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Research Article

Effect of solvent polarity of phytochemical analysis of different Moroccan sites of *Opuntia ficus-indica* (L.) Mill. pads

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Abstract: The current study focuses on the study of phenolic compounds in methanol, dichloromethane, and chloroform extracts as solvents with increasing polarity, in order to quantify the secondary metabolites of *Opuntia ficus indica* pads collected from three Moroccan sites (Oulad Boubker, Imzouren, and Skoura). The findings showthat betalains and carotenoids were present, in varying amounts depending on the study area. The methanolic extracts had the concentrations of compounds. When analyzing the chemical composition using Gas Chromatography - Mass Spectrometry, it was found that linoleic acid was the unsaturated fatty acid accounting for 14.279%. Palmitic acid, fatty acid derivatives and alkanes were also detected in all regions. Among the extracts Imzouren had the concentration of fatty acids (24.874%) followed by Oulad Boubker (13.907%) and Skoura (13.319%). However, oleic acid was detected only in the extract of Skoura. Hence, we were able to confirm that the chemical differences among the various *Opuntia* extracts were primarily influenced by the selection of the extracting solvent and the prevailing climatic and geographical factors.

1. INTRODUCTION

Opuntia ficus-indica (OFI) popularly named prickly pear, originally from Mexico, was introduced in North Africa (Morocco, Algeria, Tunisia) around the 16th century (Boutakiout, 2015). The economic importance of this plant lies in the production of the fruit for human consumption and its use for animal feed purposes. This plant holds significance due to its adaptation to prevailing environmental and climatic conditions, as well as its ability to withstand various soil and climate variations (Hernández-Urbiola *et al.*, 2011), its nutritional properties, and the use of its waste to produce biogas (Inglese *et al.*, 2018).

The Green Morocco Plan, a novel agricultural development approach, presents a tangible chance for the advancement of the cactus industry (Bouzoubaâ *et al.*, 2014). The different parts of OFI (pads, fruits, and flowers) present a variability of shape, color, weight, content of acids

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antioxidant compounds (phenols, flavonoids, betaxanthin and betacyanine), etc. These different parameters depend on the cultivar and are strongly influenced by the environment (Bouzoubaâ *et al.*, 2014; Mouas *et al.*, 2021; Piga, 2004). The composition of polyphenols in prickly pear and its derived products have been thoroughly identified, encompassing compounds such as phenolic acids, flavonoids, and lignans (Mena *et al.*, 2018; Serra *et al.*, 2013). In addition, the fruit contains large amounts of betalains (Mena *et al.*, 2018).

The present study aims at quantifying the secondary metabolites and identifying the chemical composition of the pads using increasing polarity solvents and three sites present in the regions of Driouche, Al Houceima and Ouarzazate in Morocco.

2. MATERIAL and METHODS

2.1. Sample Collection

The mature pads of *Opuntia ficus indica* (OFI) were collected, between October and November 2021, from three sites that differ by their earth's topography and the climate. These are Imzouren's site, which is a town located in the province of Al Hoceima in the northern coast of the Mediterranean in the Tangier Tetouan Al Hoceima region, Oulad Boubkar's site, which is a Moroccan rural commune in the subdivision of Driouch, in the Oriental region, and Skoura's site, located 40 km from Ouarzazate in the South of Morocco in the Sousse Massa region.

2.2. Plant Material

Mature and healthy pads were selected, placed in a dark and humid place, and transported to the laboratory where they were cleaned and stripped of their spines, disinfected with 10% alcohol, and then rinsed using distilled water before being cut into tiny cubes. Then, the plant material was dried in the oven at 60°C under ventilation for 18 to 20 hours with the verification of the stability of the weight of the material during the drying process. The dried material was finely powdered and stored away from light and in darkness.

2.3. Preparation Of Extracts

Solid-liquid extraction is performed with methanol (MeOH), chloroform and dichloromethane as solvents of increasing polarity following the approach outlined by the International Organization for Standardization with some modifications (ISO, 2003). The powder of pads from different locations (10g) is macerated in 100ml of solvents for 48h in the cold and dark. The filtrates containing the extracts were retrieved through filtration and then preserved in opaque containers, being stored at 4°C until they were employed.

