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ESCUELA DE INGENIERÍAS INDUSTRIALES

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Máster en Ingeniería Química

OPTIMIZATION OF PROCESSING CONDITIONS ON THE QUALITY OF EXTRACTS AND FORMULATION OF *ARNICA MONTANA*

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TÍTULO:	Optimizacija postopka ekstrakcije učinkovin iz arnike *Arnica Montana* in formulacija produktov
ALUMNO:	Álvaro Pequeño Alonso
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TUTOR:	Assoc. Prof. Dr. Maša Knez Marevci

Resumen

Arnica Montana es una fuente prometedora de compuestos bioactivos, principalmente fenoles, que debido a su actividad antioxidante muestra beneficios para la salud en humanos. Para llegar a una etapa farmacéutica, las flores de *Arnica Montana* necesitan ser procesadas para obtener un producto viable. Para este propósito, se realizaron varias extracciones con solventes: maceración en frío, ultrasonidos, Soxhlet y CO2 supercrítico; los solventes, etanol, metanol y una mezcla 95:5 (v:v) de etanol y agua fueron usados. Se realizaron análisis de espectrometría ultravioleta/visible para medir la actividad antioxidante y el contenido fenólico total de los extractos. Finalmente, un organogel fue formulado con alta estabilidad con el extracto, aceite de cannabis y cera de salvado de arroz, y fue evaluado según su estabilidad. Una revisión bibliográfica exhaustiva de la literatura sobre Arnica Montana, los métodos de extracción y los solventes, compuestos fenólicos y organogeles se incluye además en este trabajo. Es necesario que el trabajo futuro se centre en la determinación de sus propiedades reológicas y solubilidad en diferentes disolventes a varias temperaturas.

Palabras clave: *Arnica Montana*, organogels, extraccion, fenoles, separación

Abstract

Arnica Montana is a promising source of bioactive compounds, mainly phenols, which, due to their antioxidant activity, show health benefits in humans. To reach a stage of pharmaceutical use, *Arnica Montana* flowers need to be processed into a viable product. For this purpose, various extractions techniques with different solvents were performed: cold maceration, ultrasounds, Soxhlet, and supercritical CO2; the solvents used were ethanol, methanol, and a 95:5 (v:v) mixture of ethanol and water. Analysis involving ultraviolet/visible spectrometry were accomplished to measure the antioxidant activity and the total phenolic content of the extracts. Finally, an organogel was formulated using the extract, cannabis oil, and rice bran wax and was tested for its stability. In the frame of this work, organogel with a high stability has been formulated for the first time, since until now, scientific literature does not provide any data of such kind of formulation. Additional studies will be required to determine its rheologic properties and solubility in different solvents at various temperature conditions.

Key words: *Arnica Montana*, organogels, extraction, phenols, isolation

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Álvaro Pequeño Alonso

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Magistrsko delo

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OPTIMIZATION OF PROCESSING CONDITIONS ON THE QUALITY OF EXTRACTS AND FORMULATION OF *ARNICA MONTANA*

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University of Maribor

Faculty of Chemistry and Chemical Engineering

Place and date: MARIBOR, 21.06.2022

On the basis of Article 330 of the Statute of the University of Maribor (Official Gazette of the Republic of Slovenia, No. 41/2021-UPB13), I issue the following

DECISION ON FINAL WORK

ÁLVARO PEQUENO ALONSO, student of 2nd cycle MS study programme Izmenjava MAG, has satisfied all requirements and is allowed to compose the final work.

The topic of the final work is predominantly from the field of the chair/department/institute: KATEDRA ZA KEMIJSKO TEHNOLOGIJO.

Supervisors:

izr. prof. dr. MAŠA KNEZ MAREVCI, univ. dipl. inž. kem. tehnol., supervisor
red. prof. dr. ŽELJKO KNEZ, univ. dipl. inž. kem. tehnol., co-supervisor

Title of final work:
Optimizacija postopka ekstrakcije učinkovin iz arnike *Arnica Montana* in formulacija produktov

Title of final work in English:
Optimization of extraction of active compounds from *Arnica Montana* and product formulation

The deadline for submission of the final work is 01.07.2022. Final work shall be prepared in line with requirements of the guidelines: *Predloge z navodili za pisanje zaključnega dela* and submitted at the Student Affairs Office. Number of copies: 0. At the same time, a statement from the supervisor is submitted (and possible co-supervisor) on final work adequacy .

Legal precept: an appeal against this decision is possible to the Senate of the faculty within 10 business days from date of receipt of the decision.

red. prof. dr. Zdravko Kravanja, Dean

To be notified:
- candidate,
- supervisor,
- co-supervisor,
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Univerza v Mariboru

Fakulteta za kemijo in kemijsko tehnologijo

Kraj in datum: MARIBOR, 21.06.2022

Na osnovi 330. člena Statuta Univerze v Mariboru (Uradni list RS, št. 41/2021-UPB13) izdajam

SKLEP O ZAKLJUČNEM DELU

ÁLVARO PEQUENO ALONSO, študent/-ka študijskega programa 2. stopnje MAG Izmenjava MAG, izpolnjuje predpisane pogoje in se mu/ji dovoljuje izdelati zaključno delo.

Tema zaključnega dela je pretežno s področja katedre/oddelka/inštituta: KATEDRA ZA KEMIJSKO TEHNOLOGIJO.

Mentorji:

izr. prof. dr. MAŠA KNEZ MAREVCI, univ. dipl. inž. kem. tehnol., mentor/-ica
red. prof. dr. ŽELJKO KNEZ, univ. dipl. inž. kem. tehnol., somentor/-ica

Naslov zaključnega dela:
Optimizacija postopka ekstrakcije učinkovin iz arnike *Arnica Montana* in formulacija produktov

Naslov zaključnega dela v angleškem jeziku:
Optimization of extraction of active compounds from *Arnica Montana* and product formulation

Rok za izdelavo in oddajo zaključnega dela je 01.07.2022. Zaključno delo je potrebno izdelati skladno z navodili: *Predloge z navodili za pisanje zaključnega dela*, in ga oddati v pristojnem referatu članice. Število izvodov: 0. Hkrati se odda tudi izjava mentorja/-ice (in morebitne/-ga somentorja/-ice) o ustreznosti zaključnega dela.

Pravni pouk: Zoper ta sklep je možna pritožba na Senat članice v roku 10 delovnih dni od dneva prejema sklepa.

red. prof. dr. Zdravko Kravanja, dekan

UNIVERZA V MARIBORU FAKULTETA ZA KEMIJO IN KEMIJSKO TEHNOLOGIJO 3

Obvestiti:
- kandidata/-ko,
- mentorja/-ico,
- somentorja/-ico,
- arhiv.

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Abstract

Arnica Montana is a promising source of bioactive compounds, mainly phenols, which, due to their antioxidant activity, show health benefits in humans. To reach a stage of pharmaceutical use, *Arnica Montana* flowers need to be processed into a viable product. For this purpose, various extractions techniques with different solvents were performed: cold maceration, ultrasounds, Soxhlet, and supercritical CO_2; the solvents used were ethanol, methanol, and a 95:5 (v:v) mixture of ethanol and water. Analysis involving ultraviolet/visible spectrometry were accomplished to measure the antioxidant activity and the total phenolic content of the extracts. Finally, an organogel was formulated using the extract, cannabis oil, and rice bran wax and was tested for its stability. In the frame of this work, organogel with a high stability has been formulated for the first time, since until now, scientific literature does not provide any data of such kind of formulation. Additional studies will be required to determine its rheologic properties and solubility in different solvents at various temperature conditions.

Key words: *Arnica Montana,* organogels, solvent extraction, phenolic compounds, isolation, formulation

UDK: 66.061.3:633.88(043.2)

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Symbols and abbreviations used

Abbreviations

CO_2	Carbon dioxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EtOH	Ethanol
EtOH+W	Ethanol and water
GA	Galic acid
MeOH	Methanol
SCE	Supercritical fluid extraction
UV	Ultraviolet

1 Introduction

1.1 Problem definition

Arnica Montana is a widely used therapeutic plant, traditionally used to treat various ailments, in the field homeopathic medicine. Recently, there has been a growing interest in its extract and its possible pharmaceutical applications. Among its many properties, it is worth highlighting the antibiotic, anti-inflammatory, and analgesic capabilities the extract of the flowers have (Kriplani et al., 2017). *Arnica Montana* does not contain a high amount of components in *Arnica Montana* (Šutovská et al., 2014) which means, if these natural remedies are going to be used in the pharmaceutical industry, it is necessary to develop effective and selective methods for the extraction and isolation of those bioactive natural products. Also important are the methods used to study the chemical properties of these extracts. Oxidation is key in numerous physiological conditions, including cellular injury, aging, and cancer, so the antioxidant capabilities of the extract are to be studied.

The first step in the isolation and purification of bioactive compounds from plant material is the pretreatment of the material, to facilitate the extraction of the compounds. The most common pretreatment is the drying and posterior grinding of the plant materials to reduce them to fine dust, increasing the surface area in contact with the solvent. Extraction is the next step, and it is considered one of the most crucial procedures in the manufacture of herbal products, since it will affect the active ingredients in the sample both qualitatively and quantitatively (Jha & Sit, 2022). While conventional solvent extraction methods, such as Soxhlet and maceration, are successful procedures in the extraction of bioactive compounds, they have problems of heat degradation, low yields, and the presence of impurities in the final samples (Zhang et al., 2018). Because of this, innovative extraction methods in the pharmaceutical industry have been extensively investigated, like ultrasound extraction, and supercritical fluid extraction. Cold maceration is the most conventional extraction method, and it involves soaking the material in the solvent at room temperature and pressure. Although it does not present degradation of the sample due to heat, it has disadvantages like long extraction times and low yield (Šutovská et al., 2014). Soxhlet extraction, although more efficient in its extraction rates, uses reflux and high temperature to obtain the extract, which makes it not recommendable for thermal sensitive products, like plant-based samples (Zhang et al., 2018).

Ultrasounds are commonly used for temperature sensitive natural products (Zhang et al., 2018), and they use ultrasonic wave energy to create cavitation on the surface of the material accelerating the dissolution of it in the solvent, as well as the heat transfer, which improves the ultrasound extraction efficiency of the biocompounds. Finally, the most interesting method is using supercritical CO_2 for the extraction. Recently, supercritical fluid CO_2 extraction has been the focus of many studies as a promising technology over other more traditional extraction methods for plant-based compounds. In this context, the properties of supercritical CO_2, like higher diffusivity, lower viscosity, and lower surface tension than conventional solvents, make the mass transfer more efficient and allow an environmentally friendly operation (Knez et al., 2019).

