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“Evaluation of the plant species Portulaca oleracea L. as raw material for cosmetic products”

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Portulaca oleracea L. is a warm-climate, herbaceous, succulent, annual plant, belonging to the Portulacaceae family. It is commonly known as purslane and is distributed worldwide. Purslane is drawing increased interest from the cosmetic industry due to the high amounts of omega-3 fatty acids and cyclo-dopa alkaloids, known as “oleraceins”. Furthermore, it contains a variety of secondary metabolites, such as flavonoids, terpenoids, polysaccharides, vitamins, sterols, proteins, and minerals. This study aimed to evaluate the phytochemical content and the antioxidant properties of leaves and stems after two drying methods, oven and freeze-drying. Plant samples were collected from the region of Patras, Greece, in September of 2022. The first step was the separation of stems and leaves, and the samples were cut into small pieces to facilitate drying. Plant samples were divided into two groups and dried using two different drying methods: freeze and oven drying (50°C). The dried samples were ground, and ultrasound-assisted extraction was conducted in the presence of n-hexane followed by 70% (aq) methanol. The hydroalcoholic extracts were analyzed with ultra-high performance liquid chromatography–diode array detector–tandem mass spectrometry (UHPLC-DAD-MS), providing data mainly for the alkaloids, a major class of chemicals of the plant, and the glycolipids; while through HPLC-DAD analysis, a total of 7 alkaloids were quantified. A direct transesterification method was used for the analysis of fatty acids. The identification was carried out using GC-FID. The results show a rich content of fatty acids, mainly in the leaves, and the presence of two major omega-3 fatty acids, namely, α-linolenic acid and linoleic acid. The analysis of the volatile compounds was conducted using GC-MS for the identification of the compounds, followed by GC-FID analysis for their quantification. Overall, the antioxidant activity of the hydroalcoholic extracts was measured using DPPH and FRAP assays, proving the higher antioxidant activity of the leaves in comparison with the stems. The results were confirmed by the measurement of the total phenolic content (TPC) and the total sugar content in the extracts.

Key words: Portulaca oleracea L., purslane, alkaloids, fatty acids, volatile compounds, antioxidant activity.
ΠΕΡΙΛΗΨΗ

Το είδος Portulaca oleracea L. είναι ένα ξεχωριστό κλίματος, ποώδες, χυμώδες ετήσιο φυτό, που ανήκει στην οικογένεια Portulacaceae. Είναι κοινώς γνωστό ως γλιστρίδα ή αντράκλα και συναντάται ευρέως σε αφθονία. Η γλιστρίδα προσελκύει αυξανόμενο ενδιαφέρον στη βιομηχανία καλλυντικών προϊόντων λόγω της υψηλής περιεκτικότητάς της σε ομέγα-3 λιπαρά οξέα και μιας οικογένειας αλκαλοειδών, γνωστών ως "oleraceins". Επιπλέον, περιέχει μια ποικιλία από δευτερογενείς μεταβολίτες, όπως φλαβονοειδή, τερπενοειδή, πολυσακχαρίτες, βιταμίνες, στερόλες, πρωτεΐνες και μέταλλα. Σκοπός αυτής της εργασίας ήταν η φυτοχημική ανάλυση και ο προσδιορισμός των αντιοξειδωτικών ιδιοτήτων των φύλλων και των βλαστών γλιστρίδας που είχε υποβληθεί σε δύο διαφορετικές διεργασίες ξήρανσης, σε υψηλή θερμοκρασία και σε λυοφιλοποίηση.

Το φυτικό υλικό συλλέχθηκε από την περιοχή της Πάτρας, τον Σεπτέμβριο του 2022. Αρχικά, το πρώτο στάδιο ήταν ο διαχωρισμός των βλαστών και των φύλλων, και ο τεμαχισμός τους σε μικρότερα μέρη για τη διευκόλυνση της ξήρανσής τους. Τα φύλλα και οι βλαστοί του φυτού χωρίστηκαν σε δύο μέρη και ξηράνθηκαν με δύο διαφορετικές μεθόδους, με τη χρήση λυοφιλοποιητή και σε φούρνο στους 50oC (4 μέρες για τα φύλλα και 5 μέρες για τους βλαστούς). Τα αποξηραμένα μέρη κονιορτοποιήθηκαν και ακολούθησε η εκχύλιση τους υποβοηθούμενη από υπερήχους, πρώτα με εξάνιο και στη συνέχεια με 70% (aq.) μεθανόλη.

Τα υδρομεθανολικά εκχυλίσματα αναλύθηκαν με τη χρήση HPLC-DAD-MS, από την οποία προέκυψε ταυτοποίηση κυρίως των αλκαλοειδών και των γλυκολιπιδίων, ενώ μέσω της ανάλυσης με HPLC-DAD ποσοτικοποιήθηκαν σε σύνολο 7 αλκαλοειδή. Για την ανάλυση των λιπαρών οξέων από τα εκχυλίσματα του εξανίου χρησιμοποιήθηκε η μέθοδος της άμεσης μετεστεροποίησης. Στη συνέχεια, η ταυτοποίηση και ποσοτικοποίηση πραγματοποιήθηκε με χρήση GC-FID. Τα αποτελέσματα υποδεικνύουν πλούσια περιεκτικότητα σε λιπαρά οξέα, κυρίως των φύλλων, και την παρουσία δύο κύριων ομέγα-3 λιπαρών οξέων, του α-λινολενικού οξέος και του λινολεικού οξέος. Η ανάλυση των πτητικών ενώσεων πραγματοποιήθηκε με τη χρήση GC-MS για την ταυτοποίηση των ενώσεων, και ακολούθησε η ανάλυση με GC-FID για την ποσοτικοποίηση τους.

Τέλος, η αντιοξειδωτική δράση των υδρομεθανολικών εκχυλισμάτων μετρήθηκε με δοκιμές DPPH και FRAP, αποδεικνύοντας την ισχυρότερη αντιοξειδωτική δράση των φύλλων σε σύγκριση με τους βλαστούς. Τα αποτελέσματα επιβεβαιώθηκαν και με μέτρηση των ολικών φαίνολικών ενώσεων (TPC) και των ολικών σακχάρων στα εκχυλίσματα.

Λέξεις κλειδιά: Portulaca oleracea L., γλιστρίδα, αλκαλοειδή, λιπαρά οξέα, πτητικές ενώσεις, αντιοξειδωτική δράση
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CHAPTER 1

1 INTRODUCTION
1.1 Introductory information

Purslane is a plant species that is widely and abundantly found, especially in tropical and subtropical climates. Its consumption is quite common in both Mediterranean countries and tropical Asian regions. Its introduction into the diets of consumers is directly linked to the high content of omega-3 fatty acids and its antioxidant properties (Petropoulos et al., 2019).

It has a wide range of biological properties, including antibacterial, antiviral, anti-inflammatory, antioxidant, and wound-healing activity. Because of all the plant's valuable beneficial properties, the World Health Organization has listed purslane as one of the most used medicinal plants and has awarded it the term "World Panacea". In their tradition, the Chinese called it "the vegetable of longevity" and for thousands of years used it as a "medicine" for various ailments (Petropoulos et al., 2019; Zhou et al., 2015).

The genotype and cultivation practices, such as planting season, fertilization use, and harvest period, determine the content of macronutrients, phenolic compounds, and organic acids contained in different parts of the plant (Petropoulos et al., 2019).

1.2 Drying process

Drying is a common preservation method for food and the final quality of the products depends largely on the method. The reduction of water through the removal of moisture leads to a reduction in weight and volume, while allowing the plant to retain its nutrient content without degradation (Nowak & Jakubczyk, 2020).

In this thesis, two different drying techniques were used, freeze drying and oven drying (hot air drying).

1.2.1 Freeze drying or cryopreservation

Freeze drying is a process in which water is evaporated by the direct transition of water from a solid (ice) to a gaseous (vapour) phase, thus omitting the liquid state and then leaching water from the "dry" layer. During the process, the quality of the dried product (biological, nutritional, and organoleptic properties) is maintained. This is because freezing the water in the material before freeze-drying inhibits chemical, biochemical, and microbiological processes. Therefore, the taste, aroma, and the content of various nutrients are not greatly altered.

Purslane has a high water content, ranging from 80% to 95%, so the use of freeze drying as a drying method would contribute to faster and more efficient drying of the dew. It is considered a particularly suitable drying method to protect the volatile compounds contained in plant
materials, it protects the color, chlorophylls, and the structure of the material from degradation. However, the protection it provides in terms of bioactive compounds depends primarily on the plant material in question (Thamkaew, Sjöholm, & Galindo, 2021a).

Drying using this method consists of two stages:

1) Primary drying: sublimation of ice under reduced pressure
2) Secondary drying: desiccation and drying to the desired final moisture content

Freeze drying is widely considered to be the best drying method. However, inappropriately selected process parameters can cause adverse changes in the material, such as shrinkage, color change, and degradation (Nowak & Jakubczyk, 2020).

![Figure 1: Schematic illustration of how a freeze dryer works](image-url)

A freeze dryer consists of four individual components:

- the drying chamber
- the vacuum pump
- a heat source
- the condenser.

The material to be dried is placed in the drying chamber, either already frozen or cooled in the chamber if the system has a cooling system. In addition, the shelves within the chamber are temperature controlled and the chamber is designed to be tightly sealed to achieve very low pressures. The vacuum pump is responsible for achieving a low pressure, ideally below 0.61 kPa, within the chamber and for removing uncondensed gases from the chamber. The heat source
contributes to the evaporation of moisture from the product under reduced pressure conditions with values ranging between 243.15 K and 423.15 K. Finally, the condenser collects the water vapors released by the evaporation of the ice and should have sufficient surface area to cool the vapors produced by the evaporation process. The ice generated in the condenser is then removed from the system (Garcia-Amezquita et al., 2015).

![Figure 2: Pressure-temperature phase diagram of clean water in a closed system. (Garcia-Amezquita et al., 2015)](image)

1.2.2 Oven drying

Hot-air drying (hot-air drying or oven-drying) provides the important advantage of controlling the drying process by regulating the temperature, thus reducing the time required for drying. However, this is also a disadvantage of this technique, as an increase in temperature can cause the degradation of bioactive compounds and volatile components of the plant material. A possible solution to improve the quality of the final product is to apply low temperatures (35-50°C) to protect thermosensitive compounds. During the drying process, the flow of hot air into the chamber causes the evaporation of moisture and volatile compounds, creating an environment suitable for oxidative reactions. In addition, another disadvantage is the high energy consumption required for complete drying, as in some cases it takes several days to complete the drying process (Thamkaew et al., 2021).

As illustrated in Figure 3, an oven consists of a drying chamber, a heat source, and a fan-driven hot air transport system to the chamber. The material to be dried is placed within the chamber where the hot air flow is introduced to vaporize the water content.
1.3 The species *Portulaca oleracea* L.

*Portulaca oleracea* L. is a succulent, annual plant that thrives in the summer months. It can be found in all regions of temperate and tropical climates and is the eighth most widespread plant in the world. It grows in gardens, fields, near rivers, and in mountainous areas where the climate is warm and sunny (Srivastava, Srivastava, & Singh, 2023).

Reports indicate that purslane was a common vegetable in the Roman Empire. Its origin is uncertain, but the existence of this plant is mentioned nearly 4,000 years ago. *P. oleracea* is mentioned by Dioscorides (40–90 AD), with the name of “andrachne”. In his “De Materia Medica”, he described the plant as an astringent and a treatment for burning in the stomach, erysipelas, headaches, inflammation of the eyes and other organs, and bladder problems. The succulent stems and fleshy leaves of purslane reflect that it may have originated and adapted to the dry climates of the Middle East and India. It can also be found in Europe, Africa, North America, Australia, and Asia (Uddin et al., 2014).

The name *Portulaca* etymology derives from New Latin, from Latin, purslane, from *portula*, diminutive of *porta* gate; from the lid of its capsule (‘Merriam-Webster.com dictionary’). According to the binomial classification, the word *Portulaca* is derived from the Latin, *portula* = diminutive of porta gate, while *oleracea* = vegetable, indicating historically the use of this species as food (Srivastava et al., 2023).

The family Portulacaceae is relatively small with 21 genera and 580 species.
The systematic classification of the plant is as follows:

**Kingdom:** Plantae (Plantae)

**Kingdom (Plantata (Plantae)):** Magnoliophyta (Magnoliophyta)

**Taxonomy:** Dicotyledons (Magnoliopsida)

**Order:** Caryophyllales

**Family:** Portulacaceae

**Genus:** Portulaca

**Species:** *P. oleracea*

Historically, the first official reference to the classification of the plant that has been recorded is in a book, shown in *Figure 4*, published by Linné, C. von. (1753) and printed in Latin (Linné & Salvius, 1753).

*Figure 4: The cover of the book SPECIES PLANTARUM by Carl Linnaeus and the first report of the classification of Portulaca oleracea (Linné & Salvius, 1753).*
1.3.1 Stages of plant growth

Purslane grows parallel to the ground (creeping habit) and its stems have a characteristic red-green or purple-green color. In addition, they are cylindrical, up to 30 cm long, 2-3 mm in diameter, with swellings at the nodes, smooth, with diffuse branching, and the midribs are 1.5-3.5 cm long. The leaves are succulent, oval, 1-5 cm long, 0.5-2 cm wide, with a blunt or slightly serrated surface, without stalks or with very short stalks, about 5-30 mm long. Flowering begins from May to September. The flowers bloom singly or in clusters of two to five at the tips of the stems. The flowers are small and orange-yellow, purple, or white-pink with five petals and usually open only on warm, sunny days from mid-morning to early afternoon (Uddin et al., 2014).

![Figure 5](image)

Figure 5: A) The plant, B) different stages of development, C) the stems, D) different stages of leaf development, E) stems with capsule endings, F) capsules after flowering, G) flowers, H) the black seeds inside the capsules, I) dry seeds (Srivastava et al., 2023).

The fruits consist of almost round to egg-shaped capsules, usually about 4-8 mm long, which open around the middle to release the seeds. The seeds are numerous, tiny, less than 1 mm in diameter, circular to ovoid in shape, flattened, and brown to black with a white spot (Uddin et al., 2014). Seeds require specific conditions of heat and light to germinate. Purslane uses the C4 photosynthetic metabolism and can survive high temperatures and stress conditions. For example, in the dry and warm climate of Australia, the plant grows quite large and produces up to 10,000 seeds, whereas in contrast in Britain, the cooler and rainy climate, even in summer, results in lower seed yields (Srivastava et al., 2023).
Enzymes mediate and regulate a complex network of chemical processes throughout all organisms, including plants. They participate in both primary and secondary metabolic pathways that produce different kinds of metabolites (Massaro Malheiros Ferreira et al., n.d.). Primary metabolism produces essential compounds for the development and the growth of the plant. The primary metabolites act as the intermediate compounds in the synthesis of more complex metabolites that comprise the secondary metabolism (Massaro Malheiros Ferreira et al., n.d.). The secondary metabolites are more lineage-specific and their production is highly dependent on the biotic and abiotic conditions, thus impact on the plant’s specific characteristics, with the aim to support the plant’s growth and survival (Massaro Malheiros Ferreira et al., n.d.).

A variety of phytochemical groups such as flavonoids, alkaloids, fatty acids, terpenoids, polysaccharides, vitamins, sterols, proteins, and minerals have been identified and isolated in *Portulaca oleracea* (Zhou et al., 2015). Different varieties, harvest times, and environmental conditions can determine the nutritional benefits of the species (Uddin et al., 2014).

Purslane is characterized by the presence of a variety of secondary metabolites that are divided in three main categories: the nitrogen-containing compounds, terpenes, and phenolic compounds (Massaro Malheiros Ferreira et al., n.d.).

Nitrogen-containing compounds include alkaloids, a main class of secondary metabolites in the plant kingdom (Wink, 2015). They are natural antioxidant agents and provide protection against predators (Patel et al., 2020; Wink, 2015). In purslane, the biosynthesis of the L-dopa alkaloids is linked to the shikimic acid pathway that also produces tyrosine, a precursor compound of the cyclo-dopa alkaloids of the plant (*Figure 6*) (Montoya-Garcia et al., 2023).

