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Development and Analysis of Analytical Methods for Determination of Catechins and Quercetin in Natural Products: A Review

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ABSTRACT

Catechins are polyphenolic compounds that have the potential as an antioxidant and antibacterial as well as safe for use in food processing, and the physicochemical properties of the catechins may be a drug dosage formulations. Catechins can be used for pharmaceutical preparations. Catechins are a group of secondary metabolites that are naturally produced by plants and are included in the flavonoid group while quercetin is a flavonoid compound that was found in many vegetables and fruits. In addition to having a very strong antioxidant activity, quercetin also has other biological activities such as antiviral, antibacterial, anti-inflammatory, anticancer, and also can serve as a hypo-allergenic, and hypertension. In addition, quercetin was also used for a number of pharmacological activities such as diabetes and wound healing drugs. Several studies have shown that quercetin has significant activity in inhibiting some cancer cells such as breast, prostate, colon, and lung. Catechins and quercetin has been widely used in Indonesia for treatment. Over the years, a variety of analytical methods have been developed.This review focuses on the latest developments in analytical techniques to analyze catechin and quercetin contained in the products of natural materials. This review provides some insight into the analytic methods, UV-Vis Spectrophotometry, High Performance Liquid Chromatography (HPLC), Mass Spectrometry High Performance Liquid Chromatography (HPLC-MS), Liquid Chromatography Vacuum (VLC) using a gradient elution and Thin Layer Chromatography (TLC). The presence of catechin compounds in each fraction of the VLC process was monitored by TLC. Catechin levels were determined by the UV-Vis spectrophotometer. Based on the results of the UV-Vis spectrophotometer, the levels of natural ingredients were adjusted to international standards. The results of Liquid Chromatography Mass spectrometry (LC-MS) showed that natural materials were analyzed which contains catechins and quercetin.

Keywords: Catechins, quercetin, analytical techniques, natural ingredients.

INTRODUCTION

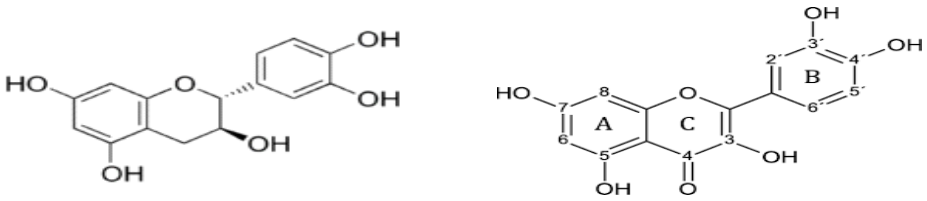
Indonesia is a country that is rich in biodiversity, especially in plants. More than 30,000 plant species found in the archipelago used for treatment. Along with the tendency of modern society to use products derived from natural ingredients for health improvement. So the safety, benefits, and quality of herbal medicinal products become important considerations. It is a challenge for developers of science and technology in the processing of medicinal plants. [1]

Catechins are flavonoid compounds that can be found in green tea, black tea, Gambir, grapes, and other food crops such as fruits and cocoa. [2] Catechins weakly acidic (pK_a^1 = 7.72 and pK_a^2 = 10.22), soluble in water, and easily oxidized in the open air and at a pH close to neutral (pH 6.9). [3]

Quercetin is categorized as flavonol, one of six subclasses of flavonoid compounds. The International Union of Pure and Applied Chemistry (IUPAC) nomenclature for quercetin mention is 3,3 ',

4', 5,7-pentahydroxyflavanone. Quercetin is the aglycone. Aglycone is not the sugar while glicon component is a sugar component. Various flavonol created by differential placement phenolic-OH group and sugar (glicon). All flavonols including quercetin have in common is a 3-hydroxy flavone. [4]

Quercetin is a flavonoid reported to show some biological activity. This activity was attributed to the antioxidant properties of quercetin, such as the ability to capture free radicals and reactive oxygen species such as superoxide anion and hydroxyl radicals. [20] And quercetin has shown the ability to prevent the oxidation of Low Density Lipoprotein (LDL) so that quercetin helps in the prevention of certain diseases, such as cancer, atherosclerosis, and chronic inflammation. [5]



(a) (b)

Figure 1.The chemical structure of (a) catechin, (b) quercetin.

