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GC–MS profiling of fatty acid composition and antioxidant evaluation of *Asphodelus microcarpus* Salzm. & Viv. seed oils collected from different Moroccan regions☆

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Abstract – This study investigates the geographical variation in oil quality and bioactivity of *Asphodelus microcarpus* seed oils collected from five Moroccan regions. Oils were extracted using Soxhlet with petroleum ether, analyzed by GC-MS after FAME preparation, and evaluated for physicochemical properties (ISO/NF standards) and antioxidant activity (DPPH, ABTS). Yields ranged from 18.93% to 21.03%, with stable physicochemical parameters. GC-MS revealed 18 fatty acids (94.82–98.59% of total lipids), showing regional variation. Casablanca and Mohammedia oils had exceptional linoleic acid content (>74%), a first report for *Asphodelus* species. Meknes and Rabat oils were richer in nervonic and tricosanoic acids. Antioxidant assays showed the strongest activity in Casablanca oil (DPPH IC_{50} = 291.5 µg/mL; ABTS IC_{50} = 308.93 µg/mL). PCA revealed clear coastal *vs*. inland chemotypes. *This study represents the first Moroccan report on the fatty acid composition and antioxidant potential of A. microcarpus seed oil. It reveals a distinctive linoleic acid-rich chemotype and highlights the species' potential as a novel bioactive source for food, health, and industrial applications*.

Keywords: *Asphodelus microcarpus* Salzm. & Viv / seed oil / fatty acid / Linoleic acid / antioxidant activity

Résumé – Profilage GC–MS de la composition en acides gras et évaluation de l'activité antioxydante des huiles de graines d'*Asphodelus microcarpus* Salzm. & Viv. collectées dans différentes régions du Maroc. Cette étude explore la variation géographique de la qualité et de la bioactivité des huiles de graines d'*Asphodelus microcarpus* collectées dans cinq régions marocaines. Les huiles ont été extraites par Soxhlet à l'aide d'éther de pétrole, analysées par GC-MS après préparation des FAME, et évaluées pour leurs propriétés physico-chimiques (normes ISO/NF) ainsi que pour leur activité antioxydante (DPPH, ABTS). Les rendements varient de 18,93% à 21,03%, avec des paramètres physico-chimiques globalement stables. L'analyse GC-MS a révélé 18 acides gras (94,82–98,59% des lipides totaux), montrant une variation régionale. Les huiles de Casablanca et Mohammedia présentaient une teneur exceptionnelle en acide linoléique (>74%), une première dans les espèces *Asphodelus*. Les huiles de Meknès et Rabat étaient plus riches en acide nervonique et en acide tricossanoïque. Les tests antioxydants ont montré la plus forte activité pour l'huile de Casablanca (DPPH IC_{50} = 291,5 µg/mL; ABTS IC_{50} = 308,93 µg/mL). L'analyse PCA a mis

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en évidence des chimiotypes distincts entre régions côtières et intérieures. Cette étude constitue le premier rapport marocain sur la composition en acides gras et le potentiel antioxydant de l'huile de graines d'*A. microcarpus*. Elle révèle un chimiotype distinct riche en acide linoléique et souligne le potentiel de l'espèce comme nouvelle source bioactive pour les domaines alimentaire, sanitaire et industriel.

Mots-clés : *Asphodelus microcarpus* Salzm. & Viv / huile de graines / composition en acides gras / acide linoléique / activité antioxydante

Highlights

- This first Moroccan study on *A. microcarpus* seed oils reveals strong regional variability, an exceptional coastal linoleic-acid–rich chemotype (>74%), and notable antioxidant activity, highlighting these oils as a promising new bioactive source for food, health, and industrial applications.

1 Introduction

Lipids constitute a broad and diverse class of naturally occurring organic compounds that play essential roles in numerous biological processes. Among them, fatty acids are particularly important due to their structural diversity and functional relevance in fields such as nutrition (Shin *et al.*, 2024), pharmacology (Sanchez *et al.*, 2024), and cosmetic science (Lu *et al.*, 2023). Beyond their roles as energy sources and essential nutrients, unsaturated fatty acids (UFA) have been extensively studied for their biological activities (Ladele *et al.*, 2016; Zheng *et al.*, 2005), including anti-inflammatory (Wall *et al.*, 2010), antioxidant (Makuria *et al.*, 2025), antimicrobial (Shin *et al.*, 2024), and anticancer effects (Jóźwiak *et al.*, 2020). These effects are closely related to their chemical structure, specifically the number, position, and configuration (cis/trans) of double bonds, which influence membrane fluidity, signaling pathways, and oxidative stability (Calder, 2015; Moustiés *et al.*, 2022; Stillwell and Wassall, 2003; Wang *et al.*, 2006). These properties, largely attributable to the presence of bioactie fatty acids, make plant-derived oils particularly attractive for the development of functional and therapeutic products across a range of sectors.

In Morocco, several oilseed species such as olive (*Olea europaea*) (Gagour *et al.*, 2024), argan (*Argania spinosa*) (Hilali *et al.*, 2020), sesame (*Sesamum indicum*) (Gharby *et al.*, 2017), sunflower (*Helianthus annuus*) (Gagour *et al.*, 2022), and hemp (*Cannabis sativa*) (Raoui *et al.*, 2024) are widely used for their high content of UFA. These oils provide essential compounds like oleic, linoleic, and α-linolenic acids, contributing significantly to their nutritional, cosmetic, and pharmacological value in both traditional and modern applications. Furthermore, physicochemical parameters such as acid value, iodine value, saponification value, and peroxide index are key indicators of oil quality, oxidative stability, and potential industrial applications (Codex, 2019; Ph. Eur, 2020).

Asphodelus microcarpus Salzm. & Viv (*A. microcarpus*), a member of the Asphodelaceae (formerly Xanthorrhoeaceae) family, is an herbaceous plant native to the Mediterranean region. Typically reaching about one meter in height, it is characterized by narrow, linear basal leaves, white flowers in radial inflorescences, a robust root system, and fruit capsules containing numerous seeds (Fig. 1) (Majeed, 2014; Teline.fr, 2024). Traditionally, this species has been used in folk medicine for the treatment of various ailments. In recent years, the phytochemical composition of *A. microcarpus* extracts from different geographical origins has been investigated (Rizk and Hammouda, 1979; Di Petrillo *et al.*, 2016; Ghoneim *et al.*, 2014; Ghoneim *et al.*, 2014; González *et al.*, 1973; Hammouda *et al.*, 1974; Fell, 1968; Hamrnouda and Rizk, 1972; Sayed *et al.*, 2017). These studies have revealed a rich diversity of bioactive secondary metabolites, including terpenes, flavonoids, phenolic acids, anthraquinones, arylcoumarins, carbohydrates, fatty acids, and triterpenoids, many of which serve as chemotaxonomic markers for the genus *Asphodelus* (Malmir *et al.*, 2018).

In this context, the present study complements our previous research on the essential oils (EOs) of *A. microcarpus* collected from various Moroccan regions (Benchama *et al.*, 2024), maintaining a focus on regional variation. Seeds were specifically targeted due to their high lipid content and their importance as a source of bioactive fatty acids. Here, we evaluate the fatty acid composition, physicochemical properties, and antioxidant activity of seed oils obtained from the same populations. Notably, this study reveals a distinctive chemotype rich in linoleic acid and highlights regional variation among populations. This integrated approach provides a comprehensive understanding of the nutritional and functional potential of *A. microcarpus* seed oils.

To the best of our knowledge, this is the first Moroccan study reporting the fatty acid profile, physicochemical traits, and antioxidant activity of seed oils from this species. Importantly, the study highlights a distinctive chemotype characterized by exceptionally high linoleic acid content (>74%) in coastal populations and reveals regional chemotypic variation, underscoring the potential of these oils for applications in the food, pharmaceutical, and cosmetic industries.

2 Material and methods

2.1 Reagents and equipment

All reagents used were of analytical grade and obtained from Sigma-Aldrich, Merck. Petroleum ether was used for Soxhlet extraction. Methanol, sulfuric acid, hexane, ethanol, toluene, chloroform, and diethyl ether employed for FAME preparation and physicochemical analyses. Standard titrants included KOH, HCl, $Na_2S_2O_3$, and KI. DPPH and ABTS reagents were used for antioxidant activity assays.

