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Article Review: Identification of Secondary Metabolites Using the TLC-Densitometry Method

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ABSTRACT

Background & Objective: This review article discusses the identification and quantification of secondary metabolites using densitometric thin-layer chromatography (TLC) based on 24 journal articles published over the past ten years. The compounds analyzed include quercetin, catechins and their derivatives (EGCG), phenolic compounds, flavonoids, and various other secondary metabolites. **Method:** Based on the overall data, densitometric TLC is an effective method for the identification and quantification of secondary metabolites because it provides good separation, clear Rf values, and stable quantification results across various natural material matrices. **Result:** The results of the study indicate that silica gel 60 F254 and GF254 are the most commonly used stationary phases, while variations in the mobile phase are the primary factors influencing Rf values and separation resolution. In the quercetin group, Rf values ranged from 0.26 to 0.89, with the highest concentration in meniran at 3.5% and the lowest in cocoa beans at 0.115%. **Conclusion:** Analysis of catechins showed Rf values ranging from 0.22 to 0.60, with the highest content in avocado seeds at 25.55% and gambir at 25.50%, while EGCG in green tea had an Rf value of 0.21 and a content of 3.33%. Phenolic and flavonoid compounds exhibited Rf values ranging from 0.24 to 0.94, influenced by differences in polarity and solvent composition. Other secondary metabolites such as syringic acid, kaempferol, chlorogenic acid, eugenol, beta-sitosterol, oleanolic acid, ursolic acid, myricetin, and rutin exhibited migration patterns consistent with their chemical properties.

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Introduction

Thin-layer chromatography (TLC)-densitometry is a method used to measure the concentration of a compound in a sample. In Thin-Layer Chromatography (TLC)-Densitometry, an adsorbent is applied to a glass plate as the stationary phase, and a chromatogram forms as the mobile phase passes through the adsorbent (Rollando et al., 2019). The measurement principle is based on the Kubelka-Munk theory, which describes the relationship between measured transmittance and sample volume on the TLC plate (Spangenberg, 2023). Therefore, TLC can be utilized in the testing of pharmaceutical preparations and herbal medicines (Prasetyawan et al., 2024). TLC densitometry is frequently used for analyzing the concentration of active compounds because this method is more cost-effective and simpler. Additionally, this method can test multiple samples simultaneously in a single process (Safitri et al., 2024). To ensure the quality, safety, and efficacy of herbal extracts, concentration determination is necessary. Quantification of herbal extracts can be performed using Thin-Layer Chromatography (TLC) because it has excellent capabilities for separating active compounds from other components and quantifying the active compounds contained in herbal extracts (Sudjarwo et al., 2019).

One of the primary flavonoids found in various plant species, including medicinal leaves, is quercetin. Quercetin is a polyphenolic flavonol compound with the chemical name 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one and the molecular formula $C_{15}H_{10}O_7$ (da Silva et al., 2023). This compound is commonly found in vegetables and fruits, such as red onions, apples, cabbage, legumes, and tea. Quercetin possesses distinctive physicochemical properties, appearing as yellow crystals with high solubility in fats and alcohol but low solubility in cold water. Several studies indicate that quercetin exhibits a wide range of biological activities. Specifically, quercetin exhibits high antioxidant activity, capable of neutralizing free radicals and preventing lipid peroxidation. Additionally, quercetin possesses several other important biological activities, such as anticancer, antiviral, antiallergic, antidiabetic, anti-inflammatory properties, and protection of the cardiovascular system (Batiha et al., 2020).

Catechins are polyphenolic compounds with 15 carbon atoms arranged in a C6-C3-Co configuration, and their carbon skeleton consists of two Co groups (substituted benzene rings) linked by three aliphatic carbon atoms (Wang et al., 2018). Catechins are secondary metabolites in tea plants with a flavan-3-ol structural framework and account for approximately 42% of the total dry leaf weight (Rabbani et al., 2019). Catechins in tea are a complex group of compounds comprising epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and gallocatechin (GC) (Anjarwati, 2016) Among the many types of catechins, EGCG is the most potent and primary compound. EGCG is the most abundant flavonoid-3-ol polyphenol found in green tea. Structurally, EGCG contains eight free hydroxyl groups responsible for its bioactive properties. EGCG has the potential to be utilized in the treatment of various diseases, including oral disorders, cancer, obesity, diabetes, and inflammatory and neurodegenerative diseases, as research indicates that EGCG possesses biological activities such as antioxidant, anticancer, and antidiabetic effects (Alam et al., 2024).