This review focuses on the latest developments in analytical techniques to analyze catechin and quercetin contained in the products of natural materials. This review provides some insight into the analytic methods, UV-VIS Spectrophotometry, High Performance Liquid Chromatography (HPLC), Mass Spectrometry High Performance Liquid Chromatography (HPLC-MS), Thin Layer Chromatography (TLC), Liquid Chromatography Vacuum (VLC), Liquid Chromatography Spectrometry Mass (LC-MS) were used to analyze catechin and quercetin in products of natural materials.

METHOD ANALYSIS

UV-VIS spectrophotometry

There are a few have used the UV-Vis spectrophotometry method for analyzing levels of catechins as a product of natural ingredients in pharmaceutical preparations (Table 1).

Table 1. Analysis of catechins using UV-Vis spectrophotometry method

No.	Sample	Solvent	Wavelength	Concentration range	References
1	Gambir	Ethanol	279 nm and 300 nm	0.02-0.06 µg/mL	[3, 4]
2	Green tea	Methanol	279 nm	5-25µg/mL	[5,6]
3	Black tea	Methanol	257 nm	5-25 µg/mL	[9]
4	Cacao fruit	Ethanol	434.2 nm	20-100 µg/mL	[11]
5	Meniran	Ethanol	434.2 nm	20-100 µg/mL	[12]

Determination of catechin levels in Gambir samples using ethanol to obtain a variety of solution concentrations, namely 0.02 µg/mL, 0.03 µg/mL, 0.04 µg/mL, 0.05 µg/mL, 0.06 µg/mL. Then the absorbance was measured by UV-Vis spectrophotometer at the wavelength of maximum. The catechin content in the solution was calculated using a calibration curve. [24] Measurement of absorbance of blank solutions, standard catechin solutions, and sample solutions was carried out by ultraviolet spectrophotometry at wavelengths of 279 nm and 300 nm. The absorbance of the sample at 300 nm is not more than 0.03. [6]

Determination of catechins in green tea leaves samples and dissolved in methanol standard solution made in order to obtain various concentrations of 5 mg/mL, 10 mg/mL, 15 µg/mL, 20 mg/ml, and 25 mg/mL. [7] Then using a UV-Vis

spectrophotometer by measuring the absorbance of standard solutions and sample extracts at a wavelength of 279 nm. [8]

The longer brewing a higher level of catechins obtained, because of the more time spent on extractable. Because catechin requires a longer time to be extracted. [7] Highest levels of catechins in the brewing temperature 85^{o}C with a 5-minute brewing time of 1.1427% and the lowest level at 100°C for 10 min brewing 0.9314%.

Determination of catechin levels in black tea leaves samples and dissolved in methanol with various concentrations of standard solutions made 5, 10, 15, 20, 25 µg/mL by UV-Vis spectrophotometry method at a maximum wavelength of 257 nm. [9] The data shows that the catechin content of fresh tea leaves is in accordance with research (Balittri, 2013) [10] which states that the catechin content of fresh tea leaves is between 13.5–31%. Assay of catechins in cocoa fruit samples were dissolved in ethanol with various concentrations of a standard solution made 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL. Absorbance was measured at λ 434.2 nm using UV-Vis spectrophotometry method. [11]

Assay of flavonoids, the addition of potassium acetate is to detect the presence of the 7-hydroxyl group while the treatment of incubation for 30 minutes performed before the measurement was intended to run perfectly reaction, thus providing maximum color intensity. Assay of the methanol extract of flavonoids from pod husks done troplo and obtained amounted to 0.2371 ± 0.0004%. [11] Assay of catechins in the sample was dissolved in ethanol meniran made various concentrations of a standard solution 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL. Absorbance was measured at λ 434.2 nm using Uv-Vis spectrophotometry method. [12]

Table 2. Analysis of quercetin using UV-Vis spectrophotometry method

No.	Sample	Solvent	Wavelength	Concentration range	References
1	Leaf wear	Ethanol	350-500 nm	2-10 µg/mL	[13]
2	Bark of wood raru	Ethanol	438 nm	20-120 µg/mL	[16]
3	Avocado rind	Ethanol	435 nm	6-14 µg/mL	[18]

