysis of Antioxidant Biomarkers in Cucumis Melo Var. Agrestis Leaves Using a Validated Hptlc Technique

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A B S T R A C T

Background: This study focused on using the HPTLC method for the simultaneous estimation of natural antioxidant markers, specifically rutin, gallic acid, and quercetin, from the plant extract of Cucumis melo var. agrestis (CME).

Aims and Objectives: The primary objective of this research was to extract and simultaneously quantify the three antioxidant markers—rutin, gallic acid, and quercetin—from CME using normal phase HPTLC.

Materials and Methods: Compounds were separated on TLC aluminum plates precoated with silica 60 F 260, followed by the detection of rutin, gallic acid, and quercetin. Densitometric scanning at 250 nm was performed using a Camag TLC scanner 3 equipped with winCATS software. This method was further validated for linearity, precision, accuracy, and sensitivity according to ICH guidelines.

Results: The calibration plots showed a strong linear relationship with R2 values of 0.982, 0.9892, and 0.9860 for rutin, gallic acid, and quercetin, respectively. Accuracy was assessed through recovery studies at three different levels, yielding an average recovery rate between 95% and 98% for all three markers.

Conclusion: The developed HPTLC method is suitable for detecting antioxidant markers in botanicals and herbal formulations.

Keywords: Bioflavonoids, Rutin, Gallic acid, Quercetin, Antioxidant and Developed method of HPTLC

Introduction

Antioxidant properties are crucial for treating various diseases, as oxidative stress and free radicals are generated during disease states, making the scavenging of radicals essential for disease treatment.[1,2] Numerous herbs and herbal extracts contain various phyto-pharmacological compounds such as flavonoids, steroids, terpenoids, glycosides, alkaloids, tannins, and phenols. Among these phytochemicals, phenolics and flavonoids exhibit strong antioxidant properties.[3,4,5,6] Flavonoid and phenol compounds, including rutin, gallic acid, and quercetin (Figure 2), are commonly used as antioxidant markers. Rutin, chemically known as quercetin-3-O-rutinoside, rutoside, and sophorin, is the glycoside of the flavonol quercetin and rutinose (a disaccharide).[7] Rutin is one of the most abundant flavonoids in the human diet and has potent antioxidant properties against various oxidizing species such as hydroxide and peroxide radicals. Quercetin,

chemically known as 3,3',4',5,7-pentahydroxyflavone, is a flavonoid found in numerous plants and exhibits antioxidant activities.[8] Gallic acid, a phenolic compound, is chemically named 3,4,5-trihydroxybenzoic acid. Most derivatives of gallic acid occur naturally in plants and possess strong antioxidant properties. Cucumis melo var. agrestis, belonging to the Cucurbitaceae family, is widely distributed in rural and coastal areas (Figure 1). This plant is commonly referred to as wild musk melon, kachari, or small gourd.[9]

Materials Methods

Chemicals

All analytical grade chemicals were obtained from Merck Specialities Private Limited, Mumbai. Antioxidant markers—rutin (98.5% purity), gallic acid (98% purity), and quercetin (97% purity)—were sourced from Sigma Aldrich Chemical Company, Steinheim, Germany.

Preparation of Plant Material

Fresh leaves of Cucumis melo var. agrestis were collected and dried in a shaded, dark room. Once dried, the leaves were powdered using a mechanical grinder. The powdered leaves were then sieved through sieve no. 40 and sieve no. 60, and the resulting material was stored in an airtight container at room temperature.

Preparation of Standard

Precisely weighed 100 mg of each rutin, gallic acid, and quercetin were separately dissolved in 100 ml standard flasks containing methanol to prepare stock solutions of 1 mg/ml. The stock solutions were filtered using Whatman filter paper no. 1, sonicated for 10 minutes, and then stored in amber-colored containers.[11]

Extraction of plant material and preparation of sample solution

A precise amount of 100 g of CME powder was extracted in 500 ml of 50% methanol (methanol: water - 50:50) using a Soxhlet apparatus for 48 hours.[10, 12] The resulting extract was concentrated by distillation and dried at room temperature until a solid mass was obtained, which was then stored in a refrigerator at 4°C for further studies. An accurately weighed 100 mg of the plant extract was dissolved in a 100 ml standard flask containing methanol to prepare a stock solution of 1 mg/ml. The prepared stock solution was filtered using Whatman filter paper no. 1, sonicated for 10 minutes, and stored in an amber-colored container.