Key equipment included a Soxhlet extractor, rotary evaporator, centrifuge (ROTINA 380), UV–Vis spectropho-

Fig. 1. Morphological appearance of Asphodelus microcarpus Salzm. & Viv. seeds collected from Moroccan populations (Teline.fr, 2024).

tometer, and GC-MS (Shimadzu QP 2010 Plus) with a DB-5 column. Density and refractive index were measured using a pycnometer and refractometer. An analytical balance (±0.1 mg) was used for weighing, and all tests were performed in triplicate.

2.2 Plant material

Seeds of *A. microcarpus* (Fig. 1) were collected in March 2023, during the flowering stage, from five different sites in Morocco: El Jadida (E), Casablanca (C), Mohammedia (M), Rabat (R), and Meknes (Mk). For each region, a parcel of approximately 200 m × 200 m was randomly selected. Within this parcel, five subplots of 20 m × 20 m were chosen (Benchama *et al.*, 2024). In each subplot, 10 individual plants at the same developmental stage were sampled to ensure reliable comparisons across populations. A total of 50 samples were collected per region, corresponding to approximately 30 kg of plant material. These 10 individual samples were then combined to form a single heterogeneous sample of about 6 kg. Consequently, for each region, five main heterogeneous samples were obtained, each treated separately. To ensure robustness and reproducibility, all extractions were performed in triplicate under identical conditions. Collections were carried out during the peak seed maturation period to minimize seasonal variation and ensure consistency across regions.The plant material was taxonomically identified by botanists of the Scientific Institute of Rabat, and voucher specimens from each site were deposited in the National Herbarium (RAB), whose Index Herbariorum code is RAB, under the following numbers: RABAM-E2023 (El Jadida), RABAM-C2023 (Casablanca), RABAM-M2023 (Mohammedia), RABAM-R2023 (Rabat), and RABAM-Mk2023 (Meknes) in accordance with the Flora of Morocco (Jahandiez *et al.*, 1931; Bellakhdar, 1997).

2.3 Extraction of oils

The Oil extraction from 100 g of dried and powdered seeds was conducted at temperatures between 60 and 80°C for 4 h using a Soxhlet extractor with petroleum ether as the solvent. The solvent was subsequently removed using a rotary evaporator. The oils obtained were stored at a temperature of 4°C.

2.4 Preparation of fatty acid methyl esters (FAMEs)

The fatty acids in the total lipid were esterified into methyl esters by esterification in an acidic medium using 100 mg of crude oil with 4 mL (2% sulfuric acid in methanol). The mixture was heated to 80 °C for 2 h. After cooling to room temperature, 2 mL of hexane were added, followed by 2 mL of distilled water. After centrifugation at 1,000 rpm, 2 min. Then, two phases were distinguished. The organic phase (hexane) was dried over anhydrous sodium sulfate. and finally evaporated to dryness and stored at -4 °C (Zheng *et al.*, 2012).

2.5 Gas chromatography/mass spectroscopy analysis of FAMEs

The apparatus used was a Shimadzu GC-MS (QP 2010 Plus, Tokyo, Japan) equipped with a DB-5 capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm; SGE Ltd) for analyte separation. Helium was utilized as the carrier gas at a flow rate of 1.7 mL/min. The flow control mode was linear velocity, with a pressure of 59.1 kPa. The injector temperature was maintained at 250°C, with a split ratio of 20:1. The column temperature gradient started at 197 °C and was held for 22 min. It was then increased by 20 °C/min to 235 °C and held for 10 min.

The mass range was set from 40 to 450 m/z with an ionizing voltage of 70 eV. The total analysis time was estimated to be 40 min. Identification of fatty acids was done matching their mass spectra with those in the computer library (Shimadzu corporation library and NIST05 database/Chem-Station data system).

2.6 Physicochemical characterization

The density (ISO & 6883, 2017), Refractive index (Faqer and Rais, 2024; International Organization for Standardization ISO & 6320, 2017), acid value (AV), saponification value (SV), iodine value (IV), peroxide value (PV), and unsaponifiable matter (UM), content was determined according to the standards NFT 60-214, NF T60-204, NFT 60-206, NF ISO 3961, and NFT 60-220, respectively.

2.6.1 Determination of acid value NF ISO 660

The acid value of the oil samples was determined according to the NF ISO 660 standard (ISO 660, 2009). Approximately 2 g of the oil sample was accurately weighed into a 250 mL Erlenmeyer flask. A solvent mixture of ethanol and toluene (1:1, v/v), previously neutralized with a drop of 1% phenolphthalein solution, was prepared. Subsequently, 50 mL of this mixture was added to the flask containing the oil sample, and the contents were gently swirled until the sample was fully dissolved. For titration, 2–3 drops of phenolphthalein solution (1% in ethanol) were added as an indicator. The solution was immediately titrated with freshly prepared 0.1 M potassium hydroxide (KOH) solution. The titration was performed under constant swirling until a pale pink color persisted for at least 30 s.

2.6.2 Determination of saponification value NF ISO 3657

The saponification value (SV) of the oil samples was determined according to the **NF ISO 3657** standard (ISO 3657, 2020), which measures the milligrams of potassium hydroxide (KOH) required to saponify one gram of oil. Approximately 2 g of the oil sample was accurately weighed and transferred into a 250 mL Erlenmeyer flask. A total of 25 mL of ethanolic potassium hydroxide solution (0.5 M) was added to the flask, and the mixture was heated under reflux for 30 min to ensure complete saponification. After cooling, a few drops of phenolphthalein indicator (1% in ethanol) were added, and the excess KOH was titrated with 0.5 M hydrochloric acid until the pink color disappeared.

2.6.3 Ester value (IE)

The ester value (IE) was calculated based on the analytical data using the formula:

$$IE = IS - IA.$$

2.6.4 Iodine value NF ISO 3961

The iodine value (IV) of the oil samples was determined according to the **NF ISO 3961** (ISO 3961, 2018), which evaluates the degree of unsaturation by measuring the amount of iodine absorbed by the double bonds present in the sample. Approximately 0.5 g of the oil sample was accurately weighed into a 250 mL Erlenmeyer flask. The sample was dissolved in 20 mL of chloroform, followed by the addition of 25 mL of Wijs reagent. The flask was gently swirled and stored in the dark at room temperature for 30 min to allow the reaction to proceed.

After the reaction period, 20 mL of 10% potassium iodide solution and 150 mL of distilled water were added to the flask. The liberated iodine was titrated with 0.1 M sodium thiosulfate solution while swirling continuously. The titration was carried out until the yellow color nearly disappeared. At this point, a few drops of 1% starch indicator solution were added, and titration was continued until the blue color disappeared completely

2.6.5 Peroxide value NF ISO 3960

The peroxide value (PV) of the oil samples was determined following the **NF ISO 3960** (ISO 3960, 2017), which quantifies primary oxidation products and is expressed in milliequivalents of active oxygen per kilogram of sample. Approximately 5 g of the oil sample was accurately weighed and placed into a 250 mL Erlenmeyer flask. A total of 30 mL of an acetic acid–chloroform mixture (3:2, v/v) was added to dissolve the sample. Subsequently, 0.5 mL of saturated potassium iodide solution was introduced, and the mixture was allowed to react in the dark for 5 min. After this reaction period, 30 mL of distilled water was added, and the liberated iodine was titrated with 0.01 M sodium thiosulfate solution under constant swirling. The titration continued until the yellow color faded, followed by the addition of 1 mL of starch indicator solution to enhance color detection. The titration proceeded until the blue color disappeared entirely.

2.6.6 Unsaponifiable matter

The unsaponifiable matter in the oil samples was determined following the improved method described by Schartz (1988) (Fontanel, 2011). Approximately 5 g of the oil sample was accurately weighed into a 250 mL Erlenmeyer flask. A total of 50 mL of ethanolic potassium hydroxide solution (2% w/v) was added, and the mixture was refluxed for 1 h to ensure complete saponification. After cooling, the saponified mixture was transferred to a separatory funnel, and 50 mL of diethyl ether was added. The unsaponifiable fraction was extracted by vigorous shaking, followed by the separation of the organic layer. The extraction process was repeated three times, and the combined ether extracts were washed with distilled water until neutral pH was achieved. The ether phase containing the unsaponifiable matter was dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure using a rotary evaporator. The residual unsaponifiable matter was weighed and expressed as a percentage of the oil sample's total mass.

