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Research Article

Chemical Composition and Physicochemical Analysis of *Opuntia dillenii* Extracts Grown in Morocco

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The chemical composition and physicochemical properties of hexane and ethyl acetate extracts of skin, juice, and seeds of *Opuntia dillenii* fruit collected from three Moroccan regions (Oujda, Nador, and Essaouira) were studied. The study revealed that the seed oil extracts presented the highest yield of 13.12%, followed by the skin fraction (1.77%) and the juice extract (0.49%). The evaluation of fatty acid compositions using GC-MS analysis revealed the presence of linoleic acid as a dominating unsaturated fatty acid with a value of 72.39%, followed by palmitic acid, oleic acid, and stearic acid in all localities. Otherwise, the juice extract of Oujda locality was richer in margaric acid (37.41%), followed by Essaouira skin extract (10.7%) and Oujda seed extract (6.18%). However, the campesterol was detected only in trace in the juice extract. The physicochemical properties of *O. dillenii* seed oils such as acid value, peroxide value, ester value, pH value, saponification value, density, and refractive index were all found to be in good agreement with the quality criteria for pure and fresh oils. In addition, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were implemented to compare the difference in the chemical composition of the different *O. dillenii* extracts.

1. Introduction

Opuntia dillenii, commonly known as prickly pear, is a perennial plant belonging to the Cactaceae, Caryophyllales, family and the Caryophyllidae subclass [1, 2]. It is extensively distributed in the desert, semidesert, and dry regions in the tropics and subtropics [3]. This plant is native to tropical America and India. However, it can be grown in Australia, Asia, and the Mediterranean region [4]. It was introduced in Europe and North Africa in the 16th century [5]. Cactus has remarkable benefits to human and animal health [6]. It can be a source of various chemical compounds of biological and pharmacological importance.

Several studies have revealed its high efficacy in the treatment of antihyperlipidemic, antiatherosclerotic effects [7]. Its ability to protect nerve cells from Alzheimer's, Parkinson's, and cerebral vascular diseases has also been reported [8]. Particularly, juice of *Opuntia* fruits is a rich source of dietary fiber, vitamins (B1, B2, and C), and natural colorants (betanin and indicaxanthin) [9–14], which are responsible for the intense red coloring of the fruit [11]. It is also known by its analgesic, antihyperglycemic [15], inflammatory [16], antifungal [17], anticancer [1, 18], hepatoprotective [19], and antidiabetic activities [18, 20]. Indeed, the seeds of *O. dillenii* may contribute to the higher antioxidant activity due to the high concentrations of polyphenols and flavonoids and the presence of a large amount of unsaturated fatty acids that provide valuable natural antioxidants for the pharmaceutical and food industry [21]. The *O. dillenii* oil is well-known for its richness

in essential fatty acids and vitamin E [6] which have efficiency potential on the skin and can be used as antiaging and antiwrinkle agents. The oil of *O. dillenii* could also be a source of linoleic acid (omega-6) [22], which is involved in the regulation of cardiovascular disorders. This oil has also shown a hepatoprotective and antidiabetic effect [23, 24]. All these properties have been able to give a positive impact on the exploration of *O. dillenii* fruits.

In this paper, continuing our previous work on *O. dillenii* plant, we report the evaluation of the chemical and physicochemical properties of hexane and ethyl acetate extracts of skin, juice, and fruit seeds of *O. dillenii* grown in three different regions of Morocco (Oujda, Nador, and Essaouira). This investigation was performed to assess the potential nutritional value of *O. dillenii* fruits for human and animal consumption. The extracts were analyzed, characterized, and identified by GC-MS. The variation of *O. dillenii* extracts parameters between the three regions was investigated using statistical analyses (hierarchical cluster analysis (HCA) and principal component analysis (PCA)).

2. Materials and Methods

2.1. Chemicals and Reagents. Hexane, ethyl acetate, and methanol were purchased from Merck (Karlsruhe, Germany). All chemicals and solvents were of highly analytical grade and were used as received from Merck without further purification.

2.2. Plant Material. The matured purple fruits of prickly pear (*O. dillenii*) were collected in February 2017 from three regions in Morocco (Figure 1): Oujda (Sidi Maafa), Nador (Kariat Arkmane), and Essaouira (Smimou). The geographical and climatic data of the studied regions are presented in Table 1. The plant species was authenticated by Prof. M. Bnouham (Faculty of Sciences, University Mohammed Premier, Oujda, Morocco).

The sampled fruits were transported to the laboratory, washed thoroughly with water to remove dust and spines, then peeled, and mixed for 5 min using a Moulinex blender (Faciclic Glass LM310E, Groupe Seb, Mayenne, France) to separate seeds from the juice by passing through a sieve with a 2 mm screen (Figure 2). The juice was dehydrated by heating at 40°C in the oven for 15 days. Seeds and skins were washed with distilled water, dried at room temperature (25°C), for one week, weighed to calculate the percentage in the edible fraction, then reduced to a fine powder using a Moulinex coffee grinder (DPA241, Groupe Seb, Lourdes, France), and stored at −20°C, as well as the juice, for a maximum of eight weeks.

2.3. Preparation of Plant Extracts. Ground seeds, skin, and juice of *O. dillenii* were macerated with hexane as a nonpolar solvent for removal of fatty acids. Each powder (50 g) was mixed with 110 mL of hexane. The mixture was stirred at room temperature (18°C) for 24 h and filtered through a glass filter crucible (50 mL, Porosity 4, Isolab, Wertheim, Germany) connected to a water aspirator. The extracts were concentrated on a rotary evaporator (Laborota 4000, Heidolph Instruments, Schwabach, Germany) under reduced pressure at 40°C to obtain the corresponding powder which was then used by repeating the process of maceration with ethyl acetate, filtration, and concentration under reduced pressure. The extracts were conserved in dark bottles and stored at 4°C until use.

