The Pharmaceutical and Chemical Journal, 2018, 5(4):42-49

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Phytochemical Content and Antioxidant Activity of Opuntia Ficus-Indicacold-Pressed Seed Oil in Tunisia

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Abstract *Opuntiaficus indica* (OFI) has been used in traditional folk medicine. Many parts of the plant were investigated. However fewer data about seeds are available.

In this study, the lipid fraction was extracted from OFI seeds in two different regions of Tunisia, then investigated for their phytochemical content and antioxidant activity in order to evaluate their importance in health field. The results showed that the content of seed oil in unsaturated fatty acids is relatively high (83 %) mainly in linoleic acid (58.14 %). Also, the content in phenolic compound and vitamin E was high which leads to significant antioxidant activity. These compounds and antioxidant activity vary between the seeds collected from two regions. The oil from region 1 (Sidi El hani) is richer than the oil from region 2 (Kasserine) in polyphenols, flavonoids and alpha-tocopherols (366.36±18.73 vs 233.82 ± 10.50 mg gallic acid / kg of oil; 205.8 ± 10.65 vs 155.24 ± 10.96 mg catechine / kg of oil, 185.90 ± 30.62vs82.12± 24.66 mg / kg of oil). In the same way, the anti-free radical activity and resistance to oxidation were higher in region 1compared to region 2 samples. The OFI oil can be used in food and pharmaceutical industry due to its special characteristics. However, the effectiveness of this oil depends on the area of collection.

Keywords Antioxidant activity, fatty acid, *Opuntiaficus indica*, phytoconstituent, seed oil

1. Introduction

It was reported that Opuntiaficus is with great nutritional and health benefits [1]. Fernandez et al. showed that the incorporation of pectins sprung from peels of the prickly pear as adjuvant in drugs, allows to exploit the capaciy of elimination of cholesterol and triglycerides in the blood, that possess these pectins [2]. Also, Jose Luis Lopez mentioned that Opuntia Cactus is used as a Hypoglycemic Agent in Managing Type 2 Diabetes Mellitus [3]. More else, Ennouri et al. showed that seeds and oils are effective in decreasing the atherogenic risk factors in rats [4]. Ammar et al. have recently shown anti-inflammatory, antioxidant, antibacterial and in vivo dermal wound healing effects of Opuntia flower extracts [5, 6].

In Tunisia, fruits of the prickly pear are generally consumed in the fresh state, but now, the nectars, the juices, jams are also made from the pulp of the prickly pear. Their preparation leads to a significant production of byproducts. It is important to develop these by products such as seeds and peel. A review of literature reveals that Prickly pear seeds were first studied by Sawaya et al. and showed that the seeds of OFI are rich in minerals and sulphur amino

acids [7]. Then, the prickly pear seed oil composition and its chemical characteristics were investigated in many countries by different researchers [8, 9, 10, 11].

Coskuner & Tekin studied the seed composition of prickly pear fruits during the maturation perio [12]. Ramadan and Morsel compared the seed and pulp oil compositions [13]. Ennouri et al. studied the physicochemical properties of the seed oil of the *Opuntiaficus indica* and *O. stricta* fruits which are the most abundant species in Tunisia [9]. In the same context El Mannoubi et al. studied the chemical and physical properties of the lipid fraction of OFI in detail [14]. Many factors influence the content of plants [15]. Matthausa and Ozcanb studied habitat effects on yield, fatty acid composition and tocopherol contents of prickly pear seed oils [16]. Also, other studies reported that the climate and the altitude have an influence on chemical composition and biologic activity of plants [17, 18, 19].

Many studies focus on the antioxidant activity of the peel and pulp of this plant, but not the seed oil [20, 21, 22].The antioxidant property of plants is responsible for their importance in health field (drugs and food). This antioxidant activity could be attributed to phytoconstituent of the plant such as polyphenol, flavonoids, vitamins. These compounds with free radical scavenging activity help in the prevention and therapeutics of many disease associated with oxidation like cardiovascular disease, cancer…

The major objective of the present work was to study the chemical composition and the antioxidant activity of OFI seed oil in order to evaluate its importance in health field (drugs and food), and whether these compounds and activity are affected or not when seeds were harvested in different regions.