2.7 Determination of Antioxidant Activity

To assess the antioxidant activity of *A. microcarpus* oil, we employed two distinct analytical methods: the 2,2-diphenyl 1-picrylhydrazyl ($DPPH^{\bullet}$), and the 2,2'-azinobis(3ethylbenzo-thiazoline-6-sulphonic) acid ($ABTS^{\bullet+}$) radical scavenging assays

Table 1. Yield and Physico-chemical characteristics of Moroccan *A. microcarpus* seed oil.

Characteristics	Regions				
	E	C	M	R	MK
yield (%)	19.79 ± 0.06[a]	18.93 ± 0.82[a]	19.27 ± 0.30[a]	19.13 ± 0.33[a]	21.03 ± 0.55[b]
Density g/cm³ (20 °C)	0.92 ± 0.80[a]	0.92 ± 0.35[a]	0.91 ± 0.32[a]	0.92 ± 0.35[a]	0.92 ± 0.17[a]
Refractive index	1.47 ± 0.08[a]	1.47 ± 0.32[a]	1.47 ± 0.04[a]	1.47 ± 0.12[a]	1.47 ± 0.04[a]
Acid value (mg KOH/g Oil)	1.86 ± 1.06[a]	2.12 ± 0.25[a]	2.23 ± 0.29[a]	2.76 ± 0.57[ab]	2.98 ± 0.51[b]
Saponification value (mg KOH/g Oil)	194.16 ± 0.40[a]	197.88 ± 0.08[ab]	201.41 ± 0.27[b]	215.09 ± 0.75[c]	191.49 ± 0.40[a]
Calculated ester value	192.30 ± 0.41[a]	195.76 ± 0.08[ab]	199.18 ± 0.27[b]	212.33 ± 0.76[c]	188.50 ± 0.41[a]
Iodine value (g d'I2/100 g)	91.03 ± 0.32[a]	107.53 ± 0.27[c]	107.53 ± 0.36[c]	97.54 ± 0.30[b]	98.64 ± 0.59[b]
peroxide value (meq O2/kg)	2.12 ± 1.25[a]	2.16 ± 0.71[a]	2.14 ± 0.54[a]	2.15 ± 0.54[a]	2.16 ± 0.71[a]
unsaponifiable matter (g/kg)	0.90 ± 1.15[a]	1.48 ± 1.01[a]	1.03 ± 1.12[a]	1.05 ± 1.08[a]	0.99 ± 1.16[a]

Values are expressed as mean ± standard deviation (n = 3). Statistical analysis was performed using ANOVA. Means followed by different letters within the same row are significantly different ($p < 0.05$). Sampling regions: El Jadida (E), Casablanca (C), Mohammedia (M), Rabat (R), and Meknes (MK).

(Faqer *et al.*, 2024; Salma *et al.*, 2020). The free radical scavenging activity in both assays was expressed as a percentage of inhibition (I%):

$$\mathbf{I}\,\% = \frac{\mathbf{A0} - \mathbf{AE}}{\mathbf{A0}}\mathbf{X}100.$$

where, **A0**: Absorbance of ABTS/DPPH solution **AE**: Absorbance of the extract

The results were expressed as IC_{50} in micrograms per milliliter (µg/mL).

2.8 Statistical analysis

The percentage composition of the identified fatty acids was determined using the peak area normalization method (Huangxian Zhang, Ting Huang, Xiaoning Liao, Yaohong Zhou, Chen, Jing, Shangxing Chen, 2022) and served as the basis for establishing correlations between the different regional seed oil samples. Cluster analysis was performed using NTSYS-pc software (version 2.2; Exeter Software, Setauket, NY, USA) (Rohlf *et al.*, 2009). The correlation coefficient was employed as a similarity index, and clustering was carried out using the unweighted pair group method with arithmetic mean (UPGMA). According to the classification proposed by Pestana and Gageiro (Pestana, 2014), correlation levels were interpreted as follows: very high (0.90–1.00), high (0.70–0.89), moderate (0.40–0.69), low (0.20–0.39), and very low (<0.20). Principal Component Analysis (PCA) was conducted using the Rstudio software for Windows, version 22.1.64970 (2025).

3 Results and discussion

3.1 Oil yields and physicochemical properties

The oil yield and physicochemical characteristics of *A. microcarpus* seed oils collected from five different Moroccan regions (El Jadida, Casablanca, Mohammedia, Rabat, and Meknes) were determined and are presented in Table 1. When comparing the physicochemical properties and oil yield of Moroccan *A. microcarpus* seed oil with those reported for other species of the *Asphodelus* genus, several notable similarities and differences emerge. In our study, the oil yield ranged from 18.93% (Casablanca) to 21.03% (Meknes), which is comparable to the yield reported for *A. tenuifolius* from the Tinghir region: 21.97% with Soxhlet extraction, 19.28% with microwave-assisted extraction, and 16.50% with ultrasound-assisted extraction (Eddaoudi *et al.*, 2023). These results highlight the influence of both extraction methods and geographical origin on lipid yield. In terms of physicochemical characteristics, the density of our *A. microcarpus* oil samples ranged from 0.91 to 0.92 g/cm^3, while the refractive index remained constant at 1.47. These values are consistent with those reported by Rizk and Hammouda (Rizk and Hammouda, 1979). For *A. microcarpus* tuber oil from Egypt, the reported density was 0.91 g/cm^3 and the refractive index was 1.46. However, its acid value was significantly higher (AV = 4.5 mg KOH/g), indicating a greater degree of lipid hydrolysis compared to our Moroccan seed oils, which showed acid values ranging from 1.86 to 2.98 mg KOH/g. In contrast, our saponification values, ranging from 191.49 to 215.09 mg KOH/g, are in good agreement with those reported by Abdel-Gawad *et al*. for *A. albus* root oil, which averaged around 200 mg KOH/g (Abdel-Gawad *et al.*, 1976). The iodine values observed in our study (91.03 to 107.53 g I_2/100 g) were higher than those reported by Madaan and Bhatia for *A. tenuifolius* seed oil (80 g I_2/100 g), indicating a greater degree of unsaturation in *A. microcarpus* seed oil (Madaan and Bhatia, 1973). The peroxide values (2.12 to 2.16 meq O_2/kg) were low and consistent with those reported for *A. fistulosus* by Fell *et al.*, indicating good oxidative stability (Fell, 1968). Finally, the unsaponifiable matter content in our oils (0.90–1.48 g/kg) falls within the range reported by Khan *et al*. for *A. fistulosus* seed oil (Khan *et al.*, 1961). Altogether, these findings underscore the high nutritional and physicochemical quality of *A. microcarpus* seed oil from Morocco.

3.2 Fatty acid composition

The GC-MS analysis of fatty acid methyl esters derived from *A. microcarpus* seed oil collected from five Moroccan regions is summarized in Table 2. Compounds are listed in the

order of their elution on a DB-5 column, with identification confirmed by both GC and GC-MS analyses. A total of 18 fatty acids were identified, representing between 94.82% and 98.59% of the total lipid content.

The sample from El Jadida Figure 2 was distinguished by an exceptionally high proportion of saturated fatty acids (SFAs), predominantly lignoceric acid (C24:0, 68.64%) and erucic acid (C22:1Δ13, 17.25%), indicating a highly stable but potentially less bioactive lipid profile. In contrast, the Casablanca and Mohammedia Figures 3 and 4 samples were rich in polyunsaturated fatty acids (PUFAs), particularly linoleic acid (C18:2Δ9,12), which accounted for 76.13% and 74.93% of the total fatty acids, respectively. This high content of linoleic acid has not previously found in any Asphodeldus species in literature. We can suggest that our edible Asphodelus is a new source of PUFA molecules which are known for their greater potential for biological activities such as antioxidant and anti-inflammatory effects. Mohammedia also exhibited a notable level of palmitoleic acid (C16:1Δ9, 28.64%), while the Rabat and Meknes Figures 5 and 6 samples showed elevated levels of monounsaturated long-**chain fatty** acids, including nervonic acid (C24:1Δ15, 18.74%) and tricosanoic acid (C23:0, 23.83%). In samples where α-linolenic acid (ω-3) was absent (C, M, R), the omega-6/omega-3 ratio could not be calculated. These oils are strongly dominated by omega-6 fatty acids, indicating a potential imbalance from a nutritional perspective. In samples E and MK, the ratio was 0.35 and 0.15, respectively, suggesting a more balanced fatty acid profile. These results highlight the importance of considering both omega-6 and omega-3 contents to properly evaluate the health potential of *A. microcarpus* seed oils, as excessively high omega-6/omega-3 ratios may be less favorable for cardiovascular and anti-inflammatory benefits. Comparative studies on the fatty acid composition of *Asphodelus* species across different countries and plant parts have revealed substantial chemical diversity within the genus. When comparing our findings on *A. microcarpus* seed oil to previously reported data, both similarities and notable divergences emerge, underscoring the influence of geographic origin and plant organ on lipid profiles. In Egypt, Rizk and Hammouda analyzed the tuber oil of *A. microcarpus*, reporting a moderately balanced fatty acid profile composed of 53.53% unsaturated fatty acids (UFAs), including oleic (25.52%), linoleic (22.38%), and linolenic acids (5.63%) and 46.47% saturated fatty acids (SFAs), primarily palmitic (16.73%), behenic (15.73%), and lignoceric acids (7.25%) (Rizk and Hammouda, 1979).