2.4. Physicochemical Analysis of O. dillenii Oil. In order to check the quality and appropriate conservation of the oil samples, the acid value and the ester value (expressed as milligram KOH per g of oil) and the peroxide value (PV) (expressed as milliequivalents of active oxygen per kg of oil) were determined. The water content in the hexane extracts has been calculated according to the standard NF V 05–108 [25]. The acid value, ester value, peroxide value, and saponification value were determined according to the Codex Stan 210–1999 [26] standard protocol. The standard protocols NF ISO 6883 (July 1995) [27] and NF ISO 6320 (January 1996) [25] were adopted to measure the density and refractive index at 20°C. The density of the oils was determined by a mass over volume measurement. The pH values were measured using a CPC-501 pH-meter (Elmetron, Chorzow, Poland). The measures were performed in triplicate for each parameter, and the results were presented as mean ± standard deviation.

2.5. Fatty Acid GC-MS Analysis of O. dillenii Extracts. The fatty acid methyl esters of hexane and ethyl acetate extracts of *O. dillenii* fruits were prepared following the standard protocol NF T60-233 [28]. Their separation and identification were performed on a Shimadzu GC system (Kyoto, Japan) equipped with a BPX25 capillary column with 5% diphenyl, 95% dimethylpolysiloxane phase (30 m × 0.25 mm inner diameter × 0.25 μm film thickness), coupled to a QP2010 MS. Pure helium gas (99.99%) was used as carrier gas with a constant flow rate of 3 mL/min. The injection, ion source, and interface temperatures were all set at 250°C. The temperature program used for the column oven was 50°C (held for 1 min), heated to 250°C at 10°C/min and held for 1 min. The ionization of the sample components was done in the EI mode (70 eV). The mass range scanned was 40–300 m/z. 1 μL of each prepared extract diluted with an appropriate solvent was injected in a splitless mode (split ratio 90 : 1). All samples were analyzed in triplicate. Finally, compounds were identified by comparison of their retention times with those of authentic standards and their mass spectrum fragmentation patterns with those found in databases or those stored on the National Institute of Standards and Technology (NIST) 147, 198 compounds. LabSolutions (version 2.5) was used for data collection and processing.

2.6. Statistical Analysis. All the results are expressed as mean ± standard deviation (SD). The obtained data of the oil components with a percentage greater than 5% were analyzed statistically using the principal component (PCA) and

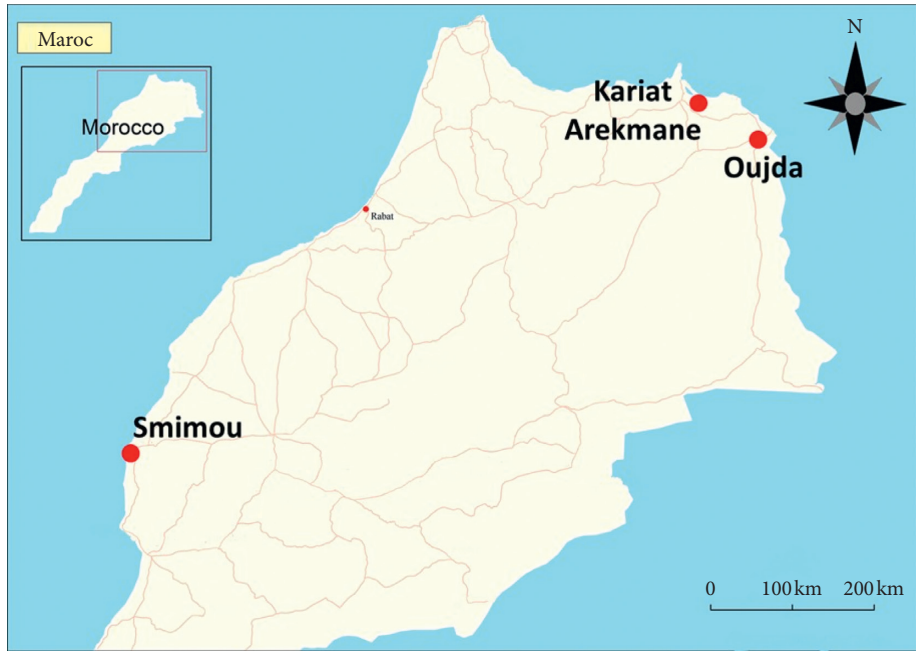

Figure 1: Sampling sites: Oujda, Nador, and Essaouira localization.

Table 1: Ecogeographic characteristics of the sampling sites.

Sampling location	Region	Latitude	Longitude	Altitude (m)	MAP (mm)	MWT (°C)	MST (°C)
Sidi Maafa	Oujda	34°38′16′	1°53′16′	450	338	10.7	23.9
Kariat Arkmane	Nador	35°06′15	2°44′04′	2	313	14.5	22.8
Smimou	Essaouira	31°12′49′	9°42′21′	237	300	8.7	24.5

MAP: mean annual precipitation; MWT: mean of winter temperature; MST: mean of summer temperature. Source: https://fr.weatherspark.com/y/31980/Météo-habituelle-à-Smimou-Maroc.

(a) (b) (c) (d) (e)

Figure 2: *O. dillenii* fruit: (a) fresh fruits; (b) pulp; (c) juice; (d) seeds; (e) seed oil.

hierarchical cluster (HCA) analyses. All the statistics were performed employing the XLSTAT software.

