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Green Extraction Technique Using Natural Deep Eutectic Solvents (NADES) And Supramolecular Solvents (SUPRAS) To Recover Bioactive Compounds from Medicinal Halophyte *Polygonum Maritimum* L.

Faculdade de Ciências e Tecnologia

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Faculdade de Ciências e Tecnologia

2025

DECLARATION OF AUTHORSHIP

I declare that I am the author of this work, which is original. The work cites other authors and works, which are adequately referred to in the text and are listed in the bibliography.

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RESUMO

A extração sustentável de compostos bioativos de alto valor a partir de recursos naturais continua sendo um desafio crítico na química verde. Este estudo avalia a eficácia dos Solventes Eutéticos Profundos Naturais (NADES) e dos Solventes Supramoleculares (SUPRAS) como alternativas inovadoras e sustentáveis aos solventes convencionais para a extração de compostos fenólicos e alcaloides de *Polygonum maritimum*, uma planta com potencial bioativo pouco explorado. Quatro sistemas NADES e dois SUPRAS, incluindo suas formas em equilíbrio e híbridas, foram avaliados utilizando extração assistida por ultrassom (UAE) e comparados com etanol e água como solventes de referência. Entre os solventes testados, a mistura Cloreto de colina: ácido lático (CC:LA) apresentou a maior atividade antioxidante no ensaio DPPH, com um valor de IC_{50} de 0,0281 ± 0,014 mg/mL, superando significativamente o Cloreto de colina: ácido málico (CC:MA). Esses resultados foram corroborados pelas medições do teor total de flavonoides (TFC) e pela análise por LC-MS, confirmando a presença de diversos compostos fenólicos. Embora o etanol tenha extraído a maior quantidade total de bioativos, os NADES e SUPRAS demonstraram enriquecimento seletivo e recuperação de compostos fenólicos específicos e alcaloides. Este estudo ressalta a integração promissora dos NADES e SUPRAS com UAE como uma abordagem ecoeficiente e escalável para a extração de produtos naturais valiosos de plantas marinhas.

Palavras-chave: NADES, SUPRAS, polyphenol, Antioxidante

ABSTRACT

The sustainable extraction of high-value bioactive compounds from natural resources remains a critical challenge in green chemistry. This study evaluates the efficacy of Natural Deep Eutectic Solvents (NADES) and Supramolecular Solvents (SUPRAS) as innovative, environmentally friendly alternatives to conventional solvents for extracting phenolic compounds and alkaloids from *Polygonum maritimum*, a plant with largely underexplored bioactive potential. Four NADES and two SUPRAS systems including their equilibrium and hybrid forms were assessed using ultrasound-assisted extraction (UAE) and compared against ethanol and water as benchmark solvents. Among the tested solvents, Choline chloride: lactic acid (CC:LA) exhibited the strongest antioxidant activity in the DPPH assay, with an IC_{50} value of 0.0281 ± 0.014 mg/mL, significantly outperforming Choline chloride:malic acid (CC:MA). In the DPPH assay, although the equilibrium mixture (5:9.5:85.5%) showed greater radical scavenging activity compared to ethanol, this

difference was not statistically significant. These findings were further supported by total flavonoid content (TFC) measurements and LC-MS analysis, confirming the presence of diverse phenolic compounds. While ethanol extracted the highest total quantity of bioactives, NADES and SUPRAS demonstrated selective enrichment and enhanced recovery of specific phenolic compounds and alkaloids. This study underscores the promising integration of NADES and SUPRAS with UAE as an eco-efficient and scalable approach for the extraction of valuable natural products from marine plants.

Keywords: NADES, SUPRAS, polyphenols, Antioxidant

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List Of Abbreviations and Acronyms

S/N	**Abbreviations**	**Meaning**
1	SUPRAS	Supramolecular solvent
2	NADES	Natural deep eutectic solvent
3	EqS	Equilibrium solution
4	CC:LA	Choline chloride: Lactic acid
5	CC:MA	Choline chloride: Malic acid
6	Gly: Urea	Glycerol: Urea
7	GLU:LA	Glucose: Lactic acid
8	UAE	Ultrasound-assisted extraction
9	IC_{50}	Half-maximal inhibitory concentration
10	DPPH	1,1-Diphenyl-2-picrylhydrazyl
11	ABTS	2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid
12	RSA	Radical scavenging activity
13	TFC	Total flavonoid content
14	LC-MS	Liquid Chromatography – Mass spectroscopy

CHAPTER 1

INTRODUCTION

1.1 Importance of natural products

Natural products are substances that occur naturally and can be obtained from different sources, including plants, animals, fungus, and microorganisms (Les et al., 2024).. A sizable portion of medications in the contemporary pharmaceutical industry can be traced back to their natural product beginnings, and many ancient medicines contain natural products. From the refined nature of quinine and morphine, which have transformed antimalarial therapy and pain alleviation (Sunita Varma, 2023). Another notable example is paclitaxel (commercially known as Taxol), a potent anticancer agent first discovered in 1967 by Dr. Mansukh C. Wani and Dr. Monroe E. Wall (National Cancer Institute, 2015). Working at the Research Triangle Institute in collaboration with the U.S. National Cancer Institute (NCI), the researchers isolated paclitaxel from the bark of the Pacific yew tree (*Taxus brevifolia*) as part of a natural products screening program (National Cancer Institute, 2015). The compound demonstrated remarkable cytotoxic activity, eventually leading to its approval for the treatment of various cancers, including ovarian, breast, and non-small cell lung cancer (Alalawy, 2024). The discovery of paclitaxel exemplifies the profound potential of plant-derived natural products in yielding life-saving pharmaceuticals and underscores the continued need for biodiversity exploration and natural compound research in drug development.

Phenolic acids, flavonoids, catechins, tannins, lignans, stilbenes, and anthocyanidins are the main components of polyphenols, a prominent class of naturally occurring phytochemicals (Rudrapal et al., 2022). They possess antioxidant, chemopreventive and a wide range of pharmacological properties (Khan et al., 2021). Phenolic compounds work as antioxidants by interacting with various free radicals. The method of antioxidant action involves either hydrogen atom transfer, single electron transfer, sequential proton loss electron transfer, or transition metal chelation (Zeb, 2020).

Antioxidant properties of phenolic compounds are of particular interest for neurodegenerative diseases whose pathophysiology strongly rely on oxidative stress at the brain level (Nájera-Maldonado et al., 2024). Neurodegenerative diseases are characterized by an imbalance and increase in reactive oxygen and nitrogen species (ROS and RNS), sometimes caused by protein

aggregation (Roda et al., 2022) In addition, phenolic compounds display other advantages such as the permeability of the blood–brain barrier (BBB) (Nájera-Maldonado et al., 2024). Phenolic metabolites (e.g., catechol-O-sulfate, pyrogallol-O-sulfate) can cross the BBB in vitro. Methylation and sulfation enhance this ability, especially for gallic acid derivatives. These compounds reduce Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), and Nuclear Factor Kappa (NF-κB) translocation, contributing to anti-inflammatory and antioxidant effects (Campos-Esparza et al., 2009).

Clinical trials have shown that phenolic compounds can exert antioxidant, anti-inflammatory, and neuroprotective effects in patients with neurodegenerative disorders, though the number of trials remains limited. A population-based study involving 2,574 middle-aged adults demonstrated that higher dietary intake of polyphenols specifically catechins, theaflavins, flavonols, and hydroxybenzoic acids was positively associated with improved linguistic and verbal memory performance, highlighting the potential cognitive benefits of regular polyphenol consumption (Kesse-Guyot et al., 2012). In a 52-week clinical study involving 119 patients with mild-to-moderate Alzheimer's disease, resveratrol treatment resulted in decreased levels of Aβ40 in cerebrospinal fluid, improved scores on the Alzheimer's Disease Cooperative Study Activities of Daily Living (ADCS–ADL) scale, and reduced concentrations of pro-inflammatory cytokines including indicating its ability to cross the blood–brain barrier and modulate neuroinflammation (Moussa et al., 2017).

Phenolic compounds exert their antitumor effects through a range of molecular mechanisms, often by targeting multiple regulatory pathways within cancer cells. Phenolic compounds have been shown to promote programmed cell death (apoptosis) in cancer cells, a key mechanism in inhibiting tumour growth. Compounds such as gallic acid, caffeic acid, rosmarinic acid, sinapic acid, curcumin, and quercetin have demonstrated the ability to induce apoptosis across a variety of cancer cell lines, highlighting their potential as effective antitumor agents (Bakrim et al., 2022). Gallic acid has strong anticancer effects due to its antioxidant characteristics (Bakrim et al., 2022) A study by Patra et al., (2020) demonstrated that gallic acid enhances the anticancer effects of chemotherapy drugs (Docetaxel, Cisplatin, Doxorubicin, 5-FU, and Paclitaxel) combined with gamma irradiation in oral squamous cell carcinoma cell lines (FaDu and Cal33) by inducing superoxide-mediated apoptosis through lipophagy inhibition via an NRF2-dependent pathway.

1.2 Importance of halophytes as sources of natural products

Halophytes are plant species that thrive in saline environments, such as beaches, post-industrial wastelands, and salt flats (Ben Hsouna et al., 2022). For centuries, halophytes have been utilized in traditional medicine due to their rich phytochemical content (Fan et al., 2019). They contain various phytochemicals, including polyphenols, flavonoids, terpenes, and alkaloids, which are linked to their therapeutic potential (Sun & Shahrajabian, 2023).

The biological potential of halophytes is closely associated with their ability to produce secondary metabolites, particularly phenolic compounds, that help them cope with environmental stress and oxidative damage caused by reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide generated under saline conditions (Stanković et al., 2023)).The synthesis of these metabolites, especially polyphenols, underpins the strong antioxidant activity found in many halophytes, which is critical for mitigating oxidative stress in saline environments (Azeem et al., 2023). Salt accumulation in halophytes involves the sequestration of excess Na^+ and Cl^- ions into vacuoles, helping maintain osmotic balance and protect the cytoplasm from ion toxicity. This process relies on key ion transporters, including vacuolar Na^+/H^+ exchangers (NHX) and the plasma membrane-bound SOS1 (Na^+/H^+ antiporter), which facilitate efficient ion compartmentalization (Nikalje et al., 2017).

The roots and seed extracts of *Foeniculum vulgare* have been documented for their effectiveness in treating gastrointestinal, urological, and gynecological infections (Alqethami et al., 2017). Additionally, *Polygonum aviculare* is known to be a rich source of phenolic compounds, which implies its antioxidant properties and potential applications in managing neurodegenerative conditions like Alzheimer's disease (Mahnashi et al., 2022), Furthermore, it has also demonstrated its anti-diabetic, anti-cancer, and dermato-protective activities (Benrahou et al., 2023). *Haloxylon salicornicum*, also halophytic plant, has been shown to contain bioactive phytochemicals specifically coumarins, sterols, and flavonoids that exhibit significant anti-inflammatory activity by modulating the NF-κB and iNOS signaling pathways and suppressing the release of pro-inflammatory mediators such as nitric oxide, TNF-α, and IL-6 in LPS-stimulated macrophages (Alruhaimi RS, 2025). The genus *Salsola*, widely distributed in arid and temperate regions, is a rich source of phytochemicals such as flavonoids, alkaloids, and phenolic acids. Traditionally used for antihypertensive and anti-inflammatory purposes, *Salsola* species have demonstrated diverse

pharmacological activities, including antioxidant, antimicrobial, hepatoprotective, and neuroprotective effects, making them promising candidates for pharmaceutical and nutraceutical applications (ElNaggar et al., 2022).

Salicornia ramosissima extracts, rich in phenolic compounds and antioxidants, demonstrate significant potential for use in cosmetic formulations due to their antioxidant properties and enzyme-inhibitory effects related to skin hyperpigmentation and aging (Hulkko et al., 2023). *Limonium tetragonum*, a halophyte with strong antioxidant properties, shows anti-melanogenic effects by inhibiting tyrosinase activity and melanin production in B16-F10 cells. Its active flavonoids, myricetin 3-galactoside and quercetin 3-O-β-galactopyranoside, support its potential use in natural skin-whitening cosmetics (S. G. Lee et al., 2017). *Atriplex halimus* extract-based cream demonstrated significant wound healing activity in Wistar rats, accelerating wound contraction and epithelialization, likely due to its rich content of flavonoids, phenolic acids, tannins, and minerals highlighting its potential as a natural wound care agent (Missoun et al., 2024).

1.3 Polygonum maritimum

1.3.1 Botanical Description and Ecological Distribution of *Polygonum maritimum*

Polygonum maritimum (common name: sea knotgrass) (Figure 1.1) is a low-growing shrublet, typically prostrate or sprawling, reaching up to 50 cm in height. It is characterized by conspicuous, papery stipules that become lacerate with age. The leaves are elliptic, imbricate, greyish, with revolute margins. The white to pink flowers are 5-merous and usually appear singly or in groups of up to three in the leaf axils. The perianth is shorter than the fruit, a distinctive trait of the species. (Source: Plants of the World Online, 2025). Table 1.1 shows the botanical classification of *Polygonum maritimum.*

Figure 1.1: Image of Polygonum maritimum *source: iNaturalist 2025*

Table 1.1:Classification of Polygonum.maritimum according to the Global Biodiversity Information Facility (GBIF) 2023 (Polygonum Maritimum Classification, n.d.)

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Polygonaceae
Genus	Polygonum L.
Species	*Polyogonum maritimum*

Habitat and Distribution

Sea knotgrass occurs in habitats with high soil salinity and is classified as a halophyte in the eHALOPH database (https://ehaloph.uc.pt/listplants). Sea knotgrass can be found on sandy shores across America, Europe, South Africa, and the Mediterranean region (Kazantzoglou et al., 2009). It is native to Albania, Algeria, Azores, Baleares, Bulgaria, Cape Provinces, Corse, Cyprus, East

Aegean Is., Egypt, France, Great Britain, Greece, Ireland, Italy, Kriti, Krym, Lebanon-Syria, Libya, Madeira, Morocco, Netherlands, Palestine, Portugal, Romania, Sardegna, Sicilia, Spain, Tunisia, Turkey, Yugoslavia (Plants of the World Online, 2025). In Portugal, sea knotgrass is distributed along the entire coastline, from north to south (Figure. 1.2).

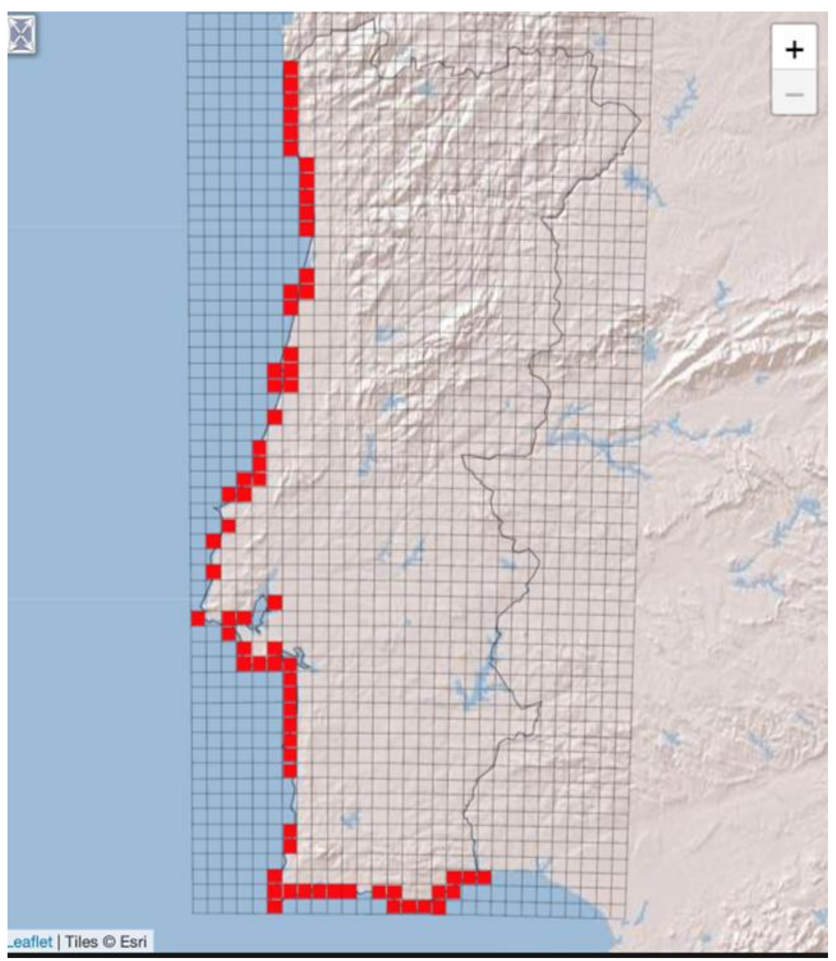


Figure 1.2: Geographical distribution of Polygonum maritimum, in Portugal. (source: Flora-on, https://flora-on.pt)

1.3.3 Phytochemical Profile of *Polygonum maritimum*

Sea knotgrass has attracted increasing attention due to its production of diverse phytochemicals with potential pharmacological properties. This section reviews the major classes of phytochemicals reported in *P. maritimum*, their biological relevance, and methods employed in their identification.

a. Phytochemical Studies

Several studies have identified phenolic compounds, flavonoids, organic acids, and other secondary metabolites in various parts of sea knotgrass, including aerial parts, and roots. These compounds are largely responsible for the plant's reported biological activities, such as antioxidants, antimicrobial, and anti-inflammatory effects. Table 1.2 summarizes the main phytochemicals found in sea knotgrass, along with their chemical classification, associated biological activities, and supporting references.

