A review of extraction and HPLC methods used for Melastomataceae and Asteraceae leaves in the analysis of flavonoids

A review of extraction and HPLC methods used for Melastomataceae and Asteraceae leaves in the analysis of flavonoids

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Abstract. HPLC (high-performance liquid chromatography) is one of the accurate chromatographic techniques that is widely used for analysis. This present review submitted the extraction and HPLC methods for determining the compound in Melastomataceae and Asteraceae families that related with insecticidal compounds. This review discusses the standard used for the compound and chromatographic conditions. There are several species under both families. As for the Melastomataceae family, the species are *Melastoma malabathricum* and *Miconia albicans* meanwhile, the species under the Asteraceae family are *Santolina semidentata, Eupatorium littorale*, and *Vernonia condensata*. This review focus on the flavonoid and phenolic compound as these compounds are categorized as an insecticidal compound. HPLC can be used in conjunction with several detection technologies to identify flavonoids and phenolics in the plant, food, and biological samples because it is a method for separating these molecules. As a result, the HPLC procedure for the chosen molecule was reviewed in this article, which will aid in further analysis.

1. Introduction

Plants consist of a lot of different families such as Melastomataceae and Asteraceae families. These families consist of many species. Plants are categorized as the crucial source for new natural products [1]. Natural products are known to have potential such as antioxidants, enzyme inhibitors, hypocholesterolemic agents, herbicides, immunosuppressive agents, anti-migraine, insecticidal activity, and agents for anti-parasitic [2]. The biological potential actions of the Melastomataceae family include analgesic, antioxidant, anti-inflammatory, and antibacterial capabilities [3-6]. These activities could be linked to the presence of secondary metabolites such as tannins, flavonoids, terpenes, and anthocyanidins [7-8,6]. The main constituents of this family are simple phenolics, terpenoids, lignans quinones, tannins, some flavonoids and acylated anthocyanins groups [8]. Asteraceae family have biological potentials such as antibacterial, anti-inflammatory, antifungal, insecticide, and antitumor capacities that have been demonstrated by several studies [9]. According to [10], the Asteraceae family has importance for ornamental, medicinal, and economic reasons. Flavonoids, glycosides, tannins,

mucilage, and carbohydrates have been detected in preliminary phytochemical screening [11]. Flavonoid composition in the Asteraceae family has been established. The most present flavonoids are the 7-glycosides of luteolin and apigenin [12-13]. Pharmacological activity from many members of Asteraceae shows to have crucial phytochemical compounds such as diterpenoids, flavonoids, and polyphenols, and this shows to come [14-15].

Melastomataceae is shrubs, herbs, woody climbers, or trees. Their flowers are bisexual, radially symmetric, diplostemonous. One of the characteristics of Melastomataceae is they have berries and can be found about 2150-2350 of the species in 38 genera. Capsules are found in 2000-2200 species throughout 112 genera. The Melastomataceae family, which is mostly found in tropical and subtropical regions of the world, has 166 genera and 4,570 species [16]. According to [17], the characteristics of Melastomataceae leaves are generally hairy, opposite or opposite-crossed, petiolate, ovate or oblong blades and with smooth or serrated margins.

In the world, there are many flowering plants family, and the Asteraceae family is one the largest family that reported. The family contains approximately 1,600 genera and 23,000 species. Asteraceae is botanically highly and one among the specialized families of generally herbaceous plants. Warm temperate, arid, and semi-arid subtropical, lower temperate, and tropics of South, South-East, and East Asia, as well as Africa, including Madagascar and central South America, are all home to this family. In the Asteraceae family, there are approximately 950 genera and 20000 species worldwide [18]. Hairy and odors scents are two characteristics of the plant's leaves. Near the apex of the flower stalk, flat clusters of little flowers can be seen.

