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Composés bioactifs

Bioactive compounds and antioxidant activity of parsley leaves powder

Composés bioactifs et activité antioxydante de la poudre des feuilles de persil

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Abstract ***Introduction.*** Parsley is a widely consumed culinary herb and it is employed for the treatment of various diseases. ***Objective***. Evaluation of bioactive compounds and antioxidant activity of parsley leaves powder. ***Material and methods*****.** Parsley leaves were dried, ground and then sieved to produce parsley leaves powder. Polyphenols, flavonoids, and carotenoids contents as well as the antioxidant activity (DPPH radical scavenging activity and Ferric-reducing antioxidant power) were determined. ***Results*****.** The determination of polyphenols, flavonoids, and carotenoids revealed respective contents of 10.06 mg GAE/ g, 2.51 mg QE/ g, and 4.32 mg EβC/ g of dry plant material. Parsley leaves powder exhibited also a strong antioxidant activity as revealed by both methods used in the present study. ***Conclusion*****.** Parsley leaves powder shows an interesting nutraceutical potential and it can successfully be used in the formulation of functional food products.

Key words: *Parsley leaves, Bioactive compounds, Antioxidant activity*

Résumé ***Introduction.*** Le persil est une herbe culinaire largement consommée et il est utilisé pour le traitement de diverses maladies. ***Objectif.*** Évaluation des composés bioactifs et de l'activité antioxydante de la poudre de feuilles de persil. ***Matériel et méthodes*****.** Les feuilles de persil ont été séchées, broyées puis tamisées pour produire de la poudre de feuilles de persil. Les teneurs en polyphénols, flavonoïdes et caroténoïdes ainsi que l'activité antioxydante (activité de piégeage des radicaux DPPH et pouvoir antioxydant réducteur ferrique) ont été déterminées. ***Résultats.*** Le dosage des polyphénols, flavonoïdes et caroténoïdes a révélé des teneurs respectives de 10,06 mg EAG/g, 2,51 mg EQ/g et 4,32 mg EβC/g de matière végétale sèche. La poudre de feuilles

de persil a aussi présenté une forte activité antioxydante, comme l'ont révélé les deux méthodes utilisées dans la présente étude. ***Conclusion.*** La poudre de feuilles de persil montre un potentiel nutraceutique intéressant et elle peut être utilisée avec succès dans la formulation des produits alimentaires fonctionnels.

Mots clés: *Feuilles de persil, Composés bioactifs, Activité antioxydante*

Introduction

Since antiquity, herbs and spices have been used to preserve, flavor and color foods as well as for medicinal and cosmetic purposes. They are an excellent source of bioactive compounds and they have been considered as potential antioxidant additives [1,2]. Therefore, there is an increasing interest in aromatic herbs and spices both in scientific research and industrial applications [1].

Parsley (*Petroselinum crispum*) is a biennial herb, usually grown as a perennial herb in the Apiaceae family. Parsley originates from Sardinia, but nowadays is cultivated in USA, in almost the whole of Europe, and in North Africa (mainly in Algeria) [3].

Parsley is a popular culinary herb widely consumed due to its specific aroma and taste. Its dried and/or fresh leaves are largely used as a condiment, garnish and flavoring food additive [4,5]. Due to its high content of different bioactive components (e.g. vitamins, flavonoids, and essential oil components), it is used also in medicines and dietary supplements, as well as in cosmetics [3,6].

Parsley is employed in traditional and folklore medicine for the treatment of various diseases (urinary diseases, gastrointestinal disorders, cardiovascular diseases, hypertension, diabetes, dysmenorrheal, amenorrhea, sniffle, otitis, and also various dermal diseases) [6,7] due to its bioactive compounds that exhibit a wide range of activities including antioxidant, cytoprotective, gastro-protective, gastrotonic, carminative, hepato-protective, antiseptic of urinary tract, diuretic, brain protective, spasmolytic, analgesic, anti-inflammatory, estrogenic, immune-suppressant, laxative, hypotensive, anti-diabetic, anti-urolithiasis, anti-platelet, anti-dote, antibacterial and antifungal activity [7].

With growing awareness of parsley health benefits and the recent trend toward healthy eating, demand for parsley materials has seen steady growth. Parsley leaves are the main parts used in commercial applications and they are used not only as a food, but also as a dietary supplement, contributing to the demand [6]. Therefore, the objective of the present study was to evaluate the bioactive compounds and the antioxidant activity of parsley leaves powder.