In contrast, our study focusing on seed oil revealed a significantly higher proportion of PUFAs, suggesting pronounced organ-specific metabolic differences. Similarly, *A. albus* root oil, as reported by Abdel-Gawad *et al.*, showed a predominance of UFAs (67.5%), mainly linoleic, oleic, and linolenic acids, with SFAs accounting for 31.9%, reinforcing the nutritional relevance of the genus (Abdel-Gawad *et al.*, 1976). In line with our findings, seeds of *A. fistulosus* from Pakistan contained 88.0% UFAs particularly linoleic (54.9%) and oleic acid (33.1%) and only 9.8% SFAs (Fell, 1968; Khan *et al.*, 1961). In contrast, *A. aestivus* seeds from Turkey displayed an atypical lipid profile dominated by butyric acid (76.26%) and a total SFA content of 76.84%, with very low UFA levels (PUFAs: 0.54%) (Fafal *et al.*, 2016). Meanwhile, *A. tenuifolius* seeds collected in Pakistan exhibited a favorable fatty acid profile, with 80.82% UFAs (mainly linoleic, oleic, and linolenic acids) and only 17.80% SFAs (Madaan and Bhatia, 1973). These values align closely with our data from *A. tenuifolius* seeds harvested in the Tinghir region of southeastern Morocco, where UFA content exceeded 90%, dominated by linoleic acid (78.50%), highlighting the strong nutritional potential of this local population (Eddaoudi *et al.*, 2023).

To the best of our knowledge, no previous study has examined the fatty acid composition of *A. microcarpus* seeds from Morocco.Hence, this investigation provides the first comprehensive report, offering valuable baseline data on this species' lipid profile in Moroccan populations. These findings support the potential of *A. microcarpus* seed oil as a promising source of essential fatty acids for both dietary and pharmaceutical applications. **However, it is important to acknowledge that the observed variability in fatty acid composition among the different regional samples may partly reflect natural intra-specific chemical diversity or, alternatively, possible limitations in botanical attribution. In the absence of molecular confirmation (*e.g.*, DNA barcoding), the results presented here should be interpreted with caution and considered provisional until further taxonomic verification is achieved.**

3.3 Cluster analysis of fatty acid profiles

A hierarchical cluster analysis was performed to assess the similarity among seed oil samples of *A. microcarpus* collected from five Moroccan regions. The resulting Dendrogram, shown in Figure 7, illustrates distinct clustering patterns based on fatty acid composition, using Pearson correlation coefficients as the similarity metric. The strongest correlation was observed between Casablanca (C) and Mohammedia (M), which formed a tight cluster with a similarity coefficient (Scorr) of 0.98, indicating nearly identical fatty acid profiles. This close similarity is likely attributable to their shared coastal environments, which may influence lipid biosynthesis pathways in a similar manner. El Jadida (E), although also a coastal region, clustered with this pair at a much lower correlation level (Scorr = 0.10), suggesting significant divergence in chemical composition possibly due to local microclimatic or edaphic (soil-related) factors.

Conversely, Rabat (R) and Meknes (MK) formed a separate cluster, though they exhibited a relatively low correlation between themselves (Scorr = 0.20). These two inland locations are characterized by distinctive fatty acid profiles, which may result from variations in altitude, temperature, and ecological stressors. Notably, comparison between the ECM group (El Jadida, Casablanca, Mohammedia) and the RMK group (Rabat, Meknes) revealed a complete lack of correlation (Scorr = 0.00), indicating the presence of two clearly distinct chemotypes of *A. microcarpus* in Morocco. The ECM chemotype, associated with coastal regions, is marked by a very high content of linoleic acid (C18:2, >74%) and palmitoleic acid (C16:1, up to 28.64%), reflecting a highly unsaturated lipid profile. In contrast, the RMK chemotype, found in inland mountainous areas, is characterized by a dominance of long-chain saturated and monounsaturated fatty acids, including

Table 2. Fatty acids composition of *A. microcarpus* seed oil.

N	Chemical Name	Common or systemic names	RT min	Formula	Saturated/ unsaturated	Shorthand notation (Δ)	% Percentage of total fats				
							E	C	M	R	MK
1	Dodecanoic acid, methyl ester	Lauric acid	5.0	$C_{12}H_{24}O_2$	S	12 :00	-	0.05	0.11	0.93	0.38
2	Tetradecanoic acid, methyl ester	Myristic acid	6.5	$C_{14}H_{28}O_2$	S	14 :00	-	-	-	0.68	0.34
3	Tridecanoic acid, 12-methyl, methyl ester	Isomyristic acid	6.8	$C_{14}H_{28}O_2$	S	14 :00	-	0.02	0.09	0.33	-
4	Pentadecanoic acid, methyl ester	Pentadecanoic acid	9.5	$C_{15}H_{30}O_2$	S	**15 :00**	-	0.09	0,33	-	-
5	(Z)-9-Hexadecenoic acid, methyl ester,	Palmitoleic acid	10.0	$C_{16}H_{30}O_2$	U	16: 1Δ9	-	**6.82**	**8.06**	**28.64**	**3.55**
6	Hexadecanoic acid, methyl ester	Palmitic acid	15.0	$C_{16}H_{32}O_2$	S	16 :00	-	0.08	-	-	-
7	Heptadecanoic acid, methyl ester	Margaric acid	19.8	$C_{17}H_{34}O$	S	17 :00	-	**8.35**	**13.59**	6.97	1.14
8	(Z,Z)-9,12-Octadecadienoic acid, methyl ester	Linoleic acid	20.1	$C_{18}H_{32}O_2$	U	18: 2Δ9,12	1.15	**76.13**	**74.93**	4.76	1.06
9	(Z)-9-Octadecenoic acid, methyl ester	Oleic acid	21.0	$C_{18}H_{34}O_2$	U	18 :1Δ9	-	**3.28**	1.48	4.75	0.89
10	Octadecanoic acid, methyl estere octadécanoïque	Stearic acid	23.0	$C_{18}H_{36}O_2$	S	18 :00	-	-	-	-	1.8
11	(Z)-6-Octadecenoic acid, methyl ester	Petroselinic acid	23.7	$C_{18}H_{34}O_2$	U	18 :1Δ6	-	-	-	-	**17.16**
12	(Z,Z,Z)-octadeca-9,12,15-trienoic acid	α-Linolenic acid	25.0	$C_{18}H_{30}O_2$	U	18: 3 Δ9,12,15	3.3	-	-	-	6.97
13	Eicosanoic acid, methyl ester	Arachidic acid	26.5	$C_{20}H_{40}O_2$	S	20 :00	-	-	-	**20.57**	-
14	Docosanoic acid, methyl ester	Behenic acid	27.5	$C_{22}H_{44}O_2$	S	22 :00	-	-	-	5.68	**16.02**
15	(Z)-13-Docosenoic acid, methyl ester	Erucic acid	30.5	$C_{22}H_{42}O_2$	U	22 :1Δ13	**17.25**	-	-	3.25	**-**
16	Tricosanoic acid, methyl ester	Tricosanoic acid	32.5	$C_{23}H_{46}O_2$	S	23 :00	-	-	-	**17.08**	**23.83**
17	Tetracosanoic acid, methyl ester	Lignoceric acid	34.9	$C_{24}H_{48}O_3$	S	24 :00	**68.64**	-	-	2.07	3.1
18	15-tetracosenoic acid, methyl ester	Nervonic acid	38.5	$C_{24}H_{46}O_2$	U	C24 :1 Δ15	**5.38**	-	-	-	**18.74**
	Total identified		95.72	94.82	98.59	95.71	94.98				
	Saturated fatty acids (SFAs)		68.64	8.59	14.12	54.31	46.61				
	Unsaturated fatty acids (UFAs)		27.08	86.23	84.47	41.4	48.37				
	Monounsaturated fatty acids (MUFAs)		22.63	10.1	9.54	36.64	40.34				
	Polyunsaturated fatty acids (PUFAs)		4.45	76.13	74.93	4.76	8.03				
	Others		4.28	5.18	1.41	4.29	5.02				
	Ratio ω6/ω3		0.35	NA	NA	NA	0.15				

Values are expressed as percentages (% of total fatty acids) based on a single determination (n = 1). Sampling regions: El Jadida (E), Casablanca (C), Mohammedia (M), Rabat (R), and Meknes (MK). NA: not applicable.

