

Physicochemical Properties and Antioxidant Activity of the Seeds Oil of Two Cucurbita Species From Bejaia (Algeria): Comparative Study

Propriétés physicochimiques et activité antioxydante de l'huile de graines de deux espèces de Cucurbita de Bejaia (Algérie) : étude comparative

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Abstract Cucurbita species are delicious, nutritious, and delightful products. Cucurbita seeds remain in large quantities as a waste product that could be valorized since they are excellent sources of oil. The aim of this study was to compare the seed oil of two Cucurbita species (*Cucurbita pepo* and *Cucurbita moschata*) harvested in Bejaia (Algeria). The oil quality was evaluated by the determination of some physicochemical parameters, and the content of phenolic compounds. The antiradical capacity of the antioxidants present in the oils was also assessed using two methods. The oil yield was 42.85% and 40.47% from the seeds of *Cucurbita pepo* and *Cucurbita moschata*, respectively. The determined physicochemical parameters were close to those defined by the international standards. The phenolic contents of the methanolic extracts of both oils were 5.53 and 4.45 mg GAE/100 g for *Cucurbita moschata* and *Cucurbita pepo*, respectively. The best anti-DPPH power was attributed to the oil of *Cucurbita moschata* (44.7%), while the methanolic extract of the seed oil of *Cucurbita pepo* showed the highest percentage (41.02%) of the ABTS^{•+} radical inhibition. By this study we confirmed that the Cucurbita seeds oil are highly nutritious and offer some medicinal benefits.

Keywords Seeds oil · Physicochemical properties · *Cucurbita pepo* · *Cucurbita moschata* · Phenolic contents · Radical scavenging activity

Résumé Les espèces du genre Cucurbita ont des fruits délicieux et nutritifs. Les graines de ces fruits produites en grande

quantité sont considérées comme des déchets qui pourraient être valorisées, car elles sont d'excellentes sources d'huile. Le but de cette étude est de comparer l'huile de graines de deux espèces du genre Cucurbita (*Cucurbita pepo* et *Cucurbita moschata*) récoltées à Bejaia (Algérie). La qualité des huiles a été évaluée par la détermination de certains paramètres physicochimiques et la teneur en composés phénoliques. La capacité antiradicalaire des antioxydants présents dans les huiles a également été évaluée à l'aide de deux méthodes. Le rendement en huiles était de 42,85 et 40,47 % pour les graines de *Cucurbita pepo* et de *Cucurbita moschata*, respectivement. Les paramètres physicochimiques déterminés étaient proches de ceux définis par les normes internationales. Les teneurs en composés phénoliques des extraits méthanoliques des deux huiles étaient respectivement de 5,53 et 4,45 mg EAG/100 g pour *Cucurbita moschata* et *Cucurbita pepo*. Le meilleur pouvoir anti-DPPH a été attribué à l'huile de *Cucurbita moschata* (44,7 %), tandis que l'extrait méthanolique de l'huile de graines de *Cucurbita pepo* a montré le pourcentage le plus élevé (41,02 %) de l'inhibition de radical ABTS^{•+}. Par cette étude, nous avons confirmé que l'huile de graines des espèces du genre Cucurbita est très nutritive et offre certains effets médicinaux.

Mots clés Huiles des graines · Propriétés physicochimiques · *Cucurbita pepo* · *Cucurbita moschata* · Contenus phénoliques · Activité scavenger

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Introduction

The vegetable kingdom comprises natural resources of active substances with multiple applications. The protective effects of fruits and vegetables against free radicals that react with tissues causing oxidative damage have been attributed to the presence of phyto-chemical compounds, including phenolic acids, carotenoids, and vitamin E. These compounds have a

range of demonstrated biological activities including anti-inflammatory, antibacterial, and antioxidant effects [1].

