AN OVERVIEW: THE BOTANICAL EXTRACT PRODUCED WITH SC -CO2 EXTRACTION FOR THE DETERMINATION OF FLAVONOIDS

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Abstract

Fruits, vegetables, grains, bark, roots, stems, flowers, tea, wine, and other naturally occurring foods as well as medicinal plants include flavonoids, a class of compounds with varied phenolic structures. The health benefits of these natural chemicals are well known, and attempts are being done to identify the so-called flavonoid components. Flavonoids are regarded as a crucial component in a wide range of nutritional, pharmacological, therapeutic, and aesthetic applications.

Analyzing the occurrence of these chemicals in medicinal plants was the aim of this study. After using the SC-CO_2 extraction method, an analysis was conducted. Using this novel technique, we could recognize the flavonoids found in almost 26 different plants with various characteristics.
The chemical profile of these plants was evaluated with the help of GC-FID, and with the aid of HPLC we were able to determine whether the presence of flavonoids. According to the data gathered, the medicinal plant with the lowest amount of flavonoids is Mentha piperita (10.2 mg/g extract), and Cistus incanus (650 mg/g extract) followed by Melissa officinalis (480 mg/g extract) is the plants with the highest amount of flavonoids.

Keywords: *medicinal plants, flavonoids, SC-CO_2 extraction,*

Introduction

Flavonoids belong to an important class of natural products, particularly a class of secondary plant metabolites with a polyphenolic structure, widely present in fruit, vegetables and some beverages. In response to microbial diseases, plants in particular summarize them.

Chemically: The flavonoids have fifteen carbon atoms and are composed of two aromatic rings joined by a bridge of three carbon atoms as seen in Figure 1.

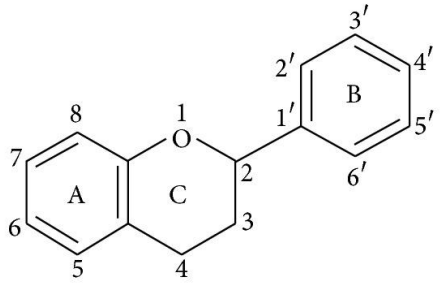

Figure 1. The chemical structure of a flavonoid.[1]

While the individual compounds within a class differ in the model of replacing rings A and B, the various classes of flavonoids differ in the extent of oxidation and the replacement model of ring C.

Flavonoids, flavonols, anthocyanidins, and isoflavonoids are the four primary families of flavonoids (see Figures 2, 3, and 4).

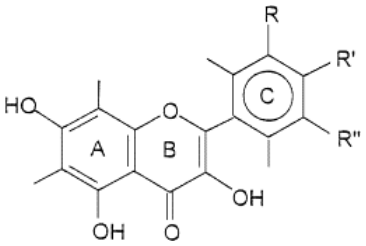

Figure 2. The chemical formula of a flavonol. [2]

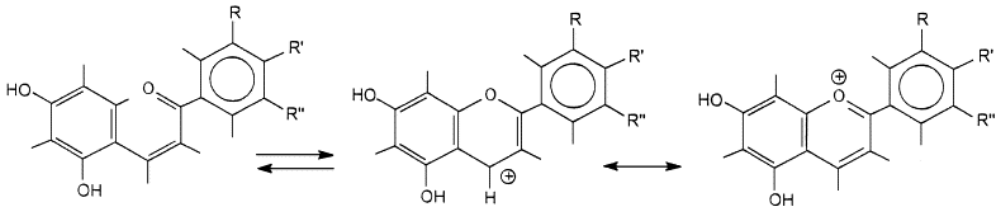

Figure 3. Structure, tautomerism and mesomerism of anthocyanidins. [2]

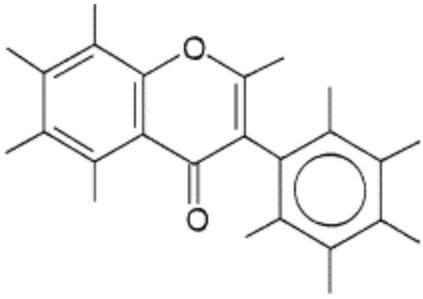

Figure 4. The chemical structure of an isoflavone [2.]