The selection of the solvent is an important part in solvent extraction. Selectivity, solubility, cost, and safety should be considered in selection of solvents. Based on the law of similarity and intermiscibility, solvent with a polarity value near to the polarity of the solute are likely to perform better and vice versa. The main compounds of interest in phytochemical investigation are polar, like phenolic compounds, so alcohols, sometimes diluted in water, are universal solvents in solvent extraction in plant related extraction (Daud et al., 2022), although as previously mentioned, new applications are being developed with supercritical CO_2, which is non-polar.

Plants are known to contain a variety of natural antioxidants that protect and preserve their physical and metabolic integrity as well as their heredity by way of their seeds. Many of these extracts and compounds from plants are emerging as candidates for moderating the effects of the aging process on skin by limiting biochemical consequences of oxidation (Madhujith et al., 2022). Antioxidant activity is also important in regulating the redox state of the body and thereby, reducing the damage caused by diseases or drugs (Koo et al., 2000). Phenolic compounds have demonstrated several beneficial properties by acting as antioxidant agents due to the hydroxylated aromatic rings contained in their molecules (do Carmo et al., 2021). Because of this, the main study of the chemical properties of *Arnica Montana* will focus on the antioxidant activity, as well as the total phenol content of the extracts and the incorporation of the extracts in the suitable formulations with a high bioavailability.

Individual parts of *Arnica Montana* plant contain various constituents, like sesquiterpene lactones, thymol derivates, and phenolic acids (Šutovská et al., 2014), which are of interest for the potential pharmaceutical applications, due to their antioxidant activity. The study of the chemical properties of the *Arnica Montana* extract can be done with a spectrophotometer

working in the Ultraviolet/Visible range (UV/Vis). This is a popular and widely used technique in analytical chemistry; it is a powerful analytical technique with very high sensitivity and specificity, and being relatively inexpensive and easily implemented, it is widely used in diverse applied and fundamental applications. With this process, information about the antioxidant capabilities and total phenols content, of the extract can be obtained.

Gels are defined as semi-solid systems, formed by a three-dimensional structure composed of gelator molecules, with a liquid solvent phase immobilized (Zeng et al., 2021). Organogels use as an organic phase to for the 3D structure; this allows them to have higher boiling points, which confers them higher stability, than standard gels. Also, organogels exhibit a series of unique properties and functionalities, such as topical deliveries for hydrophobic drugs (Zeng et al., 2021) which makes them interesting for pharmaceutical applications. Common organic solvents as well as mineral and vegetable oils are commonly reported to be used as organic solvents for organogel formulations (Esposito et al., 2018); in this case, cannabis oil and rice bran wax were used as the organic phase and the gelator, respectively.

1.2 Objectives

The general objective of this thesis is to study the pharmaceutical viability of *Arnica Montana*. For this, the aforementioned methods of extraction and solvents will be used for the obtention of *Arnica Montana* extract. An analysis of the antioxidant activity and total phenol content of the extract and phenolic profile, using UV/Visible spectrometry, will be performed. After, an organogel formed from the cannabis oil, rice bran wax, and the *Arnica Montana* extract will be produced and formulated.

2 Arnica Montana

2.1 Description

Arnica Montana, member of the *Asteraceae* family, one of the largest flowering plant families, is an herbaceous perennial plant growing on mountains to alpine meadows, pastures and in light forests, it has bright green leaves with rounded tips at the base that are level with the ground. Their surface is veined and aggregated in rosettes. The top leaves are opposing, and smaller (Figure 1). They bloom into a star or sunflower formation from June through August (Hanrahan, 2018). The flower is native to the mountains of Siberia and central Europe, but it has spread over most of Europe (Figure 2) and can be found in North America and Russia. However, this species is considered endangered in different European countries so its cultivation is increasing but its harvest is not allowed in most countries to protect the species (Kriplani et al., 2017).

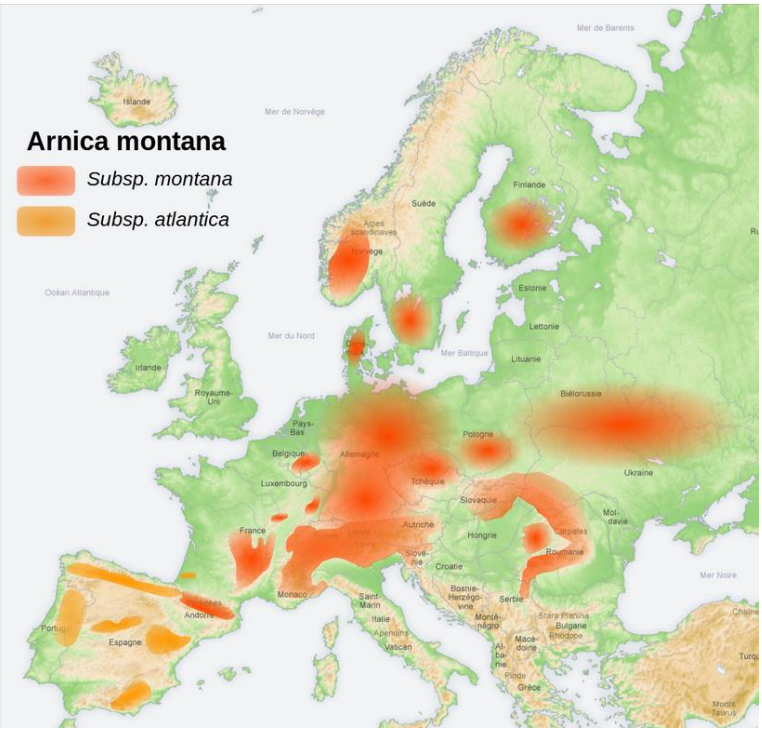

Figure 1.- Map of the population of *Arnica Montana* in Europe (Wikipedia, 2007)

Figure 2.- *Arnica Montana* growing in the mountains of Switzerland (Studer, 2006)

Arnica Montana is popular as a traditional homeopathic material. The herb has been used in folk remedies since the sixteenth century. A wound healing effect was already attributed to Arnica in a manual of Tabernaemontanu in 1613, and it was a popular remedy for numerous ailments during the coming centuries. Since the middle of the 20th century, in countries like Germany, Arnica is used to treat hematomas, sprains, and contusions, in homoeopathic systems of medicine. A monograph on Arnica was established by the German Commission E, a scientific advisory board of the Federal Institute for Drugs and Medical Device specializing on herbal medicines, indicating, and limiting, the application to external use, due to possible risks with the ingestion of *Arnica Montana* based solutions (EMA, 2013). Also, detailed written studies elaborated by the European Pharmacopoeia, a regional book which provides common quality standards throughout the pharmaceutical industry, indicate Arnica's flowers as an appropriate topical remedy with the right elaboration. It is clearly a widespread herb in the homeopathic field, since there are over one hundred medicinal preparations using Arnica extracts commercially available in Germany. Romania is the main producer of wild-collected *Arnica Montana* flowers, exporting mainly to Germany, Italy, France and Switzerland (Robertson et al., 2007). A research from the University of Koblenz-Landau in Germany estimated that 50 metric tons of dried Arnica flowers are used annually in Europe (Ucenic & Mastorakis, 2007) It is estimated that the demand in Germany is 10 metric tons for the pharmaceutical sector alone. In the United States, arnica is widely used in topical application, like inflammations (Fiume, 2001).

2.2 Properties and chemical composition

It has been investigated by many researchers that flowers of the plant are mainly rich in active constituents (Hanrahan, 2018; Kriplani et al., 2017; Pawlaczyk et al., 2014). *Arnica Montana* contains up to 1% of a volatile oil that is partly composed (approximately 50%) of fatty acids, especially palmitic, linoleic, myristic, and linolenic acids (Bandaiphet & Kennedy, 2004). The flowers of Arnica species contain especially sesquiterpene lactones. Sesquiterpene are a class of terpenes that consist of three isoprene units, and occur naturally in insects and plants as semiochemicals, that is, as chemicals that affect the behavior of the individual and those around it when released. Sesquiterpene lactones have a lactose ring and occur mainly in in plants of the family Asteraceae.

Beside sesquiterpene lactones (with a natural variability of 0.3 to 1%), other components include essential oil compounds (0.2 to 0.35%), flavonoids (0.4 to 0.6%), hydroxycoumarins and phenyl acrylic acids (Kriplani et al., 2017). The flowers also contain 13 helenanolides, which are sesquiterpene lactones; these have been identified as helenalin, 11,13-dihydrohelenalin , and 11 ester derivatives. In vitro studies have shown that the most active components of Arnica, as well as of other members of the *Asteraceae* family, are helenalin and other sesquiterpene lactones such as 11a,13-dihydrohelenalin and chamissonolid. (Fiume, 2001). The most relevant constituents that have been isolated are the helenalins (Figure 3). Helenalin is a sesquiterpene responsible for the toxicity of the *Arnica Montana,* causing skin irritation and especially severe gastroenteritis and internal bleeding of the digestive tract, if ingested in large amounts. Because of this, Arnica extracts are not recommended for internal administration, although helenalins possess some anti-inflammatory and anti-neoplastic effects (Schröder et al., 1990).

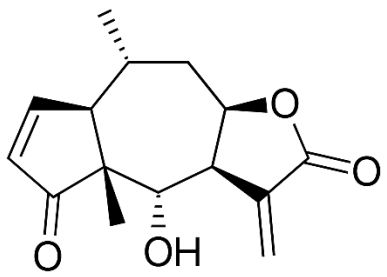

Figure 3.- Skeletal formula of helenalin (Concellos, 2008)

Phenolic compounds are a group of small molecules characterized by their structures having at least one phenol unit. Phenol itself is a benzene ring that is substituted with a hydroxyl group

(Figure 4). Thus, its systematic name is hydroxybenzene. Based on their chemical structures, phenolic compounds can be divided into different subgroups, such as phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbens, and curcuminoids. The categories of phenolic compounds and their representative compounds are shown in Figure 5.

