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Review Article

Various Analytical Techniques for the Isolation and Identification of Flavonoid Compounds: A Descriptive Review

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Abstract

Flavonoids are phytochemical compounds that can be found in a wide range of plants, including vegetables, fruits, and leaves. This vast set of phenolic plant elements can be split into numerous classes based on their diverse structures, including Flavanones, Flavanols, Flavonols, Flavones, Isoflavones, and Anthocyanins. Interestingly, they possess various applications such as natural dyes, medicinal uses, and food sources. Flavonoids have been shown to have anti-cancer, antioxidant, anti-inflammatory, and anti-viral properties in clinical studies. They also have cardio-protective and neuroprotective effects. In addition, they are responsible for the presence of different colors and flavors in various fruits, flowers, and food sources. Multiple spectroscopic techniques, including Infrared spectroscopy (IR), Ultraviolet spectroscopy (UV), and Nuclear magnetic resonance (NMR) spectroscopy, are being used to identify the structure of flavonoids. UV-Vis spectroscopy data can be used to estimate the position, type, and number of substituents present in a conjugated system. IR spectroscopy is primarily used to determine the type of functional groups and aromatic ring substitutions. The structure of Flavonoids, their type, number of protons, and carbons can be determined by NMR spectroscopy. The current review was based on searches of the Scopus, Web of Science, and Google Scholar databases for literature reviews. The purpose of this review article is to demonstrate the structure, function, and different extraction methods of flavonoids. It also summarizes the isolation and analytical identification techniques for flavonoids.

1. Introduction

Polyphenols are compounds generally present in plants and are essential components of food. Flavonoids are one of the most common types of polyphenols. Flavonoids are the principal bioactive ingredients of many medicinal and food plants, making them a significant class of natural products. They have been shown to provide a wide range of health advantages, including antioxidant, anti-inflammatory, and anti-cancer properties coupled with their capacity to modulate vital cellular enzyme functions [1].

These compounds serve as natural pigments that are primarily stored in plant edible parts such as vegetables and Fruits. The reason for the red and dark blue colored berries and the color of different citrus species is the presence of flavonoid compounds. Flavonoids have been used for medicinal purposes, which have the same activity as Vitamins, mainly to protect capillaries from damage and are beneficial for blood vessel function. In addition, many flavonoids have antioxidant properties to remove free radicals in the body. Flavonoids have a basic chemical structure of 15 carbons (C6-C3-C6 skeleton), which contains two aromatic rings (A & B) connected to a non-aromatic pyran ring [1, 3, 4].

Flavonoids are found in two forms; free form and glycoside form. From a total of 4000 flavonoid compounds, more than 500 types are considered in the free form. The most abundant observed flavonoids are Polygonaceae, Rutaceae, Leguminosae, Umbelliferae, and Compositae.Flavonoids have varying degrees of Solubility, but they are typically soluble in water and alcohols but not in organic solvents [5, 6]. The motivation for developing flavonoid component analysis methods in natural products has shown to be complex. Hundreds of papers on flavonoid analysis have been published in the last two decades. For sample preparation, isolation, and identification, traditional and modern analytical techniques have gotten attention [2]. Therefore, the purpose of this review article is to demonstrate the structure, function, and different extraction methods of flavonoids. It also tends to summarize the modern and traditional extraction methods of flavonoids, along with isolation and analytical identification techniques.

2. Different structures of Flavonoids

Flavonoids as a common type of phenolic plant are introduced as 2-phenyl-benzo-γ-pyrone derivatives. These molecules contain two benzene rings, generally referred to as A & B, linked by a heterocyclic pyran ring (C), see the generic structure of Figure 1.

The carbon backbone of the flavan system is a common component of all flavonoids' chemical structures (C6-C3-C6 skeleton). Chalcone is formed when A and B rings are condensed. The formed chalcone then undergoes cyclization isomerization and the Flavanone-initial compound is formed, which is a building block for flavonoids and other groups [1, 4, 7]. The majority of Flavonoids are Chromone derivatives with the key structure of 2-phenylchromone, Figure 2 [2].

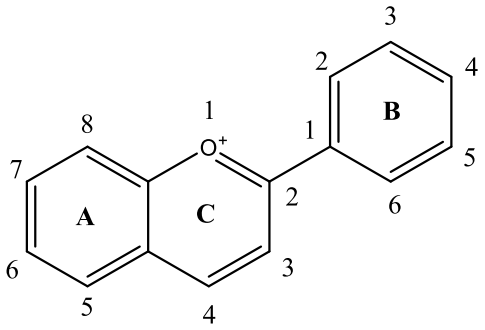
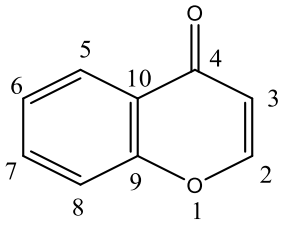
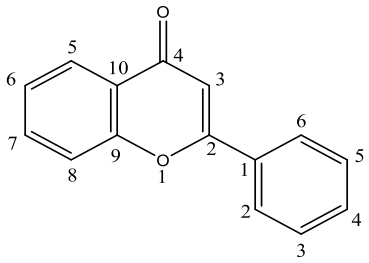