Flavonoid classes are divided into subclasses, representing that class's many benefits in human health. The figure shows the classes and subclasses, and their natural source. [3]

[1] Shashank Kumar, Abhay K. Pandey, "Chemistry and Biological Activities of Flavonoids: An Overview. Scientific World Journal, 2013 Dec 29;2013:162750. doi:10.1155/2013/162750. eCollection 2013.

[2] Bent H Havsteen, "The biochemistry and medical significance of the flavonoids", Pharmacol. Ther, 2002 Nov-Dec;96(2-3):67-202. doi: 10.1016/s0163-7258(02)00298-x.

Chalcones, flavones, flavonols, and isoflavones are some of the subcategories of flavonoids. Unique important sources define these groupings. For example, onions and tea are prominent dietary sources of flavonols and flavones. [3]

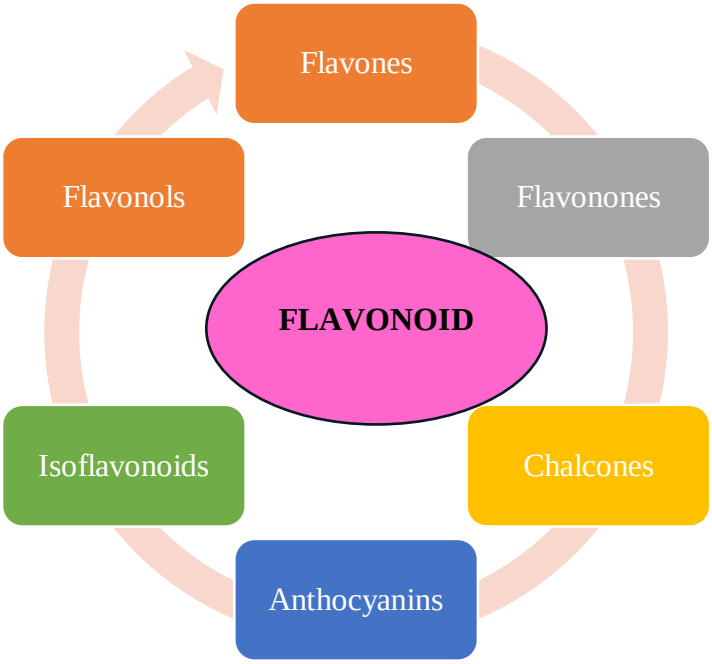

Figure 2. Flavonoid classes

Part of e flavonoid classes there are even subclasses:

a) **Chalcone subclasses**: With a wide spectrum of pharmacological properties, including anti-inflammatory, antibacterial, antioxidant, cytotoxic, and anticancer effects, chalcones and their derivatives play a significant role in medicinal chemistry.

b) **Anthocyanin subclasses**: red and purple berries, grapes, apples, plums, cabbage, or foods with high quantities of natural colorants are dietary sources of anthocyanins. The six common anthocyanidins are cyanidin, delphinidin, malvidin, peonidin, petunidin, and pelargonidin.[4]

[3] A. N. Panche, A. D. Diwan,and S. R. Chandra 'Flavonoids: an overview' J Nutr Sci, v.5; 2016 , PMC5465813

[4] Anthocyanins: A Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases, Roberto Mattioli, Antonio Francioso, Luciana Mosca, and Paula Silva' Molecules. 2020 Sep; 25(17): 3809. doi: 10.3390/molecules25173809

c) Flavonols subclasses: The primary flavonols include quercetin, kaempferol, myricetin, isorhamnetin, and fisetin. Fruits, vegetables, red wine, tea, and red wine are all notable sources of flavonol.[5]

d) Flavones - exhibit antioxidant, antiproliferative, anticancer, and antibacterial effects in a variety of pathological situations. In addition to luteolin (3',4',5,7-tetra hydroxy flavone), tangeritin (4',5,6,7,8-penta methoxy flavone), chrysin (5,7-dihydroxyflavone), and 6-hydroxy flavone, other common flavones include apigenin (4', 5,7-trihydroxy flavone), and luteolin.

e) **Isoflavones:** Daidzin, genistin, biochanin A, and formononetin are the four distinct chemical forms of isoflavones that are found in soybeans and soy-based foods.[6]

f) **Flavanones:** Another significant class, flavanones, are typically found in all citrus fruits, including oranges, lemons, and grapes. Examples of this group of flavonoids include hesperetin, naringenin, and eriodictyol.[7]

The flavonoids and their subclasses are present in the medicinal plant, and in animals, and microbes, flavonoids are involved in a wide range of biological processes. The color and perfume of flowers as well as the ability of fruits to attract pollinators and, as a result, fruit dispersion to aid in seed and spore germination as well as the growth and development of seedlings are all attributed to flavonoids in plants[8].