Table 1.2: Identified Phytochemicals in *Polygonum maritimum*

S/N	**Compound**	**Chemical Class**	**Plant part**	**Bioactivity**	**References**
1.	ß-type(epi) catechin	Favan-3-ol	Aerial part	antioxidant, antiproliferative, antiapoptotic	Oliveira et al., 2023), Osorio-Cruz et al., 2024
2.	Epigallocatechin	flavan-3-ol	Aerial part	Antioxidant, anti-inflammatory	Capasso et al., 2025), Rodrigues et al., 2018a.
3.	Gallic acid	Phenolic acid	Aerial part	Antioxidant, anti-inflammatory	Rodrigues et al., 2018a
4	Quercetin	Flavonol	Aerial part	Antioxidant, neuroprotective,	Rukavina et al., 2021
5	Caffeic acid	Phenolic acid	Aerial part	Antioxidant, anti-inflammatory	Rukavina et al., 2021, Pavlíková, 2023
6	linoleic acid	Omega-6 polyunsaturated fatty acid	Seed	Anti-inflammatory, Immunomodulatory	O'shea et al., 2004

1.3.4 **Bioactive Constituents of *P. maritimum:* Pharmacological Relevance**

I. Anti-inflammatory potential: The phytochemical profile of *P. maritimum* indicates a potential anti-inflammatory activity, possibly due to the presence of phenolic compounds (Rodrigues et al., 2019) and flavonoids such as epigallocatechin, myricetin (Capasso et al., 2025).

Rodrigues et al. (2019) confirmed the anti-inflammatory potential of the ethanol leaf extract of *P. maritimum* through similar in -vitro assays using LPS-stimulated macrophages, where the extract

significantly reduced nitric oxide (NO) production marker of inflammation. Additionally, the study assessed the expression of pro-inflammatory cytokines and found a downregulation, further supporting the extract's anti-inflammatory activity.

ii. Antidiabetic potential: Alpha-glucosidase inhibitors, such as acarbose and miglitol, help manage diabetes by slowing the breakdown of carbohydrates in the small intestine. This results in a slower release of glucose into the bloodstream, preventing rapid spikes in blood sugar levels after meals, thus aiding in better postprandial glucose control (Salehi et al., 2019). A study by Rodrigues et al. (2017) investigated the inhibitory effects of *P. maritimum* methanolic extracts on key enzymes linked to hyperglycemia, namely α-amylase and α-glucosidase. The extracts showed strong inhibition of baker's yeast α-glucosidase, with IC_{50} values of 19 µg/mL for root extracts and 29 µg/mL for leaf extracts. These values were markedly lower than that of the reference drug acarbose (IC_{50} = 3144 µg/mL), highlighting the potential antidiabetic properties of the plant.

iii. Anti-genotoxic effects: Oxidative stress resulting from prolonged exposure to dietary xenobiotics can lead to DNA damage and contribute to cancer development (Oliveira et al., 2023). A study by Oliveira et al. (2023) demonstrated that the ethanol extract of leaves and stems of *P. maritimum* exhibits strong *in vitro* antioxidant activity, largely attributed to its high polyphenol content. Furthermore, the extract showed antigenotoxic properties by effectively protecting *Saccharomyces cerevisiae* cells against DNA damage induced by hydrogen peroxide (H_2O_2).

i.v Skin care applications

Natural products are gaining increasing prominence in the cosmetics industry, driven by growing consumer awareness of the potential risks associated with synthetic chemicals and the perceived health benefits of plant-derived ingredients (Liu, 2022). The demand for "green, natural, and environmentally friendly" products continues to rise. In this context, *P. maritimum* has emerged as a promising source of bioactive compounds for skincare applications. A study by Rodrigues et al. (2019) demonstrated that leaf extracts of *P. maritimum* exhibit significant antioxidant capacity, delayed lipid peroxidation, and potent superoxide radical scavenging activity surpassing even conventional antioxidants like catechin. These findings underscore the potential of incorporating *P. maritimum* extracts into cosmetic formulations to combat oxidative stress, a major contributor to skin aging and cellular damage. Additionally, the extract displayed strong inhibitory activity against tyrosinase, exceeding that of arbutin, and effectively reduced melanin production in

melanoma cells. This positions *P. maritimum* as a potential ingredient for the development of skin-whitening formulations targeting hyperpigmentation. Moreover, the polyphenolic compounds abundant in *P. maritimum* extracts have demonstrated multifunctional biological properties, including antioxidant, anti-inflammatory, antimicrobial, and antiallergic activities (Silva et al., 2025). These attributes support its potential as a valuable natural activity for promoting and maintaining skin and hair health.

1.3.5 Limitations in the extraction of natural products

The choice of solvent plays a critical role in the efficiency and selectivity of bioactive compound extraction. Inappropriate solvent selection can result in suboptimal yields and the co-extraction of undesirable compounds. Common organic solvents such as methanol, ethanol, and acetone are widely employed for the extraction of natural products (Peng et al., 2016). However, their use presents several limitations, including high solvent consumption, prolonged extraction times, and low reusability, which contribute to increased processing costs (Rukavina et al., 2021). Additionally, these solvents are often toxic, volatile, and environmentally hazardous (Cvetanović, 2019), reducing extract purity and necessitating further purification steps.
In contrast, ionic liquids (ILs), which are salts in the liquid state at room temperature (RT), have emerged as promising green alternatives. Comprising various cations (e.g., alkyl-imidazolium, pyridinium, ammonium, phosphonium) and anions (e.g., acetate, trifluoroacetate, trifluoromethyl sulfate), ILs are characterized by low volatility, high thermal and chemical stability, tunability, and enhanced solubility (Rukavina et al., 2021b). Despite their advantages, ILs still face challenges such as high cost, limited biodegradability, and potential toxicity (Mbous et al., 2017).

Degradation of Sensitive Compounds

Many bioactive compounds are highly sensitive to extraction conditions such as temperature, light exposure, and pH. These factors can lead to structural degradation and loss of activity during the extraction process (Mungwari et al., 2025). For example, certain phenolic compounds like anthocyanins are particularly susceptible to thermal degradation at temperatures above 70°C (Tazi et al., 2024). To preserve the integrity and functionality of flavonoids and other thermolabile compounds, it is essential to optimize extraction conditions and use gentle reduced temperatures that maintain compound stability.

1.4 Polyphenols and Flavonoids

Flavonoids are naturally occurring molecules with chemical characteristics like phenolic compounds (Singla et al., 2019). They are classified into classes and subclasses based on their chemical structure, number of phenol rings, functional group positions, and carbon skeleton (Šamec et al., 2021). Thus, polyphenols can be easily categorized as flavonoids and nonflavonoids, or they can be further separated into numerous sub-classes based on the type of linkage between phenol units, the number of phenol units in their molecular structure, and substituent groups (Singla et al., 2019).

Variations in the hydroxylation pattern and oxidation state of the core pyran ring give rise to the structural variety of flavonoid molecules, which produce a broad spectrum of compounds: flavones, isoflavones, flavonols, flavanones, flavanonols, anthocyanidins, anthocyanins, and flavonols (Singla et al., 2019). Some of these structures are shown in figure 1.3

Figure 1.3: Some subclasses of flavonoids: anthocyanidins, anthocyanins, flavonols, flavones and isoflavones.

Table 1.3 presents an overview of the major flavonoid groups, including representative compounds and their associated therapeutic potential based on recent scientific literature. These compounds are categorized by their structural class, and the corresponding biological activities are summarized. **Figure 1.4** illustrates the basic chemical structures of representative flavonoids from each group, highlighting their common backbone and functional group variations.

Table 1.3: Classification of Flavonoids with Representative Compounds and Their Therapeutic Potentials.

	Group	Structure	Example	Therapeutic Potential
1	**Flavones**		baicalein, luteolin, quercitol, apigenin, kaempferol, tricin (Leonte et al., 2023)	Antitumor activity, (Moreira et al., 2019). Antibacterial and antifungal (Bollikolla et al., 2023)
2	**Flavonol**		Quercetin, Kaempferol, Fisetin, Myricetin, Apigenin, Rutin (Mahmud et al., 2023)	Antioxidant, anticancer, anti-inflammatory, hepatoprotective (El-Saber Batiha et al., 2020) (Periferakis et al., 2022)
3.	**Flavanone**		Hesperetin, Naringenin	Antioxidant, anticancer (Madureira et al., 2023)
4.	**Anthocyanidins**		Cyanidin, delphinidin, malvidin, petunidin	Antioxidant, anti-inflammatory (Salehi et al., 2020)

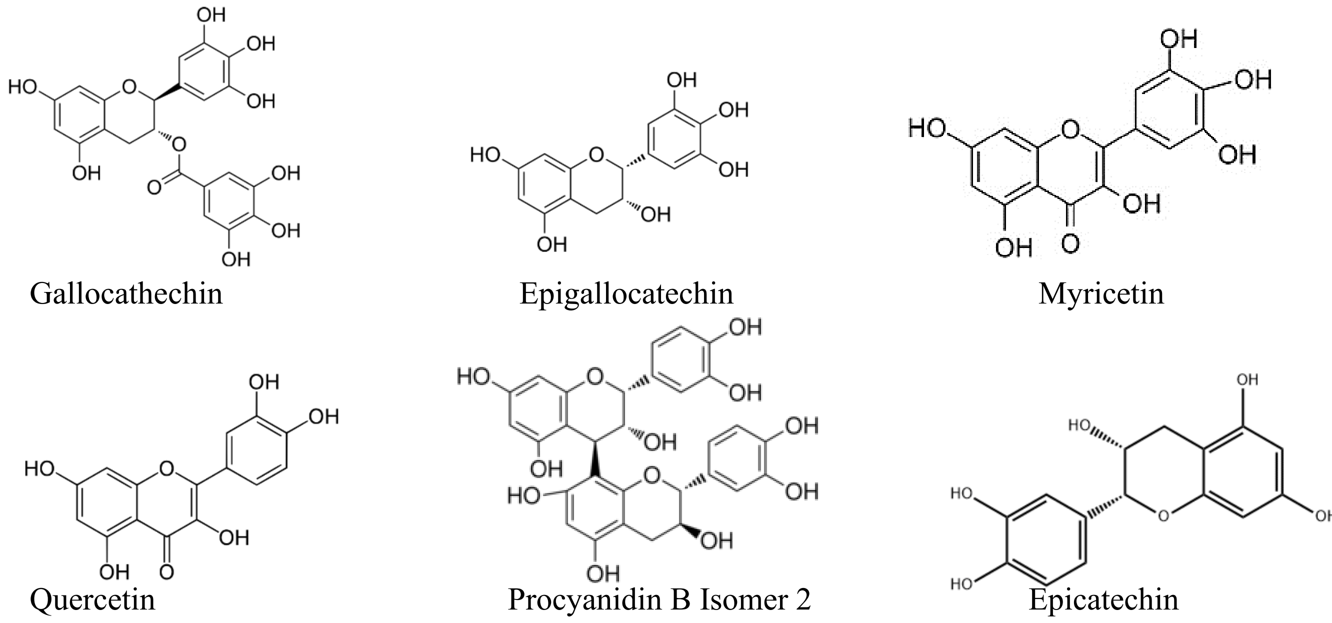


Figure 1.4: Chemical Structures of Representative Flavonoids Across Major Subclasses

1.5 Extraction techniques

Solvents play a crucial role in extraction by selectively dissolving bioactive compounds from plant matrices. The choice of solvents ranging from conventional organic solvents to greener alternatives like natural deep eutectic solvents (NADES) significantly influences yield, selectivity, and sustainability of the process.

1.5.1 Conventional solvents used in the extraction of bioactive compounds from medicinal plants.

In the process of extracting bioactive compounds or phytochemicals from plant biomass, various conventional solvents have been used. While these solvents offer certain advantages, they also come with some drawbacks. Table **1.4** below presents a list of these solvents along with their disadvantages as extraction agents.

Table 1.4: Advantages and Disadvantages of Conventional Solvents Used in the Recovery of Bioactive Compounds

Solvent(s)	Advantages	Disadvantages
Ethanol	- Broad solubility range; capable of extracting a wide variety of compounds. - Antimicrobial qualities help to maintain the quality of extracts and lower the danger of contamination. - Evaporates quickly after extraction to facilitate concentration processes (J. E. Lee et al., 2024)	- Ethanol is highly flammable and evaporates quickly, necessitating strict safety and containment measures (Abubakar & Haque, 2020) - Ineffective at dissolving non-polar compounds such as lipids and gums.
Methanol	- Broad solubility range; capable of extracting a wide variety of compounds (Gil-Martín et al., 2022) - Low cost of solvent offering some economic advantages (Lee et al., 2024)	- Methanol's severe toxicity precludes its use in applications involving human touch or ingestion (Gil-Martín et al., 2022) - High volatility and ability to ignite vapour (Lee et al., 2024) - Strict Environmental regulatory adherence and safety management (Lee et al., 2024).

Acetone	- Effective for extracting phenolic compounds (Downey & Hanlin, 2010) (Lee et al., 2024) - Due to its small molecular size and polarity, acetone can penetrate plant tissues efficiently, facilitating the release of intracellular compounds (Azwanida, 2015).	- Highly flammable and volatile, increasing health and fire hazards (PubChem, 2025)
Water	- Cost effective, safest, most environmentally friendly polar solvent (Lee et al., 2024)	- Low extraction efficiency for apolar compounds. It is mainly used to extract polar compounds (Lee et al., 2024).
Ionic liquids	- Good tunability and solubility across a wide variety of temperatures (Padinhattath et al., 2025)	- Toxicity profile due to bioaccumulation, posing safety and environmental concerns (Płotka-Wasylka et al., 2020).

1.5.2 Importance of Green Extraction Techniques for the Recovery of Natural Products

The global interest in green chemistry and the need to meet some of the Sustainable Development Goals (SDGs), such as responsible consumption (SDG 12), climate action (SDG 13), clean water protection (SDG 6) and promoting good health and well-being (SDG 3) (World Health Organization Sustainable Development Goals., 2023), as well as the increase in public interest towards non-hazardous materials (Picot-Allain et al., 2021) made scientists to shift their perspectives and approaches towards using sustainable extraction practices to recover bioactive compounds from natural products. In addition, the importance of more environmentally friendly extraction techniques has grown due to the UN's emphasis on a more sustainable future (R. Martins et al., 2023). Researchers from both academia and business describe "green extraction" and lay forth its six guiding principles, (Chemat et al., 2012) which includes:

Principle 1: Innovation by selection of varieties and use of renewable plant resources.
Principle 2: Use of alternative solvents and principally water or agro-solvents.
Principle 3: Reduce energy consumption by energy recovery and using innovative technologies.

Principle 4: Co-product production instead of waste, including the bio-and agro-refining industry.

Principle 5: Reduce unit operations and favour safe, robust and controlled processes.

Principle 6: Aim for a non-denatured and biodegradable extract without contaminants.

Innovative green extraction techniques have increased extraction yields with high concentrations of heat-labile and active chemicals at a reduced environmental cost, quickly, and with effective solvent use (Awad et al., 2021). It has been shown that different combinations of extraction technologies can work together or as an additional support (Awad et al., 2021) Some of the green extraction technologies includes: Ultrasound + enzymatic extraction technique (UAEE), Ultrasound + microwave assisted extraction technique (UMAE), Ultrasound + supercritical fluid extraction technique (USFE), Microwave + enzymatic extraction technique (MAEE), Microwave + supercritical fluid extraction (MSFE), Microwave + subcritical water extraction (MSWE) (Kumar et al., 2023), Ultrasound + Natural Deep Eutectic Solvents (NADES) (Mansinhos et al., 2021a) .

To extract biologically active chemicals from natural matrices, the most used solvents are water and traditional organic solvents, such as ethanol Liu et al., 2019). Water based on its polarity can only extract hydrophilic and water-soluble compounds which could be a limitation to its extraction potential, since extracting as many target chemicals from the matrix as possible is the main objective of any extraction procedure. Traditional organic solvents such as acetone, ethanol and methanol have issues with their volatility, and properties which limit their application especially in natural compound processing (Liu et al., 2019). Overall, green extraction techniques are essential for promoting sustainability and reducing the ecological footprint of extraction processes.

1.5.3 NATURAL DEEP EUTECTIC SOLVENTS (NADES)

a. **Definition and composition**

NADES are made from naturally existing compounds such as sugars, alcohols, amino acids, organic acids, and choline derivatives, and fully adhere to green chemistry principles. NADES are formed by combining a hydrogen-bond acceptor (HBA) with a hydrogen bond donor (HBD) molecule, resulting in a significantly lower melting point (Gomez et al., 2018). The network of hydrogen bonds makes the system more stable and permits charge delocalization in the case of ionic ingredients. The outcome of these effects is a lower melting point (Kovács et al., 2020). Choi et al. (2011) was the first to report natural deep eutectic solvents (NADES) as a Deep eutectic solvent (DES) subclass. According to these authors, NADES may exist in living things as a third liquid phase in addition to water and lipids. This could explain why organisms can survive in arid environments or why poorly water-soluble macromolecules can be biosynthesized in cells that are otherwise aqueous environments.

There are numerous advantages to switching from typical organic solvents to NADES because they are nonflammable and nonvolatile. NADES are less expensive, more biodegradable, and less hazardous than ILs. NADES are less susceptible to impurities than ILs and can be produced with 100% atom economy (Kovács et al., 2020). Figure 1.5 shows the NADES formation between choline chloride and urea. While table 5 shows different NADES combinations.

Figure 1.5: NADES obtained by combining choline chloride (the hydrogen-bond acceptor) with urea (the hydrogen-bond donor). The dashed circle indicates complicated hydrogen bonding between the constituents. (Kovács et al., 2020)

Table 1.5: Examples of different composition and combinations of NADES used in the extraction of mainly hydrophilic compounds. (Dai et al., 2013a)

Component 1	Component 2	Molar ratio
Choline chloride	Lactic acid	1:1
Choline chloride	Malonic acid	1:1
Choline chloride	Maleic acid	1:1, 2:1
Choline chloride	Citric acid	1:1, 2:1
Choline chloride	Aconitic acid	1:1
Choline chloride	Glycerol	1:1, 3:2
Choline chloride	d-(+)-Glucose	1:1 ,2:1
Choline chloride	d-(+)-Glucose	5:2
Choline chloride	d-(−)-Fructose	1:1, 1:2,1:1.5
Glycerol	Urea	1:5
Glucose	Lactic acid	1:2
Citric acid	xylose	1:1
Citric acid	Sorbose	1:1
Citric acid	Maltose	2:1
Citric acid	Sucrose	1:1
Beta-Alanine	Malic acid	1:1, 3:2

Some of the commonly used HBDs and HBAs used in the preparation of NADES are shown in Figures 1.6 and 1.7.