The most abundant chemical compounds found in plants are phenolics, where phenolic compounds are known to play a significant role in antioxidant activity; the higher the content of phenolic compounds, the greater the antioxidant activity. Phenolic compounds encompass a diverse range of plant-derived compounds characterized by an aromatic ring containing one or two hydroxyl groups. Phenolic compounds tend to be highly soluble in polar solvents because they are often bound to sugar as glycosides and are typically found within plant cell vacuoles (Syarif et al., 2015).

Method

In writing this review article, the author employed a literature review method by searching for sources or literature in the form of primary data through electronic databases such as Google Scholar, NCBI, ScienceDirect, PubMed, ResearchGate, and other electronic sources. The literature review was conducted on several national and international research journals based on the keyword "compound identification using TLC-densitometry," with journal criteria including:

1. Published within the last ten years (2015–2025)
2. The data used consists of journals related to the identification of secondary metabolite compounds using the TLC-densitometry method

Results

LC-Densitometry is an analytical method that can be used to determine the concentration of active ingredients in natural materials or pharmaceutical products. Densitometry is an instrumental analytical method based on the interaction of electromagnetic radiation (EMR) with the analyte, which appears as a spot on the TLC plate (Grantica, et al. 2020). Densitometry places greater emphasis on quantitative analysis. In studies that have been conducted, TLC-Densitometry can be applied to determine the concentration of a secondary metabolite, such as phenolic compounds. In the TLC-Densitometry method, the quantitative parameters used include the peak height and the area under the densitometric curve. A densitometer has two modes: reflectance (reflection) mode and transmission mode. In reflectance mode, the UV/Vis, fluorescence, and fluorescence quenching spectral ranges are used. This analysis is performed by measuring the absorption or fluorescence properties of a substance directly on a thin-layer chromatogram using an instrument with one or more light sources, or as a function of light reflected from points on the plate. The mechanism of TLC-Densitometry involves a light source directed toward a monochromator to convert polychromatic light into monochromatic light, which is then directed toward the sample on the plate and reflected back. The reflected light is detected by a detector, yielding the results (Puspitasari & Handayani, 2020).

LC-Densitometry distinguishes analytical approaches based on the presence of chromophore groups in the analyte's molecular structure. For secondary metabolites rich in conjugated double-bond systems—such as flavonoids, phenolics, certain alkaloids, and anthraquinone derivatives—analysis is performed directly (direct scanning) by utilizing the intrinsic absorbance of these compounds. The quantification mechanism in TLC-densitometry is based on the conversion of chromatogram spots into analog peak profiles, where the area under the curve (AUC) or peak height is directly proportional to the analyte concentration according to the Kubelka -Munk law, which provides a quantitative description of the absorption, reflection, and

scattering of light in media such as TLC plates (Ramadhani et al., 2023). For specific secondary metabolites such as flavonoids, alkaloids, or terpenoids, scanning is performed at the maximum absorption wavelength of the target analyte. The main advantage of this method lies in its ability to analyze many samples simultaneously with minimal sample preparation, making it an efficient method for fingerprint analysis or chromatographic profiling of medicinal plant extracts (Laksono & Hayati, 2021).

