Study of the antioxidant properties of some aromatic plant extracts

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Abstract. In this study the effect of extraction conditions (time - temperature-type of aromatic plants-extraction method) on the antioxidant activity, pH extracts was investigated. The extraction process was carried out using water as a solvent, and also through using the ultrasonic treatment. Aromatic plants that have been used are rosemary, a mixture of herbs (black cumin+ oregano). The extracts were prepared by the solvent extraction method, and the ultrasonic treatment was under the following conditions: time - (10-20-30 min), temperature - (40-60-80 °C ), then pH extracts were estimated using pH meter, and the antioxidant activity of all extracts was determined by scavenging of DPPH (1,1-diphenyl 2-picrylhydrazyl) radicals. The results have showed that the values of pH extracts increase in the use of ultrasonic treatment. Also it was revealed that the highest value of antioxidant activity (95.43 %) was when extracts were obtained from rosemary using ultrasonic treatment according to the following parameters: (60 °C, 30 min). Therefore, these conditions could be applied for further extraction for their use in the preparation of medicinal products or functional food.

1. Introduction
In an effort to reduce the undesirable effects of synthetic food preservatives on human health, food industry experts and scientists have turned their attention to natural substances that have a preservative effect. Aromatic plants contain phenolic compounds, commonly called polyphenols, which are natural components of plants and herbs, and they play the role of antioxidant and antimicrobial to maintain food quality [1].

Antioxidants are originally defined as substrates capable of preventing or significantly slowing the oxidation of easily oxidized nutrients such as fats in small amounts [2].

Consequently, there is growing interest in using plant antioxidants as food ingredients to prevent food oxidation [3].

A plant extract is a substance or active substance with the desired properties that are removed from the tissue of a plant, usually by treating it with a solvent, for use for a specific purpose. Extracts are called preparations obtained by extracting substances from plant materials by solvents. Water, alcohol, glycerin, ether, and their mixtures are used as solvents; respectively, extracts are subdivided into aqueous, alcoholic, glycerin, ethereal, aqueous-alcoholic, water-glycol (propylene glycol), oil, CO2-extracts. Plant extracts can be dry, liquid, and thick [4].

A large group of cultivated and wild-growing plants belongs to spicy-aromatic (spicy-flavoring) plants, which, due to the content of volatile, pleasantly smelling substances, are used for flavoring food products [5]. There are several subgroups among aromatic plants that include herbs and spices such as
grape seed, thyme, rice bran, white peony, red peony, angelica, sedge, marjoram, wild marjoram, cumin, basil extract, ginger, plum concentrates, aloe vera, oregano and rosemary extract [6].

Aromatic plants that have been used for this research are rosemary, cumin, and oregano. Rosemary is a widely used food ingredient for flavoring and is known as a traditional medicinal plant for its beneficial properties such as antibacterial, anti-alcoholic, and antirheumatic effects [7]. It also contains the highest concentration of phenolic substances [8].

Black cumin seeds contain fixed and essential oils, alkaloids, proteins, and saponin. Most of the biological activity was attributed to these essential components - thymoquinone (24.5-57%), p-cymene (10.7-40.3%), α-thuyene (1.9-8.2%), carvacrol (2, 2-4.5%), 4-terpineol (1.9-4.5%) [9].

Some studies have shown that oregano has the highest total antioxidant capacity and has the highest phenol content compared to some other herbs such as thyme, sage, rosemary, mint, and sweet basil [10].

Phenolic components, the main ingredients with antioxidant and antimicrobial properties, can be extracted from plants by traditional methods such as Soxhlet extraction, maceration, and hydrodistillation using polar solvents such as methanol, ethanol-water, glycerin, or mixtures thereof.

The main problems of traditional extraction are long extraction times, the use of an expensive high-purity solvent, evaporation, and accordingly, consuming a large quantity of solvent, lack extraction yield, and decomposition of biodegradable compounds under the influence of heat [11]. Therefore, in this study, aqueous plant extracts were obtained using ultrasonic extraction. This method is one of the most important recently used methods since the technology does not require complex instruments, it gives the highest yield of some flavonoids, in addition, it has some advantages such as shorter reaction times, use of small amounts of material, efficient and minimal solvent consumption [12].

The aim of this study is to determine the content of antioxidants in various aromatic plants and determine the optimal extraction for obtaining and further use of the extract in the food industry.

2. Objects and methods of research

Dried, ground leaves of (rosemary- oregano), dried black cumin seeds were used for research. Samples were prepared from different mixtures of these herbs, the amount of herbs (10 g). Then they were mixed with 200 ml of distilled water, placed in a water bath at (60°C) for (10-20-30 min). After determining the ideal extraction time (30 min), this time used for other temperatures (40-80 °C).