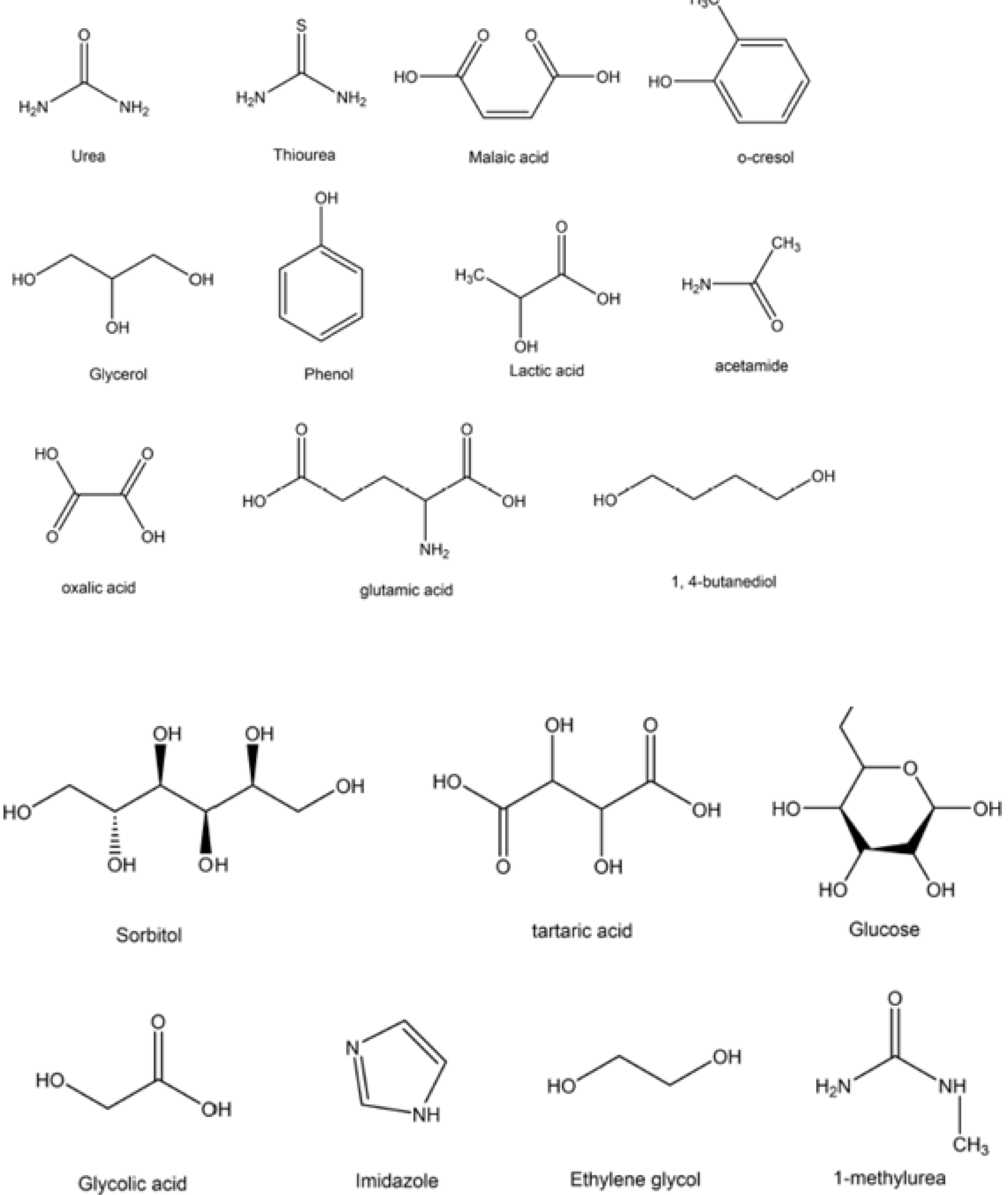


Figure 1.6: Commonly used HBDs for the preparation of NADES (Satija et al., 2024)

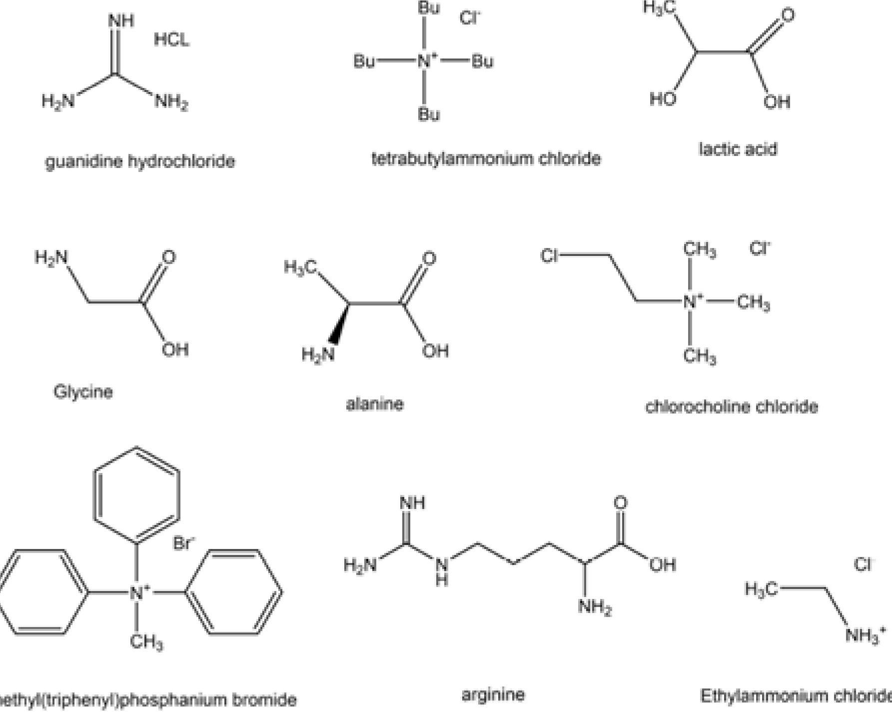


Figure 1.7: Commonly used HBAs for the preparation of NADES.

1.6 Preparation for NADES

NADES can be prepared using several methods, depending on the specific materials, available equipment, and desired outcomes. Table 1.6 shows some of the commonly employed preparation techniques.

Table 1.6: NADES preparation

Entry	Method	Description	Preparation	Reference(s)
1	Heating and stirring	• Classical procedure involves carefully combining HBA and HBD in specific molar ratios. • To achieve eutectic formation, begin with thorough weighing and mixing before gradually heating	• In a water bath below 323 kelvins, mix two ingredients until a clear liquid forms (30-90 minutes). • To ensure a homogeneous liquid, heat the two ingredients	(Satija et al., 2024), (Wei et al., 2015)

		beyond melting temperatures. • Continuous stirring helps maintain homogeneity, prevent phase separation, and improve intermolecular interactions. • Advantages of this method include its simplicity and universality, making it adaptable to a variety of NADES compositions. However, energy consumption and probable longer reaction times should be considered carefully.	to 353 Kelvins and stir regularly.	
2	Freeze drying	• The dehydration method is essential to the synthesis of NADES. • To avoid crystal formation, the frozen liquid is rapidly frozen before being sublimated at low pressure to generate a porous solid matrix. • Secondly, drying removes remaining water, preserving the structural and chemical integrity of NADES components. • Although beneficial for maintaining characteristics and stability, the technique's dependency on specialized equipment	• To create a transparent viscous liquid, mix the components, freeze the resulting aqueous solution, then freeze-dry it. This process removes excess water, resulting in the formation of a stable, homogeneous Natural Deep Eutectic Solvent (NADES) with improved viscosity and solubility characteristics suitable for bioactive	(Satija et al., 2024), (Dai et al., 2014)

		and time-consuming operations may limit its scalability.	compound extraction.	
3	Vacuum evaporation	• After mixing HBAs and HBDs, the solution evaporates under reduced pressure, lowering the solvent's boiling point. • Condensation recovers the evaporated solvent, producing a concentrated NADES.	• Components should dissolve in water and then evaporate. To maintain uniform weight, the liquid is placed in a desiccator using silica gel.	(Satija et al., 2024)
4.	Ultrasound-assisted heating	• Ultrasonication involves applying ultrasound waves to a component mixture to accelerate molecular interactions and produce homogeneous NADES without the need for high temperatures.	• The weighed components are mixed in a vessel and subjected to ultrasound (via bath or probe) at room temperature or slightly elevated temperatures until a clear, uniform liquid is formed.	(Bajkacz & Adamek, 2018)

1.6.1 Structure - Property Relationship of NADES

The intermolecular hydrogen-bonding mechanism between the constituents gives NADES its unique characteristics. The degree of this interaction's power largely dictates the physicochemical characteristics of the eutectic mixture, which are determined by the composition's structure and ratio (Kovács et al., 2020).

a. ***Melting point***

The eutectic point, or the system's lowest melting point, and its corresponding composition dictate how a eutectic system is applied (Kovács et al., 2020). Temperature and component ratio are two major determinants in a binary eutectic system (figure 1.8) (Liu et al., 2018). These components

interact by intermolecular forces, not covalent or ionic bonds. NADES including amides, carboxylic acids, and sugar-derived polyols with organic salts frequently have melting points lower than room temperature (Kovács et al., 2020).

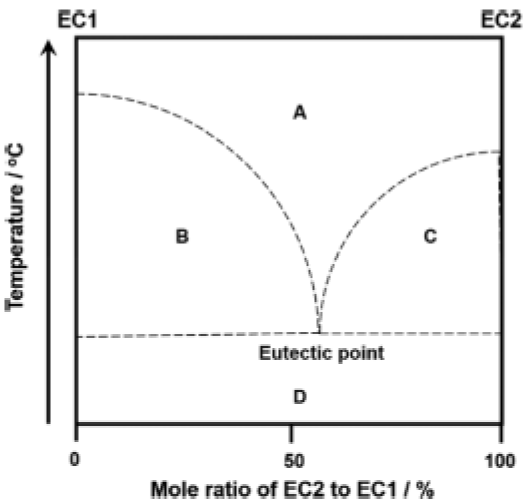


Figure 1.8: Schematic representation of the eutectic point in a two-component (1 and 2) phase, where EC means eutectic component. The dashed curve depicts the melting points of a binary NADES. family with varying molar ratios. A contains all unified liquid media, and the applied NADES species are at or below ambient temperature. This area should resemble a del operator, with the eutectic point located at one of the valley's angles. B and C are combinations of EC1 and EC2 (solid/liquid or liquid/solid), whereas D is a combination of EC1 and EC2 (solid/solid). The eutectic point allows for exact and fortuitous combination of chemicals, resulting in mutual compatibility and a significantly lower melting point (Y. Liu et al., 2018) .

The system's enthalpy (H) and entropy (S) vary as the NADES constituents melt (fuse) (Kovács et al., 2020). This can be illustrated through the Gibbs free energy equation:

$$\Delta G = \Delta H - T \cdot \Delta S$$

At the melting temperature of the eutectic, $\Delta G = 0$

At different temperatures, the ΔG value determines whether the solid ($\Delta G>0$) or liquid ($\Delta G<0$) state is thermodynamically stable (Kovács et al., 2020). Figure 1.9 illustrates this

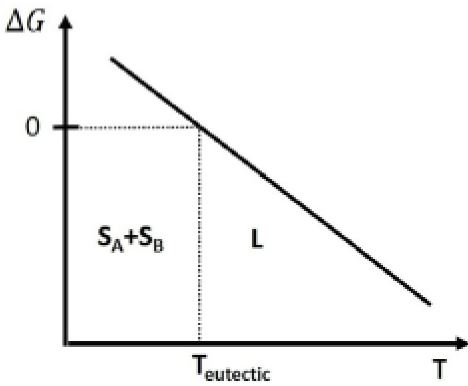


Figure 1.9: SA + SB are solid phases of constituents A and B, $T_{eutectic}$ is the melting point of the eutectic mixture, L is the liquid phase.

b. **Density, viscosity and surface tension**

The flowability of NADES at different shear rates is determined by their apparent viscosity, which indicates their intended application and use (Elhamarnah et al., 2024). NADES possess significantly higher viscosities than organic solvents. For instance, the viscosity of water and toluene are 0.89 and 0.56 mPaS at 25°C respectively while the viscosity of a mixture of choline chloride and urea is around 750 mPas at 25°C (Kovács et al., 2020). Selecting smaller component molecules with fewer hydrogen-bond donating/accepting groups and weaker interactions is required to decrease the viscosity of NADES since high viscosity implies substantial restrictions to mass and energy transfer during chemical processes (Kovács et al., 2020). However, there is a trade-off between transport and solvation qualities because high intermolecular forces lead to low melting temperatures and good solubility. Eutectic solvents have higher surface tension than most molecular solvents and are comparable to imidazolium-based ILs (Zhang et al., 2012). Most NADES have densities higher than water, ranging from 1.1 to 1.4 gcm (Dai et al., 2013b). Higher temperature decreases the density, viscosity and surface tension of NADES. In addition, the stronger the hydrogen-bond interaction the lower the mobility of NADES, it is for this reason that NADES with stronger bonds such as choline chloride – urea or choline chloride – ethylene glycol have higher surface tension (Kovács et al., 2020). Disrupting the hydrogen bond network, such as adding a quaternary component, decreases all three values (Dai et al., 2013).

1.6.2 Applications of NADES in Bioactive Compound Extraction

NADES have emerged as a promising alternative for bioactive compound extraction, offering a safer, customizable, and eco-friendly solution for isolating these valuable molecules. NADES have demonstrated the ability to enhance the solubility, stability, bioactivity, and bioavailability of plant metabolites (Hikmawanti et al., 2021).

a. ***Improved solubility***

NADES components have varying polarities, viscosities, and dissolving abilities. This has to do with the effectiveness of extraction (Duan et al., 2016). High solubility is associated with the interactions between NADES and phytochemicals, including dipole-dipole interactions and hydrogen bonding (Cao et al., 2020). For example, gallic acid, an important phenolic compound, was extracted in greater amounts from *P. maritimum* using ChCl: fructose compared to conventional solvents like acetone and ethanol (Rukavina et al., 2021). Another important phenolic acid is chlorogenic acid, has been reported to be optimally extracted from several different plants using NADES that consist of various types of HBA-HBD components, including betaine and triethylene glycol in a 1:2 ratio (Fanali et al., 2020). The extraction of aglycone flavonoids, apigenin and naringenin, was favored using NADES, with concentrations being 2-fold and 3.5-fold higher, respectively, in the NADES extracts compared to conventional hydroethanolic extracts (Tzani et al., 2022).

b. ***Improved stability***

The characteristics of NADES, including their chemical and thermal stability, contribute to the effective storage of natural compounds (Mbous et al., 2017). NADES have demonstrated the ability to stabilize various natural pigments and plant metabolites (Jauregi et al., 2024).

Certain NADES, particularly those based on sugars and polyalcohols like choline chloride: xylose, significantly enhanced stability and extend the half-life of ascorbic acid, acting as protective agents against degradation (Gomez-Urios et al., 2024).

The durability of curcumin powder, a yellow pigment derived from turmeric, in methanol and choline chloride-glycerol NADES after exposure to artificial sunshine was examined by Jeliński

et al. (2019) Curcumin's concentration in methanol solution dropped to 5% of its initial level after 120 minutes, however it remained stable and did not degrade in NADES solution.

Stupar et al. (2021) investigated the stability of additional hydrophobic pigments, including beta-carotene, in NADES. Caprylic acid-capric acid (3:1) fatty acid NADES efficiently retrieved beta-carotene from pumpkin (96.74 g/mL), according to the authors. Compared to 107 g/mL with n-hexane, these NADES enhanced the solubility of β -carotene to 200.77 g/mL. Furthermore, the NADES extract demonstrated excellent stability after 180 days of dark storage. In another work, Jeong et al. (2017) assessed EGCG's stability in NADES over time. According to the scientists, there was less of a drop in EGCG levels in NADES than in the traditional solvent employed as a comparison.

1.6.3 Impact of viscosity Parameters on NADES Properties and Extraction Yields

One of the most crucial properties and one of the biggest barriers to the use of NADES is viscosity (Dai et al., 2013d). Extraction efficiency is reduced when the viscosity of the NADES is too high, as it restricts compound mobility (Fernández et al., 2018). The viscosity of NADES is influenced by the percentage of water and temperature, as demonstrated in a study by Dai et al., (2013d) showed that diluting a glucose: choline chloride NADES with varying percentages of water (v/v) led to a decrease of one-third with 5% water and a decrease of 10% with 10% water. Additionally, raising the temperature from 20 to 40 ºC reduced the viscosity by two-thirds. The optimal percentage of water, based on several studies, to enhance the mass transfer of solutes during the extraction process and thus improve extraction efficiency was determined to be 30% (Mansinhos et al., 2021b) (Satija et al., 2024).

1.7 SUPRAMOLECULAR SOLVENTS (SUPRAS)

Definition and Composition

SUPRAS are nanostructured liquids formed spontaneously by adding a coacervation-inducing agent to colloidal suspensions of amphiphiles (Krivošija et al., 2024). Coacervation-induction agents, such as organic or inorganic salts, pH variations, cause amphiphilic aggregates to develop in the colloidal suspension and separate into a new denser or lighter liquid phase (Hem et al., 2019). This interaction typically involves non-covalent bonds, including dipole-dipole interactions, π-π interactions, Van der Waals forces, hydrogen bonding, and metal-ligand interactions (Jagirani & Soylak, 2021a).

