Journal of Food and Dairy Sciences

Journal homepage & Available online at: www.jfds.journals.ekb.eg

Phenolic Compound Profiles and Bioactive Properties of Parsley Leaves Extract and Seeds Oil

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ABSTRACT

Parsley is a popular dietary plant appreciated for its ability to increase flavor. It is one of the earliest plants to be used as a food spice and popular medicine. The goal of this study is to thoroughly examine the phenol compounds and antioxidant capacity, and antifungal activities of parsley leaves extracts and oil. Results revealed seventeen phenolic compounds; Chlorogenic acid at 220.3 µg/ml in parsley leaves powder and Cinnamic acid at 119.16 in oil. as well as, twenty different essential oil; the predominant compounds were myristcin (13.70%) and apiole (15.28%). Additionally, the results of the antifungal experiments showed positive inhibitory effects against the several strains of fungus, indicating parsley's possible use as a natural antifungal agent. In conclusion, this research reveals fascinating details about the chemical constitution of parsley leaves and its oil. Parsley may find use in the food preservation and pharmaceutical industries because to its proven antioxidant and antifungal qualities.

Keywords: Parsley; antioxidant; antifungal

INTRODUCTION

Parsley (*Petroselinum crispum*) is an aromatic biennial plant belongs the Apiaceae family. Many different types of plants are classified as aromatic because of their ability to add flavor to food and drink as well as aroma to commercial and medicinal products. They are offered all year round because they are best when sold either fresh or frozen, and dry. These days, the demand for aromatic plants is growing for both industrial and fresh markets due to their immense popularity. Additionally, to its medicinal properties, parsley is widely used as a flavor worldwide, primarily in dishes such as salads pancakes, soups, sauces, and herb cream preparations, but it's also used as a side dish for a variety of other foods (Teuscher *et al*., 2006).

One of the most prevalent ways that food deteriorates is oxidation; in fact, antioxidants are commonly utilized in food. There is an increasing need for natural sources of antioxidants, since customers choose natural products, even though some of them are created by chemical synthesis. Strategic plans are in place at the European Commission to support organic farming throughout the EU. Recently, essential oils are mostly used as flavouring agents in the food company, but they are also used in the medicinal, cosmetic, and hygienic fields. (Hyldgaard *et al*. 2012). The food industry gains from the preservation qualities of essential oils as well (Hyldgaard *et al*. 2012 and Oussalah *et al*., 2007). Soups, meat products, dairy products (cheeses, creams), flavored oils and fermented vegetables and vinegars, among others, usually contain essential oils or other extracts or plant parts. Investigating the antimicrobial characteristics of essential oils, particularly concerning food spoilage and pathogenic microbes, as well as the relationships between food, essential oils, and microorganisms and potential combinations of antibacterial substances.

The majority of essential oils antimicrobial research has focused on bacteria, with a limited number of studies also involving moulds and yeasts. Gram-negative bacteria are often less sensitive to extracellular organic compounds (essential oils) than gram-positive bacteria (Trombetta *et al*., 2005), mostly because of the properties of their membranes, which serve as barriers against hydrophobic chemicals and macromolecules. Gram-negative bacteria are in some way protected against Essential oils because they are hydrophobic substances (Nikaido 2003).

There have also been reports of Essential oils' antioxidant qualities. By inhibiting the beginning or progress of oxidation chain reactions, antioxidant chemicals have the power to postpone or prevent the oxidation of lipids and other molecules (Velioglu *et al*., 1998). The principal component of Essential oils, phenolic compounds, is what give them their antioxidant qualities that can be utilized as a substitute for synthetic antioxidants, according to Zeng and Wang (2001). This makes Essential oils useful as food preserving agents.

The in vitro properties of organic Essential oils are of tremendous interest due to their potential as antioxidants and antimicrobials. Their understanding could enable their appropriate application in organic foods and provide a substitute for artificial antioxidants in foods produced conventionally.

Parsley is an abundant source of vitamins C, E, b-carotene, thiamin and natural minerals (Okos *et al*., 1992 and Doymaz *et al*., 2006). Parsley is typically dried before being sold because of its high-water content (78–82%, w/w), which prevents the growth of microorganisms and avoids deterioration from biochemical reactions. Furthermore, drying significantly reduces weight and volume, which lowers the cost of packing, storing, and shipping (Soysal, 2004).