3. Results and Discussion

3.1. Yield Percentage of Skin, Pulp, and Seeds in Fruit. The length and width (diameter) as well as the skin, juice, and seed content of the *O. dillenii* fruits, according to their locality, are summarized in Table 2. The morphological study showed that the Essaouira fruits were bigger than those of Oujda and Nador, while no large differences were observed between the morphological characteristics (length and width) of the *O. dillenii* belonging to Oujda and Nador locality. On the other hand, the percentage yield analysis of each dry part (skin, juice, and seeds) obtained from fresh Essaouira fruits revealed that the seed content was found to be the highest (14.7%), followed by skin (11.6%) and juice (4.4%). The Essaouira fruit contained the highest content of seeds, skin, and juice. In the literature, Ali Alsaad reported lower fruit seed content, 9.5%, in Iraq [22].

Table 2: Morphological characteristics, pH juice, and yield of the dry skin, juice, and seeds.

Locality	Length (cm)	Width (cm)	Yield			pH (juice)
			Dry skin (%)	Dry juice (%)	Dry seeds (%)	
Oujda	5.00 ± 0.14	2.50 ± 0.34	8.50 ± 0.17	4.00 ± 0.33	13.30 ± 0.18	3.90 ± 0.28
Nador	4.70 ± 0.15	2.30 ± 0.09	9.16 ± 0.15	3.03 ± 0.30	12.80 ± 0.24	3.20 ± 0.26
Essaouira	7.00 ± 0.10	4.00 ± 0.47	11.60 ± 0.14	4.40 ± 0.10	14.70 ± 0.16	4.80 ± 0.21

These findings demonstrated clearly that the fresh *O. dillenii* fruits contain more than 75% of water, which was in good agreement with previous data reported in the literature [29, 30]. Furthermore, the juice fruit of Essaouira was less acidic compared to that of the two other localities, pH = 4.8, 3.9, and 3.2 for Essaouira, Oujda, and Nador, respectively.

3.2. Physical and Chemical Parameters of O. dillenii Seed Oils. The study of the physicochemical properties of any product is a very useful way to measure its quality before its use or consumption. These different parameters can be influenced by climatic conditions as well as cultural practices. In this context, some physicochemical parameters of *O. dillenii* seed oils (for the three sites) such as acid value, ester value, peroxide value, saponification value, pH value, density, and refractive index were measured following the appropriate protocols. The obtained results were collected and are presented in Table 3.

According to the obtained data, the water content in seed oil for the three localities of *O. dillenii* was found to be below the max value proposed by Codex Stan 210–1999 and COI standards (max 0.2%). The higher content was shown by Nador sample with 0.189%, followed by Essaouira and Oujda samples with values of 0.154% and 0.148%, respectively. The peroxide value, which is the most used indicator to describe the relative stability to lipid oxidation of oils, was calculated [31]. Values greater than 10 meq/kg are highly susceptible to autooxidation as a consequence of moisture or presence of trace elements [32]. In our case, the peroxide values of the three localities ranged from 5.60 to 6.35, less than 10 meq O_2/kg of oil. These outcomes suggested that our oils possess a high shelf life. However, these values are higher than those obtained by El Mannoubi et al. [33] for *O. ficus-indica* seed oil and by Alsaad et al. [22] for *O. dillenii*.

The saponification values of the studied samples ranging from 210.37 to 252.45 were higher than those of *O. stricta* and *O. ficus-indica* reported by Ennouri et al. [29], but they always stayed in the range of Codex Stan 210–1999 and COI standards. This parameter indicates the presence of saturated fatty acids in the studied oil, and a low value points to the predominance of long-chain fatty acids, as suggested by Akintayo and Bayer [34]. The refractive index is another parameter that can be used to measure the quality of the oil. Low indices characterize oils of high quality. The refractive indices of our oils were found to be higher compared to the results of El Mannoubi [33] and Ennouri [29], but in accordance with the norm (Codex Stan 210–1999 and COI standards). The density of the *O. dillenii* seed oils at 20°C compared favorably with that of *O. stricta* seed oil but was higher than that of *O. ficus-indica* seed oil [18]. Essaouira oil was found to be less acidic with a pH value of 6.96, higher than that of Oujda (pH = 6.54) and Nador (pH = 6.50) samples.

The evaluation of the acid value is another possible way to determine the quality of fatty acids present in the oil, which could in turn explain the shelf life and stability of such oil. Its value gives an idea of the free acid level. A value below 2 presents proof of good conservation (low amount of free acids) [32]. In the present work, the acid values found in *O. dillenii* seed oils for the three samples were higher than 2. These high acidity levels could be attributed to the advanced maturity of the fruit and/or the harvesting period, as previously reported by Terouzi et al. [35]. This high acidity value could indicate strong enzymatic hydrolysis of the seeds during oil harvesting, handling, or processing [36]. It could also be explained by bad preservation. According to [37], when oil was subjected to poor storage conditions, its quality could deteriorate in various ways, but most often by hydrolysis or oxidation. The ester index test is an indirect method for determining the ester content of the oil. In fact, oils of excellent qualities have a large quantity of esters.

Generally, the studied oils extracted from *O. dillenii* seeds have been found to meet all quality criteria that confirm an excellent application. However, the observed differences between the samples could be due to geographical location, soil, climatic conditions, and maturation [38].

3.3. Chemical Composition of Hexane and Ethyl Acetate O. dillenii Extracts. In this study, we compared the yields and the chemical composition of the hexane and ethyl acetate extracts of skin, juice, and seeds of *O. dillenii* fruits harvested from three geographical locations in Morocco (Tables 4–6).