SUPRASs are formed through a two-step self-assembly process of amphiphilic molecules first into nanostructures such as micelles or vesicles once the critical aggregation concentration is reached, and then into larger aggregates that separate into a new immiscible phase via coacervation. This self-assembly is driven by non-covalent interactions and influenced by the amphiphile's molecular geometry, environmental conditions, and mobility, resulting in a reversible, adaptive, and thermodynamically favorable system (Ballesteros-Gómez et al., 2010), as shown in figure 1.10.

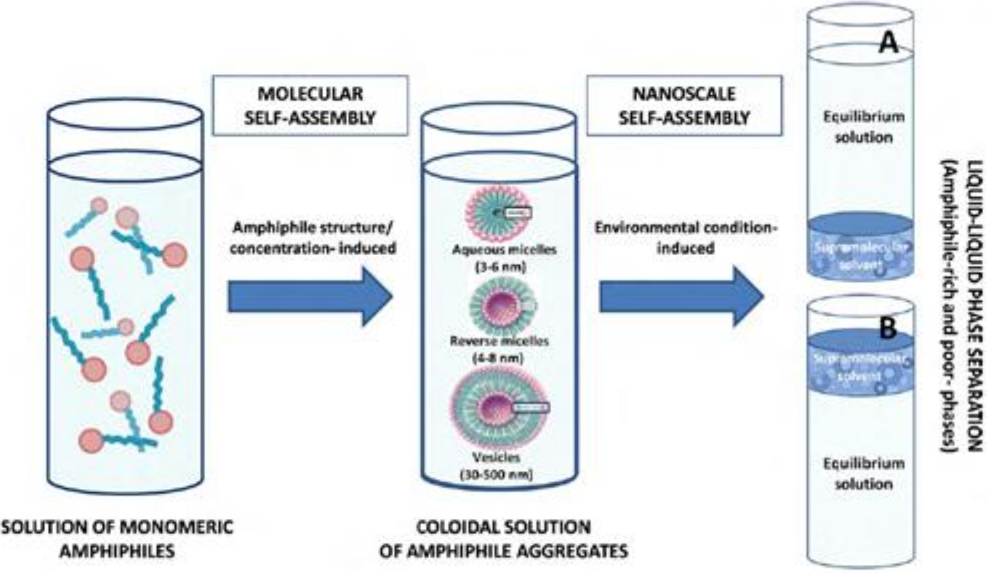


Figure 1.10: Self-assembly processes in supramolecular solvent formation (Ballesteros-Gómez et al., 2010).

The simultaneous extraction of bioactive compounds from biomass using organic solvents is often inefficient, as the extraction yields of these phytochemicals are highly dependent on the polarity of the solvent. An innovative approach is the use of SUPRAS, which can facilitate the simultaneous extraction of phytochemicals across a wide range of polarities (Keddar et al., 2020) This is made feasible by the differential polarity areas seen in supramolecular aggregates, which are made up of distinct hydrophobic and polar regions provided by the hydrocarbon chains and amphiphile head groups, respectively (Ballesteros-Gómez et al., 2019) . Amphiphiles such as octanoic acid, 1-dodecanol, lecithin, and cetyltrimethylammonium bromide, when combined with co-solvents or additives like ethanol and water, sodium lauryl sulfate, water, or salt and water, form various structures including vesicles, coacervates, reverse micelles, and micelles, as reported by Keddar et al. (2020) and Ballesteros-Gómez et al. (2010).

1.7.1 Bioactive Compound Extraction Using SUPRAS

SUPRASs offer a green and efficient alternative for the extraction of bioactive compounds due to their tunable polarity, self-assembling nature, and ability to selectively solubilize target analytes (Keddar et al., 2020).

A study by Keddar et al. (2020), proposed the use of SUPRAS as an innovative approach for the simultaneous extraction of a wide range of hydrophilic and lipophilic antioxidants from freeze-dried biomass of the microalgae *Scenedesmus sp.* They synthesized SUPRAS from octanoic acid, ethanol, and water, which spontaneously separated from an equilibrium solution (EqS). The experimental conditions, including SUPRAS composition and the SUPRAS:EqS ratio, were optimized for the extraction process. Under optimal conditions using only SUPRAS, they reported achieving maximum extraction yields of up to 10.3 ± 0.3 mg GAE/g dw for polyphenols and 1.04 ± 0.07 mg carotenoids/g dw.

Ballesteros-Gómez et al., (2023) also demonstrated the application of SUPRASs for the liquid-liquid extraction of hydroxytyrosol, a phenolic compound found in olive brines. The research focused on valorizing table olive brines, which are abundant but underutilized waste streams containing valuable phenolic compounds. In comparison to existing methods such as nanofiltration and sorption onto resins which are often expensive, slow, and require large volumes of organic solvents the SUPRAS extraction method proved to be more efficient than conventional methods.

SUPRAS has been effectively combined with an ultrasound-assisted method for extracting phenolic acids from *Prunella vulgaris.* This combination demonstrated significantly higher extraction efficiency and resulted in extracts with enhanced antioxidant capacity compared to conventional organic solvents and deep eutectic solvents (Xia et al., 2024). The experimental results, which included yields and antioxidant assays, were supported by theoretical molecular dynamics simulations. These simulations explain the superior performance of SUPRAS through favorable molecular interactions and its unique droplet structure (Xia et al., 2024).

Phenolic compounds were extracted from Tamarillo pill utilizing SUPRAS, which demonstrated higher extraction of total phenolic compound and higher antioxidant yield compared to several conventional organic solvents (ethanol, acetone, diethyl ether, methanol) commonly used for this purpose (Torres-Valenzuela et al., 2025). The total phenolic content (TPC) of octanoic acid based SUPRAS was (0.0031 mg g^{-1}), while that of conventional solvents were methanol (0.0021 mg g^{-1}), ethanol (0.0012 mg g^{-1}), diethyl ether (0.0009 mg g^{-1}), and acetone (0.0005 mg g^{-1}) (Torres-Valenzuela et al., 2025).

Sánchez-Monedero et al. (2025) evaluated the extraction efficiency of SUPRAS/EqS and NADES consisting of menthol and octanoic acid in a 1:1 ratio, as well as conventional solvents like water and ethanol, for recovering bioactive compounds from *Olea europaea* L. leaves. Their results indicated that the highest extraction yield was achieved using SUPRAS formulated with octanoic acid (C8), whereas the pure NADES tested were the least effective solvents when compared to both SUPRAS and ethanol/water mixtures.

1.7.2 Challenges Towards the Use of Supramolecular Solvents

1. High viscosity for analysis: After the extraction process, enriched SUPRASs can exhibit very high viscosity. This increased viscosity complicates analysis, especially with techniques like High-Performance Liquid Chromatography (HPLC) (Jagirani & Soylak, 2021b). Moradi et al. (2021) also noted compatibility issues with gas chromatography, highlighting that the amphiphile-rich SUPRAS phases can pose a risk of coagulation, potentially blocking the capillary column in gas chromatography systems. Furthermore, injecting the SUPRAS phase into a gas chromatography system can lead to irreversible negative effects (Moradi et al., 2021).

2. Time-Consuming Centrifugation: The typical phase separation process in SUPRAS-based microextraction often relies on centrifugation, which is a time-consuming step (Keddar et al., 2020), (Jagirani & Soylak, 2021b).

3. **Environmental and toxicity concerns:**

- Some SUPRAS precursors are toxic to aquatic or terrestrial organisms. For example, alkylphenol ethoxylates (APEs) used as surfactants in SUPRAS is a known endocrine disruptor; degrades slowly and can bioaccumulate (Acir & Guenther, 2018).
- Tetrahydrofuran (THF)-based SUPRAS possess some issues in terms of biodegradability. THF is a volatile organic compound that is moderately biodegradable but problematic at high concentrations (Ren et al., 2020).
- The use Sodium dodecyl sulfate (SDS)-based SUPRAS in high volumes on an industrial scale, even though it's biodegradable, in high concentrations, it becomes toxic to fish and plants (Feng et al., 2023).

1.8 Statement of the Problem and Research Objectives

The increasing demand for bioactive compounds from natural sources, particularly plants, has highlighted the need for efficient and sustainable extraction methods. Conventional solvents such as ethanol and ionic liquids, while widely used, often result in lower extraction yields, degradation of sensitive compounds, and environmental concerns due to their toxicity and volatility (Patrice Didion et al., 2023) Martins et al., 2021). NADES and SUPRAS have emerged as promising green alternatives that enhance extraction efficiency while minimizing environmental impact (Mansinhos et al., 2021b) (Keddar et al., 2020). However, their efficacy in extracting bioactive compounds from *P. maritimum* remains largely unexplored. This research addresses this gap by evaluating and comparing the extraction efficiency of NADES and SUPRAS against conventional solvents (water and ethanol), assessing their antioxidant activity, and identifying the extracted compounds using LC-MS analysis.

1.8.1 Research Justification / Rationale

The study of green extraction solvents is essential for advancing sustainable extraction techniques in natural product research. Halophytes are a rich source of bioactive compounds with potential applications in pharmaceuticals, cosmetics, and functional foods. However, traditional extraction methods often involve toxic organic solvents that pose environmental and health risks. NADES and SUPRAS offer eco-friendly alternatives with the potential to improve extraction efficiency and compound stability. By comparing these solvents with conventional methods, this research contributes to the development of greener, more effective extraction strategies, aligning with the growing global emphasis on sustainability and green chemistry. Additionally, understanding the antioxidant potential of these extracts can support their application in nutraceutical and pharmaceutical industries.

1.8.2 Research Aims and Objectives

Research Aim: To evaluate the efficiency of NADES and SUPRAS as green extraction solvents for recovering bioactive compounds from *P. maritimum* and compare their performance with conventional solvents (water and ethanol) in terms of yield, antioxidant activity, and phytochemical profile.

Research Objectives:

1. To compare the extraction efficiency of NADES, SUPRAS, water, and ethanol in recovering bioactive compounds from *P. maritimum.*

2. To assess the antioxidant activity of the extracts using *in-vitro* assays.

3. To identify and characterize the bioactive compounds extracted using LC-MS analysis.

4. To evaluate the sustainability and applicability of NADES and SUPRAS as alternative green solvents in natural compound extraction.

Research Questions

1. Are NADES and SUPRAS comparable to water and ethanol in terms of extraction efficiency for bioactive compounds from *Polygonum maritimum?*

2. What are the antioxidant properties of extracts obtained using NADES and SUPRAS compared to conventional solvents?
3. What bioactive compounds are present in the extracts, as identified by LC-MS analysis?
4. How do NADES and SUPRAS contribute to green and sustainable extraction processes compared to traditional solvents?

Hypothesis:

Null Hypothesis (H_0): There is no significant difference in the extraction efficiency and antioxidant activity of bioactive compounds from *P. maritimum* using NADES, SUPRAS, water, and ethanol.

Alternative Hypothesis (H_1): NADES and SUPRAS exhibit higher extraction efficiency and antioxidant activity compared to conventional solvents (water and ethanol).

Scope of the Study

This study focuses on the extraction of bioactive compounds from *P. maritimum* using NADES, SUPRAS, water, and ethanol. The research encompasses the following:

- Extraction Methodology: Extraction of bioactive compounds from P. maritimum using NADES, SUPRAS, water and ethanol
- Antioxidant Assay: *In vitro* evaluation of the antioxidant potential of the different extracts
- Compound Identification: Characterization of extracted compounds via LC-MS analysis.

Study Limitations: The study is limited to in-vitro analysis and does not extend to *in-vivo* biological validation. The research also does not explore the large-scale industrial application of these solvents but provides insights into their feasibility at a laboratory scale.

Significance of Study

The findings of this research have broad implications for various stakeholders:

Scientific Community: Provides insights into the potential of NADES and SUPRAS as green solvents, contributing to sustainable extraction research.

- **Pharmaceutical & Nutraceutical Industries:** Highlights eco-friendly methods for extracting bioactive compounds with antioxidant properties, which can be applied in drug development and functional foods.
- **Environmental and Green Chemistry Advocates:** Supports the shift toward sustainable extraction practices by reducing reliance on toxic organic solvents.
- **Policymakers & Regulatory Bodies:** Offers data that can guide policies promoting green chemistry in natural product extraction.

2.0 MATERIALS AND METHODS

2.1 Chemicals and Reagents

Choline chloride, urea was provided by VWR Chemicals, USA). D- (+)- glucose, glycerol (≥99%), glacial acetic acid (≥99%), potassium persulfate, octanoic acid ≥98%, 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), lactic acid (≥ 88%) was acquired from Sigma-Aldrich, USA). DL-Malic acid (≥99%) (Acros Organics Belgium). Bismuth subnitrate, ethanol (≥96%) were from Aga, Portugal). Ammonium chloride hexahydrate (Riedel-de Haen). Potassium iodide and hydrochloric acid 37% were from Fischer Chemicals, UK).

2.2 Plant Material

Aerial organs (leaves and stems) of *P. maritimum* were collected in spring 2024 in "Praia de faro", Southern Portugal. After collection, biomass was washed with tap and distilled water, dried in an oven (40ºC) for 3 days and ground to powder (< 2 mm particle size). The dried biomass was stored in a dark container at RT (approx. 20°C) until required.

2.3 NADES Preparation

NADES were prepared following the method reported by Mansinhos et al. (2021) with minor modifications. The mixtures were heated on a magnetic stirrer set to a constant temperature between 50 and 70°C, with a specific amount of distilled water added to facilitate dissolution. Water was incorporated during the formation of the NADES to reduce viscosity, as lower viscosity enhances mass transfer (Mitar & Prlić Kardum, 2020). The optimal water content, determined to increase the extraction yield of bioactive compounds, was found to be 30% (v/v) (Shang et al., 2019). A total of four different combinations of NADES were used, as detailed in Table 2.1 and illustrated in Figure 2.1.

Table 2.1: NADES compositions and appearance

S/N	Abbreviation	Component A (HBA)	Component B (HBD)	Molar ratio	Appearance
1	CC:LA	Choline chloride	Lactic acid	1:2	Transparent colourless liquid
2	CC:MA	Choline chloride	Malic acid	1:1	Transparent light golden colourless liquid
3	GLU:LA	Glucose	Lactic acid	1:5	Transparent light colourless liquid
4	GLY: Urea	Glycerol	Urea	1:1	Transparent light colourless liquid

HBD: Hydrogen Bond Donor, HBA: Hydrogen Bond Acceptor

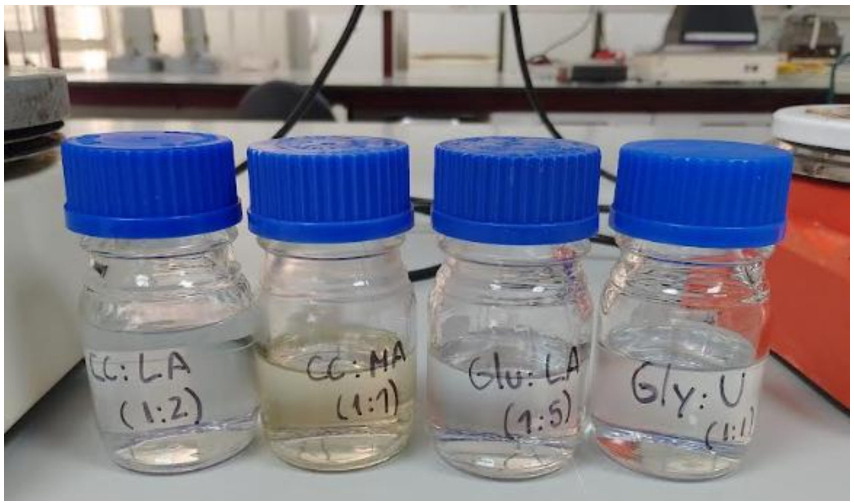


Figure 2.1: General aspects of prepared NADES

2.4 SUPRAS Preparation

SUPRAS were prepared according to Keddar et al., 2020) with slight modifications. SUPRAS were created by dissolving octanoic acid in ethanol and then adding 0.01 M hydrochloric acid as a self-assembly trigger. The octanoic acid concentration was 5% (v/v), while the concentration of ethanol and 0.01M HCl varied between 36% - 9.5%v/v and 59% - 85.5% respectively. The mixtures were stirred using a magnetic stirrer at 400 rpm, and RT. After stirring, mixtures were transferred into 50 mL Falcon tubes and centrifuged in an Eppendorf Centrifuge 5810 R at 4000 rpm for 20 minutes, at RT. The mixture produced SUPRAS which separated as a top layer from the bulk solution, designated as the equilibrium solution (EqS) (Fig. 2.2). The SUPRAS and EqS phases were collected independently and kept in airtight containers at RT, until needed. For each 500 ml of solvent mixture, the volume of SUPRAS collected ranged from 22 to 25 ml. Additionally, a mixture was prepared by combining 19 mL of SUPRAS (formulated at a volume ratio of 5:36:59% v/v) with 1 mL of EqS, which was also prepared using the same 5:36:59% v/v ratio. This resulting mixture (SUPRAS + EqS) maintained the original formulation ratio across both components.