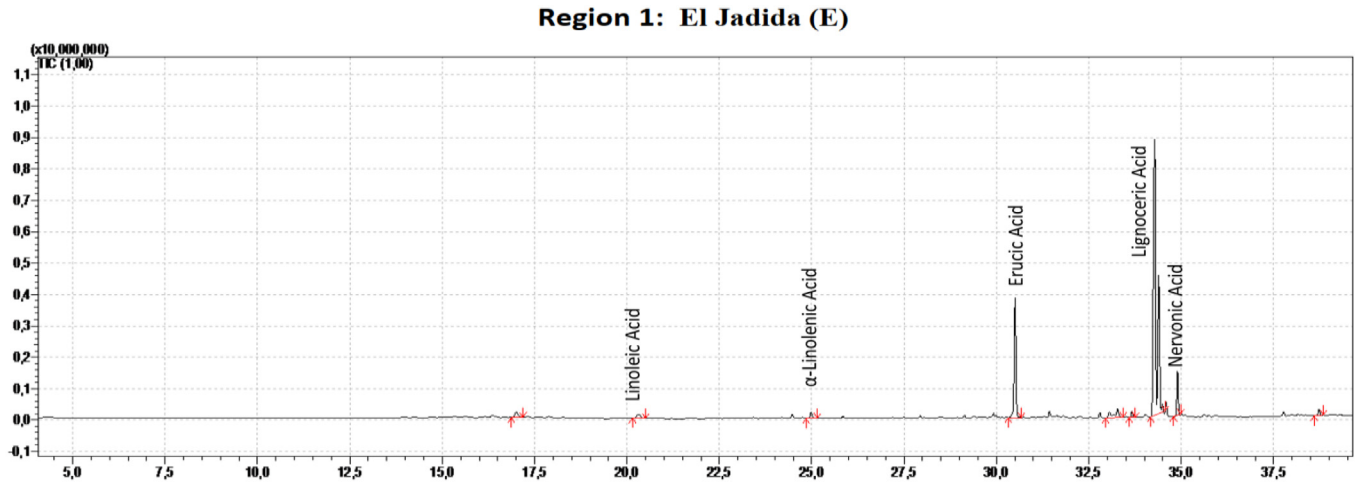

Fig. 2. GC-MS chromatogram of fatty acid methyl esters (FAMEs) from *Asphodelus microcarpus* seed oil collected in El Jadida.

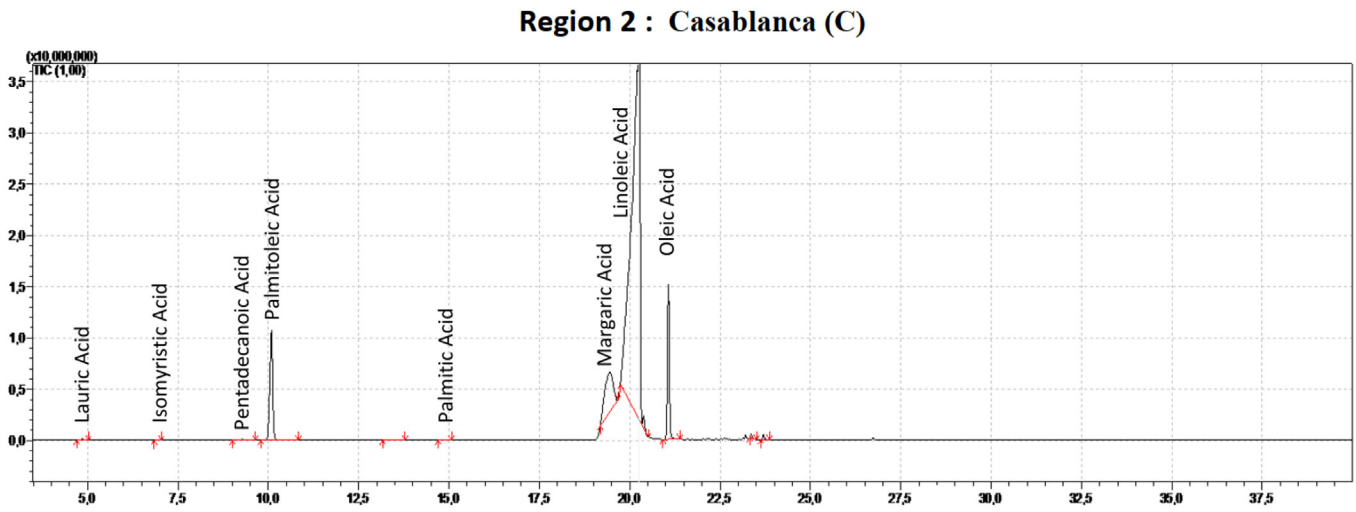

Fig. 3. GC-MS chromatogram of fatty acid methyl esters (FAMEs) from *Asphodelus microcarpus* seed oil collected in Casablanca.

tricosanoic acid (C23:0, up to 23.83%), behenic acid (C22:0, up to 16.02%), and nervonic acid (C24:1Δ15, 18.74%).

This pronounced chemical divergence likely reflects ecological adaptation to contrasting environmental conditions, as well as potential underlying genetic differentiation within the species.

3.4 Antioxidant activity

Antioxidant assays are essential tools for assessing the capacity of natural compounds to neutralize reactive oxygen species (ROS) and reduce oxidative stress, which is often associated with inflammation, bacterial infections, and cancer. These assays also contribute significantly to the development of safer and healthier products, including functional foods, dietary supplements, and natural alternatives to synthetic antioxidants. In this current study, the antioxidant potential of *A. microcarpus* oil extracted from five Moroccan regions El Jadida, Casablanca, Mohammedia, Rabat, and Meknes was evaluated using two well-established assays: DPPH and ABTS. The findings are presented in Figure 8 and Table 3.

Both assays revealed a clear dose-dependent antioxidant response. In the DPPH assay, oil from Casablanca displayed the strongest radical scavenging activity (68.04%), followed by Meknes (62.05 ± 0.44%), Mohammedia (61.25 ± 0.08%), Rabat (56.01 ± 0.05%), and El Jadida (52.22 ± 0.14%) (Fig. 8A). These trends were supported by the IC_{50} values, which confirmed the superior activity of the Casablanca sample (291.5 µg/mL), whereas the El Jadida oil exhibited the weakest effect (853.63 ± 13.44 µg/mL), indicating a relatively low antioxidant capacity. Based on these results, the oils can be ranked in decreasing order of effectiveness as follows: Casablanca > Meknes > Mohammedia > Rabat > El Jadida ($p < 0.001$; Tab. 3). A similar pattern was observed in the ABTS assay (Fig. 8B). Again, Casablanca oil showed the highest level of inhibition (70.35 ± 0.15%), closely followed by Meknes (66 ± 0.31%) and Mohammedia (64.62 ± 0.10%). These outcomes were consistent with the IC_{50} values, where Casablanca oil demonstrated the strongest and significant antioxidant effect (308.93 ± 0.37 µg/mL), and El Jadida remained the least active (924.76 ± 5.71 µg/mL) ($p < 0.001$; Tab. 3).