Among these foods, pumpkin is a very nutritious fruit that belongs to genus *Cucurbita*. The most cultivated species world-wide are *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita pepo* [2]. The seeds of these species are an excellent source of beneficial elements, helping to treat illnesses related to the bladder and prevent the formation of kidney stones and are known for their natural preventive effect against osteoporosis [3]. They have also been used as an antihelminthic, taeniocide, diuretic and as an alternative treatment for Benign Prostatic Hyperplasia (BPH) stage I and II [4].

These seeds belong to the oleaginous family; they are rich in oils, in which we are interested in through this study. Seeds of *Cucurbita* species are considered as a waste product for food processing, but their oils are known for their richness in phenolic compounds, carotenoids, vitamin E, phytosterols and also for their different biological properties such as antimicrobial and antioxidant [5].

Expanded research on the physico-chemical and biological properties of these oils is limited, especially in Algeria [6,7]. Besides, the most available and consumed varieties in Algeria are pumpkin (*Cucurbita pepo*) and the musk gourd (*Cucurbita moschata*). The two studies realized on the seed oils of these varieties concerned each variety apart; *Cucurbita moschata* harvested in the Bouira location [7] and *Cucurbita pepo* L from the Laghouat location [6].

In this line, the goal of this study is to compare the seed oil of two *Cucurbita* species (*Cucurbita pepo* and *Cucurbita moschata*) harvested in another location in Algeria (Bejaia). The oils are extracted by petroleum ether and their physico-chemical parameters, phenolic contents and antioxidant activity were compared.

Materials and methods

Oils extraction

Two species of *Cucurbita* were used in this study: *Cucurbita pepo* and *Cucurbita moschata*, harvested in the Amizour and Bouzegane regions of Bejaia (North-east of

Algeria), during October 2012. The characteristics of these two species are shown in Table 1.

The seeds were separated from the flesh, then dried at room temperature. The oil was extracted according to the method described by Ortiz Grisales et al. [8], with some modifications; 300 dried seeds were weighed and crushed. The ground material obtained was extracted with 150 mL of petroleum ether under reflux in a Soxhlet extractor at 100 °C for 8 hours; after removal of the solvent by distillation and drying at 60 °C for 15 minutes, the resulting oil was weighed and stored at +4 °C. The yield of the extracted oil from both species was determined.

Physicochemical analysis of the oils

The Brix degree was determined according to Marek et al. [9] while the acid index using the protocol reported by Drian et al. [10] and the acidity by adopting the standard: ISO660 [11]. The peroxide index of the oils was also evaluated according to UICPA [12].

Extraction of phenolic compounds

An aliquot of 2.5 g of each species seed oil was added with 2.5 mL of methanol/water (80:20 v/v), followed by centrifugation at 3500 rpm for 15 minutes; the supernatant was recovered and this operation was repeated twice for the pellet [13].

Determination of total phenolic content

The total phenolic content was determined by the method of Al-Farsi et al. [14]. A volume of 200 µL of methanolic extract (80%, v/v) of the oil was added with 1.5 mL of the Folin–Ciocalteu reagent (diluted 10 times); after 5 min of incubation, 1.5 mL of sodium carbonate (6%) was added. After 90 min of incubation, the absorbance was measured at 725 nm. The concentration of total phenolic was expressed in milligram gallic acid equivalent per 100 g of the oil (mg GAE/100 g of the oil), with reference to the calibration curve.

Table 1 Morphological characteristics of <i>Cucurbita pepo</i> and <i>Cucurbita moschata</i>					
Species	Fruit color and form	Width (cm)	Length (cm)	Weight (kg)	Number and color of seeds
<i>Cucurbita pepo</i>	Orange and round	27	23.5	7.54	710 Grey
<i>Cucurbita moschata</i>	Yellow and lengthened	15.5	90	7.70	386 Beige

Determination of the condensed tannins content (proanthocyanidins)

The content of condensed tannins was determined as reported by Hecimovic et al. [15]. A volume of 500 µL of methanolic extract (80%, v/v) of the seed oils was added with 3 mL of butanol-HCl (95:5, v/v) and 100 µL of the ferric reagent (2% ferric ammonium sulfate in 2 M HCl). The mixture was incubated at 90 °C for 15 min and the absorbance was measured at 550 nm. The content of condensed tannins (proanthocyanidins) was calculated by the following formula:

$$C = \frac{Abs * M * DF}{\epsilon * L} * L$$

DF: Dilution factor.