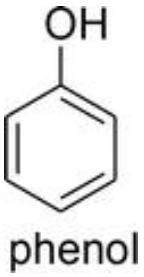

Figure 4.- Phenol unit (Mamari, 2021)

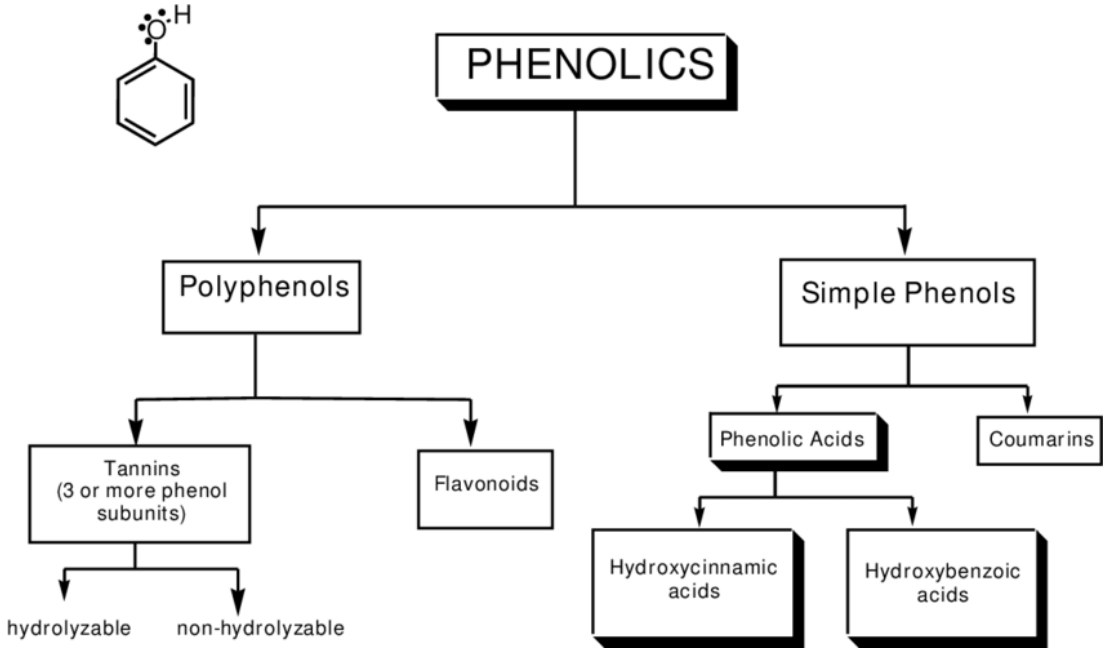

Figure 5.- Categories of phenolic compounds (Cooper & Nicola, 2014)

Flavonoids (Figure 6) are some of the most common phenolics, widely distributed in plant tissues, and often responsible alongside the carotenoids and chlorophylls for their blue, purple, yellow, orange and red colors. The flavonoid family includes flavones, flavonols, iso-flavonols, anthocyanins, anthocyanidins, proanthocyanidins and catechins. All flavonoids are derived from the aromatic amino acids, phenyalanine and tyrosine, and have three-ringed structures. Phenolic acids are one of the other main phenolic classes within plants and occur in the form of esters, glycosides, or amides, but rarely in free form. Variation in phenolic acids is in the number and location of hydroxyl groups on the aromatic ring. Phenolic acids have

two parent structures: hydroxycinnamic and hydroxybenzoic acid (Figure 6). Hydroxycinnamic acid derivatives include ferulic, caffeic, p-coumaric and sinapic acids, while hydroxybenzoic acid derivatives consist of gallic, vanillic, syringic and protocatechuic acids (Khoddami et al., 2013).

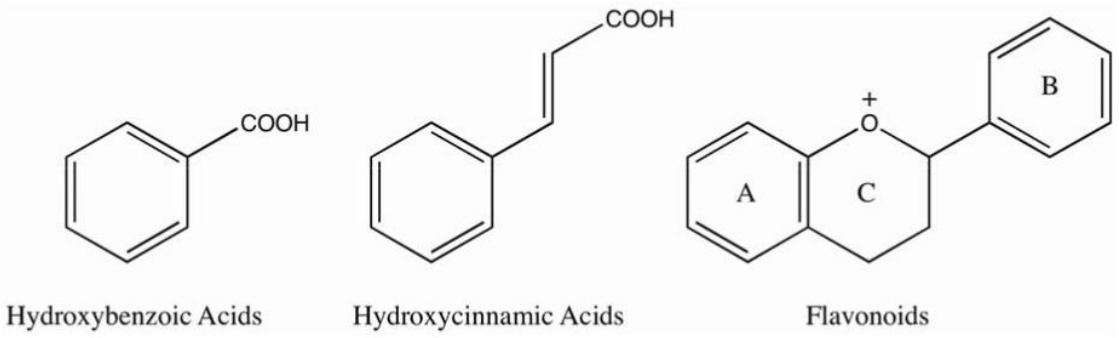

Figure 6.- Structure of phenolic acids and flavonoids (Khoddami, 2013)**.**

They are very important antioxidants due to their hydroxyl groups that confer free radical scavenging ability to plant products. This means they can neutralize harmful free radicals that would otherwise damage body cells and increase the risk of conditions like cancer, diabetes, and heart disease (Ćujić et al., 2016). Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. A study done in 2004 showed that ethanolic extracts of *Arnica Montana* contain high concentrations of phenolic acids and flavonoids content, conferring them a good antioxidant activity, which justifies its use in the treatment in skin disorders (Craciunescu et al., 2012). According to epidemiological studies, the intake of phenolic compounds is inversely correlated with the risk of coronary heart disease (do Carmo et al., 2021). A number of polyphenolic compounds, especially catechins, have been found to be potent antioxidants and to be effective in preventing cancer (Šutovská et al., 2014) while tannins have been reported to exert other physiological effects; they can reduce blood pressure, accelerate blood clotting, lower serum-lipid levels, modulate immunoresponses and cause liver necrosis (Khoddami et al., 2013).

Variation in the chemistry of phenolics in a sample is related to the concentration of simple and complex polyphenolic compounds and the different proportions of phenolic acids, flavonoids, anthocyanins and proanthocyanins (among others). Thus, it is difficult to choose a single method of preparation and extraction for phenolics for many plant products. The most common techniques to extract phenolics employ solvents, either organic or inorganic. Several parameters may influence the yield of phenolics, including extraction time, temperature,

solvent-to-sample ratio, the number of repeat extractions of the sample, as well as solvent type. Solvents, such as methanol, ethanol, propanol, acetone, ethyl acetate, and their combinations have also been used for the extraction of phenolics, often with different proportions of water. For example, phenolic compounds can be efficiently extracted from legumes using an ethanol/water (70:30 v:v) system (Casazza et al., 2010). Extraction of phenolics from aerial parts of *Potentilla atrosanguinea* showed that 50% aqueous ethanol was more efficient than pure or 50% aqueous forms of methanol, and acetone (Kalia et al., 2008).

Supercritical fluid extraction has proven to be a promising technique for obtaining high-quality phenolic compounds with minimal loss of its properties (Žitek et al., 2022). Carbon dioxide is the most widely used solvent due to its characteristics, such as moderate critical conditions and high availability. It is also nontoxic, inflammable and chemically stable. However, CO_2 as the extracting solvent is not efficient for the extraction of phenolic compounds because of its low polarity in comparison to most phenols, which can be solved using a polar co-solvent, as elaborated in the next section.

Despite a very large number of published investigations, quantification of various phenolic structural groups remain difficult. While lower molecular weight compounds are quite easily analyzed by UV–visible spectrophotometry and mass spectrometry, analysis of polymeric compounds remains a challenge, as their diversity results in poor resolution and detection. Tannins are major examples of such structures for which method development is needed (Khoddami et al., 2013). Spectrophotometry is one of the relatively simple techniques for quantification of plant phenolics. The Folin-Ciocalteu method is a classic method used in spectrophotometry to measure total phenolics in plant materials (Hrnčič et al., 2019).

2.3 Current and potential applications

Focusing on the medical aspects of the plant, it is widely used worldwide as a homeopathic remedy. The flowers of the plant show greater medicinal value and are used as antiphlogistic, inotropic, antibiotic, anti-inflammatory, immunomodulatory, antiplatelet, uterotonic, antirheumatic and analgesic in febrile conditions (Kriplani et al., 2017). Clinical studies conducted in humans have worked on the topical and internal applications of *Arnica Montana* extracts, and their homeopathic products. They have shown great potential in the treatment of various inflammatory and microbial conditions. Arnica extracts have been studied primarily for their topical use while studies on internal uses are limited to homeopathic products, due to Arnica's toxicity (Koo et al., 2000; Kriplani et al., 2017).

In the European Union, several alcoholic preparations from *Arnica Montana*, are under regulation as traditional herbal medicinal products, this means, requiring registration and an authorization before accessing the market. This solutions are of various strengths; the ones prepared from the whole plant are 1:5, ethanol 60% (v/v); and 1:10, ethanol 60% or 70% (v/v) and the liquid extract of fresh flowers are 1:20, ethanol 50% (m/m) (Gayle & Brinckmann, 2015). These preparations are marketed for cutaneous use only and are indicated for the relief of bruises, sprains, and localized muscular pain. In Germany alone, there are 843 registered medicinal products listing *Arnica Montana* as an active ingredient, of which 360 products are homeopathic medicines (Hanrahan, 2018).

3 Extraction procedures

The initial stage in separating the desired natural products from the raw ingredients is extraction. The most common approach in plant extraction is solvent-based methods. The following phases are involved in the extraction of natural products: (1) the solid matrix of the raw plant material is penetrated by the solvent; (2) the solvents dissolve the solute; (3) in the solid matrix, the solute diffuses following the mass transfer mechanisms; and (4) the solutes are collected as the final product extracted. A lot of factors are there to be considered when working with the extraction of plant matter in an efficient manner.

The selection of the solvent is crucial for solvent extraction. Alcohols (ethanol and methanol) are universal solvents for phytochemical investigation, since they are polar solvents, and most of the compounds of interests in plant extractions, like polyphenols, are polar too (EMA, 2013). According to law of similarity, the closer the polarity values of the solvents and solutes, more effective the extraction will be.

Normally, increasing time and temperature promote analyte solubility; however, plant phenolics are generally degraded or undergo undesirable reactions such as enzymatic oxidation by extended extraction times and high temperatures. This will result in a lower yield and the presence of impurities in the final extract (Huang et al., 2013).

Extraction efficiency is usually directly correlated to how fine the particles are. A smaller solute particle size increases the surface area the solvent can act on, improving solvent diffusivity in the solute. If the particle size is too small, however, there will be trouble in the following filtering. Usually, the methods to reduce the particle size of the material are mechanical (grinding, milling, cutting, etc.).

Polyphenol recovery from plants may be accomplished using a variety of extraction processes, which can be broadly classified as conventional and contemporary. Classic extraction procedures include cold maceration, and Soxhlet extraction. With increasing energy consumption and the drive to improve efficiency, industries and research institutions are challenged to find ways which can simplify operation procedure, meet low-cost requirements, and achieve good quality. In recent years, novel approaches for extracting bioactive substances have been developed, such as ultrasound-assisted extraction, and supercritical fluid extraction.

3.1 Cold maceration

This is a very simple extraction method, where the plant matter is put into contact with the solvent and its stirred, with the purpose of transferring the solid compounds into the liquid solution. The extraction is done at room temperature and pressure, so the extractions times are longer than the other methods, but it is useful for the extraction of thermolabile components, like plant matter.