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DOI: 10.21608/jfds.2024.263204.1149

In recent decades, individuals have favored using natural products over conventional preservatives due to the adverse effects of chemical preservatives. Because of these, customers become attracted to natural products, especially those made from extracts of plants and their essential oils. Foods have been treated with spices and herbs to improve their flavor, color, and smell. They are also well-known for their therapeutic and preservation properties (Wu *et al*., 2011). Plant phenolics, which can be found in all regions of plants, including fruits, vegetables, nuts, seeds, roots, leaves, and barks, are the main source of natural antioxidants (Yakoob *et al*., 2016). Plants that have antioxidant and antimicrobial properties may have a variety of compounds, such as peptides, aldehydes, alkaloid compounds, volatile oils, phenols, and other soluble substances. Then, it has been revealed that these plants contain substances with significant medicinal potential against infections affecting humans (Moldovan *et al*., 2014 & Nickavar *et al*., 2008). Thus, the objectives of this research were to identify the chemical constituents of parsley volatile oils and investigate the oils' antifungal and antioxidant properties.

Thus, this study was designed to investigate the Essential oils composition, phenolic content, antioxidant properties, and antifungal properties of parsley leaves extract and parsley seeds oil against fungal food spoilage.

MATERIALS AND METHODS

Material

1- Fresh parsley (*Petroselinum crispum*) was obtained from local market in Assiut Governorate, Egypt.

2. Parsley seeds oil (PSO) was obtained by cool pressing of parsley seeds from a local commercial pressing unit.

Methods

Parsley leaves powder extract preparation (PLPE)

Fresh parsley leaves were air dried immediately on 40ºC for 48 h. Then, they were crushed and milled to obtain parsley leaves powder and kept under -80 ºC for further analyses. Aqueous ethanol 70 % was used as extraction solvent. A mixture of dried parsley leaf powder (5.0 g) and 70 % ethanol (100 mL) were stirred in a shaking incubator at (25°C) and 250 rpm for 1 h and then centrifuged at 10000 rpm for 10 min. The supernatant was distilled in a vacuum at 50°C using a rotating evaporator, and the residue was freeze-dried (Gnintoungbe *et al*., 2023).

Determination of phenols and flavonoids components by HPLC

HPLC analysis of Parsley seeds oil and Parsley leaves powder extract preparation was used by an Agilent 1260 series. The Zorbax Eclipse Plus was utilised for the separating process C8 column (4.6 mm x 250 mm i.d., 5 µm). Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were used as the mobile phase, with a flow rate of 0.9 ml/min. The mobile phase was computing sequential in a linear gradient as follows: 0 min (82% A); 0–1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A) ; 22-24 min (82% A). Monitoring of the multi-wavelength detector was place at 280 nm. For every sample solution, there was one volume for injection of 5 µl. At 40 °C (Stan *et al*., 2012).

GC-MS Analysis of Parsley seeds oil (PSO)

The GC model 7890B from Agilent Technologies was installed with flame ionization detectors in Cairo, Egypt's National Research Centre. A Zebron ZB-FAME column was used to carry out separating (60 m x 0.25 mm internal diameter x 0.25 µm film thickness). Hydrogen was used as the carrier gas during the analyses, using a split-1:50 mode and a flow rate of 1.8 ml/min1 µl of injection volume and the subsequent temperature programme: 3 minutes at 100 °C, followed by a 2.5 °C/min rise to 240 °C and a 10-minute hold. The injector and detector (FID) were maintained at 250 °C and 285 °C, respectively (Aziz *et al*., 2013).

DPPH Free Radical Scavenging Activity of PLPE and PSO

Extracted solution dissolved in methanol. The mixture was good shake, it was allowed to stand at room temperature for fifty minutes in the dark. Using a spectrophotometer, the absorbance was measured at 517 nm in relation to a control. The results were displayed as IC50 µg/mL sample, which indicates the concentration of each sample required to scavenge 50% of the DPPH radicals (Valko *et al*., 2007).

The following equation was used to determine the percentage of DPPH discoloration:

$$\textbf{Percentage inhibition} = [(Abs_{0control} - Abs_{sample}) / Abs_{0control}] \times 100$$

Determination of antimicrobial activity of PLPE and PSO

The antifungal activity of PLPE and PSO was determined using laboratory reference strains obtained from fungal center in Assiut University, Egypt. Nine different fungal types were prepared in a sterile 15-cm Petri plate filled with Trypticase soy agar (TSA) and Sabouraud dextrose agar (SDA) media according to Pfaller *et al*., 2004. Using a sterile cork borer, 6 mm wells were made in the agar, and the agar was removed, leaving empty wells that had been filled with the oil and powder emulsion. After leaving the plates at ambient temperature for about two hours, incubate them for twenty-four hours at 37ºC. The inhibitory zones that resulted were measured in millimetres, and averages values were obtained (Khalil, 2018).