In the case of Nador variety, the hexane seed extract showed the highest yield with a value of 13.12%, followed by Oujda extract (9.25%) and then Essaouira extract (5.85%). However, in the literature, Liu et al. [39] obtained a lower yield of 6.65% by using a supercritical carbon dioxide extraction of seed oil. Recently, Alsaad et al. [22] reported relatively the same yield of 6.5% by hydrodistillation extraction. Labuschagne and Hugo [40] reported that the oil content in cactus *Opuntia dillenii* seeds from South Africa was 5.69%, while Chang [21] reported that the oil content in cactus pear seeds from China was 6.01%.

The preliminary report of gas-chromatography coupled with mass spectrometry analysis of the hexane extract revealed the presence of numerous fatty acids. Their amount varied according to the locality and the part of cactus fruit.

Table 3: Physicochemical properties of *O. dillenii* seed oils from the three localities.

Properties	Locality			Codex Stan 210–1999 and COI standards [26]
	Oujda	Nador	Essaouira	
Acid value (mg KOH/g of oil)	5.61 ± 0.04	7.85 ± 0.20	8.97 ± 0.41	—
Saponification value (mg of KOH/g of oil)	238.42 ± 1.73	252.45 ± 1.54	210.37 ± 0.98	168–265
Ester value (mg KOH/g of oil)	232.82 ± 1.92	244.60 ± 1.34	201.40 ± 1.63	—
Peroxide value (Meq O_2/kg of oil)	5.85 ± 0.46	6.35 ± 0.92	5.60 ± 0.91	Max (10)
Density (20°C)	0.926 ± 0.04	0.925 ± 0.18	0.913 ± 0.37	0.881–0.927
Refractive index (20°C)	1.476 ± 0.93	1.478 ± 0.82	1.477 ± 0.62	1.447–1.477
Water content (%)	0.148 ± 0.03	0.189 ± 0.09	0.154 ± 0.10	Max 0.2%
pH	6.54 ± 0.4	6.50 ± 0.34	6.96 ± 0.94	—

Table 4: Chemical composition of hexane and ethyl acetate extracts from *O. dillenii* seeds fruits.

Compounds	Retention time (min)	Relative content (%) in seeds					
		Oujda		Nador		Essaouira	
		H. E	EAc. E	H. E	EAc. E	H. E	EAc. E
Butanoic acid (C4:0)	12.53 ± 0.04	nd	nd	nd	21.68 ± 0.02	nd	nd
Acetic acid	12.89 ± 0.04	nd	nd	nd	7.37 ± 0.11	nd	nd
Capric acid (C10 : 0)	14.69 ± 0.04	nd	nd	nd	4.41 ± 0.03	nd	nd
2,4-Decadienal	15.08 ± 0.03	0.71 ± 0.12	0.95 ± 0.05	nd	10.26 ± 0.05	nd	1.28 ± 0.07
Myristic acid (C14 : 0)	20.40 ± 0.02	2.91 ± 0.13	0.75 ± 0.12	2.41 ± 0.07	1.58 ± 0.08	0.97 ± 0.02	0.54 ± 0.12
Methoxyacetic acid	22.58 ± 0.01	nd	nd	nd	nd	nd	1.14 ± 0.45
Palmitic acid (C16 : 0)	22.62 ± 0.06	24.27 ± 0.17	9.40 ± 0.08	21.50 ± 0.41	12.98 ± 0.07	19.81 ± 0.07	20.17 ± 0.18
Margaric acid (C17 : 0)	23.10 ± 0.01	nd	6.18 ± 0.33	nd	nd	nd	nd
Oleic acid (C18 : 1)	24.41 ± 0.02	nd	5.04 ± 0.04	nd	nd	nd	nd
Linoleic acid (C18 : 2)	24.45 ± 0.08	66.57 ± 0.08	58.50 ± 0.21	72.39 ± 0.11	36.21 ± 0.33	69.60 ± 1.01	58.32 ± 2.04
Stearic acid (C18 : 0)	24.77 ± 0.05	5.49 ± 0.17	19.1 ± 0.40	2.37 ± 0.14	5.29 ± 0.12	9.61 ± 0.81	18.04 ± 0.21
SFA[a]		32.67	35.43	26.28	45.94	30.39	39.89
UFA[b]		66.57	63.54	72.39	36.21	60.69	58.32
UFA/SFA[c]		2.04	1.79	2.75	0.78	2.29	1.46
Yield of extracts %		9.25 ± 0.07	0.92 ± 0.04	13.12 ± 0.02	0.96 ± 0.20	5.85 ± 0.47	1.34 ± 0.20

nd: not detected; H. E: hexane extract; EAc. E: ethyl acetate extract; a: saturated fatty acids (SFA); b: unsaturated fatty acids (UFA); c: unsaturation ratio = UFA/SFA.

The major constituents of the seed oils were linoleic acid with amounts varying from 66.57 to 72.39%, followed by palmitic acid (19.81 to 24.27%), stearic acid (2.37 to 9.61%), and myristic acid (0.97 to 2.91%). The Nador locality oil presented the highest percentage of linoleic acid (72.39%) followed by Essaouira and Oujda localities with 69.60 and 66.57%, respectively. A high unsaturated ratio of 2.75 was detected for the Nador locality oil, followed by Essaouira and Oujda localities, 2.29 and 2.04, respectively. These values showed that the seed oils were rich in unsaturated fatty acids and confirmed their importance as nutritional value for human consumption.