A study in 2015 achieved a high yield of total phenols chokeberry fruit using 50% ethanol, a solid–solvent ratio of 1:20, which might mean that maceration was a simple and effective method for the extraction of phenolic compounds from chokeberry fruit (Ćujić et al., 2016). In 2018, Soxhlet extraction was compared with cold maceration to find out which one gave a better antimicrobial activity of licorice extract against Streptococcus mutans; the findings suggested that using 100% ethanol as the solvent, licorice extract kept its antimicrobial activity better when it was obtained using cold maceration (Sankeshwari et al., 2018). The volatile fractions obtained from *Lonicera macranthoides* using different extraction methods were compared in a study done in 2015. Although the preferred method was hydrodistillation, cold maceration showed advantages over the variety of constituents obtained in the final extract (Wu et al., 2015).

3.2 Ultrasound extraction

An extraction method where high-power, low-frequency ultrasound waves are used in a solution of the plant matter and the solvent. These waves create cavitation bubbles in the solution which implode on the surface of the solid, breaking it down and creating a breakage in the plant cell walls, releasing the bioactive compounds from the plant matter to the solvent in the presence of mass transfer gradient. This cavitation accelerates the dissolution and diffusion, as well as improving the mass and heat transfer, which improves the extraction efficiency. Ultrasound-assisted extraction frequently uses less solvent, takes less time, and yields a greater extraction yield, and it is a safer option for thermos-sensitive materials than other heat-based methods. It is a promising processing technology to extract plant bioactive compounds such as phenolics, flavonoids, thymols, saponins and proteins (Yusoff et al., 2022).

Many researchers prefer to use ultrasonic energy for the extraction of phenolic compounds from plant material. Methanol was showed to be the most appropriate solvent in extracting

phenolic compounds from the leaves from *Centaurea* sp., and followed by ethanol (Bouafia et al., 2021). A study in 2019 varied the concentration of ethanol from 50 to 100% to extract phenolic compounds from lime peel waste, finding that using ethanol and ultrasounds in combination is a potential technique for extracting phenolic compounds in an ultrasonic-assisted extraction system (Rodsamran & Sothornvit, 2019). The frequency and intensity of the ultrasounds is a factor to keep in mind. The results of Lim et. al. (Lim et al., 2019) showed that high intensity at 700 W could extract mangiferin effectively from the fruits of *Phaleria Macrocarpa*. Time wise, research shows that if the intensity is high enough, the extraction times can be as low as 0.5 min. Arruda et al. (2019) (Arruda et al., 2019) correlated a low intensity ultrasound extraction to longer extraction times of phenolic compounds from *araticum* peel. The range used was 160–640 W for 0.5–5 min, respectively. The high-intensity ultrasound technology, the study showed, proved to be an efficient and low environment impact method to extract phenolic compounds from *araticum* peel.

3.3 Soxhlet extraction

It is a conventional extraction method that utilizes the principle of reflux and siphoning to continuously extract the herb with fresh solvent. Originally designed for extracting lipids from a solid, its use has extended to any extraction application. As it can be seen in Figure 7, the extractor is connected to a flask containing the solvent, and to a condenser. Inside the extractor there is a filter paper thimble containing the raw material. The solvent is evaporated, ascending to the condenser, where it condenses and falls in the raw material. When the chamber containing the test is almost full, it is emptied by siphon action, back down to the flask. This cycle then is repeated as many times as necessary. This is the main advantage of Soxhlet extraction, only clean warm solvent is used to extract the solid in the thimble, which improves the efficiency of the extraction if compared to other methods, like just heating up the solvent and solute in a flask. The main drawback is that it uses high temperature during long periods of time, so if the components to be extracted are sensitive to heat, as plant matter is, it will degrade, and impurities will appear in the final extract.

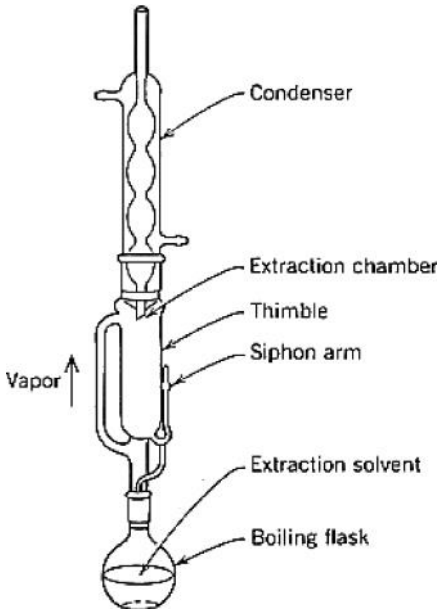

Figure 7.- Soxhlet extractor (Shithole, 2010)

As previously mentioned, in a study performed by Sankeshwari et al. (Sankeshwari et al., 2018), Soxhlet extraction didn't not perform better than cold maceration when keeping the antimicrobial activity of licorice against *Streptococcus mutans*; this is due to the high temperature used in the Soxhlet extraction inhibited the antimicrobial active compounds. In this line of study, it was observed that catechins in tea degraded due to the high temperature applied. When compared to maceration, which used temperatures under 40 ºC, the concentrations of total polyphenols were higher than the Soxhlet extraction, which was operating at 70 °C, degrading the bio compounds of the plant matter. Similarly, *Lagenaria siceraria* was extracted using ethyl acetate with ultrasounds and Soxhlet extraction (Abbas et al., 2021), showing less yield of phenolic compounds when the extraction performed was the Soxhlet type.

3.4 Supercritical fluid extractions

Supercritical fluid extraction is a non-conventional extraction technique that has been developing the last decades, and it is a promising method to use in the extraction of natural products, like plant matter. In supercritical fluid extractions, the main solvent used is a fluid in its supercritical point. This superfluid has similar solubility to liquid and similar diffusivity to gas and can dissolve a wide variety of natural products. Carbon dioxide is the most common supercritical solvent. With critical conditions of 30.9 ºC and 73.8 bar, it is inexpensive, safe, and widely accepted by the FDA (United States Food and Drug Administration) and EFSA

(European Food Safety Authority) (Leitner & Poliakoff, 2008). More benefits of supercritical CO_2 include the high diffusivity and its gaseous state at ambient temperature and pressure, making analyte recovery simple and allowing for solvent-free analytes. In the context of extracting plant matter, its capacity to work at low temperatures using a non-oxidant medium, allows the extraction of thermally labile or readily oxidized chemicals. The main drawback of using CO_2 as the solvent is its non-polarity; if polar compounds, like phenols, need to be extracted, a polar co-solvent (an alcohol for example) will need to be used during the process (Knez et al., 2019).

The use of co-solvent might not be necessary. Some studies have been carried out with co-solvents. The essential oil of rosemary was extracted using this technique with supercritical CO_2, hydrodistillation, and steam distillation. Yields of essential oil and antioxidant activity of the supercritical extraction method were higher than those from other two methods (Conde-Hernández et al., 2017). A variety of Brazilian fruits extracts were obtained using supercritical CO_2 and a co-solvent. The highest antioxidant activity was obtained when using ethanol as the co-solvent (Veggi et al., 2014). Ramirez et al. fractionanted carnosic acid and carnosol, the main antioxidants compounds found in rosemary, using supercritical CO_2 and 10% ethanol, at 130 bar and 353 K (Vázquez et al., 2006). Using a co-solvent is not always necessary, as a study showed when extracting *Myrmecodia pendans* using a co-solvent free CO_2 supercritical extraction (Sanjaya et al., 2014). This claim is supported by other studies, like a 2008 study showing high yields of phenolic compounds when obtaining extracts of roasted wheat germ without the use of a co-solvent with pure CO_2 at 442 bar and 313.15 K (Gelmez et al., 2009).

One of the main aspects that should be considered in supercritical fluid extraction is the parameter optimization. The use of the optimum values for the different variables influencing the supercritical fluid extractions could significantly enhance the recovery or extraction yield of a target compound. The main variables affecting the extraction are pressure, and temperature. Temperature has a couple of effects on the solute solubility: at a constant rate of increase, at constant pressure, the density of the fluid decreases. In the other hand, increasing the temperature at constant density, influences the vapor pressure of the solute increasing it. The pressure increase contributes to higher fluid densities, which implies a higher solubility of the solute (Knez et al., 2019). *Myrmecodia pendans* was extracted in a range of parameters of 9 to 22.5 MPa and 313.15 to 343.15 K for 6 to 7 h. It was found that the extraction yieldsand solubilities of phenolic compounds are strongly affected by the changes of pressure and

temperature. The solubilities were increased significantly with increasing pressure and temperature (Sanjaya et al., 2014).

4 Organogels

A gel is a semi-solid material with various phases. The gelator is the solid compound, usually at low concentrations (<15% w/v) (Zeng et al., 2021) used to immobilize a continuous phase, the solvent, in an elastic or viscoelastic crosslinked network. Depending on the nature of the involved solvent, gels can be classified as either hydrogels or organogels. Hydrogels contain hydrophilic solvents while organogels possess an organic solvent as their continuous phase.

Organogels are viscoelastic systems constituted by a continuous liquid phase, typically an organic solvent, a mineral or vegetable oil, immobilized in a three-dimensional network. The wide functionality of organogels has been studied especially as drug and vaccine delivery platforms via various routes such as transdermal, oral, ophthalmic and parenteral administrations (Li et al., 2022). Organogels are attractive due to the highly tunable properties driven by the inherent advantages of diversity in the gel components. Although they have well-recognized performances, there has not been a successful attempt of taking these applications into actual clinical products due to a lack of biocompatibility with these formulations, since the used organic solvents were found to be significantly toxic (Debnath et al., 2014). Hopefully, in the recent years, major advances have been made in the pharmaceutical organogel field. More biocompatible, biodegradable organic solvents and organogelators prove more pharmaceutically acceptable and environmental-friendly organogels. Although a lot of advances have been made in recent years, the research on organogels is lackluster; even with limited reviews on organogels, the studies mainly focus on supramolecular organogels for drug delivery function, not into its properties or possible formulations.

4.1 Description of organogels

Organogels exhibit a series of unique properties and functionalities, such as topical deliveries for hydrophobic drugs and ultralow adhesion features for anti-icing and anti-fouling application, which are due to the combination of hydrophobic polymer networks and organic liquid with low surface tension

Organogelators may be organized in two groups based on the molecular weight range of the gelling molecules: polymeric organic gelators or low-molecular weight organogelators. The chemical structures of the most common organogelators used in organogel formulations for

current applications in both cosmetic and pharmaceutical fields, amongst others, are presented in figure 8.