In addition to acting as special UV filters[9], signal molecules, allopathic substances, phytoalexins, detoxifying agents, and antimicrobial defensive compounds, flavonoids shield plants from a variety of biotic and abiotic challenges.

Due to their antioxidant properties, flavonoids are of great interest to researchers. In fact, there are flavonoids' functional hydroxylic groups, which are in charge of neutralizing free radicals and chelating metal ions to stop the production of new free radicals. These radicals cause cell damage and ageing and may also play a role in the metabolic processes that trigger the beginning of many diseases.

Protection against *cardiovascular diseases* appeared as one of these chemicals' advantageous traits from various epidemiological research; in fact, they are involved in controlling blood pressure and cholesterol levels. The total flavonoid fraction of Astragalus Complanatus was found to have an antihypertensive impact in hypertensive rats, while other research has linked the qualities of flavonoids found in berries with a favourable effect on memory (positive effects associated with *Alzheimer's disease*).

A molecular mechanism for treating *diabetes* has also been revealed. In reality, flavonoids and saponins regulate glucose and lipid metabolism, which is changed in the diabetic patient, in addition to their antioxidant and anti-inflammatory properties. Oxidative stress does, in fact, mediate hyperglycemia.

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[5] Bioactive Natural Products, Sümeyra ÇetinkayaKevser Taban AkçaIpek Süntar, in Studies in Natural Products Chemistry, 2022

[6] G. Bultosa, in Encyclopedia of Food Grains (Second Edition), 2016

[7] Flavonoids: an overview, A. N. Panche, A. D. Diwan, and S. R. Chandra**,** J Nutr Sci,v.5; 2016 ,PMC5465813

[8] Griesbach R (2005) Biochemistry and genetics of flower color. *Plant Breed Rev* 25, 89–114. [Google Scholar] [Ref list]

[9] Takahashi A & Ohnishi T (2004) The significance of the study about the biological effects of solar ultraviolet radiation using the exposed facility on the international space station. *Biol Sci Space* 18, 255–260. [PubMed] [Google Scholar] [Ref list]

Different researchers have studied the interaction, the metabolism of flavonoids and their derivates, with glucose, and how the administration of different natural sources of flavonoids can help with the reduction of oxidative stress and regulation of glucose metabolism.

Numerous flavonoids derived from plants have been shown to decrease the activity of -amylase and -glucosidases in vitro and to enhance postprandial glycemia in diabetic animal models and in a few human investigations [10],[11]. But very few researchers have discussed isomaltase inhibition. In industrial-scale manufacture, the disaccharide isomaltose is frequently added as low-calorie food sweeteners or is created from amylopectin hydrolysis to limit dextrin [12],[13]. Isomaltose is a rare naturally occurring disaccharide.

In this paper, the aim is to highlight the presence of flavonoids in different plants, 26 medicinal plants, which were purified, went under an extraction process using an innovative methodology such as supercritical fluid extraction with carbon dioxide as a solvent. The importance of this method is that the extract is almost identic to the main structure of the plant. Once the extraction is performed the chemical profile of the plants is investigated, but under specific requirements, the extraction process is very important to be followed the rules after the extract was collected, for these plants, in the ratio of 1:2 they were diluted with n-hexane and were analyzed with gas chromatography for the chemical composition. The second step was the quantitative analysis with HPLC, for the determination of flavonoids. From the data collected, it was interesting to view the higher presence of flavonoids and their derivate present in the Cistus incanus (650 mg/g extract) and the lowest concentration in the Mentha piperita (10.2 mg/g extract),

Materials and methods

2.1 Materials

- 26 different plants
- Supercritical CO_2 extraction machine Model 50x2/40 Mpa.
- GC -FID chromatogram (SCION 436 GC).
- Sartorius PTFE 0.20 m syringe filter.
- Buffer Solutions
- Sigma Aldrich flavonoids standards
- HPLC - ISO/CD 11843-7

2.2 Method

26 different medicinal plants, gathered, and collected according to quality storage standards, were cleaned of physical impurities and subjected to the extraction process by the Supercritical Fluid Extraction method using CO_2 as a solvent.