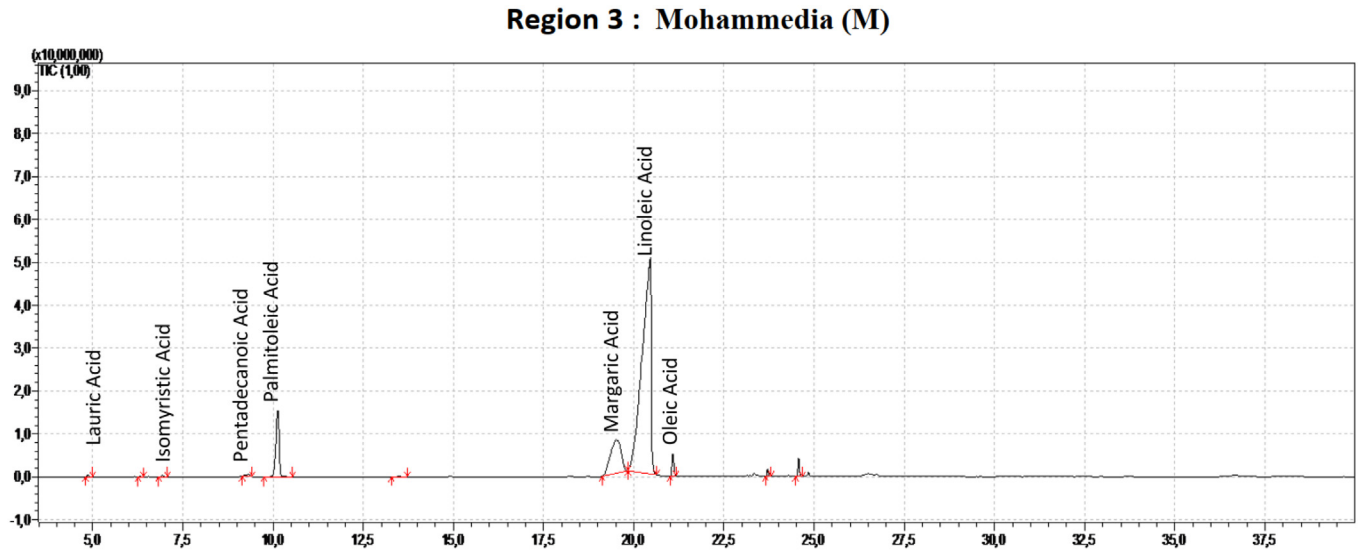

Fig. 4. GC-MS chromatogram of fatty acid methyl esters (FAMEs) from *Asphodelus microcarpus* seed oil collected in Mohammedia.

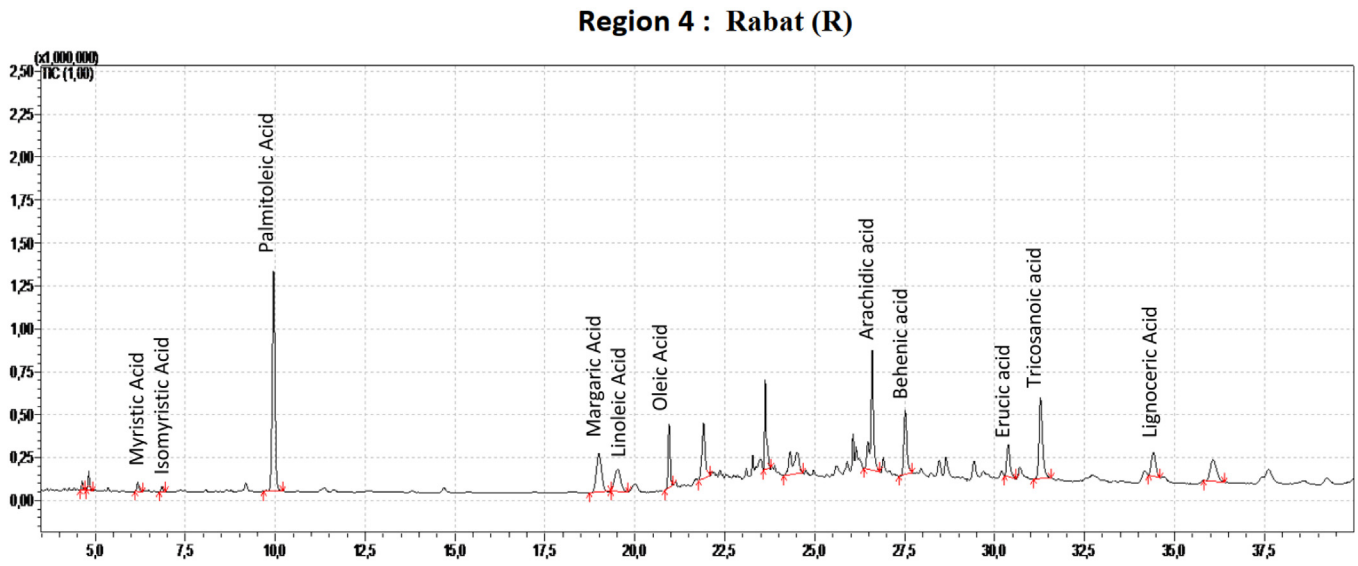

Fig. 5. GC-MS chromatogram of fatty acid methyl esters (FAMEs) from *Asphodelus microcarpus* seed oil collected in Rabat.

The differences in antioxidant capacity among the oils seem closely tied to their fatty acid composition. Analysis showed that Casablanca oil is particularly rich in SFAs, which make up 68.64% of its total fatty acids. In contrast, it contains very little PUFAs just 4.45%. On the other hand, the oils from El Jadida and Mohammedia are dominated by PUFAs, accounting for 74.93% and 76.13% respectively, with linoleic acid being especially abundant. While PUFAs are known for their nutritional benefits, they are also chemically unstable and prone to oxidation (Shahidi and Ambigaipalan, 2018). This likely explains why the El Jadida and Mohammedia samples showed higher IC_{50} values, indicating weaker antioxidant activity. By contrast, the Casablanca and Meknes oils, which contain higher levels of SFAs and certain MUFAs like nervonic and petroselinic acids, appear to be more resistant to oxidation contributing to their stronger antioxidant performance (Jacobsen, 2016).

To better understand these patterns, a principal component analysis (PCA) was performed and the results are showed in Figure 9**.** The results supported the observed trends: in the PCA biplot, the Casablanca oil appeared in the quadrant opposite the DPPH and ABTS vectors, which reflects its low IC_{50} values and stronger antioxidant activity. In comparison, the El Jadida sample clustered in the direction of these vectors, confirming its lower radical scavenging ability. The positions of the other samples on the PCA plot also aligned well with their fatty acid profiles and antioxidant behaviors. Notably, the first two principal components (PC1 and PC2) together explained nearly 70% of the total variation, highlighting the reliability and strength of the model.

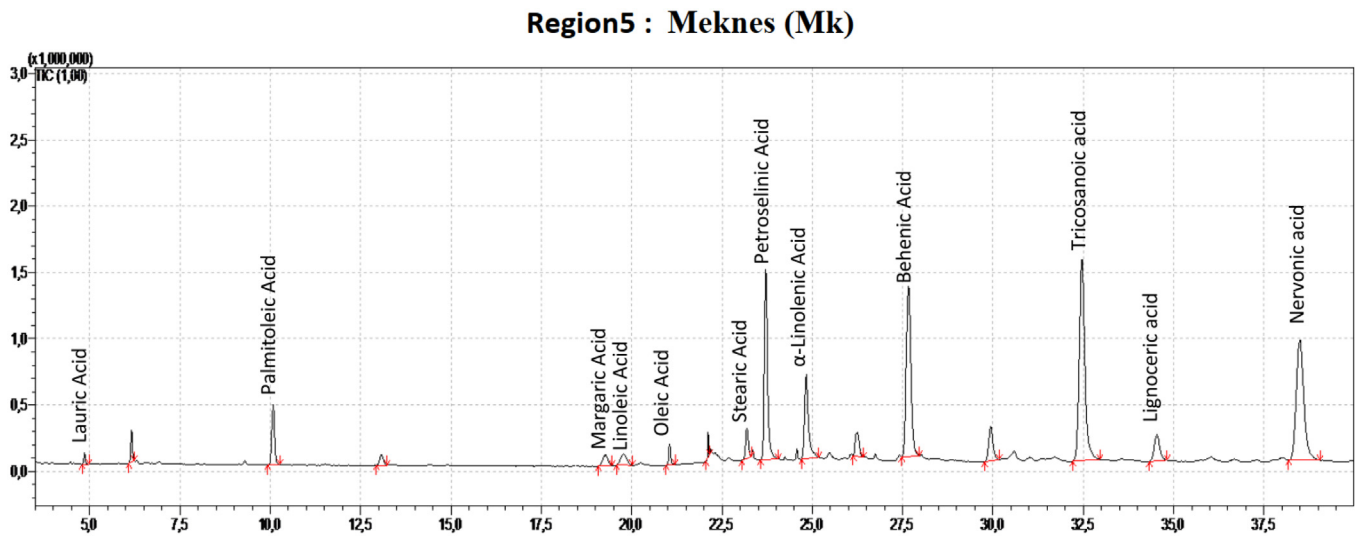

Fig. 6. GC-MS chromatogram of fatty acid methyl esters (FAMEs) from *Asphodelus microcarpus* seed oil collected in Meknes.

