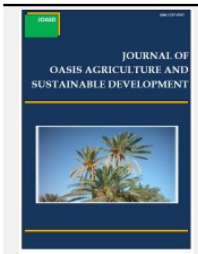

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Seed oil physicochemical characterization from non-edible *Pancratium maritimum*

Marwa Jouaidi, Rami rahmani & Mohamed Debouba*

Laboratory of Biodiversity, Molecules and Applications (LR22ES02), Higher Institute of Applied Biology Medenine, University of Gabes, 4119 Medenine, Tunisia

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***Corresponding author**
mohamed.debouba@univgb.tn

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Abstract

The oil extracted from *Pancratium maritimum* seeds was subjected to qualitative (acidity, K_{232}, K_{270}, chlorophyll content) and quantitative (polyphenol content, fatty acid composition) characterization. The *P. maritimum* seed oil showed an acidity of 0.22 mg/g, a k_{232} extinction coefficients of 2.6% and total polyphenol contents of 89.5 mg EGA/Kg. The chlorophyll contents were similar to that commonly recorded for edible vegetable oils. Interestingly, the *P. maritimum* seed oil was enriched in fatty acids distributed into 11 fatty acids with the most abundant fatty acids being the linoleic acid (41.1%), oleic acid (39.9%) and palmitic acid (14.95%). Taken together, *P. maritimum* oil contains antioxidant compounds (chlorophyll and polyphenols) and fatty acid composition potentially useful for pharmaceutical and cosmetic application.

1. INTRODUCTION

Over the past two decades, interest in non-food applications of vegetable oils has increased as new renewable industrial products and substantially displaced petroleum products in new markets (Avato and Tava 2022). Many industries, including cosmetics and pharmaceuticals, frequently use vegetal oils, emphasizing their importance due to their emollient properties, sustainability and bioactive potential (Abdalla et al, 2024). Thanks to their richness in triacylglycerol, essential fatty acids, tocopherols, sterols, vitamins, minerals, and low cholesterol, they have a significant impact on the function and development of the body and contribute to the maintenance of health (Monika and Anna 2019; Ben Haj Koubaier et al, 2024). In recent decades, the demand for naturally bioactive, eco-friend, and safe molecules has increased in the market (Khanal et al, 2024). Tunisian flora is rich in many plant species that are valuable for food, pharmaceutical and cosmetic industries (Bourgou et al., 2021). Therefore, multiple studies have been carried out on common vegetable oils to investigate their physicochemical characterization and fatty acid composition (Karoui et al, 2020). However, many indigenous and spontaneous non-edible seeds' oils have not yet been studied, such as *Pancratium maritimum*. *Pancratium maritimum*, commonly known as sea daffodil, is a native plant to coastal regions, especially in Mediterranean climates (Molina-Venegas and Verano 2024). As far as we know, many studies in the literature have focused on the biochemical properties of leaves, roots, and bulb extracts or essential oil of *P. maritimum* (Carfagna et al, 2022; Melliti et al, 2024; Jouaidi et al, 2024), but there are scarce reports on the chemical characterization of the *P. maritimum* seed's oil. Therefore, a study on the qualitative (acidity tests and extinction coefficients) and quantitative characterization (chlorophyll and total phenolic content) as well as the fatty acid

composition of the seed oil of *P. maritimum* species was conducted in this work.

2. MATERIAL AND METHODS

2.1. Plant material

The seeds of *P. maritimum* were collected on the coast of Zarzis delegation in south-eastern Tunisia (33°30'14" N 11°06'43" E), which is characterized by an arid climate (average rainfall 150 mm/year). The plant was authenticated by Professor Mohamed Debouba and a voucher specimen (under the number PM18sd) was deposited at the research department of the High Institute of Applied Biology (Medenine, Tunisia). The seeds were harvested during the summer season (June-July 2018), the endosperm was separated from the coat, dried and conserved in a refrigerator at 4°C to avoid any oxidation or degradation of lipids.

2.2. Seed oil extraction

Twenty g of *P. maritimum* seeds were grounded to a fine powder using an electric blender. The oils were obtained by continuous extraction in a Soxhlet apparatus for 8 hours using 200 mL of n-hexane (50-60°C) as solvent. The solvent was removed using a rotary vacuum evaporator at 35°C. The obtained oil was stored in dark glass-bottles at 4°C, until analyzed for fatty acids and triacylglycerols. The yield was determined as follows:

$$\text{Yield (\%)} = (m/M) \times 100$$

where m: weight of seed oil (g); M: weight of plant material (g).