TABLE 1. Identification of Quercetin Using TLC-Densitometry

No.	Compound	Stationary Phase	Mobile Phase	Result	Reference
1.	Quercetin	Silica gel GF254	Chloroform : Ethyl acetate : Formic acid (5:4:1)	Concentration: 3.5%	(Ihsan *et al.*, 2022)
2.	Quercetin	Silica gel plate 60 F254	Chloroform : Ethyl acetate : Formic acid (5:4:1)	Rf: 0.5, concentration: 1.46%	(Ihsan *et al.*, 2019)
3.	Quercetin	Silica gel plate GF254	Toluene : Ethyl acetate : Formic acid (5:4:0.2)	Rf: 0.388, concentration: 0.12%	(Sulistyowati *et al.*, 2021)
4.	Quercetin	Silica gel GF254	Ethyl acetate : methanol (9:1)	Rf: 0.89	(Atiku *et al.*, 2019)
5.	Quercetin	Silica gel plate GF254	toluene, ethyl acetate, and formic acid (5:4:0.4).	Rf: 0.553, concentration: 0.115%	(Arbi *et al.*, 2025)
6.	Quercetin	Silica gel plate 60 F254	Toluene: Ethyl Acetate: Formic Acid (7:3:1)	Rf: 0.26, concentration: 0.5841 ± 6.93 (w/w)	(Sudjarwo *et al.*, 2023)
7.	Quercetin	Silica gel plate GF254	Chloroform: methanol: dichloromethane: acetonitrile: formic acid (6:2:2:0.05:0.05)	Rf value: 0.48 Concentration: 3.26%	(Purwani *et al.*, 2024)
8.	Quercetin	Silica gel plate 60 F254	Chloroform: methanol: acetic acid (8:1.5:0.5)	Rf value: 0.56 Concentration: 0.44%	(Rollando *et al.*, 2025)
9.	Quercetin	Silica gel plate 60 F254	Toluene: ethyl acetate: formic acid: methanol (5.5:4:1:0.5)	Rf values: 0.54 & 0.55 Concentration: 0.929%	(Nithya & Kamalam, 2018)
10.	Quercetin	Silica gel plate 60 F254	chloroform: ethyl acetate: formic acid (5:4:1)	Rf value of quercetin and guava extract: 0.68; Rf value of star apple extract: 0.85	(Primadiastri *et al.*, 2021).

The identification of quercetin using TLC-densitometry in various studies has shown that the stationary phases used were silica gel GF254 and 60 F254. These stationary phases produce similar separation patterns because both are highly polar. However, silica gel 60 F254 tends to produce a wider range of Rf values, indicating higher sensitivity to changes in the mobile phase composition. Differences in Rf values are primarily influenced by the mobile phase used. The mobile phase chloroform:ethyl acetate:formic acid yields medium to high Rf values (0.5–0.68) and provides stable separation, whereas the toluene:ethyl acetate:formic acid system yields lower Rf

values due to toluene's highly non-polar nature, causing quercetin to be more strongly retained in the stationary phase. In more polar mobile phases, such as ethyl acetate:methanol, quercetin migration slows down, resulting in very low Rf values. Based on Table 1, as the mobile phase becomes less polar, the Rf value increases, and the addition of organic acids has been shown to enhance spot sharpness. Thus, variations in the mobile phase are the primary factor determining the effectiveness of quercetin separation in the TLC-densitometry method.

Of the ten journals compared, nine reported the Rf value of quercetin, while one journal, namely Ihsan et al. (2022), only reported the concentration without including Rf data, so it cannot be compared in this section. In general, the Rf values of quercetin in most studies tend to fall within the medium range, particularly when the mobile phase used is a mixture of semi-polar solvents such as chloroform or toluene with ethyl acetate and a small amount of organic acid. This similarity is evident in the study by Ihsan et al. (2019) with an Rf of 0.50; Arbi et al. (2025) with an Rf of 0.553; Rollando et al. (2025) with an Rf of 0.56; and Nithya and Kamalam (2018) with an Rf of 0.54–0.55. This close range of values indicates that the ratio of semi-polar to non-polar solvents plays a dominant role in quercetin migration; thus, even though the types of solvents differ, the relative polarity of the mixtures still produces similar migration patterns.

On the other hand, there are several studies that report Rf values outside this range. For example, Sulistyowati et al. (2021) reported an Rf value of 0.388. This lower value is influenced by a more non-polar mobile phase, causing quercetin to migrate a shorter distance. The opposite condition is observed in the study by Atiku et al. (2019) with an Rf value of 0.89, which is considered high because the mobile phase is more polar (ethyl acetate–methanol), causing quercetin to be carried farther from the starting point. A low value also appears in the study by Sudjarwo et al. (2023) with an Rf of 0.26, while the study by Purwani et al. (2024) obtained an Rf of 0.48, which is still close to the general range but slightly lower, likely because the mobile phase consists of five solvents, resulting in a more complex mixture polarity.