2.4. Phenolic Compounds Analysis

The method for determining the total polyphenol content of the extracts, as described by Singleton *et al.* (1999), utilizes gallic acid as a standard. The results are expressed as gallic acid equivalents per gram of the dry matter (mg GAE/g DM).The quantification of total flavonoid content was carried out following the procedure outlined by Djeridane *et al.*, while the flavones and flavonols were measured using the method of (Kosalec *et al.*, 2005). The results of these flavonoids are stated as mg quercetin equivalent per gram of pad's dry matter (mg QE/g DM) referring to the calibration curve of quercetin as a standard. The quantification of condensed tannins was determined using the acidified vanillin method adapted by (Boutakiout *et al.*, 2015), and the hydrolysable tannins were measured using the Willis method (Willis *et al.*, 1998), and the results are presented in terms of milligrams of catechin equivalent per gram of dry plant material (mg CE/g DM) with reference to the catechin calibration curve.

2.5. Extraction And Quantification of Betalains

Extraction of betalains was performed according to the protocol described by Castellanos-Santiago and Yahia, which consists of shaking a mixture of 100 mg of pad's powder and 20 ml of citrate-phosphate buffer (pH 6.5) for 10 min in the dark and then centrifuging at 12000xg at

15 °C for 15 min (Castellanos-Santiago & Yahia, 2008). The recovered supernatant is filtered and the absorbance is measured at 538 nm for betacyanins and at 480 nm for betaxanthantins.

2.6. Extraction And Determination of Carotenoids

The protocol for carotenoid extraction used was the one outlined by (Sass-kiss *et al.*, 2005). 4g of pad's powder was homogenized for 15min with a solvent mixture (10 ml) (hexane/acetone/ethanol) (1V:2V:2V), and then centrifuged at 5500rpm at 4 °C for 15 min. The upper hexane layer, which holds the pigment, is removed and its absorbance is measured at 430 nm.

2.7. GC-MS

In the analysis of the pad powder extract using GC-MS, 1 µL of each extract was automatically injected via an autoinjector into a BRUKER 456 GC EVOQ gas chromatograph. This system was equipped with an RXL-5SIL MS column from BURKER, GERMANY, and coupled to a GC mass spectrophotometer (3Q: triple Quadrupole) operating in electron impact mode. The scan range covered m/z values from 10 to 600 atomic mass units. The column temperature was initially set at 35 °C and gradually increased to 300 °C at a rate of 5 °C/min, remaining at that temperature for 10 minutes. Helium (He) was used as the carrier gas at a flow rate of 1.5 mL/min, and the injection chamber was maintained at a temperature of 300 °C (Lahlou *et al.*, 2014).

2.8. Statistical Analysis

The experimental analysis of the data was done using IBM SPSS version 23. The data were presented as the mean and standard deviation (SD) from three separate experiments conducted in triplicate (ANOVA), followed by Tukey test. We considered $p \leq 0.05$ to be statistically significant.

3. RESULTS

In this research, the content of total phenolic compounds measured in OFI pads is significantly close between the three extraction solvents, as well as between the three study sites (Tables 1, 2, and 3). MeOH extracts are richer in total polyphenols compared to dichloromethane and chloroform extracts. However, chloroform provided the lowest concentrations of these compounds for all three study sites.

The content of condensed and hydrolyzable tannins in OFI pads revealed fairly close values for the three extraction solvents as well as for the three study sites (Table 4, 5). The pads contain an amount of betalains ranging from 48.98±0.02 to 49.07±1.32 mg per 100 g dry weight (DW) depending on the study site (Table 6).

We contrasted the chemical makeup of extracts obtained using MeOH, dichloromethane, and chloroform from pads of the OFI cactus. These pads were sourced from three distinct Moroccan geographical areas (as shown in Tables 7, 8, and 9. The initial findings from the GC-MS examination of the MeOH extract indicated the existence of various fatty acids and their derivatives. Their amounts varied depending on the location, climatic conditions and maturity of the plant. The primary components of these pad extracts consisted of linoleic acid, present in quantities varying from 6.281 % to 14.279 %, subsequent to palmitic acid (3.077 to 4.740 %), fatty acid derivatives (28.160 to 65.567 %) including alcohol functions and fatty acid esters, terpenes (2.486 to 15.054 %), phytosterols (1.273 to 15.615 %) and other compounds (6.950 to 17.531 %). Among the samples, Imzouren's extract exhibited the greatest proportion of linoleic acid (14.279 %), succeeded by Oulad Boubker and Skoura with 8.614 % and 6.281 % respectively. Oleic acid was detected only in the extract of Skoura with a value of 1.260 %.