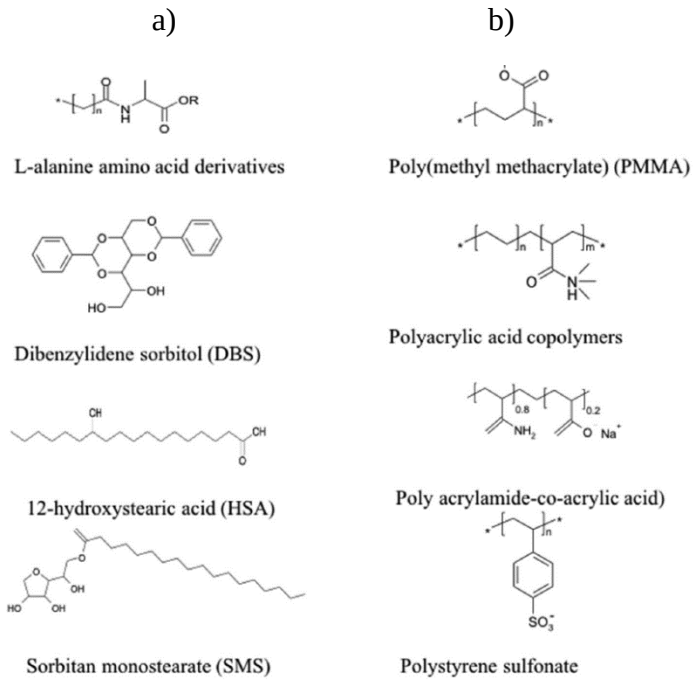

Figure 8.- Commonly used organogelators chemical structures: monomers, (a) , or polymers (b). (Esposito et al., 2018)**.**

Low-molecular weight organogelators are low molecular weight compounds like fatty acids and n-alkanes. They the principal class of organogelators used to produce physical organogels. These organogel networks can either be qualified as strong or weak. Strong physical gels are characterized by long-lasting three-dimensional networks, presenting similar physicochemical properties with solids. Weak organogels, on the contrary, are three-dimensional networks presenting liquid-like viscoelastic behavior (Kirilov et al., 2015).

Polymeric organic gelators are high molecular weight gelators. They present good gelation ability with many types of organic solvents at low concentrations. These organogels are either defined as chemical organogels, when the network is maintained by actual chemical bounds, or physical organogels, when the network is induced by weaker, non-covalent bounds. These gels generally present specific properties, such as higher gel strength leading to the formation of supramolecular crosslinking gelation points (Kirilov et al., 2015).

In addition to the nature of networks or solvents, the crosslinking mechanism of organogels is also an important classification basis for organogels categorizing organogels into physical and chemical organogels. Organogels are formed by crosslinking junction points and 3D networks. Physical organogels are formed by strong non-covalent interactions (hydrogen bonding, van der Waals forces, electrostatic...) in the crosslinking points. Chemical organogels are constructed with covalent crosslinks during the gelation process, using processes like copolymerization reactions. With physical organogels, the intermolecular non-covalent interactions are their primary mechanisms for gelation, which give rise to weak polymer networks. This organogels are generally less stable with low mechanical strength. In the other hand, chemical organogels are more stable and have better mechanical properties due to the strong covalently crosslinked network (Zeng et al., 2021).

4.2 Properties and characterization of organogels

Organogels present viscoelastic properties generally following the Maxwell model of visoelasticity. At lower shear rates, organogels behave like solid-like formulations (Kobori et al., 2007). They are thermoreversible, that is, they have the capacity to recover the stable thermodynamic solid-like structure after being heated. When an organogel is heated up above their melting temperature, there is an increase in the thermal energy inside the low energy thermostable matrix, disrupting the molecular connections in the network. When the systems are cooled down, the physical interactions between organogelator molecules reverse back to their stable form (Zeng et al., 2021). Organogels with pharmaceutical applications are biodegradable in nature, especially the most recent formulations based on biomolecules, like vegetable oils, cocoa butter, or mango butter (Sagiri et al., 2013). The bio-degradation rate can be regulated by adapting the concentration of biocompatible components and the used of advanced formulation technologies.

Recently synthesized organogels are intensively characterized thorough various techniques and methodologies to confirm their stability and efficiency. The most common and simple is a qualitative evaluation method based on a simple inversion (upside down) of the container with the formulation; the gel state is determined when the formulated system does not flow anymore under the effect of its own weight. Various parameters, such as the gravitational flow, time, temperature and concentration of the gelling agent can then affect the visualization and the determination of the gel formation. This method has been used in multiple studies related

to the characterization of organogels using vegetable oils as solvents (Li et al., 2022; Lupi et al., 2016; Patel et al., 2015)

4.3 Applications of organogels

The applications of organogels are strongly dependent on their properties. The relatively poor stability of physical organogels makes them appropiate for drug delivery. For example, some biocompatible molecules/ingredients could release from the gelled matrix in a controlled manner by simple disintegration of the gel network. The chemical organogels, with a more stable internal structure, makes them more appropriate for long-lasting and demanding applications such as industrial anti-fouling and anti-icing applications (Zeng et al., 2021).

Focusing on the pharmaceutical applications of the physical organogels, they have an advantage in drug delivery due to their softness, biocompatibility, and bioactivity. Organogels allow entrapping a variety of bioactive agents and allow for various delivery modes, suggesting great potential for such use of drug delivery platforms. Lecithin organogels, for example, have been extensively investigated as vehicles of cutaneous administration due to the low skin irritation and the biocompatible nature of lecithin. Also, it was found that lecithin organogles have high and compatibility with a wide range of organic liquid including various edible oils and nonpolar solvents as well as pharmaceutical molecules (Brinksma et al., 2000). Organogels prepared with soy bean, vegetable oil proved to be an effective cutaneous drug delivery systems, thanks to the hydrophobic anti-inflammatory nature of the structure, the active compound encapsulated in this organogel was slowly and safely released in the absence of light (Park et al., 2016).

5 Experimental work

The general purpose of the experimental work was to obtain *Arnica Montana* extract to measure its antioxidant activity and total phenol content. Also, an organogel formed by cannabis oil and rice bran wax was formulated, with the extract obtained by supercritical CO_2 extraction. *Arnica Montana* extracts were obtained with different methods and solvents: cold maceration, ultrasound extraction, Soxhlet, and supercritical CO_2 extraction, using ethanol, methanol, and a 95:5 (v/v) mixture of ethanol and water. After its antioxidant activity and total phenol content were measured using UV–visible spectrophotometry. After, the extract was immobilized in an organogel formed by rice bran wax and cannabis oil.

5.1 Extraction of compounds from *Arnica Montana* plant

Dried *Arnica Montana* flowers were crushed into dust using an electrical blender. The resulting material was then diluted in the solvent in a flask and stirred to favor the mixing. The solvents used were ethanol, methanol, and a 95:5 solution of ethanol and water; in every case, the amount used was 150 mL of solvent.

The cold maceration extraction was performed at room temperature and with magnetic stirring, for 90 minutes. The ultrasound extraction (figure 10) was performed at room temperature and pressure for 60 minutes. The Soxhlet extraction (figure 9) was performed heating the solution up to 80ºC degrees for 180 minutes. The supercritical CO_2 extraction was performed in an autoclave at 250 bar, which was submerged in a water bath at 40º C, for 180 min. After each type of extraction, the extract was collected in a round flask, and an evaporation was performed using a rotavapor to separate the solvent from the extract. After completion, the extract was removed from the round flask using a metal spoon and transferred to a smaller container (figure 11), which were stored in a fridge at 2 ºC.

Figure 9.- Set-up for the Soxhlet extraction

Figure 10.- Set-up for the ultrasound extraction

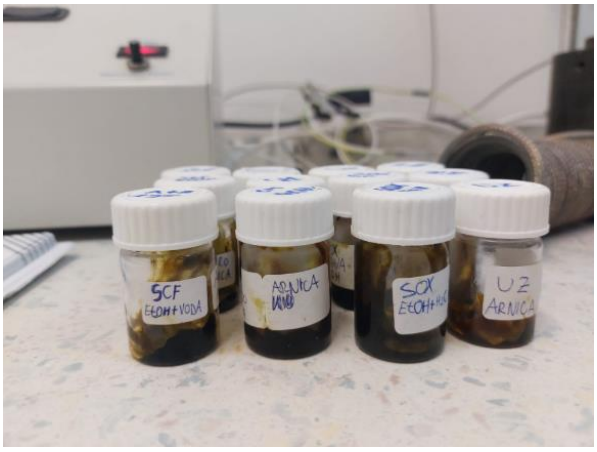

Figure 11.- Final extract of *Arnica Montana*

5.2 UV/Visible spectrophotometry

The UV/Visible spectrometry was performed using the equipment in figure 12. For the antioxidant activity, a solution of 0.01 grams of extract and DPPH (2,2-diphenyl-picryl-hydrazil) diluted in distilled water were used; for the total phenols content, a solution of 0.05 grams of extract with Folin-Ciocalteu reagent diluted in distilled water was used. After letting the solutions rest in a dark chamber for 2 minutes, they were heated to trigger the reaction. The spectrophotometry analysis begins with a blank sample of pure methanol, and then the total phenols and antioxidant activity of each sample were measured, in that order.

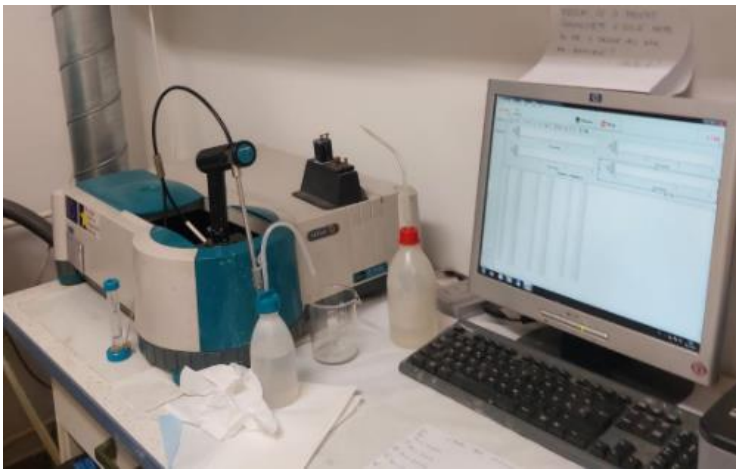

Figure 12.- Uv/Visible spectrometer used in the analysis

5.3 Organogel formulation

The organogel was formulated using the extract obtained with the supercritical CO_2 using ethanol, since it had the highest amount of antioxidant activity. Cannabis oil was used as organic phase, and rice bran wax was used for the gelator: first, the oil and extract were mixed, and then, the wax was introduced in the mixture. The compoenents were mixed at 40ºC during 5 min. The organogels obtained were tested qualitatively by their texture, color, and stability over time (Figure 13).