In the study by Primadiastri et al. (2021), the Rf values obtained depended on the sample. Guava leaf extract showed an Rf value of 0.68, which is still close to the quercetin standard value, while guava fruit extract showed an Rf value of 0.85. This higher Rf value is likely influenced by the presence of other compounds in the plant matrix that have similar polarity, causing the quercetin spot to shift.

Overall, the variation in the Rf values of quercetin across these nine studies is primarily due to differences in the polarity of the mobile phase, solvent ratios, and sample composition. When the mobile phase is semi-polar, quercetin generally falls within the Rf range of 0.50–0.56. Lower values usually appear when the mobile phase is more non-polar, while higher values appear if the mobile phase is too polar or the sample contains compounds that interfere with spot positioning. Meanwhile, Ihsan et al. (2022) could not be included in the comparison because they did not include Rf values.

Based on the data in the table, the quercetin content obtained in each sample varies. In meniran (Phyllanthus niruri L.) using the maceration extraction method, a quercetin content of 3.5% was obtained; in guava leaves (Psidium guajava L.) using maceration extraction, a quercetin content of 1.46% was obtained; and in kenikir leaves (Cosmos caudatus H. B. K) using the maceration extraction method yielded a quercetin content of 0.12%, cocoa beans (Theobroma cacao L.) using the maceration

extraction method yielded a quercetin content of 0.115%, star fruit leaves (Averrhoa bilimbi L.) using the maceration extraction method yielded a quercetin content of 0.5841%, tempuyung leaves using digestion extraction yielded a quercetin content of 3.26%, avocado leaves using maceration extraction yielded a quercetin content of 0.44%, and E. odoratum leaves using Soxhlet extraction yielded a quercetin content of 0.929%. Based on the results of these studies, the highest quercetin content was obtained from the meniran (Phyllanthus niruri L.) sample, and the lowest from the cocoa (Theobroma cacao L.) sample. Variations in quercetin content can occur due to differences in plant species and various factors. Quercetin is a flavonoid compound, and the concentration of flavonoids varies among different plant species. These differences can be influenced by both internal and external factors. Internal factors include genetic factors, while external factors include humidity, light, temperature, pH, and nutrient content in the soil (Sholekah, 2017). Additionally, variations in quercetin levels are influenced by other factors such as differences in the extraction method used, the balance between the amount of soluble quercetin and the volume of solvent used, the absorption of quercetin into the crude extract, and the presence of other chemical compounds in the extract that can affect the stability of quercetin levels (Aisyah et al., 2025; Maryam et al., 2023).

Overall, variations in Rf values and quercetin content across various studies indicate that differences in the polarity of the mobile phase, solvent composition, sample type, and extraction method significantly influence analytical results. The Rf values of quercetin generally fall within the range of 0.50–0.56 when the mobile phase is semi-polar, while lower values appear in more non-polar mobile phases and higher values are observed in overly polar mobile phases or in samples with complex matrices that shift the spot positions. Similar variations are also observed in quercetin content, with the highest levels found in meniran (3.5%) and the lowest in cocoa beans (0.115%), reflecting differences in plant characteristics, environmental factors, genetic conditions, and the efficiency of extraction methods such as maceration, digestion, or Soxhlet extraction. Thus, both Rf values and quercetin levels are significantly influenced by a combination of chemical, biological, and technical factors in each study.

TABLE 2. Identification of Catechin & EGCG Compounds Using TLC-Densitometry

No.	Compound	Stationary Phase	Mobile Phase	Result	Reference
1.	Catechin	Silica gel plate 60 F254	Toluene: Ethyl acetate: Formic acid: Methanol (3:6:1.6:0.4)	Rf value 0.6 Concentration 25.55%	(Sanjaya *et al*., 2020).
2.	Catechin	Silica gel plate 60 F254	Chloroform: Ethyl acetate: Glacial acetic acid (4:4:2)	Rf value 0.22 Concentration 25.50%	(Kamal *et al*., 2022)
3.	EGCG	Silica gel plate 60 F254	Chloroform: Acetic acid: Formic acid: Isopropanol (16:2:2:8)	Rf value 0.21 Concentration 3.33%	(Chatimah *et al*., 2020)

Analysis of catechins and their derivative, EGCG, using thin-layer chromatography (TLC) densitometry on three different samples—green tea, avocado seeds, and gambir—shows that although the stationary phase used was uniform

(Silica Gel F254), differences in the matrices of the three samples required significant adjustments to sample preparation and the mobile phase system. These differences demonstrate how the mobile phase and the polarity of the analyte strongly influence the results of thin-layer densitometric chromatography.