Table 1. Total polyphenol contents in extracts of *O. ficus indica* pads (mg GAE/g DM).

Solvents	Oulad Boubker	Imzouren	Skoura
Dichloromethane	24.02±0.05 ^{d}	22.97±0.08 ^{b}	23.25±0.03 ^{c}
Chloroforme	23.06±0.06 ^{bc}	22.25±0.09 ^{a}	22.82±0.04 ^{bc}
MeOH	24.24±0.22 ^{d}	24.15±0.07 ^{d}	24.32±0.15 ^{d}

Letters a-d signify that there is a statistical difference ($p < 0.05$) among each individually tested parameter.

Table 2. Contents of flavonoids in the extracts of *O. ficus indica* pads (mg QE/g DM)

Solvents	Oulad Boubker	Imzouren	Skoura
Dichloromethane	11.10 ± 0.09 ^{abc}	11.25 ± 0.04 ^{bc}	11.40 ± 0.01 ^{c}
Chloroforme	10.95 ± 0.06 ^{ab}	10.85 ± 0.06 ^{a}	10.90 ± 0.09 ^{a}
MeOH	12.66 ± 0.08 ^{e}	12.61 ± 0.12 ^{e}	12.10 ± 0.08 ^{d}

Letters a-e signify that there is a statistical difference ($p < 0.05$) among each individually tested parameter.

Table 3. Contents of flavones and total flavonols in the extracts of *O. ficus indica* pads (mg QE/g DM).

Solvents	Oulad Boubker	Imzouren	Skoura
Dichloromethane	6.23±0.05 ^{ab}	6.48±0.02 ^{b}	6.51±0.02 ^{b}
Chloroforme	6.08±0.07 ^{a}	5.94±0.05 ^{a}	6.02±0.04 ^{a}
MeOH	7.79±0.17 ^{d}	7.29±0.04 ^{c}	6.98±0.13 ^{c}

Letters a-d signify that there is a statistical difference ($p < 0.05$) among each individually tested parameter.

Table 4. Contents of condensed and hydrolysable tannins in the extracts of *O. ficus indica* pads (mg CE/g DM).

Solvants	Oulad Boubker	Imzouren	Skoura
Chloroforme	0.14±0.03 ^{a}	0.15±0.04 ^{a}	0.15±0.03 ^{a}
Dichloromethane	0.17±0.03 ^{a}	0.14±0.03 ^{a}	0.13±0.03 ^{a}
Methanol	0.18±0.02 ^{a}	0.16±0.04^{a}	0.16±0.04 ^{a}

The letter "a" indicates that there is no statistical difference ($p < 0.05$) between the tested samples.

Table 5. Contents of hydrolysable tannins in the extracts of *O. ficus indica* pads (mg CE/g DM).

Solvants	Oulad Boubker	Imzouren	Skoura
Chloroforme	0.07±0.01 ^{a}	0.08±0.004 ^{a}	0.07±0.01 ^{a}
Dichloromethane	0.09±0.03 ^{a}	0.07±0.01 ^{a}	0.06±0.01 ^{a}
Methanol	0.10±0.02 ^{a}	0.09±0.01 ^{a}	0.09±0.01 ^{a}

The letter "a" indicates that there is no statistical difference ($p < 0.05$) between the tested samples.

Table 6. Betalains and carotenoids contents of *O. ficus indica* pads (mg/100g DW).

Parameters	Oulad Boubker	Imzouren	Skoura
Betalains (betacyanins and betaxanthantins)	49.07±1.32 ^{a}	48.98±0.02 ^{a}	49.01±0.01 ^{a}
Carotenoids	13.71±0.15 ^{b}	13.52±0.08 ^{b}	13.09±0.07 ^{c}

Letters a-c signify that there is a statistical difference ($p < 0.05$) among each individually tested parameter.

Table 7. Chemical composition of methanolic extracts of *O. ficus indica* pads.