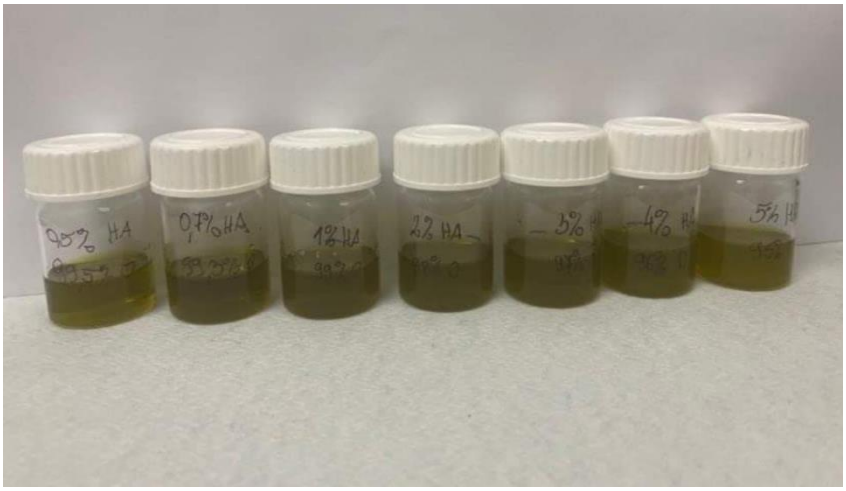

Figure 133.- Organogels formulated from rice bran wax, cannabis oil, and *Arnica Montana* extract

6 Results

6.1 Extraction of *Arnica Montana*

The laboratory extraction results are summarized in Table 1 and Figure 14. The collected data shows the raw *Arnica Montana* used for the extraction, the amount of extract obtained after the extractions and evaporations, and the yield obtained. The best solvent overall was the methanol, with the highest yields of extract in each type of extraction. The most effective extraction method, independently of the solvent, when looking at quantitative yield of extract obtained was Soxhlet extraction amongst all the extraction techniques; followed closely by the supercritical extraction method. Cold maceration and ultrasounds are the least effective methods of them all, with the lowest yields.

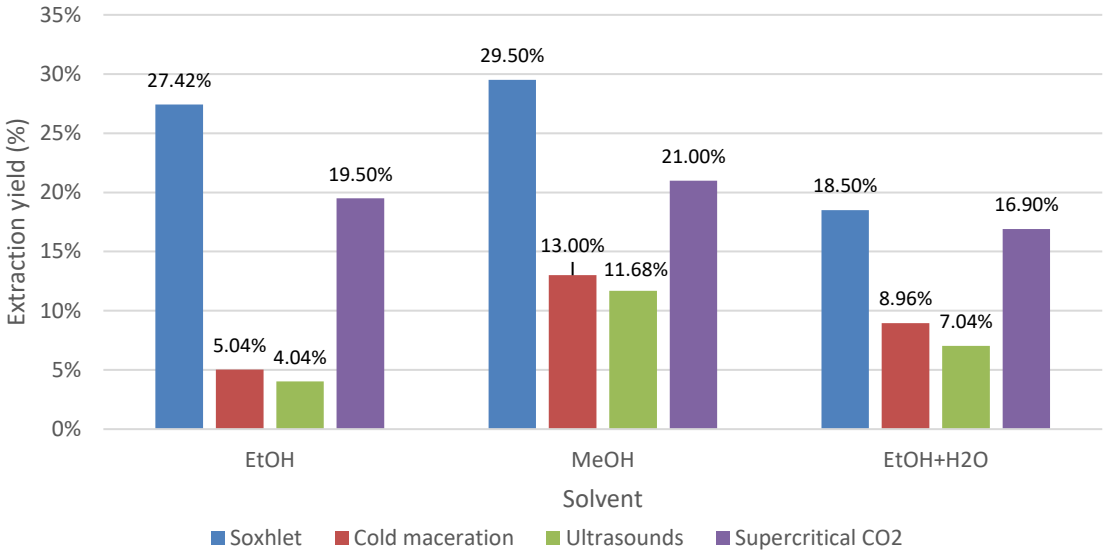

Figure 14.- Extraction yield in % of the *Arnica Montana* samples

6.2 UV/Visible spectrophotometry

The spectrometer measures the light absorbed by the sample, calculating the transmittance and absorbance of the *Arnica Montana* extract. The apparatus works in the range of 800 to 200 nm to detect ultraviolet light (100 to 400 nm) and visible light (380 to 700 nm) spectrum.

The antioxidant activity and total phenol content are presented in figures 15 and 16, respectively. A calibration curve was prepared using galic acid diluted in distilled water. The total phenolic content was expressed as mg GA per g of extract. The extract obtained with the

supercritical CO_2 using ethanol as cosolvent had the highest antioxidant activity, however the highest content of phenolic compounds was present in the samples using a mixture of ethanol and water as a solvent, being the Soxhlet extraction the one that yielded more of these phenolic compounds.

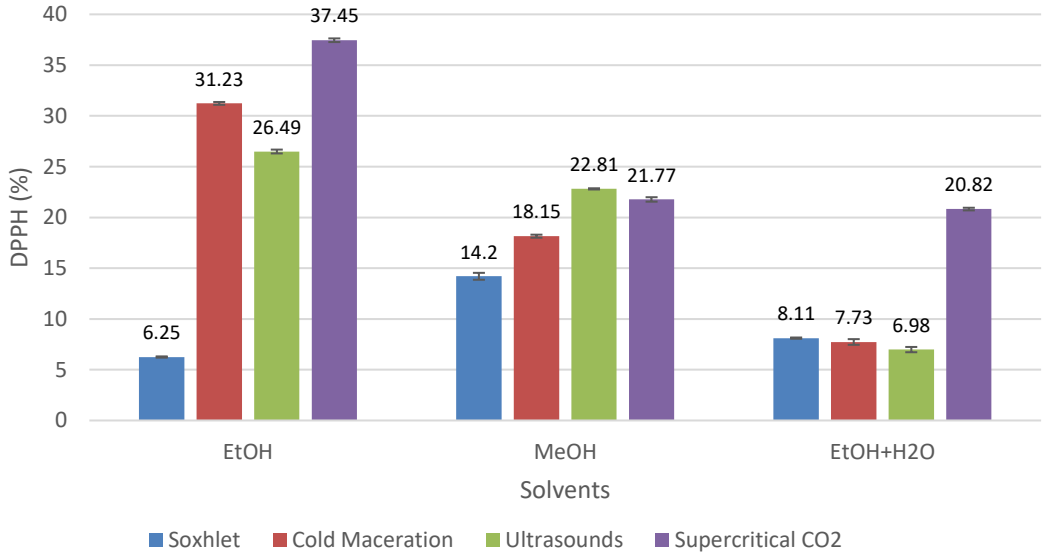

Figure 15.- Antioxidant activity (DPPH)

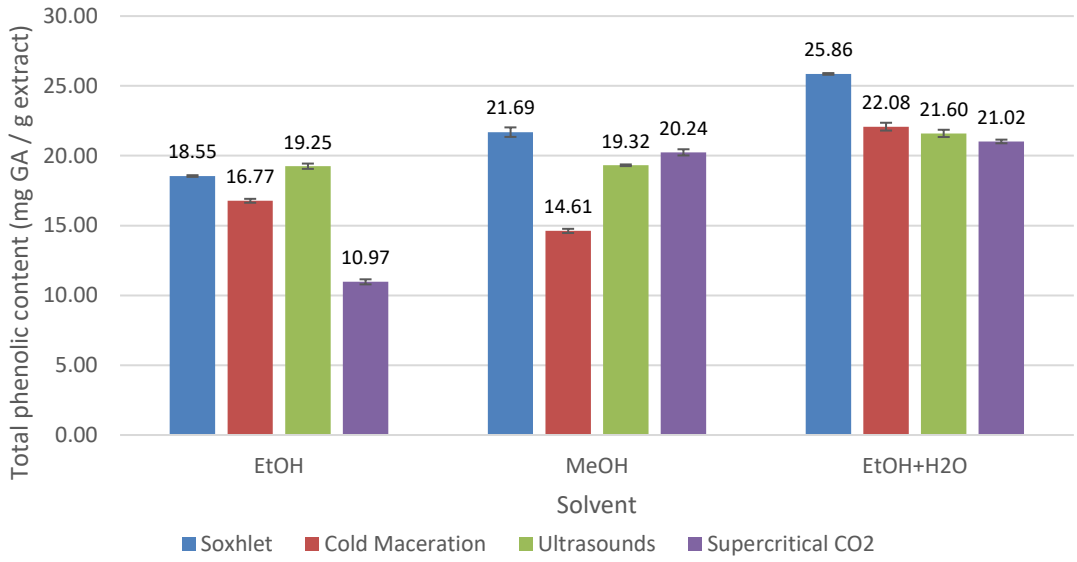

Figure 16.- Total phenol content (mg GA / g extract)

Results in Figure 14 indicate that the best solvent in the terms of the highest yield was methanol, with the highest yields of extract in each type of extraction, with the highest yield, 29.5%, in Soxhlet extraction. Ethanol gave a relatively high yield in Soxhlet extraction too, 27.42%. This confirms that polar solvents are a good choice for the solvent in the extraction of *Arnica Montana* due to their high polarity, making them the indicated solvents for polyphenolic components methanol being the most effective solvent due to its high polarity and selectivity, as other studies confirm (Žitek et al., 2022). Apparently, the high temperature (80 ºC) of the Soxhlet extraction, contributed to the highest yield of extract; low temperature methods, like ultrasound extraction and cold maceration, yielded the lowest amount of extract when using any solvent.

6.3 Organogel formulation

The most stable organogel resulted to be the one with a ratio 90:9:1 (cannabis oil: rice bran wax, extract), meaning 4.6 grams of oil, 0.46 grams of wax, and 0.051 grams of extract. Rice wax was heated up to the melting temperature and the mixture of compounds has been homogenized at 40ºC for 5 min.

Organogel has been tested for its stability after exposure to the light at air temperature for 10 days. No separation of phases or color change has been observed.

7 Discussion of results

In the experimental part of the study, different extraction methods and solvents were used to obtain *Arnica Montana* extracts, and their antioxidant activity and phenol content was measured using UV/Vis spectrophotometry. The combination of a highly polar and selective solvent like methanol and a high temperature high extraction time method like Soxhlet extraction showed the best results in extraction yield (29.5%), but the high temperatures of Soxhlet degraded the phenolic compounds, resulting in low antioxidant activity (14.2). Supercritical extraction using CO_2 and ethanol was a more effective way to preserve antioxidant activity on the extracts (37.45), due to the moderate temperatures and shorter extraction times. The total phenol content was higher with the Soxhlet extraction, using ethanol and water (25.86), but was closely followed by the other techniques, indicating that the choice of solvent is more important than the technique if a high amount of phenolic compounds is the main objective; still, this solvent combination was the one with the lowest antioxidant activity (8.11), making it not so desirable for the pharmaceutical use of the extract. Focusing on the antioxidant activity, it can be seen in Figure 15 that supercritical CO_2 extraction using a co-solvent presented the highest DPPH activity independent on co-solvents used; in the case of methanol, it is slightly lower for ultrasound extraction (22.81). Soxhlet extraction gave low antioxidant activity, probably due to the high temperatures and times (80 ºC for 180 minutes) used during the extraction, which resulted in thermal degradation of the phenolic compounds conferring the antioxidant activity to the extracts. All of this is to be used as a reaffirmation that supercritical fluid extraction is a gentler, more efficient extraction, requiring only moderate temperatures, and avoiding the use of harmful organic solvents, while preserving the properties of the extract phenolic compounds.