2. Discussions

2.1 Flavonoids

Flavonoids are a type of secondary metabolite found in many plants [19-20]. Flavonoids are one of the most important categories of specialized metabolites, which also contain approximately 9,000 additional chemicals [21]. Flavonoids are hydroxylated phenolic compounds that are generated from plants and are among the most beneficial secondary metabolites. Flavonoids have several classes like flavones, flavanones, flavonols, anthocyanidins, catechins, and isoflavones. According to [22], besides flavonoids flavones, flavonols, and flavanones, flavonoids can also be divided according to their molecular structure classes such as xanthones, chalcones, isoflavones and biflavones. Flavonoids also contribute to biological activities [23]. Flavonoids are widely distributed in plants and give many functions. Flavonoids have an important effect in scavenging oxygen of free radicals. In vitro experimental study also showed that flavonoids possess antioxidant, anti-viral, anti-inflammatory, and anti-carcinogenic properties [24]. Leaves, floral tissues, and woody components such as bark and stems are all rich in flavonoids. They're crucial in defense against infection and injury and for plant normal growth [25]. Fruits and flowers colors come from this flavonoid group of plant pigments and over 4,000 flavonoid compounds are classified and characterized according to chemical structure [26].

2.2 The molecular structures of four main groups of flavonoids are flavones, flavanones, catechins, and anthocyanins. The best-known member of each group is as stated [27]:

2.2.1. The first group is the flavones. one of the best-described flavonoids and Quercetin is one of the members of this group.