[10] Kumar, S.; Mittal, A.; Babu, D.; Mittal, A. Herbal medicines for diabetes management and its secondary complications. Curr. Diabetes Rev. 2020. [CrossRef]
[11] Williamson, G. Possible effects of dietary polyphenols on sugar absorption and digestion. Mol. Nutr. Food Res. 2013, 57, 48–57. [CrossRef] [PubMed]
[12] Van Beers, E.H.; Al, R.H.; Rings, E.H.H.M.; Einerhand, A.W.C.; Dekker, J.; Buller, H.A. Lactase and sucrase-isomaltase gene expression during Caco-2 cell differentiation. Biochem. J. 1995, 308, 769–775. [CrossRef] [PubMed]
[13] Miyake, T.; Sakai, S.; Shibuya, T. Process for Producing a High-Purity Isomaltose. U.S. Patent 4,521,252, 26 October 1981

The method of extraction is a common procedure performed at the Esencial Laboratory. The extraction was performed with the use of the *Supercritical CO2 extraction machine Model 50x2/40 Mpa.*

The image above schematically depicts a typical supercritical CO_2 extraction system. The working tank, where the CO_2 is kept for the operation, is where the process begins. It is then heated to the proper extraction temperature and compressed in a high-pressure pump. The carrier material is then placed in one of the extraction containers where it is then guided. The extractors have quick-opening lids that make filling and emptying simple and secure. The extractor is typically supplied solid raw materials (medicinal plants), through a basket. Despite the extractors' batch operation, many extraction vessels can be coupled into a useful system to enable continuous extraction.

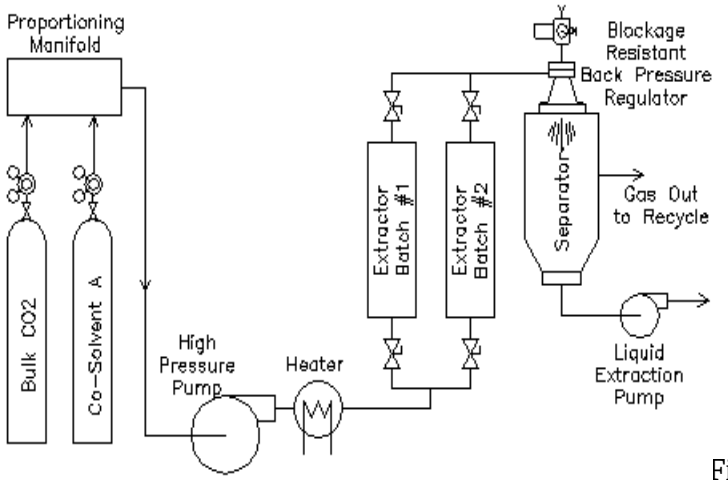


Figure 4. SC-CO_2 extractor.

The loaded carbon dioxide travels to the separators after exiting the extractors, where a change in temperature or pressure provides circumstances that allow the extract to separate from the carbon dioxide. At the bottom of the separators, the extract can then be collected. At this point, the extract can also be fractionated into several separators thanks to CO_2's solvent characteristics. To remove all the residual chemicals, the CO_2 is typically expanded into a gas in the final separator. This pressure level is crucial since it is best to avoid recompression, which uses a lot of energy. The gas is liquefied in a condenser after separation and then supplied back into the operating tank. It is now usable once more.

For each extract obtained, a quantity was received and in a ratio of 1: 2 n-hexane was diluted, then analyzed any essential oil in the GC -FID chromatogram (SCION 436 GC). After the extract of each plant is collected, the next step is the analysis with HPLC.