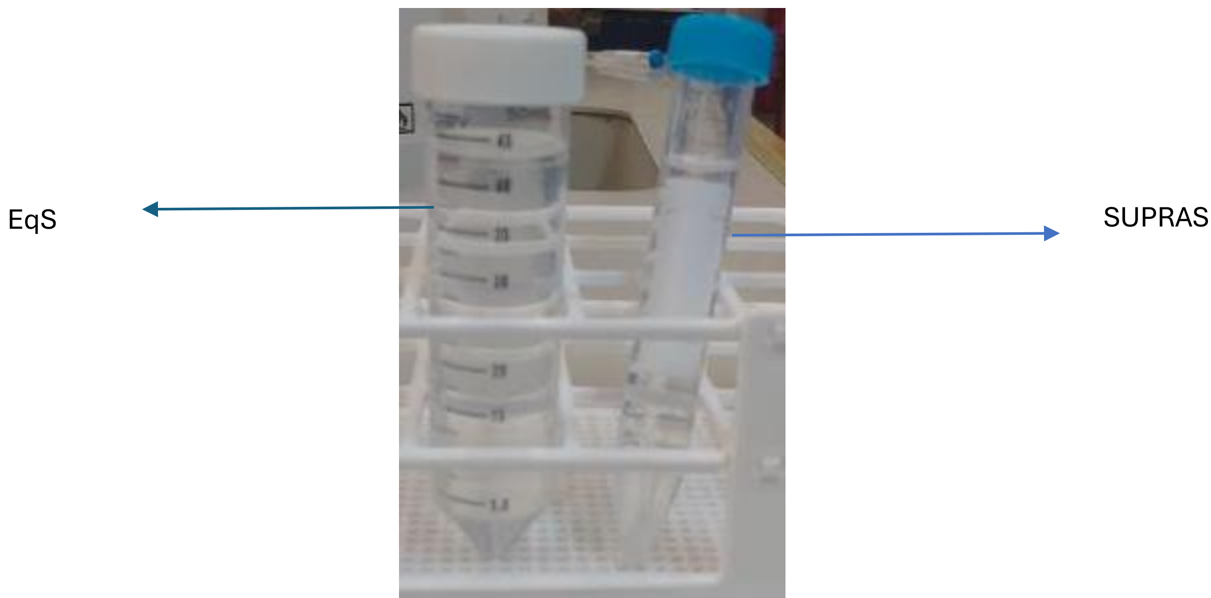


Figure 2.2: General aspect of prepared SUPRAS and EqS

2.5 Preparation of the Extracts

The extracts were prepared by mixing the dried biomass with the solvents at a ratio of 1:40 (w/v). The used solvents were distilled water, ethanol (96%), NADES (CC:LA, 1:2, CC:LA 1:5, CC:MA, 1:1 and Gly: Urea, 1:1), SUPRAS, EqS and SUPRAS+EqS. The resulting mixture was vortexed for 30 seconds and placed in an ultrasonic bath (model VWR Ultrasonic Cleaner USC-TH) for 30 minutes, at RT, in the dark. The extracts were then filtered using Whatman No. 1 filter paper and kept at -20°C until use.

2.6 Determination of the total flavonoid content (TFC) of the extracts

The TFC was determined by a spectrophotometric method as described by Pirbalouti et al., (2014).The assay involves forming a flavonoid-aluminium complex with maximum absorptivity at 420 nm. Fifty µL of the extracts at different dilutions, and the standard (quercetin), were mixed separately with 50 µL of a 2% $AlCl_3$-ethanol solution, and incubated for 10 minutes at RT. After incubation, the absorbance was measured at 420 nm using a microplate reader (Biochrom EZ Read 400 UK). The flavonoid content was calculated using a linear equation based on the calibration curve obtained with different concentrations of quercetin $Y= mx + c$, where Y is the absorbance, m=8.4002, x = flavonoid content in mg/ml and c= 0.099. The total flavonoid content was expressed as mg of quercetin equivalents (QE) per mL of extract. All experiments were conducted in six replicates.

2.7 **Test for the presence of alkaloids**

The presence of alkaloids was tested using the Drangendorff's reagent test as described previously (KANCHERLA et al., 2019). The reagent was prepared by dissolving 0.4 g of bismuth nitrate, Bi $(NO_3)_3$, in 5 ml of glacial acetic acid. This mixture was then diluted with 20 ml of distilled water to create solution (A). In a separate step, 0.4 g of potassium iodide (KI) was dissolved in 5 ml of water to form solution (B). Equal volumes of solutions A and B were combined with 5 ml of glacial acetic acid and further diluted with 20 ml of water. Finally, the resultant mixture was filtered using Whatman No. 1 filter paper to obtain the final reagent. For the assay, 1 ml of Drangendorff's reagent was added to 2 ml of extracts in a clear test tube. The formation of an orange precipitate indicates the presence of alkaloids.

2.8 **Phenolic profile analysis by LC-MS**

To characterize the phenolic compounds, present in the extracts, liquid chromatography–mass spectrometry (LC-MS) analysis was conducted. The extracts were diluted at a ratio of 1:4 prior to analysis. Separation of secondary metabolites was carried out using a Dionex Ultimate 3000RS UHPLC system (Thermo Scientific) equipped with a Phenomenex Kinetex XB-C18 column (100 mm × 2.1 mm i.d., 2.6 µm), maintained at a constant temperature of 25 °C (±1 °C). The mobile phases consisted of water (A) and methanol (B), both acidified with 0.1% formic acid. A flow rate of 200 µL/min was applied throughout the analysis. The gradient program was as follows: 0–3 min, 95% A; 3–43 min, linear decrease to 0% A; 43–61 min, 0% A; 61–62 min, return to 95% A; and 62–70 min, re-equilibration at 95% A.

2.9 **In vitro antioxidant properties**

a. 1,1-Diphenyl-2-picrylhydrazyl *(DPPH) Free Radical Scavenging Assay (RSA)*

The RSA towards the DPPH radical was measured by the protocol described by Jeeno et al. (2023). The extracts (22 µL) at different concentrations were mixed, in 96 well plates, with 200 µL of DPPH solution (120 mM, in ethanol) and incubated in the dark at RT, for 30 minutes. After incubation, the absorbance (**A_1**) was measured at 492 nm using a microplate reader.

A colour control was prepared following the same procedure, but 200 µL of ethanol was used in place of the DPPH solution to account for any background absorbance (**A_0**) of the extract. The negative control (**A^-**) included the solvents used for the extraction, while gallic acid was used as the positive control.

The RSA (%) was determined using the equation.

$$RSA = 100 - ((A_1 - A_0) * (100)) / A^-$$

The IC_{50} (half-maximal inhibitory concentration), which represents the concentration of an antioxidant needed to scavenge 50% of DPPH free radicals, was determined for all the extracts and standard using GraphPad Prism.

b. 2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Free Radical Scavenging Assay

The ABTS free RSA was performed according to the method described by Re et al., (1999). To produce the $ABTS^{\cdot+}$ radical cation, 7 mM ABTS was prepared using potassium persulfate 2.45mM ($K_2S_2O_8$) and distilled water and stored in the dark in a refrigerator for 12 to 16 hours. This solution was then diluted with water to obtain a working solution with an absorbance of 0.700 ± 0.02 at 734 nm. For the assay, 10 µL of the extracts were mixed with 190 µL of $ABTS^{\cdot+}$, and the absorbance was immediately recorded at 734 nm. Butylated hydroxytoluene (BHT, 1mg/ml) was used as the reference standard.

The RSA (%) was determined using the equation.

$$RSA = 100 - ((A_1 - A_0) * (100) / A^-)$$

The IC50, which represents the concentration of an antioxidant needed to scavenge 50% of free radicals, was determined for all the extracts using GraphPad Prism.

3.0 RESULTS AND DISCUSSION

3.1 SUPRAS Yield from Solvent Mixture

The supramolecular solvent (SUPRAS) phase was recovered following the formation process using a solvent mixture. For each 500 mL of solvent mixture, the volume of SUPRAS obtained ranged between 22 and 25 mL. The yield of SUPRAS was calculated using the formula:

Yield (%) = (Volume of SUPRAS (ml)) / (Total volume of solvents (ml)) * 100

Accordingly, the SUPRAS yield ranged between:

(22/500) * 100 = 4.4% & (25/500) * 100 = 5%

Thus, the SUPRAS recovery yield from the solvent mixture ranged between **4.4% and 5.0%**.

3.2 Antioxidant activity

In this study, two assays were used to appraise the antioxidant properties of extracts of *P. maritimum,* namely RSA towards DPPH and ABTS free radicals, which use hydrogen-atom transfer and single-electron transfer mechanisms, respectively (Mansinhos et al., 2021).

In the DPPH assay, the extracts SUPRAS 9.5, SUPRAS 36, and choline chloride: malic acid (1:1) exhibited the **lowest radical scavenging activity (RSA)**, as indicated by their **highest IC_{50} values** of 10.652, 2.152 mg/mL, and 0.7280 mg/mL, respectively. These values were significantly higher than those recorded for the ethanol, water, EqS 36, EqS 9.5, and SUPRAS 36 + EqS 36 extracts, as well as for all other NADES, except for choline chloride: malic acid (1:1) (Table 3.1).

In the ABTS assay, SUPRAS 36 and SUPRAS 36 + EqS 36 also showed the lowest RSA, confirming their reduced ability to scavenge ABTS free radicals (Table 3.1).

A previous study on the same species by Rukavina et al. (2021a) reported that NADES formulations based on choline chloride: fructose and choline chloride: sucrose exhibited lower antioxidant activity than ethanolic extracts, which contrasts with the performance of several NADES observed in the present study.

Among the various NADES tested, the combinations of **choline chloride:lactic acid (1:2)** and **glucose: lactic acid** consistently exhibited the **highest antioxidant activity**, as demonstrated by their **lowest IC_{50} values** in both DPPH and ABTS assays. This NADES (CC:LA) outperformed the conventional ethanol and water extracts, indicating a superior efficiency in extracting antioxidant compounds from the plant material. Similarly, among the SUPRAS-based systems, **EqS 36 and EqS 9.5** achieved **greater RSA** than ethanol and water in both assays. These findings suggest that these solvents could be used in replacement of ethanol and water in the extraction od antioxidant compounds from *P. maritimum* leaves and stems.

The extracts that exhibited the highest radical scavenging activity (RSA) notably choline chloride:lactic acid (1:2) also showed strong antioxidant potential in both DPPH and ABTS assays. LC-MS profiling **(table 3.4**) revealed that this extract contained key antioxidant compounds such as epicatechin, and quercetin glycosides , which are well-documented for their radical scavenging properties (Martinović et al., 2022). This indicates a strong correlation between RSA and the phytochemical profile, particularly the presence of flavan-3-ols and flavonols.

Table 3.1: Radical scavenging properties of extracts from Polygonum maritimum leaves and stems, on DPPH and ABTS radicals. Results are expressed as half maximal inhibitory concentration (IC_{50}, mg/mL).

Extracts	**DPPH IC_{50} (mg/ml)**	**ABTS IC_{50} mg/ml**
Ethanol	**0.024 ± 0.001^{a}**	0.2451 ± 0.099^{a}
Water	**0.093 ± 0.021^{a}**	0.1162 ± 0.015^{a}
SUPRAS 36	2.152 ± 0.150^{C}	**1.3325 ± 0.729^{b}**
SUPRAS 9.5	10.652 ± 4.852^{D}	**0.828 ± 0.262^{a}**
EqS 36	0.063 ± 0.012^{a}	**0.0102 ± 0.002^{a}**
EqS 9.5	0.0176 ± 0.015^{a}	**0.0667 ± 0.008^{a}**
SUPRAS 36 + EqS 36	**0.1117 ± 0.037^{a}**	2.2168 ± 0.765^{bc}

Glycerol: Urea (1:1)	0.7280 ± 0.358 [a, b]	0.0142 ± 0.006[a]
Choline Chloride: Malic acid (1:1)	1.0997 ± 0.665[b]	**0.0536 ± 0.028[a]**
Choline chloride: Lactic acid (1:2)	0.0281 ± 0.014[a]	**0.0022 ± 0.001[a]**
Glucose: Lactic acid (1:5)	0.0779 ± 0.057[a]	**0.0044 ± 0.002[a]**

Values represent mean ± standard deviation. Results were compared by ANOVA using Tukey post hoc test and different letters in each column corresponds to statistically significant differences at $p < 0.05$.

Previous studies on NADES have also supported our findings. In a study by Rukavina et al., (2021) NADES containing choline chloride combined with sucrose or fructose were evaluated for extracting antioxidants from *Polygonum maritimum* L. The authors found that these NADES extracts exhibited comparable or superior antioxidant activities measured by DPPH compared to conventional solvents like ethanol and acetone. Although ethanol showed the highest radical scavenging activity overall, NADES (especially ChCl:fructose) outperformed acetone in most antioxidant tests. Moreover, NADES extracts retained key antioxidant compounds such as flavonols and flavan-3-ols in similar proportions to conventional extracts, despite having a less complex phenolic profile. These findings suggest NADES as effective, green alternatives for antioxidant extraction with promising potential in sustainable application. Another study by Martinović et al., (2022), NADES were used to extract phenolic compounds and flavonoids from bilberry leaves, and green tea leaves. Compared to conventional solvents such as water and ethanol, NADES showed higher extraction efficiency and yielded extracts with significantly greater antioxidant activity, as demonstrated by DPPH, and ABTS assays. These results suggest that NADES are effective alternatives for obtaining bioactive compounds with potent antioxidant properties from plant materials.

3.3 Total flavonoid content (TFC)

The total flavonoid content (TFC) of the extracts was determined to evaluate the presence of flavonoid compounds, a major class of bioactive molecules known to occur in *P. maritimum* (Rukavina et al., 2021a). The results are presented in **Table 3.2.**

Significant differences were observed among the extracts obtained with different solvent systems ($p < 0.05$), with TFC values ranging from 0.0088 to 0.1390 mg QE/mL. The ethanol extract had a higher TPC than water.

Ethanol demonstrated the highest efficiency in extracting flavonoids, outperforming both NADES and SUPRAS. Among these, SUPRAS extracts showed higher flavonoid content than water extracts, while within the NADES group, the glycerol:urea combination yielded the highest flavonoid content.

Among the equilibrium solvents, EqS 9.5 (0.1029 ± 0.0063, group B) and EqS 36 (0.0883 ± 0.0168, groups A, B) showed comparably high TFC values. These were statistically like ethanol (0.0719 ± 0.0086, group A), suggesting similar or even exceed ethanol's extraction efficiency.

Intermediate TFC values were observed with SUPRAS 36 (0.0495 ± 0.0085) and SUPRAS 36 + EqS 36 (0.0388 ± 0.0055), both falling within group F. SUPRAS 9.5 (0.0379 ± 0.0037) and glycerol: urea (1:1) (0.0413 ± 0.0034) were also placed in group E and F, indicating no significant difference among these supramolecular and these eutectic blends.

Water (0.0225 ± 0.0044, groups C, E) and choline-based DESs such as choline chloride: lactic acid (1:2) (0.0113 ± 0.0017, group C) and choline chloride: malic acid (1:1) (0.0088 ± 0.0049, groups C, E) showed significantly lower TFC, with choline chloride: malic acid being the lowest. This may reflect the limited flavonoid solubility in highly polar or viscous solvents.

Extracts with the highest TFC EqS 9.5 also had richer flavonoid profiles based on LC-MS data (**table 3.5**), particularly flavonols and catechins. This aligns with their strong antioxidant performance. In contrast, choline chloride: malic acid and water extracts showed both low TFC and weaker antioxidant activity, confirming a link between TFC and flavonoid composition.

Table 3.2: Total flavonoid content (TFC) of extracts from Polygonum maritimum leaves and stems, expressed as a mg of quercetin equivalents (QE) per ml of extract.

Extracts	**Total flavonoid content (TFC, mg QE/ml)**
Ethanol	0.0719 ± 0.0086 [a]
Water	0.0225 ± 0.0044 [c, e]
SUPRAS 36	0.0495 ± 0.0085 [f]
SUPRAS 9.5	0.0379 ± 0.0037 [e, f]
EqS 36	0.0883 ± 0.0168 [a, b]
EqS 9.5	0.1029 ± 0.0063[b]
SUPRAS 36 + EqS 36	0.0388 ± 0.0055 [e, f]
Glycerol: Urea (1:1)	0.0413 ± 0.0034 [e, f]
Choline Chloride: Malic acid (1:1)	0.0088 ± 0.0049 [e, c]
Choline chloride: Lactic acid (1:2)	0.0113 ± 0.0017[c]
Glucose: Lactic acid (1:5)	0.1390 ± 0.028[d]

Values represent mean ± standard deviation. Results were compared by ANOVA using Tukey post hoc test and different letters corresponds to statistically significant differences at $p < 0.05$.

3.4 Qualitative Analysis of Alkaloids in the Extracts

The presence of alkaloids in the extracts of *P. maritimum* was evaluated qualitatively using the Dragendorff's test. To our knowledge, alkaloids have not been previously reported to be in *P.maritimum.* The classical assay to detect alkaloids is through the formation of an orange precipitate upon reaction with Dragendorff's reagent (Raal et al., 2020). Among all the solvent systems tested, only SUPRAS 36, the combination of SUPRAS 36 with EqS 36 (**Figure 3.1**), and the NADES composed of choline chloride:malic acid (1:1) yielded a positive result, as indicated by the formation of the characteristic orange precipitate. This outcome suggests that these SUPRAS-based extraction systems, along with the selected NADES, may be particularly effective in extracting or revealing the presence of alkaloid-like compounds in *P. maritimum*.

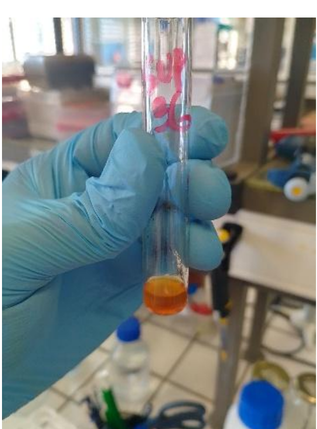


Figure 3.1: Alkaloid detection: orange-red precipitate in SUPRAS 36

3.5 LC-MS-Based Profiling of Bioactive Compounds in *Polygonum maritimum* Extracts

To further elucidate the influence of the type of used solvent on the extraction of natural compounds from *P. maritimum*, LC-MS analysis was performed on all extracts. This technique allowed for the high-resolution identification of a wide range of secondary metabolites based on their mass-to-charge (m/z) ratios and retention times. The resulting chromatograms displayed a complex phytochemical profile that was analysed and categorized into four main groups: phenolic compounds, flavonoids, organic acids, and other miscellaneous metabolites.