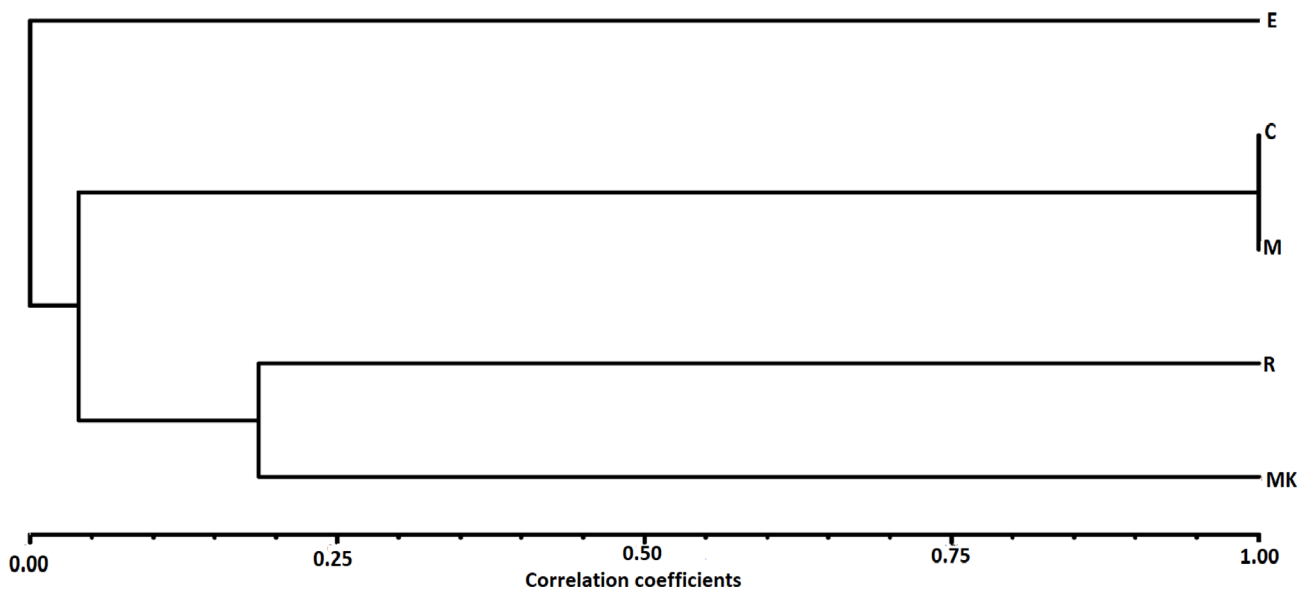

Fig. 7. Dendrogram illustrating the hierarchical cluster analysis of *Asphodelus microcarpus* seed oils from five Moroccan regions (E: El Jadida, C: Casablanca, M: Mohammedia, R: Rabat, MK: Meknes).

In comparison, the study by Eddaoudi *et al*. (Eddaoudi *et al.*, 2023) on *Asphodelus tenuifolius* reported the antioxidant activity of oils extracted using three different methods, Soxhlet extraction (SE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) with IC_{50} values ranging from 982.47 µg/mL (SE) to 503.19 µg/mL (UAE) in the DPPH assay. The UAE method produced the extract with the highest antioxidant activity, attributed to its superior retention of polyphenolic compounds (TPC = 18.51 mg GAE/g extract). The authors reported a strong positive correlation between TPC and antioxidant activity (r = 0.994), emphasizing the contribution of phenolics beyond fatty acids alone.

While both studies confirm the antioxidant potential of *Asphodelus* seed oils, their underlying mechanisms appear to differ. In *A. microcarpus*, antioxidant activity correlates more closely with lipid composition particularly the balance between SFA, MUFA, and PUFA whereas in *A. tenuifolius*, it is primarily driven by phenolic content and influenced by the extraction technique. The substantially lower IC_{50} values observed in *A. microcarpus* oil (*e.g.*, 291.5 µg/mL) compared to *A. tenuifolius* (503.19–982.47 µg/mL) suggest that *A. microcarpus* seed oil may possess a stronger intrinsic radical-scavenging capacity, potentially due to the synergistic effect of its unique fatty acid profile.

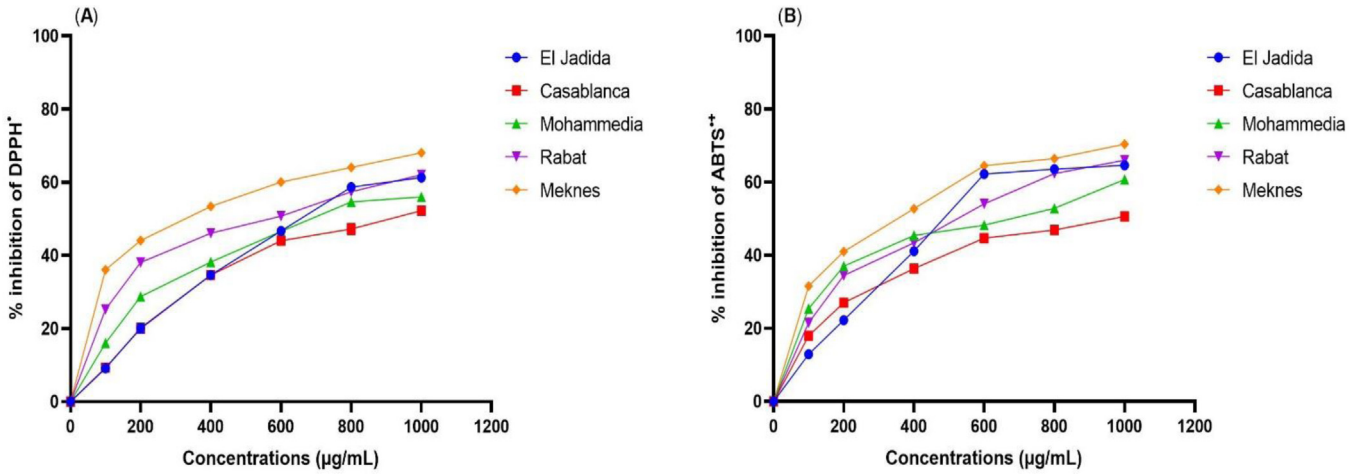

Fig. 8. Antiradical activity of *Asphodelus microcarpus* oil, expressed as a percentage of DPPH• radical inhibition (A) and ABTS•□ radical inhibition (B).

Table 3. Antioxidant activities of *A. microcarpus* oil expressed as IC_{50} values.

		A. microcarpus oil					Ascorbic acid
		M	E	R	MK	C	
Antioxidant assays	DPPHIC$_{50}$ (µg/mL)	652.66 ± 1.64$^{a, b}$	853.63 ± 13.44^{a}	695.83 ±1.95^{a,b}	501.8± 13^{a}	291.5^{a}	14.67 ± 0.01
	ABTSIC$_{50}$ (µg/mL)	507.43 ± 1.44^{a}	924.76 ± 5.71^{a}	576.36 ± 6.08^{a}	473.7 ± 3.6^{a}	308.93 ± 0.37^{a}	12.82 ± 0.04

Results are expressed as mean ± SD of three different experiments. Column with different superscript letters differs significantly ($p < 0.05$). E, El Jadida; C, Casablanca; M, Mohammedia; R, Rabat; MK; Meknes.

A notable fact is that edible oils rich in fatty acids can also exhibit antioxidant activity. In this context, the antioxidant activity of enriched argan oil was studied by Bakrim *et al.* (Bakrim *et al.*, 2025): artisanal argan oil exhibits remarkable antioxidant activity (IC_{50} = 1.5 mg/mL, FRAP assay), associated with a high content of unsaturated fatty acids (80%), mainly oleic acid (48%) and linoleic acid (30%).

Similarly, the IC_{50} values obtained for Asphodelus microcarpus seed oil fall within the same range, suggesting that the high proportion of polyunsaturated and monounsaturated fatty acids directly contributes to the observed antioxidant capacity. Moreover, the richness in linoleic acid, known for its role in stabilizing free radicals, gives this oil a potential comparable or even superior to other edible oils such as olive, argan, sunflower, and hemp oils.