Terpenes are the largest class of phytoconstituents that display multiple functions in the growth and development stages of the plant life cycle. Terpenes consist of isoprene units and can be further categorized according to the number of C5 units into monoterpens (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), tetraterpenes (C40), and polyterpenes (Wink, 2015). Monoterpenes possess aromatic properties, have strong antioxidant activities, and are part of the essential oils of the plants (Patel et al., 2020; Wink, 2015).

Phenolic compounds are a large group of plant constituents that include flavonoids, tannins, coumarins, lignans/lignins, and phenylpropanoids (Patel et al., 2020). Polyphenols are characterized by the presence of phenolic rings with attached hydroxyl groups, which provide antioxidant, anti-inflammatory, wound-healing, and a plethora of other properties. The class of flavonoids involves the flavones, isoflavones, flavanones, proanthocyanidins, anthocyanins, and chalcones (Patel et al., 2020).

Considering the plethora of secondary metabolites in *P. oleracea*, its ethnobotanical importance underlines its value for use in the food, drug, and cosmetic industries.
Figure 6: Purslane’s metabolic paths (Montoya-García et al., 2023).
The flavonoid content of purslane is largely dependent on the part of the plant, as the roots are the richest in flavonoids, followed by the stems, and finally, the leaves of the plant seem to have the lowest content. Their typical chemical structure consists of a 15-carbon skeleton with two phenyl rings and one heterocyclic ring (Li et al., 2024). The predominant flavonoids identified are kaempferol, myricetin, lutein, luteolin, apigenin, quercetin, genistein and genistein. However, in the ethanolic extracts of the plant, kaempferol and genistein are the major flavonoids, and mainly in the stems (Zhou et al., 2015). Another class of valuable flavonoids of purslane are the isoflavones, namely, Portulacanones. Interestingly, Portulacanones B-D have displayed cytotoxic activities against the human cancer cell lines SF-268, NCI-H460, and SGC-7901 (Zhou et al., 2015). Other studies revealed the presence of quercetin, apigenin, luteolin, kaempferol, isorhamnetin, kaempferol-3-O-glucoside, and rutin in both dried and fresh purslane leaves. Among them, quercetin was the most abundant flavonoid (6.02 mg/kg DW), followed by rutin (4.12 mg/kg DW) and kaempferol (1.85 mg/kg DW) (Sicari, Loizzo, Tundis, Mincione, & Pellicanò, 2018). Other novel flavonoids that were recently discovered in the species are Oleracones that belong to the subclass of homoisoflavones. Interestingly, Oleracone C can prevent the photoaging of human keratinocytes induced by ultraviolet B (UVB), by inhibiting UVB-mediated reactive oxygen species (ROS) activation (Li et al., 2024).
Figure 8: Chemical structures of purslane’s main flavonoids (Li et al., 2024).
Alkaloids are precisely defined by Pelletier as: “cyclic organic compounds containing nitrogen in a negative oxidation state which are of limited distribution among living organisms” (Coqueiro & Verpoorte, 2019). Most of them are highly biologically active, have complex cyclic structures with nitrogen, and are among the key active ingredients in traditional herbal medicine; in Greece purslane’s tea treats sore throat and earache (Iranshahy et al., 2017). Like many other members of the order Caryophyllales, *P. oleracea* contains betalains. These compounds are water-soluble pigments containing nitrogen atoms in their molecules. A subclass is betacyanins, red-purple compounds, which are found in an acylated form in purslane under the name 'Oleraceins'. Oleraceins are glycosylated indole amides consisting of a 5,6-dihydroxyindolyl-2-carboxylic acid group with N-acetylation of cinnamic acid derivatives (Voynikov et al., 2021). In studies (Voynikov Y. et al., 2019) that were conducted, the antioxidant potential of three Oleraceins - A, B and E was demonstrated. The free radical binding activities using the DPPH assay, expressed as EC$_{50}$ (μM), of Oleraceins A, B and E (8.96 ± 0.19 μM, 5.56 ± 0.11 μM and 9.87 ± 0.08 μM, respectively) were slightly lower than those of caffeic acid (4.97 ± 0.09 μM), but higher than those of ascorbic acid and α-tocopherol (11.70 ± 0.22 μM and 13.14 ± 0.11 μM, respectively).

Few studies have conducted a quantitative analysis of oleraceins. One study found that the concentration of Oleracine C in the leaves (21.2-143 mg/100 g DW) was significantly higher than the stems (3.34-15.2 mg/100 g DW), and its concentration was the highest at the early harvest stages (29 days after sowing) (S. A. Petropoulos et al., 2019). These unique alkaloids are mainly detected in the leaves of the plant and are one of the main groups of secondary metabolites that confer antibacterial, antioxidant, wound-healing, anti-inflammatory, and many other properties.

Depending on the type of substitution of the cinnamic acid derivatives, such as coumaric acid, ferulic acid, and caffeic acid, and the number of glucose moieties attached, there are various Oleraceins as shown in Figure 9.

![Figure 9: The names, prosthetic groups, and chemical structures of cyclo-dopa alkaloids from *P. oleracea* (Jiao et al., 2015).](image-url)
1.3.2.3 Fatty acids

Purslane is rich in omega-3 fatty acids, such as alpha-linolenic acid known for its role in preventing cardiovascular disease and boosting the immune system. Omega-3 fatty acids belong to the group of polyunsaturated fatty acids that are essential for human development, the prevention of numerous cardiovascular diseases, and help maintain a healthy immune system. Our body does not synthesize omega-3 fatty acids, so omega-3 fatty acids should be consumed from dietary sources. Omega-3 fatty acids contain 18 to 24 carbon atoms and have three or more double bonds within the fatty acid chain. In the plant kingdom, purslane has the highest concentration of fatty acids of any leafy vegetable, even spinach (Uddin et al., 2014). Alpha-linolenic acid is the predominant fatty acid in purslane and is a precursor compound for the biosynthesis of other omega-3 fatty acids, such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosahexaenoic acid (DPA) (L. Liu et al., 2000). Purslane contains approximately 4 mg/g fresh weight of alpha-linolenic acid (ALA) (Uddin et al., 2014).

![Chemical structures of the main fatty acids in P. oleracea](https://example.com/figure11.png)

*Figure 11: Chemical structures of the main fatty acids in P. oleracea (a. a-linolenic acid, b. linoleic acid, c. palmitoleic acid d. oleic acid e. palmitic acid) (S. A. Petropoulos et al., 2019).*

1.3.2.4 Vitamins

High levels of tocopherols, vitamin C, and certain vitamins of the B complex are contained in purslane. Considering that these nutrients are present in significant quantities, many health benefits can be harnessed by using the plant nutritionally and in other exploitable ways. Specific to purslane, alpha-tocopherol (12.2 mg/100 g of fresh leaves) is the most abundant tocopherol, while the ascorbic acid content is about 38.56 mg/100 g. Several types of B vitamins are also found in *P. oleracea*, such as B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6
(pyridoxine) and B9 (folates) (Nemzer, Al-Taher, & Abshiru, 2020). However, it is important to note that there is a wide variation in the content of these vitamins in purslane, depending on their place of origin and growing conditions, the time of harvest, and the genotype.

1.3.2.5 Carotenoids

Carotenoids are antioxidant compounds that can protect humans from disease and boost the immune system. They reduce the risk of diseases, especially certain cancers, and eye diseases (Nemzer et al., 2020).

Purslane contains a plethora of carotenoid compounds, such as alpha-carotene (0.009 mg/100 g), beta-carotene (3.5 mg/100 g), lutein (5.4 mg/100 g), and zeaxanthin (0.19 mg/100 g). The highest concentration of carotenoids appears to be in the leaves and the lowest in the stems. However, the content of those phytochemicals depends not only on the growing season but also on the growing conditions (environmental factors, soil characteristics, area/location). In particular, a study conducted in Australia showed that lutein was the most abundant carotenoid (7.0 mg/100 g), followed by beta-carotene (1.6 mg/100 g), while there were no detectable amounts of alpha-carotene and zeaxanthin (S. Petropoulos, Karkanis, Martins, & Ferreira, 2016).

1.3.2.6 Metals

Dietary minerals, i.e., iron (Fe), zinc (Zn), potassium (K), boron (B), nitrogen (N), manganese (Mn), calcium (Ca), copper (Cu) and magnesium (Mg) are the most abundant in P. oleracea, while other minerals (e.g. phosphorus (P), sulphur (S), sodium (Na)) are present in relatively smaller quantities (S. Petropoulos et al., 2016). The correlation between the place of origin and the plant part for the mineral content is important, as between leaves and seeds; leaves seem to be the plant part of purslane with the highest content of active substances (S. Petropoulos et al., 2016). In addition, significant differences in mineral content have also been observed between different varieties of the species, as well as between different growth stages, and even the planting period and soil salinity contribute to the different composition from plant to plant (S. Petropoulos et al., 2016).

1.3.2.7 Sterols

Sterols are derivatives of isoprenoid compounds and are essential structural elements of biological membranes. Plant sterols come in a variety of forms, including free sterols, fatty acid-rich sterol esters, sterol glycosides, and acylsterol glycosides, which are not found in animal cells.

Two known sterols that have been extracted and identified in Portulaca oleracea are daucosterol and β-sitosterol (Li et al., 2024). These sterols have a key role in decreasing blood cholesterol levels. Therefore, the sterols may also help to some extent in lowering blood lipids. For the past few decades, most preclinical research has shown that β-sitosterol has a variety of anticancer actions against different types of cancer. Studies showed that both daucosterol and β-sitosterol efficiently induced apoptosis and upset the cell cycle in A549 cells, suggesting that they could be considered as safe and effective cancer treatments (Li et al., 2024).
1.3.2.8 Polysaccharides

Polysaccharides are carbohydrates made up of more than ten monosaccharide units. Numerous biological processes are regulated by polysaccharides, such as blood sugar regulation, blood circulation, and immune function. *P. oleracea* contains many polysaccharides, with a yield of about 6.45% for water-soluble crude polysaccharides. Gas chromatography (GC) monosaccharide analysis revealed that polysaccharides of the species contained mannose, arabinose, glucose, and galactose. *P. oleracea*’s polysaccharides have shown effects in metabolism, gastroprotection, antibacterial, anticancer, and other areas. Due to their abundance in purslane and because they exhibit a range of biological functions, they should also be considered as important bioactive constituents of purslane (Li et al., 2024).

1.3.2.9 Terpenes

Terpenoids are compounds that consist of isoprenoid moieties. The anti-hypoxia activity of purslane’s extracts is linked to the synergistic effects of the terpenes and unsaturated fatty acids of the species (D. Chen et al., 2019a). Terpenes that have been identified in *P. oleracea* include lupeol, linalool, taraxerol, friedelin, and portuloside A and B, and a diterpenoid, portulene, that has been isolated from the species (Elkhayat, Ibrahim, & Aziz, 2008; Kumar, Sreedharan, Kashyap, Singh, & Ramchiary, 2022).

![Chemical structures of the terpenoids portulene (left) and lupeol (right) from *P. oleracea*](image)

*Figure 12: Chemical structures of the terpenoids portulene (left) and lupeol (right) from *P. oleracea* (Li et al., 2024).*

1.4 Biological activities of purslane

Purslane is an important medicinal herb, as it possesses several valuable phytochemicals. Its use as medicine and food in China has a history of thousands of years (Kumar et al., 2022). There is also evidence of its use for respiratory-related diseases in ancient medical books in Iran (Kumar et al., 2022). Due to the importance of the plant in various cultures as an important medicinal plant, research interest is increasingly turning to purslane to investigate its pharmacological potential against various diseases such as diabetes, cancer, nervous diseases, asthma, obesity, and bacterial and viral diseases using various models including in vivo studies and cell lines (Kumar et al., 2022).
Figure 13: The multiple benefits and uses of P. oleracea (Srivastava et al., 2023).

1.4.1 Antioxidant activity

Antioxidants are very important for human health, as they reduce the risk of cell damage from free radicals. Various parts of purslane including leaves, stems and flowers have been used to test its antioxidant potential. Studies have shown that the methanolic extract of six different varieties of Portulaca oleracea had strong antioxidant activity (Lim & Quah, 2007). Also, other findings revealed that the flowers exhibited higher antioxidant activity, which was associated with higher content of total phenolics, ascorbic acid, beta-carotene, and omega-3 fatty acids (Kumar et al., 2022). Several compounds that have been isolated from purslane and studies have proven their antioxidant activities (D. Liu, Shen, & Xiang, 2011; X. Yang, Ying, Liu, Ying, & Yang, 2019; Z. Yang, Liu, Xiang, & Zheng, 2009). Phenolic alkaloids, such as oleracein A, oleracein B and oleracein E showed antioxidant activities (Z. Yang et al., 2009). Specifically, the EC₅₀ value of Oleracein A, B and E has been determined to be 8.96 μM, 5.56 μM, and 9.87 μM, respectively, while a known antioxidant agent, butylated hydroxyanisole (BHT) has an EC₅₀ value of 9.08 μM (Z. Yang et al., 2009). The acylated groups enhance the antioxidant activity of these cyclo-dopa alkaloids. Oleracein K contains a caffeoyl moiety and displays higher radical scavenging activity than Oleracein N (15.3 μM and 29.05 μM, respectively), that has an attached feruloyl moiety, suggesting that the number of hydroxyl groups of the phenolic acyl at the glucose moiety, influences the antioxidant activity of these compounds (Jiao et al., 2015). Another interesting finding is that the substitution of a diglucoside in the C-6 atom of the Oleraceins H and P (42.89 μM and 43.52 μM, respectively), showed no significant difference in the radical scavenging activity, when compared to the monoglucoside Oleraceins A and C (35.11 μM and 38.28 μM,
respectively) (Jiao et al., 2015). Moreover, the growth stage as well as the plant part are directly related to the antioxidant capacity of the plant, as the mature leaves (at the flowering stage) can serve as a good source of fatty acids (mainly \(\alpha\)-linolenic acid and linoleic acid) and phenolic compounds with suitable antioxidant activities (Saffaryazdi, Ganjeali, Farhoosh, & Cheniany, 2020). Polysaccharides, which remove hydroxyl free radicals following a quantity-efficiency relationship, also participate in the antioxidant capacity of purslane. When the concentration of polysaccharides is 3.5 mg/ml, the free radical binding rate was 50%, while at a concentration of 17.6 mg/ml, the binding reached up to 90% (D. Chen et al., 2019a). Also, higher amounts of alpha-tocopherol, ascorbic acid and beta-carotene were observed in the leaves of purslane grown both in the growth chamber and in the wild, compared to their spinach counterparts. As a result, purslane contained higher concentration of \(\alpha\)-tocopherol (22.2mg and 130mg/100g fresh and dry weight, respectively) and ascorbic acid (26.6mg and 506mg/100g fresh and dry weight, respectively), while \(\beta\)-carotene was slightly higher in spinach. These findings demonstrate the significant antioxidant activity of \(P\). \(o\)leracea’s extracts that can find diverse applications in pharmaceutical and cosmetic industries (Uddin et al., 2014).

1.4.2 Antimicrobial action

\textit{Portulaca oleracea} has antibacterial, antifungal, and antiviral activities (Kumar et al., 2022). The 70% methanol extract of purslane shows antibacterial activity against Gram-negative bacteria: \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, and \textit{Neisseria gonorrhoea} with inhibition zones of 14, 15 and 15 mm, respectively (Zhou et al., 2015). Also, it is effective against Gram-positive bacteria: \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, and \textit{Streptococcus faecalis} with inhibition zones of 13, 14, and 15 mm, respectively, and displays antifungal activity against \textit{Candida albicans} with an inhibition zone of 12 mm (Zhou et al., 2015). The antibacterial mechanism of action of the flavonoid compounds of purslane is mainly carried out through the destruction of bacterial cell membranes, inducing vaporization (D. Chen et al., 2019a). When erythromycin and oleic and linoleic acids from \(P\). \(o\)leracea are combined, they demonstrated synergistic antibacterial efficacy against methicillin-resistant \textit{Staphylococcus aureus} (MRSA) (Iranshahy et al., 2017).