A total of 36 phytochemicals were identified across eleven extracts representing a wide array of bioactive classes including phenolic acids, flavan-3-ols (catechins), flavonols (quercetin/myricetin derivatives), and proanthocyanidins. (Tables 3.3 – 3.5). Key compounds such as gallic acid, epicatechin, epigallocatechin gallate, and quercetin glycosides were detected, many of which are well-known for their antioxidant and therapeutic potential. Most of the identified compounds are consistent with those found on earlier works on *P. maritimum* NADES extract (Rukavina et al., 2021a) and acetone extract (Rodrigues et al., 2019a). These identified compounds are known for their diverse bioactivities, particularly antioxidant (Rukavina et al., 2021b), anti-inflammatory (Sun et al., 2024) and antimicrobial properties (Manso et al., 2022) may contribute to the biological activities observed in previous assays. Representative chromatograms are presented alongside the list of annotated compounds to provide insight into the chemical diversity of the extracts.

The extraction efficiency varied significantly across different solvents, which is influenced by their polarity and ability to solvate compounds. Relative peak areas were used as a proxy to determine the efficiency and selectivity of each solvent system toward extracting different classes of bioactive compounds.

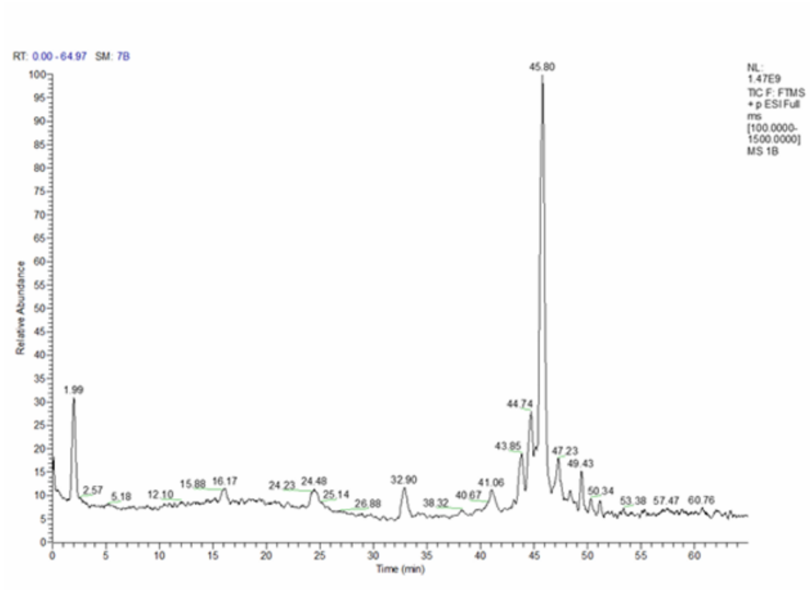
3.5.1. Phytochemical profile of the ethanol extract

Among all solvents evaluated, the ethanol extract (**Table 3.3)** showed the highest extraction performance, yielding the highest number of identified phytochemicals. For most compounds, ethanol extracted approximately 5 to 10 times more than other solvents, indicating its broad polarity compatibility and high solvation power. Importantly, ethanol was the only solvent able to extract both Procyanidin B isomer 1 and isomer 2, with isomer 1 detected at 4.5 times the peak area of isomer 2 highlighting its ability to solubilise larger, dimeric flavan-3-ols. Additionally, only ethanol and EqS 9.5 were capable of extracting p-coumaric acid, with EqS 9.5 extracting approximately 50% of the amount extracted by ethanol. The chromatogram is shown in **figure 3.2**

Table 3.3: Phytochemical profile of the ethanol extract of *Polygonum maritimum* by LC-MS

Chemical class	Chemical Compound	M/Z	Rt	Peak area
Phenolic Acids and Derivatives	Gallic acid	191.05	2.12	111309
	Galloylhexose	331.0	3.82	138229
	Galloylshikimic acid	325.05	12.55	20484
	Digalloylhexose	483.07	17.28	4616
	Caffeic acid-*O*-hexoside	341.08	17.12	20022
	Caffeic acid-*O*-sulfate	258.99	20.09	122471
	p-Coumaric acid	163.03	20.80	8299
	trans-4-*O*-Glucosyl-4-hydroxycinnamate	325.09	16.86	50461
	5-*O*-(4-Coumaroyl) quinic acid	337.09	19.98	42103
	5-*O*-(4-Coumaroyl) quinic acid cis isomer	337.09	21.45	14974
	2-Glucopyranosyloxy-3-phenylpropanoic acid	327.10	18.55	10030
Flavonoids (Flavanols, Flavonols, and Procyanidins)	Gallocatechin	305.06	10.75	126322
	Epigallocatechin	305.06	16.1	54485
	Catechin	289.07	16.05	435384
	(Epi)gallocatechin-(epi)catechin gallate	745.14	17.58	29343
	Procyanidin B isomer 1	577.13	14.86	27327
	Procyanidin B isomer 2	577.13	19.10	5974
	Procyanidin B gallate	729.14	18.97	13610
	Prodelphinidin B gallate	761.13	15.28	39646
	Epicatechin	289.07	19.14	35174
	Epicatechin-3-*O*-gallate	441.08	21.74	94994

	Epigallocatechin-3-O-gallate	457.07	19.21	185061
	Pentahydroxyflavanone-O-hexoside	465.10	21.94	29018
	Myricetin-O-galloylhexoside	631.09	22.20	6995
	Myricetin-3-O-glucoside (Isomyricitrin)	479.08	23.21	73075
	Quercetin-O-galloylhexoside	615.09	23.93	12514
	Myricitrin (Myricetin-3-O-rhamnoside)	463.08	24.46	1723132
	Isoquercitrin (Quercetin-3-O-glucoside)	463.08	25.19	420071
	Quercitrin (Quercetin-3-O-rhamnoside)	447.09	26.89	121383
Organic acids and other compounds	Quinic acid	191.05	2.12	111309
	Shikimic acid	173.04	2.27	163016
	Malic acid	133.01	2.49	47943
	Citric acid	191.01	2.64	7461
	Azelaamic acid (9-Amino-9-oxononanoic acid)	186.11	20.86	55006
	Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	327.21	33.64	19347
	Pinellic acid (9,12,13-Trihydroxy-10E-octadecenoic acid)	329.23280	34.88	9435



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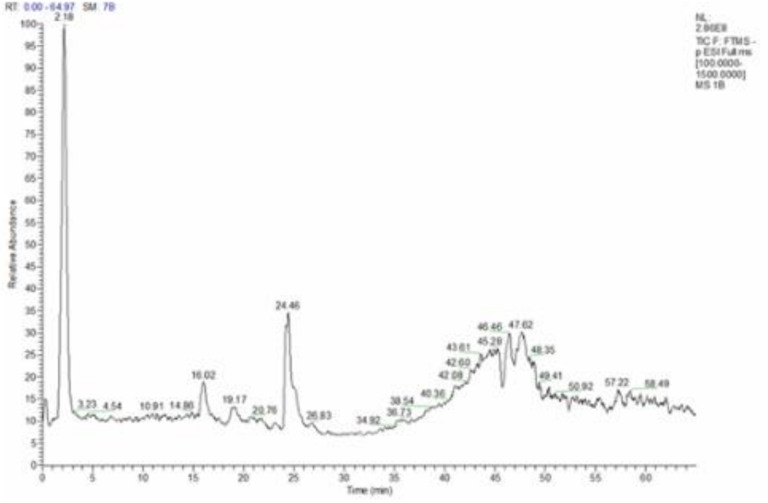

Figure 3.2: Ethanol extract chromatogram

3.5.2. Phytochemical Profile of the NADES

The extraction efficiency of NADES varied significantly based on the specific composition of hydrogen bond donors and acceptors (**Table 3.4**). For caffeic acid, the combination of choline chloride and lactic acid (CC:LA) (the chromatogram is shown in **figure 3.3**), proved to be the most effective, extracting 6.5 times more than the glycerol and urea (Gly: Urea) mixture, and 8.7 times more than the combination of choline chloride and malic acid (CC:MA) (Table 3.4). In contrast,

for gallocatechin, the Gly: Urea system outperformed the others, extracting twice as much as CC:MA and four times more than glucose and lactic acid (Glu:LA). CC:LA extracted seven times more myricetin-*O*-galloylhexoside, than Gly: Urea, and 108 times more than Glu: LA. Only Glu:LA was able to extract gallic acid as well as both isomers of 5-*O*-(4-coumaroyl) quinic acid, with the first isomer occurring at twice the abundance of the cis isomer, showing a rare ability among NADES to solubilize both free phenolic acids and their derivatives.

Table 3.4: Phytochemical profile of the Choline chloride: lactic acid extract of *Polygonum maritimum* by LC-MS

Chemical class	Chemical Compound	M/Z	Rt	Peak area
Phenolics and derivatives	Galloylhexose	331.06	4.19	4898
	Caffeic acid-*O*-sulfate	258.99	21.0	26108
	trans-4-O-Glucosyl-4-hydroxycinnamate	325.09	16.86	6464
Flavonoids (Flavanols, Flavonols, and Procyanidins)	Gallocatechin	305.06	10.75	9728
	Epigallocatechin	305.06	16.04	2816
	Catechin	289.07	16.07	35844
	Procyanidin B isomer 1	577.13	14.94	3960
	Epicatechin	289.07	19.36	35844
	Epicatechin-3-*O*-gallate	441.08	21.88	7008
	Epigallocatechin-3-*O*-gallate	457.07	19.36	11573
	Myricitrin (Myricetin-3-*O*-rhamnoside)	463.08	24.46	169220

	Isoquercitrin (Quercetin-3-*O*-glucoside)	463.08	25.22	42264
	Quercitrin (Quercetin-3-O-rhamnoside)	447.09	26.87	14754
Organic acids and other compounds	Azelaamic acid (9-Amino-9-oxononanoic acid)	186.11	20.86	74197

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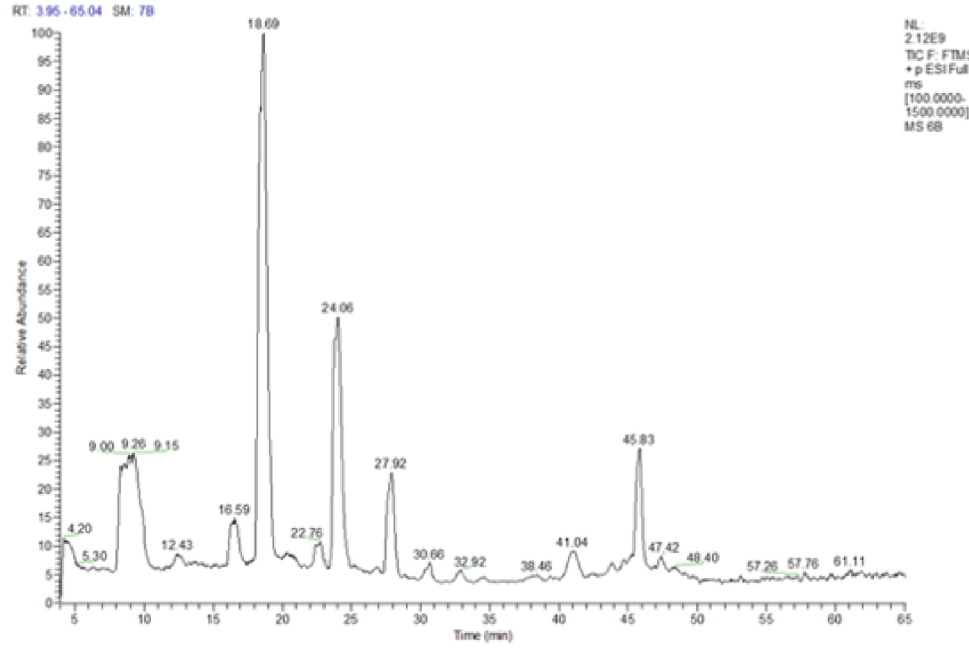

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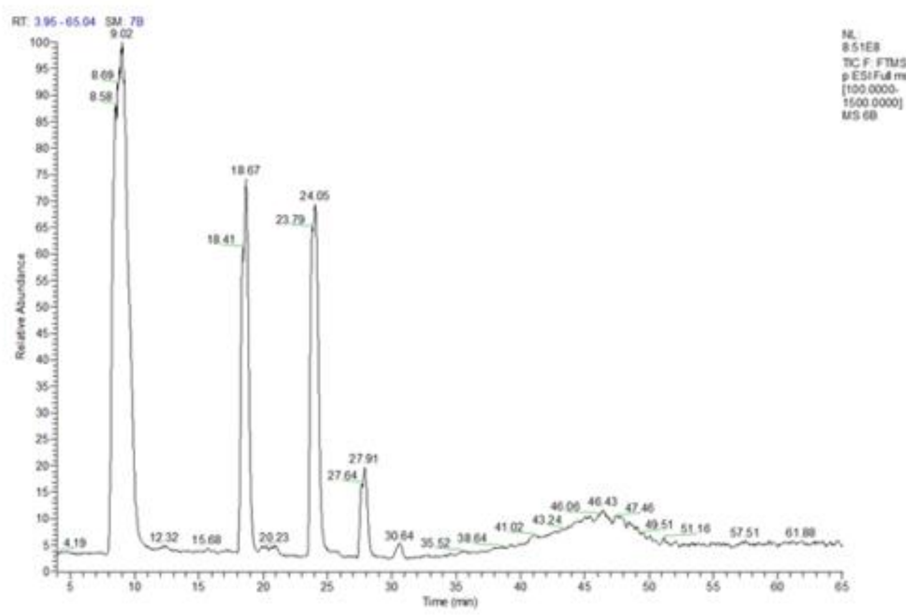


Figure 3.3: Choline chloride: lactic acid chromatogram

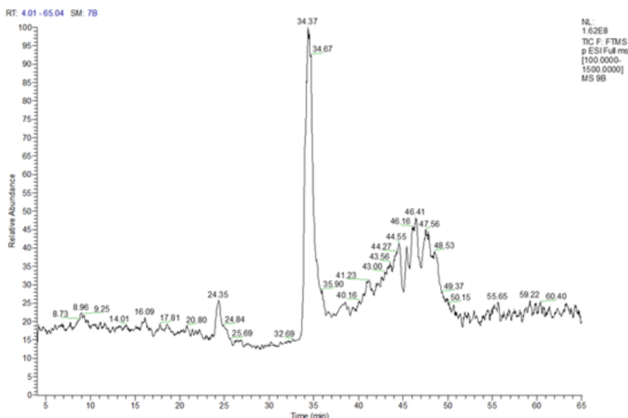
3.5.3. Phytochemical profile of SUPRAS

SUPRAS displayed a narrower extraction profile but showed selective efficiency for specific compounds: SUPRAS 36 extracted the least total number of phytochemicals amongst the 11 solvents (**Table 3.5**). It was most effective at extracting epicatechin, with levels 2–3× higher than those found in EqS 9.5, EqS 36. This suggests that despite its lower overall efficiency, SUP 9.5 has a distinct affinity for certain flavan-3-ols. EqS 9.5 demonstrated the highest extraction of gallic acid, with 3× more than EqS 36. Caffeic acid-*O*-sulfate was most abundantly extracted by EqS 9.5 (The chromatogram is shown in figure 3.4.). For isomyricitrin (myricetin-3-*O*-glucoside): EqS 9.5, SUP 36, and SUP+EqS all extracted 1.5–2× more than the NADES. However, SUPRAS 9.5 failed to extract isomyricitrin, suggesting selectivity limitations for flavonol glycosides in this system.