Material and methods

Plant material

Parsley (*Petroselinum crispum* Mill.), purchased from a local market (Constantine, Algeria), was firstly cleaned by washing and rinsing with tap water, and then the leaves were dried at 40°C in a fluid bed dryer Retsch TG 200 (Retsch, Haan, Germany) until constant weight. The dried leaves (6% of residual moisture) were subsequently ground and sieved to produce parsley leaves powder (PLP) with particle size below 0.5 mm. the obtained PLP was stored in hermetically sealed plastic bags.

Extracts preparation

Approximately 1 g of PLP was extracted with 20 mL of 75% acetone [8]. The mixture was stirred for 30 min, centrifuged at 1700×g for 5 min (Sigma 3-30K, Osterode am Harz, Germany) and paper filtered. The filtrates (extracts) were used for the assessment of polyphenols, flavonoids, and antioxidant activities.

Determination of polyphenols content

Polyphenols content was determined in duplicate as described by Singleton and Rossi [9] by mixing 150 µL of extract with 750 µL of Folin–Ciocalteu reagent and 600 µL of sodium carbonate (7.5%). After 30 min of reaction in a dark place at room temperature, the absorbance was measured at a wavelength of 750 nm with a UV spectrophotometer UV-1800 (Shimadzu, Kyoto, Japan). The polyphenols content was expressed as a gallic acid equivalent (GAE) in mg/g of dry weight (dw).

Determination of flavonoids content

Flavonoids content in extracts was estimated in duplicate by the method of Quettier-Deleu *et al.*, [10], based on the formation of flavonoids–aluminium complex. Equal volumes of extract and aluminium chloride solution (2%) were mixed. The absorbance of the reaction mixture was measured at 430 nm after 15 min of incubation. Total flavonoids

content was expressed as mg quercetin equivalents (QE) per g dw.

Determination of carotenoids content

Carotenoids were quantified in double according to the procedure described by Kuti [11]. Approximately, 0.5 g of PLP were homogenized and extracted with 10 mL of hexane/acetone/ethanol (2: 1: 1) for 30 min before being centrifuged for 15 min at 2250 × g. The top layer of hexane was recovered and transferred to a 10 mL volumetric flask. The volume of recovered hexane was then adjusted to 10 mL with hexane. The absorbance was measured at 450 nm. Carotenoids content was expressed as mg equivalents of β-carotene per g dw.

Evaluation of antioxidant activities

DPPH assay

The method described by Brand-Williams *et al*., [12] with minor modification was used to measure the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of PLP. Briefly, the extract (0.1 mL) was added to DPPH solution (1 mL, 60 µM), and after 30 min of incubation, the absorbance was measured at 517 nm. Results were expressed as ascorbic acid equivalents (AAE) in mg/g dw. Tests were performed in duplicate.

Ferric-reducing antioxidant power

The ferric-reducing antioxidant power (FRAP) of PLP was evaluated in duplicate according to the method described by Chaalal *et al.,* [8]. A mixture containing 1 mL of extract, 1 mL of phosphate buffer (200 mM, pH 6.6) and 1 mL of potassium ferricyanide (1%) was prepared and incubated at 50°C for 20 min. After the addition of 1 mL of 10% trichloroacetic acid, the obtained mixture was centrifuged for 10 min at 1700 ×g; then an aliquot of supernatant (1 mL) was mixed with distilled water (1 mL) and 0.2 mL of ferric-chloride (0.1%). After 10 min of incubation, the absor-bance was measured at 700 nm. FRAP was expressed as ascorbic acid equivalents (AAE) in mg/g dw.

Statistical analysis

Results were expressed as mean ± standard deviation. Statistical analysis was performed using Microsoft Excel 2013.

Results

Bioactive compounds of PLP

Bioactive compounds of PLP are shown in **Table 1**.

Polyphenols and flavonoids contents of PLP were 10.06mg GAE/g dw and 2.51mg QE/g dw respectively. As regard carotenoids content of PLP, it was 4.32 mg EβC/g dw.

Table 1. Bioactive compounds of parsley leaves powder

	Concentration
Polyphenols	10.06± 1.37 mg GAE/g dw
Flavonoids	2.51± 0.75 mg QE/g dw
Carotenoids	4.32± 0.04 mg EβC/g dw

GAE: gallic acid equivalent ; QE: quercetin equivalent. Results are presented as mean ± SD (n = 2).