Determination of quercetin levels in the leaves of the leaves dissolved with ethanol so that the solution was made with various concentrations of 2, 4, 6, 8, 10 µg/mL then absorbance and absorption were measured on UV-Vis spectrophotometry at wavelengths 350-500 nm. Assay of the ethanol extract of leaves flavonoid kelakai done by colorimetry with aluminum chloride. The analysis was done by making the mother liquor quercetin, a solution of the standard series, the wavelength determination, the determination of the absorbance levels of flavonoids, and calibration of the measurement results with the standards that have been made. [13]

In a study (Suhartono et al., 2012) [14] found that levels of flavonoids contained in the water extract of leaves kelakai has a flavonoid content average of 14.5 mg/mL ± 0.7 or ± 0.00145 0.00007%. The results showed that levels of flavonoids contained in the ethanol extract of the leaves kelakai has a flavonoid content average of 2.2159 ± 0.083%. The ethanol extract resulted in higher levels of flavonoids than the water extract, suggesting that ethanol can extract flavonoids. [15] Determination of quercetin levels in raru bark was dissolved with ethanol and then made various concentrations of 20, 40, 60, 80, 100, and 120 µg/mL and their absorbance was measured in UV-Vis spectrophotometry with a wavelength of 438 nm. [16]

Qualitative results on the ethanol extract of raru bark formed a red color indicating the sample contained flavonoids. The results of a quantitative analysis of the average levels of flavonoids in stem bark ethanol extract timber raru is 3.6922%. [16] According to (Ministry of Health of the Republic of Indonesia, 2014) [17] The total flavonoid concentration range based on absorbance values ranged from 0.2 to 0.8.

Consecutive absorbance values obtained in the ethanol extract samples of 0,039; 0.038; 0.037. The results obtained from the ethanol extract of raru bark contained flavonoids of 4.3939%.

Determination of quercetin levels in the skin of an avocado was diluted with ethanol and then made the concentration that is, 6, 8, 10, 12, and 14 mg/mL and measured absorbance determined using UV-Vis spectrophotometry method at a wavelength of 435 nm. [18] In the measurement of the total flavonoid compound, the sample solution was added to $AlCl_3$ to form complexes, resulting in a shift in wavelength toward visible (visible) which was marked with a solution that produces a more yellow color. The results of this study obtained a total flavonoid content of the ethanol extract of the skin of an avocado (Persea Americana Mill.) Of 4.0122 mg.QE/g extract.

High Performance Liquid Chromatography (HPLC)

There are a few have been using the HPLC method for analyzing levels of quercetin as a product of natural ingredients in pharmaceutical preparations (Table 3). HPLC quantitative analysis performed at a wavelength of 190 to 400 nm, Separation was performed using Zorbax column Eclipse XDB-C18 (250 mm x 4.6 mm, which is 5m), from Agilent Technologies (USA). The column temperature is maintained at 25^{o}C. Elution was carried out using a mobile phase A (0.1% TFA in acetonitrile 5%) and mobile phase B (0.1% TFA in acetonitrile) with a gradient elution program at a flow rate of 0.8 mL/min. The following gradient was applied: 0-10 minutes 88-82% A, 10-15 min 82% A, 15-30 min 50% A. The UV Absorbance was monitored at a wavelength of 280 nm. The solvent for the mobile phase was filtered through a 0.45 µm membrane filter. 10 mL of each sample and standard was injected manually into the HPLC system. [19]

Results of testing the total phenol at Gambir extract shows that there are real differences in the use of different solvents on the content of phenol. The ethanol extract: ethyl acetate (1:1) has the highest phenol content than any other extract, amounting to 62.13% ± 0.53%. This shows that the use of ethanol: ethyl acetate (1:1) more effectively dissolve the components of phenolic Gambir compared to other solvents were distilled water, distilled water: ethanol (1:1), ethanol and ethyl acetate, which each have a total phenol by 45.57% ± 0.45%, 43.69% ± 0.62%, 43.75% ± 0.0% and 47.44% ± 0.08%. [20] Determination of quercetin content in the ethanol extract of the leaves miana by HPLC with C18 column, the maximum wavelength of 369.11 nm with a mobile phase methanol: aquabidest (59:41) flow rate of 1 mL/min. [21] Determination of the maximum wavelength done merunning quercetin solution concentration of 3 mg/mL using UV-Vis spectrophotometer in the wavelength range 300-700 nm in order to obtain maximum wavelength of 369.11 nm. Qualitative analysis was done by comparing the retention time of the same (identical) of the sample solution chromatogram with the chromatogram of reference standard solution of quercetin on the same HPLC conditions. [22]