Table 3.5: Phytochemical profile of the SUPRAS 36 extract of *Polygonum maritimum* by LC-MS

Chemical class	Chemical Compound	M/Z	Rt	Peak area
Phenolic Acids and Derivatives	Gallic acid	191.05	4.85	14814
	Galloylhexose	331.06	4.23	3721
	Galloylshikimic acid	325.05	12.58	3251
	Caffeic acid-*O*-hexoside	341.08	17.06	4415
	Caffeic acid-*O*-sulfate	258.99	20.09	32007
	trans-4-O-Glucosyl-4-hydroxycinnamate	325.09	16.83	12729
	5-O-(4-Coumaroyl) quinic acid	337.09	19.98	9487
	5-O-(4-Coumaroyl) quinic acid cis isomer	337.09	21.45	3363
	2-Glucopyranosyloxy-3-phenylpropanoic acid	327.10	18.55	3490
Flavonoids (Flavanols, Flavonols, and Procyanidins)	Gallocatechin	305.06	10.75	16907
	Epigallocatechin	305.06	16.1	5397
	Catechin	289.07	16.05	114061
	Procyanidin B isomer 1	577.13	14.86	2013
	Epicatechin	289.07	19.14	7569
	Epicatechin-3-*O*-gallate	441.08	21.74	94994
	Epigallocatechin-3-*O*-gallate	457.07	19.21	18471
	Pentahydroxyflavanone-*O*-hexoside	465.10	21.94	7597
	Myricetin-*O*-galloylhexoside	631.09	22.20	1525
	Myricetin-3-*O*-glucoside (Isomyricitrin)	479.08	23.21	12012

	Quercetin-*O*-galloylhexoside	615.09	23.83	2484
	Myricitrin (Myricetin-3-*O*-rhamnoside)	463.08	24.35	377277
	Isoquercitrin (Quercetin-3-*O*-glucoside)	463.08	25.15	112366
	Quercitrin (Quercetin-3-O-rhamnoside)	447.09	26.89	36126
Organic acids and derivatives	Azelaamic acid (9-Amino-9-oxononanoic acid)	186.11	20.86	63736
	Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	327.21	33.64	7113
	Pinellic acid (9,12,13-Trihydroxy-10E-octadecenoic acid)	329.2328 0	34.88	6723



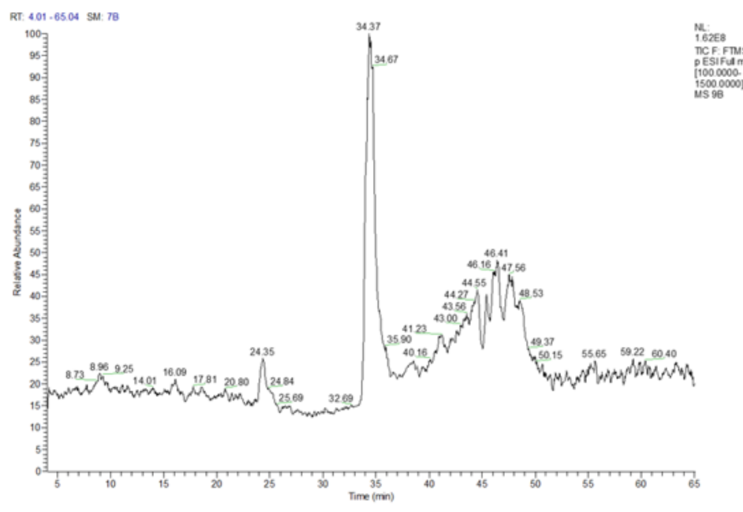


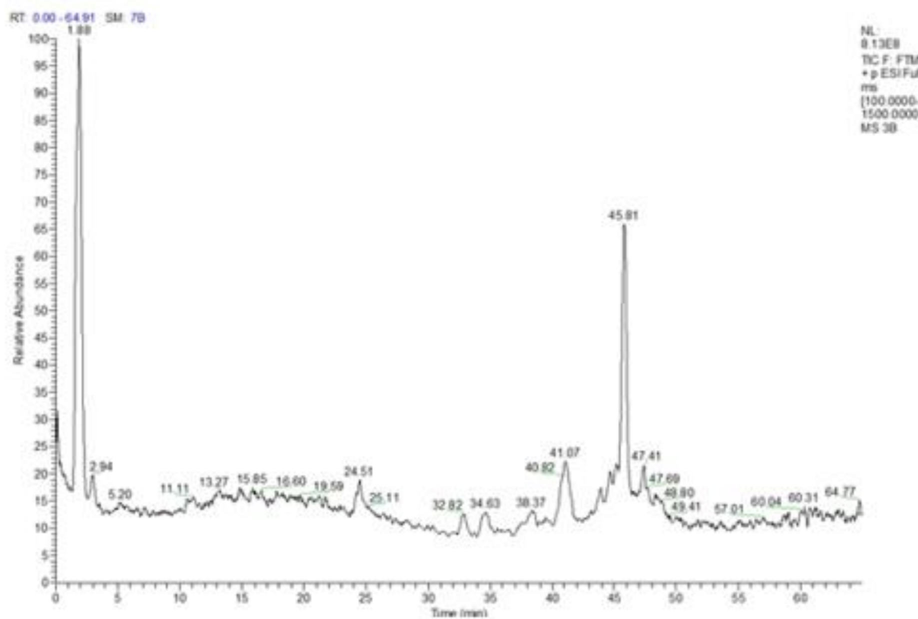
Figure 3.4: SUPRAS 36 extract chromatogram

Table 3.6: Phytochemical profile of the EqS 9.5 extract of *Polygonum maritimum* by LC-MS

Chemical class	Chemical Compound	M/Z	Rt	Peak area
Phenolic acids and derivatives	Galloylshikimic acid	325.05	12.57	18707
	Caffeic acid-*O*-hexoside	341.08	17.12	19496
	p-Coumaric acid	163.03	20.82	4581
	trans-4-O-Glucosyl-4-hydroxycinnamate	325.09	16.86	47804
	5-O-(4-Coumaroyl) quinic acid	337.09	19.90	30327
	5-O-(4-Coumaroyl) quinic acid cis isomer	337.09	21.37	9508
	Caffeic acid-o-sulfate	258.99	21.08	126280
	Digalloyhexose	483.07	17.27	3665
Flavonoids (Flavanols, Flavonols, and Procyanidins)	Gallocatechin	305.06	10.75	49872
	Epigallocatechin	305.06	16.12	13162
	Catechin	289.07	16.04	133134
	(Epi)gallocatechin-(epi)catechin gallate	745.14	17.53	31824

	Procyanidin B isomer 1	577.13	14.89	21054
	Procyanidin B gallate	729.14	18.97	14218
	Prodelphinidin B gallate	761.13	15.25	38076
	Epicatechin	289.07	19.12	35174
	Epicatechin-3-O-gallate	441.08	21.74	8809
	Epigallocatechin-3-O-gallate	457.07	19.06	103439
	Pentahydroxyflavanone-O-hexoside	465.10	21.96	17303
	Myricetin-O-galloylhexoside	631.09	22.20	5650
	Myricetin-3-O-glucoside (Isomyricitrin)	479.08	23.30	30898
	Quercetin-O-galloylhexoside	615.09	23.89	7799
	Myricitrin (Myricetin-3-O-rhamnoside)	463.08	24.49	1042033
	Isoquercitrin (Quercetin-3-O-glucoside)	463.08	25.22	209303
	Quercitrin (Quercetin-3-O-rhamnoside)	447.09	26.88	72214
Organic acids	Azelaamic acid (9-Amino-9-oxononanoic acid)	186.11	20.85	67803
	Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	327.21	33.67	4421

TIC POSITIVE



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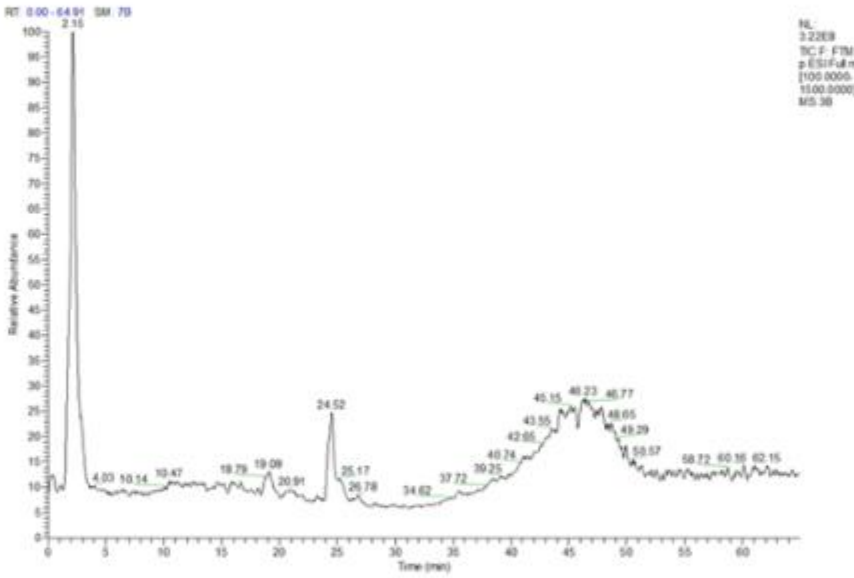

Figure 3.5: EqS chromatogram

3.6 Comparative Identification of Polyphenolic Compounds in *Polygonum maritimum*

Previous phytochemical analyses of *P. maritimum* have reported various classes of polyphenolic compounds. For instance, (Oliveira et al., 2023) identified β-type (epi)catechin dimers and trimers, along with compounds such as (+)-catechin, myricetin-O-deoxyhexoside, and quercetin-O-deoxyhexoside following ethanol extraction of the aerial parts. Similarly, Rodrigues et al., (2018), through methanolic extraction, reported the presence of epigallocatechin, catechin/epicatechin, epigallocatechin gallate, procyanidin, quercetin-3-O-glucoside, avicularin, myricitrin, and several quercetin and myricetin derivatives.

Rodrigues et al., (2019b) using acetone as the extraction solvent, identified a broad range of phenolic acids (e.g., gallic acid, caffeic acid derivatives, glucogallin), flavan-3-ols (e.g., catechin, epicatechin, gallocatechin), proanthocyanidins (e.g., dimeric procyanidins and their galloylated forms), and flavonol glycosides such as myricetin-3-O-galactoside, quercetin-galloylgalactoside, and myricitrin. Unique components such as mudanoside B, phlorizin, and polygonophenone were also detected, reflecting the solvent's capacity to extract both polar and mid-polar compounds effectively.

In the current study, based on ethanol extracts of *P. maritimum* aerial parts, several bioactive phenolic compounds were also identified, including:

- **Phenolic acids**: Gallic acid, galloylhexose, galloylshikimic acid, caffeic acid-O-hexoside, caffeic acid-O-sulfate, and trans-4-O-glucosyl-4-hydroxycinnamate;
- **Flavan-3-ols**: Gallocatechin, epigallocatechin, catechin, epicatechin, epicatechin-3-O-gallate, and epigallocatechin-3-O-gallate.
- **Procyanidins**: Procyanidin B isomer 1.
- **Flavonol glycosides**: Myricetin-O-galloylhexoside, myricetin-3-O-glucoside (isomyricitrin), quercetin-O-galloylhexoside, myricitrin, isoquercitrin, and quercitrin.
- **Other derivatives**: Pentahydroxyflavanone-O-hexoside, 5-O-(4-coumaroyl) quinic acid and its cis-isomer, and 2-glucopyranosyloxy-3-phenylpropanoic acid.

This overlap in identified compounds particularly in flavan-3-ols and flavonol glycosides reinforces the consistent presence of key bioactive constituents in *P. maritimum* across different extraction solvents and methodologies.

3.7 Effect of solvents on extraction capacity

The solvent's polarity, viscosity, and hydrogen-bonding ability directly influenced extraction efficiency and selectivity.

- Ethanol, a moderately polar organic solvent, extracted a wide range of phenolic compounds and had the highest TFC among conventional solvents.
- Highly Viscous NADES such as glycerol: Urea and Choline chloride: malic acid (Martinović et al., 2022) (Carbonell-Rozas et al., 2025) showed lower extraction efficiency, likely due to limited compound diffusion.
- SUPRAS, known for their amphiphilic nature, performed poorly in RSA despite showing intermediate TFC, likely due to inefficient extraction of the most active antioxidants as seen from the LC-MS results.

4.0 CONCLUSION

This study aimed to evaluate the efficiency of NADES and SUPRAS as green alternatives to conventional solvents (ethanol and water) for the extraction of bioactive compounds from *Polygonum maritimum*, assessing their antioxidant activity and phytochemical profiles. Among all solvents tested, ethanol consistently proved to be the most effective for broad-spectrum phytochemical extraction, yielding the highest concentrations of phenolic compounds, flavonoids, and other bioactives. It was also the only solvent capable of extracting both isomers of procyanidin B and the highest amount of *p*-coumaric acid, reflecting its strong solvating power and well-balanced polarity.

LC-MS analysis revealed a diverse array of phytochemicals, with ethanol extracts containing the highest number of identified compounds. However, NADES exhibited composition-dependent selectivity, showing strong potential for targeted recovery of specific compounds, particularly when using choline chloride: lactic acid (1:2), which demonstrated the highest antioxidant activity. SUPRAS-based systems also showed notable selectivity for certain flavonoids and phenolic acids.

Interestingly, this study provides the first indication of alkaloid-like compounds in *P. maritimum*, although their presence could not be confirmed by LC-MS, likely due to the method's optimization for phenolic detection. Future analyses using alkaloid-specific techniques are recommended.

In summary, NADES, particularly choline chloride: lactic acid and selected SUPRAS systems demonstrated extraction efficiencies that, while not surpassing ethanol in overall breadth, were comparable or superior for specific compound classes, especially antioxidants. These findings suggest that NADES and SUPRAS can, indeed, be used as effective and sustainable alternatives to ethanol and water, especially when the goal is to enrich extracts in selected bioactive targets. Their green chemistry profile further reinforces their value as safer, biodegradable, and tunable solvents for natural product extraction in pharmaceutical, nutraceutical, and cosmetic applications.

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ANNEX 1.0 - LC-MS Analysis of Bioactive Compounds in Solvent Extracts

Figure 1.0 LC-MS Analysis of *Polygonum maritimum* Extract Using CC:MA Solvent System

Name	Formula	Rt	$[M+H]^+$	$[M-H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Area/1000
Gallocatechin	C15H14O7	10.69		305.06613	219.0652	179.0333	137.0224	125.0230		8842
Procyanidin B isomer 1	C30H26O12	14.92		577.13460	425.0881	407.0763	289.0712	161.0233	125.0228	4201
Epigallocatechin	C15H14O7	16.02		305.06613	219.0654	179.0335	137.0224	125.0230		2806
Catechin	C15H14O6	16.06		289.07121	245.0821	203.0710	151.0387	125.0232	109.0280	30145
trans-4-O-Glucosyl-4-hydroxycinnamate	C15H18O8	16.86		325.09235	163.0390	119.0486				6782
Caffeic acid-O-hexoside	C15H18O9	16.99		341.08726	179.0344	135.0435	107.0487			2998
Epicatechin	C15H14O6	19.13		289.07124	245.0822	203.0709	151.0391	125.0227	109.0281	1572
Epigallocatechin-3-O-gallate (Teatannin II)	C22H18O11	19.22		457.07709	305.0631	169.0131	161.0240	125.0230		13234
Caffeic acid-O-sulfate	C9H8O7S	20.74		258.99125	179.0342	135.0438	96.9586			21933
Azelaamic acid (9-Amino-9-oxononanoic acid)	C9H17NO3	20.84		186.11302	125.0959	123.0803	97.0647			74277
Epicatechin-3-O-gallate	C22H18O10	21.84		441.08218	289.0728	245.0818	169.0132	125.0230		6662
Pentahydroxyflavanone-O-hexoside	C21H22O12	22.01		465.10331	313.0943	303.0515	151.0024			2918
Myricetin-3-O-glucoside (Isomyricitrin)	C21H20O13	23.34		479.08257	317.0309	316.0228	271.0255			5341
Myricitrin (Myricetin-3-O-rhamnoside)	C21H20O12	24.48		463.08765	317.0304	316.0226	287.0197	271.0248	178.9978	162411
Isoquercitrin (Quercetin-3-O-glucoside)	C21H20O12	25.21		463.08765	301.0358	300.0278	271.0247	178.9972	151.0029	41106
Quercitrin (Quercetin-3-O-rhamnoside)	C21H20O11	26.89		447.09274	301.0358	300.0276	271.0247	151.0029		14043

Figure 1.1 LC-MS Analysis of *Polygonum maritimum* Extract Using GLU:LA Solvent System

Name	Formula	Rt	$[M+H]^+$	$[M-H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Area/1000
Galloylhexose	C13H16O10	4.18		331.06653	271.0466	211.0242	169.0132	151.0025	101.0229	8633
Gallic acid (3,4,5-Trihydroxybenzoic acid)	C7H6O5	4.82		169.01370	125.0231	97.0281	69.0325			26813
Lactic acid lactate (Lactic acid dimer)	C6H10O5	8.86		161.04500	89.0228	71.0122				60806176
Gallocatechin	C15H14O7	10.68		305.06613	219.0655	179.0338	137.0224	125.0229		4432
Procyanidin B isomer 1	C30H26O12	14.86		577.13460	425.0879	407.0752	289.0724	161.0231	125.0229	1499
Epigallocatechin	C15H14O7	16.01		305.06613	219.0650	179.0331	137.0221	125.0227		1182
Catechin	C15H14O6	16.05		289.07121	245.0819	203.0708	151.0388	125.0231	109.0280	13855
trans-4-O-Glucosyl-4-hydroxycinnamate	C15H18O8	16.83		325.09235	163.0389	119.0486				9615
Caffeic acid-O-hexoside	C15H18O9	17.05		341.08726	179.0341	135.0440	107.0483			4012
Lactic acid lactate - anhydroglucose adduct	C9H14O7	18.57		233.06613	161.0445	89.0229				6872090
Epicatechin	C15H14O6	19.18		289.07126	245.0823	203.0709	151.0393	125.0228	109.0281	330
Epigallocatechin-3-O-gallate (Teatannin II)	C22H18O11	19.31		457.07709	305.0647	169.0132	161.0237	125.0230		12912
5-O-(4-Coumaroyl)quinic acid	C16H18O8	19.98		337.09235	191.0557	173.0443	163.0386	93.0326		6987
Azelaamic acid (9-Amino-9-oxononanoic acid)	C9H17NO3	20.85		186.11302	125.0959	123.0798	97.0644			51359
Caffeic acid-O-sulfate	C9H8O7S	20.97		258.99125	179.0341	135.0438	96.9587			26042
5-O-(4-Coumaroyl)quinic acid cis isomer	C16H18O8	21.40		337.09235	191.0555	173.0444	163.0388	93.0332		2766
Epicatechin-3-O-gallate	C22H18O10	21.75		441.08218	289.0718	245.0817	169.0133	125.0232		8109
Pentahydroxyflavanone-O-hexoside	C21H22O12	21.95		465.10331	313.0931	303.0506	151.0025			5382
Myricetin-3-O-glucoside (Isomyricitrin)	C21H20O13	23.30		479.08257	317.0317	316.0223	271.0255			9095
Lactic acid tetramer	C12H18O9	24.02		305.08726	161.0444	89.0229				12794738
Myricitrin (Myricetin-3-O-rhamnoside)	C21H20O12	24.48		463.08765	317.0303	316.0225	287.0203	271.0241	178.9979	280597
Isoquercitrin (Quercetin-3-O-glucoside)	C21H20O12	25.12		463.08765	301.0355	300.0277	271.0239	178.9975	151.0021	64255
Quercitrin (Quercetin-3-O-rhamnoside)	C21H20O11	26.90		447.09274	301.0356	300.0277	271.0250	151.0022		25229
Unidentified lactic acid derivative	C18H22O13	27.84		445.09822	301.0547	229.0327	183.0267	161.0444	89.0229	397754
Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	C18H32O5	33.69		327.21715	291.1964	239.1282	229.1439	211.1334	171.1022	3728