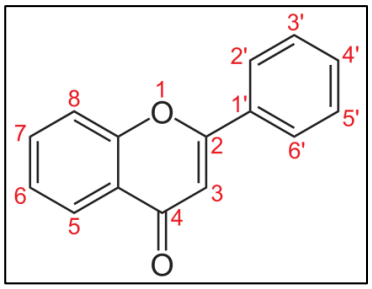

Figure 1. Flavone

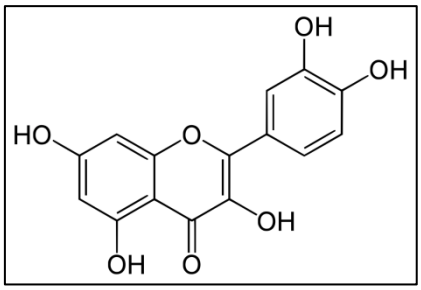

Figure 2. Quercetin

2.2.2. The second group is flavanones. Naringenin is an example of this group of flavonoids.

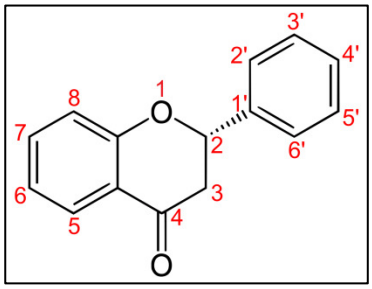

Figure 3. Flavanones

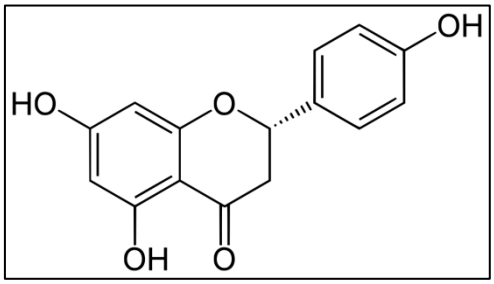

Figure 4. Naringenin

2.2.3. The third group is the catechins. Epicatechins is an example of this group of flavonoids

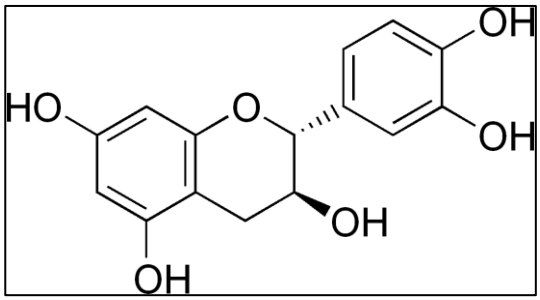

Figure 5. Catechins

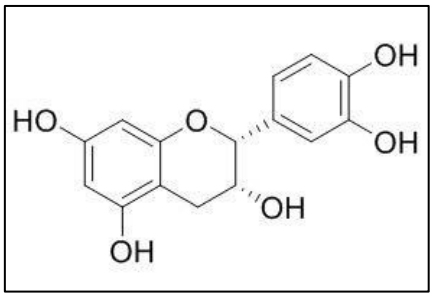

Figure 6. Epicatechin

2.2.4. The fourth group is anthocyanins. Cyanidin is an example of this group of flavonoids

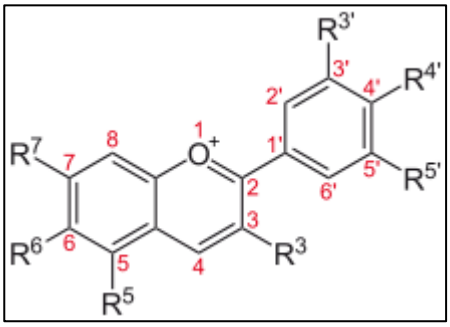

Figure 7. Anthocyanin

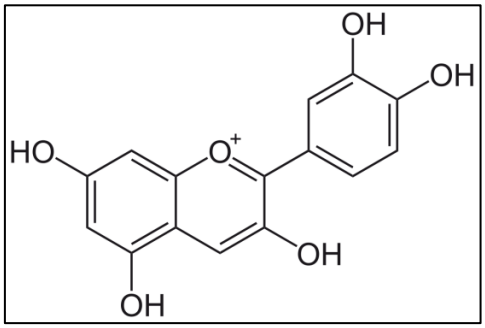

Figure 8. Cyanidin

2.3. Sample preparation and extraction method

Sample preparation is done with the sampling according to the selected area. Preparation of samples continues with grinding, homogenization and milling. Before moving on to the analytical procedure, the major process for recovering and isolating bioactive phytochemicals from plant sources is extraction. The chemical nature of the materials, sample particle size, and the presence of interfering chemicals will all influence the extraction process. Extraction solvents include alcohols (ethanol, methanol), acetone, ethyl acetate, and diethyl ether. Pure organic solvents could not completely extract polar solvents like (benzoic, cinnamic acids), which are classified as phenolic acids. It is preferable to employ alcohol-water or acetone–water mixtures. Nonpolar extraneous compounds (waxes, sterols, chlorophyll, oils, etc.) will be extracted from the plant matrix using less polar solvents (hexane, dichloromethane, benzene, chloroform).

We'll start by pre-treating the samples before extracting them. A more homogenous plant matrix can be developed for solvent extraction research by grinding dried plant material at room temperature. If the ground plant material comes in an inhomogeneous state, it will be sieved to obtain a particle size fraction of 0.10 to 0.30 cm. This sample will be kept for extraction research. Next, for extraction parameters, we will choose extraction techniques such as maceration and ultrasonic agitation. Different solvent extraction methods will be assayed for good optimization conditions by maceration and ultrasonic agitation methods for the whole extraction procedure. The preliminary maceration procedure was used to see how extracting solvents affected the recovery process. From the results obtained, sonication extractions will be carried out at different times using optimum extractant concentration for better performance [28].

For extraction procedures, we will follow the procedures for the maceration technique. For extraction method optimization of flavonoids, the solvent used will be functioning according to the required type of flavonoid. The extractant's polarity is critical in this case. Lower-polar flavonoids are commonly extracted using dichloromethane, diethyl ether, chloroform, or ethyl acetate, whereas flavonoid glycosides and more polar aglycones are usually extracted with alcohol-water combinations [29-31,21]. Because it is extensively utilized in pharmaceutical preparations, aqueous ethanol can be used as an extracting solvent [32]. Using the optimized HPLC method, the effect of extractant polarity on recovery can be assessed.

Using HPLC techniques is one of the only ways to separate all of the studied components, as well as any derivatives or degradation products, all at the same time. They enable the detection of low analyte concentrations in the presence of various other interfering and coeluting components in many conditions. HPLC provides several advantages, including the flexibility to switch between two or more columns and the availability of a large variety of commercially accessible columns, including new generation sorbents that have been employed in fit for properties designs. The sample collected must be transformed into a form appropriate for analysis in most chemical analyses [33]. The extraction methods are listed in table 1, along with the types of extract (crude and powder).