2.3. Free fatty acids (FFAs %)

The oil was tested for acidity using the American Oil Chemists' Society (AOCS) method (Japir et al, 2017). In brief, 5 g of seed oil is added to 20 ml of alcohol, which serves as a solvent. After stirring, dosing is performed using a NaOH solution in the presence of a few drops of phenolphthalein. The end of the assay is indicated by the appearance of a light pink color that lasts for at least 10 seconds. Finally, the acidity is estimated as the quantity of sodium hydroxide used until the color changes (mg/g).

2.4. Determination of Specific UV Extinction Coefficients (K_{232} and K_{270})

The specific extinction coefficients (K_{232} and K_{270}) were determined according to the method used by Kozłowska et al. (2025) with some modifications. A 1% oil solution was prepared in cyclohexane and the absorbance was measured at 232 and 270 nm using a UV/VIS spectrophotometer. The two coefficients were calculated using the following equation:

$$K\lambda = E\lambda / c \times s$$

where Kλ is the specific extinction coefficient at the wavelength λ, Eλ is the measured absorbance at the wavelength λ, c is the concentration of the oil solution (g/100 mL) and s is the cuvette thickness (cm).

2.5. Determination of chlorophyll content

The chlorophyll pigments in the oil were determined in cyclohexane 1:10 (v/v) at 630, 670 and 710 nm using the specific extinction coefficients according to the method of Gharby et al. (2018). Pure carbon tetrachloride was used as a reference. Chlorophyll content was determined as follows: Chlorophylls (ppm) = $(A_{670} - (A_{630} + A_{710}) / 2) / 0.1086x\ L$.

Where A is the absorbance at the respective wavelength (nm), and L is the thickness of the spectrophotometer cell (mm).

2.6. Determination of total polyphenol content

The total phenolic content was determined according to the method of Deme et al. (2021) with some modifications. In brief, 2.5 g of the seed oil was mixed with 5 ml of hexane and 5 ml of methanol-water (60/40). The resulting mixture was vigorously shaken and then added with 500 µL Folin-Ciocalteu reagent and 4300 µL of distilled water. Then 1000 µL of sodium carbonate (Na_2CO_3) and distilled water were added to obtain a final volume of 10 mL. The samples were incubated for 2 hours in the dark at room temperature. The absorbance was measured using a UV spectrophotometer at 726 nm. The amount of polyphenols was sorted from a curve plotted using caffeic acid as standard. The content of total polyphenols is expressed in ppm (mg/kg) equivalent of caffeic acid according to the following formula of the method:

$$\text{Total polyphenol content} = (833.32xA) + 10.25$$

2.7. Determination of the oil composition in fatty acids

For the determination of the fatty acid composition, the methyl esters are analyzed

according to REG. CEE n° 2568/91 of the European Regulation for the analysis of olive oil and other fats. The *P. maritimum* seed oil (100 mL) was added to 2 mL of hexane and 200 µL of a methanolic KOH solution (2N). After stirring for 3 minutes and decanting, the upper layer containing the methyl esters is removed for analysis. The analysis was performed with a SHIMADZU GC-MS equipped with a QP 2010 Ultra Capillary column: supelcowaxtm10/fused silica capillary column 30 m x 0.25 mm x 0.25 µm film thickness.