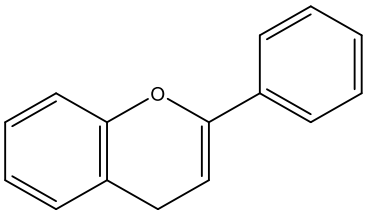
Figure 1. The structure of Flavylium cation [3]



Chromone



2-Phenylchromone



C6-C3-C6 Skeleton

Figure 2. Basic structure of Flavonoids [4]

3. Classification of Flavonoids

Flavonoids can be classified into six main types, including flavanols, anthocyanins, flavanones, flavonols, isoflavones, and flavones. These different classes are classified depending on their structure differentiation. Figure 4 shows their basic chemical structure. Furthermore, flavonoids can be classified further

based on the location of the connection between rings A and B, the oxidation degree of the C3 base structure, and the polymerization degree. Other kinds of flavonoids are also found such as Biflavonoids, Prenylflavonoids, Flavonolignans, Flavonoid glycosides, Proanthocyanins, and Chalcone [1]. The main type of flavonoids are classified and presented in Figure 3.

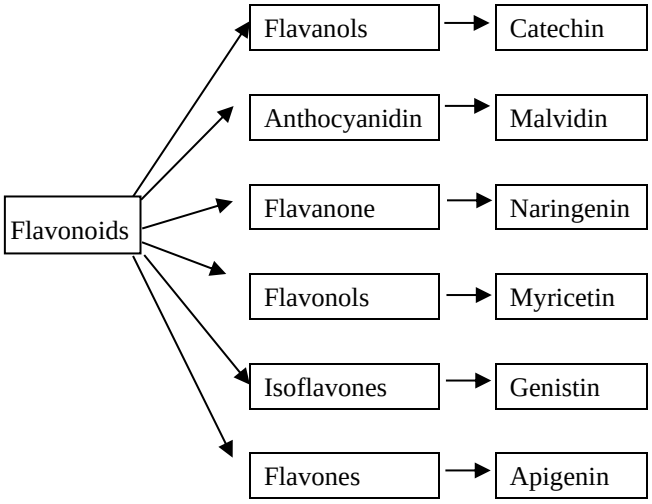


Figure 3. The distribution of Flavonoids found in plants [3]

3.1.Flavonols (3-Hydroxyflavones)

Flavonols are the most prevalent flavonoids found in plants. There is a double bond among Carbon2 & Carbon3 in their structures, which are bound to hydroxyl groups. Myricetin, Quercetin, Isorhamnetin, and Kaempferol are the most important phytochemicals that belong to flavonols. Flavonols can be found in a variety of colors (White to pale yellow). They exist in two states in nature: Aglycones and Glycosides Kaempferol and Quercetin [5, 6].

3.2.Flavones (2-phenyl-4H-chromen-4-one)

Flavones are found in the majority of land-plant families. Flavones can be found in over 70 different plant families. Flavone is formed when two hydrogen atoms are subtracted and a double bond is formed between the C2 and C3 rings. Flavones can exist in many components of plants, atop and underneath the ground, in generative and vegetative organs, such as the trunk, leaves, flowers, bark, thorns, roots, rhizomes, flour, fruit, and nuts, as well as leaf and root exudates or resins [5, 7].