ϵ : Extinction factor (34 700 mol/cm.l).

L: Optical path (1 cm).

Abs: Absorbance of the sample.

M: Molar mass of cyanidine (g/mol).

C: Concentration (g/L).

The results were expressed in equivalent microgram of cyanidine per 100 g of the oil (µg EC/100 g of the oil).

Determination of antioxidant activity

The antiradical power of the antioxidants present in the seed oils was evaluated by the scavenging effect of the radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) and 2,2'-azino-bis(acid 3-ethylbenzthiazoline-6-sulfonic acid) (ABTS^{•+}).

Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•])

The percentage inhibition of the DPPH[•] radical was determined for the oils and their methanolic extracts. For these later, it was estimated by the method described by Milardović et al. [16]. A volume of 0.1 mL of the extract was added to 2.9 mL of 2,2-diphenyl-1-picrylhydrazyl solution. Concerning the oils, a volume of 0.1 g of oil was dissolved in 4 mL of toluene. After stirring, 1 mL of this mixture was added to 3.9 mL of 2,2-diphenyl-1-picrylhydrazyl solution.

The mixtures were incubated for 30 minutes and the absorbances were measured at 515 nm; the inhibition potential of the DPPH[•] radical was expressed as a percentage and calculated as follows:

$$\text{Percent inhibition of the DPPH}^{\bullet} \text{ radical (\%)} = \frac{(A_C - A_E)}{A_C} \times 100$$

A_C : Absorbance of the control, A_E : Absorbance of the sample.

Scavenging effect on the cationic radical 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS^{•+})

The cationic radical ABTS^{•+} is generated by a mixture of equal volumes of potassium persulfate (K₂S₂O₈) at 3 mM and ABTS^{•+} at 8 mM. This solution was kept in the dark and at room temperature for 16 hours. At the end of incubation, this solution was diluted with ethanol.

A volume of 0.1 mL of the methanolic extract of the oil was added to 2.9 mL of ABTS^{•+} freshly prepared, after 30 min; the absorbance was measured at 734 nm [17]. The inhibition potential of the ABTS^{•+} radical by the methanolic extract of the seed oils was expressed as a percentage and calculated as described in the DPPH assay.

Statistical analysis

The extracted oils from the two studied species and the reported parameters were evaluated using three assays. One-way (ANOVA) and two-factor (MANOVA) variance analysis, followed by LSD (Least Significant Difference), they were performed using Statistica 5.5 software.

The degree of significance of the results was taken at a probability of $P < 0.05$, in order to highlight the significant differences between the extracted oils from both species for each parameter.

Results and discussion

Extraction of the oil from Cucurbita species seed

The mass of 300 seeds of *Cucurbita pepo* and *Cucurbita moschata* after grinding and shelling is equal to 35.95 and 39.72 g, while the weight of the oil extracted from them is equal to 15.41 and 16.82 g, respectively. According to Ortiz Grisales et al. [8], the weight of 300 seeds and extracted oil from *Cucurbita moschata* harvested in Santa Marta (Magdalena, Spain) was equal to 41.4 and 17.11 g, respectively. These values are slightly higher than those found in this study. This is probably due to the shape of the seeds that differ within the different species and the studied varieties of *Cucurbita*.

The oil extracted from the seeds of *Cucurbita* species is of a yellow color, with a liquid consistency and a characteristic odor like that of its seeds (Table 2).