The procedure is as follows:

1. The extract, which we will test for a broad range of flavonoids and have already been methanol digested.
2. Next, we performed 10,000 rpm centrifugation.
3-After that, we filter it using a Sartorius PTFE 0.20 m syringe filter.
4-Manually inject into HPLC
5- We previously performed standards by injecting the total flavonoid standards that were bought from Sigma Aldrich.
6-The calibration graph of the procedure is the graph produced with the corresponding bake.

7. Slowly inject the syringe into the space where the septum of the apparatus is 100 l.
8-Control Buffer Solutions that promote health by delivering the substance's peak signal
9- Add one ml/min of juice.
10- Pressure between 250 PSI and 400 PSI is stored.
11- After Detector separates and catches molecules from the extract, we compare it with the reference graph to compute the total flavonoids at a quantitative level.
12- There will be 5–6 matching bakes with derivatives of flavonoids on the graph.

The calibration standard for HPLC equipment is ISO/CD 11843-7, which helps to prevent errors in the rectification.

3. Result and discussion

After the extraction with SC CO_2, it was able to obtain the chemical profile of each plant, using the GC – FID apparatus, but before that, the extract was diluted with n-hexane in the ratio 1:2, then was performed analysis for the profile. A secondary analysis was performed, the one for the detection of the secondary metabolites such as flavonoids, using the HPLC. (Shimadzu UV-VIS).

The UV index was decent reads at wavelengths of 485 nm harassment and up to 535 nm emission in a column that is 4.6 mm and 30 cm long. The results are shown in Table nr 1.

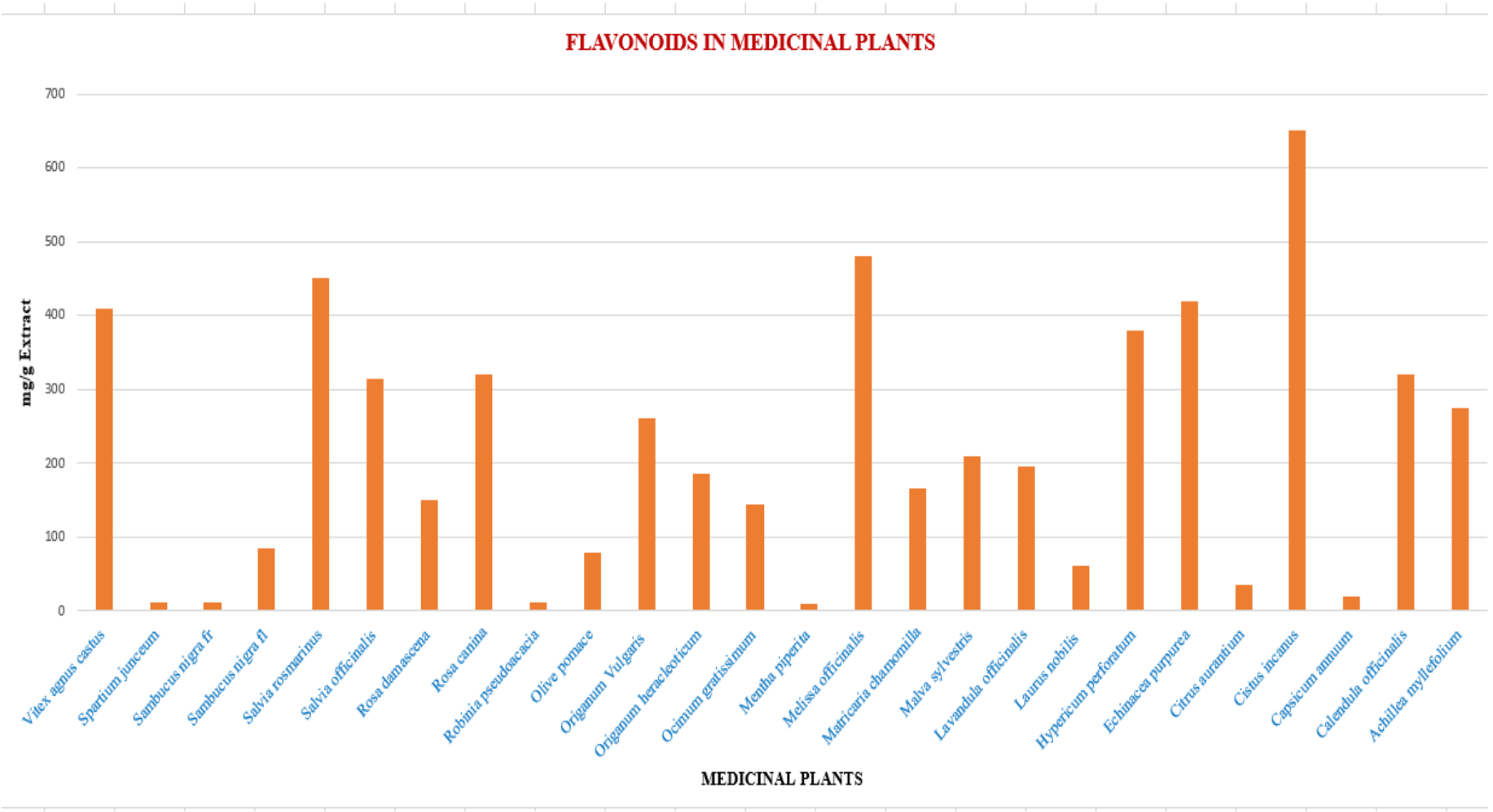
Table 1. The presence of Flavonoids in medicinal plants.