Figure 1.2 LC-MS Analysis of *Polygonum maritimum* Extract Using Gly:urea Solvent System

Name	Formula	Rt	$[M+H]^+$	$[M-H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Area/1000
Galloylhexose	C13H16O10	4.20		331.06653	271.0463	211.0241	169.0134	151.0021	101.0230	5482
Gallocatechin	C15H14O7	10.48		305.06613	219.0659	179.0356	137.0231	125.0230		17459
Procyanidin B isomer 1	C30H26O12	14.89		577.13460	425.0885	407.0783	289.0713	161.0226	125.0229	4667
Epigallocatechin	C15H14O7	16.06		305.06613	219.0654	179.0338	137.0226	125.0230		5015
Catechin	C15H14O6	16.09		289.07121	245.0817	203.0709	151.0386	125.0230	109.0282	65261
trans-4-O-Glucosyl-4-hydroxycinnamate	C15H18O8	16.83		325.09235	163.0391	119.0490				9854
Caffeic acid-O-hexoside	C15H18O9	17.12		341.08726	179.0343	135.0437	107.0486			3993
Epicatechin	C15H14O6	19.15		289.07124	245.0814	203.0705	151.0384	125.0228	109.0281	4892
Epigallocatechin-3-O-gallate (Teatannin II)	C22H18O11	19.31		457.07709	305.0667	169.0132	161.0235	125.0232		33467
5-O-(p-Coumaroyl)quinic acid	C16H18O8	20.12		337.09235	191.0555	173.0455	93.0332			6074
Azelaamic acid (9-Amino-9-oxononanoic acid)	C9H17NO3	20.60		186.11302	125.0959	123.0799	97.0643			65631
Caffeic acid-O-sulfate	C9H8O7S	20.77		258.99125	179.0342	135.0438	96.9586			29221
Epicatechin-3-O-gallate	C22H18O10	21.90		441.08218	289.0708	245.0804	169.0132	125.0231		19006
Pentahydroxyflavanone-O-hexoside	C21H22O12	21.93		465.10331	313.0924	303.0513	151.0024			4387
Myricetin-3-O-glucoside (Isomyricitrin)	C21H20O13	23.35		479.08257	317.0316	316.0226	271.0255			5568
Myricitrin (Myricetin-3-O-rhamnoside)	C21H20O12	24.49		463.08765	317.0305	316.0226	287.0204	271.0251	178.9977	242970
Isoquercitrin (Quercetin-3-O-glucoside)	C21H20O12	25.19		463.08765	301.0357	300.0279	271.0248	178.9979	151.0021	58792

Figure 1.3 LC-MS Analysis of *Polygonum maritimum* Extract Using Water Solvent System

Name	Formula	Rt	$[M + H]^+$	$[M - H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Area/1000
Quinic acid	C7H12O6	2.15		191.05557	173.0442	127.0388	111.0435	93.0333	90655
Shikimic acid	C7H10O5	2.30		173.04500	155.0339	137.0232	111.0438	93.0331	76475
Malic acid	C4H6O5	2.33		133.01370	115.0022	89.0229	87.0073	72.9915	47292
Citric acid	C6H8O7	3.08		191.01918	173.0084	111.0074	87.0072	85.0280	103878
Galloylhexose	C13H16O10	3.93		331.06653	271.0463	211.0241	169.0130	151.0026	14508
Gallic acid (3,4,5-Trihydroxybenzoic acid)	C7H6O5	4.84		169.01370	125.0230	97.0280	69.0330		133364
Gallocatechin	C15H14O7	10.81		305.06613	219.0655	179.0342	137.0233	125.0231	23362
Galloylshikimic acid	C14H14O9	12.58		325.05596	169.0134	155.0335	137.0236	125.0231	6160
Procyanidin B isomer 1	C30H26O12	14.93		577.13460	425.0882	407.0793	289.0723	161.0233	6586
Prodelphinidin B gallate	C37H30O18	15.32		761.13539	423.0722	305.0674	177.0184	125.1230	5806
Catechin	C15H14O6	16.07		289.07121	245.0819	203.0707	151.0388	125.0231	81280
Epigallocatechin	C15H14O7	16.07		305.06613	219.0649	179.0332	137.0221	125.0227	6160
trans-4-O-Glucosyl-4-hydroxycinnamate	C15H18O8	16.85		325.09235	163.0389	119.0488			12466
Caffeic acid-O-hexoside	C15H18O9	17.11		341.08726	179.0342	135.0439	107.0487		5390
Digalloylhexose	C20H20O14	17.27		483.07749	313.0555	271.0460	169.0127	125.0230	724
2-Glucopyranosyloxy-3-phenylpropanoic acid	C15H20O8	18.53		327.10799	165.0545	147.0438			3129
Epicatechin	C15H14O6	19.15		289.07124	245.0812	203.0703	151.0388	125.0227	3524
Epigallocatechin-3-O-gallate (Teatannin II)	C22H18O11	19.18		457.07709	305.0680	169.0133	161.0247	125.0231	19567
5-O-(4-Coumaroyl)quinic acid	C16H18O8	19.99		337.09235	191.0552	173.0439	163.0395	93.0333	7332
Azelaamic acid (9-Amino-9-oxononanoic acid)	C9H17NO3	20.87		186.11302	125.0959	123.0803	97.0647		51747
Caffeic acid-O-sulfate	C9H8O7S	21.07		258.99125	179.0342	135.0440	96.9589		46856
5-O-(4-Coumaroyl)quinic acid cis isomer	C16H18O8	21.39		337.09235	191.0555	173.0441	163.0387	93.0330	2832
Epicatechin-3-O-gallate	C22H18O10	21.82		441.08218	289.0721	245.0810	169.0134	125.0232	9276
Pentahydroxyflavanone-O-hexoside	C21H22O12	21.98		465.10331	313.0937	303.0512	151.0023		3599
Myricetin-3-O-glucoside (Isomyricitrin)	C21H20O13	23.32		479.08257	317.0296	316.0224	271.0255		4314
Myricitrin (Myricetin-3-O-rhamnoside)	C21H20O12	24.46		463.08765	317.0306	316.0226	287.0211	271.0255	208555
Isoquercitrin (Quercetin-3-O-glucoside)	C21H20O12	25.20		463.08765	301.0356	300.0279	271.0253	178.9974	51072
Quercitrin (Quercetin-3-O-rhamnoside)	C21H20O11	26.87		447.09274	301.0357	300.0277	271.0245	151.0027	15273
Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	C18H32O5	33.70		327.21715	291.1978	239.1285	229.1440	211.1341	3762
Pinellic acid (9,12,13-Trihydroxy-10E-octadecenoic acid)	C18H34O5	34.91		329.23280	311.2235	229.1436	211.1331	99.0800	1909

Figure 1.4 LC-MS Analysis of *Polygonum maritimum* Extract Using EqS 9.5 Solvent System

Name	Formula	Rt	$[M + H]^+$	$[M - H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Area/1000
Quinic acid	C7H12O6	2.21		191.05557	173.0441	127.0390	111.0442	93.0331	85.0280	211047
Shikimic acid	C7H10O5	2.36		173.04500	155.0338	137.0231	111.0438	93.0330	73.0280	112829
Malic acid	C4H6O5	2.55		133.01370	115.0023	89.0229	87.0074	72.9916	71.0123	53397
Citric acid	C6H8O7	2.99		191.01918	173.0077	111.0074	87.0072	85.0280		454807
Galloylhexose	C13H16O10	3.98		331.06653	271.0466	211.0243	169.0132	151.0025	101.0231	97676
Gallic acid (3,4,5-Trihydroxybenzoic acid)	C7H6O5	4.79		169.01370	125.0231	97.0279	69.0329			94361
Gallocatechin	C15H14O7	10.77		305.06613	219.0662	179.0343	137.0229	125.0231		49872
Galloylshikimic acid	C14H14O9	12.57		325.05596	169.0132	155.0340	137.0225	125.0230	111.0438	18707
Procyanidin B isomer 1	C30H26O12	14.89		577.13460	425.0875	407.0777	289.0723	161.0231	125.0231	21054
Prodelphinidin B gallate	C37H30O18	15.25		761.13539	423.0726	305.0668	177.0184	125.1231		38076
Epigallocatechin	C15H14O7	15.99		305.06613	219.0658	179.0341	137.0230	125.0230		13162
Catechin	C15H14O6	16.04		289.07121	245.0817	203.0709	151.0389	125.0231	109.0281	133134
trans-4-O-Glucosyl-4-hydroxycinnamate	C15H18O8	16.81		325.09235	163.0390	119.0489				47804
Caffeic acid-O-hexoside	C15H18O9	17.10		341.08726	179.0342	135.0440	107.0483			19496
Digalloylhexose	C20H20O14	17.27		483.07749	313.0555	271.0458	169.0132	125.0230		3665
(Epi)gallocatechin-(epi)catechin gallate	C37H30O17	17.53		745.14047	423.0727	407.0769	289.0716	177.0182	125.0230	31824
2-Glucopyranosyloxy-3-phenylpropanoic acid	C15H20O8	18.57		327.10799	165.0546	147.0440				10782
Procyanidin B gallate	C37H30O16	18.93		729.14556	407.0763	289.0717	169.0130	161.0224	125.0230	14218
Epigallocatechin-3-O-gallate (Teatannin II)	C22H18O11	19.06		457.07709	305.0667	169.0132	161.0234	125.0231		103439
Epicatechin	C15H14O6	19.12		289.07124	245.0814	203.0708	151.0387	125.0231	109.0279	8809
5-O-(4-Coumaroyl)quinic acid	C16H18O8	19.90		337.09235	191.0555	173.0445	163.0391	93.0331		30327
p-Coumaric acid	C9H8O3	20.82		163.03952	119.0489	93.0331				4581
Azelaamic acid (9-Amino-9-oxononanoic acid)	C9H17NO3	20.85		186.11302	125.0959	123.0798	97.0646			67803
Caffeic acid-O-sulfate	C9H8O7S	21.08		258.99125	179.0341	135.0440	96.9588			126280
5-O-(4-Coumaroyl)quinic acid cis isomer	C16H18O8	21.37		337.09235	191.0555	173.0446	163.0391	93.0333		9508
Epicatechin-3-O-gallate	C22H18O10	21.76		441.08218	289.0721	245.0818	169.0132	125.0231		44347
Pentahydroxyflavanone-O-hexoside	C21H22O12	21.96		465.10331	313.0932	303.0519	151.0025			17303
Myricetin-O-galloylhexoside	C28H24O17	22.19		631.09352	479.0828	317.0295	316.0231			5650
Myricetin-3-O-glucoside (Isomyricitrin)	C21H20O13	23.30		479.08257	317.0307	316.0228	271.0234			30898
Quercetin-O-galloylhexoside	C28H24O16	23.89		615.09861	463.0863	301.0368	300.0277			7799
Myricitrin (Myricetin-3-O-rhamnoside)	C21H20O12	24.49		463.08765	317.0305	316.0225	287.0206	271.0252	178.9978	1042033
Isoquercitrin (Quercetin-3-O-glucoside)	C21H20O12	25.22		463.08765	301.0356	300.0278	271.0247	178.9973	151.0020	209303
Quercitrin (Quercetin-3-O-rhamnoside)	C21H20O11	26.88		447.09274	301.0357	300.0279	271.0241	151.0021		72214
Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	C18H32O5	33.67		327.21715	291.1969	239.1292	229.1442	211.1335	171.1015	4421
Caprylic acid (Octanoic acid)	C8H16O2	34.68		143.10721						19363

Figure 1.5 LC-MS Analysis of *Polygonum maritimum* Extract Using SUPRAS 9.5 Solvent System

Name	Formula	Rt	$[M+H]^+$	$[M-H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Area/1000
Gallic acid (3,4,5-Trihydroxybenzoic acid)	C7H6O5	4.87		169.01370	125.0231	97.0282	69.0325			10917
Catechin	C15H14O6	16.00		289.07121	245.0816	203.0708	151.0385	125.0230	109.0281	26237
trans-4-O-Glucosyl-4-hydroxycinnamate	C15H18O8	16.85		325.09235	163.0392	119.0488				5731
2-Glucopyranosyloxy-3-phenylpropanoic acid	C15H20O8	18.48		327.10799	165.0542	147.0434				1192
Epicatechin	C15H14O6	19.04		289.07124	245.0815	203.0706	151.0390	125.0233	109.0281	26237
Azelaamic acid (9-Amino-9-oxononanoic acid)	C9H17NO3	20.83		186.11302	125.0958	123.0797	97.0641			4734
Caffeic acid-O-sulfate	C9H8O7S	21.06		258.99125	179.0343	135.0429	96.9587			4071
Myricitrin (Myricetin-3-O-rhamnoside)	C21H20O12	24.33		463.08765	317.0305	316.0226	287.0200	271.0253	178.9982	135051
Isoquercitrin (Quercetin-3-O-glucoside)	C21H20O12	25.02		463.08765	301.0356	300.0278	271.0248	178.9976	151.0020	57124
Quercitrin (Quercetin-3-O-rhamnoside)	C21H20O11	26.76		447.09274	301.0359	300.0278	271.0232	151.0022		28485
Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	C18H32O5	33.46		327.21715	291.1962	239.1283	229.1437	211.1334	171.1011	7773
Caprylic acid (Octanoic acid)	C8H16O2	34.23		143.10721						9056634
Ethyl caprylate (Ethyl octanoate)	C10H20O2	39.86	173.15415		145.1224	127.1120	103.0758	89.0602	57.0706	3518653

Figure 1.6 LC-MS Analysis of *Polygonum maritimum* Extract Using SUPRAS + EqS Solvent System

Name	Formula	Rt	$[M+H]^+$	$[M-H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Area/1000
Galloylhexose	C13H16O10	4.23		331.06653	271.0462	211.0242	169.0132	151.0022	101.0230	10388
Gallic acid (3,4,5-Trihydroxybenzoic acid)	C7H6O5	4.79		169.01370	125.0231	97.0282	69.0326			28283
Gallocatechin	C15H14O7	10.75		305.06613	219.0657	179.0346	137.0234	125.0231		40304
Galloylshikimic acid	C14H14O9	12.58		325.05596	169.0135	155.0335	137.0234	125.0229	111.0437	4837
Procyanidin B isomer 1	C30H26O12	14.89		577.13460	425.0882	407.0768	289.0723	161.0230	125.0230	10222
Prodelphinidin B gallate	C37H30O18	15.25		761.13539	423.0719	305.0664	177.0180	125.1229		14649
Epigallocatechin	C15H14O7	16.03		305.06613	219.0659	179.0342	137.0235	125.0230		14464
Catechin	C15H14O6	16.06		289.07121	245.0817	203.0707	151.0389	125.0231	109.0281	142413
trans-4-O-Glucosyl-4-hydroxycinnamate	C15H18O8	16.84		325.09235	163.0390	119.0489				14820
Caffeic acid-O-hexoside	C15H18O9	17.07		341.08726	179.0343	135.0442	107.0483			6328
Digalloylhexose	C20H20O14	17.29		483.07749	313.0554	271.0460	169.0123	125.0229		1130
(Epi)gallocatechin-(epi)catechin gallate	C37H30O17	17.52		745.14047	423.0759	407.0772	289.0723	177.0180	125.0230	10833
2-Glucopyranosyloxy-3-phenylpropanoic acid	C15H20O8	18.52		327.10799	165.0542	147.0438				3504
Procyanidin B gallate	C37H30O16	18.94		729.14556	407.0764	289.0725	169.0127	161.0220	125.0228	5184
Epicatechin	C15H14O6	19.14		289.07124	245.0820	203.0707	151.0393	125.0237	109.0281	9594
Epigallocatechin-3-O-gallate (Teatannin II)	C22H18O11	19.24		457.07709	305.0670	169.0132	161.0231	125.0232		44217
5-O-(4-Coumaroyl)quinic acid	C16H18O8	19.99		337.09235	191.0553	173.0443	163.0385	93.0330		9280
Azelaamic acid (9-Amino-9-oxononanoic acid)	C9H17NO3	20.86		186.11302	125.0956	123.0796	97.0642			3512
Caffeic acid-O-sulfate	C9H8O7S	21.09		258.99125	179.0342	135.0439	96.9586			59613
5-O-(4-Coumaroyl)quinic acid cis isomer	C16H18O8	21.35		337.09235	191.0557	173.0445	163.0392	93.0332		3856
Epicatechin-3-O-gallate	C22H18O10	21.75		441.08218	289.0723	245.0811	169.0132	125.0229		18225
Pentahydroxyflavanone-O-hexoside	C21H22O12	21.81		465.10331	313.0944	303.0519	151.0025			8070
Myricetin-3-O-glucoside (Isomyricitrin)	C21H20O13	23.25		479.08257	317.0300	316.0227	271.0255			11653
Myricitrin (Myricetin-3-O-rhamnoside)	C21H20O12	24.40		463.08765	317.0304	316.0225	287.0202	271.0255	178.9978	414641
Isoquercitrin (Quercetin-3-O-glucoside)	C21H20O12	25.18		463.08765	301.0357	300.0278	271.0246	178.9975	151.0024	111950
Quercitrin (Quercetin-3-O-rhamnoside)	C21H20O11	26.77		447.09274	301.0358	300.0277	271.0251	151.0022		39095
Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	C18H32O5	33.50		327.21715	291.1962	239.1284	229.1439	211.1327	171.1009	6221
Caprylic acid (Octanoic acid)	C8H16O2	34.48		143.10721						5014554
Ethyl caprylate (Ethyl octanoate)	C10H20O2	39.94	173.15415		145.1223	127.1114	103.0758	89.0602	57.0706	11612254