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QUANTITATIVE ANALYSIS OF FIVE FLAVONOIDS IN URTICA DIOICA L. AND MELISSA OFFICINALIS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Abstract

In this thesis, we describe quantitative determination of five flavonoids: Gallic acid, rutin, quercetin apigenin and kaempferol in two medicinal plants Urtica dioica L. and Melissa officinalis by high performance liquid chromatography (HPLC). Plant samples were extracted by optimized methods with 96% ethanol, and flavonoids separation was performed using a Perkin Elmer C18 column and gradient elution. The flavonoid concentrations were measured based on the calibration with standard solutions. Those results showed that the two species had different flavonoid profiles. In particular, Urtica dioica L. had the higher concentration of gallic acid, rutin and quercetin while Melissa officinalis revealed significant levels of rutin and kaempferol. These results offer an idea of the phytochemical content these to be a potentially used source for further natural product examination and characterization in pharmaceutical and nutraceutical sectors.

Introduction

Flavonoids, which belong to the well- known polyphenols, are well -known for their many biological activities such as antioxidant, anti-inflammatory and antimicrobial [1]. Flavonoids, which are important secondary metabolites in plant defense responses [2], have received much attention for their pharmaceutical and nutraceutical interests owing to health-promoting properties such as anti-cancer effects [3]. On the other hand, from flavonoid classes in particular gallic acid, rutin, quercetin, apigenin and kaempferol are found to be significant for therapeutic application [4]. Lemon balm (Melissa officinalis) [5] and stinging nettle (Urtica dioica L.) [6] are among the medicinal plants that have been widely used over both

history and traditions. Urtica dioica L. is known for its anti-inflammatory, diuretic, and antioxidant activities [7], while Melissa officinalis is freely taken for calmness, antiviral activity, and cognitive enhancement properties [8]. These are known to be rich in bioactive compounds, particularly flavonoids, which are thought to play a prominent role in their pharmacological effects [9]. High-performance liquid chromatography (HPLC) is a powerful analytical technique commonly utilised for the identification, separation, and quantification of flavonoids in complex plant matrices. Its high sensitivity, reproducibility and precision make it an ideal method for the accurate determination of flavonoid content in medicinal plants. This study objects to quantify five flavonoids such as gallic acid, rutin, quercetin, apigenin, and kaempferol in Urtica dioica L. [10] and Melissa officinalis [11] using the HPLC method [12]. The study offers a deep insight into the flavonoids present in these plants that enhances the knowledge on their phytochemical profile and augments their use in pharma and nutraceuticals.

Materials and methods

To qualitatively and quantitatively determine the flavonoid content in finely ground plant samples, the following method was employed: For this purpose, the sample matrix was extracted with 96% ethanol as a solvent. For 2 gram sample, 20 milliliters of ethanol was added. This mixture was extracted at 90°C for 75 minutes with a magnetic stirrer in place. Dispersive solid-phase extraction was carried out using an Agilent Zorbax cartridge (4.6 mm ID x 12.5 mm) and a PerkinElmer C18 column (250 x 4.6 mm, 5 µm particles, USA) as stationary phases. The analysis was performed on LC 2030 C 3D Plus liquid chromatography system (Shimadzu). Quantitative determination of rutin, gallic acid and quercetin in the samples was performed on the column. For a standard solution in acetonitrile, 0.025 mg/mL and 0.05 mg/mL of the solutions were prepared in an aqueous solution containing some of the constituent as well as 0.5% acetic acid for the analysis result respectively The analysis was performed at 40°C under isothermal conditions with a flow rate of 0.8 mL/min and an 18 min gradient elution program, over the potential range of −1,000 to +1,000 mV.

Time (min)	Phase A (%) (0.5% acetic acid in water)	Phase B (%) (Acetonitrile)
1	60	40
3	70	30
6	55	45
10	80	20
12	Stop	

Results and Discussions

The amounts of flavonoids in the extracted and purified sample (in dry mass, mg/100g) are presented in the following [Table-2].