The results obtained by quantitative analysis of quercetin levels in samples R1 is 3.449 mg/g, while levels of quercetin in the replication sample R2 is 2.796 mg/g and the final results of quantitative analysis on a sample of the ethanol extract of the leaves miana (*Plectranthus scutellarioides* (L.) R.Br.) containing average levels of quercetin that is 3.122 mg/g and quercetin levels in percent of 0.312%. [21] The results showed the retention time to quercetin as a comparison, the standard solution of quercetin 50 mg/mL in 4.183 minutes, a sample with a concentration 1000 mg/mL in 4.563 minutes, and additional sample (concentration of 1000 ug/ml + 50 mg/mL) in 4.363 minutes. [21]

The chromatogram spectrum of the compound quercetin was detected at a wavelength of 273 nm with a retention time of 6.116 minutes. The content of quercetin

in leaves of starfruit according to research done by (Cabiri et al., 2011) [25] The highest levels of quercetin were found in the ethyl acetate fraction of 6.37% and 4.49%, while the content of quercetin in ethanol extract of 0.48% and 0.83%.

The HPLC system consisting of a Jasco system AS-1555 autosampler with detector UV/Vis-2077, UV-Plus, and pump PU-2080 Plus. The data were recorded and analyzed using Azur 5.0 software installed on a Dell computer system 5150. The analysis was performed under an isocratic reversed-phase system, using Waters C18 Symmetry Shield 5 µ 4.60 x 250 mm column. The cellular phase consists of 10 mM SDS, 10 mM TBAA, and 25 mM citric acid made with 60:40 acetonitrile: water. The injection volume was 20 mL and the detection myricetin, quercetin, and kaempferol were measured at 370 nm. The flow rate was set at 1 mL/minute and every round. Samples were extracted, derived from the leaves treated with the reflux temperature in dilute hydrochloric acid, is for a period of 30 minutes at room temperature. [26]

The results of physicochemical analysis such as moisture, water, and ethanol soluble extractive materials. It was found that extracts from the Moringa oleifera moderate altitude (Sigi, ± 70 m) indicate the content of dissolved water and ethanol extractive higher problems than other areas. Further identification of the ethanol extract by thin-layer chromatography using the mobile phase n-hexane: ethyl acetate (5: 2) showed that all extracts contain flavonoids (two yellow spots at R_f 0.64 and 0.51), triterpenoids (one brown spot at R_f 0.81), steroids (one green spot at Rf 0.94), tannin (one blackish-green spot on R_f 0.72), saponins (one blue spot) at R_f 0.92) and phenolic (one blue spot blackish at Rf 0.77). Alkaloids are not found in all extract identification. [27]

Measurement of concentrations of quercetin in moringa oleifera leaf extract was carried out by using the HPLC method with quercetin as standard. Separation of the ethanol extract of quercetin on moringa oleifera owned peak resolution which indicates a feasible method used for identification quercetin. Having linearity with a correlation coefficient (R^2) of 0.993. Quercetin content is in the range of 0.0638 to 0.0648 mg/g. Statistical analysis showed that the concentrations of quercetin differ significantly between regions ($p < 0.05$). [27]

Quantitative determination of quercetin done using Reverse-phase high-performance chromatography (RP-HPLC) with a UV-visible detector. Simple and fast chromatographic separation was performed by using the C18 Zorbax column (4.6 × 150 mm 5µm) using isocritical flow with a mixture of water and methanol (60:40) with a flow rate of 1 mL/min. Detection was carried out at 370 nm. Linear regression data for the calibration plot showed a good linear relationship with R^2 = 0.996. The comparative study methanol and ethanol extract of the leaves, bark, and flowers Tecomella undulata (seem.). The results support the view that the arial part of Tecomella undulata (seem) can be a potential source of natural antioxidant, anticarcinogenic, and drugs other essential drugs. [28]