	Flavonoids	
	Plant	**Total Flavonoids mg/g Extract**
1	Vitex agnus castus	410
2	Spartium junceum	12
3	Sambucus nigra fr	10.5
4	Sambucus nigra fl	85
5	Salvia rosmarinus	450
6	Salvia officinalis	315
7	Rosa damascena	150
8	Rosa canina	320
9	Robinia pseudoacacia	12
10	Olive pomace	78
11	Origanum Vulgaris	260
12	Origanum heracleoticum	185
13	Ocimum gratissimum	145
14	Mentha piperita	10.2
15	Melissa officinalis	480
16	Matricaria chamomilla	165
17	Malva sylvestris	210
18	Lavandula officinalis	195

19	Laurus nobilis	60
20	Hypericum perforatum	380
21	Echinacea purpurea	420
22	Citrus aurantium	35
23	Cistus incanus	650
24	Capsicum annuum	20
25	Calendula officinalis	320
26	Achillea myllefolium	275

From the results, it is shown that the medicinal plant with the highest amount of flavonoids containing is Cistus incanus (650 mg/g extract) followed by Melissa officinalis (480 mg/g extract) and Salvia Rosmarinus (450 mg/g extract).

The medicinal plant with the lowest amount of flavonoids is Mentha piperita (10.2 mg/g extract), followed by Sambucus nigra fr (10.5 mg/g extract) and Robinia pseudoacacia (12 mg/g extract),



Graphic 1. The presence and amount of flavonoids in the medicinal plant.

4. Conclusion

The medicinal plants under study display their primary components in their chemical profiles, but secondary metabolites from these plants are also abundant in flavonoids, which is also the primary aim of this article.
The effects of flavonoids are well recognized, but it is crucial to analyze the presence of these compounds in the essential oils of these medicinal herbs that have already undergone Super Critical Fluid Dioxide Extraction.
A process that, in addition to being quite clean, allows the extract to be free of any chemical or microbiological impurities (supported by pertinent analysis), and that also produces an extract that is almost entirely true to the character of the plant itself.
Compared to extracts made using other methods, the CO_2 extract is the closest to oil as it naturally occurs in botanical plants. Without digestion residues, notes, more keynotes, more back notes, and higher solubility, it is best as a lovely aroma and component of perfumes. Due to its non-toxic, odourless, free, colourless, and non-flammable qualities, CO_2 is an excellent solvent.

They are highly concentrated since they are whole extractions of the plants, which also comprise water- and fat-soluble compounds and essential oils. This means that when you use whole CO_2 extracts, you are utilizing both the plant's essential oil and additional chemicals that were also removed from CO_2 Supercritical.

Given the importance of flavonoids as a composition with high antioxidant properties in pharmaceutical applications, their natural source is the botanical extract produced with SC-CO_2 extraction.

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