Figure 16 indicates a high quantity of phenolic compounds in the samples obtained using a mixture of ethanol and water as extraction solvent. Soxhlet had the highest content of 25.86 mg GA / g extract out of all the extraction methods using that solvent, but it was closely followed by the rest of the extraction methods at around 22 mg GA / g extract for all of them. This might indicate that the mixture of ethanol and water is a good solvent overall for the extraction of phenolic compounds. This is probably due to the glycosidic bond that should be interrupted to extract the phenolics.

Although is worth considering that this mixture of solvents had the lowest antioxidant activity out of all the solvents. Further investigation on the determination of antioxidant activity and applying different methods should be performed for the evaluation of antiradical scavenger activity of extract and compounds from *Arnica Montana*.

8 Conclusions

This work is composed of two parts: a review, and the experimental work. In the review, various topics were covered: the morphology, pharmacological properties, and applications, and phytochemistry of the plant, *Arnica Montana*; the different extraction methods used in the experimental work; and a brief review of organogels, their structure, and their properties. The focus of the literature related to *Arnica Montana* possible applications is on the homeopathic use of its flowers. Many studies have been conducted to analyze the possibility of *Arnica Montana* as a homeopathic remedy, but there is a lack of more rigorous pharmaceutical works on it, and with other part of the plant that are not its flowers. *Arnica Montana* chemistry and composition is well documented, as well as the many possible extraction methods, being cold maceration the most found in the bibliography. Supercritical extraction is a novel extraction method which is gaining popularity in the plant extraction field, but there is still room for more studies covering different supercritical solvents, conditions, and procedures. Gels are a well-known drug delivery method, but the specifics of organogels are still not well researched, being most of the works on them practical without studying the structure and mechanism behind them; still, they promise great results in the pharmaceutical field.

In the experimental part, extraction of the plant was obtained using different methods and solvents: cold maceration, Soxhlet, ultrasounds, and supercritical CO_2 extraction; and using ethanol, methanol, and a 95:5 mixture of water and ethanol. This extract was analyzed using an ultraviolet/visible range spectrometer to obtain the antioxidant activity and total phenol content of the extracts. Also, an organogel was formulated using the extract, cannabis oil, and rice bran wax. The best solvent overall was the methanol, and the highest quantity of extract obtained was with the Soxhlet extraction. Looking at the spectrometry results, the highest antioxidant activity was found in the extract obtained with the supercritical CO_2 using ethanol. The highest content of phenols was in the samples using ethanol and water as a solvent and Soxhlet as the extraction method.

All in all, extraction of *Arnica Montana* compounds is a well-researched topic, with traditional methods being the focus of the studies. More novel methods, like ultrasounds and superfluid extraction are not that well represented or utilized, but there is a growing interest on them. The same happens with organogels, with proven benefits for the pharmaceutical industry as drug delivering methods, but with not enough research behind them to make them a viable

alternative to more traditional methods. More work needs to be done, especially in the field of promising technologies, like supercritical extraction and organogels formulation.

9 Bibliography

Abbas, M., Ahmed, D., Qamar, M. T., Ihsan, S., & Noor, Z. I. (2021). Optimization of ultrasound-assisted, microwave-assisted and Soxhlet extraction of bioactive compounds from Lagenaria siceraria: A comparative analysis. *Bioresource Technology Reports*, *15*(June), 100746. https://doi.org/10.1016/j.biteb.2021.100746

Arruda, H. S., Silva, E. K., Pereira, G. A., Angolini, C. F. F., Eberlin, M. N., Meireles, M. A. A., & Pastore, G. M. (2019). Effects of high-intensity ultrasound process parameters on the phenolic compounds recovery from araticum peel. *Ultrasonics Sonochemistry*, *50*(August 2018), 82–95. https://doi.org/10.1016/j.ultsonch.2018.09.002

Bandaiphet, C., & Kennedy, J. F. (2004). Encyclopedia of Common Natural Ingredients used in Food, Drugs and Cosmetics (2nd Edition). In *Carbohydrate Polymers* (Vol. 58, Issue 2). https://doi.org/10.1016/j.carbpol.2004.05.002

Bouafia, M., Colak, N., Ayaz, F. A., Benarfa, A., Harrat, M., Gourine, N., & Yousfi, M. (2021). The optimization of ultrasonic-assisted extraction of Centaurea sp. antioxidative phenolic compounds using response surface methodology. *Journal of Applied Research on Medicinal and Aromatic Plants*, *25*(June), 100330. https://doi.org/10.1016/j.jarmap.2021.100330

Brinksma, J., Feringa, B. L., Kellogg, R. M., Vreeker, R., & Van Esch, J. (2000). Rheology and thermotropic properties of bis-urea-based organogels in various primary alcohols. *Langmuir*, *16*(24), 9249–9255. https://doi.org/10.1021/la000839s

Casazza, A. A., Aliakbarian, B., Mantegna, S., Cravotto, G., & Perego, P. (2010). Extraction of phenolics from Vitis vinifera wastes using non-conventional techniques. *Journal of Food Engineering*, *100*(1), 50–55. https://doi.org/10.1016/j.jfoodeng.2010.03.026

Concellos, F. (2008). *Skeletal formula of helenalin*. https://en.wikipedia.org/wiki/Helenalin#/media/File:Helenalin.svg

Conde-Hernández, L. A., Espinosa-Victoria, J. R., Trejo, A., & Guerrero-Beltrán, J. Á. (2017). CO2-supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (Rosmarinus officinalis). *Journal of Food Engineering*, *200*(C), 81–86. https://doi.org/10.1016/j.jfoodeng.2016.12.022

Craciunescu, O., Constantin, D., Gaspar, A., Toma, L., Utoiu, E., & Moldovan, L. (2012). Evaluation of antioxidant and cytoprotective activities of Arnica montana L. and Artemisia absinthium L. ethanolic extracts. *Chemistry Central Journal*, *6*(1), 1. https://doi.org/10.1186/1752-153X-6-97

Ćujić, N., Šavikin, K., Janković, T., Pljevljakušić, D., Zdunić, G., & Ibrić, S. (2016). Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chemistry*, *194*, 135–142. https://doi.org/10.1016/j.foodchem.2015.08.008

Daud, N. M., Putra, N. R., Jamaludin, R., Md Norodin, N. S., Sarkawi, N. S., Hamzah, M. H. S., Mohd Nasir, H., Abang Zaidel, D. N., Che Yunus, M. A., & Md Salleh, L. (2022). Valorisation of plant seed as natural bioactive compounds by various extraction methods: A review. *Trends in Food Science and Technology*, *119*(March 2021), 201–214. https://doi.org/10.1016/j.tifs.2021.12.010

Debnath, S., Vanitha, G., Bindu, H. P., & Babu, N. M. (2014). Applications of organogels in drug delivery. *Indian Journal of Research in Pharmacy and Biotechnology*, *2*(1), 976.

do Carmo, M. A. V., Granato, D., & Azevedo, L. (2021). Antioxidant/pro-oxidant and antiproliferative activities of phenolic-rich foods and extracts: A cell-based point of view.

In *Advances in Food and Nutrition Research* (1st ed., Vol. 98). Elsevier Inc. https://doi.org/10.1016/bs.afnr.2021.02.010

EMA. (2013). Assessment report on Arnica montana. *Committee on Herbal Medicinal Products (HMPC)*, *44*(May), 1–25.

Esposito, C. L., Kirilov, P., & Roullin, V. G. (2018). Organogels, promising drug delivery systems: an update of state-of-the-art and recent applications. *Journal of Controlled Release*, *271*(October 2017), 1–20. https://doi.org/10.1016/j.jconrel.2017.12.019

Fiume, M. Z. (2001). Final report on the safety assessment of arnica Montana extract and arnica Montana. *International Journal of Toxicology*, *20*(2_suppl), 1–11. https://doi.org/10.1080/10915810152902547l1

Gayle, E., & Brinckmann, J. (2015). Herb Market Report : Arnica Montana. *American Botanic Council*, *107*.

Gelmez, N., Kincal, N. S., & Yener, M. E. (2009). Optimization of supercritical carbon dioxide extraction of antioxidants from roasted wheat germ based on yield, total phenolic and tocopherol contents, and antioxidant activities of the extracts. *Journal of Supercritical Fluids*, *48*(3), 217–224. https://doi.org/10.1016/j.supflu.2008.11.002

Hanrahan, C. (2018). *Arnica Montana, an encyclopedia*. https://www.encyclopedia.com/plants-and-animals/plants/plants/arnica

Hrnčič, M. K., Cör, D., Kotnik, P., & Knez, Ž. (2019). Extracts of white and red grape skin and rosehip fruit: Phenolic compounds and their antioxidative activity. *Acta Chimica Slovenica*, *66*(4), 751–761. https://doi.org/10.17344/acsi.2019.5253

Huang, H. W., Hsu, C. P., Yang, B. B., & Wang, C. Y. (2013). Advances in the extraction of natural ingredients by high pressure extraction technology. *Trends in Food Science and Technology*, *33*(1), 54–62. https://doi.org/10.1016/j.tifs.2013.07.001

Jha, A. K., & Sit, N. (2022). Trends in Food Science & Technology Extraction of bioactive compounds from plant materials using combination of various novel methods : A review. *Trends in Food Science & Technology*, *119*(November 2021), 579–591. https://doi.org/10.1016/j.tifs.2021.11.019

Kalia, K., Sharma, K., Singh, H. P., & Singh, B. (2008). Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of potentilla atrosanguinea lodd. and quantification of its phenolic constituents by RP-HPLC. *Journal of Agricultural and Food Chemistry*, *56*(21), 10129–10134. https://doi.org/10.1021/jf802188b

Khoddami, A. (2013). *Basic structures of phenolic acids and flavonoids*. https://doi.org/doi:10.3390/molecules18022328

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, *18*(2), 2328–2375. https://doi.org/10.3390/molecules18022328

Kirilov, P., Le Cong, A. K., Denis, A., Rabehi, H., Rum, S., Villa, C., Haftek, M., & Pirot, F. (2015). Organogels for cosmetic and dermo-cosmetic applications. *Classification, Preparation and Characterization of Organogel Formulations Part I & II. H&PC Today*, *10*, 15–21.