Table-2.

Plant	Gallic Acid (mg/100g)	Rutin (mg/100g)	Quercetin (mg/100g)	Apigenin (mg/100g)	Kaempferol (mg/100g)
Urtica dioica L	85.689	28.985	27,9540	-	13,2290
Melissa officinalis	81,0030	60,7400	-	-	13,2770

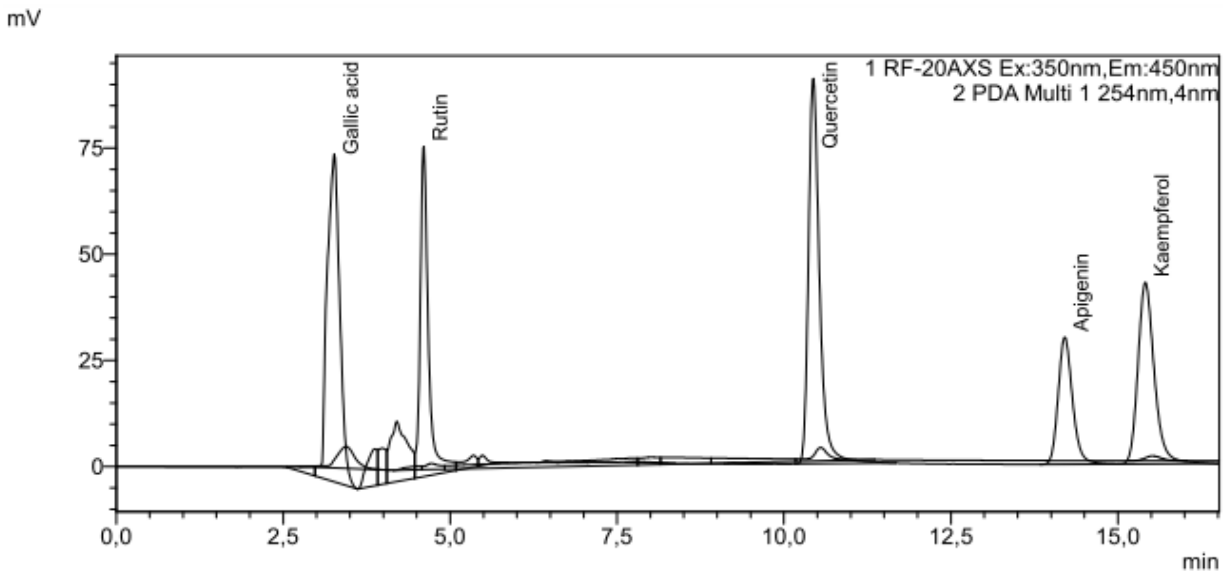

Figure 2. Chromatogram obtained from standard flavonoid samples.