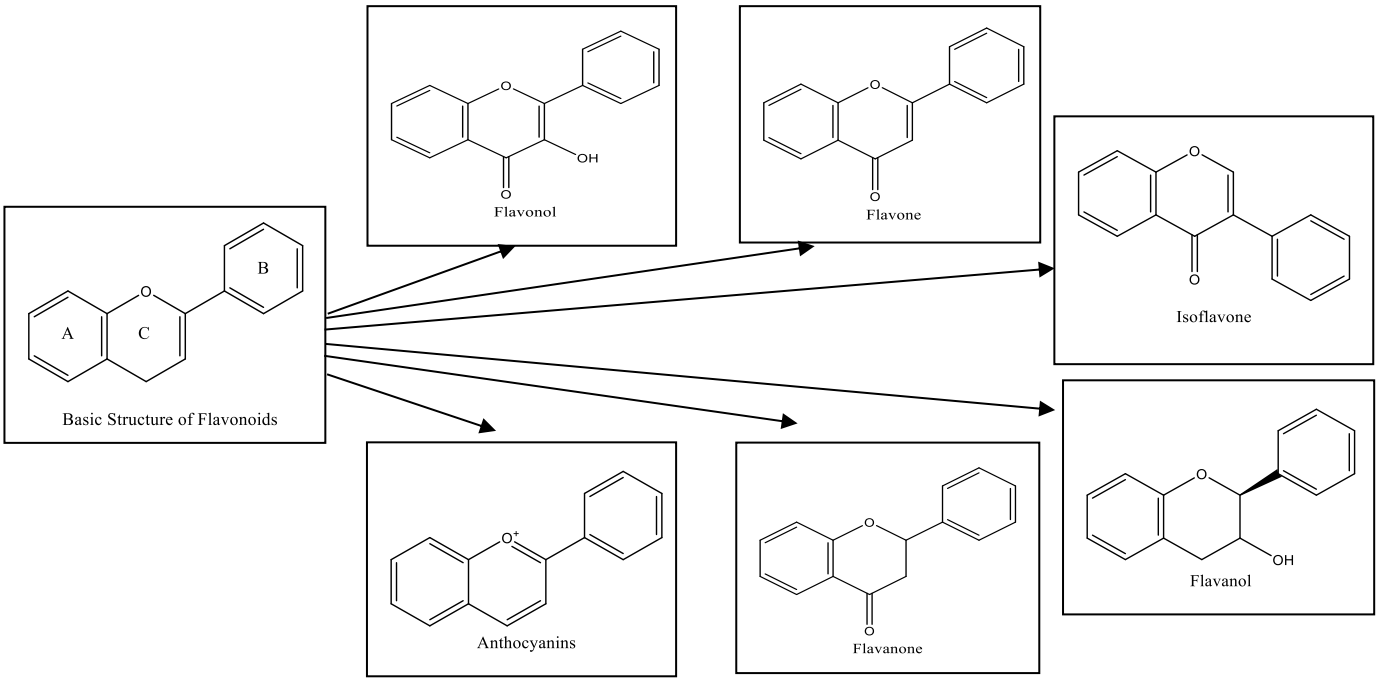


Figure 4. Flavonoids' basic chemical structures and classification

3.3.Isoflavones (3-phenylchromen-4-one)

Isoflavones are another type of flavonoids, and owing to their strong estrogen activity they are generally referred to as phytoestrogens. They are formed when ring B is fused with the Carbon-3 of ring C. Isoflavones can be discovered in plants such as the Leguminosae family (Soybeans, Red clover leaves, and Alfalfa sprouts). They have important roles in different fields for instance cosmetics, medicine, and nutrition [5, 8].

3.4. Flavanols (Flavan-3-ols)

Flavanols are also considered as Catechin. They share a flavonoid chemical structure but have distinctive hydroxylation patterns in rings B and A, as well as asymmetrical carbon stereochemistry in ring C (Carbon 2 & Carbon 3). There are two classes of Catechins: free Catechins and esterified Catechins. Because of their size, monomers (catechin), or forms of polymers (Proanthocyanidins), Flavanols are the most complex classes of flavonoids. They are the main components of Green tea [5, 9].

3.5. Flavanones (Dihydroflavones)

The absence of the C2=C3 double bond in the ring C results in a different Flavonoid structure known as Flavanones (Dihydroflavones). Glycones and Glycosides are forms of flavonoids in nature, which are

considered Naringenin, Hesperetin, and Eriodictyol. This class of flavonoids could be present in aromatic plants for example mint, tomatoes, and citrus such as grapefruit [5, 10].

3.6. Anthocyanins (Flavylium)

Anthocyanins are mainly present in the glycosidic type in nature. The appearance of different kinds of plant pigments is due to this type of flavonoids. There are over 500 different types of anthocyanins, which are the result of glycosylation, hydroxylation, and methoxylation patterns in the B ring. Pelargonidin, Cyanidin, and Delphinidin are the most common compounds that represent this class of flavonoids [5, 11].

4. Flavonoids Natural Sources

Flavonoids can be found in every part of the plants. Vegetables, Fruits, Seeds, Flowers, Beer, Wine, Green, and Black tea are the main sources of flavonoids, which are used by humans in their diets. They provide taste, fragrance, and color to the flowers, fruits, and seeds, also attracting insects, birds, and mammals to aid in the transfer of pollen or seeds.