RESULTS AND DISCUSSION

Determination of phenols and flavonoids compounds by HPLC analysis

The HPLC analysis was conducted parsley leaves powder extract (PLPE) and parsley seeds oil (PSO) to identify seventeen phenolic compounds in the form of symmetrical peaks split apart from one another. PLPE has polyphenol concentrations ranging from 0.00 to 220.3 µg/ml in parsley leaves powder, while in PSO vary from 0.00 to 119.16.

Table 1 reveals a relatively high concentration of Chlorogenic acid at 220.3 µg/ml in parsley leaves powder and Cinnamic acid at 119.16 in parsley oil, which is followed by Naringenin at 82.25 µg/ml in parsley leaves powder and Querectin at 110.9 µg/ml in parsley oil. In contrast, Tadros *et al.* (2017) reported that rosmarinic acid was the most abundant ingredient in the methanolic extract of parsley seeds (1948.59 µg/ml), although it was found in the methanolic extract of green portions (1078.79 µg/ml). Additional study has demonstrated that parsley contains quercetin (Plazonić *et al*., 2009), myricetin (Yıldız *et al.*, 2008), and ferulic acid. Therefore, our findings support the parsley plant's high phenolic component content.

Table 1. Phenols and flavonoids compounds in PLPE and PSO by HPLC

Phenols and flavonoids compounds	PLPE (µg/ml)	PSO (µg/ml)
Gallic acid	7.39	21.02
Chlorogenic acid	220.31	8.63
Catechin	0.49	0.00
Methyl gallate	0.36	1.01
Coffeic acid	10.51	9.12
Syringic acid	1.21	17.49
Pyro catechol	19.10	0.00
Rutin	0.00	2.91
Ellagic acid	0.15	0.97
Coumaric acid	0.09	0.80
Vanillin	0.36	10.77
Ferulic acid	2.00	8.59
Naringenin	82.25	14.16
Rosmarinic acid	6.48	13.51
Daidzein	0.19	1.24
Querectin	1.05	110.99
Cinnamic acid	0.08	119.16
Kaempferol	1.30	16.62
Hesperetin	0.00	30.15

GC-MS Analysis of Parsley seeds oil (PSO)

Twenty different chemicals were found in parsley seeds oil, as shown in Table 2.

Table 2. Oil content in parsley seeds.

Compounds	Parsley seed(GC-MS Peak Area %)
α-pinene	11.07
β-pinene	11.68
Myrcrene	0.39
β-phellandrene	12.24
Myrtenal	0.83
Myristcin	13.70
Cis-aserone	7.86
Cis-6-octadecenoic acid	9.80
Apiole	15.28
Linoleic acid (C18:2)	2.43
Butyric acid (C4:0)	2.29
Palmitic acid (C16:0)	7.72
Oleic acid (C18:1)	4.8
caproic acid (C6:0)	2.78
Caprylic acid (C8:0)	3.3
Capric acid (C10:0)	3.67
Lauric acid (C12:0)	3.97
Linolenic acid (C18:3)	4.57
Arachidic acid (C20:0)	2.23
Margaric acid (C17:0)	2.34

When the chemical composition of parsley seed oil was investigated, the predominant compounds were myristcin (13.70%) and apiole (15.28%). Significant levels were also detected for α-pinene (11.07%), β-pinene (11.68%), and cis-6-octadecenoic acid (9.80%). Myristicin (between 36% and 42%) is the most prevalent component in PSO, according to Louli *et al.* (2004) analytical investigation. The authors additionally found that the amounts of β-pinene (2% and 0.5%), α-pinene (2.7%), and apiole (26.7% and 34.6%). The researchers found that myristicin was the most common compound in parsley seeds with 42.7 % for fermented seeds and 36.7 % for native seeds. Additionally, it was found that apiole (5.4%), β-pinene (16.7%), and α-pinene (20.22%) were the other three of the most common compounds (Stankovic *et al.,* 2005). However, if it was examined quantitatively constituent of essential oil from parsley seeds, it disagreed with previous research. According to Okan *et al.* (2018), the reason for this is that different species of plants reveal variations in the synthesis of essential oils caused by factors such as genetics, weather, and environmental conditions.

DPPH radical-scavenging activity of PLPE and PSO

Antioxidant molecules act by reducing or removing free radicals to prevent oxidation in organisms. There are numerous techniques for determining a natural product's antioxidant potential (Okan *et al.,* 2019). Parsley seed oil's capacity to scavenge free radicals was assessed using the DPPH test (IC50: 5.11 µg/ml). In a different study, using DPPH techniques, the parsley oil's antioxidant properties were investigated. As a result, 87-91% of the radicals in the reaction mixture were quenched in 10 minutes, according to the DPPH value (Parry *et al.,* 2006). High antioxidant capacity for parsley seed oil was confirmed using the DPPH antioxidant activity (Wei and Shibamoto, 2007). Researchers have observed that high concentrations of parsley seed oil contain myristicin, α-pinene, β-pinene, and apiole, all of which are useful in enhancing antioxidant activity.