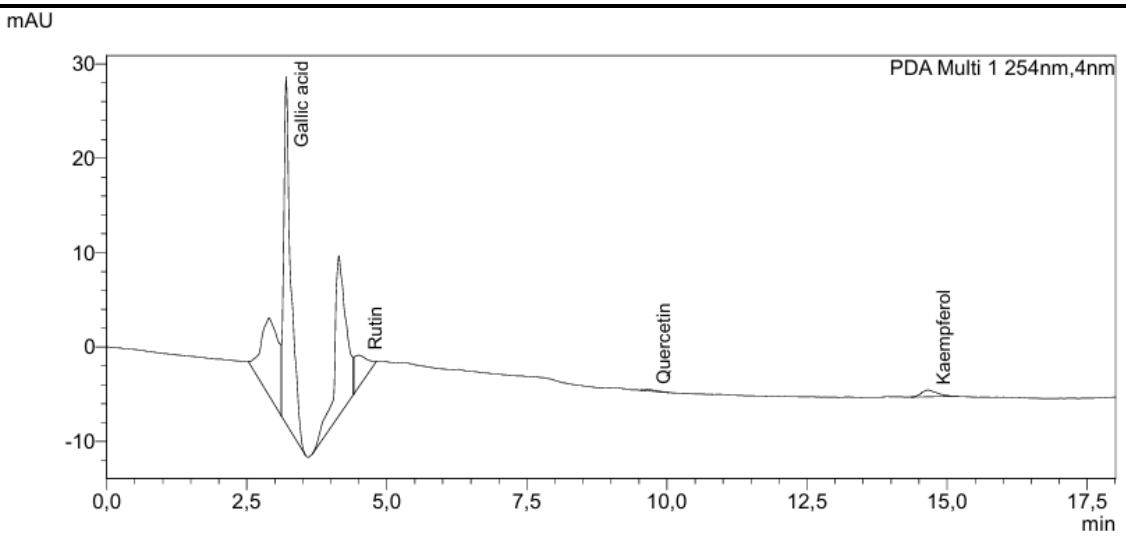

Figure-2. Urtica dioica L xromatogrammasi

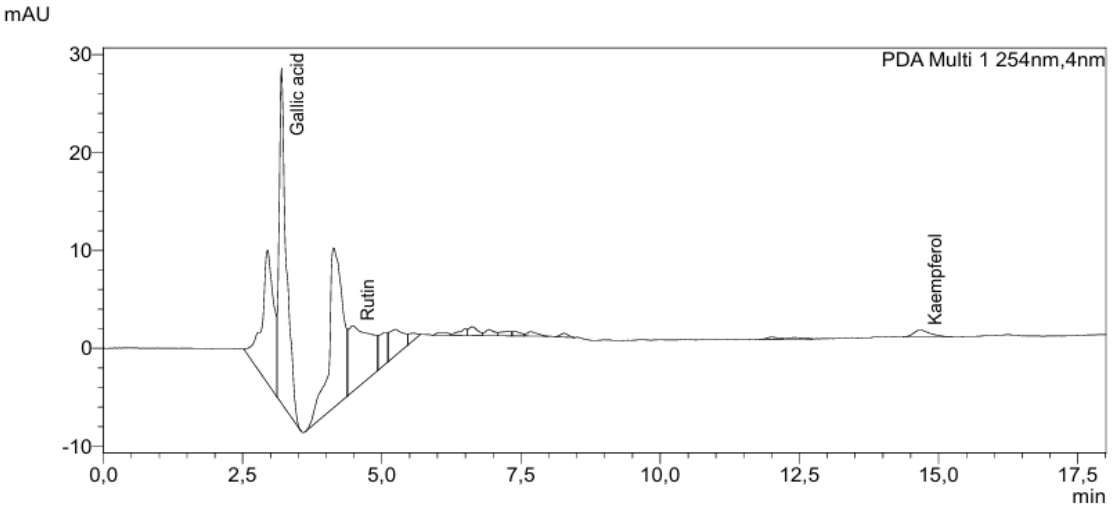

Figure-3. Melissa officinalis xromatogrammasi

Table-2 summarizes results from high-performance liquid chromatography (HPLC) analysis for quantification of a selection of flavonoids in the two plant species Urtica dioica L. and Melissa officinalis. The identified and quantified compounds include apigenin, rutin, gallic acid, quercetin, and kaempferol, with concentrations expressed as mg per 100 g of dry mass. The data reflect the differences in flavonoid profiles between species that may hint to their particular phytochemical and pharmacological properties.

Conclusion

In this work, the quantification of gallic acid, rutin, quercetin, apigenin, and kaempferol was done using HPLC in Urtica dioica L. and Melissa officinalis. It was determined that the content of gallic acid, rutin, and quercetin was higher in Urtica dioica L., while the content of rutin and kaempferol was dominant in Melissa officinalis. Therefore, this study determines the plants' features in terms of phytochemical profiling, which supports their potential use for pharmaceuticals and nutraceuticals. The study also confirmed that HPLC was a viable and reliable method for flavonoid analysis in medicinal plants.

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