1.4.3 Anti-inflammatory action

One of the defense mechanisms against damaging stimuli is inflammation. Inflammation itself damages cells and tissues after an extended period of time. When LPS-stimulated macrophages are exposed to the alkaloids oleracone and oleracimine from \(P\). \(o\)leracea, they significantly reduce inflammation. These compounds have remarkable anti-nitric oxide (NO) activity (Rahimi, Ajam, Rakhshandeh, & Askari, 2019). Many studies have demonstrated the anti-inflammatory activity of purslane. The aqueous extract of purslane demonstrates an important role in suppressing the vascular inflammatory process associated with the development of atherosclerosis (Zhou et al., 2015). The aqueous extract of \textit{Portulaca oleracea} inhibits tumor necrosis factor-a (TNF-a) by induced production of intracellular reactive oxygen species (ROS) and overexpression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion protein 1 (VCAM-1) and E-
selectin in human endothelial cells (HUVECs) in a dose-dependent manner (Zhou et al., 2015). This extract also suppresses nuclear factor-kB (NF-kB) p65 translocation to the nucleus, TNF-α-induced binding of NF-kB, and degradation of inhibitor molecule (IκB)α (Zhou et al., 2015). In addition, polysaccharides isolated from the aerial parts of purslane, stimulated the human T-cells (CD4+/CD25+ and CD8+/CD25+), activated phagocytes (CD14+ and CD64+ cells), and enhanced the production of IL-6 from the human white blood cells and Peyer's patch cells (Kumar et al., 2022). Although purslane’s anti-inflammatory properties are well demonstrated, the underlying mechanism need to be elucidated.

Two valuable phytoconstituents of the species, namely α-linolenic acid and quercetin display anti-inflammatory effects. Specifically, when LPS-stimulated human corneal epithelial (HCE) were treated with α-linolenic acid, a notable reduction of the stimulation-induced rise in TNF-α, IL-1β, IL-6, and IL-8 mRNA and protein levels occurred (Ghorani et al., 2023). Also, treatment of ALA (500 and 2000 mg/kg, daily, for 13 days) to mice in an OVA-induced allergic rhinitis model resulted in a decrease in the expression of IL-6 and IL-1β in the nasal mucosa, indicating the anti-inflammatory properties of this essential fatty acid (Ghorani et al., 2023). Also, Quercetin (40 µM) inhibited T-cell receptor (TCR) stimulation-induced production of IFN-γ and IL-2 in primary cells. When exogenous recombinant human IL-2 and TCR are triggered, quercetin dramatically reduced the rise in IL-2Ra expression (Ghorani et al., 2023).

1.4.4 Anticancer effects

Cancer is one of the most common causes of death worldwide. In the last decades, researchers have developed and investigated anticancer drugs, many of which have a plant origin, including taxol, epipodophyllotoxin, vincristine, vinblastine, paclitaxel, docetaxel, camptothecin, and irinotecan (Kumar et al., 2022). Purslane has promising effects against a variety of cancer types. Polysaccharides from P. oleracea affect immune processes in rats with ovarian cancer and scavenge the formation of free radicals (Zhou et al., 2015). The proliferation of human lung adenocarcinoma cancer cells (A-549), a particular type of throat epidermoid cancer cell (Hep-2), and a cervical cancer cell line (Hela) was clearly inhibited by purslane alkaloids in vitro (D. Chen et al., 2019a). Hela cervical cancer cells were significantly inhibited by purslane polysaccharides. Considerable suppression of Hep-2 cells, a form of laryngeal epidermoid carcinoma, was demonstrated by fatty acids (D. Chen et al., 2019a). Remarkably, the seed oil from purslane demonstrated notable cytotoxic effects against human lung cancer (A-549) and liver cancer (HepG2) cell lines, as well as inhibiting cell proliferation (Kumar et al., 2022). Purslane’s anticancer properties highlight its potential utilization to develop new anticancer agents for various cancer types.
1.4.5 Wound-healing

Physical injuries that cause an opening or break in the skin are called wounds. For the skin's compromised functional state and damaged anatomical integrity to be restored, wounds must heal properly. A substance that promotes the healing process is considered a wound healing stimulator. Based on study results, applying fresh homogenized crude extract of *Portulaca oleracea* to wounds caused by skin excision in white Swiss mice has been shown to accelerate the healing process (Li et al., 2024). As compared to the control group, treated excision wounds had a higher rate of wound contraction, which accelerated healing (Li et al., 2024). There is a long history of using plant-based medicinal mixtures to treat skin diseases, especially burns and wounds. The most active and promising chemicals from plants that have been shown to have wound-healing properties have been described and experimentally examined on a variety of animal models. The use of extracts of purslane in the cosmetics industry is increasingly expanding. The isoflavones and bioflavonoids contained in the plant, such as quercetin and myricetin, have proven anti-aging activity (Srivastava et al., 2023). In addition, the dry extract has wound-healing activity in *Mus musculus* JVI-1, since it significantly accelerates the wound healing stages through stimulation of contraction in the wound site and demodulation of the resection wound surface (Zhou et al., 2015). Various free radicals in the human body increase over time. Due to the quantitative degradation and lower activity of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), the content of malondialdehyde (MDA), free radical metabolites, will also increase, along with cellular damage and decreased tissue and organ function activity. If the activities of GSH-Px and SOD can be enhanced, the level of MDA may decrease. When α-linolenic acid is dosed into the human body, the antioxidant and anti-aging effects are improved (D. Chen et al., 2019b). Other studies revealed that in vitro UVB-induced damage to human fibroblast and keratinocyte cells was prevented by *P. oleracea* extracts. Specifically, following UVB irradiation, *P. oleracea*’s extracts efficiently decreased apoptotic DNA cleavage and cell death (Lee, Kim, Park, Lee, & Kim, 2014). These findings suggest that these extracts may prevent UVB-induced apoptosis in human skin cells. Consequently, it is proposed that purslane’s extracts might be a useful cosmetic material to stop UVB-induced skin damage (Lee et al., 2014).
1.6 Aim of thesis

Although it is considered a weed by many, the extract of *Portulaca oleracea* L. has soothing properties for the skin and has strong antioxidant action. Even though it is commonly consumed as part of most Western diets, the nutritional profile of this plant classifies it as a true superfood. Ongoing research consistently shows that this plant has remarkable benefits for the skin and body, as it helps to treat many skin problems, including wrinkles and other signs of aging. Purslane is a very good source of skin-beneficial minerals such as potassium, magnesium, calcium, and phosphorus, as well as omega-3 fatty acids, amino acids, and sugars that contribute to its moisturizing action. Recommended levels of use of the plant in skin care range from 1-3% of the pure plant and 1-5% if part of a blend. It is not known to be an irritant or otherwise problematic for the skin, although no formal safety assessment of this plant has yet been conducted. Therefore, the increasing trend in cosmetic products to use ingredients of natural origin and plant extracts, and the need for the cosmetic industry to find cheap and bioavailable plant raw materials, but at the same time effective, leads to the need to investigate the action and composition of widely used plants.

The purpose of this thesis was to compare two drying methods (oven-drying and freeze-drying) and to investigate the individual extracts of the leaves and stems of *Portulaca oleracea* L., in terms of their composition and antioxidant activity, with the aim of their use in cosmetic products. Initially, stems and leaves were separated to compare the phytochemical content of these aerial parts. After the completion of the drying processes, the dry extracts were extracted at first with hexane as a solvent, to obtain the non-polar metabolites, followed by 70% (aq) methanol to extract the semi-polar and more polar metabolites. The identification of the individual constituents of the extracts was carried out using UHPLC-MS-DAD for the hydro-methanolic extracts and GC-MS for the hexane extracts, while their quantification was carried out using HPLC-DAD and GC-FID, respectively. The fatty acid composition was conducted using a GC-FID apparatus, and their quantification was carried out using external standards. Finally, the antioxidant activity was evaluated using two different methods (FRAP and DPPH), and their total phenolic compound (TPC) and sugar composition were determined.
CHAPTER 2

2 EXPERIMENTAL PART
2.1. Collection of the plant

The plant was collected from a privately owned field in the region of Patras. It was collected in September 2022.

*Figure 14: The collection site of Portulaca oleracea (GoogleEarth).*

2.2. Pre-treatment of the plant

Immediately after the collection of the plant material, the leaves were washed and separated from the stems. The stems were cut into small pieces of 2-3 cm to facilitate their drying. The leaves and stems were then divided into two individual parts to carry out drying in the oven and pulverized. Once both drying processes were completed, the leaves and stems from each drying process were pulverized in an electric grinder and then placed in sealed bags under a nitrogen atmosphere, and in a dark place for further use.

*Figure 15: The pre-treatment of the plant prior to drying (a) and the freeze-drying chamber with the plant inside (b).*
2.3 Drying of the plant material

2.3.1 Freeze drying

2.3.1.1 Technical data for freeze drying

- Freeze Dryer: CryoDryer 5 equipped with CPS 18 m3/h pump from Gellert Engineering (Langweid a. Lech, Germany)
- Pressure sensor: Pirani
- Product temperature sensor: calibrated PT 100
- Tray material: aluminium, Hardness H14, Thickness 1.8mm

2.3.1.2 Drying cycle data

Stage 1: Freezing the material

- Condenser temperature: -50°C to -55°C
- Cooling temperature: -45°C
- Cooling time: 4 hours
- Product temperature: -40°C

<table>
<thead>
<tr>
<th>Part</th>
<th>Tshelf (°C)</th>
<th>Pc (mbar)</th>
<th>Tprmax (°C)</th>
<th>Duration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°</td>
<td>-24.0</td>
<td>0.250</td>
<td>-26.7</td>
<td>3</td>
</tr>
<tr>
<td>2°</td>
<td>6.5</td>
<td>0.250</td>
<td>-14.5</td>
<td>5</td>
</tr>
<tr>
<td>3°</td>
<td>22</td>
<td>0.250</td>
<td>2.0</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Tshelf: shelf temperature, Tprmax: maximum recorded product temperature, Pc = chamber pressure

- Total primary drying time: 14.5 hours
- Condenser temperature: -50°C to -55°C

Stage 2: Primary drying

Table 1: Parameters and conditions during the primary drying of the plant material.

<table>
<thead>
<tr>
<th>Part</th>
<th>Tshelf (°C)</th>
<th>Pc (mbar)</th>
<th>Tprmax (°C)</th>
<th>Duration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°</td>
<td>-24.0</td>
<td>0.250</td>
<td>-26.7</td>
<td>3</td>
</tr>
<tr>
<td>2°</td>
<td>6.5</td>
<td>0.250</td>
<td>-14.5</td>
<td>5</td>
</tr>
<tr>
<td>3°</td>
<td>22</td>
<td>0.250</td>
<td>2.0</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Stage 3: Secondary drying

Parameters and conditions during secondary drying of plant material:

- Condenser temperature: -50°C to -55°C
- Tshelf = 50.0 °C
- Pc = 0.250 mbar
- Tprmax = 32.4 °C
- Duration: 9.5 hours
\( T_{shelf} \): shelf temperature, \( T_{\text{pmax}} \): maximum recorded product temperature, \( P_c \) = chamber pressure

Total duration of the drying cycle: 28 hours

### 2.3.2 Oven drying

#### 2.3.2.1 Oven drying technical data

Oven: 2000210 from J.P. Selecta, s.a. (Highway A-2, Km 585.1 – 08630 Abrera, Barcelona)

#### 2.3.2.2 Drying data

- Chamber temperature: 50 °C
- Drying duration: 4 days for leaves and 5 days for stems

### 2.4 Ultrasonic extraction (UAE)

#### 2.4.1 Materials and instruments

- Methanol HPLC grade 99.8% (Fischer Scientific, LE11 5RG Loughborough, UK)
- H\(_2\)O HPLC grade (Fischer Scientific, LE11 5RG Loughborough, UK)
- Hexane \( \geq 95\% \) (SIGMA-ALDRICH, 3050 Spruce St., Saint Louis, MO, United States, 63103)
- Sodium sulfate anhydrous (Lach-Ner, s.r.o, Karásek 1, 62133 Brno, Czech Republic)
- Reflux condenser
- Ultrasonic bath: 621.05.001 from ISOLAB (Laborgeräte GmbH Am Dillhof 2 - 63863 Eschau, Germany)
- High-precision analytical balance with four decimal places and safety glass cage: 870 from Kern & Sohn GmbH (Ziegelei 1 Balingen, BW 72336, Germany)
- Rotavapor: R-200/R-205, Büchi Labotechnik AG (Postfach, CH-9230 Flawil, Switzerland)
- Freeze Dryer: FreeZone 6 Freeze Dryer System 7753000 G from Labconco (8811 Prospect Avenue Kansas City, MO 64132-2696)
- Pasteur pipettes
- 50ml and 100ml spherical bottles
- Funnel
- Büchner's funnel
- 100ml Büchner flask
2.4.2 Parameters of ultrasonic extraction

Table 2: Parameters applied for the extraction of leaves and stems for the two drying methods.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Hexane</th>
<th>70% (aq) methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>30 min</td>
<td>30 min</td>
</tr>
<tr>
<td>g dry plant material: ml solvent</td>
<td>1:30</td>
<td>1:30</td>
</tr>
<tr>
<td>Number of repetitions</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Temperature</td>
<td>29°C</td>
<td>40°C</td>
</tr>
</tbody>
</table>

*Figure 16: The experimental set-up for ultrasonic extraction.*
2.4.3 Experimental process of ultrasonic extraction

First part of the extraction: extraction of lipophilic compounds from the leaves and stems with hexane as solvent.

First, approximately 0.2 g of initial dry material is weighed and placed in a 5 mL spherical flask. Next, 6 mL of hexane is added to the spherical flask and immersed in the ultrasonic bath set at 29°C for 30 minutes. A vertical freezer is applied to the spherical flask and ultrasounds are initiated. After 30 minutes, the extract is collected by means of a pasteur pipette and stored in a sealed glass vial. This procedure is repeated two more times. After the three hexane extractions, the collected hexane extract is filtered through a filter crucible and a quantity of anhydrous sodium sulphate (drying agent) is added. The extract, when clear after filtration, is then transferred into a clean and pre-weighed 50 ml spherical flask. Next step is the evaporation of the solvent in a rotary evaporator until dry. The spherical flask after the evaporation is weighted and the difference from the pre-weighed flask is the mass of the dry hexane extract.

Second part of the extraction: obtain hydrophilic and moderately polar compounds of the leaves and stems with 70% (aq) methanol solvent.

The extraction process starts with the addition of 6 ml of 70% (aq) methanol to the same plant material from the previous hexane extraction. This is followed by ultrasonic extraction for 30 minutes at 40 °C. At the end of the extraction, the extract is collected by filtration under vacuum and 6 ml of 70% (aq) methanol is added again to the plant material for the second extraction. The procedure is repeated a total of three times and the resulting extract is filtered through a pleated filter crucible into a clean 100 ml spherical flask. Evaporation of methanol is then carried out in a rotary evaporator and the remaining volume of water is removed using a freeze dryer to obtain the dry methanol extract.
2.4.3 Extraction yield

The yield is calculated as the ratio of the mass of dry extract after concentration to the original mass of plant material weighed for extraction.