Table 1. Methods of extraction with the solvent used

No	Plant	Parts of the plant	Type of extract	Extraction solvent	Methods of extraction	References
1	*Melastoma malabathricum* (Melastomataceae)	Leaves	Crude	Methanol	1. With 40 g of leaf powder, the macerated technique was employed. The sample was then immersed in 800 ml of methanol at a 1:20 (w/v) ratio for 72 hours. 2. Cotton wool, a cloth filter, and Whatman No. 1 filter paper were used to filter the methanol supernatant. 3. The sample was evaporated to dryness using a rotary evaporator at 40°C and a decreased pressure (204 mbar). 4. The residue was gathered and subjected to two more extraction and evaporation procedures.	[34]
2	*Melastoma malabathricum* (Melastomataceae)	Leaves	Crude	Methanol (1:20; w/v), distilled water, petroleum ether, ethyl acetate	1. The leaves were macerated by immersing them in a methanol solution containing 1:20 (w/v) for 72 hours. 2. These steps were done three times with the same residue each time. 3. Cotton wool, steel filters, and Whatman no. 1 filter paper were used to filter the supernatant. 4. The supernatant from each extraction was collected and pooled, then evaporated to dryness using a rotary evaporator at 40 °C under reduced pressure.	[35]

No	Plant	Parts of the plant	Type of extract	Extraction solvent	Methods of extraction	References
3	*Miconia albicans* (Melastomataceae)	Leaves	Crude	Ethanol	1. The dried crushed leaves were extracted twice with ethanol extract, each time for 72 hours, and then dried using a rotary evaporator to yield 100 g.	[36]
4	*Santolina semidentata* (Asteraceae)	Leaves and stalks	Powder	Hydroetha-nolic solvent (50% ethanol/ water), (v/v)	1. The leaves and stalks of plants are used to extract phytomolecules. 2. For each gram of lyophilized powder, 12 mL hydroethanolic solvent (50 percent (v/v) ethanol/water) was added, and the mixture was shaken for 30 minutes at room temperature. 3. The supernatant was filtered via filter paper before being passed through cellulose acetate membrane filters with 0.2m hole sizes. 4. The mixture was centrifuged at 12,400 rpm for 10 minutes. 5. Solid-phase extraction (SPE) was used to fractionate the hydroethanolic extracts using Giga tubes 2g/12 mL, C18-E units (Phenomenex®), resulting in a (poly)phenol-enriched fraction (PEF)	[37]
5	*Eupatorium littorale* (Asteraceae)	Leaves	Crude	Methanol	1. 1 kilogram of leaves are pulverized and extracted twice in methanol at 40°C. 2. The sample was evaporated to dryness in a rotary evaporator under reduced pressure, yielding 142.57 g of crude extract.	[38]
6	*Vernonia condensata* Baker	Leaves	Crude	Ethanol	1. The material was extracted for 3 weeks at room temperature using a static maceration	[39]

No	Plant	Parts of the plant	Type of extract	Extraction solvent	Methods of extraction	References
					procedure in 2.5L of 95 percent ethanol, with the solvent being replaced every 2 days. 2. In a rotary evaporator, filtered the ethanol extract and evaporated the sample at a controlled temperature of 50 to 60 degrees Celsius. 3. Desiccate the material in a silica desiccator to get approximately 27 g. 4. The ethanol extract was suspended in water:ethanol (9:1), followed by a liquid/liquid partition with increasing polarity organic solvents: hexane, ethyl acetate, dichloromethane, and butanol. Hexane (HF), ethyl acetate (EF), dichloromethane (DF), and butanol (BF) fractions were obtained using this method.	