In the article by Chatimah et al. (2020), the determination of EGCG in green tea utilized a mobile phase of chloroform: acetic acid: formic acid: isopropanol (16:2:2:8). This system is chemically more nonpolar due to the high proportion of chloroform. Therefore, polar compounds such as EGCG will have stronger interactions with the silica stationary phase, which is also polar. Consequently, EGCG migrates a shorter distance and has a low Rf value of approximately 0.21, which is optimal for the identification and separation from other green tea components. This system was selected after testing other mobile phases that resulted in tailing or unclear separation; thus, this phase was found to be the most selective for EGCG. Furthermore, method validation demonstrated excellent linearity with a correlation coefficient close to 1 (r = 0.9996), low LOD and LOQ, and accuracy and precision within the required ranges, making the procedure highly suitable for routine EGCG quantification. Using this mobile phase, the EGCG content in the analyzed green tea sample was found to be approximately 3.33%, consistent with the general characteristics of commercial green tea, which typically contains EGCG in the range of 3–10%.

Meanwhile, in the article by Sanjaya et al. (2020), catechin analysis in avocado seeds utilized a mobile phase with higher polarity, namely toluene : ethyl acetate : formic acid : methanol (3:6:1.6:0.4). The higher proportions of ethyl acetate and methanol increase the polarity of the mobile phase, causing the polar catechin compounds to migrate farther than EGCG. This is reflected in the reported Rf value of the standard catechin at 0.6, where this Rf indicates ideal migration for separation from nonpolar compounds such as lipids and resins present in avocado seeds. This relatively high Rf value indicates that the eluent used is capable of carrying the catechins over an appropriate distance without mixing with other components in the matrix. In this article, the highest catechin content was reported in the extract macerated using ethyl acetate as the solvent, at approximately 25.55%. This demonstrates that, in addition to valid TLC-densitometric analysis, the choice of solvent during the extraction process also significantly influences the final quantification results. Compared to green tea EGCG, the catechin content in avocado seed extract is significantly higher due to the matrix's nature—which does not undergo severe oxidation like tea leaves—and minimal processing.

Meanwhile, the catechin content of gambir blocks examined in the article by Kamal et al. (2022) yielded results falling between these two values, both in terms of Rf values and the obtained concentrations. The mobile phase used—chloroform: ethyl acetate: acetic acid (4:4:2)—is a more non-polar eluent compared to the mobile phase used in the analysis of catechins in avocado seeds. This mobile phase resulted in the migration of the standard catechin at an Rf of 0.21. This Rf value is very close to the Rf of EGCG from the green tea leaf study, as EGCG is a derivative of catechins; however, the gambir matrix has a more complex flavonoid composition and often undergoes degradation due to heating processes during gambir block production. The catechin content obtained in gambir block products is in the range of 25.50%, nearly the same as that in avocado seeds but higher than EGCG in green tea. This indicates that the catechin content in a sample matrix is clearly higher than EGCG, since EGCG constitutes only half of the catechin compound itself.

Overall, the differences in Rf values and concentrations observed in the three samples reflect variations in the mobile phase composition, the polarity of the analytes, and the sample matrix conditions. More polar analytes like EGCG yield low Rf values when using a nonpolar mobile phase, whereas catechins exhibit varying Rf values depending on the polarity of the mobile phase used. In terms of concentration, the highest values were found in the avocado seed and gambir block extracts, while the concentration in green tea was the lowest because the composition of catechins and EGCG is significantly influenced by harvesting and heating processes during processing.