The equation used to calculate the yield is as follows:

\[ \text{yield} \% = \frac{\text{mass of dry extract}}{\text{mass of original plant material}} \times 100 \] (1)

2.5 Identification of non-polar and polar extract compounds

2.5.1 GC-MS characterization of hexane extracts

2.5.1.1 Reagents and instrumentation

The analysis and identification of the volatile compounds of leaves and stems of Portulaca oleracea were carried out at the Centre for Instrumental Analysis, University of Patras. The analysis was carried out using GC-MS, specifically an Agilent model 6890N GC, with an HP-5MS polar capillary column (30 m × 0.25 mm × 0.25 µm) and an Agilent 5975 B single quadrupole mass spectrometer (Santa Clara, CA, USA). Prior to analysis, the samples were filtered through a Millex Syringe Filter, Durapore® (PVDF), non-sterile, pore size 0.22 µm, diameter 33 mm, hydrophobic.
2.5.1.1 GC-MS analysis parameters

- Ionisation energy: 70 eV
- Carrier gas: He
- Flow rate: 1 mL/min
- Injection volume: 1μL
- Split: 1:5

Table 3: The temperature program used for the identification of compounds in GC-MS and for their quantification in GC-FID.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Rate of increase (°C/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 (2 min)</td>
<td>5</td>
</tr>
<tr>
<td>200</td>
<td>8</td>
</tr>
<tr>
<td>230</td>
<td>10</td>
</tr>
<tr>
<td>280 (hold for 20 min)</td>
<td></td>
</tr>
</tbody>
</table>

The obtained results were analyzed using AMDIS (Automated Mass spectral Deconvolution & Identification System, NIST Institute) and the identification of the compounds of the extracts was carried out by comparing the mass spectra as well as the retention indices (RI) for the non-polar columns with the literature (NIST Chemistry Webbook, Adams R., 2017). The retention index (RI) was determined based on a series of linear C8-C20 and C21-C40 alkanes and the van den Dool and Kratz equation (2):

\[
RI(x) = 100 \times P_z + 100 \times \left[ \frac{RT(x) - RT(P_z)}{RT(P_{z+1}) - RT(P_z)} \right] \quad (2)
\]

where, \( x \): the compound under investigation, \( P_z \): the alkane eluted before the compound, \( P_{(z+1)} \): the alkane eluted after the compound, \( RT(x) \): the retention time (min) of the compound, \( RT(P_z) \): the retention time (min) of the alkane eluted before the compound and \( RT(P_{z+1}) \): the retention time (min) of the alkane eluted after the compound.

2.5.2 Esterification of fatty acid methyl esters – GC-FID quantification

2.5.2.1 Reagents and instrumentation

- GC-FID 7890A, Agilent Technologies Inc. (Santa Clara, CA, USA)
- DB-WAX capillary column (10 m x 100 μm x 0.1 μm)
- Thermostatic water bath
- Vortex
2.5.2.2 Experimental process of fatty acid esterification

The method is based on the transesterification of fatty acids in the presence of methanol and high temperature (90°C) (Levine, Costanza-Robinson, & Spatafora, 2011).

First, the digestion reagent consisting of methanol, chloroform, and concentrated sulphuric acid in a ratio of 100 / 10 / 3 (v/v/v), respectively, is prepared. The sulphuric acid is added last and under stirring. The sulfuric acid is used to hydrolyse the biomass, the methanol creates the methyl esters of fatty acids, and the chloroform is used to dissolve the fatty acids. Then, to approximately 80 mg of initial dry herb or seeds that have been milled, 5 ml of digestion reagent is added. Vortex and incubate in a water bath at 90 °C for two hours. After two hours, the samples are left at RT and 2 ml of H2O 3D are added to terminate the hydrolysis reaction. The fatty acids are then extracted with a solution of hexane in chloroform (4:1, v/v), where the hexane phase containing the esterified fatty acids is recovered. The extraction procedure is carried out a total of three times and the hexane extracts were filtered through a WHATMAN® Nylon Syringe Filter, no-sterile, 0.2 μm pore size, diameter 25 mm, hydrophobic. Finally, the resulting samples are analyzed in GC-FID and the quantification of fatty acids is carried out using external standards.
2.5.2.3 GC-FID parameters for fatty acid analysis

- Carrier gas: He
- Flow rate: 0.3 mL/min
- Injection volume: 1 μL
- Split ratio: 1:30

Table 4: The temperature program used for the quantification of fatty acids in GC-FID.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Rate (°C /min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 (0.5 min)</td>
<td>25</td>
</tr>
<tr>
<td>195</td>
<td>3</td>
</tr>
<tr>
<td>205</td>
<td>8</td>
</tr>
<tr>
<td>230</td>
<td></td>
</tr>
</tbody>
</table>

Figure 20: The fatty acid extracts of the freeze-dried leaves (1a), the oven-dried leaves (2a), the freeze-dried stems (1b), the oven-dried stems (2b) and the seeds (c).
2.5.2 HPLC-DAD-MS characterization of the 70% (aq) methanol leaf extracts

The analysis of the hydro-methanolic extracts of the leaves was performed using a HPLC-DAD-MS system at the Laboratory of Instrumental Analysis of the University of Patras, consisting of a Thermo Scientific Dionex Ultimate 3000 HPLC instrument (Waltham, MA, USA) and a Bruker MS Amazon SL spectrometer (Billerica, MA, USA). A C-18 reverse phase column from Phenomenex®, Kinetex, 2.6 μm, 100 Å, 100 x 3.0 mm, was used. Prior to analysis, the leaf samples were filtered through a CHROMAFIL® RC Syringe Filter, color-coded, pore size 0.2 μm, diameter 15.6 mm, hydrophilic.

2.5.2.2 Analysis parameters for HPLC-DAD-MS

- Thermostat temperature: 30°C
- Flow rate: 0.3 mL/min
- Injection volume: 12 μL
- Wavelengths (nm): 273, 280, 330, 330, 430
- Mobile phase: H₂O + 0.2% formic acid (A) and MeCN + 0.2% formic acid (B)

The gradient elution program used to identify the compounds in the hydro-methanolic extract of the leaves is listed in Table 5.

Table 5: The gradient elution program used for both HPLC-DAD-MS identification and HPLC-DAD quantification.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>88</td>
<td>12</td>
</tr>
<tr>
<td>35</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>45</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>55</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>65</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>70</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

2.5.2.3 Identification procedure for the hydro-methanolic extracts of the leaves

Metabolite identification were based on the retention time, UV-vis spectra, and mass data (exact mass, isotopic distribution, and fragmented negative and positive ions) of the detected compounds and their comparison with the literature. To facilitate the identification of the compounds an in-house library of data exclusively related to the specific plant species was created based on literature data and relevant publications.
For the development of method analysis, relevant publications from the literature using a two solvent system, namely, water with acetonitrile with a small percentage of formic acid (Farag & Shakour, 2019a) (Jaafari et al., 2021) were taken into consideration for the analysis method developed. The addition of a low concentration of formic acid to the mobile phase is known to improve the shape of the resulting separation peaks, while also contributing to proton generation in positive ionization, producing \([M+H]^+\) ions (Núñez & Lucci, 2014). The leaf extract was analyzed in both positive and negative ionization, as \(P. \text{oleracea}\) is known to contain alkaloids (Yue et al, 2005) which are best ionized in positive ionization, and flavonoids, which are best ionized in negative ionization (Farag M., 2019).

2.6 HPLC-DAD alkaloids quantification

2.6.1 Reagents and instrumentation

Following the identification of the compounds, the quantification of the alkaloids detected in the hydro-methanolic extract of the leaves was carried out with an Agilent HPLC-DAD system. The reason for choosing a DAD photodiode array detector for the quantification of alkaloids is its high sensitivity (LLOD, LLOQ), while at the same time these alkaloids, known as oleraceins, have a high molecular weight and their extensive fragmentation with a mass detector proved to be particularly difficult. However, through their identification it was shown that these compounds absorb strongly at wavelengths of 300-330 nm, so quantification based on UV-vis absorption was chosen. The column on which the separation of the compounds was performed is a reverse phase C-18 100 Å column of Phenomenex® Kinetex 2.6 µm, 100*3mm. Prior to analysis, the leaf samples were filtered through a CHROMAFIL® RC Syringe Filter, color-coded, pore size 0.2 µm, diameter 15.6 mm, hydrophilic.

2.6.2 Chromatographic conditions and calibration curve

The method used is the same as that for the identification in Table 5, but the flow rate was increased to 0.4 mL/min to improve the separation of compounds and the temperature was maintained at 30°C. Quantification of alkaloids was performed at a wavelength of 330 nm. \(\text{Trans-}p\)-coumaric acid was used as the reference compound as it was the closest compound structurally available in the laboratory, therefore the alkaloids were quantified as equivalents of this compound. The calibration curve was constructed with a concentration range of 0.5-50 µg/ml, from which the linear equation \(y = 50.088 \times x + 22.607\) with \(R^2 = 0.9997\) was obtained.
Figure 21: trans-p-coumaric acid standard curve.

2.7 Determination of antioxidant capacity

To evaluate the antioxidant activity of leaves and stems by the two drying methods, the reduction activity by converting Fe$^{3+}$ to Fe$^{2+}$ (FRAP, Ferric Reducing Antioxidant Power) and 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) binding capacity were measured, which are fast, simple and with reliable result (Pisoschi & Negulescu, 2012). The principle of the DPPH assay relies on the reduction of the DPPH radical, in the presence of a hydrogen donor, such as an antioxidant agent. As a result, the violet color of the DPPH radical diminishes, and the reduction in absorbance is proportional to the concentration of the antioxidant (Pisoschi & Negulescu, 2012). Ferric reducing antioxidant power, or FRAP, is a technique that is based on the antioxidants reducing power of the complex ferric ion-TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine). A vivid blue color is produced when Fe$^{2+}$ binds to the ligand. The absorbance can be used to determine the amount of iron that has been reduced, and is associated with the concentration of the antioxidant (Pisoschi & Negulescu, 2012).

2.7.1 FRAP assay

2.7.1.1 Reagents and instrumentation

- Acetic acid (Honeywell Fluka™, 8008 Corporate Center Dr, Charlotte, NC)
- Water for analysis (CARLO ERBA Reagents GmbH. 79312 Emmendingen Germany)
- TPTZ: 2,4,6-Tris(2-pyridyl)-1,3,5-triazine, (Alfa Aesar, MA 01835, USA)
- FeCl$_3$ x 6H$_2$O (BDH), 99+ % for analysis (Acros Organics Bvba, 2440 Geel, Belgium)
- FeSO$_4$.7H$_2$O, Riedel-de Haën (CH-9471 Buchs SG, Switzerland)
- Sunrise-Basic Tecan microplate reader (Tecan Group Ltd, 8708 Männedorf, Switzerland)
- pH/EC Meter (Consort™ C830-series, B-2300 Turnhout, Belgium)
- Hydrochloric acid (Petr Švec - PENTA s.r.o., Radiová 1122/1 102 00 Prague 10)
2.7.1.2 Experimental procedure for the FRAP assay

The method is based on the trivalent iron reduction reaction of the 2,4,6-tri-(2-pyridyl)-triazine (TPTZ) complex to divalent iron. For the evaluation of the antioxidant capacity of the hydro-methanolic extracts of leaves and stems from the two drying methods, the preparation of five solutions is required:

Solution 1: 300 mM acetate buffer prepared from acetate sodium trihydrate with adjustment to pH = 3.6, using acetic acid and water for analysis.

Solution 2: 40 mM HCl

Solution 3: 10 mM TPTZ which is dissolved in solution 2

Solution 4: 20 mM FeCl₃x6H₂O (BDH) dissolved in water

Solution 5: FRAP reagent consisting of solutions 1, 3 and 4 in a 5/1/1/1 volume ratio.

Figure 22: The reduction of Fe³⁺ in the TPTZ complex in the presence of an antioxidant.
The following volumes are then added to a 96-well ELISA plate:

- 55 μL of acetate buffer
- 80 μL of FRAP solution
- 60 μL of sample or standard

The plate is allowed to stand for 5 minutes in a dark place and then the absorbance is measured at 595 nm. FeSO₄·7H₂O in a concentration range of 0.05-0.4 mM was used as the standard compound, with the equation of the standard curve represented in Figure 22, and the antioxidant capacity result of the extracts was expressed in equivalent mg of FeSO₄·7H₂O per dry initial plant material and per g of dry extract.

![Figure 22: Standard curve of FeSO₄·7H₂O](image)

**2.7.2 DPPH assay**

**2.7.2.1 Reagents and instrumentation**

- DPPH: 2,2-diphenyl-1-picryl-hydrazyl (SIGMA-ALDRICH, Saint Louis, MO, USA, 63103)
- Water for analysis, (CARLO ERBA Reagents GmbH. 79312 Emmendingen Germany)
- Methanol HPLC grade (SIGMA-ALDRICH, Saint Louis, MO, United States, 63103)
- Trolox: (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97% (SIGMA-ALDRICH, Saint Louis, MO, USA, 63103)
- Sunrise-Basic Tecan microplate reader (Tecan Group Ltd, 8708 Männedorf, Switzerland)
- 96 cell ELISA plate (Greiner Bio-One International GmbH, A-4550 Kremsmünster Österreich)
2.7.2.2 Experimental procedure for the DPPH method

This method of measuring antioxidant capacity is based on the ability of an antioxidant to bind the DPPH free radical and reduce it. The experimental procedure for this method involves the addition to an ELISA plate of 96 cells of the following volumes:

- 5 μL of sample or standard
- 195 μL of DPPH reagent in 0.1 mM MeOH

The plate was then incubated at room temperature and in the dark for 30 minutes, followed by measurement of the absorbance at 540 nm. Ascorbic acid in a concentration range of 0.005-2 mg/ml was used as the standard compound.

![Figure 24: The neutralization of the DPPH radical in the presence of an antioxidant.](image)

The antioxidant activity of the hydro-methanolic extracts of leaves and stems, from the two drying methods, was calculated with the equation:

\[
\% \text{ RSA (Radical Scavenging Activity)} = \left[1 - \left(\frac{A_s - A_c}{A_b - A_c}\right)\right] \times 100
\]

where, \(A_b\): the absorbance of the DPPH, \(A_c\): the absorbance in the absence of DPPH, and \(A_s\): the absorbance of the sample or standard.
Using the GraphPad Prism 6.0 program, the curve of % inhibition of DPPH radical is obtained (Figure 25) and the IC\textsubscript{50} value is calculated, which is the concentration of the antioxidant that causes 50% inhibition of DPPH radical activity. The lower the IC\textsubscript{50} value, the higher the antioxidant capacity, as a small concentration of antioxidant is required to neutralize the DPPH radical. Ascorbic acid was used as a positive control, for which the IC\textsubscript{50} value of 0.1430 mg/ml was obtained.

### 2.8 Determination of total phenolic content (TPC)

#### 2.8.1 Reagents and instrumentation

- Folin-Ciocalteu's Reagent for clinical diagnosis (AppliChem GmbH., D-64291 Darmstadt, Germany)
- Sodium carbonate anhydride (Na\textsubscript{2}CO\textsubscript{3}) (ChemLab NV, West Flanders, Belgium)
- Gallic acid (Fluka Chemie GmbH, Buchs 9471, Switzerland)
- Water for analysis (CARLO ERBA Reagents GmbH. 79312 Emmendingen Germany)
- Sunrise-Basic Tecan microplate reader (Tecan Group Ltd, 8708 Männedorf, Switzerland)
- 96 cell ELISA plate (Greiner Bio-One International GmbH, A-4550 Kremsmünster Österreich)

#### 2.8.2 Experimental procedure for the determination of total phenolics of hydro-methanol extracts

The measurement of the phenolic compound content of leaf and stem extracts was carried out by the Folin-Ciocalteu method (V. L. Singleton, 1965). According to this method, the oxidation of phenolic components by the Folin-Ciocalteu reagent and its reduction immediately afterwards, results in the production of colored reaction products which are then quantified by a spectrophotometer microplate reader (Pérez, Domínguez-López, & Lamuela-Raventós, 2000).

*Figure 25: The % scavenging curve of the DPPH radical by various concentrations of ascorbic acid.*
Antioxidants reduce the anionic derivatives of phosphotungstic and phosphomolybdic acids, resulting in a color shift from yellow to blue. The amount of color shift that occurs at the end of the reaction is directly related to the phenolic compounds’ reducing activity (Pérez et al., 2023). More specifically, blue complexes are produced in an alkaline solution by the transfer of electrons from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes; these complexes are identified spectroscopically at a wavelength of 760 nm. At basic pH conditions, phenolic compounds stimulate a sequence of reversible one- or two-electron reactions that transform the initial yellow Folin-Ciocalteu reagent into blue species (Pérez et al., 2023).