2. Materials and Methods

2.1. Prickly pear seed

Two samples of wild mature Prickly pear fruits, *Opuntiaficus-indica* (OFI) were collected, from two regions of Tunisia Sidi El Hani, Kairouan (latitude 35º 40' 41" N, longitude 10º 05' 46" E, altitude 68 m) and Kasserine (latitude 35º 10' 03" N, longitude 8º 50' 11" E, altitude 674m) in the same season (month of September).

The fruits were immediately sorted, washed with running water and crushed at the end. Then seeds were separated, washed many times and dried at ambient temperature.

2.2. Oil extraction

The extraction of oil from the seeds of prickly pear is made by cold pressure.

The oil obtained from Kasserine (K) is of greenish color, and that obtained from Sidi El Hani (S.H) is of brown orange color. Oils were stored at low temperature (4°C) and in the darkness.

2.3. Physicochemical analyses

The analysis was carried out in triplicate.

The analyses of the physico-chemical characteristics of two samples of oil of prickly pear, such as free acidity percentage (%), peroxide value, coefficient of extinction K232 and K270 were made according to the European community-regulations for analytical methods [23]. The content in colored pigments was analyzed by colorimetric method according to Wolf [24] for chlorophyll and carotenoid pigments and the method of Psomiadou & Tsimidou for the analysis of pheophytine pigment [25].

2.4. Fatty acid analysis

The fatty acid compositions of both oil samples were analyzed by gas chromatography after transesterification. Fatty acid methyl esters (FAMES) were prepared in the presence of 0.2 N potassium hydroxide in methanol and analyzed on a Hewlett-Packard model 5890 series II gas chromatograph equipped with a flame ionisation detector and a fused silica capillary column HP - INNOWAX 30 m length x 0.25 mm i.d. and 0.25 µm of film thickness. The temperature was programmed to increase from 170 to 270°C at a rate of 5°C per minute. Nitrogen ultra was used as carrier gas. The results were expressed as relative area percent of the total FAMES [26].

2.5. Amount of the total phenolic content

The amount of the total polyphenols (PT) is evaluated by spectrometry [27]. In an alkaline medium and in the presence of polyphenols, the reagent of Folin-Ciocalteu is reduced to oxide of tungsten and molybdenum to give a blue color. The intensity of the color is proportional to the concentration of phenols. The absorbance is measured in 765 nm by referring to the Gallic acid as standard.

2.6. Determination of flavonoïdes content

The total flavonoïdes is measured by a colorimetric method [27] in the presence of $AlCl_3$ (10 %), $NaNO_2$ (5 %), NaOH (1M) and in H_2O. The standard graph was prepared by using catechine in different concentrations and the absorbance was measured at 510 nm.

2.7. Analysis of α-tocophérol (vitamin E) by high performance liquid chromatography (HPLC)

The determination of the tocophérols content was carried out as described by Dabbou et al. [28]. HPLC analysis was conducted using an agilent Technologies system model 1100 (Agilent Technology, DE, Germany).

2.8. Determination of the anti-free radical activity by the DPPH test

The current method to estimate the antioxidant activity (Radical Scavenging Activity) is by using the synthetic free radical 1.1-diphenyl-2-picrylhydrazyl radical (DPPH*). The estimation of this anti-free radical activity was measured as described by Brahmiet al. [29]. 20 µl of the methanolic extract was mixed with 980 µl of methanol and 1ml of the solution of DPPH (0.1 mM). The mixture was kept at room temperature for 30 minutes in the darkness. The reading was measured at 517 nm. The blank is without extract (without any antioxidant).

$$PI = \% = (1 - (\text{abs sample} / \text{abs blank})) \times 100$$

2.9. Acceleratedoxidation test

A Rancimat apparatus, model 734 (Metrohm, Herisau, Switzerland) was used to measure the oxidative stability of the oils as described by Dabbou et al. [28]. The principle of the test consists of premature ageing of fats by thermal decomposition. The results were expressed as induction time in hours of hydroperoxides decomposition.

3. Results

Many studies were done on OFI in order to see the composition of different parts (flowers, peels, cladode, seeds…). Other studies aim to determine the biological activities of those components. The present study was designed to investigate the oil seed of OFI. To obtain the seed oil, cold pressure method was used. As per our literature review, the most used method of oil extraction is solvent extraction [9, 14].