Compounds	RT (min)	Formula	Area %		
			Oulad Boubker	Imzouren	Skoura
3-Furaldehyde	8.158	$C_5H_4O_2$	3.879	0.254	14.326
2-Methyltetrahydrothiophene	10.89	$C_5H_{10}S$	-	11.345	-
4-oxo-5-methoxy-2-penten-5-olide	14.403	$C_6H_6O_4$	-	-	0.882
Undecane	22.203	$C_{11}H_{24}$	1.179	1.792	0.585
Oleic Acide	24.71	$C_{18}H_{34}O_2$	-	-	1.260
5-Hydroxymethylfurfural	28.418	$C_6H_6O_3$	62.570	23.124	24.818
Thymol	31.779	$C_{10}H_{14}O$	0.362	-	-
2-methoxy-4-vinyl-phenol	32.049	$C_9H_{10}O_2$	-	1.929%	-
Stearyl alcohol	49.245	$C_{18}H_{38}O$	0.700	-	-
Dibutyladipate	50.09	$C_{14}H_{26}O_4$	1.340	2.204	1.974
Neophytadiene	52.588	$C_{20}H_{38}$	1.980	1.257	12.170
Phytol	53.354	$C_{20}H_{40}O$	1.629	1.228	2.884
Palmitic acid	56.705	$C_{16}H_{32}O_2$	3.077	4.740	4.149
Methyl 9,10-octadecadienoate	60.53	$C_{19}H_{34}O_2$	-	-	0.374
Methyl 8,11,14-heptadecatrienoate	60.689	$C_{18}H_{30}O_2$	-	-	0.513
Linoleic acid	61.758	$C_{18}H_{32}O_2$	8.614	14.279	6.281
Elaidamide	67.942	$C_{18}H_{35}NO$	-	0.918	0.992
11,14-Octadecadienoic acid, methyl ester,	69.573	$C_{19}H_{34}O_2$	-	-	0.743
methyl hydroxylinolenate	69.741	$C_{19}H_{32}O_3$	-	-	0.824
Octocrylene	75.256	$C_{24}H_{27}NO_2$	0.956	1.915	2.180
methyl hydroxylinolenate	75.638	$C_{19}H_{32}O_3$	-	-	0.328
Oleamide	78.337	$C_{18}H_{35}NO$	2.216	5.954	-
Cholesterol	80.232	$C_{27}H_{46}O$	-	-	0.457
Clionasterol	84.512	$C_{29}H_{50}O$	1.273	-	4.643
α,-Tocopheryl acetate	85.416	$C_{31}H_{52}O_3$	-	-	0.479
E Vitamin	85.436	$C_{27}H_{46}O_2$	0.714	2.211	-
β,-Sitosterol	88.749	$C_{29}H_{50}O$	-	10.406	10.972

Table 8. Chemical composition of dichloromethane extracts of *O. ficus indica* pads.

Compounds	RT (min)	Formula	Area %		
			Oulad Boubker	Imzouren	Skoura
Nonadecane	47.756	$C_{19}H_{40}$	5.298	3.128	5.915
Dibutyl adipate	50.031	$C_{14}H_{26}O_4$	7.285	4.727	7.427
Neophytadiene	52.535	$C_{20}H_{38}$	4.148	-	6.693
Octadecane	55.16	$C_{18}H_{38}$	-	2.666	-
Palmitic acid	56.677	$C_{16}H_{32}O_2$	4.197	4.301	7.902
Butylated Hydroxytoluene	57.501	$C_{15}H_{24}O$	-	3.643	8.808
Octocrylene	75.025	$C_{24}H_{27}NO_2$	-	2.897	-
Elaidamide	78.077	$C_{18}H_{35}NO$	-	-	12.176
9-Octadecenamide	78.16	$C_{18}H_{35}NO$	39.387	-	-
1-Dotriacontanol	83.048	$C_{32}H_{66}O$	-	25.484	-
Triacontane	85.196	$C_{30}H_{62}$	5.193	23.316	19.430
Vitamine E	85.383	$C_{27}H_{46}O_2$	4.496	5.331	6.258
β-Sitosterol	88.62	$C_{29}H_{50}O$	9.620	12.849	22.798
Hentriacontane	89.05	$C_{31}H_{64}$	4.252	-	-
Octatriacontyl pentafluoropr	90.796	$C_{41}H_{77}F_5O_2$	-	3.661	-

Table 9. Chemical composition of chloroform extracts of *O. ficus indica* pads.