3. RESULTS AND DISCUSSION

3.1. Yield extraction and free fatty acidity (FFA)

The extraction yield of *P. maritimum* seeds using the Soxhlet method was 27.1 % (Table 1). The oil yield from these seeds using this method was relatively high, considering that most commercial oleaginous seeds have a yield of 30 to 40% (Alenyorege et al, 2015). This makes these seeds a practical choice for regular oils for production. Our results, also, showed that seeds of *P. maritimum* are rich in fat (oil). Interestingly, the oil yield obtained from our seeds (27.1%) was about twice as high as that reported by Quílez et al. (2020) for Spanish oregano (15.1%). In addition, the yield was consistent with that found in the literature for cotton seeds (22%) and close to the yield of olive seeds, which varies between 20% and 27% (Djenotin et al. 2006). According to the literature, the oil extraction from *P. maritimum* flowers showed that the yield was higher when the plant was growing in the sand in the spontaneous state, while the it was lower in sand-free soil (Citanava 1957). As the free fatty acid (FFA) content correlates with the degree of deterioration of the oil, it is an important quality indicator (Bahadi et al, 2016). The FFA content of an oil, which is formed when lipolytic enzymes hydrolyze the triglycerides of the oil, is measured by its acid value or free acidity (García Martín 2022). Due to its inedibility, *Pancratium maritumum* oil is only used in non-food applications and is therefore unsuitable for the food industry. According to Table 1, the oil of *P. maritumum* seeds had a low free acidity (0.2%). A low free acidity indicates that the oil is less degraded, which indicates increased chemical stability. This is important to extend the shelf life of the end product and avoid the formation of undesirable by-products such as free radicals.

Table 1 : Oil quality parameters

Parameters	Value
Yield (%)	27.1
Free fatty acids (%)	0.2
Total chlorophyll (mg/Kg)	0.6
Total phenolic content (mg CAE /Kg)	89.5
K_{232} (nm)	2.6
K_{270} (nm)	0.4

3.2. K_{232} and K_{270}

The coefficients K_{232} and K_{270} are among the indicators of oil quality that respectively evaluate primary and secondary oxidation of oils (Gharby et al, 2021). The maximum allowable values are 2.6 for K_{232} and 0.4 for K_{270} (Table 1). While the obtained value of K_{232} indicated some primary oxidation, the value of K_{272} indicated minimal secondary oxidation. According to Mancebo-Campos et al (2022), our results were within the range for extra virgin olive oil. These results indicated that *P. maritumum* oil has good oxidative stability and is suitable for cosmetic use.

3.3. Chlorophyll and total phenolic contents

In the first step to determine the antioxidant activity, the oils were analyzed for their polyphenol and chlorophyll contents. The total chlorophyll and total phenol contents are listed in Table 1. Chlorophylls are responsible for the green color of vegetable oils and are among the parameters that indicate the quality of seed oils (Brahmi et al, 2023). The total chlorophyll and total phenol contents showed values of 0.6 mg/kg and 89.5 mg GAE/kg, respectively. Compared to the literature, the chlorophyll content of *P. maritimum* seeds was similar to that of olive oil (0.5 to 0.7 ppm). In our study, the total phenolic contents (TPC) of *P. maritimum* seed oil were about 90 mg CAE/kg. The obtained

TPC was similar to that reported by Al Juhaimi et al. (2021) for *Capparis ovata* seed oils (95 mg GAE/kg). In addition, the actual TPC were much higher than for that recorded for *Origanum vulgare* and *Majorana hortensis* seed oils (Daga et al, 2022).

3.4. Fatty Acid Composition

The data presented in Fig. 1 and Table 2 on the fatty acids determined by GC-MS analysis of the oil from the seeds of *P. maritimum* showed that this oil is rich in unsaturated fatty acids. In particular, oleic acid and linoleic acid (39.52 and 41.40%, respectively) are the major fatty acids in *P. maritimum* seed oil, followed by palmitic acid (14.95%), which together account for 95.87% of the total fatty acids. Li et al. (2022) reported that linoleic acid and oleic acid are a natural product that acts as a pancreatic lipase inhibitor and has become a novel strategy to combat obesity. Arachidic acid, gadoleic acid, linolenic acid, and stearic acid make up about 4%. The relatively high stearic acid content (3%) makes it undesirable for human consumption (Li et al, 2020). However, thanks to its high essential fatty acid content, *P. maritimum* seed oil can be used as a cosmeceutical active ingredient applied topically to the skin, as a carrier oil, as a penetration enhancer, and in oil-based skin creams and lotions. Based on previous studies, these compounds play a key role in various