Table 1. Major classes of Flavonoids, Representative compounds, and Dietary sources [14]

Subclass	Color	Representative flavonoids	Dietary sources	Remarks
Anthocyan	Red, Blue,	Cyanidin	Fruits and Flowers	Natural dyes
s	Violet			
Flavanols	Colorless	Catechins, Epicatechin, Gallocatechi	Hops, Apples, Beer, Tea	Astringent
	Yellow	Epigallocatechin gallate,		taste
		Procyanidin	Fruit juice, Wine	
		Theaflavins	Black tea	
Flavanones	Colorless	Hesperidin	Citrus fruits	Bitter taste
	Pale	Eriodictyol, Naringenin	Oranges, Grapefruits, Cumin, Peppermi	
	Yellow	Neohesperidin		
Flavones	Pale yellow	Apigenin, Luteolin, Chrysin	Cereals, Herbs, Parsley, Fruits, Thyme	Bitter taste
		Diosmetin, Luteolin	Flowers, Vegetables	
Flavonols	Pale yellow	Isorhamnetin, Kaempferol, Quercetir	Apples, Cherries, Berries, Broccoli,	
		Myricetin, Rutin	Onions, Kale, Tomatoes, Red wine, Gre	
			buckwheat, Tea	
Flavanonol		Taxifolin	Aurantium, Limon	
Isoflavones	Colorless	Daidzein, Glycitein, Genistein,	Legumes (e.g. Soybeans)	
		Formononetin		

Flavonoids are mostly found in flowers and leaves. As a result, they are the most commonly extracted parts. Fresh leaves and bark are far less suitable sources and more difficult to handle for flavonoids extraction because they contain waxes and resins. Agro-industrial wastes are another source of these phenolic compounds [12, 13]. Humans and animals cannot produce flavonoids, however, their existence in animals is due to eaten plants rich in flavonoids and not synthesized by animals [14]. The food source and representative color of the various flavonoid subgroups are presented in Table 1.

5. Applications and Health effect of Flavonoids natural sources

Flavonoids are used in agriculture as pesticides and medicine as therapeutics. They are vital constituents in different application fields such as medicinal, pharmaceutical, and cosmetics. Flavonoids perform important biological functions in the human body. This is because they have antioxidant, anti-carcinogenic, and anti-inflammatory properties, as well as the ability to modulate essential cellular enzyme functions. Several studies have shown that flavonoids have anti-allergenic, antiviral, anti-inflammatory, anti-diabetic, and vasodilating biological activities. As well as having a significant influence on human health in different areas such as physiological consequences, the nervous system, the prevention of Alzheimer's disease (AD) and the prevention of Stroke. Flavonoids also possess some actions in plants including antioxidants, antimicrobials, visual attractors, photoreceptors, and feeding deterrents. [15, 16].

Flavonoids are widely used as food coloring and sweetening agents in the food industry. Moreover, they are used in the cut flower industry and horticulture due to their characteristic role in flower pigment. The unique flavor of Citrus, as well as common drinks such as wine, beer, and tea, is due to flavonoids' behavior and glycoside structure. The quality of wine is influenced by flavonoids in grapes. Anthocyanins are the main group inherent in wine, which has the responsibility of the grape's color. By controlling the genetics of vacuolar, pH, flower color modification may be obtained. Besides, natural flavonoid-containing plant dyes are sometimes benefitted as mordant dyes, except for Catechins, which are known as direct dyes [3, 17].

6. Separation and Extraction Methods

A variety of modern methods have been used to isolate and extract flavonoids as a bioactive component from natural sources.

6.1. Separation (Isolation) methods

The separation of flavonoids from a mixture of different components is referred to as Flavonoid isolation. The isolation methods are chosen based on acidity, polarity, special structure, and differential molecular weight. The first approach for isolating flavonoids is chromatography.

6.1.1. Silica Gel Chromatography

Silica gel chromatography is a purification technique that uses two phases in a column, one solid and one liquid. The solid phase is usually referred to as the stationary phase, such as silica gel, and the liquid phase is referred to as the mobile phase. When an impure sample is added to the column, fractions of the sample are separated based on the polarity of the stationary phase. Silica gel chromatography is more convenient for isolating low or medium-polar substances. It is a preferred method for the identification and isolation of flavonoids. However, For the isolation of flavonoid glycosides, the reversed-phase C18 silica gel is usually used.

6.1.2. Polydextran Gel Chromatography

Polydextran gel chromatography has the same principle work as Silica gel chromatography but instead of silica gel, polydextran gel is used. Sephadex is the polydextran gel that is most widely used for the isolation of flavonoids. The main process for isolating free flavonoids is adsorption, and the adsorption strength is determined by the phenolic hydroxyl groups. Nevertheless, throughout the separation of flavonoid glycosides, the molecular sieve effect has a great role.

6.1.3. Polyamide Chromatography

Polyamide chromatography is a column chromatography in which polyamide is used as a stationary phase. The isolation process relies on the formation of strong hydrogen bonds between flavonoids and polyamide, which is dependent on the number as well as the location of -OH groups in the flavonoid molecules.