Furthermore, recent studies on other seed oils support these observations: Mesembryanthemum forsskalii seed oil (Aljouf, Saudi Arabia) showed DPPH IC_{50} = 3.43 ± 0.19 mg/mL (Bilel *et al.*, 2020), while (Ficus carica L.) seed oils from Morocco exhibited DPPH IC_{50} values of 19.27 ± 1.15 mg/mL for the "White Adriatic" cultivar and 60.7 ± 7 mg/mL for "Bourjassotte Noir," with lower ABTS IC_{50} values for some cultivars (*e.g.*, "White Adriatic" 17.60 ± 1.52 mg/mL(Hssaini *et al.*, 2020).

These findings underscore the species-specific nature of antioxidant mechanisms within the genus *Asphodelus* and highlight the critical roles of both extraction strategy and targeted compound classes in optimizing biological activity. Future investigations should therefore focus on the identification and quantification of other bioactive constituents such as tocopherols, sterols, and phenolic compounds, in order to establish a more comprehensive understanding of the antioxidant mechanisms in *A. microcarpus* seed oils.

Overall, the findings of this study reveal substantial chemical diversity among Moroccan populations of *A. microcarpus*, with fatty acid profiles ranging from SFA-rich to PUFA-dominant chemotypes. In particular, the exceptionally high linoleic acid content observed in the Casablanca and Mohammedia samples highlights the existence of a novel omega-6 chemotype within the species, unprecedented in the Asphodelus genus. This PUFA-rich profile enhances the nutritional relevance of *A. microcarpus* seed oils and supports their potential value for dietary and functional applications. Conversely, the SFA-dominant profile of the El Jadida population underscores the importance of geographic and environmental factors in shaping lipid biosynthesis. Collectively, these results provide a clear overview of the main contributions of this work and strengthen the positioning of

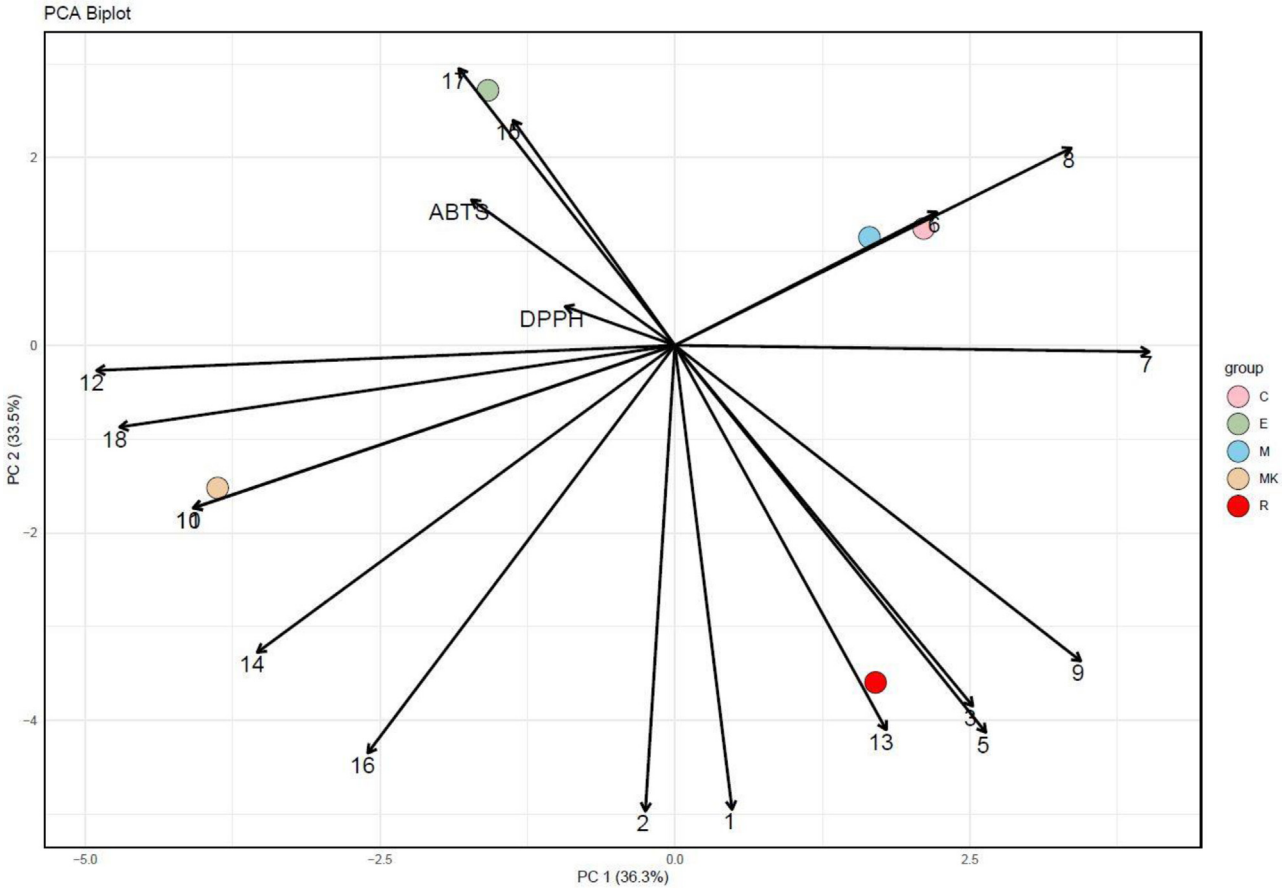

Fig. 9. Principal component analysis of chemical and antioxidant profiles of *Asphodelus microcarpus* oil. 1, Lauric acid; 2, Myristic acid; 3, Isomyristic acid; 4, Pentadecanoic acid; 5, Palmitoleic acid; 6, Palmitic acid; 7, Margaric acid; 8, Linoleic acid; 9, Oleic acid; 10, Stearic acid; 11, Petroselinic acid, 12, α-Linolenic acid; 13, Arachidic acid;14, Behenic acid; 15, Erucic acid; 16, Tricosanoic acid; 17, Lignoceric acid; 18, Nervonic acid; C, Casablanca; E, El Jadida; M, Mohammedia; Mk, Meknes; R, Rabat.

A. microcarpus seed oils as a promising source of bioactive fatty acids.

4 Conclusion

This study presents the first comprehensive analysis of the fatty acid composition, physicochemical properties, and antioxidant potential of *A. microcarpus* seed oils collected from five distinct regions of Morocco. The oils showed good yields, high unsaturation, and strong oxidative stability. Fatty acid profiles varied by region, with linoleic acid dominant in coastal samples and saturated/monounsaturated fats in inland ones. Antioxidant activity appeared to be influenced by fatty acid composition, while chemometric analyses identified two ecotypes coastal and inland shaped by environmental conditions. These results underscore the value of *A. microcarpus* seed oil as a bioactive lipid source with potential applications in the food and nutraceutical industries, it may serve as a functional ingredient or omega-6 enriched supplement. In the cosmetic sector, its antioxidant properties and favorable lipid composition could be exploited for skin-protective formulations. Additionally, these bioactive compounds present potential for pharmaceutical applications, particularly in developing products aimed at preventing oxidative stress or modulating inflammation. Limitations of the study include the lack of data on other bioactive lipid-soluble compounds, such as tocopherols, phytosterols, and phenolic esters, which are known to contribute to antioxidant and therapeutic properties. Future research will focus on the identification and quantification of these compounds to better understand the full nutritional and pharmacological potential of this underexplored species.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

Zakaria Benchama: Conceptualization, Methodology, Investigation, and Writing – original draft.

Othman El Faqer: Formal analysis, Writing, and editing.

Achraf Abdou: Formal analysis, Conceptualization, and Visualization

Aouatif Guedmioui: Investigation, and Resources.

Mohamed Dakir: Conceptualization, and Visualization.

Abdelhakim Elmakssoudi: Conceptualization, and Visualization.

José Antonio Cayuela-Sánchez: Conceptualization, Visualization and Validation.

Jamal Jamal Eddine: Resources, and Supervision.

Abdelmjid Cherif: Conceptualization, and Visualization.

Anass El Ouaddari: Conceptualization, Methodology and Supervision.

Abdelaziz El Amrani: Conceptualization, Writing & editing, and Supervision.

Data availability statement

Data will be made available on request.

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