In contrast, a very low amount of oil was recovered in the case of ethyl acetate seed extracts. The yield was about 1.35% for Essaouira, followed by 0.96 and 0.92% for Nador and Oujda localities, respectively. This oil was also dominated by linoleic acid whose values ranged from 36.21 to 72.39%, followed by butanoic acid, 0 to 21.68%, stearic acid, 5.49 to 23.25%, and palmitic acid, 9.40 to 20.17%. Oujda and Essaouira extracts were richer in linoleic acid, with a value of 72.39%, than Nador extract with 36.21%. However, a moderate quantity of butanoic acid as saturated fatty acid was found only in Nador extract with approximately 21.68%. The ethyl acetate seed extract showed the presence of less than 5.04% of oleic unsaturated fatty acid in only Oujda locality and a low amount of margaric acid (6.18%) in the same locality. On the other hand, the extract from Nador showed a higher value of unsaturated ratio (2.75), followed by Essaouira extract (2.29) and Oujda extract (2.04). Our results are in concordance with those reported in the literature by Alsaad et al. [22]. The major constituents of the seed oil were linoleic acid (72.9%), palmitic acid (15.12%), and stearic acid (7.51%). However, Liu et al. [39] reported a different profile of the Chinese seed oil. The main components obtained were linolenic acid (66.56%), palmitic acid (19.78%), stearic acid (9.01%), and linoleic acid (2.65%). This variation could be due to geographical location, soil, climatic conditions, and maturation.

The yields of hexane and ethyl acetate extracts of *O. dillenii* juice from the three localities presented low values less than 0.86%. The chemical composition analysis of hexane extract (Table 5) showed that palmitic acid was the dominant fatty acid with a value of 55.89% for Oujda sample followed by linoleic (39.09%), oleic (24.82%), and stearic acid with less than 4.47%.

Table 5: Chemical composition of hexane and ethyl acetate extracts from *O. dillenii* juice fruits.

Compounds	Retention time (min)	Relative content (%) in juice					
		Oujda		Nador		Essaouira	
		H. E	EAc. E	H. E	EAc. E	H. E	EAc. E
1,4-Butanedioic acid	12.68 ± 0.03	nd	nd	nd	1.08 ± 0.01	nd	nd
Acetic acid	12.89 ± 0.03	nd	nd	nd	nd	nd	2.01 ± 0.10
Capric acid (C10 : 0)	14.95 ± 0.03	nd	nd	2.29 ± 0.02	nd	nd	0.85 ± 0.09
Citric acid	17.23 ± 0.04	nd	10.18 ± 0.01	1.61 ± 0.03	2.55 ± 0.03	3.49 ± 0.02	17.01 ± 0.21
Lauric acid (C12 : 0)	17.89 ± 0.04	3.96 ± 0.41	1.64 ± 0.31	nd	2.45 ± 0.05	1.62 ± 0.04	7.61 ± 0.17
9-Eicosene	18.74 ± 0.01	4.69 ± 0.01	nd	1.18 ± 0.01	nd	nd	nd
Lauryl ethoxylate	20.32 ± 0.01	2.76 ± 0.51	2.08 ± 0.17	1.6 ± 0.05	nd	nd	nd
Myristic acid (C14 : 0)	20.40 ± 0.02	nd	nd	nd	nd	4.04 ± 0.08	12.54 ± 0.28
11-Hexadecenoic acid (C16 : 1)	22.42 ± 0.01	nd	nd	nd	nd	4.73 ± 0.07	nd
Palmitic acid (C16 : 0)	22.96 ± 0.06	55.89 ± 0.31	6.81 ± 0.11	31.46 ± 0.11	17.77 ± 0.24	23.56 ± 0.41	9.65 ± 0.08
Margaric acid (C17 : 0)	23.00 ± 0.01	0.15 ± 0.17	37.41 ± 0.08	nd	13.18 ± 0.02	nd	34.15 ± 0.09
Oleic acid (C18 : 1)	23.89 ± 0.03	1.01 ± 0.11	nd	22.57 ± 0.17	nd	24.82 ± 0.32	5.15 ± 0.09
Linoleic acid (C18 : 2)	24.39 ± 0.08	31.31 ± 0.21	16.04 ± 0.25	39.09 ± 0.22	47.13 ± 0.35	32.84 ± 1.01	10.46 ± 0.21
Stearic acid (C18 : 0)	24.75 ± 0.02	nd	23.25 ± 0.08	nd	13.11 ± 0.06	4.47 ± 0.09	nd
Campesterol	25.44 ± 0.01	nd	2.48 ± 0.01	nd	2.15 ± 0.02	nd	nd
SFA[a]		59.85	69.11	33.75	46.51	33.69	55.15
UFA[b]		32.32	16.04	61.66	47.13	62.39	15.61
UFA/SFA[c]		0.53	0.23	1.96	1.01	1.85	0.27
Sterols		0	2.48	0	2.15	0	0
Yield of extracts (%)		0.49 ± 0.01	0.27 ± 0.08	0.40 ± 0.12	0.46 ± 0.07	0.45 ± 0.19	0.86 ± 0.08

nd: no detected; H. E: hexane extract; EAc. E: ethyl acetate extract; a: saturated fatty acids (SFA); b: unsaturated fatty acids (UFA); c: unsaturation ratio = UFA/SFA.

Table 6: Chemical composition of hexane and ethyl acetate extracts from *O. dillenii* skin fruits.