Antioxidant activities of PLP

Dried parsley leaves showed a DPPH scavenging activity at 0.82mg AAE/g dw and FRAP at 5.35mg AAE/g dw (**Table 2**).

Table 2. Antioxidant activities of parsley leaves powder

	Concentration
DPPH scavenging activity (mg AAE/g dw)	0.82± 0.90
Ferric-reducing antioxidant activity (mg AAE/g dw)	5.35± 0.67

AAE: Ascorbic acid equivalent. *Results are presented as mean±SD (n = 2).*

Discussion

The objective of this study was to evaluate the bioactive compounds, and the antioxidant activity of parsley leaves powder. It is interesting to determine the concentration of the various bioactive compounds contained in parsley leaves which are linked to biological effects of the plant. According to the obtained results, the content of polyphenols was 10.06 mg GAE/g dw, that of flavonoids 2.51 mg QE/g dw, and that of carotenoids 4.32 mg EβC/g dw. The comparison of our results with those found by other authors was difficult since different factors could be involved, such as methods of extraction, calculation methods, and standards used for the calibration curve drawing. The differences could also be due to the variation of bioactive compounds within varieties, place of production, time of harvest and environmental stressors [13].

Characteristic constituents of parsley are flavonoids (apiin, luteolin-, apigenin glycosides), cumarines (bergapten, imperatorin), and essential oil (apiol, miriszticin) [14].

Kaiser *et al*., [1] separated up to 11 phenolic compounds in parsley which were assigned to flavonoid derivatives (mostly represented by flavones apiosyl-

glucosides) and phenolic acid (mainly *p*-coumaric acid derivatives). Farzaei *et al.*, [7] reported that the active components identified in parsley are phenolic compounds particularly flavonoids (such as apigenin, apiin and 6"-Acetylapiin), essential oil components (mainly myristicin and apiol), coumarins and furocoumarins. More recently, Sęczyk *et al.*, [15] discovered that all identified phenolic compounds of parsley leaf extracts belonged to the flavonoids (flavones and flavonols sub-classes). These include derivatives of apigenin, isorhamnetin, diosmetin and catechin. According to the same authors, parsley leaf flavonoids are potentially bioaccessible.

Flavonoids are the main phenolics compounds of pars -ley and these can be found in relatively large amounts in the leaves [15]. The major phenolic compound of parsley is apiin (apigenin-7-apiosylglucoside) as reported in previous studies [1,2,14,16].

As regards carotenoids, Francis [17] detected in parsley leaf various carotenoids including β-carotene, lutein, violaxanthin and neoxanthin.

Polyphenols are considered as the main antioxidants in diet. Their functional properties are related to antibacterial, antiviral, anti-inflammatory and anti-carcinogenic activities that impact positively the human health [18]. In fact, investigations have demonstrated that consumption of polyphenols prevents the development of chronic diseases like cancers, diabetes and cardiovascular diseases [19-21].

The antioxidant activity of parsley leaves powder was assessed by two different methods: the scavenging of the free radical DPPH and the reducing power of ferrous iron. The antioxidant activity of PLP using the first method was 0.82 mg AAE/g dw, while it was 5.35 mg AAE/g dw using the second method.

The antioxidant activity of parsley leaf has been reported in several studies [13,22-24], and some *in vitro* and *in vivo* studies indicate that parsley leaves are a good source of antioxidants that exhibit different mechanisms of action [7,14,25]. Phenolic compounds and other compounds (e.g. flavones, xanthophylls, apiol and myristicin) are considered as the main components for parsley antioxidant activity [15, 26] and Nielsen *et al.*, [25] demonstrated that apigenin was the main compound responsible for this.

Many mechanisms contribute to the antioxidant efficacy of polyphenols: capacity to quench singlet oxygen, chelate transition metal ions, reduce hydrogen peroxides to stable compounds, disrupt the propagation of chain auto-oxidation reactions, inhibit lipid radical formation, and endogenous pro-oxidative enzymes and activate endogenous antioxidant enzymes [27,28].

The antioxidant activity can be affected by several factors as the botanical origin, the amount of antioxidants in the material, and the solvent used for extraction [29].

Conclusion

Parsley leaves powder has an interesting nutraceutical potential as it has high amounts of phenolic compounds, with high antioxidant activity. The outcomes of the present research will promote the use of a commonly available raw material (parsley leaves) in food formulation, and widen the categories of food products with an improved nutritional quality and health benefits.

Conflict of interests

The authors declared no conflict of interests.

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