Compounds	RT (min)	Formula	Area %		
			Oulad Boubker	Imzouren	Skoura
2,4-Di-tert-butylphenol	40.487	$C_{14}H_{22}O$	-	4.031	5.976
Docosane	47.755	$C_{22}H_{46}$	-	3.661	-
Nonadecane	47.756	$C_{19}H_{40}$	-	-	5.456
Dibutyl adipate	50.027	$C_{14}H_{26}O_4$	11.778	4.925	-
Hexanedioic acid, dibutyl ester	50.031	$C_{14}H_{26}O_4$	-	-	6.236
Neophytadiene	52.518	$C_{20}H_{38}$	3.173	-	3.897
Tricosane	54.911	$C_{23}H_{48}$	0.967	3.116	-
Hexacosane	54.914	$C_{26}H_{54}$	1.298	7.714	8.199
Eicosane	62.211	$C_{20}H_{42}$	2.431	-	-
Phytol	71.438	$C_{20}H_{40}O$	1.100	-	-
Hentriacontane	72.897	$C_{31}H64$	2.173	-	-
Heneicosanoic acid, methyl ester	73.717	$C_{22}H_{44}O_2$	1.257	-	-
palmitic acid	75.735	$C_{16}H_{32}O2$	2.759	-	-
7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	76.459	$C_{17}H_{24}O_3$	1.452	-	-
Elaidamide	78.083	$C_{18}H_{35}NO$	8.932	-	12.529
Tetratriacontane	82.935	$C_{34}H_{70}$	0.316	-	-
Linoelaidic acid	83.168	$C_{18}H_{32}O_2$	1.345	-	-
Heptadecane	84.427	$C_{17}H_{36}$	0.743	-	-
9-Octadecenoic acid	84.624	$C_{18}H_{34}O_2$	6.553	-	-
Triacontane	85.121	$C_{30}H_{62}$	-	33.559	36.894
β—Sitosterol	88.62	$C_{29}H_{50}O$	-	10.525	20.814
Octocrylene	102.944	$C_{24}H_{27}NO_2$	3.380	-	-
Heptacosane	105.481	$C_{27}H_{56}$	4.001	-	-
Nonacos-1-ene	108.749	$C_{29}H_{58}$	1.593	-	-
Squalene	108.999	$C_{30}H_{50}$	7.001	-	-

4. DISCUSSION and CONCLUSION

The total polyphenol content of pads from the Oulad Boubker site is the highest, with a concentration of 24.24±0.22 mg GAE/g DM, followed by those from the Skoura and Imzouren sites, which have concentrations of 24.32±0.15 and 24.15±0.07 mg GAE/g DM, respectively. These values are close considerably with those found by (Msaddak, 2018) (24.85 mg EAG/g DM), for the methanolic extracts of pads of the same species. On the other hand, a much higher value (63.54±1.13 mg GAE/g DM) was reported by Moussaoui, (2020). Alternative research carried out by Gallegos-Infante *et al.*, on Algerian pads of the same species showed a total polyphenol content of 63.54±1.13 mg GAE/g DM (Gallegos-Infante *et al.*, 2009). According to De Santiago *et al.* (2018), this value ranged from 1.7 mg/g DM to 180 mg GAE/g DM.

Concerning solvents, the results presented in Table 2 reveal that the total flavonoid contents of MeOH extracts of OFI pads are the highest ranging from 12.10±0.08 mg QE/g DM to 12.66±0.08 mg QE/g DM followed by those of dichloromethane extracts which are between 11.10±0.09 mg EQ/g DM and 11.40±0.01 mg QE/g DM; on the other hand the lowest values are those obtained from chloroform extracts, and the same for flavones and flavonols.

In addition, all three sites have significantly close values for each solvent separately. Comparing the locations, the lowest concentrations of total flavonoids are those obtained by pads from the Imzouren site with values ranging from 10.85±0.06 mg QE/g DM to 12.61±0.12 mg QE/g DM, followed by those of Skoura's site with values between 10.90±0.09 and 12.10±0.08 mg QE/g DM, while the pads from Oulad Boubker's site are the richest in flavonoids with concentrations ranging from 10.95±0.06 mg QE/g DM to 12.08±0.53 mg QE/g DM. when it comes to flavones and flavonols, Oulad Boubker's site has the highest values from 6.08±0.07 to 7.79±0.17 mg QE/g DM (Table 3).

In other similar studies, a higher value was revealed by Moussaoui (18.07±0.77 mg QE/g DM) for flavonoids and (12.20±0.07 mg QE/g DM) for flavones and flavonols (Moussaoui, 2020), while Guevara-Figueroa *et al.* (2010), who worked on pads of two varieties of OFI, found lower values (9.8 mg and 5.9 mg QE/g DM).