6.1.4. High-Performance Liquid Chromatography

The HPLC is a widely used technique for the isolation of flavonoids as well as other natural products. The selection of the mobile phase and stationary phase affects the isolation's efficiency. The stationary

phase in normal-phase HPLC is polar, and the mobile phase is non-polar; in reversed-phase (RP-HPLC), the stationary phase is non-polar, and the mobile phase is polar. During the normal-phase HPLC process, silica gel and amino columns are frequently used in stationary phases. In RP-HPLC, Phenyl or Amino columns could be used, with C8 and C18 columns being the most convenient. Moreover, Acetonitrile-water and Methanol-water systems are used as mobile phases in RP-HPLC. Minimum amounts of an organic acid (e.g., 2,2,2-Trifluoroacetic acid) should be applied to the mobile phase to increase the separation efficiency [18].

6.1.5. High-speed counter-current chromatography

The HSCCC is utilized to divide the solutes amid the two immiscible liquid phases. A liquid mobile phase, free liquid stationary phase, and centrifugal force field are required. Free liquid means the liquid is not affected by outside forces. The centrifuge's force field keeps the liquid stationary phase in place while forcing the liquid mobile phase through it. The solubility of each compound is different in each liquid phase, thus it can be separated between the two phases efficiently. Flavonoids are isolated effectively by HSCCC. For example, the HSCCC method was used to separate 7 flavonoids from a methanolic extract of Oroxylum Indicum leaves using a two-phase system of $CHCl_3$:CH_3OH:H_2O (9.5:10:5) in a one-step isocratic elution [4].

6.2. Extraction methods

Several approaches for extracting flavonoids have been established in order to boost the extraction yields of these important bioactive chemicals.

6.2.1. Percolation method

Percolation is an extraction method in which a plant is placed in a cone-shaped narrow vessel open at both sides is called a Percolator and a liquid (solvent) is passed through it. It is an extraction process carried out at room temperature by passing a liquid among solid substances drop by drop. It means that the solvent is passed through the plant gradually, stacked with active ingredients when another pure solvent is added above it pushing away slowly [19, 20]. This method is time-consuming because the process involves retaining the interaction of the liquid (solvent) and the plant for a while [5].

6.2.2. Maceration process

Maceration is an extraction method consists of a liquid (water, oil, alcohol, etc.) that a plant immersed in it inside an airtight container. The process was carried out at room temperature with different time consuming according to the liquid and plant material used. This simple extraction method has the drawbacks of taking a long time and having a low extraction efficiency. Total anthocyanins and total phenols were extracted from chokeberry with 50% Ethanol [19, 21], while flavonoids were extracted from Solanum Scabrum leaves immersed in acetone for 72 hours [5].

6.2.3. Refluxing method

Refluxing is an extraction method that includes heating and boiling the chemical materials for a fixed time to become vapor, a vapor is then condensed and move through the condenser and then collected from a container. Reflux extraction requires less time for extraction and also less solvent is needed, thus it is more efficient than percolation or maceration. Thermolabile natural products could not be extracted by this method. Reflux extraction of total flavonoids content from Ginkgo biloba leaves with 70% Ethanol is one example. It is thought that the yield of the product was considerably greater than with the Decoction method [4, 19, 22].

6.2.4. Extraction by Soxhlet

The Soxhlet extractor employs the reflux plus siphoning principles to extract the plant materials via a fresh solvent on a continuous basis. It possesses advantages over reflux and percolation extraction methods. Soxhlet extraction is a continuous automated technique with great extraction efficiency; it consumes less solvent and time than percolation and maceration. Extracting flavonoids from Vernonia cinerea leaves with Ethanol (60%) in 2 hrs is an example of this method. In this method, thermal degradation possibilities will increase due to high temperature as well as long extraction time, such as degradation of catechins in tea [5, 19, 23].

6.2.5. Supercritical fluid extraction (SFE) method

The extraction solvent is a supercritical fluid (SF) in this method. SFs has the same diffusivity as gas and equal solubility to liquid and are capable of dissolving a wide range of natural products. The solvent

properties of SFs alter drastically near their critical points due to slight variations in temperature and pressure. The purpose of the chosen isolation, separation, or purification method may be accomplished by regulating the pressure, temperature, solvent forms, and use of co-solvents during the supercritical fluid extraction process. Ethanol is an example of co-solvent which is typically applied to promote production yield. For instance, the supercritical Carbon dioxide extraction of flavonoids from Liquorice increased production by 2.2 times over conventional alcohol solvent extraction [4].

6.2.6. Microwave-assisted technique

The application of Microwave-assisted extraction (MAE) offers many benefits, such as rising the extraction yield, reducing the thermal deterioration as well as specific heating of plant material. The MAE is often called a green extraction method because it eliminates the use of organic solvents. Good results have been obtained in the flavonoids extraction.

However, it has been confined to laboratories yet, it could also be used in conjunction with other approaches to increase the yield. For example, after microwave operation, reflux extraction was used for a short period in the flavonoids extraction from Ophiopogon japonicas, and the production yield was dramatically increased as a result [4, 19, 24].