Compounds	Retention time (min)	Relative content (%) in skin					
		Oujda		Nador		Essaouira	
		H. E	EAc. E	H. E	EAc. E	H. E	EAc. E
Propanoic acid	12.02 ± 0.01	nd	nd	nd	2.09 ± 0.10	nd	nd
Capric acid (C10 : 0)	14.99 ± 0.01	nd	nd	nd	nd	nd	2.1 ± 0.09
Citric acid	17.23 ± 0.04	0.89 ± 0.10	nd	0.64 ± 0.02	nd	2.18 ± 0.09	nd
Lauric acid (C12 : 0)	17.89 ± 0.01	0.98 ± 0.01	nd	1.27 ± 0.31	nd	3.43 ± 0.21	nd
4-Fluorobenzoic acid	17.52 ± 0.04	2.14 ± 0.2	16.48 ±.31	nd	1.06 ± 0.28	nd	0.6 ± 0.01
9-Eicosene (E)	18.78 ± 0.01	nd	nd	nd	nd	nd	3.5 ± 0.09
Lauryl ethoxylate	20.32 ± 0.01	nd	3.28 ± 0.04	1.33 ± 0.09	nd	nd	nd
Myristic acid (C14 : 0)	20.38 ± 0.02	2.34 ± 0.02	nd	nd	nd	6.37 ± 0.32	nd
Pentadecanoic acid (C15 : 0)	21.48 ± 0.03	5.08 ± 0.09	nd	nd	nd	1.88 ± 0.51	nd
11-Hexadecenoic acid (C16 : 1)	22.39 ± 0.04	nd	nd	nd	nd	15.39 ± 1.26	nd
Palmitic acid (C16 : 0)	23.60 ± 0.09	28.15 ± 0.31	13.01 ± 0.41	45.28 ± 0.28	28.34 ± 0.89	17.49 ± 0.75	14.17 ± 0.29
Margaric acid (C17 : 0)	23.77 ± 0.07	3.15 ± 0.09	nd	nd	nd	nd	10.7 ± 0.35
Oleic acid (C18 : 1)	24.13 ± 0.05	16.67 ± 0.23	nd	nd	nd	nd	nd
Linoleic acid (C18 : 2)	24.40 ± 0.09	40.55 ± 1.56	67.07 ± 2.01	51.39 ± 0.91	68.09 ± 0.78	52.98 ± 0.51	38.27 ± 0.86
Stearic acid (C18 : 0)	24.98 ± 0.01	nd	nd	nd	nd	nd	3.22 ± 0.03
SFA[a]		39.70	13.01	46.55	28.34	29.17	25.39
UFA[b]		57.22	67.07	51.39	68.09	68.37	58.27
UFA/SFA[c]		1.44	5.15	1.09	2.43	2.34	2.29
Yield of extracts (%)		1.77 ± 0.10	0.98 ± 0.10	1.16± 0.37	0.91 ± 0.47	1.06± 0.20	1.11 ± 0.37

nd: no detected; H. E: hexane extract; EAc. E: ethyl acetate extract; a: saturated fatty acids (SFA); b: unsaturated fatty acids (UFA); c: unsaturation ratio = UFA/SFA.

In the ethyl acetate extract, linoleic acid was found to be the most abundant fatty acid detected with 47.13% in Nador juice extract followed by stearic (23.25%) and palmitic acid (17.77%). However, citric acid was found especially in ethyl acetate extract with a moderate percentage (17.01%) in Essaouira locality. Interesting, the study showed the presence of small quantities of campesterol, which is a well-known beneficial agent with cholesterol-lowering and anticarcinogenic properties [41], concentrated especially in ethyl acetate extracts of Oujda and Nador varieties with contents of 2.48% and 2.15%. It is also

important to note the presence of margaric acid in ethyl acetate extract with a moderate amount of 37.41% and 34.15% in Oujda and Essaouira extracts, respectively. This compound was detected in a low value of 13.18% in Nador variety and was only present in trace amount, 0.15%, in the hexane extract of Oujda locality.

Concerning the skin extract, the highest yield of 1.77% was detected in the hexane extract of Oujda variety. However, the yield obtained in the ethyl acetate was about 1% in all the localities. Similar to previous results of seeds and juice extracts, the skin extracts were also found to be rich in linoleic and palmitic acids (Table 6).

The highest percentage of linoleic acid, 68.09%, was found in Nador ethyl acetate extract. This compound was also abundant in the hexane extract with a quantity of 52.98% in the Essaouira locality. This study also showed that the Nador variety contained a higher amount of palmitic acid, 45.28% and 28.34% in hexane and ethyl acetate extracts, respectively. In contrast, oleic acid (16.67%) and 11-hexadecenoic acid (15.39%) were present, as unsaturated fatty acids, only in the hexane extracts of both samples of Oujda and Essaouira.

In addition, the 4-fluorobenzoic acid was found in the ethyl acetate extract, with a percentage of 16.48%, as well as in the hexane extract, with a low content of 2.14%, of Oujda sample. This compound was also detected in ethyl acetate extract of Nador and Essaouira samples but only in trace amounts.

Furthermore, a low quantity of margaric acid was identified only in the ethyl acetate extract of Essaouira. Pentadecanoic acid and margaric acid have a positive effect on health in several disease etiologies [42]. Holman [43] showed that both pentadecanoic and margaric acids have a role in reducing the development of multiple sclerosis, suggesting that fatty acids could increase membrane fluidity [44] to a similar degree to polyunsaturated fatty acids. Besides and based on previous studies, some of the constituents identified in *O. dillenii* fruits were biologically active compounds. They were proven to possess pharmacologic activities which may contribute to the healing potential of the plant. Linoleic acid has beneficial properties for the skin, and, for this purpose, it is widely used in the cosmetics and pharmaceutical industry. Stearic acid has neutral effects on the concentration of LDL cholesterol in blood serum and no cholesterol-lowering impact on human health [45]. Based on UFA/SFA ratio, it is essential to mention that there is a higher amount of unsaturated fatty acids (UFA) in the skin and seed parts of the studied *O. dillenii* fruits than the juice part, which was found to be richer in saturated fatty acids (SFA). The ethyl acetate extract of Oujda skin extract presented the highest unsaturated ratio of 5.15. However, the hexane extract was less rich in unsaturated acids. Particularly, the study reported that the amount of linoleic acid is higher in comparison with other vegetable oils such as potato oil (52.69%), pomegranate oil (3.84%), and sesame oil (44.5%). These findings were in accordance with recently published studies by Ghazi, where linoleic acid was the dominant fatty acid with an exceptional level up to 79.83% [46–49]. Finally, the evaluation of the chemical composition as well as the physicochemical properties of *O. dillenii* fruit extracts demonstrated that this plant could be an interesting natural source of edible oil containing high amounts of unsaturated fatty acids. In addition, it is interesting to note that the difference between the chemical compositions of the different extracts from the three varieties of *O. dillenii* fruits could be a consequence of many factors such as the degree of maturation, the growing conditions, and the nature of the solvent used during the extraction [35, 36]. On the other hand, further detailed analyses and studies, along with isolation of active constituents, are needed to investigate this plant further.