High-Performance Thin-Layer Chromatographic Analysis Instrumentation and operating conditions

The analysis was conducted using a CAMAG HPTLC system equipped with a Linomat V Automatic Sample Spotter (Camag Muttenz, Switzerland) and a CAMAG TLC Scanner III, along with winCATS planar chromatography manager software version 1.4.3. Samples were applied on TLC plates precoated with silica gel 60 F254 (E. Merck #5554), with a thickness of 0.2 mm on aluminum sheets, in 7 mm bands positioned 10 mm from the bottom, 15 mm from the sides, and with a 7 mm space between bands. The plates were developed in a twin trough chamber pre-saturated with a mobile phase of Toluene: Ethyl acetate: Formic acid: Methanol (3:6:1.6:0.4) for 30 minutes, to a height of 8.5 cm from the base. After development, quantitative evaluation of the plate was performed in reflectance mode at 254 nm using a tungsten lamp in conjunction with WINCATS software.[13]

Method Validation

The validation of the developed analytical method followed the International Conference on Harmonisation (ICH) guidelines.[14] The evaluated parameters included linearity, precision, specificity, accuracy, sensitivity, and ruggedness.[15]

Specificity

The specificity of the technique was determined by comparing the bands of the sample solutions with those of specific reference standards in terms of their Rf values.

Sensitivity

Sensitivity was determined to limit of detection (LOD) and limit of quantification (LOQ). Different dilutions (1-5 µg/ml) of standard solutions of rutin, gallic acid and quercetin were applied on TLC plates along with methanol as blank. LOD was determined at S/N of 3:1 and LOQ at S/N of 10:1.

Calibration and quality control samples

The calibration curves were prepared from the stock solutions to get desired concentrations in the quantification range. The employed standards in the range of 1-5 µg/ml, 1-5 µg ml, 1-5 µg/mL for rutin, gallic acid and quercetin, respectively, were applied on TLC plate for obtaining a 5 point linear calibration curve.[16,17]

Precision Instrumental precision

Instrumental precision was assessed by repeatedly scanning (n = 3) samples containing 5 µg/ml of rutin, gallic acid, and quercetin, and the results were expressed as the relative standard deviation (% RSD).

Repeatability

The repeatability of the method was verified by analyzing 5 µg/mL of rutin, gallic acid, and quercetin on an HPTLC plate (n = 3) and expressing the results as % RSD (relative standard deviation).[18]

Inter- and intra-day precision

The variability of the method was assessed by spotting

quality control samples of rutin, gallic acid, and quercetin on the same day (intra-day precision) and on different days (inter-day precision). The results were expressed as % RSD (relative standard deviation).

Accuracy

Accuracy was determined by adding known amounts (spiking) of rutin, gallic acid, and quercetin to quality control samples in the plant matrix.

Assay

The content of all antioxidant biomarkers in the extract of Cucumis melo var. agrestis (CME) was determined by applying samples (10 µL) in triplicate along with pure standards of rutin, gallic acid, and quercetin.[19,20]

Results

Three bioflavonoids—rutin, gallic acid, and quercetin—were eluted using various solvent systems, specifically Toluene: Ethyl acetate: Formic acid: Methanol (3:6:1.6:0.4), on normal phase HPTLC. This method effectively resolved rutin (Rf = 0.18), gallic acid (Rf = 0.78), and quercetin (Rf = 0.87), allowing for their simultaneous estimation from the plant extract of Cucumis melo var. agrestis (CME). Visualization of the plate was done at 254 nm (Figure 3). The developed method demonstrated good baseline resolution of each compound (Figure 4).