6.2.7. Extraction by Ultrasound

Ultrasonic-assisted extraction (UAE), also known as Sonication extraction or Ultrasonic extraction, employs ultrasonic wave energy for extraction. Extraction is carried out by using ultrasonic wave energy. It produces cavitation in the solvent which accelerates the solute to dissolve and diffuse in the solution, and thus enhances the efficiency of extraction. The low extraction time and temperature, the minimum amount of solvent, and energy used are other advantages of UAE.This method is useful for the extraction of natural products, and unstable, and thermolabile substances. UAE is applicable in the quality analysis and extraction of flavonoids even if they are present in a small amount. It is still rarely used in manufacturing goods. However, for example, flavone extraction by ultrasonic from Sophora japonica bud produces a greater yield than that of extraction by reflux method. UAE has advantages over the reflux method in time-saving, energy-saving, and technology properties.

6.2.8. Enzyme approach

During Enzymolysis, impurities can be eliminated such as pectins, proteins, and starches. Mild operating conditions may resolve the imperfections that certain bioactive components may be disintegrated due to high temperature. However, the longtime extraction is the limitation of this process [4]. Flavonoid extraction by this method from the roots of Impatiens glandulifera plant using 80% methanol solvent at 80 C° for 30 min yields 257.34 µg Phenolic acid content/g of dry weight [5].

7. Identification of Flavonoids

There are several analytical identification procedures for the chemical structural analysis of different flavonoids including UV-, IR-, and NMR-spectroscopy.

7.1. Ultraviolet spectroscopy (UV)

The principle of Ultraviolet spectroscopy (UV) works by interacting a sample with light to produce a UV spectrum. Almost all types of Flavonoids are observed by detectors because they are capable of absorbing ultraviolet radiation. The locations, forms, and quantity of substitution groups in the conjugated structures may be estimated by a spectrum of UV. There are two main bands absorbed for flavonoids in methanol. Band I is induced by the Cinnamoyl group electron transition and is observed at 300-400 nm. Electron transition of benzoyl group induced observation of Band II at 240-280 nm, as displayed in Figure 5.

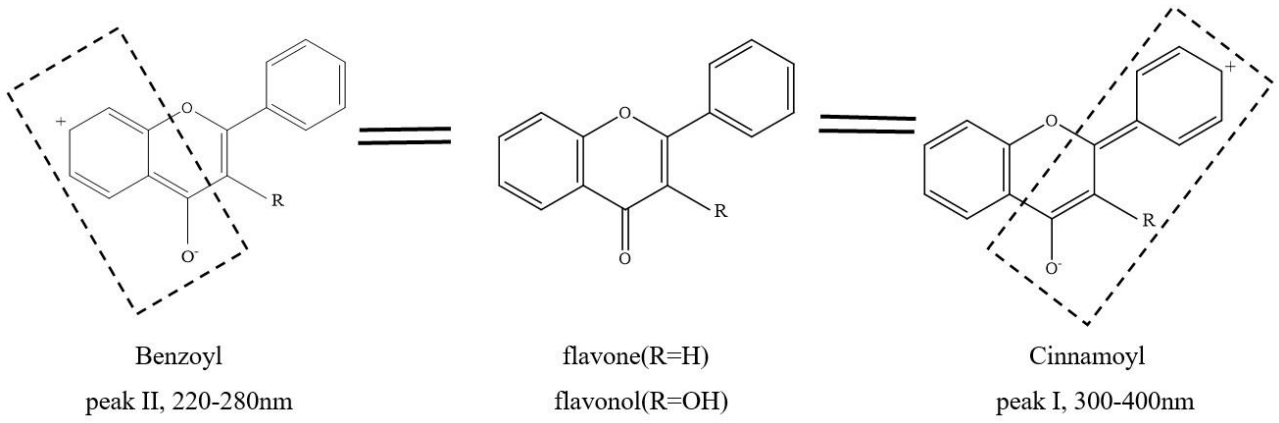


Figure 5. Absorption bands of Flavonoids in the UV region

The determination of structure forms and flavonoids oxygen-bearing groups is shown in Table 2 by the position of peaks, strength, and shapes of Bands I & II. The substituents attached to rings B and A will

influence the locations as well as shapes of Bands II and I. Normally, as the number of -OH groups at Ring B increases, so does the redshift of Band I. Similarly, as the number of -OH groups at Ring A increases, so does the redshift of Band II, but it has only a minor effect on Band I, except for 5-OH. If the hydroxyl is glycosylated, the corresponding bands will be violet shifted 5-15 nm. Besides, if the -OH groups are acetylated, their influence almost disappears. [4].