The experimental procedure for this method involves the addition to an ELISA plate of 96 cells of the following volumes:

- 20 μL of Folin-Ciocalteu reagent (1:10 dilution)
- 20 μL dilutions of leaf or stem extracts or gallic acid standard (10-200 μg/ml)
- 180 μL water for analysis
- 20 μL sodium carbonate 13,75 % (Na₂CO₃)

The plate is then incubated for 30 minutes, at RT and in the dark. The absorbance is then measured at 750 nm. Based on the standard gallic acid curve constructed (Figure 25), the quantification of dry extracts of leaves and stems was carried out. The sample concentrations used were 0.3 and 0.4 mg/ml of dry extract.

![Gallic acid curve](image)

*Figure 26: Standard curve of gallic acid.*
2.9 Determination of total carbohydrates

2.9.1 Reagents and instrumentation

- Sulfuric acid 96% A.G. at 4 °C (Petr Švec - PENTA s.r.o., 102 00 Prague 10)
- Anthrone (SIGMA-ALDRICH, Saint Louis, MO, United States, 63103)
- D(+)-Saccharose (Riedel-de Haën, CH-9471 Buchs SG Switzerland)
- Sunrise-Basic Tecan microplate reader (Tecan Group Ltd, 8708 Männedorf, Switzerland)
- 96 cell ELISA plate (Greiner Bio-One International GmbH, A-4550 Kremsmünster Österreich)
- Water for analysis (CARLO ERBA Reagents GmbH. 79312 Emmendingen Germany)

2.9.2 Experimental procedure for total sugar content in the hydro-methanolic extracts

The carbohydrate content of leaf and stem extracts was measured according to the laboratory protocol developed based on the method of Laurentin and Edwards, 2003 (Laurentin & Edwards, 2003). The principle of the method is based on the use of heat and strongly acidic conditions to carry out simultaneous hydrolysis of glycosidic bonds and dehydration of monomers to obtain furfural derivatives. These compounds react with anthrone to produce colored products. Sucrose was used as a positive control to quantify the dry extracts, as based on literature (Petropoulos et al., 2019), the stems and leaves of purslane contain glucose and fructose, which are its monomers.

The experimental procedure of this method involves adding 40 μL of dilutions of leaf or stem extracts or sucrose standard (0.15-1.8 mg/ml) to a 96-cell ELISA plate and standing at 4°C for 15 min in the dark. Then, 100 μL of anthrone is added followed by incubation of the plate in a water bath at 92°C for 5 min. Finally, the plate is briefly left at RT and the absorbance is measured at 620 nm. The quantification of carbohydrates in leaves and stems was performed using the standard sucrose curve (Figure 26) and the results were expressed as saccharose equivalents. The sample concentrations used were 5 mg/ml of dry extract for leaves, and 2 and 4 mg/ml of dry extract for stems from freeze drying and oven drying, respectively.
Figure 27: Standard curve of saccharose.
CHAPTER 3

3 RESULTS AND DISCUSSION
3.1 Moisture content and dry matter determination

The calculation of the moisture content (MC) for each drying process of the plant parts was carried out using the formula:

\[
\% \text{ MC} = \frac{\text{Weight before drying} - \text{Weight after drying}}{\text{Weight before drying}} \times 100
\]

\[
\% \text{ dry matter} = 100 - \% \text{ MC}
\]

Table 6: The % moisture content and % dry matter from the freeze-dried and oven-dried leaves and stems.

<table>
<thead>
<tr>
<th></th>
<th>Leaves FD</th>
<th>Leaves OD</th>
<th>Stems FD</th>
<th>Stems OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>% MC</td>
<td>87.99</td>
<td>87.04</td>
<td>91.38</td>
<td>88.53</td>
</tr>
<tr>
<td>% dry matter</td>
<td>12.01</td>
<td>12.96</td>
<td>8.62</td>
<td>11.47</td>
</tr>
</tbody>
</table>

(Leaves OD: leaves oven-dried, leaves FD: leaves freeze-dried, Stems OD: Stems oven-dried, Stems FD: Stems freeze-dried)

In both aerial parts, the % moisture content from the two drying techniques did not significantly differ (Table 6). Subsequently, the stems' and leaves' percentage of dry matter because of the drying procedures, did not differ noticeably. The % amount of moisture determined in both aerial parts from the two drying methods, are in the same range as other publications (S. A. Petropoulos et al., 2019). The oven-drying's extensive duration is probably the cause of the non-significant differences between the two drying methods, as the leaves took four days to fully dry in the oven, and the stems required five days.

3.2 Extraction Yields

3.2.1 Yields of hexane extractions in leaves and stems

Extractions using hexane were carried out to make an initial separation between non-polar and volatile compounds from the more polar compounds contained in the leaves and stems of Portulaca oleracea.

Table 7: The hexane and 70% (aq) methanol extraction yields of leaves and stems from the two drying methods. The table shows the mean values ± standard deviation of the replicates (n=3).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Leaves FD</th>
<th>Leaves OD</th>
<th>Stems FD</th>
<th>Stems OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>5.80 ± 1.40</td>
<td>8.77 ± 1.52</td>
<td>8.90 ± 1.56</td>
<td>7.35 ± 0.92</td>
</tr>
<tr>
<td>70% (aq) methanol</td>
<td>19.15 ± 0.50</td>
<td>18.53 ± 2.53</td>
<td>14.6 ± 2.26</td>
<td>12.95 ± 2.19</td>
</tr>
</tbody>
</table>

(OD: oven-dried, FD: freeze-dried)
From the data in Table 7, we observe small, non-significant differences at the hexane extraction yields of the leaves. The stem yields from the hexane extractions for both drying methods were quite close, due to the larger area of the stems that contributed to their better pulverization in an even manner. In addition, we can observe that the yields of the 70% methanol extractions are relatively close and almost twice as high as the corresponding yields of hexane. This is probably due to the higher content of polar or moderately polar compounds in the leaves that are extracted with an aqueous polar solvent (water and methanol).

![Hexane extraction yields](image)

*Figure 28: Hexane extraction yields of the freeze-dried (FD) and oven-dried (OD) leaves and stems.*

![70% (aq) methanol extraction yields](image)

*Figure 29: The hydro-methanolic extraction yields of the freeze-dried and oven-dried leaves and stems.*
3.3 Phytochemical composition of the hexane extracts

Figure 30: Chromatograms of oven-dried (below) and freeze-dried (above) leaves using GC-MS displaying the identified peaks.

Figure 31: Chromatograms of oven-dried (below) and freeze-dried (above) stems using GC-MS displaying the identified peaks.
The GC-MS analysis of the leaves and stems (Figures 30, 31) are presented in Table 8 and Table 9, respectively.

Table 8: The identified compounds of the leaves from freeze-drying (L. FD) and oven-drying (L. OD), the retention times of the compounds (RT), the theoretical and experimental retention indices (RI), and the source of the theoretical retention index value.

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Compound</th>
<th>RT (min)</th>
<th>RІ_experimental</th>
<th>RІ_theoretical</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L. FD</td>
<td>Palmitic acid</td>
<td>22.342</td>
<td>1968</td>
<td>1971</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>L. FD</td>
<td>(E)-Phytol</td>
<td>24.772</td>
<td>2111</td>
<td>2122</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>24.855</td>
<td>2121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>L. FD</td>
<td>Linoleic acid</td>
<td>25.108</td>
<td>2139</td>
<td>2144</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>L. FD</td>
<td>α-Linolenic acid</td>
<td>25.236</td>
<td>2148</td>
<td>2158.9</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>L. FD</td>
<td>Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-</td>
<td>28.535</td>
<td>2430</td>
<td>2398</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>L. FD</td>
<td>Pentacosane</td>
<td>29.186</td>
<td>2498</td>
<td>2500</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>29.205</td>
<td>2500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>L. FD</td>
<td>Heptacosane</td>
<td>30.888</td>
<td>2698</td>
<td>2700</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>30.904</td>
<td>2700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>L. FD</td>
<td>Squalene</td>
<td>32.094</td>
<td>2832</td>
<td>2835.8</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>32.103</td>
<td>2833</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>L. FD</td>
<td>Nonacosane</td>
<td>32.737</td>
<td>2897</td>
<td>2900</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>32.742</td>
<td>2897</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>L. FD</td>
<td>(+/-)-gamma-Tocopherol</td>
<td>34.842</td>
<td>3064</td>
<td>3074.2</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>34.844</td>
<td>3323</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>L. FD</td>
<td>Hentriacontane</td>
<td>35.313</td>
<td>3097</td>
<td>3100</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>35.308</td>
<td>3097</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>L. FD</td>
<td>dl-α-Tocopherol</td>
<td>36.096</td>
<td>3144</td>
<td>3149.4</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>36.090</td>
<td>3143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>L. FD</td>
<td>Tritriacontane</td>
<td>39.104</td>
<td>3298</td>
<td>3300</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>39.096</td>
<td>3297</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>L. FD</td>
<td>γ-Sitosterol</td>
<td>39.762</td>
<td>3323</td>
<td>3351.3</td>
</tr>
<tr>
<td></td>
<td>L. OD</td>
<td>39.746</td>
<td>3323</td>
<td></td>
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</tr>
</tbody>
</table>

*a: Theoretical RIs from NIST Chemistry Webbook, based on Van Den Dool and Kratz equation with active phases HP-5MS and DB-5MS, and Adams, R. P. (2017) with active phases HP-5MS and DB-5.*

60
Table 9: Includes the identified compounds of the stems from freeze-drying (S. FD) and oven-drying (S. OD), the retention times of the compounds (RT), the theoretical and experimental retention indices (RI) and the source of the theoretical retention index value.

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Compound</th>
<th>RT (min)</th>
<th>R\textsubscript{E}</th>
<th>R\textsubscript{T}</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pentadecane</td>
<td>12.468</td>
<td>1500</td>
<td>1500</td>
<td>Adams, R. P. (2017)</td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Palmitic acid</td>
<td>22.383</td>
<td>1970</td>
<td>1977</td>
<td>nist</td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>(E)-Phytol</td>
<td>22.341</td>
<td>1967</td>
<td>1967</td>
<td>nist</td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>S. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Phenol, 2,2'-(1,1-dimethylethyl)-4-methyl</td>
<td>28.536</td>
<td>2398</td>
<td>2398</td>
<td>nist</td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
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<tr>
<td>9</td>
<td>Pentacosane</td>
<td>29.188</td>
<td>2500</td>
<td>2500</td>
<td>Adams, R. P. (2017)</td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
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<td>S. OD</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Squalene</td>
<td>32.102</td>
<td>2833</td>
<td>2835.8</td>
<td>nist</td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>S. OD</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>S. OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. FD</td>
<td>S. OD</td>
<td>dl-α-Tocopherol</td>
<td>36.099</td>
<td>3144</td>
</tr>
<tr>
<td>---</td>
<td>-------</td>
<td>-------</td>
<td>----------------</td>
<td>--------</td>
<td>------</td>
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<tr>
<td>16</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39.085</td>
<td>3297</td>
</tr>
<tr>
<td>18</td>
<td>S. FD</td>
<td>S. OD</td>
<td>γ-Sitosterol</td>
<td>39.752</td>
<td>3323</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39.790</td>
<td>3325</td>
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</tbody>
</table>

*Theoretical RIs from NIST Chemistry Webbook, based on Van Den Dool and Kratz equation with active phases HP-5MS and DB-5MS, and Adams, R. P. (2017) with active phases HP-5MS and DB-5.*

**Figure 32**: Chromatograms of oven-dried (above) and freeze-dried (below) leaves using GC-FID displaying the identified peaks.
Figure 33: Chromatograms of oven-dried (above) and freeze-dried (below) stems using GC-FID displaying the identified peaks.

Table 10: Volatile compounds of *P. oleracea*’s leaves and stems samples from freeze-drying and oven-drying methods and their % composition. The table shows the mean values of the replicates (n=3).

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compounds</th>
<th>FID Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FD</td>
</tr>
<tr>
<td>1</td>
<td>Palmitic acid</td>
<td>9.39** ± 1.49</td>
</tr>
<tr>
<td>2</td>
<td>(E)-phytol</td>
<td>0.28** ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>Linoleic acid</td>
<td>6.79*** ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>α-linolenic acid</td>
<td>17.77* ± 5.04</td>
</tr>
<tr>
<td>5</td>
<td>Phenol, 2,2'-methylenebis [(6-(1,1-dimethylethyl)-4-methyl terminated)]</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>Pentacosane</td>
<td>1.65* ± 0.19</td>
</tr>
<tr>
<td>7</td>
<td>Heptacosane</td>
<td>2.04* ± 0.39</td>
</tr>
<tr>
<td>8</td>
<td>Squalene</td>
<td>1.30 ± 0.35</td>
</tr>
<tr>
<td>9</td>
<td>Nonacosane</td>
<td>3.08** ± 0.72</td>
</tr>
</tbody>
</table>
The volatile compounds from the hexane leaf extracts include a variety of compounds, i.e. fatty acids, tocopherols, squalene and γ-sitosterol. The % area of the compounds is a comparative index of their concentration (Table 10). The major constituents of the non-polar extracts of leaves and stems are tritriacontane, hentriacontane, γ-sitosterol. E-Phytol is a product of the chlorophyll degradation pathway (Thamkaew, Sjöholm, & Galindo, 2021b). Its presence in relatively higher amounts in the oven-dried parts of purslane is a strong indicator that the chlorophylls were degraded during the heating process of the plant. E-Phytol is not present in the freeze-dried stems, while in the freeze-dried leaves, the relative abundance is seventy times higher than the oven-dried leaves. The only vitamin E isoforms in the leaves were α- and γ-tocopherols, but in stems γ-tocopherol is absent. Squalene and γ-sitosterol are also present in the leaves. Phytosterols can reduce the oxidative stress by increasing the activity of antioxidant enzymes (Anghel et al., 2015). Previous reports showed that about 73.1 mg of β-sitosterol can be found in 100 g of dried herbal product from Portulaca oleracea (Anghel et al., 2015), while another study reported a value of 11.88% for β-sitosterol in the leaves’ hydro-methanolic extracts (Saxena & Rao, 2021). Squalene content has been reported about 0.77% in the leaf hydro-methanolic extract of P. oleracea (Saxena & Rao, 2021), while the results from the GC-FID analysis (Table 10) showed a higher range (1.15% - 1.30%) of squalene in the leaves.

Also, all the alkanes, except from pentacosane, have a higher abundance in the oven-dried leaves, which along with the lower percentage of unsaturated (linoleic and α-linolenic acid) and saturated (palmitic acid) fatty acids shows that probably saturated fatty acids have been degraded in the oven-drying process to produce saturated carbon chains.

In comparison to the leaves, the concentration of the fatty acids in stems is significantly lower than the leaves. Also, γ-sitosterol is more abundant in the freeze-dried stems than the freeze-dried leaves, while the oven drying method significantly decreased the β-sitosterol content in the stems.

In general, the oven-drying method produces relatively higher amount of alkanes and E-Phytol, because of the degradation of the aroma compounds and pigments. Tocopherols and fatty acids, in particular, palmitic acid, linoleic acid and α-linolenic acid, of both freeze-dried aerial parts are significantly higher than the oven-dried ones. As a result, the freeze-drying method appears to preserve the fatty acids and the vitamins from degradation.
3.4 Phytochemical composition of the hydro-methanolic extracts

3.4.1 Qualitative analysis of the leaf extracts by UPLC-DAD-MS

The analysis of the oven dried leaves with UPLC-DAD-MS aided the identification of the phytoconstituents of the 70% hydro-methanolic extract (Figure 34). Using an in-house library for the species, the identification of the components is presented in Table 1.

Figure 34: Chromatogram of oven-dried leaves at 273 nm using HPLC-DAD-MS, displaying the numbered peaks.