We determine the seed oil yield of the various samples of seeds from Kasserine and Sidi El Hani. According to table 1 the prickly pears from Kasserine give a yield in seeds little lower when compared to the prickly pears from Sidi El Hani, and a yield in oil obtained by cold pressure (2.4 %) roughly twice more important that the return found for the seeds fromSidi El Hani (1.08 %).But this yield is lower than that found by Ennouri et al. [9] (10.9 %) and El mannoubi et al. [14] (11.75 %) who used the soxhlet as mode of extraction.

3.1. Physico-chemical stability of oil

Physico-chemical properties of our samples (table 1) showed acceptable values for free acidity, peroxide value, specific extinction at 232 nm and at 270 nm.

Table 1: Physico-chemical characteristics of *Opuntificus-indica* oil from Sidi El hani and Kasserine

Analyses made	Oil of origin of Sidi El Hani	Oil of origin of Kasserine
Return on seeds / fruits	3.6 %	3.3 %
Return on oil / dry seeds	1.08 %	2.4 %
Free acidity (%)	1.46±0.16	1.69±0.11
Peroxide value (milliequivalent of oxygen / kg of oil)	10.33±1.76	13.71±1.11*
K232	2.40±0.03	3.08±0.04*
K270	0.26±0.02	0.50±0.01*

*P < 0.05

There was no significant difference in the acidity value of the two samples :1.46 % for the OFI oil of Sidi El Hani and 1.69 % for the OFI oil of Kasserine. However, the peroxide value, K232 and K270 were higher in kasserine oil sample when compared to Sidi El Hani.

3.2. Fatty acid composition of prickly pear seed oil

The composition of fatty acids represents a criteria for classification of vegetable oil (saturated, mono or polyunsaturated) determined according to the dominance of a given fatty acid. The results obtained for the composition in fatty acids of the studied samples are represented in table 2. The results were similar in many of reported studies done on OFI. The linoleic acid is the major fatty acid, followed by oleic acid then palmitic acid. The composition in fatty acids of the oil of cactus does not vary between both samples. Both oils are exceptionally rich in linoleic acid (w6) (until 58.14 %) and their contents in unsaturated fatty acids are relatively high (83 %) .

Table 2: Fatty acid composition of *Opuntiaficu indica* oils from Sidi El Hani and Kasserine

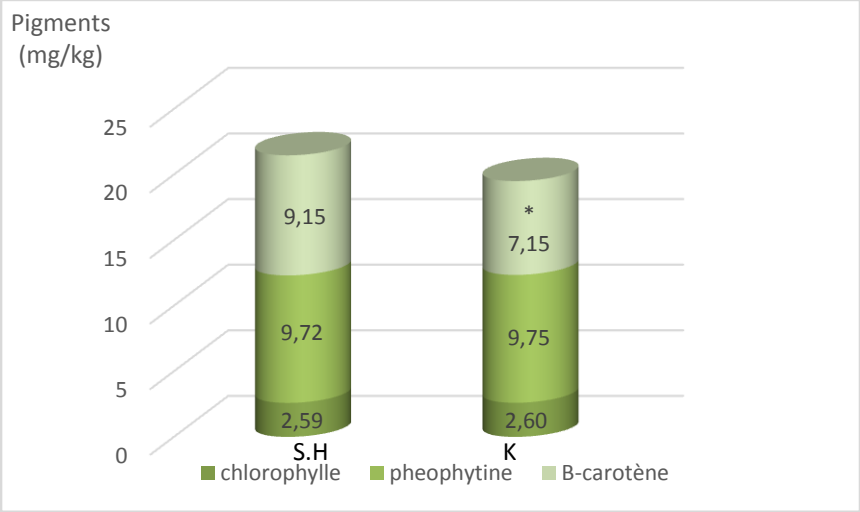
		% of Fatty acids	
Sign	**Name**	**Sidi El Hani**	**Kasserine**
C 12 : 0	Lauric Acid	0.10	0.10
C 14 : 0	Myristic Acid	0.03	0.05
C 14 : 1	Myrstoleic Acid	0.08	0.06
C 16 : 0	Palmitic Acid	11.08	11.53
C 16 : 1	Palmitoleic Acid	0.78	0.84
C 17: 0	Margaric Acid	0.24	0.17
C 17: 1	Margaroleic Acid	0.15	0.12
C 18 : 0	Stearic Acid	3.42	3.41
C 18 : 1	Oleic Acid	21.26	21.95
C 18 : 2	Linoleic Acid	57.04	58.14
C 18 : 3	Linolenic Acid	0.58	0.49
C 20 : 0	Arachidic Acid	1.00	0.71
C 20 : 1	Gondoic Acid	1.53	0.71
C 20 : 3	Homo-gamma-linoleic acid	1.89	0.94
C 22 : 0	Behenic Acid	0.28	0.25
C 22 : 1	Erucic Acid	0.21	0.22
C 24 : 0	Lignoceric Acid	0.11	0.12
C 24 : 1	Nevronic acid	0.22	0.19
Total saturate (%)		16.26	16.33
Total insaturate (%)		83.74	83.67
total mono insaturate (%)		24.23	24.09
total poly-insaturate (%)		59.51	59.56