MeOH extracts always contained higher amounts of tannins than those determined by dichloromethane and chloroform extracts with values ranging from 0.16±0.04 to 0.18±0.02 mg CE/g DM, from 0.13±0.03 to 0.17±0.03 mg CE/g DM and 0.14±0.03 to 0.15±0.03 mg CE/g DM for condensed tannins, and 0.9±0.1 to 0.10±0.02 mg CE/g DM, 0.06±0.01 to 0.09±0.03 mg CE/g DM, and 0.07±0.01 to 0.08±0.004 mg CE/g DM for hydrolyzable tannins. The quantity of tannins found in the Oulad Boubker site is between 0.14±0.03 to 0.18±0.02 mg CE/g DM for condensed tannins, 0.07±0.01 to 0.10±0.02 mg CE/g DM for hydrolysable tannins, followed by Imzouren's site with values ranging from 0.14±0.03 to 0.16±0.04 mg CE/g DM, from 0.07±0.01 to 0.09±0.01 mg CE/g DM for condensed and hydrolysable tannins respectively, while the lowest values concern Skoura's site with contents ranging from 0.13±0.03 to 0.16±0.04 mg CE/g DM for condensed tannins and from 0.06±0.01 to 0.09±0.01 mg CE/g DM for hydrolysable tannins.

These findings align with the research conducted by Boutakiout in 2015, on pads from five different regions in Morocco (Khouribga, Beni Mellal, Bejaad, Oued Zam and Kelaa), where he found values that varied between 12.10±0.21 to 18.23±0.36 mg ATE/100ml for condensed tannins and 1.24±0.02 to 1.33±0.15 mg ATE/100ml for hydrolysable tannins. Similarly, Moussaoui revealed values of 0.18±0.02 and 0.1±0.03 mg ATE/g DM (Moussaoui, 2020), while the content of condensed tannins in pad juice, recorded by Boutakiout, was 0.01 mg CE/mL (Boutakiout, 2015).

The content of Betalaines and carotenoids in pads from Imzouren site is the lowest compared to other sites with a value equal to 48.98±0.02 mg/100g DW. These values are very close to those found by Moussaoui (48.13±2.2 mg/100g DW) for Algerian pads of the same species (Moussaoui, 2020). The extraction yield of betalains is proportional not only to the variety, part or tissue selected and its growth stage, but also to the extraction technique used and its parameters. These factors even cause heterogeneous distribution and variation of betalains, individual structures and betacyanin/ betaxanthin ratios in different parts of *Opuntia* (Cai *et al.*, 1998; Sanchez-Gonzalez *et al.*, 2013; De Wit *et al.*, 2019). In 2019 De Wit *et al.*, also identified the presence of betalains in pads of *Opuntia*, South African, as they reported that the levels of these pigment depend on the cultivar.

The value of carotenoids in Moroccan pads of OFI observed in this study ranged from 13.09±0.07 to 13.71±0.15 mg/100g DW depending on geographical locations (Table 6). The highest value was found in Oulad Boubker (13.71±0.15 mg/100g DW), followed by Imzouren (13.52±0.08 mg/100g DW) and Skoura (13.09±0.07 mg/100g DW), noting that these values are significantly close. In a similar analysis carried out by (Jaramillo-Flores *et al.*, in 2003; and Bensadón *et al.*, in 2010, the authors reported the presence of carotenoids in *Opuntia* pads with values ranging from 2 to 23.18 mg/100g DW, and they indicated that these values can be increased depending on the heat treatment used. For the variants related to the development conditions of the plants, the polarity of the carotenoids influences their solubilities in the extraction solvents and the extraction itself (Tsao *et al.*, 2004; Dias *et al.*, 2009). Ethanol remains the best solvent for extraction of polar carotenoids while hexane is the best choice for extraction of apolar carotenoids (Amorim-Carrilho *et al.*, 2014).

However, a very small amount of fatty acids was present in the dichloromethane extracts with values ranging from 4.197 to 7.902% of palmitic acid, fatty acid derivatives (19.609 to 46.671 %), alkanes (14.744 to 29.109 %), phytosterols (9.620 to 22.798 %), tocopherols (4.496 to 6.258 %) and other compounds (3.661 to 15.501%). The extract of Oulad Boubker has the lowest amount of fatty acids (4.197%), followed by Imzounren (4.301 %) and Skoura (7.902 %). The extracts of Oulad Boubker and Imzouren are richer in fatty acid derivatives with 46.671% to 33.108 %, than the extract of Skoura with 19.609 %.