TABLE 3. Identification of Phenolic Compounds and Flavonoids Using TLC-Densitometry

N	Compound	Stationary Phase	Mobile Phase	Result	Reference
1.	Ellagic acid	Silica gel plate 60 F254	Ethyl acetate: formic acid: water (10 : 2 : 3)	Ellagic acid content: 2.96 ± 0.05 µg/g	(Altemimi *et al.*, 2015).
2.	Gallic acid	Silica gel plate 60 F254	Chloroform: ethyl acetate: n-butanol: formic acid (15:2:2:1)	The phenolic compound content of the water fraction was 18.76236 mg GAE/g sample, the ethyl acetate fraction was 58.9238 mg GAE/g sample, and the n-hexane fraction was 19.95158 mg GAE/g sample.	(Pratiwi, 2024).
3.	Gallic acid	Silica gel plate GF254	n-butanol: acetic acid: distilled water (4:1:5)	Rf value 0.94 Concentration 35.280 µg or 0.004%	(Ahmad *et al.*, 2020)
4.	Flavonoids	chloroform: methanol (2:8)	TLC plate	RFa: 0.81; 0.83; 0.80. Flavonoid content: 0.73 gQE/g	
5.	Flavonoids	Silica gel plate 60 F254	Ethyl acetate: n-hexane (9:1)	Before spraying with $AlCl_3$: 254 nm = Rf 0.54, 0.42, and 0.24. 366 nm = N.A. After spraying with $AlCl_3$: 254 nm = Rf 0.54; 0.42; and 0.24 366 nm = Rf 0.84; 0.78; 0.54; 0.42; and 0.24	Hadi, S., *et al*, A. (2023)

				Concentration: 31.19 mg QE/g.
6.	Flavonoids	Silica gel plate GF 254	Petroleum ether: acetone (7:3) and (2:1)	Rf: 0.83 Up to: 5.7% by weight

In the identification of phenolic compounds and flavonoids, two examples were selected: ellagic acid from pumpkin (Cucurbita pepo) flesh by Altemimi et al. (2015) and gallic acid from the peel of Binjai rambutan (Nephelium lappaceum L.) by Pratiwi (2024). In both studies, the same stationary phase was used, namely silica gel 60 F254 plates; however, there was a difference in the stationary phase preparation stage. In the study by Altemimi et al. (2015), the TLC plates were placed in an oven at 110 °C for 20–30 minutes until completely dry to remove moisture adsorbed within the TLC plates (Mahdalena et al., 2022).

Ellagic acid has four hydroxyl groups and two lactone groups (Dewi, 2021), which tend to be more non-polar than gallic acid, which has three hydroxyl groups and one carboxyl group (Antasionasti et al., 2020); thus, it requires a less polar mobile phase or one containing components capable of specific interactions. The mobile phase for ellagic acid (ethyl acetate : formic acid : water, 10:2:3) is dominated by ethyl acetate, a semi-polar solvent, with the addition of polar formic acid and water to control elution appropriately. Conversely, the mobile phase for gallic acid (chloroform: ethyl acetate: n-butanol: formic acid, 15:2:2:1) contains a high proportion of chloroform as a non-polar solvent, indicating that this mixture is more suitable for separating gallic acid from the mixture due to gallic acid's tendency to be more polar than ellagic acid.

In a study by Altemimi et al. (2015), TLC plate images were analyzed using Quantity One™, a densitometry software that evaluates the area of separated spots by comparing the intensity of the spot color with the background color of the TLC plate, yielding an ellagic acid content in the sample of 2.96 ± 0.05 µg/g. Meanwhile, in a study by Pratiwi (2024), gallic acid was measured at ultraviolet wavelengths of 200–400 nm. The measured spectral peaks for gallic acid in the study were 223 nm (first peak) and 280 nm (second peak). The measurement of phenolic compound content was performed using the AUC of the sample at a specific Rf (close to the Rf of gallic acid) with the standard gallic acid linear regression equation y = 0.009531x + 0.00293, yielding a phenolic compound content of 18.76236 mg GAE/g sample for the water fraction, 58.9238 mg GAE/g sample for the ethyl acetate fraction, and 19.95158 mg GAE/g sample for the n-hexane fraction.

Ellagic acid was specifically analyzed and quantified using densitometry, while gallic acid was analyzed using a more complex mobile phase with total phenolic (GAE) measurement. Both methods highlight the importance of adjusting TLC techniques to achieve effective resolution and accurate quantification based on the polarity of the analytes.