2.4. HPLC

HPLC is a chromatographic technique for quantifying, identifying, and purifying the constituent components of a mixture. It is used in analytical chemistry and phytochemistry to separate a mixture of substances. HPLC is a robust, dependable, and widely used method for the isolation of natural substances [40-41]. For measuring and characterizing secondary metabolites in plant extracts, such as phenol compounds, flavonoids, alkaloids, steroids, and other steroid-like substances, HPLC has been advocated in various articles [42-46]. The HPLC column is at the heart of any commercial chromatographic system [47]. In HPLC, there are stationary phases and mobile phases [48]. As in this review, we concentrate on the use of HPLC in the analysis of flavonoids, including all stationary phase, mobile phase, and column components that are specific to flavonoids. The stationary phases are a thorough separation of flavonoid combinations found in nature. Because of the vast range of polarities and the tendency for flavonoids with comparable polarities to elute in groups, it poses issues. For the stationary phase, C18 is usually used. Columns are often employed and have an internal diameter of 2 to 5 mm, particle sizes of 3 to 5 mm, and a length of 75–250 mm. More polar flavonoids are separated using the C8 stationary phase.

For mobile phases, the composition of the eluent parameters is one of the most crucial parameters for good flavonoids separation. An important factor in determining the combination of solvents is controlling the flavonoids solubility in the eluent. Hydrophobicity is the property that will be checked for retained analytes on the stationary phase in RP-HPLC. As a result, flavonoids are eluted in decreasing polarity order in RP-HPLC. The polarity of hydroxyls in the fourth position is the largest, followed by

those in the second and third places. Polarity is reduced and retention periods can be extended by adding methoxy groups or removing polar hydroxyl groups. The solubility of flavonoids has a big impact on how well they interact with the mobile phase in HPLC. As a result, flavonoid properties such as hydrogen bonding, hydrophobicity, dipole moment, ionization, and steric effects must be taken into account while selecting a mobile phase for effective separation.

Unattached sugar units in flavonoids are pH sensitive and have low water solubility. The solubility of rutin, isoquercitrin, quercetin, naringenin, chrysin, and hesperetin in three different organic solvents (acetone, tert-amyl alcohol, and acetonitrile) was examined, and no evident relationship between flavonoids' solubility and their thermodynamic properties was discovered [49]. To extract flavonoids from powdered plant sources, a solvent such as methanol, ethanol, water, acetone, acetonitrile, ethyl acetate, or a mixture of these solvents is commonly used [50]. Acidified water, such as acetic acid or formic acid, is commonly used as the aqueous mobile phase. The organic mobile phase is either methanol or acetonitrile [51]. Figure 1 shows the HPLC components as well as how HPLC works. In table 2, the HPLC profiling of flavonoids was stated to see the solvents, stationary phases, mobile phases, flow rate, wavelength settings and also flavonoid standards from the leaves sample.

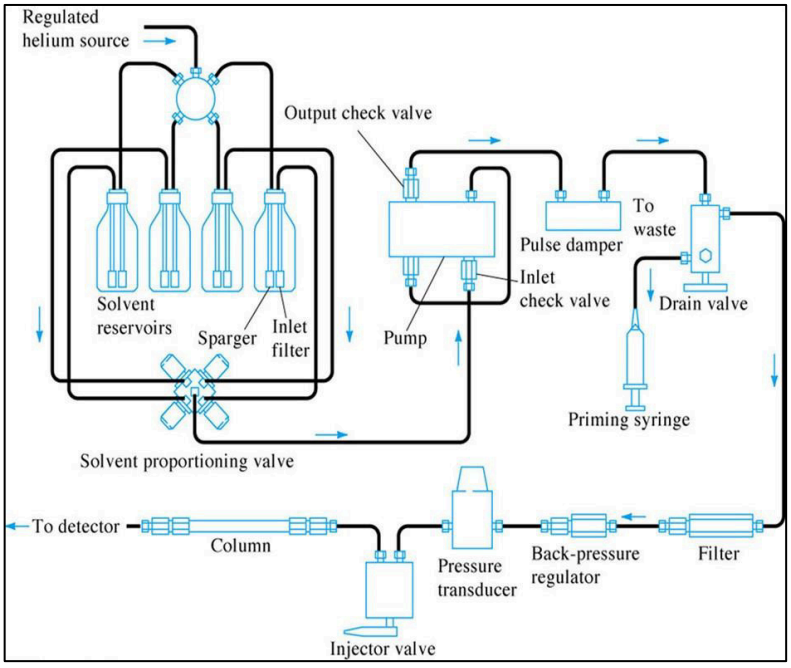

Figure 9. HPLC Parts [54]