Table 2. Spectral characteristics of the UV-VIS spectrum of Flavonoids [4]

Type of Structure	Band II (nm)	Band I (nm)
Flavone	250-280	304-350
Flavonol (substituted 3-OH)	250-280	328-357
Flavonol (Free 3-OH)	250-280	358-385
Isoflavone	245-270	310-330 (shoulder peak)
Flavanone & Flavanonol	270-295	300-330 (shoulder peak)
Chalcone	220-270 (weak peak)	340-390
Aurone	230-270 (weak peak)	370-430
Anthocyanidin	270-280	465-560

7.2. Infrared spectroscopy (IR)

The absorption of light by a compound in the infrared region (IR) of the electromagnetic spectrum induces a change in the dipole moment of the molecule and detects functional groups which give IR spectrum. It gives information about different functional groups, as all functional groups correspond to IR absorptions such as phenolic hydroxyl, carbonyl, glycosyl, and phenyl. Figure 6 shows the IR spectrum of flavonol (Quercetin), in which carbonyl groups [C=O] are observed in 1660-1680 cm^{-1}, hydroxyl groups are in the region of 3200–3650 cm^{-2} and 1500, 1580, and 1600 cm^{-1} absorption vibrational regions for benzene rings [4].

7.3. Nuclear magnetic resonance spectroscopy (NMR)

When an external magnetic field is applied to a molecule, the spins of the nuclei of protons and carbons are changed and generate a magnetic field. An energy transfer occurred between the ground state to the excited state which then gives the NMR spectrum. During NMR experiments different kinds of solvents are used such as CDCl3, C5D5N, CD3OD, (CD3)2CO, and DMSO-d6. To perform flavonoids

NMR spectroscopy, the appropriate solvent is DMSO-d6. The signals of flavonoids are scarcely overlappe with solvent peaks (about δ2.5) because all flavonoids are dissolved in this solvent excellently. Otherwise, phenolic hydroxyl group signals are seen clearly in DMSO-d6 solvent. The high boiling point is the main disadvantage of this solvent and thus the sample recovery is difficult at the end of the analysis [4].

7.3.1. ^{1}H-NMR

The accurate information about the proton's structural environment on molecules is given by 1H-NMR spectroscopy [25]. The number of protons, chemical shifts, and coupling constants is informed by this technique. 1H-NMR spectrum could expect different kinds of flavonoids, number and structure of glycosyls as well as alternative modes [4]. Figure 7 represents a 1H-NMR spectrum of quercetin which is a type of flavonols [26].

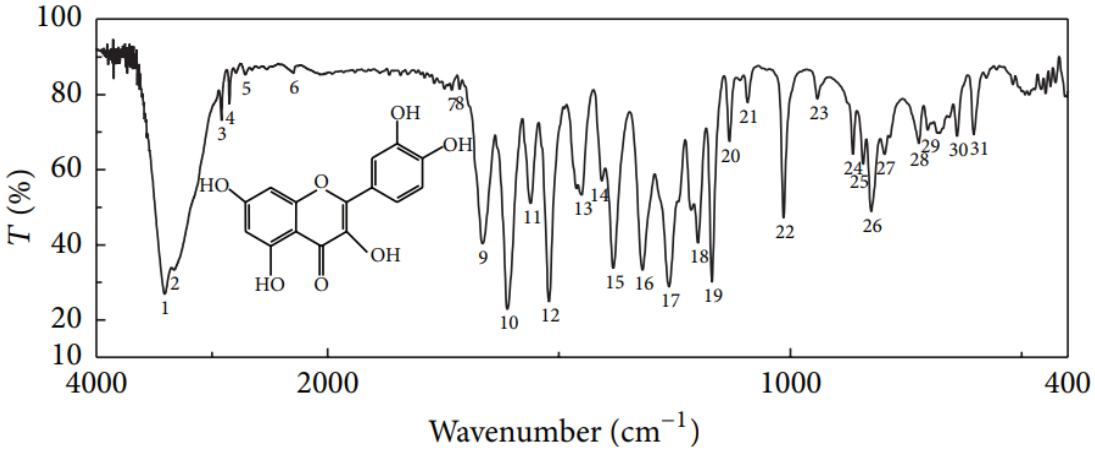


Figure 6. FT-IR spectrum of Quercetin [26]