The method was validated for specificity, precision, sensitivity, ruggedness, and accuracy. Specificity was confirmed by matching the Rf values of bands from the CME sample with those of the reference antioxidant markers. The absence of interfering bands confirmed the method's specificity.

The five-point calibration curves for the three standard reference compounds were linear in the range of 1-5 µg/ml for rutin (Figure 5), gallic acid (Figure 6), and quercetin (Figure 7). The regression equations and coefficients for the reference standard compounds were as follows: y = 3131.6x + 1564.3, R2 = 0.9957 for rutin; y = 4264.4x - 248.72, R2 = 0.9909 for gallic acid; and y = 4309.7x - 3715, R2 = 0.9920 for quercetin

The LOD (Limit of Detection) and LOQ (Limit of Quantification) values obtained were 1 µg/ml and 5 µg/ml, respectively, for rutin, gallic acid, and quercetin, indicating that the developed method was more sensitive for rutin compared to gallic acid and quercetin

The % RSD (Relative Standard Deviation) for rutin, gallic acid, and quercetin was found to be 1.03, 1.16, and 1.28, respectively, which was less than 2%. This demonstrated good ruggedness of the method, even with slight variations in mobile phase composition and spotting volume of the samples.

Intra-day and inter-day precision were within the range of 0.9% to 2%, indicating excellent precision and repeatability. Good recovery values were obtained for the standard reference markers.

Rutin, gallic acid, and quercetin were simultaneously estimated from the hydroalcoholic extract of Cucumis melo var. agrestis (CME). Rutin was found to be present in the highest amount compared to the other antioxidant markers. The amounts of rutin, gallic acid, and quercetin in the extract were determined to be 18.83 mg/g, 6.78 mg/g, and 9.72 mg/g, respectively.

Discussion

Antioxidant properties are crucial in treating various diseases, as oxidative stress and free radicals are generated during disease stages, making scavenging of these radicals essential for treatment. Rutin, quercetin, and gallic acid are well-documented in the literature for their potent antioxidant properties. These antioxidant biomarkers were simultaneously estimated in Cucumis melo var. agrestis.

Conclusion

The developed and validated HPTLC method pioneered the simultaneous estimation of rutin, gallic acid, and quercetin from Cucumis melo var. agrestis. This method will be utilized for the simultaneous quantification of these three antioxidant markers from various other herbal extracts and formulations.

References

1. L.C.N. Abraham, T. Masakuni, H. Isao, T. Hajime, Antioxidant flavonoid glycosides from the leaves of Ficus pumila L, Food Chem. 10 (2008) 415–420.
2. G.S.Jonatas, R.L.S. Mariana, C.O.Aline, R.S.D.Jurandir, M.P.V.Carlos, S.P.C.Clarissa, Cathodic adsorptive stripping voltammetric determination of rutin in soybean cultivars, Journal of Food Composition and Analysis. 25 (2012) 25:1–8.
3. L.Z.Hou, Y.T.Ze, X.Y.Jing, Z.Ying, L.Yue, L.Yuan, Bi-directional regulation of emodin and quercetin on smooth muscle myosin of gizzard, FEBS Letters. 580 (2006) 469–473.
4. Yang, J., Guo, J., Yuan, J., 2008. In vitro antioxidant properties of rutin. LWT - Food Sci. Technol. 41, 1060–1066.
5. K. Khoddami, M.A. Wilkes, T.H. Roberts, Techniques for analysis of plant phenolic compounds, Molecules 18 (2013) 2328–2375.
6. A.S. Nasir, A.M. Ramzi, J.A.R. Adnan, A. Perwez, Y. Muhammad, A. Sarfaraz, A. Abdulrahman, High-performance thin-layer chromatography based concurrent estimation of biomarkers entphyllanthidine and rutin in the dried aerial parts of Flueggea virosa, Saudi Pharmaceutical Journal. 25 (2017) 696–702.