Chromatographic separation was performed at Acclaim C18 column (5 µm particle size, 250 x 4.6 mm), liquid chromatography Dionex Ultimate 3000 and the detection is made at three different wavelengths (272, 280, and 310 nm) using a mobile phase of acetonitrile and 1% solution dilute acetic acid with gradient elution. HPLC analysis was carried out with Dionex Ultimate 3000 liquid chromatography (Germany) with four solvent pump delivery system quarter (LPG 3400 SD) including a detector array diode (DAD 3000) with a 5 cm flow cell, manual injection valve sample equipped with a 20 l loop and Chromeleon 6.8 system manager as a data processor. The separation was accomplished by Acclaim TM 120 column reversed-phase C18 (5 µm particle size, Indo. 4.6 x 250 mm). [29]

Table 3. Analysis of quercetin High Performance Liquid Chromatography (HPLC) method

No.	Sample	Column	Mobile Phase	Detector	Chromatographic conditions	References
1	Gambir	Zorbax Eclipse XDB-C18 column (250 mm x 4.6 mm, i.e. 5 µm)	The mobile phase A (0.1% TFA in acetonitrile 5%) and the mobile phase B (0.1% TFA in acetonitrile)	190nm-400 nm	Flow rate: 0.8 mL/min Column temperature: 25°C	[19]
		C18 reversed-phase column (250 x 4.6 mm/5 µm)	0.1% Tri Floro Acetic Acid (F_3COOH) in water (eluent A), acetonitrile: A (50:50) (eluent B)	Detected at a wavelength of 280 nm	Flow rate: 1 mL / minute Column temperature: 40°C	[20]
2	Miana leaves	C18 column	Metanol: aquabides (59:41),	369.11 nm	Flow rate: 1 mL/minute	[21]
3	Rosa Roxburghii Tratt	Biopearl-HC C18 column (4.6 mm × 200 mm × 5 m)	Methanol and pure water	270 and 368 nm	Flow rate: 1.0 mL/min Column temperature: 30°C	[23]
4	Star fruit	Sun Fire C18 column size (4.6 × 150 mm)	Acetonitrile: water: acetic acid (10: 90: 0.2, v/v)	Photodiode Array Detector (PDA) at 273 nm	Flow rate: 1.0 mL/min	[24]
5	Moringa	Waters C18 SymmetryShield 5 µ 4.60 x 250 mm column	10 mM SDS, 10 mM TBAA and 25 mM citric acid were made with 60:40 acetonitrile: water	UV/Vis UV 370 nm	Flow rate: 1.0 mL/min	[26]
		Column C18 measures 250 mm × 4.6 mm	Methanol: water (90:10, v/v)	Wavelength detection at 370 nm	Flow rate: 1.0 mL/min	[27]
6	Rohida/honey tree	Zorbax -C18 (5µm 4.6 × 150mm)	Mixture of water and methanol (60:40)	UV-VIS detector 370 nm	Flow rate: 1.0 mL/min Column temperature: 30°C	[28]
7	Tempuyung	Acclaim column C 18 (particle 5 µm size, 250 x 4.6 mm)	The mobile phase of acetonitrile and 1% dilute acetic acid solution (1: 9)	Wavelengths (272, 280, and 310 nm)	Flow rate: 1.0 mL/min	[29]

Identification and quantitative standards that a significant recovery rate of phenolics and flavonoids worth mentioning. Therefore, the method under consideration was characterized by precision, accuracy, precision, and can be used for qualitative as well quantitative test. HPLC established the testing showed good separation of compounds and methods developed are linear, sensitive, accurate, precise, and reproducible. A high recovery percentage (96-103%), a low coefficient of variation (R^2> 0.99), and low limits of detection (LOD) and the limit of quantitation (LOQ). [29]

Mass Spectrometry High Performance Liquid Chromatography (HPLC-MS)

Agilent 1260 HPLC Diamonsi C18 (2.1 mm × 150 mm × 5 µm) was used in the analysis. The mobile phase consisted of methanol (solvent A) and pure water (solvent B), and the flow rate set at 0.80 mL/min. The optimal gradient program starts with 50% methanol at the time of injection and increases linearly to 70% methanol for 2 minutes, which was maintained for 5 minutes min. Furthermore, the gradient increased linearly to 90% methanol for 2 minutes and held for 5 minutes, followed by a decline back to 50% acetonitrile for 0.1 minutes and held for 5 minutes until the next injection.