Table 1: The identified metabolites of the hydroalcoholic oven-dried leaf extract by HPLC-DAD-MS at 273 nm. Contains the retention times, the molecular weight, the ions in the positive or/and negative ionization with the ion origin, their relative abundance in parentheses, and the UV–vis max absorption wavelengths.

<table>
<thead>
<tr>
<th>Peak No</th>
<th>RT (min)</th>
<th>M.W.</th>
<th>Negative Ionization m/z (% Relative Intensity)</th>
<th>Positive Ionization m/z (% Relative Intensity)</th>
<th>λmax (nm)</th>
<th>Class</th>
<th>Compound</th>
<th>Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>212</td>
<td>273 [M+AcOH-H]⁻ (100%)</td>
<td>213 [M+H]⁺ (100%)</td>
<td>203, 214</td>
<td>-</td>
<td>Unknown</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>111 [M-2H2O-CO2]⁻ (83%)</td>
<td>291 [M+DMSO+H]⁺ (29 %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>192</td>
<td>191 [M+H]⁺ (100%)</td>
<td>193 [M-H]⁻ (7%)</td>
<td></td>
<td></td>
<td>Organic acid</td>
<td>Isocitric acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>111 [M-2H2O-CO2]⁻ (83%)</td>
<td>231 [M+K]⁺ (40%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>165</td>
<td>-</td>
<td>166 [M+H]⁺ (100%)</td>
<td></td>
<td></td>
<td>Amino acid</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>183 [M+NHL]⁺ (25%)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>229 [M+MeCN+Na]⁺ (21%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
<td>192</td>
<td>191 [M-H]⁻ (100%)</td>
<td>-</td>
<td>-</td>
<td>Organic acid</td>
<td>Citric acid</td>
<td>(Farag &amp; Shakour, 2019a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>-------------</td>
<td>--------------</td>
<td>------------------</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>267</td>
<td>312 [M+FA-H]^+</td>
<td>(100%) 266 [M-H]^−</td>
<td>100%)</td>
<td>268 [M+H]^+</td>
<td>(100%)</td>
<td>190, 259</td>
</tr>
<tr>
<td>5</td>
<td>2.2</td>
<td>283</td>
<td>282 [M-H]^− (100%)</td>
<td>284 [M+H]^+ (42%)</td>
<td>164 [M+2Na]^2+ (100%)</td>
<td>251</td>
<td>Amino acid Guanosine</td>
<td>(Ahmed et al., 2022)</td>
</tr>
<tr>
<td>6</td>
<td>7.7</td>
<td>204</td>
<td>203 [M-H]^− (100%)</td>
<td>205 [M+H]^+ (16%)</td>
<td>188 [M-H+O+H]^+ (100%)</td>
<td>218, 280</td>
<td>Amino acid Tryptophan</td>
<td>(Farag &amp; Shakour, 2019a)</td>
</tr>
<tr>
<td>7</td>
<td>12.7</td>
<td>827</td>
<td>826 [M-H]^− (100%) 664 [M-glu-H]^− (6%)</td>
<td>828 [M+H]^+ (87%)</td>
<td>666 [M-glu-H]^+ (17%)</td>
<td>308</td>
<td>Alkaloids Oleracein P</td>
<td>(Jiao et al., 2015)</td>
</tr>
<tr>
<td>8</td>
<td>13.5</td>
<td>857</td>
<td>856 [M-H]^− (21%)</td>
<td>858 [M+H]^+ (13%) 696 [M-glu-H]^+ (12%)</td>
<td>332</td>
<td>Alkaloids Oleracein Q</td>
<td>(Jiao et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>14.1</td>
<td>665</td>
<td>664 [M-H]^− (100%) 502 [M-glu-H]^− (48%)</td>
<td>666 [M+H]^+ (100%) 504 [M-glu-H]^+ (20%)</td>
<td>308</td>
<td>Alkaloids Oleracein C</td>
<td>(Farag &amp; Shakour, 2019a)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>695</td>
<td>694 [M-H]^− (41%) 532 [M-glu-H]^− (100%)</td>
<td>696 [M+H]^+ (100%) 534 [M-glu-H]^+ (20%)</td>
<td>335</td>
<td>Alkaloids Oleracein D</td>
<td>(Farag &amp; Shakour, 2019a)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>16.5</td>
<td>665</td>
<td>664 [M-H]^− (100%)</td>
<td>666 [M+H]^+ (34%) 504 [M-glu-H]^+ (13%)</td>
<td>328</td>
<td>Alkaloids Oleracein C isomer</td>
<td>(Farag &amp; Shakour, 2019a)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>17.4</td>
<td>695</td>
<td>694 [M+H]^+ (100%)</td>
<td>696 [M+H]^+ (38%) 534 [M-glu-H]^+ (11%)</td>
<td>223, 338</td>
<td>Alkaloids Oleracein D isomer</td>
<td>(Farag &amp; Shakour, 2019a)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>18</td>
<td>402</td>
<td>401 [M-H]^− (100%)</td>
<td>403 [M+H]^+ (10%) 465 [M-MeCN+Na]^+ (100%)</td>
<td>227</td>
<td>Flavonoid Nobiletin</td>
<td>(X. Liu et al., 2019)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>18.3</td>
<td>452</td>
<td>-</td>
<td>453 [M+H]^+ (100%) 475 [M+Na]^+ (37%) 485 [M+CH3OH+H]^+ (34%) 159 [M+2H+Na]^3+ (52%)</td>
<td>221</td>
<td>-</td>
<td>Unknown</td>
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<tr>
<td>15</td>
<td>18.9</td>
<td>430</td>
<td>-</td>
<td>453 [M+Na]^+ (100%) 475 [M+2Na-H]^+ (40%) 491 [M+IsoProp+H]^+ (15%)</td>
<td>224</td>
<td>-</td>
<td>Unknown</td>
<td>-</td>
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</table>

66
<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Molecular Mass (g/mol)</th>
<th>Adduct</th>
<th>Mass Spectra Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>212</td>
<td>-</td>
<td>Unknown</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>192</td>
<td>-</td>
<td>Isocitric acid</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>192</td>
<td>-</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Peak (1), corresponding to an Unknown compound with molecular mass (M.W. = 212 g/mol), has a retention time (RT) of 1.1 min. The molecular ion with m/z 213—an ion of addition of one mole of hydrogen to the molecule of [M+H]^+—along with the fragment with the fragment with m/z 291 [M+DMSO+H]^+, are used to identify the chemical in positive ionization. The molecular with m/z 273, appears in the negative ionization, which is probably the result of the proton loss following the addition of an acetic acid molecule to the compound [M+AcOH-H]^-. Two λ max were observed in the UV absorbance at 203 and 214 nm.

The peak (2) with retention time (RT) = 1.3 min, shows fragments only in the negative MS ionization with fragments at m/z 191 [M+H]^+, 231 [M+K]^+ and a fragment at m/z 111 [M-2H2O-CO2]^-. Therefore, it has a molecular weight of 192 g/mol and it has been identified as Isocitric acid.

Peak (3) with retention time (RT) = 1.8 min corresponds to elution of Phenylalanine (M.W. = 165 g/mol) and Citric acid with molecular mass (M.W. = 192 g/mol). Identification of the citric acid conducted only in negative ionization, mainly with the molecular ion with m/z 191, corresponding to the loss of a proton from the unidentified compound [M-H]. Also, there is a molecular ion with m/z 111 corresponding to the loss of two water molecules and one atom of hydrogen [M-2H2O-CO2]^− to the molecule of the compound and the ion with m/z 173 that is linked...

Abbreviations: FA: formic acid, MeCN: acetonitrile, DMSO: Dimethyl sulfoxide, AcOH: acetic acid, glu: glucose, and IsoProp: Isopropanol.
to the fragment [M-H2O-H]. In the positive ionisation, the detection of Phenylalanine took place, with a fragment of m/z 166, corresponding to the addition of a hydrogen atom [M+H]+ to the molecule. Other fragments appear with m/z 183 and 229 that correspond to the ions [M+NH4]+ and [M+MeCN+Na]+, respectively.

Retention time (RT) = 2 min for peak (4) indicates a nitrogenous **Unknown** compound (M.W. = 267 g/mol) is present. The molecular ion with m/z 268, which is an ion of addition of one hydrogen molecule [M+H]+ to the molecule, is used to identify the molecular weight upon positive ionization. There is a molecular ion with m/z 266 in the negative ionization, which indicates the loss of a proton [M-H]. Also, there is a fragment with m/z 312 which indicates an addition of a formic acid molecule and the loss of a hydrogen atom [M+FA-H] to the molecule. Using the ultraviolet/visible spectral information, peak (4) demonstrated the highest absorption at \( \lambda \) max of 193 nm and the second highest absorption at 259 nm.

The peak (5) with retention time (RT) = 2.2 min corresponds to the amino acid **Guanosine** (M.W. = 283 g/mol). The identification of the compound in positive ionisation is done with m/z 284, which is an ion of addition of one mole of hydrogen to the molecule of [M+H]+, and the fragment with m/z 164 which corresponds to the addition of two sodium molecules to the molecule [M+2Na]+. The negative ionisation shows the molecular ion with m/z 282 corresponding to the loss of a proton from the guanosine molecule [M-H]-. The UV absorbance showed a maximum at 251 nm.

The peak (6) with retention time (RT) = 7.7 min corresponds to the amino acid **Tryptophan** (M.W. = 204 g/mol). The identification of the compound on positive ionisation is carried out by the molecular ion with m/z 288, which is an ion of addition of one molecule of hydrogen and loss of one molecule of water [M-H2O+H]+ to the molecule. Also, there is the molecular ion with m/z 205 corresponding to the addition of one mole of hydrogen to the [M+H]+ molecule of the compound. In the negative ionisation there is the molecular ion with m/z 203 corresponding to the loss of a proton [M-H]. In ultraviolet/visible spectrophotometry (HPLC-DAD) peak (6) showed maximum absorption at \( \lambda \) max at 218 nm, and second at 280 nm.

Peak (7) with retention time (RT) = 12.7 min corresponds to the compound **Oleracein P** (M.W. = 827 g/mol). The determination of the molecular mass of the compound in positive ionisation is carried out with the molecular ion with m/z 828, which is an ion of addition of one molecule of hydrogen and loss of one molecule of water [M-H2O+H]+ to the molecule. Also, there is the molecular ion with m/z 666 corresponding to the loss of a glucose molecule (-162 Da) and the addition of a hydrogen molecule [M-glu+H]+. In the negative ionisation, the molecular ion with m/z 826, corresponds to the loss of a proton from the compound [M-H]. The molecular ion initially cleaves a glucose molecule (-162 Da) and loses a hydrogen atom, after which the resulting fragment at m/z 664 is formed [M-glu-H]-. Peak (7) demonstrated the highest absorption at \( \lambda \) max of 308 nm.

Peak (8) (M.W. = 857 g/mol) corresponds to co-elution of **Oleracein Q** and an **Unknown** compound, with a retention time (RT) of 13.5 min. In positive ionization, the molecular ion with m/z 858, which is an ion of addition of a hydrogen molecule to the [M+H]+ molecule is used to determine the molecular mass of the compound in positive ionization. Also, there is a fragment with m/z 696 that corresponds to the loss of a glucose molecule and of a hydrogen atom (-162 Da) [M-glu-H]-. The
molecular ion with m/z 856 in the negative ionization is associated with the proton being removed from the molecule [M-H]. Regarding the Unknown compound, in the negative ionization, the main peak with m/z 387 is linked to the ion [M-H]. In positive ionization, the fragments with m/z 214, 217, 256 and 452, are linked to the ions [M+H+K]^+, [M+2Na]^{2+}, [M+3MeCN+2H]^{2+} and [M+MeCN+Na]^+, respectively. Peak (8) showed absorption with λ max at 332 nm.

Peak (9), with a retention time (RT) = 14.1 minutes, is associated with the compound **Oleracein C** (M.W. = 665 g/mol). The molecular mass in positive ionization is found using the molecular ion with m/z 666, which is an ion of addition of a hydrogen molecule to the [M+H]^+ molecule. Also, there is the molecular ion with m/z 504 corresponding to the loss of one molecule of hexose (-162 Da) and the addition of a hydrogen atom resulting to the fragment [M-glu+H]^+ of the compound. The removal of the proton from the molecule [M-H]^− is linked to the molecular ion with m/z 664 in the negative ionization. The fragment at m/z 502 is formed when the molecular ion first loses a glucose molecule (-162 Da) and a hydrogen atom [M-glu-H]. Peak (9) demonstrated that the absorption peaked at λ max 308 nm.

Peak (10) is linked to the compound **Oleracein D** (M.W. = 695 g/mol), with a retention time (RT) of 15.0 minutes. An ion of addition of a hydrogen molecule to the [M+H]^+ molecule, the molecular ion with m/z 696, is used to determine the molecular mass in positive ionization. The compound's fragment [M-glu+H]^+ is also represented by the molecular ion with m/z 534, which is associated with the addition of a hydrogen atom and the loss of one glucose molecule (-162 Da). In the negative ionization, the molecular ion with m/z 694 is associated with the proton removal from the molecule [M-H]. The molecular ion initially loses a glucose molecule (-162 Da) and a hydrogen atom [M-glu-H], which results in the fragment at m/z 532. Using the UV/Vis spectral information a high peak with λ max at 335 nm was observed.

Peak (11) has a retention time (RT) of 16.5 minutes and is associated with the compound **Oleracein C**, most likely another isomer (M.W. = 665 g/mol). The molecular mass in positive ionization is found using the molecular ion with m/z 666, which is an ion of addition of a hydrogen molecule to the [M+H]^+ molecule. The molecular ion with m/z 504, which is linked to the addition of a hydrogen atom and the loss of one glucose molecule (-162 Da), is another representation of the compound's fragment [M-glu+H]^+. The elimination of a proton from the molecule [M-H]^− is linked to the molecular ion with m/z 664 in negative ionization. A peak with λ max at 328 nm was discovered using the UV/Vis spectral information.

Peak (12) is linked to the compound **Oleracein D**, which is another isomer (M.W. = 695 g/mol) and has a retention time (RT) of 17.4 minutes. The molecular ion with m/z 696—an ion of addition of a hydrogen molecule to the [M+H]^+ molecule—is used to determine the molecular mass in positive ionization. Another compound's fragment [M-glu+H]^+ is the molecular ion with m/z 534, which is associated with the addition of a hydrogen atom and the loss of one glucose molecule (-162 Da). In negative ionization, the molecular ion with m/z 694 is associated with the removal of a proton from the molecule [M-H]. Peak (12) showed that the absorption was highest at λ max of 223 nm and second highest at 338 nm.
Peak (13) is associated with the compound **Nobiletin**, which has a retention time (RT) of 18.0 minutes with M.W. = 402 g/mol. The molecular mass in positive ionization is found using the molecular ion with m/z 403, which is an ion of addition of a hydrogen molecule to the [M+H]^+ molecule. The molecular ion with m/z 465, which is linked to the addition of a sodium atom (+23 Da) and an acetonitrile molecule (+40 Da), is another compound's fragment [M-MeCN+Na]^+. The elimination of a proton from the molecule [M-H]^− is linked to the molecular ion with m/z 401 in negative ionization. A λ max at 227 nm was discovered from the UV/Vis spectral information.

Peak (14) is linked to an **Unknown** compound, which has an M.W. = 452 g/mol and an 18.3-minute retention time (RT). The molecular ion with m/z 453, which is an ion of addition of a hydrogen molecule to the [M+H]^+ molecule, is used to determine the molecular mass in positive ionization. The fragment with m/z 475, is associated with the addition of a sodium atom (+23 Da), and the m/z 489 is formed due to the addition of a hydrogen atom and a methanol molecule [M+CH3COH+H]^+. The m/z 159 is linked to the addition of two hydrogen atoms and one sodium atom to the molecule [M+2H+Na]^3+, and the m/z 485 corresponds to the ion [M+MeOH+H]^+. In negative ionization, no fragments were detected regarding the molecule. Using the UV/Vis spectral data, a λ max at 221 nm was discovered for the compound.