According to Vujasinovic et al. [18], the oil extracted from the seeds of *Cucurbita pepo* harvested in Serbia presents a sweet and fruity flavor (flavor of raw seeds). This is due to the pyrazine, the flavoring substance of the oil of these seeds, while Borhade [19] observed an oil of a

Table 2 Characteristics of the oils extracted from the seeds of <i>Cucurbita pepo</i> and <i>Cucurbita moschata</i>		
Appearance/ Designation	Seed oil of <i>Cucurbita pepo</i>	Seed oil of <i>Cucurbita moschata</i>
Consistency	Liquid	Liquid
Color	Pale yellow	Dark yellow
Odor	Smell of seeds	Smell of seeds

yellowish color from the seeds of *Cucurbita mixta* harvested in India.

The best oil yield was attributed to *Cucurbita pepo* (42.85%), while the yield given by the seeds of *Cucurbita moschata* was 40.47%, which is almost similar to the one given by the seeds of *Cucurbita moschata* harvested in Bouira (Algeria) which was 41.61% [7]. In the same way, the results found by Benalia et al. [6] indicated that *Cucurbita pepo* seeds from south Algeria (Laghout) were rich in oil (15.8–33.5%).

Ortiz Grisales et al. [8] showed that the extracted oil yield from 300 seeds of *Cucurbita moschata* from Santa Marta (Magdalena, Spain) was 41.34%. Gohari-Ardabili et al. [20], revealed that the oil yield of seeds of *Cucurbita pepo* extracted with hexane (1:4 *m/v*) for 36 hours was $41.59 \pm 2.71\%$. Borhade [19] found a rate of 42.71% for the oil extracted from seeds of *Cucurbita mixta* by hexane at a ratio of 1:4 *m/v* for 12 hours, at room temperature. In another study, according to Vujasinovic et al. [18], the oil yield recorded for *Cucurbita pepo* seeds harvested in Serbia extracted by cold pressing was 43.94%. All these results are consistent with those observed in this study.

Physico-chemical characteristics

The results of the physico-chemical parameters which are the Brix degree, the acid index, the acidity and the peroxide index of the oils extracted from the seeds of *Cucurbita pepo* and *Cucurbita moschata* are shown in table 3.

The contents of the sugars present in the oil extracted from the seeds of *Cucurbita pepo* and *Cucurbita moschata* were 72 and 72.2%, respectively. Gohari-Ardabili et al. [20] showed that the sugar content in *Cucurbita pepo* seed oil from Iran extracted by hexane was 25.19%. Borhade [19] found that the percentage of sugar in the oil extracted by cold pressing from the seeds of *Cucurbita mixta* harvested in India was 27%. These percentages are much lower than those obtained in the present study. This can be explained by the difference in the used species varieties, the stage of maturity of the fruits and their seeds.

The acid values of the oils of *Cucurbita pepo* and *Cucurbita moschata* were 2.81 ± 1.40 and 2.52 ± 1.26 mg KOH/g

Table 3 Physico-chemical characteristics the seed oils of <i>Cucurbita pepo</i> and <i>Cucurbita moschata</i>			
	<i>Cucurbita pepo</i>	<i>Cucurbita moschata</i>	Standard values
Brix degree (%)	72	72.2	–
Acid index mg KOH/g	2.81 ± 1.40	2.52 ± 1.26	< 3
Acidity (%)	15.82 ± 3.16	14.23 ± 2.74	< 24
The peroxide index meq/kg	18.67 ± 9.64	18.67 ± 9.91	< 5

respectively. These values are in conformity with the standard value (<3).

The acid value (0.56%) obtained from the oil extracted from the seeds of *Cucurbita moschata* growing in the Bouira location of Algeria is largely inferior [7]. However, according to Borhade [19] and Vujasinovic et al. [18], the acid values of the oils extracted from the seeds of *Cucurbita mixta* and *Cucurbita pepo* harvested in India and Serbia were 2.04 and 2.55 mg KOH/g oil, respectively. These values are similar to our results and are conform to the standard. Nevertheless, Murkovica et al. [21] recorded an acid value of 7.54 ± 0.03 mg KOH/g for the oil extracted from the seeds of *Cucurbita pepo* from Australia, which does not conform to the standard. This is probably due to hydrolysis of triglycerides and release of free fatty acids into the oil.