Knez, Ž., Pantić, M., Cör, D., Novak, Z., & Knez Hrnčič, M. (2019). Are supercritical fluids solvents for the future? *Chemical Engineering and Processing - Process Intensification*, *141*(May). https://doi.org/10.1016/j.cep.2019.107532

Kobori, T., Iwamoto, S., Takeyasu, K., & Ohtani, T. (2007). Biopolymers Volume 85 / Number 4 295. *Biopolymers*, *85*(4), 392–406. https://doi.org/10.1002/bip

Koo, H., Gomes, B. P. F. A., Rosalen, P. L., Ambrosano, G. M. B., Park, Y. K., & Cury, J. A. (2000). In vitro antimicrobial activity of propolis and Arnica montana against oral pathogens. *Archives of Oral Biology*, *45*(2), 141–148. https://doi.org/10.1016/S0003-9969(99)00117-X

Kriplani, P., Guarve, K., & Baghael, U. S. (2017). Arnica montana L. – a plant of healing: review. *Journal of Pharmacy and Pharmacology*, *69*(8), 925–945. https://doi.org/10.1111/jphp.12724

Leitner, W., & Poliakoff, M. (2008). Supercritical fluids in green chemistry. *Green Chemistry*, *10*(7), 730. https://doi.org/10.1039/b809498p

Li, W., Zhang, J., Niu, J., Jin, X., Qian, X., Xiao, C., & Wang, W. (2022). Self-powered and high sensitivity ionic skins by using versatile organogel. *Nano Energy*, *99*, 107359. https://doi.org/https://doi.org/10.1016/j.nanoen.2022.107359

Lim, Y. P., Pang, S. F., Yusoff, M. M., Abdul Mudalip, S. K., & Gimbun, J. (2019). Correlation between the extraction yield of mangiferin to the antioxidant activity, total phenolic and total flavonoid content of Phaleria macrocarpa fruits. *Journal of Applied Research on Medicinal and Aromatic Plants*, *14*(January). https://doi.org/10.1016/j.jarmap.2019.100224

Lupi, F. R., Greco, V., Baldino, N., de Cindio, B., Fischer, P., & Gabriele, D. (2016). The effects of intermolecular interactions on the physical properties of organogels in edible oils. *Journal of Colloid and Interface Science*, *483*, 154–164. https://doi.org/10.1016/j.jcis.2016.08.009

Madhujith, T., Wedamulla, N. E., & Gamage, D. A. S. (2022). *Chapter 6 - Biological macromolecules as antioxidants* (A. K. Nayak, A. K. Dhara, & D. B. T.-B. M. Pal (eds.); pp. 139–164). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-323-85759-8.00006-3

Mamari, H. H. Al. (2021). *Phenol unit*. https://doi.org/10.5772/intechopen.98958

Park, H., Yang, S., Kang, J. Y., & Park, M. H. (2016). On-Demand Drug Delivery System Using Micro-organogels with Gold Nanorods. *ACS Medicinal Chemistry Letters*, *7*(12), 1087–1091. https://doi.org/10.1021/acsmedchemlett.6b00293

Patel, A. R., Mankoč, B., Bin Sintang, M. D., Lesaffer, A., & Dewettinck, K. (2015). Fumed silica-based organogels and "aqueous-organic" bigels. *RSC Advances*, *5*(13), 9703–9708. https://doi.org/10.1039/c4ra15437a

Pawlaczyk, I., Czerchawski, L., Pilecki, W., Lamer-Zarawska, E., Gancarz, R., Šutovská, M., Capek, P., Kočmalová, M., Pawlaczyk, I., Zaczyńska, E., Czarny, A., Uhliariková, I., Gancarz, R., & Fraňová, S. (2014). Polyphenolic-polysaccharide compounds from selected medicinal plants of Asteraceae and Rosaceae families: Chemical characterization and blood anticoagulant activity. *International Journal of Biological Macromolecules*, *69*(3), 214–221. https://doi.org/10.1016/j.carbpol.2009.01.030

Robertson, A., Suryanarayanan, R., & Banerjee, A. (2007). Homeopathic Arnica montana for post-tonsillectomy analgesia: a randomised placebo control trial. *Homeopathy*, *96*(1), 17–21. https://doi.org/10.1016/j.homp.2006.10.005

Rodsamran, P., & Sothornvit, R. (2019). Extraction of phenolic compounds from lime peel waste using ultrasonic-assisted and microwave-assisted extractions. *Food Bioscience*, *28*(January), 66–73. https://doi.org/10.1016/j.fbio.2019.01.017

Sagiri, S. S., Sethy, J., Pal, K., Banerjee, I., Pramanik, K., & Maiti, T. K. (2013). Encapsulation of vegetable organogels for controlled delivery applications. *Designed Monomers and Polymers*, *16*(4), 366–376. https://doi.org/10.1080/15685551.2012.747154

Sanjaya, R. E., Tedjo, Y. Y., Kurniawan, A., Ju, Y. H., Ayucitra, A., & Ismadji, S. (2014). Investigation on supercritical CO2 extraction of phenolic-phytochemicals from an epiphytic plant tuber (Myrmecodia pendans). *Journal of CO2 Utilization*, *6*, 26–33. https://doi.org/10.1016/j.jcou.2014.03.001

Sankeshwari, R., Ankola, A., Bhat, K., & Hullatti, K. (2018). Soxhlet versus cold maceration: Which method gives better antimicrobial activity to licorice extract against Streptococcus mutans? *Journal of the Scientific Society*, *45*(2), 67. https://doi.org/10.4103/jss.jss_27_18

Schröder, H., Lösche, W., Strobach, H., Leven, W., Willuhn, G., Till, U., & Schrör, K. (1990). Helenalin and 11α,13-dihydrohelenalin, two constituents from Arnica montana L., inhibit human platelet function via thiol-dependent pathways. *Thrombosis Research*, *57*(6), 839–845. https://doi.org/10.1016/0049-3848(90)90151-2

Shithole, B. (2010). *Schematic of a Soxhlet extractor*. https://www.researchgate.net/figure/Schematic-of-a-Soxhlet-extractor_fig1_233447758

Studer, B. (2006). *Arnica Montana photo*. https://species.wikimedia.org/wiki/Arnica_montana#/media/File:Arnica_montana.JPG

Šutovská, M., Capek, P., Kočmalová, M., Pawlaczyk, I., Zaczyńska, E., Czarny, A., Uhliariková, I., Gancarz, R., & Fraňová, S. (2014). Characterization and pharmacodynamic properties of Arnica montana complex. *International Journal of Biological Macromolecules*, *69*, 214–221. https://doi.org/10.1016/j.ijbiomac.2014.05.051

Ucenic, C. I., & Mastorakis, N. (2007). The impact of environmental issues in the supply chain for a natural resource : the case study of Arnica Montana from Romania. *WSEAS International Conference on Energy & Environment (EE'07),* 257–263.

Vázquez, L., Torres, C. F., Fornari, T., Grigelmo, N., Señoráns, F. J., & Reglero, G. (2006). Supercritical fluid extraction of minor lipids from pretreated sunflower oil deodorizer distillates. *European Journal of Lipid Science and Technology*, *108*(8), 659–665. https://doi.org/10.1002/ejlt.200600035

Veggi, P. C., Cavalcanti, R. N., & Meireles, M. A. A. (2014). Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction: Experimental data and economic evaluation. *Journal of Food Engineering*, *131*, 96–109. https://doi.org/10.1016/j.jfoodeng.2014.01.027

Wikipedia. (2007). *Map of Arnica montana*. https://en.wikipedia.org/wiki/File:Location_of_Arnica_montana.svg

Wu, C., Wang, F., Liu, J., Zou, Y., & Chen, X. (2015). A comparison of volatile fractions obtained from Lonicera macranthoides via different extraction processes: ultrasound, microwave, Soxhlet extraction, hydrodistillation, and cold maceration. In *Integrative Medicine Research* (Vol. 4, Issue 3, pp. 171–177). https://doi.org/10.1016/j.imr.2015.06.001

Yusoff, I. M., Mat Taher, Z., Rahmat, Z., & Chua, L. S. (2022). A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. *Food Research International*, *157*(February), 111268. https://doi.org/10.1016/j.foodres.2022.111268

Zeng, L., Lin, X., Li, P., Liu, F. Q., Guo, H., & Li, W. H. (2021). Recent advances of organogels: from fabrications and functions to applications. *Progress in Organic Coatings*, *159*(March), 106417. https://doi.org/10.1016/j.porgcoat.2021.106417

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products : a comprehensive review. *Chinese Medicine*, 1–26.

https://doi.org/10.1186/s13020-018-0177-x

Žitek, T., Postružnik, V., Knez, Ž., Golle, A., Dariš, B., & Knez Marevci, M. (2022). Arnica Montana L. Supercritical Extraction Optimization for Antibiotic and Anticancer Activity. *Frontiers in Bioengineering and Biotechnology*, *10*(May), 1–9. https://doi.org/10.3389/fbioe.2022.897185

10 Anex

10.1 Data tables

Table 1.- Extraction yield (%)

	Material	Extract	Yield
	g	g	%
		Soxhlet	
EtOH	12.07	3.31	27.42%
EtOH+W	12	3.54	29.50%
MeOH	12	2.22	18.50%
		Cold maceration	
EtOH	25	1.26	5.04%
EtOH+W	25	3.25	13.00%
MeOH	25	2.24	8.96%
		Ultrasounds	
EtOH	25	1.01	4.04%
EtOH+W	25	2.92	11.68%
MeOH	25	1.76	7.04%
		Supercritical CO2	
EtOH	10	1.95	19.50%
EtOH+W	10	2.1	21.00%
MeOH	10	1.69	16.90%

Table 2.- Antioxidant activity of the extracts (DPPH, %)

		DPPH (%)	Standard Deviation
Soxhlet	**EtOH**	6.25	0.06
	EtOH+W	14.2	0.34
	MeOH	8.11	0.06
Cold Maceration	**EtOH**	31.23	0.14
	EtOH+W	18.15	0.15
	MeOH	7.73	0.28
Ultrasounds	**EtOH**	26.49	0.19
	EtOH+W	22.81	0.06
	MeOH	6.98	0.26
Supercritical CO_2	**EtOH**	37.45	0.18
	EtOH+W	21.77	0.22
	MeOH	20.82	0.13

Table 3.- Total phenol content (mg GA / g extract)

		Total phenol content mg GA / g extract
Soxhlet	EtOH	18.55
	EtOH+W	21.69
	MeOH	25.86
Cold Maceration	EtOH	16.77
	EtOH+W	14.61
	MeOH	22.08
Ultrasounds	EtOH	19.25
	EtOH+W	19.32
	MeOH	21.60
Supercritical CO_2	EtOH	10.97
	EtOH+W	20.24
	MeOH	21.02

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(ime članice UM)

IZJAVA O AVTORSTVU ZAKLJUČNEGA DELA

Ime in priimek študenta/-ke: Álvaro Pequeno Alonso

Študijski program: Izmenjava MAG

Naslov zaključnega dela: Optimizacija postopka ekstrakcije učinkovin iz arnike arnica montana in formulacija produktov

Mentor/-ica: Maša Knez Marevci

Somentor/-ica: Željko Knez

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