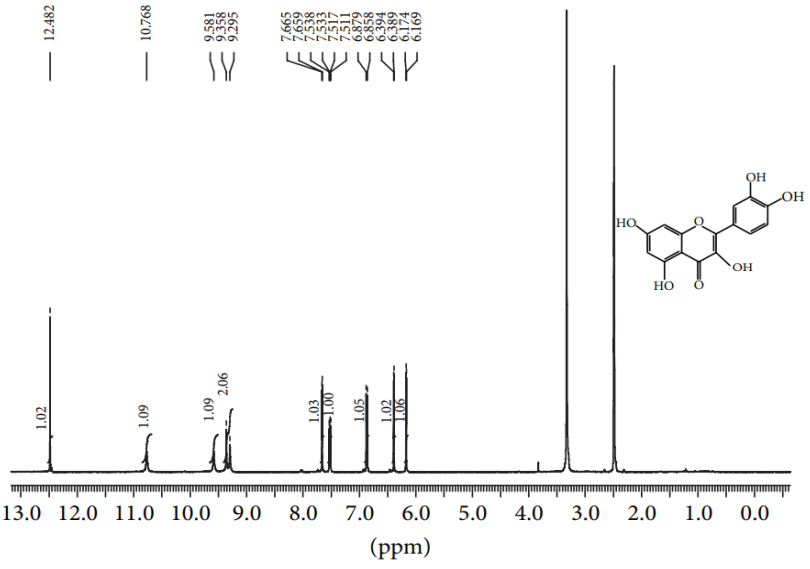


Figure 7. ^{1}H-NMR spectrum of Quercetin [26]

7.3.2. ^{13}C-NMR

The information about the molecule's carbon 'backbone' is provided by ^{13}C-NMR [25]. ^{13}C-NMR spectra can detect different kinds of flavonoids [4]. An example of flavonoids 13C-NMR is elucidated below, and Figure 8 is the 13C-NMR spectrum of quercetin [26].

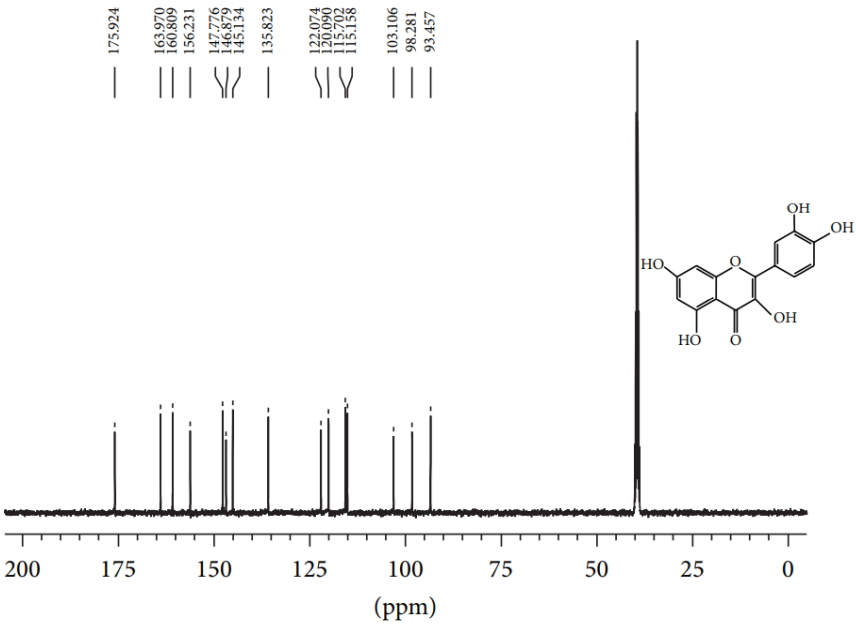


Figure 8. ^{13}C-NMR spectrum of Quercetin [26, 27]

8. Conclusion

Flavonoids are bioactive compounds that are found in fruits and vegetables that people consume daily. Flavonoids have been linked to a variety of health benefits as they are high in antioxidants and provide the human body with natural immune protection against environmental and endogenous toxins. Chromatography appears to be the best choice for purifying impure flavonoids since it is a cost-effective, sensitive, and fast process that yields very pure products. Among different types of chromatography, column and high-speed counter-current chromatography (HSCCC) are the two most widely employed methods for the isolation of flavonoids. Among researchers, HSCCC is preferred over column chromatography due to its ease of use, speed of operation, and potential for high purity product. Traditional extraction methods of Flavonoids frequently result in inefficiency, high energy consumption, and increased solvent consumption. Percolation and Maceration extraction methods need too much solvent in the process and are time-consuming techniques. In contrast, refluxing and soxhlet extraction methods require less time and solvent. Supercritical fluid, microwave-assisted, and sonication extraction have a great capability for inducing a high

product yield. Sonication is the best extraction method for flavonoids because it has great advantages over other extraction methods such as great extraction efficiency, minimal use of solvent, less time and temperature, and higher yield of product. Flavonoid functional groups can be analyzed by IR spectroscopy, number and location of substituents can be analyzed by UV Spectroscopy. The NMR is the most effective method to interpret the structure of Flavonoids as the number plus type of carbons and protons can be determined by ^{13}C-NMR and ^{1}H-NMR spectroscopy. Hence, using information from UV, IR, and NMR spectrums, the chemical structure of the main Flavonoid compounds may be reliably analyzed. Because of their link to human health, further literature reviews and studies are needed to understand better and compare the best analytical procedures for the isolation and analytical identification of flavonoids and their derivatives.

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