3.4. Statistical Analyses. The statistical study was based on the hierarchical cluster analysis (HCA) and principal component analysis (PCA). The intermediate correlation matrices, correlation coefficients between the variables and the two axes F1 and F2, and the projection of the variables in the space of the axes F1 and F2 were obtained with XLSTAT software.

From the dendrogram produced by HCA (Figure 3), based on the Euclidean distances between collected samples, *O. dillenii* populations can be classified into five main clusters, which shows that there is a significant difference in the composition of *O. dillenii* extract harvested from three Moroccan regions (Oujda, Nador, and Essaouira). The samples linked by short distances are more similar than those connected by long ones. In the closer distances (18 units), the populations examined were divided into five groups (Gr 1 to 5). The first group (Cluster I), represented by five samples from the three regions of Morocco (Oujda, Nador, and Essaouira), SO1, SN1, SKN2, SKO2, and SE1, was rich in linoleic acid (32.27–72.39%). These populations in the vicinity have a relatively different climate and soil, resulting in different oil contents. The second group (Cluster II), containing the following chemotypes: SN2, JN2, SKE1, SO2, and SE2, has been characterized by a significant percentage of linoleic acid (58.32–58.50%). The ethyl acetate juice extracts from Oujda and Essaouira samples (JE2 and JO2) were found to construct the third group (Cluster III). This latter group was found to be rich in margaric acid (34.15–37.41%), citric acid (10.18–17.01%), and stearic acid (5.29–13.11%). In the fourth group (Cluster IV), which was represented by SKE2, SKO1, JN1, and JE1 samples, palmitic acid (14.17–28.46%), oleic acid (5.15–22.57%), and linoleic acid (58.32–58.50%) were found to be the most abundant components. The group also contains significant to moderate levels of oleic acid (5.04–16.67%). Finally, the fifth group (Cluster V), represented by the two samples JO1 and SKN1, was found to be rich in linoleic acid (30.31–50.39%) and palmitic acid (45.28–55.89%).

The analysis of the results showed that most of the information is explained by the first two factorial axes. In the F1 × F2 factorial design, the eigenvalues of the two components F1 and F2 and their contribution to the total inertia are represented in Table 7. For the PCA, it was found that the first two main components accounted for 56.21% of the phytochemical variance. As shown in Figure 4, by considering the PCA1 axis, it is possible to note that the extracts of *O. dillenii*, JO2, JE2, and JN2, are distinguished by a greater quantity of margaric acid (0.854), citric acid (0.977), and stearic acid (0.147). On the other hand, the extracts SN1, SO1, SE1, SE2, SN2, SO2, SE2, SO2, SKN2, SKE1, and JN2 are characterized by a low content of these constituents. Meanwhile, SN1, SO1, SE1, SE2, SN2, SO2, SE2,

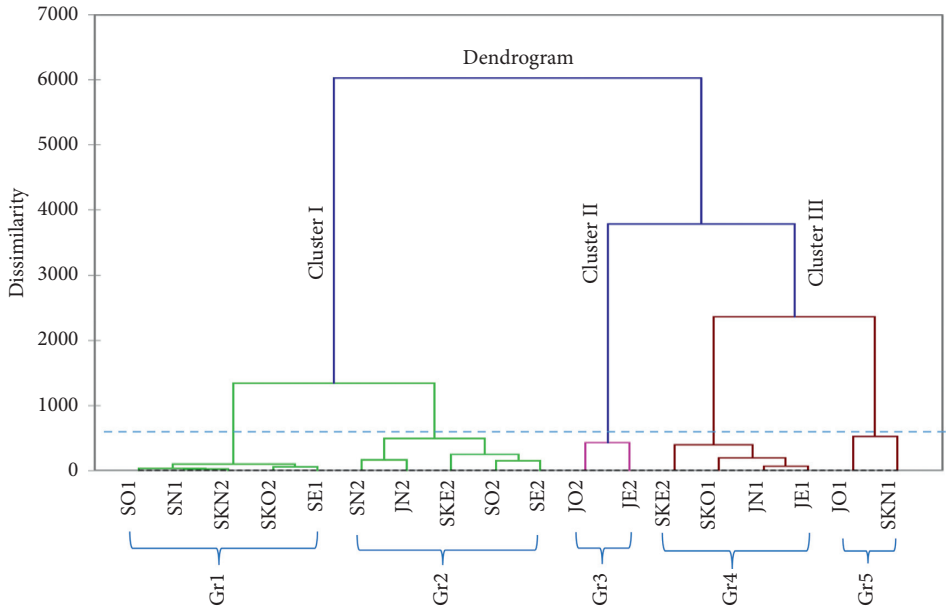

Figure 3: Dendrogram of *O. dillenii* extracts produced by the hierarchical cluster analysis.

Table 7: Distribution of inertia between the two axes (F1 × F2).