The acidity values of the oil seeds of *Cucurbita pepo* and *C. mushnata* were $15.82 \pm 3.16\%$ and $14.23 \pm 2.74\%$, respectively. The seed oils of both *Cucurbita* species have an acidity less than 24%, which is conform to the standard (< 24%). Gohari-Ardabili et al. [20] showed that the acidity of the seed oil of *Cucurbita pepo* was $0.39 \pm 0.01\%$ and according to Vujasinovic et al. [18], the acid value of *Cucurbita pepo* oil ranged from 0.50 to 1.1 mg KOH/g. Moreover, the acid value of 0.53 mg KOH/g oil of *Cucurbita maxima* was detected by Alfawaz [22] which is in accordance with the standard.

The peroxide index values of the seed oils of *Cucurbita pepo* and *Cucurbita moschata* were 18.67 ± 9.64 and 18.67 ± 9.91 meq/kg, respectively. These results do not comply with the standard (< 5). According to the Codex Alimentarius [23], a peroxide value ranging from 20 to 40 meq/kg of the oil denotes rancid oil. This is probably due to the oxidation of the oil following poor conservation by exposure to air and light (photooxidation), as well as to the mode of extraction of the oil.

Rezig et al. [24] and Vujasinovic et al. [18] reported that the peroxide values obtained from the oils extracted by cold pressing from the seeds of *Cucurbita pepo* harvested in Tunisia and Serbia were 2.33 ± 0.65 meq/kg and

5.56 meq/kg, respectively, and Alfawaz [22] found a peroxide value of 0.85 meq peroxide/kg of *Cucurbita maxima* oil. These indices are much lower than those found in this study and they conform to the standards. Besides, Gohari-Ardabili et al. [20] showed that the peroxide value in the oil extracted by hexane at room temperature for 36 hours, protected from light, from the seeds of *Cucurbita pepo* from Iran was 10.85 ± 0.62 meq/kg of the oil; the extraction solvent was evaporated at 40 °C and the oil was stored under vacuum in bottles. This result is lower than those obtained in this study. This may be due to the applied parameters of the extraction (time, temperature of extraction and evaporation of the solvent) and the conditions of conservation.

Phenolic contents

The antioxidant content (total phenolics and condensed tannins) and the antioxidant activity of the oils extracted from the seeds of *Cucurbita pepo* and *Cucurbita moschata* are depicted in table 4. The concentration of total phenolics in the methanolic seed oils of both *Cucurbita* species revealed no significant difference ($P < 0.05$). But the best content was attributed to *Cucurbita moschata* which was 5.53 ± 1.98 mg GAE/100 g while *Cucurbita pepo* contains 4.45 ± 0.88 mg GAE/100 g of oil.

So, phenolic acids like protocatechuic, syringic, caffeic, p-coumaric, vanillic, and ferulic were revealed in *Cucurbita* seed oil [25].

Table 4 Antioxidant contents and antioxidant activity of the seed oils of <i>Cucurbita pepo</i> and <i>Cucurbita moschata</i>			
	<i>Cucurbita pepo</i>	<i>Cucurbita moschata</i>	
Antioxidant contents			
Total phenolics (mg GAE/100 g)	4.45 ± 0.88^a	5.53 ± 1.98^a	
Condensed tannins (μg CE/100 g)	33.38 ± 3.44^a	34.49 ± 4.22^a	
Antioxidant activity			
DPPH assay (%)			
Oils	34.30 ± 6.32^b	44.66 ± 2.16^a	
Methanolic extracts	13.75 ± 4.14^b	28.69 ± 3.13^a	
ABTS assay (%)	41.02 ± 3.27^a	31.61 ± 5.17^b	
GAE: Gallic Acid Equivalent, CE: Catechin Equivalent			
Each value in the table is the mean $\pm$ standard deviation ($N = 3$)			
Values in the same line sharing different letters are significantly different ($P < 0.05$)			