TABLE 4. Identification of Other Secondary Metabolites Using TLC-Densitometry

No.	Compound	Stationary Phase	Mobile Phase	Result	Reference
1.	Syringic acid	Silica gel plate 60 F254	Toluene:ethyl acetate:formic acid:methanol (10:15:10:5)	Rf value 0.7, concentration 123.84 mg/100g	(Sharma *et al*., 2022).
2.	Kaempferol	Silica gel plate 60 F254	Toluene:ethyl acetate:glacial acetic acid (5.5:4:0.5)	Rf value 0.95, concentration 24.06 mg/100g	
3.	Chlorogenic acid	Silica gel plate 60 F254	Ethyl acetate:acetic acid:water (7:1.5:1.5)	Rf value 0.35, concentration 188.49 mg/100g	
4.	Eugenol	Silica gel plate 60 F254	Cyclohexane:chloroform:ethyl acetate (20:5:8)	Rf value 0.77, concentration 0.029 mg/g	(Ghani & Khan, 2015).
5.	Beta-sitosterol	Silica gel plate 60 F254	Cyclohexane:chloroform:ethyl acetate (20:5:8)	Rf value 0.49, 0.051 mg/g	
6.	Oleanolic acid	Silica gel plate 60 F254	Cyclohexane:chloroform:ethyl acetate (20:5:8)	Rf value 0.56, 0.016 mg/g	
7.	Ursolic acid	Silica gel plate 60 F254	Cyclohexane:chloroform:ethyl acetate (20:5:8)	Rf value 0.56, 0.052 mg/g	
8.	Myricetin	Silica gel plate 60 F254	Toluene:ethyl acetate:formic acid:methanol (10:15:10:5)	Rf value 0.63, content 121.99 mg/100g	(Tessema *et al*., 2023).
9.	Rutin	Silica gel plate 60 F254	Toluene:ethyl acetate:formic acid:methanol (10:15:10:5)	Rf value 0.075, content 72.05 mg/100g	

In addition, other secondary metabolites were identified, such as syringic acid, kaempferol, chlorogenic acid, eugenol, beta-sitosterol, oleanic acid, ursolic acid, myricetin, and rutin. These compounds were analyzed using TLC with the same stationary phase, namely silica gel 60 F254, so that differences in Rf values were primarily influenced by the composition of the mobile phase and the properties of each compound. The mobile phases used ranged from non-polar (toluene, cyclohexane) to semi-polar (ethyl acetate, methanol, water), so the mobility of each compound varied according to its degree of polarity.

Polar compounds such as syringic acid, chlorogenic acid, myricetin, and rutin (Ganeshpurkar and Saluja, 2017) tend to have low to moderate Rf values, especially when the mobile phase is more non-polar, because polar compounds are more strongly retained in the stationary phase, which is also polar. This is evident in rutin (Rf 0.075) and chlorogenic acid (Rf 0.35). Conversely, relatively less polar flavonoid compounds such as kaempferol exhibit high mobility with Rf values reaching 0.95 when the mobile phase is sufficiently polar.

Meanwhile, for non-polar compounds such as eugenol, β-sitosterol, oleanolic acid, and ursolic acid, the mobile phase containing cyclohexane–chloroform–ethyl acetate yields moderate Rf values (0.49–0.77). These values are consistent with the compounds' tendency to be more readily carried by a non-polar mobile phase.

Additionally, the concentration values show significant variation depending on the natural content of the compounds in the sample as well as the solvent's ability to extract them. Polar compounds such as chlorogenic acid (188.49 mg/100 g) and syringic acid (123.84 mg/100 g) exhibit very high concentrations because they are abundant in phenol-rich plants. Myricetin (121.99 mg/100 g) and rutin (72.05 mg/100 g) also have significant concentrations. In contrast, triterpenes such as oleanolic acid (0.016 mg/g) and ursolic acid (0.052 mg/g) are present at much lower concentrations, consistent with their naturally low abundance.