Peak (15) is associated with two **Unknown** compounds, which have a retention time (RT) of 18.9 minutes with M.W. = 430 g/mol and M.W. = 400 g/mol. The molecular mass of the first one in positive ionization is found using the molecular ion with m/z 453, which is an ion of addition of a sodium atom to the [M+Na]^+ molecule. There can be detected also the fragments with m/z 475 and 491, which are linked to the ions [M+2Na-H]^+ and [M+IsoProp+H]^+, respectively. The other Unknown compound was identified through the main fragment in the negative ionization with m/z 399 that corresponds to the loss of a hydrogen atom from the molecule [M-H]^−. Also, there is the fragment with m/z 141 that is linked to the ion [M+2H+Na]^3+. A λ max at 224 nm was discovered from the UV/Vis spectral information.

Peak (16) is associated with the chemical **Oleracein B**, which has a retention time (RT) of 19.9 minutes and is another isomer with M.W. = 533 g/mol. The molecular mass in positive ionization is found using the molecular ion with m/z 534, which is an ion of addition of a hydrogen molecule to the [M+H]^+ molecule. The elimination of a proton from the molecule [M-H]^− is linked to the molecular ion with m/z 532 in negative ionization. The UV/Vis spectral data was used to identify two peaks with λ max at 223 and 332 nm.

Peak (17) has a retention time (RT) of 21.1 min, indicates a co-elution of an **Unknown** compound (M.W. = 597 g/mol) and **Oleracein O** (M.W. = 871 g/mol). Utilizing the molecular ion with m/z 598—an ion that adds one mole of hydrogen to the [M+H]^+ molecule—one may identify the molecular weight of the unknown compound in positive ionization. The first molecular ion to appear in the negative ionization process is the molecular ion with m/z 596; this ion is related to the proton loss from the unknown compound [M-H]^−. Regarding the compound Oleracein O, the main fragment is an ion of addition of a hydrogen molecule to the [M+H]^+ molecule with m/z 872, which is used to determine the molecular mass in positive ionization. In the negative ionization, the molecular ion
with m/z 870 is associated with the proton removal from the molecule \([M-H]^-\). A peak of \(\lambda_{\text{max}}\) 224 nm was observed for this compound in the UV spectrum.

**Ox-DGMG (18:3 10)** (M.W. = 692 g/mol) is the glycolipid represented by peak (18) with retention time (RT) = 23.1 min. In positive ionization, the molecular ion with m/z 693—an ion of addition of one hydrogen molecule \([M+H]^+\) to the molecule—is used to identify the chemical. The molecular ion with m/z 691, which represents the loss of a proton \([M-H]^-\), is present in the negative ionization. A maximum absorption at \(\lambda_{\text{max}}\) at 227 nm has been observed in peak (18) of ultraviolet/visible spectrophotometry (HPLC-DAD). At the same retention time (RT) = 23.1 min, an **Unknown** compound co-elutes with Ox-DGMG (18:3 10), with an ultraviolet spectrum peak at \(\lambda_{\text{max}}\) 315 nm.

The glycolipid indicated by peak (19) with retention time (RT) = 23.5 min is **Ox-DGMG (18:3 10)** (M.W. = 692 g/mol). The molecular ion with m/z 693, which is an ion of addition of one hydrogen molecule \([M+H]^+\) to the molecule, is utilized in positive ionization to identify the chemical. The negative ionization contains the molecular ion with m/z 691, which denotes the loss of a proton \([M-H]^-\). UV/visible spectrophotometry (HPLC-DAD) has detected a maximum absorption at \(\lambda_{\text{max}}\) at 229 nm.

Peak (20) corresponds to the compound **Desmethyl tocopherol**, with a M.W. = 388 g/mol and a 25.1-minute retention time (RT). Using the molecular ion with m/z 389, which is an ion of addition of a hydrogen molecule to the \([M+H]^+\) molecule, one may determine the molecular mass in positive ionization. The negative ionization of a molecule with m/z 387 is associated with the removal of a proton from the molecule \([M-H]^-\). A maximum absorption with \(\lambda_{\text{max}}\) 232 nm has been determined using UV/visible spectrophotometry (HPLC-DAD).

Peak (21) has a retention time (RT) of 27.1 min, indicating an **Unknown** compound (M.W. = 537 g/mol). Positive ionization allows for the identification of the compound using the molecular ion with m/z 538, which adds one atom of hydrogen to the \([M+H]^+\) molecule. The molecular ion at m/z 536 is the only one to emerge during the negative ionization process; it is associated with the proton loss from the unidentified molecule \([M-H]^-\). For this compound, an ultraviolet spectrum peak at \(\lambda_{\text{max}}\) 224 nm was observed.

A glycolipid, di-galactosyl-monoacylglycerol (**DGMG (18:3)**) (M.W. = 676 g/mol) is indicated by peak (22) with retention time (RT) = 27.7 min. When using positive ionization, no fragments related to the compound were detected. There is a molecular ion with m/z 675 in the negative ionization, indicating a proton \([M-H]^-\) loss.

Peak (23) displays an **Unknown** compound (M.W. = 658 g/mol) with a retention time (RT) of 32.8 min. It is possible to identify the compound via positive ionization by using the molecular ion with m/z 659, which adds one atom of hydrogen to the \([M+H]^+\) molecule. The fragment with m/z 657 is the molecular ion to emerge during the negative ionization process; it is associated with the proton loss from the unidentified compound \([M-H]^-\).
3.4.2 Analysis of the hydro-methanolic leaf extracts by HPLC-DAD

**Figure 35:** Chromatogram of freeze-dried leaves at 330 nm using the HPLC-DAD technique, displaying the numbered peaks of the cyclo-dopa alkaloids.

**Figure 36:** Chromatogram of oven-dried leaves at 330 nm using the HPLC-DAD technique, displaying the numbered peaks of the cyclo-dopa alkaloids.

In the hydro-methanolic extracts of the freeze-dried and oven-dried leaves 7 characteristic cyclo-dopa alkaloids of *P. oleracea* were identified. Their UV/vis spectra from the HPLC-DAD analysis (Figures 35, 36) and their chemical structures are displayed in Table 12. The chemical structures were designed using ChemDraw and information from previous studies (Jiao et al., 2015) (Xiang et al., 2005) (Farag & Shakour, 2019b).

Table 12: Metabolites of the hydro-methanolic extract of oven-dried leaves, their chemical structures, UV absorption spectra at 320 nm, and relevant literature.
<table>
<thead>
<tr>
<th>Compound (peak No)</th>
<th>Chemical Structure</th>
<th>UV Spectra</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleracein P (1)</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td><img src="image2" alt="UV Spectra" /></td>
<td>305, 340</td>
<td>(Jiao et al., 2015)</td>
</tr>
<tr>
<td>Oleracein Q (2)</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td><img src="image4" alt="UV Spectra" /></td>
<td>290, 335</td>
<td>(Jiao et al., 2015)</td>
</tr>
<tr>
<td>Oleracein C (3)</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td><img src="image6" alt="UV Spectra" /></td>
<td>300, 340</td>
<td>(S. A. Petropoulos et al., 2019) (Xiang et al., 2005)</td>
</tr>
<tr>
<td>Oleracein D (4)</td>
<td><img src="image7" alt="Chemical Structure" /></td>
<td><img src="image8" alt="UV Spectra" /></td>
<td>290, 330</td>
<td>(Xiang et al., 2005)</td>
</tr>
</tbody>
</table>
Oleracein C/isomer (5)  
(Xiang et al., 2005)

Oleracein D/isomer (6)  
(290, 330)  
(Xiang et al., 2005)

Unknown (7)  
(240, 340)

Oleracein B (8)  
(Farag & Shako ur, 2019a)
Combining the identification with a UPLC-DAD-MS apparatus, that was conducted and analyzed previously, the quantification of the alkaloids that were observed at the 70% hydro-methanolic extracts of the leaves, from the two drying methods, was conducted using a system of liquid chromatography with UV/visible spectrophotometry (HPLC-DAD).
Most of the cyclo-dopa amides (Oleraceins) exhibit a similar UV max, as the max wavelengths are between 300 - 340 nm. At the same time, each of these compounds has a characteristic UV absorbance spectrum, as seen in Table 12, which enables to distinguish them. Oleracein C displays a UV spectrum with a minor shoulder at 300 nm, which does not appear at its isomer. Shoulders in peaks are common in highly conjugated systems, in this case oleraceins, that consist of several π-bonds due to the aromatic rings and therefore act as chromophores, thus forming a shoulder in some cases. Also, the appearance of shoulders is solvent dependent. Similarly, Oleracein D displays two λ max at 290 nm and 330 nm, with the first one appearing as a shoulder in both isomers.

The quantification performed by HPLC-DAD technique of the phenolic compounds of leaf extracts was carried out with an external calibration curve using trans-p-coumaric acid as the reference compound, because there are no commercial standards of these phenolic alkaloids and therefore, was considered the most suitable standard available in the laboratory according to its UV/vis spectrum. The linearity (R²) of the standard calibration curve was 0.9997.

Table 13: Quantitative alkaloid composition of the leaf extracts from the two drying methods. The table shows the mean values of the replicates (n=4).

<table>
<thead>
<tr>
<th>Compound</th>
<th>mg/100g DW</th>
<th>Leaves FD</th>
<th>Leaves OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleracein P</td>
<td>245.65 ± 0.61**</td>
<td>342.39 ± 1.83**</td>
<td></td>
</tr>
<tr>
<td>Oleracein Q</td>
<td>174.07 ± 0.41*</td>
<td>151.60 ± 1.24*</td>
<td></td>
</tr>
<tr>
<td>Oleracein C</td>
<td>70.46 ± 0.58***</td>
<td>381.60 ± 1.81***</td>
<td></td>
</tr>
<tr>
<td>Oleracein D</td>
<td>47.31 ± 0.19**</td>
<td>185.38 ± 1.83**</td>
<td></td>
</tr>
<tr>
<td>Oleracein C/isomer</td>
<td>23.38 ± 0.16**</td>
<td>142.38 ± 1.68**</td>
<td></td>
</tr>
<tr>
<td>Oleracein D/isomer</td>
<td>17.12 ± 0.06***</td>
<td>92.40 ± 0.51***</td>
<td></td>
</tr>
<tr>
<td>Oleracein B</td>
<td>21.15 ± 0.22***</td>
<td>166.92 ± 0.98***</td>
<td></td>
</tr>
<tr>
<td>SUM (mg/100g DW)</td>
<td>599.14</td>
<td>1462.67</td>
<td></td>
</tr>
</tbody>
</table>

OD: Oven-Dried; FD: Freeze-Dried
Symbols (*,**,***) within columns indicate significant differences (*p value < 0.05, **p value < 0.01, ***p value<0.001) between the same aerial parts of the plant with different drying methods.

According to the results from Table 13, the leaves from the oven-drying process contain a significantly higher concentration of cyclo-dopa alkaloids. The freeze-drying method generally protects the aroma compounds from degradation (Thamkaew et al., 2021b). In this case, oven-drying resulted in higher amounts of phenolic alkaloids than freeze-drying (Thamkaew et al., 2021b). A possible explanation is the low temperature (50°C) during the drying process that enables the intercellular spaces to collapse, thus releasing more phenolic compounds.

Oleracein P has the highest concentration in the freeze-dried leaves (245.65 ± 0.61 mg/100g DW), while in the oven-dried leaves Oleracein C is the most abundant (381.60 ± 1.81 mg/100g DW).
Oleracein B has the lowest concentration in the freeze-dried leaves (21.15 ± 0.22 mg/100g DW), while in the oven-dried leaves an isomer of Oleracein D is the less abundant alkaloid (92.40 ± 0.51 ± 0.22 mg/100g DW). The most significant differences ($p<0.001$) in the concentrations of the two leaf samples can be observed between the Oleraceins C, D (isomer) and B.

While in most cases freeze-drying protects the sensitive compounds, the loss of phenolics is often observed (Thamkaew et al., 2021b). Interestingly, another study demonstrated that hot-air drying (or oven-drying) at 50 ºC preserved the phenolics and flavonoids from purslane’s leaves compared to freeze-drying (Youssef & Mokhtar, 2014).

### 3.5 Quantification of the fatty acid methyl esters by GC-FID

After the one-step in situ transesterification, for the determination of the lipid content, a GC coupled with a flame ionization detector and a capillary column was used to examine the resulting fatty acid methyl esters.

Table 14: Fatty acid profile and content on dry weight (mg/g DW) basis for the leaves and stems from the two drying methods and for the seeds.

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Leaves OD</th>
<th>Leaves FD</th>
<th>Stems OD</th>
<th>Stems FD</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6:0</td>
<td>-</td>
<td>-</td>
<td>trace</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C8:0</td>
<td>14.27 ± 7.22</td>
<td>9.18 ± 10.7</td>
<td>8.25 ± 1.93</td>
<td>6.14 ± 8.75</td>
<td>0.81 ± 0.42</td>
</tr>
<tr>
<td>C12:0</td>
<td>0.6 ± 0.19</td>
<td>0.57± 0.20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C13:0</td>
<td>0.36 ± 0.49</td>
<td>0.37 ± 0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C14:0 (Myristic acid)</td>
<td>0.7 ± 0.63</td>
<td>0.77 ± 0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C14:1</td>
<td>-</td>
<td>-</td>
<td>0.38 ± 0.44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C15:1</td>
<td>0.33 ± 0.18</td>
<td>0.32± 0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C16:0 (Palmitic acid)</td>
<td>3.27** ± 1.76</td>
<td>4.28 ± 0.78</td>
<td>1.64 ± 2.61</td>
<td>1.39 ± 0.73</td>
<td>7.92 ± 0.16</td>
</tr>
<tr>
<td>C16:1 (Palmitoleic acid)</td>
<td>0.39 ± 0.02</td>
<td>0.83 ± 0.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.43 ± 0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C18:0 (Stearic acid)</td>
<td>0.77** ± 0.19</td>
<td>1.36 ± 0.49</td>
<td>0.8 ± 0.79</td>
<td>0.75 ± 0.39</td>
<td>9.66 ± 1.15</td>
</tr>
<tr>
<td>C18:1 (Oleic acid)</td>
<td>0.95 ± 0.19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.5 ± 0.43</td>
</tr>
<tr>
<td>C18:2 (Linoleic acid)</td>
<td>3.37* ± 0.02</td>
<td>6.91 ± 2.07</td>
<td>-</td>
<td>3.19 ± 2.24</td>
<td>64.2 ± 2.34</td>
</tr>
<tr>
<td>C18:3n3 (α-Linolenic acid)</td>
<td>5.54 ± 2.09</td>
<td>12.17 ± 2.07</td>
<td>-</td>
<td>1.22 ± 0.68</td>
<td>21.07 ± 0.55</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.92 ± 0.22</td>
<td>0.97 ± 0.33</td>
<td>-</td>
<td>-</td>
<td>0.86 ± 0.30</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.53 ± 0.08</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C20:5n3</td>
<td>1.98 ± 0.68</td>
<td>1.79 ± 0.46</td>
<td>0.93 ± 0.18</td>
<td>0.73 ± 0.57</td>
<td>-</td>
</tr>
<tr>
<td>C24:0 (Lignoceric acid)</td>
<td>2.81 ± 0.72</td>
<td>2.87 ± 1.05</td>
<td>1.88 ± 1.95</td>
<td>2.1 ± 0.67</td>
<td>0.61 ± 0.27</td>
</tr>
</tbody>
</table>
In general, the leaves have a greater fatty acid composition than the stems. In particular, the freeze-dried aerial parts showed a higher content in unsaturated fatty acids in contrast with the oven-dried ones. The use of the freeze-drying method showed a protection from degradation of the polysaturated fatty acids. For instance, the content of linoleic acid and α-linolenic acid in the freeze-dried leaves (6.91 mg/g DW and 12.17 mg/g DW, respectively) is almost twice as the ones from the oven-dried leaves (3.37 mg/g DW and 5.54 mg/g DW, respectively). Freeze-dried stems contain a small amount of α-linolenic acid (1.22 mg/g DW), while it’s absent in the oven-dried stems. Other studies have also reported α-linolenic acid to be most abundant in leaves (41.4% - 66.4% of total fatty acids) than stems (2.4% - 5.9% of total fatty acids) (Uddin et al., 2014). According to previous publications, the results seem to differ (Nemzer et al., 2020). In particular, α-linolenic acid in wild leaves has been found around 1.19 mg/g DW, whereas in this study the α-linolenic acid content was 5.54 mg/g DW for the oven-dried leaves, and 12.17 mg/g DW for the freeze-dried leaves (Nemzer et al., 2020). A rise in the concentration can also be observed for the stems, where wild stems resulted in around 0.30 mg/g DW of α-linolenic acid (Nemzer et al., 2020), while in the present study, the concentration was found to be 1.22 mg/g DW.