The column oven temperature was maintained at 30°C, and the sample injection volume was 5 mL. The detection wavelengths used for catechins and quercetin are λ_1 = 270 nm, and λ_2 = 368 nm, each MS analysis was performed using Agilent 1100 series ion trap mass spectrometers with electrospray ionization sources in the positive ion detection method. Follower parameters used: spray voltage, 4,000 V; ionization temperature, 350°C; pressure sprayers, 35 psi; nitrogen flow rate, 8 L/min; and bulk scan range, 200 - 800 m/z. [23]

Based on the results of UV, Rosa roxburghii Tratt content is 73.85%, which is

in accordance with national standards. The results of LC-MS showed that Rosa roxburghii Tratt contains flavonoids quercetin, 34.26%, with a Relative Standard Deviation (RSD) of 2.88% and 2.97% catechin content with RSD 1.49%. [23]

Vacuum Liquid Chromatography (VLC)

Isolation catechins from Gambir extract conducted by the percolation method using ethyl acetate solvent and the solvent evaporated. Purification of catechins was conducted using a gradient elution VLC using hexane-ethyl acetate. The presence of catechin compounds in each VLC process fraction is monitored by TLC. The fraction with R_f same and single TLC spot was collected and evaporated. Catechin levels were determined by UV spectrophotometer. The dried isolates were identified and characterized by HPLC, LC-MS, and NMR. [30]

Percolation performed using ethyl acetate, the resulting isolates of catechins as much as 80.74% to 99.80% ± 0.132 level. The results of monitoring by TLC shows there are impurities in these isolates, so the purification process is carried out with the VLC. The VLC process produces fractions, which were then monitored by TLC using the chloroform: ethyl acetate: formic acid (5: 4: 1) eluent. The fractions that have the same spot with R_f were grouped together and compared with the catechin standard and evaporated. There are at least 4 fractions aggregated results of the VLC. The results of monitoring using TLC, the catechins are in the fraction. Pure catechins obtained from the VLC process in this study is 74.79%. [30]

Thin Layer Chromatography (TLC)

On the identification of compounds of catechins in the extract Gambir performed using TLC with the following parameters: mobile phase: acetic acid 15%, stationary phase: a plate of cellulose with test solutions: 0.1% solution of catechins, which is equivalent to 1 gram of extract Gambir in methanol (0, 1% catechins in methanol). Identification of chromatograms using UV light at a wavelength of 254 nm and detection solutions: Solution 1% $FeCl_3$. [6]

Shows that the standard catechins have equal chromatograms with catechins in the extract, either in terms of the increase in the chromatogram with equal heights and colors that appear once identified with 254 nm UV light and reagent $FeCl_3$. Standard catechins have equal chromatograms with catechins in the extract, either in terms of the increase in the chromatogram with equal heights and colors that appear once identified with 254 nm UV light and reagent $FeCl_3$. [6]

On the results of the identification of the compound quercetin in an apple, peel extract waste by TLC, with the observation of the chromatogram under UV light visible 254 nm yellow stains that can be used as a positive guide their quercetin. The results of the identification of the compound quercetin on Thin Layer Chromatography extract used in pure quercetin as a comparison as well as the determination of the standard f value of the compound quercetin. This identification using TLC Silica Gel 60 fluoresces at a wavelength of 254 nm as the stationary phase. Eluent or mobile phase at this identification is used MeOH and $CHCl_3$ ratio (9:1), eluent election is because given the nature of the solubility of the compound quercetin. [31]

The results were determined by comparing the value of R_f on pure quercetin and R_f value in waste ethanol extract of apple skin, the pure quercetin obtained an R_f value of 0.87 and the ethanol extract of apple peel waste obtained R_f value of 0.85. R_f value differences between the standards and values of quercetin R_f ethanol extract of apple peel waste that is not much different can prove that the ethanol extract of apple peel waste was compounded quercetin. [31]

CONCLUSION

Overall, a variety of analytical methods have been used to determine the levels of catechin and quercetin in the products of natural materials. UV-VIS

spectrophotometry is easy to apply. However, high performance liquid chromatography (HPLC), high performance liquid chromatography mass spectrometry (HPLC-MS), and thin layer chromatography (TLC) method were often used in biological matrices of biological research because they can detect samples with low concentrations.

Authors' Contribution:
Both the authors contributed equally in the preparation of the manuscript.
Conflict Of Interest: No conflict of interest.

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