	F1	F2
Eigenvalue	3.502	2.120
Variability (%)	35.016	21.196
Cumulative variance (%)	35.016	56.212

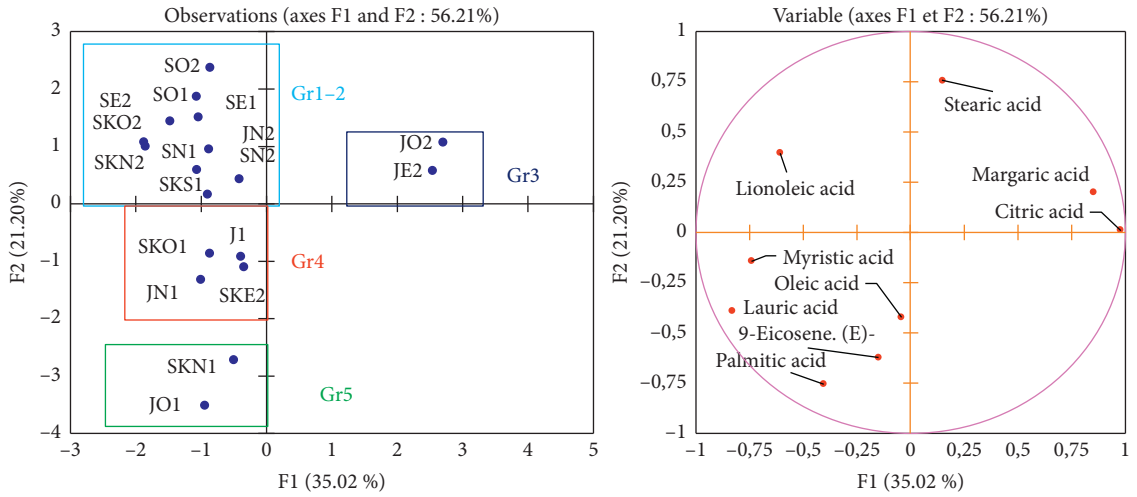

Figure 4: Principal component analysis of the composition variables of *O. Dillenii* extracts collected in the different regions of Morocco.

SO2, SKN2, SKE1, and JN2 are aligned on the PCA2 axis, where the most correlated variable is linoleic acid, which means that these extracts are rich in linoleic acid, and the reverse is true for JO2 and JE2. Table 8 shows that the higher the correlation is, the more the variable is related to the component. With the inertia of 21.02%, the second component (F2) was negatively correlated with palmitic acid (0.754) which existed in moderate to large quantities in the different *O. dillenii* extracts collected from three different regions (SKN1 and JO1). These PCA data lead to the hierarchical upward classification of the AHA into four main groups that represent the following chemotypes: citric acid (0.977) (JO2 and JS2), myristic acid (0.738) (JN1, JS1, SKS2, and PO1), palmitic acid (0.754) (SKN1 and JO1), and linoleic acid (0.610) (SN1, SO1, SE1, SN2, SO2, SE2, SKO2, SKN2, SKS1, and JN2); their correlation matrices are represented in Table 9. This classification confirmed the results of HCA.

Table 8: Correlation coefficients between variables and main axes.

	F1	F2
Citric acid	0.977	−0.008
Lauric acid	−0.832	−0.390
9-Eicosene	−0.150	−0.623
Myristic acid	−0.738	−0.141
Palmitic acid	−0.405	−0.754
Margaric acid	0.854	0.201
Oleic acid	−0.040	−0.420
Linoleic acid	−0.610	0.397
Stearic acid	0.147	0.755

Table 9: Correlation matrix between the variables.

Variables	Citric acid	Lauric acid	9-Eicosene	Myristic acid	Palmitic acid	Margaric acid	Oleic acid	Linoleic acid	Stearic acid
Citric acid	**1**								
Lauric acid	0.794	**1**							
9-Eicosene	−0.182	0.080	**1**						
Myristic acid	0.701	0.754	−0.264	**1**					
Palmitic acid	−0.368	0.023	0.461	−0.299	**1**				
Margaric acid	0.844	0.546	0.016	0.350	−0.497	**1**			
Oleic acid	0.033	−0.068	0.328	0.039	0.046	−0.002	**1**		
Linoleic acid	−0.574	−0.547	−0.220	−0.184	−0.180	−0.554	−0.210	**1**	
Stearic acid	0.131	−0.187	−0.240	−0.263	−0.458	0.416	−0.271	−0.022	**1**

4. Conclusion

In this work, the GC-MS analysis showed that the studied extracts of *O. dillenii* fruit from the three localities contained a high percentage of unsaturated fatty acids and a low percentage of saturated fatty acids. Linoleic acid was the major fatty acid detected with a maximum content of 72.39%, followed by palmitic acid (maximum value of 55.89%). Otherwise, the juice extract of Oujda locality was richer in margaric acid (37.41%), followed by the Essaouira skin extract (10.7%) and Oujda seed extract (6.18%). However, the campesterol was detected only in trace in the juice extract. The physicochemical properties of *O. dillenii* seed oils such as acid value, peroxide value, ester value, pH value, saponification value, density, and refractive index were all found to be in good accordance with the quality criteria for pure and fresh oils, which opens the way for their use in various cosmetic applications and as sources of dietary lipids. Moreover, the presence of campesterol in the juice extract demonstrated that the *O. dillenii* fruits contained nutrients that are nutritionally beneficial to human health. Besides, the study also indicated that the chemical variation between the different extracts of *O. dillenii* depended especially on the environmental factors, climatic and geographical conditions, and the domestication of the species. Furthermore, statistical methods such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed based on the obtained results to highlight the existing correlation between the different parameters and the distribution of the variables in groups.

Data Availability

All data generated or analyzed during this study are included within this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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