The content in monosaturated fatty acids with small carbon chains (C8:0, C12:0 and C13:0) is higher in the oven-dried leaves, where in the freeze-dried leaves is lower or not present. This indicates that polysaturated fatty acids with longer carbon chains have been deteriorated, probably due to the heat in the drying process, and produced saturated small-chain fatty acids. In addition, palmitic acid and stearic acid in the freeze-dried leaves (4.28 mg/g DW and 1.36 mg/g DW, respectively) are significantly higher than the oven-dried leaves (3.27 mg/g DW and 0.77 mg/g DW, respectively). Previous studies have proven the abundance of palmitic acid (20.2–21.8%) and linoleic acid (23.02–27.11%) in stems (S. A. Petropoulos et al., 2019), that are in accordance with the present results for the freeze-dried stems (1.39 mg/g DW and 3.19 mg/g DW, respectively). A significant lower concentration, in comparison with the present results, of palmitic acid, stearic acid and linoleic acid (0.52 mg/g DW, 0.08 mg/g DW, and 0.56 mg/g DW, respectively) for wild leaves and stems (0.20 mg/g DW, 0.05 mg/g DW, and 0.35 mg/g DW, respectively) has been previously reported (Nemzer et al., 2020).

Regarding the seeds of Portulaca oleracea, the abundance of fatty acids exceeds the leaves and stems. Palmitic acid (7.92 mg/g DW), stearic acid (9.66 mg/g DW) and especially, α-linolenic acid (21.07 mg/g DW) and linoleic acid (64.2 mg/g DW) have a high concentration in relation to the leaves and

| sum (mg/g DW) | 37.22 ± 0.92 | 42.4 ± 1.63 | 13.88 ± 1.31 | 15.52 ± 2 | 106.63 ± 0.7 |
| sum (% dry)  | 3.72%        | 4.24%        | 1.39%        | 1.55%     | 10.66%       |
| Total MUFAs (mg/g DW) | 2.21   | 1.15   | 0.38   | 0      | 1.50       |
| Total PUFAs (mg/g DW) | 10.9   | 20.86  | 1.88   | 5.15   | 85.88      |
| Total SFAs (mg/g DW) | 24.12  | 20.38  | 12.58  | 10.38  | 19.25      |

MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; SFAs: saturated fatty acids.

OD: Oven-Dried; FD: Freeze-Dried

Symbols (*,***) within columns indicate significant differences (*p value < 0.05, **p value < 0.01) between the same aerial parts of the plant with different drying methods.
stems from both drying methods. Especially there is a significant difference of linoleic acid in the seeds (64.2 mg/g DW), is twenty times higher than the oven-dried leaves and freeze-dried stems (3.37 mg/g DW and 3.19 mg/g DW, respectively), and ten times higher than the freeze-dried leaves (6.91 mg/g DW). Polyunsaturated fatty acids (PUFAs) were shown to be most abundant in seeds (85.88 mg/g DW), followed by leaves (10.9 - 20.86 mg/g DW), and stems (1.88 - 5.15 mg/g DW). This suggests that the seeds and the leaves are a great source of ω-3 fatty acids. Overall, linoleic acid (18:2n6), palmitic acid (16:0), and LNA (18:3n3) were the main fatty acids found in all tissues. Additionally, smaller concentrations of 12:0, 13:0, 14:0, 15:1, 18:0, 20:0, 20:5n3, and 24:0 were found.

3.6 Antioxidant potential and total phenolic content of the hydro-methanolic extracts

A great deal of research has been done on the distinct health advantages of natural antioxidants as well as the substantial impacts of free radicals and active oxygen species on cellular damage and age-related disorders.

We used three distinct photometric techniques—FRAP, DPPH, and FOLIN—to evaluate the antioxidant potential of leaves and stems (Table 15). The extracts' antioxidant reducing property was measured using the FRAP method, and the results were expressed in FeSO₄·7H₂O equivalents. The extracts' ability to bind DPPH radicals was calculated using the DPPH method, and the results were expressed as the IC₅₀ value, or the concentration that causes 50% free radical binding.

A popular technique for determining a sample's total polyphenolic content or researching antioxidant activity is the Folin-Ciocalteu reaction, which is based on electron transfer and analyzes a sample's reducing power.

Table 15: The antioxidant activity of the leaves and stems samples and their total phenolic content from the two drying methods.

<table>
<thead>
<tr>
<th></th>
<th>Leaves OD</th>
<th>Leaves FD</th>
<th>Stems OD</th>
<th>Stems FD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAP assay¹</td>
<td>54.10** ± 1.57</td>
<td>50.36** ± 0.88</td>
<td>22.24*** ± 0.59</td>
<td>37.25*** ± 1.41</td>
</tr>
<tr>
<td>DPPH assay²</td>
<td>7.26 ± 1.66</td>
<td>8.53 ± 0.93</td>
<td>82.99** ± 8.00</td>
<td>11.16** ± 0.31</td>
</tr>
<tr>
<td>TPC³</td>
<td>36.86** ± 0.87</td>
<td>32.47** ± 0.25</td>
<td>11.10*** ± 0.33</td>
<td>18.98*** ± 0.67</td>
</tr>
</tbody>
</table>

OD: Oven-Dried; FD: Freeze-Dried

1: mg FeSO₄·7H₂O equivalent/g dry extract (DE) (y = 3.5475x – 0.0479, R² = 0.9936)
2: IC₅₀ values (mg DE/ml)
3: mg GA equivalent/g DE (y = 0.0026x + 0.0037, R² = 0.987)

Symbols (**, ***): within columns indicate significant differences (**p value < 0.01, ***p value < 0.001) between the same aerial parts of the plant with different drying methods.
The results from both antioxidant activity assays show the higher antioxidant activity of the leaves compared to the stems, regardless of the drying method. The hydro-methanolic extract of the oven-dried leaves displayed the lowest IC\textsubscript{50} value (7.26 mg DE/ml), followed by the freeze-dried leaves (8.53 mg DE/ml). On the other hand, the lowest IC\textsubscript{50} was observed in the oven-dried stems (82.99 mg DE/ml) with a significant difference (p<0.001) to the freeze-dried stems (11.16 mg DE/ml). In comparison with previous studies (Sicari V. et al., 2018b), where the IC\textsubscript{50} value of the dried leaves was found 53.92 mg/ml, with a similar extraction system (MeOH:H\textsubscript{2}O, 80:20 v/v), the antioxidant activity seems to be higher in the hydro-methanolic extracts of leaves from this study (7.26 mg/ml for oven-dried leaves, and 8.53 mg/ml for freeze-dried leaves). Similarly, the FRAP assay results indicate that the leaves, in both drying methods, have a higher ferric reducing activity power than the stems, and in particular, the oven-dried stems have the lowest antioxidant activity (22.24 mg FeSO\textsubscript{4} 7H2O eq/g DE). These findings are further supported by the total phenolic content of the plant parts. The leaves exerted higher antioxidant activity than the stems, which is in line with the phenolic content of the oven-dried leaves (36.86 mg GA eq/g DE), followed by the freeze-dried leaves (32.47 mg GA eq/g DE). The significantly (p<0.001) lower phenolic content of the oven-dried stems (11.10 mg GA eq/g DE), interprets the low ferric reducing activity power activity and the high IC\textsubscript{50} value.

This study showed that the antioxidant activity varied greatly throughout purslane’s parts and drying methods. It can be concluded that the freeze-drying method provides protection of the bioactive compounds, resulting in a higher antioxidant potential of the extracts. Between the plant parts, the leaves showed a higher antioxidant activity than the stems, probably due to the higher total phenolic content, the presence of tocopherols, and alkaloids, as demonstrated before.

### 3.7 Determination of total sugar content

Using the anthrone-sulfuric acid technique, the total sugar content was determined and expressed as mg saccharose equivalents per g of dry extract. Saccharose, a disaccharide, is a sugar composed of glucose and fructose subunits and was used as a reference compound for this measurement, due to the presence of these sugars in the leaves and stems (S. A. Petropoulos et al., 2019).

<table>
<thead>
<tr>
<th>mg saccharose/ g dry extract</th>
<th>Leaves OD</th>
<th>Leaves FD</th>
<th>Stems OD</th>
<th>Stems FD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD: Oven-Dried; FD: Freeze-Dried</td>
<td>65.28** ± 8.83</td>
<td>89.89** ± 4.23</td>
<td>61.04*** ± 7.97</td>
<td>334.65*** ± 11.36</td>
</tr>
</tbody>
</table>

Symbols (**,***) within columns indicate significant differences (**p value < 0.01, ***p value < 0.001) between the same aerial parts of the plant with different drying methods.

The free sugar composition of leaves and stems in relation to the drying method showed an abundance of sugar content in the freeze-dried aerial parts. In particular, the freeze-dried leaves have a higher sugar concentration (89.89 mg/g DE) than the oven-dried (65.28 mg/g DE). The most significant difference is in the sugar content of the stems, where the freeze-dried stems contain almost five times more...
carbohydrates (334.65 mg/g DE) than the oven-dried stems (61.04 mg/g DE). The total sugar content in stems is higher than the leaves and depends on the harvest stage, as the fructose and glucose content increases in later harvest stages (S. A. Petropoulos et al., 2019). Previous reposts demonstrated the dose-dependent antioxidant activity of purslane’s polysaccharides (M. Chen et al., 2024). Specially, the scavenging activity against the DPPH radical resulted in an IC$_{50}$ value of 1.97 μg/mL, while the scavenging activity against the OH radical had an IC$_{50}$ value of 4.29 μg/mL (M. Chen et al., 2024). Since $P$. oleracea’s polysaccharides are among the primary constituents of the crude extract (M. Chen et al., 2024), their antioxidant contribution is well established, thus indicating their contribution to the overall evaluation of purslane’s extracts.
CHAPTER 4

4 CONCLUSIONS
The current study aimed to investigate the use of *Portulaca oleracea* as a raw material for cosmetics. For the first time, a thorough examination of the plant was conducted, comparing two distinct aerial parts of the same plant that were dried using two different techniques. This comparison examines the polar and non-polar extracts' composition and content as well as their antioxidant activity, the sugar and phenolic content. The study's conclusions are as follows:

- The comparison of the drying methods leads to the conclusion that freeze-drying protects most of the plants' constituents from degradation. This is shown especially from the fatty acid content, where polysaturated fatty acids have a higher concentration in both aerial parts from the freeze-drying process. Especially, linoleic acid, α-linolenic acid and are most abundant in freeze-dried stems. In addition, the polysaturated fatty acids' content of the seeds is 10-20 times higher than the leaves and stems from both drying methods. Purslane (*Portulaca oleracea*), has a high potential as a cosmetic raw material, due to the omega-3, -6 and -9 fatty acids, which display wound-healing and antibacterial properties. Its historic usage in the local treatment of inflammatory disorders further supports this theory (Popescu et al., 2018).

- The hexane extracts of the leaves revealed the presence of Vitamin E isoforms, fatty acids, squalene and γ-sitosterol. Tocopherol -a and -γ display antioxidant properties, (S. A. Petropoulos et al., 2019) while squalene, is considered a humectant in cosmetic industry that resemble human sebum membrane and can replenish the lipids between cells and on the surface of the cuticle. It can also enter the skins' cuticle quickly and combine with water to lock in moisture, thereby enhancing the function of the skin barrier (Zhao, Wang, Jiang, & Mu, 2020).

- The analysis of the hydro-methanolic extracts of the leaves lead to the identification and quantification of seven cyclo-dopa alkaloids, namely oleraceins. From the data obtained, the concentration of the alkaloids is a bit higher in the oven-dried leaves than the freeze-dried leaves, which indicates that the drying method with the usage of heat doesn’t affect the leaves' content in oleraceins. Oleracein P followed by oleracein Q were the most abundant in the freeze-dried leaves, whereas oleracein C followed by oleracein P had the highest concentration in the oven-dried leaves. These unique alkaloids have proven antioxidant activity (Z. Yang et al., 2009) and their potential biological activities are yet to be discovered.

- The measurements of the total sugar content showed that freeze-dried leaves and stems have a higher content in carbohydrates than the oven-dried ones. The most significant difference appears in the stems, where the freeze-drying method protected the carbohydrates from degradation and their concentration is five times higher than the oven-dried stems. These results further support the findings regarding the total phenolic content and antioxidant activity of the freeze-dried stems compared to the oven-dried stems, as the sugar content contributes to the antioxidant capacity and the phenolic content of the extracts.
Regarding the antioxidant activity of the hydro-methanolic extracts, the leaves from the two drying methods exhibit increased level of antioxidant activity, in both FRAP and DPPH assays than the stems. These findings are also confirmed from the TPC assay, where the total phenolic content in the leaves is 2-3 times higher than the stems, in both drying methods. The antioxidant capacity between the leaf samples from the two drying methods, display a slight significant difference, while in the stems, there is a noteworthy significant difference in the antioxidant activity assays and in the total phenolic content. This can be explained by the considerably higher sugar content of the freeze-dried stems in relation to the oven-dried stems (D. Chen et al., 2019a).

In conclusion, this research, as far as we know, was the first time that two distinct aerial parts of Portulaca oleracea were studied throughout the comparison of two drying methods, namely freeze-drying and oven-drying. The hydro-methanolic extracts of the freeze-dried leaves has the highest content in ω-3 and ω-6 fatty acids. The antioxidant activity of the leaves is significantly higher than the stems, probably due to the presence of the cyclo-dopa alkaloids and to the higher phenolic content, in comparison to the stems. Overall, the freeze-drying method in general protects the fatty acids and the carbohydrates of both leaves and stems.

Purslane is a highly promising candidate for use as a cosmetic ingredient due to its high nutritional constituents, particularly antioxidants agents like vitamin E, omega-3 fatty acids, and oleraceins, as well as its traditional use in the topical treatment of inflammatory conditions, wound-healing and antimicrobial properties. Additionally, cosmetics composed of P. oleracea’s extracts have anti-aging, wrinkle improvement, skin-whitening, and acne improvement effects (Chen et al., 2019a). However, further studies on skin wound-healing and skin-whitening, through tyrosinase inhibition, properties of the extracts would complete the study.


Linné, C. von, & Salvius, L. (1753). *Caroli Linnaei ... Species plantarum :exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas...*


CHAPTER

6 ANNEX
6.1 Mass spectra of the hexane extracts

Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-, MW: 340 g/mol, CAS#: 119-47-1

n-Hexadecanoic acid, MW: 256 g/mol, CAS#: 57-10-3

(E)-Phytol, MW: 296 g/mol, CAS#: 150-86-7

Linoleic acid, MW: 280 g/mol, CAS#: 60-33-3
α-Linolenic acid, MW: 278 g/mol, CAS#: 463-40-1

Squalene, MW: 410 g/mol, CAS#: 7683-64-9

(+/−)-gamma-Tocopherol, MW: 416 g/mol, CAS#: 148-03-8

dl-α-Tocopherol, MW: 430 g/mol, CAS#: 59-02-9
γ-Sitosterol, MW: 414 g/mol, CAS#: 83-47-6