Differences in thin-layer chromatography (TLC) behavior clearly indicate that there are differences in polarity between the flavonoid quercetin and the catechin/EGCG. Quercetin, a member of the flavonol group, tends to have semi-polar characteristics. It can be observed that the Rf values of quercetin tend to fall within the intermediate range of 0.50–0.56 when the mobile phase used is a semi-polar mixture (such as chloroform or toluene mixed with ethyl acetate and a small amount of organic acid). These moderate Rf values indicate that quercetin exhibits a relatively balanced attraction between the polar silica stationary phase and the semi-polar mobile phase. In contrast, catechins and their derivatives, particularly EGCG (flavan-3-ol), exhibit substantially more polar properties. This high polarity is evident from the Rf values, where to achieve ideal or moderate migration—i.e., an Rf of 0.6—catechin requires a mobile phase with a much higher degree of polarity (such as a mixture of toluene, ethyl acetate, formic acid, and methanol with a high proportion of polar components) (Latos-Brozio & Masek, 2019).

In addition to the compounds mentioned above, differences are also observed in several other compounds contained in flavonoids and phenolic acids. The mobile phase consisting of toluene: ethyl acetate: formic acid: and methanol (20:12:8:4) for flavonoids and toluene: ethyl acetate: formic acid: and methanol (10:15:10:5) for phenolic acids demonstrated adequate resolution and separation of sample components. Based on the mobile phase used, the flavonoids—comprising eugenol, beta-sitosterol, and oleanolic acid—yielded Rf values ranging from 0.56 to 0.77 with concentrations of 0.016 mg/g to 0.051 mg/g. Meanwhile, for phenolic acids consisting of myricetin and rutin, Rf values ranged from 0.075 to 0.63 with concentrations of 72.05 mg/100 g to 121.99 mg/100 g. For phenolic acids, the Rf values and concentrations tend to be lower compared to flavonoids; differences in concentration in the mobile phase are the primary factors influencing the fluorescence process (Tessema et al., 2023).

The TLC-densitometry method has proven highly effective for the identification and quantification of secondary metabolites in plant extracts. This is evident from the consistent Rf values observed for each compound, thereby facilitating the identification process. Based on the results, consistent and well-defined Rf values for various classes of compounds—including flavonoids (e.g., quercetin, myricetin), phenolic compounds (gallic acid, ellagic acid), sterols (beta-sitosterol), and triterpenoids (such as oleanolic acid), indicate that this method is capable of separating compounds with high resolution.

The TLC-Densitometry method offers several advantages, such as high specificity, reliable results, and ease and speed of execution. Additionally, the TLC-Densitometry method allows for the simultaneous determination of the concentrations of multiple samples (Fatimah et al., 2020). Several studies have been conducted to develop and validate the TLC-Densitometry method for the qualitative

analysis of various types of samples, including extracts from natural materials. The results of these studies indicate that the TLC-Densitometry method exhibits good precision and can produce valid and reliable analytical results (Savitri & Megantara, 2019).

However, the TLC-Densitometry method also has limitations, one of which is the lack of direct visualization of the analyzed compounds. This can lead to limitations in compound identification, especially if the compounds do not have characteristic absorption bands. Furthermore, this method requires sophisticated and expensive equipment, as well as specialized expertise in interpreting the results. Therefore, further research is needed to address these shortcomings in order to improve the validity and reliability of the TLC-Densitometry method in pharmaceutical analysis (Savitri & Megantara, 2019).

Conclusion

Thin-Layer Chromatography (TLC)-Densitometry is an efficient quantitative analytical technique based on the principles of chromatographic separation and instrumental quantification according to the Kubelka-Munk Law by measuring light absorption or reflection. This method uses a polar stationary phase to separate compounds, where the migration of each compound, indicated by the Rf value, is highly dependent on the interaction between the compound's polarity and the composition of the mobile phase. Generally, more polar compounds, such as EGCG and Rutin, tend to have low Rf values because they are more strongly retained in the stationary phase, while semi-polar compounds, such as Quercetin, exhibit intermediate Rf values (0.50–0.56) in a semi-polar mobile phase, and non-polar compounds, such as Beta-Sitosterol, migrate farther in a non-polar mobile phase. With the appropriate adjustment of the mobile phase solvent composition, TLC-Densitometry has proven capable of analyzing multiple samples simultaneously, providing specific and valid results in determining the levels of various classes of secondary metabolites, including flavonoids, phenolics, and triterpenoids, in pharmaceutical and herbal preparations.

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