The linoleic acid rate is equal to that found by Ghazi et al. [10] (58.79 %). However it remains low with regard to that found by Ennouri et al. [9] which is of the order of 70 %. The values were similar for oleic acid in both samples which were of the order of 22 %, but this value was found to be higher than that reported by Ennouri et al. [9] which is of the order of 16.8 %. The amount of palmitic acid was found to be in the range of 11 % and 12 % for both samples. It is similar to that reported (11.18 %) by Ghazi et al. [10] but more from that reported (9.3 %) by Ennouri et al. [9]. The difference in fatty acid composition between our results and the results found by Ennouri et al. [9] which was made in Tunisia could be contributed to the period of collection (August and February in Ennouri study and September in our study) or to the region of collection (Sfax in Ennouri study). Coskuner et al. have reported that the composition in fatty acid could be influenced by the degree of maturity [12]. However, another study showed that in both seed and pulp oils, linoleic acid was the dominating fatty acid, followed by palmitic and oleic acids, respectively [13]. Globally, the oil of cactus consists about 84 % of unsaturated fatty acids which are valuable in improving the youth and to slow down the ageing of the skin (mainly oleic and linoleic acids).From the results we can conclude that although the yield of oil is low on extraction the composition in fatty acids are a good source of

the nutritionally essential linoleic acid and polyinsaturated fatty acids which help in ameliorating lipid profile toward protection against cardiovascular diseases.

3.3. Contents in pigments

Chlorophylls, pheophytines and carotene play an important role in the oxidative stability of the oil of prickly pear due to their anti-oxidant activity in obscurity and pro- oxidant in the light. The contents in those pigments are expressed in mg per Kg of oil (Fig.1).



* p value < 0.05 ; S.H Sidi El Hani; K Kasserine

Figure 1: Variation of the content in pigments in the oil according to the origin of the seeds of Opuntiaficus indica

By comparing the content in pigments of two samples, it is seen that both are rich in pheophytines (9.72 mg / kg of oil of S.H and 9.75 mg / kg of oil of K) and also same content in chlorophyll i.e. 2.60 mg / kg. It was also observed that the amount of β-carotene was more in the sample of Sidi El Hani when compared to Kasserine (9.15 ppm against 7.15 ppm).

3.4. Phenolic compounds

The phenolic compounds present in the vegetable oil are antioxidants. It is well known that phenolic compounds have high antioxidant efficiency and they are effective in care of degenerative diseases [30]. There was significant difference in the amount of total phenols and flavonoids in the different oil samples from different regions (table 3).

Table 3: Contents in phenolic compounds of oil of prickly pear of two regions (Sidi El Hani and Kasserine)

Phenolic compounds	Oil of origin of Sidi El Hani	Oil of origin of Kasserine
Polyphenols (mg gallic acid / kg of oil)	366.36±18.73	233.82±10.50*
Flavonoïds (mg catechine / kg of oil)	205.8±10.65	155.24±10.96*

* p < 0.05

The oil of Sidi El Hani was richer than the oil of Kasserine in polyphenols (366.36±18.73 Mg / kg) in comparison to (233.82±10.50 Mg / kg). Also, it was noticed that the oil of Sidi El Hani is more rich in flavonoïdes than the oil of Kasserine. Literature review reported the determination of the phenolic compounds in defatted seed powder but not in seed oil [21, 31].

3.5. Content in Vitamin E

The result of vitamin E analysisis as summarized in fig. 2. The result showed significant difference between the amount of α-tocopherol in the two samples. It was higher in Sidi El Hani than in Kasserine sample (185.90±30.62 mg / kg and 82.12±24.66 mg / kg for the oil respectively; p<0.05).