Table 2. HPLC profiling of flavonoid

	Plant	Plant parts	Extraction solvent	Stationary phase	Mobile phase composition	Flow-rate, wavelength (nm) settings	Flavonoids	References
1	*Melastoma malabathricum* (Melastomataceae)	Leaves	Methanol	5 µm C18 column with 250 x 4.60 mm dimensions	Aqueous formic acid, 0.1 percent, as a solvent A and acetonitrile as solvent B	1.0 ml/min at wavelengths 254 and 336 nm,	Quercetin, Quercitrin and Rutin	[34]
2	*Melastoma malabathricum* (Melastomataceae)	Leaves	Methanol (1:20; w/v), distilled water, petroleum ether, ethyl acetate	5 µm column (4.6 mm x 250 mm)	Aqueous formic acid, 0.1 percent, as a solvent A and acetonitrile as solvent B	1.0 ml/min at wavelengths of 210, 254, 280, 300, 330, and 366 nm	Pinostrobin, Flavanone, Hesperetin, 4',5,7-trihydroxy flavanone, 2,4,4'-trihydroxy chalcone, Quercitrin, Fisetin, Dihydroquercitin, Rutin, Quercetin, Silibinin, Naringenin, and Genistein	[35, 52]
3	*Miconia albicans* (Melastomataceae)	Leaves	Ethanol	5 µm column C18 with 250 mm × 4.6 and 5.0 µm and C18 pre-column with 3.0 mm x 4.6	Water (0.1 percent phosphoric acid) and acetonitrile (77:13, v/v) as solvents (A) and (B)	0.6 ml/min, 356 nm	Rutin	[35, 52]

4	*Miconia albicans* (Melastomataceae)	Leaves	Ethanol	5 mm C18 100 Å with 250 × 4.6 mm, analytical column	Solvents (A) and (B): 0.1 percent formic acid in water and methanol, respectively	8 mL/min	Rutin	[36]
5	*Santolina semidentata* (Asteraceae)	Leaves and stalks	50 percent (v/v) ethanol/water hydro-ethanolic solution	5 µm column C18 with (250 × 4.6 mm)	Solvents (A) and (B) are 0.1 percent (v/v) formic acid in water and acetonitrile, respectively	1 mL/ min, 280, 320, 370 and 520 nm	Hydroxicinammic acids, Flavone, Simple phenolic acids, Flavonol, Coumarin	[53]
6	*Eupatorium littorale* (Asteraceae)	Leaves	Methanol	5 µm column C18 with (250 × 4.6 mm)	Methanol/water 1:1 (0-10 min) and 7:3 (10-20 min)	1 mL/ min, 339 nm	Rutin, Quercetin, Eupafolium and Hispidulin	[38]
7	*Vernonia condensata* Baker (Asteraceae)	Leaves	Ethanol	5 µm column C18 with (250 × 4.6 mm)	Solvent (A) – water pH adjusted to 4.0 with phosphoric acid Solvent (B) - acetoni-trile	0.6mL/ min, 210, 230, 254, 280, and 330 nm	Apigenin and Luteolin	[39]

3. Conclusion

This review describes extraction and HPLC methods used for flavonoids. We only choose the leaves sample from Melastomataceae and Asteraceae family for this review as we aim is to analyze flavonoid as flavonoid has insecticidal activity towards insect. The preparation of the sample and the methods used for analysis in HPLC is very important as every compound needs to use different solvents, stationary phases, mobile phases, flow rate, wavelength settings and also different standards. During the literature search, HPLC is a good technique for analyzing flavonoids. The present article summarizes the procedure used for extraction of sample and HPLC profiling procedures that will help handle HPLC in the future.

Acknowledgments

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