7. P. Jandera, V. Skerikova, L. Rehova, et al., RP-HPLC analysis of phenolic compounds and flavonoids in beverages and plant extracts using a Coul Array detector, J. Sep Sci. 28 (2005) 1005–1022.
8. K. Sharad, N. Ajay, M. Piyush, Development and validation of HPTLC method for simultaneous estimation of curcumin and galangin in polyherbal capsule dosage form, Journal of Taibah University for Science. 11 (2017) 775–781.
9. K. Shankar, S.K. Singh, D. Kumar, S. Varshney, A. Gupta, S. Rajan et al., Cucumis melo ssp. Agrestis var. Agrestis Ameliorates High Fat Diet Induced Dyslipidemia in Syrian Golden Hamsters and Inhibits Adipogenesis in 3T3-L1 Adipocytes, Phcog Mag. 11 (2015) 501-10.
10. Gopalasatheeskumar K, Ariharasivakumar G, Sengottuvel T et al. Confirmation of Best Method for Detection of Alpha Amylase Activity of Cucumis Melo Var Agrestis (Wild Musk Melon). J Adv Res BioChem Pharma 2019; 2(1): 18-20
11. L. Ciesla, D. Zaluski, H.D. Smolarz, M. Waksmundzki-Hajnos, HPTLC – densitometric method for determination of eleutherosides B, E and E1 in different Eleutherococcus species, J. Chromatogr. Sci. 9 (2011) 182–188.
12. M. Attimarad, K.K. Mueen Ahmed, Bandar E. Aldhubaib, Sree Harsha, High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery, Pharm Methods. 2 (2011) 71–75.
13. International Conference on Harmonization, 2006. ICH guideline Q2B on validation of analytical procedures: Text and Methodology, Geneva.
14. G. Sen, C. Xueqin, J. Mi, B. Lu, T. Xiao, G. Tiantian, L. Qingchao, Z. Li, T.H. Chi, B. Naisheng, Simultaneous characterization and quantification of 17 main compounds in Rabdosia rubescens by high performance liquid chromatography, Journal of food and drug analysis. 25 (2017) 417-424.
15. A. Perwez, F.A. Mohamed, H.A. Ahmed, K.P. Mohammad, A.S. Nasir, I.A. Saleh et al., Comparative study of antioxidant activity and validated RP-HPTLC analysis of rutin in the leaves of different Acacia species grown in Saudi Arabia, Saudi Pharmaceutical Journal. 25 (2017) 715–723.
16. F. Soponar, A.C. Moţ, C. Sarbu, High-performance thin-layer chromatography and three-dimensional image analysis for the determination of rutin in pharmaceutical preparations, J AOAC Int. 93 (2010) 804-810.
17. K.B. Vidhya, R.D. Sunil, Validated HPTLC method for Simultaneous Estimation of Atenolol and Aspirin in Bulk Drug and Formulation, International Scholarly Research Network. 1 (2012) 1-5.
18. P. Porto-Figueira, J.A. Figueira, J.A. Pereira, J.S. Camara, A fast and innovative microextraction technique, lSPEed, followed by ultra high performance liquid chromatography for the analysis of phenolic compounds in teas, J. Chromatogr. A. 1424 (2015) 1–9.
19. S. Shailajan, S. Menon, N. Sayed, B. Tiwari, Simultaneous quantitation of three bioactive triterpenoids Lupeol, Stigmasterol and β-sitosterol from Carissa carandas using RP-HPLC method and its validation as per ICH guidelines, Int J Green Pharm.6 (2012) 241-7.
20. G. Toth, C. Barabas, A. Toth, A. Kery, S. Beni, I.Boldizsar, E.Varga, B.Nosza, Characterization of antioxidant phenolics in Syringa vulgaris L. flowers and fruits by HPLCDAD- ESI-MS, Biomed. Chromatogr. 30 (2016) 923–932.
21. Q. Tian, D. Li, B.S. Patil, Identification and determination of flavonoids in buckwheat (Fagopyrum esculentum Moench, Polygonaceae) by high-performance liquid chromatography with electrospray ionisation mass spectrometry and photodiode array ultraviolet detection, Phytochem. Anal. 13 (2002) 251–256.