Antifungal properties

Parsley oil was more effective at inducing cell damage against six fungus species; *Aspergillus flavus* and *Aspergillus niger* are enhanced effectiveness against Parsley oil (Table 3). According to Raccach (1984), phenolic antioxidants may interact with components of cellular membranes, compromising their integrity and function The growth inhibition seen in this study could be attributed to interferences caused by herbal phytochemicals that have been shown to result from lipid–protein interactions at the cell membrane level, or the interruption of nutrients' active transportation at the cytoplasmic membrane (Cerrutti Alzamora, 1996). The overall outcome of these effects is similar to the initial stages of microbial growth, during which enzymes and metabolic intermediates are produced to facilitate exponential expansion (Ogunrinola *et al.,* 1996). Our findings demonstrated that the presence of parsley inhibited the ability of fungus to proliferate exponentially.

Table 3. Antifungal activity of PLPE and PSO

Fungi	PLPE	PSO	Control (antifungal)
Aspergillus flavus	8	20	28
Aspergillus niger	7	20	24
Candida albicans	0	2	23
Cladosporium sphaerospermum	10	17	20
Debaryomyces hansenii	0	3	18
Mucor racemosus	8	15	22
Penicillium chrysogenum	8	19	28
Pichia membranifaciens	9	10	18
Rhizopus arrhizus	8	18	23

The amount added in each pore 50 ul
Inhibition zone in mm
PLPE: Parsley leaves powder extract
PSO: Parsley seeds oil

CONCLUSION

This study concludes by Parsley seed oil has strong antioxidant action because of apiole, myristicin, α-pinene and βpinene. Particularly, apiole is recognized to be nephrotoxic and hepatotoxic. The antioxidant activity of Parsley seeds oil, which makes them suitable for use in medicine. Moreover, the antimicrobial activity of parsley products depends on the phenolic profile and the

composition of essential oils, both of which can be influenced by environmental and/or genetic factors.

ACKNOWLEDGEMENT

The authors are grateful to the technicians of Central Laboratory, Assiut University, Assiut, Egypt for their support for completing this study

Conflict of interest

The authors declare that they have no competing or conflict of interest.

Funding and financial statement

This work was supported financially by the Faculty of Agriculture, Assiut University,

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التركيب الفينولي والخصائص الفعّالة لمستخلص اوراق وزيت بذور البقدونس

روفيدة فرج محمد مفتاح ، منه الله محمد الأنورالجداوي و رانيا مصطفى حمدي

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الملخص

البقدونس هو نبات غذائي شعبي مشهور لقدرته على زيادة النكهة وهو من أقدم النباتات التي استخدمت كتوابل للطعام وفي الطب الشعبي. هدف هذه الدراسة هو فحص التركيب الفينولي للبقدونس ونشاطه كمضاد واسع للاكسدة، ونشاطه كمضاد للفطريات سواء في مستخلصات أوراق البقدونس وزيته. تم تقييم المكونات الكيميائية للبقدونس باستخدام أساليب تحليلية مبتكرة. كشفت النتائج عن سبعة عشر مركبًا فنوليًا؛ حمض الكلوروجينيك عند ٢٢٠,٣ ميكروغرام/مل في مسحوق أوراق البقدونس وحمض السيناميك عند ١١٩,١٦ في الزيت، بالإضافة إلى عشرين مركبًا زيتيًا مختلفًا؛ وكانت المركبات الرئيسية هي ميرستسين (١٣,٧٠٪) وأبيول (١٥,٢٨٪). وبالإضافة إلى ذلك، أظهرت نتائج التجارب المضادة للفطريات تأثيرات مثبطة إيجابية ضد سلالات الفطريات محل الدراسة، مشيرة إلى إمكانية استخدام البقدونس كمضاد فطري طبيعي. في الختام، تكشف هذه البحث تفاصيل رائعة حول التركيب الكيميائي لأوراق وزيت البقدونس، وقد يجد البقدونس استخدامًا في حفظ الاغذيه وفي مجال الصيدلة بناءً على خصائصه المثبتة كمضاد أكسدة ومضاد للفطريات.

الكلمات الدالة: بقدونس، مضاد أكسدة، مضاد للفطريات