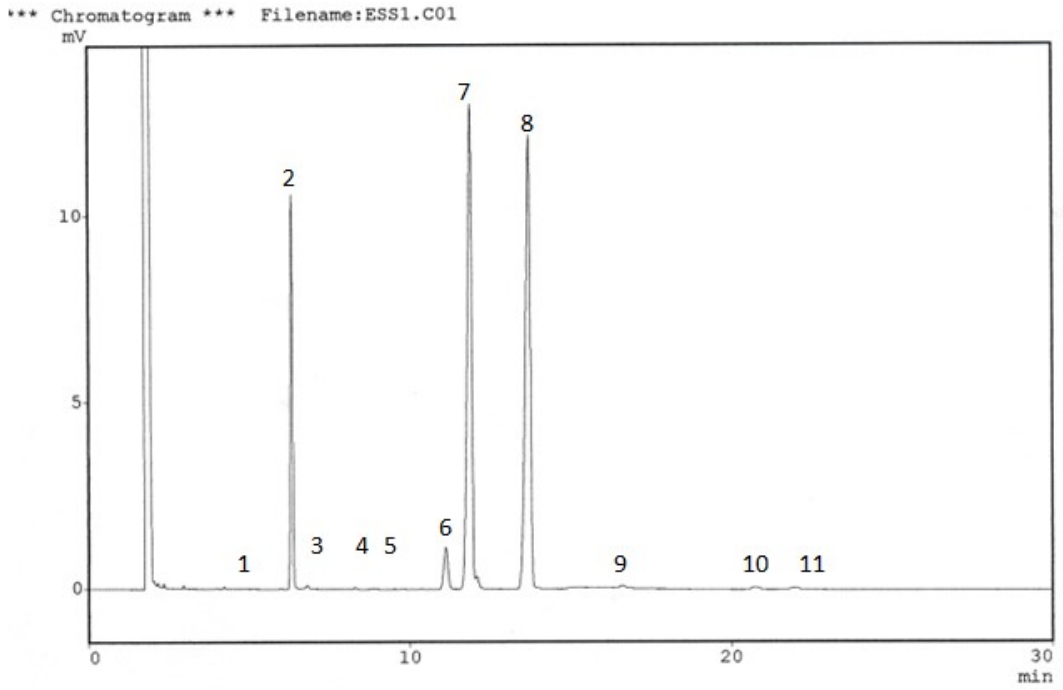

Fig. 1. GC-MS chromatogram of *P. maritimum* seeds oil.

Table 2: Fatty acid composition of *P. maritimum* seed oil.

N°	Structure	Fatty acids	Nature	Percentage
1	C14 :0	Myristic acid	saturated	0.02
2	C16 :0	Palmitic acid	saturated	14.95
3	C16 :1	Palmitoleic acid	unsaturated	0.10
4	C17 :0	Heptadecanoic acid	saturated	0.06
5	C17 :1	Octadecenoic acid	unsaturated	0.03
6	C18 :0	Stearic acid	saturated	3.12
7	C18 :1	Oleic acid	unsaturated	39.52
8	C18 :2	Linoleic acid	unsaturated	41.40
9	C18 :3	Linolenic acid	unsaturated	0.20
10	C20 :0	Arachidic acid	saturated	0.30
11	C20 :1	Gadoleic acid	unsaturated	0.20
Sum				99.9

fields, including the cosmetic and pharmaceutical industries (Saklani et al., 2023). Moreover, among the 5 saturated fatty acids detected, palmitic acid and stearic acid were the major constituents with 14.95 and 3.12%, respectively. The remaining fatty acids were present in small amounts, not exceeding 0.5%. The seed oil of *P. maritumum* was characterized by a high ratio between unsaturated and saturated fatty acids (U/S) of 4.41, comparable to that of Cupressus sempervirens (3.98) seed oil (Nehdi 2013), which is mainly used in cosmetics production.

4. CONCLUSION

Thanks to its stability and richness in fatty acids (oleic and linoleic acids) and antioxidant compounds (chlorophylls and polyphenols), *P. maritimum* seed oil is a promising ingredient, especially for cosmetic products. It can also be used in the pharmaceutical and cosmeceutical fields for skin care and in the treatment of various skin diseases. Although its use is still limited, in vitro and in vivo experiment are needed to give further insights into the natural virtues of *P. maritimum* seed oil.

AUTHORS CONTRIBUTION

Conceptualization: Marwa Jouaidi and Mohamed Debouba; Methodology: Marwa Jouaidi; Formal analysis and investigation: Marwa Jouaidi and Rami Rahmani; Writing - original draft preparation: Marwa Jouaidi; Writing - review and editing: Rami Rahmani; Supervision: Mohamed Debouba.

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