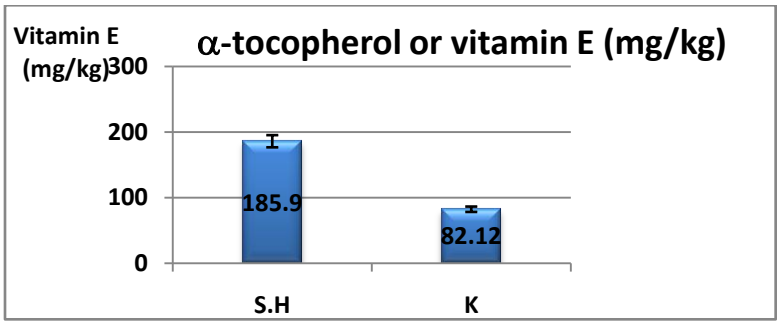


Figure 2 : Content in vitamin E of two samples of oil of Opuntiaficus indica

* p value < 0.05 ; S.H Sidi El Hani; K Kasserine

3.6. Anti-free radical activity

The anti-free radical activity is important in OFI seed oil. A decrease of such activity is associated with heart attacks, ischemic strokes, and peripheral arterial disease.

The prickly pear oil, extracted from Sidi El Hani seeds had significant anti-radical activity compared to kasserine seeds (figure 3) (31 % in front of 24.5 % of percentage of inhibition; $p<0.05$). This can be attributed to the presence of higher amount of phenolic compounds, vitamin E and β-carotene.

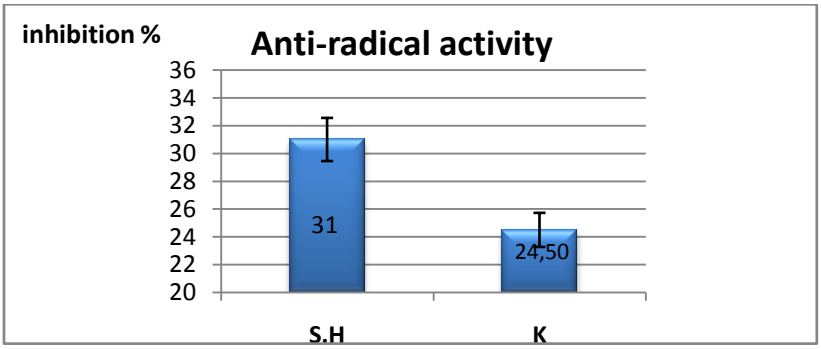


Figure 3 :Free radical-scavenging capacities of the Opuntiaficus indica oils measured in DPPH assay.

3.7. Accelerated aging test: Rancimat test

The resistance to oxidation, known often as Rancimat test, is an important parameter for the characterization of oil. This test allowed to determine the necessary time that the sample of oil begins to present symptoms of rancidity and degradation of the unsaturated fatty acids by the oxygen of the air and under a high temperature (120°C).The time of resistance of the fat in the oxidation corresponds to the inflexion point of the curve. The oil from seeds of Kasserine had an oxidative resistance which lasts 0, 48 hour in 120°C when compared to 0, 98 hour for the sample of Sidi El Hani. Hence, the oil from Sidi El Hani was found to be more resistant to oxidation than Kasserine oil. This could be explained by the presence of higher amount of phenolic compounds, vitamin E and β-carotene.

Regarding to oxidation, our results revealed that the oil from Sidi El Hani was of better quality than the oil from Kasserine, as it is more resistant to oxidation. This was confirmed by the peroxide value and the specific extinction in 232 nm and in 270 nm and could be the result of the presence of antioxidant agents like polyphenol, flavonoids, vit E and β-carotene. The difference between the amount of chemical constituents and antioxidant activity could be the result of the difference of climate and altitude between Kasserine and Sidi El Hani regions.

4. Conclusion

The powerful antiradical activity of the OFI seed oil and the presence of linoleic and oleic acids encourage the use of this oil in food and pharmaceutical fields.The oil richer in polyphenol, flavonoids, beta-caroten and alpha-tocopherol (sidi el hani) had the higher oxidative resistance and the higher anti free radical activity. However the

amount of phytoconstituents present in the plant depends on the region of collection, its climate and altitude and several other factors which in turn may affect the antioxidant activity.

Acknowledgment

This study is a part of research program of the Research Unit LR12ES05"Nutrition- functional food and Vascular Health LR-NAFS" and "DGRST-USCR- Mass Spectrometry" financed by the Ministry of Higher Education and Scientific Research (Tunisia).

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