In addition, a moderate amount of alkanes was found in the chloroform extracts with values ranging from 21.507% to 50.548% and a small amount of fatty acid derivatives (4.925 % to 25.347 %). However, a very low amount of fatty acid was exclusively detected within the extract of Oulad Boubker with nearly 10.657 % and traces of ketone with 1.452 %. The terpenes were presented only in the two extracts of Oulad Boubker and Skoura with values respectively of 13.171 % and 3.897 %. While phytosterols were found in the two extracts of Imzouren and Skoura with values of 10.525 % and 20.814 % respectively.

On a different note, Algerian pads of OFI showed the presence of seventeen alkaloids, including Isoquinoline, imidapyrazole and pyrazolo-benzothiazole derivatives (Guevara-Figueroa *et al.,* 2010). Conversely, another study identified the occurrence of palmitic acid (19.81 to 24.27 %), linoleic acid (66.57 to 72.39 %), stearic acid (2.37 to 9.61 %) and myristic acid (0.97 to 2.91 %) in another species of *Opuntia* (*Opuntia dillenii*) (Loukili *et al.*, 2021), this study is consistent with the one done by Alsaad *et al.* (2019). However, a similar study indicated an alternative fatty acid characteristic of Chinese *Opuntia dillenii* including linolenic acid (66.56 %), palmitic acid (19.78 %), stearic acid (9.01 %) and linoleic acid (2.65 %) (Liu *et al.*, 2009).

The prevalence of linoleic acid as the primary constituent in the *Opuntia ficus* plant implies potential health advantages associated with it. In human tissues, linoleic acid is chiefly transformed into arachidonic acid (ARA), α-linolenic acid (ALA), and docosahexaenoic acid (DHA) (Waleed *et al.*, 2017). Linoleic acid and its related group of fatty acids are collectively known as omega-6 fatty acids.

Multiple experimental and medical investigations have indicated the potential positive impact of including linoleic acid in a diet. Especially, if these benefits encompass the enhancement of cardiovascular well-being, notably the improvement of plasma lipid profiles, and a better glycemic control by increasing insulin resistance. Moreover, a higher dietary intake or elevated tissue levels of linoleic acid have been correlated with a decreased likelihood of cardiovascular disease and the development of metabolic syndrome or type 2 diabetes. Nevertheless, further clinical trials are necessary to comprehensively evaluate the underlying mechanisms that connect the observed health benefits with the intake of this essential fatty acid (Marangoni *et al.*, 2020).

The molecule profile reported in this study showed that pad varieties from distinct sites exhibit diverse types or compositions of molecules.

In our study, experiments on animal models are necessary to investigate and confirm the beneficial effect of the *Opuntia ficus* plant and its role in metabolism.

5. CONCLUSION

In this work, our results showed the presence of a proximal difference in phenolic compounds of OFI pads between the three geographical locations studied. Phenolic compounds were found to be higher in extracts from Oulad Boubker, followed by Skoura and Imzouren. While betalains, carotenoids and alkaloids were found to be very low. Quantification of these compounds showed that MeOH extracts had the highest concentrations, followed by dichloromethane and chloroform. Chemical composition analysis by GC-MS demonstrated that the extracts of the studied OFI pads from three locations contained a significant proportion of fatty acids derivatives and alkane, as well as a small proportion of fatty acids. Linoleic acid was the predominant fatty acid detected, with a maximum content of 14.279 %, followed by palmitic acid (maximum value of 7.902 %).

Therefore, Cladodes extracts contain compounds that may be useful for functional food and cosmetic applications. Moreover, the research also indicated that the chemical diversity of the various *Opuntia* extracts was mainly influenced by the selection of the extraction solvent and the prevailing climatic and geographic factors.

In the future, it would be prudent to continue biotechnological research, improve our grasp of its genetic variability using molecular biology techniques, and conduct more complete studies into its agronomic worth.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Sara Razzak: Conception, Methodology, Resources, Visualization, Software, Data Collection and Processing, Analysis and Interpretation, and Writing -original draft. **Marouane Aouji**: Visualization, Analysis and Interpretation, and Software. **Chaima Sabri:** Visualization, Correction and Literature Review. **Hiba Benchehida:** Writing Translating and Literature Review. **Mariame Taibi:** Methodology and Visualization. **Youness Taboz:** Supervision, Correction and validation, Critical Review

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