According to Gohari-Ardabili et al. [20], the content of total phenolics extracted by methanol from the seed oil of *Cucurbita pepo* from Iran was 6.63 mg GAE/100 g. This value is almost similar to our results. These authors found that the polyphenol content in the oil of the seeds of *Cucurbita* species differed from one oil to another and varied from 5 to 10 mg/100 g of the oil. However, the phenol contents of the seeds of *Cucurbita pepo* and *Cucurbita maxima* harvested in Poland, extracted with 50% ethanol for 24 hours, were 1.13 mg GAE/100 g and 0.66 mg GAE/100 g, respectively [26]. This difference is probably due to the concentration and nature of the antioxidants present in the oil, the solvent used for their extraction and the analyzed varieties.

The content of condensed tannins (proanthocyanidins) in the methanolic extracts of the *Cucurbita* species seed oils showed no significant difference ($P < 0.05$) between both oils with values of 33.38 ± 3.44 μg CE/100 g of oil and 34.49 ± 4.22 μg CE/100 g of oil, respectively for *Cucurbita pepo* and *Cucurbita moschata*.

Antioxidant Activities

Scavenging effect on the DPPH[•] radical

The percent inhibition of the DPPH[•] radical by the oil dissolved in toluene and the methanolic oil extracts of *Cucurbita pepo* and *Cucurbita moschata* revealed significant differences ($P < 0.05$) depending on the extraction solvent and the nature of the oil. The best antiradical activity was developed by the oils dissolved in toluene of *Cucurbita moschata*, and *Cucurbita pepo* with percentages of $44.66 \pm 2.16\%$ and $34.30 \pm 6.32\%$, respectively, while those given by their ethanolic extracts of the oils of *Cucurbita moschata* and *Cucurbita pepo* were $28.69 \pm 3.13\%$ and $13.75 \pm 4.14\%$, respectively.

The antiradical power of the DPPH[•] radical developed by the antioxidants present in the seed oil of *Cucurbita pepo* harvested in Tunisia, and dissolved in ethyl acetate, was 36.22% [24], which is consistent with our results.

The percentage of inhibition of the DPPH[•] radical of the oils dissolved in toluene and their methanolic extracts had positive linear correlations with the condensed tannins. Their correlation coefficients were 0.55 and 0.41, respectively.

Scavenging effect on the ABTS^{•+} radical

The percentage inhibition on the ABTS^{•+} radical by the methanolic extracts of both species revealed significant differences ($P < 0.05$). The high antiradical power of ABTS^{•+} was attributed to *Cucurbita pepo* extract with a percentage of $41.02 \pm 3.27\%$ whereas *Cucurbita moschata* oil methanolic extract showed a percentage of $31.61 \pm 5.17\%$.

The percentage of inhibition of the ABTS^{•+} radical of the methanolic extracts of the oil seed of both species revealed a positive linear correlation with the total phenolic content present in the oil; its correlation coefficient was 0.62.

Conclusion

The present study allowed the extraction of the oils from the seeds of two species of *Cucurbita* (*Cucurbita pepo* and *Cucurbita moschata*) from the Bejaia locality (Algeria) using petroleum ether. The evaluation of the quality of these oils by measuring some physico-chemical parameters demonstrated their compliance with standards. The estimation of the antiradical power and the determination of the phenolic content of the oils have shown that these antioxidants are primarily responsible for this activity. The seed oils of these two species of *Cucurbita* represent a good source of natural antioxidants, hence their importance in our diet.

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Links of interests: the authors have no conflicts of interest to report.

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