The selected samples were filtered and cooled to (20 °C), after that the pH of the extracts and antioxidant activity were determined.

After selecting the ideal conditions for extraction (temperature, time of extraction), these parameters were used for ultrasonic extraction.

For obtaining extracts using ultrasonic treatment: (10 g) different mixtures of herbs (rosemary-cumin-oregano) were mixed with distilled water (500 ml) at (60 °C), the extraction was carried out using an ultrasonic generator (II-10). Processing parameters are 22.5 kHz, with a power of 100 watts, sonication time (10-20-30 min). Then the antioxidant activity and pH of the extracts were measured in the prepared samples.

The pH of extracts were measured using a pH meter at 25 °C. The antioxidant activity was determined according to the slightly modified procedure described by Brand-Williams et al. [13]. Briefly, 4 ml of an alcohol DPPH solution (0.1mM in ethanol) was added to 1 ml of diluted extract (1 ml of leaf extract was taken, and diluted to 10 ml with distilled water), The mixture was then conserved at room temperature in a dark place. After 30 min. The absorbance was measured at 517 nm on a spectrophotometer.

The antioxidant activity was calculated by the following equation:

\[ \text{Antioxidant activity was} \% = \left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100 \]

Here Abs control is the absorbance of DPPH solution+ solvent; Abs sample is the absorbance of the sample extract after adding DPPH.
3. Results and Discussion

Table 1 shows the pH extracts for different samples under different parameters from various mixtures of herbs using distilled water.

**Table 1. pH extracts of herbs (rosemary, cumin + oregano) using distilled water (solvent extraction).**

| Sample | The amount of herbs (g) | Extraction time, min | Extraction temperature, ℃ | pH extract |
|--------|-------------------------|----------------------|-----------------------------|------------|
| 1 (rosemary) | 10 | 10 | 60 | 5.84 |
| 2 (rosemary) | 10 | 20 | 60 | 5.96 |
| 3 (rosemary) | 10 | 30 | 60 | 5.86 |
| 4 (rosemary) | 10 | 30 | 40 | 5.82 |
| 5 (rosemary) | 10 | 30 | 80 | 5.86 |
| 6 (5 g cumin + 5 g oregano) | 10 | 30 | 60 | 5.80 |

By measuring the pH extracts in samples after ultrasonic treatment following values were obtained (table 2).

**Table 2. pH extracts of herbs (rosemary, cumin + oregano) after ultrasonic treatment.**

| Sample | The amount of herbs (g) | Sonication time, min | Extraction temperature, ℃ | pH extract |
|--------|-------------------------|----------------------|-----------------------------|------------|
| 1 (rosemary) | 10 | 10 | 60 | 6.53 |
| 2 (rosemary) | 10 | 20 | 60 | 6.37 |
| 3 (rosemary) | 10 | 30 | 60 | 6.16 |
| 4 (5 g cumin + 5 g oregano) | 10 | 30 | 60 | 6.04 |

Results in tables (1,2) show that there is no obvious difference in the pH values for plant extracts prepared using distilled water (solvent extraction), on the other hand, pH values of extracts increased after ultrasonic treatment, the lowest pH value was obtained for extracts prepared from cumin and oregano which confirms that the pH of leaf water extracts depends on the type of plant and the length of soaking leaves [14].

The antioxidant activity of rosemary extracts at (60 ℃, 10-20-30 min) using distilled water, after ultrasonic treatment is presented in Figure 1.

**Figure 1.** Antioxidant activity of rosemary extracts (at 60 ℃ using distilled water + after ultrasonic treatment)
Figure 1 shows that by increasing the extraction time, the antioxidant activity increases, in addition, the highest value of antioxidant activity was observed when obtaining extracts using ultrasonic treatment (at 30 min). According to previous research, the ultrasonic treatment gives the highest yield of some polyphenols that act as antioxidants [12]. This explains the high values of antioxidant activity for extracts obtained using ultrasonic treatment compared to obtaining these extracts using a solvent (distilled water).

After determining the optimal extraction time (30 min), rosemary extracts were prepared at (40-80 °C) at this time (30 min) using distilled water (solvent extraction). The results of measuring antioxidant activity for these extracts are shown in Figure 2.

![Antioxidant activity](image1)

**Figure 2.** Antioxidant activity of rosemary extracts (at 40-80 °C, 30 min using distilled water)

Results in figure 2 show that antioxidant activity increases with increasing temperature. According to previous studies, the antioxidant activity increases as the extraction temperature increases due to the decreased solvent viscosity; as well as the supported mass moving and entrance of solvent into the plant matrix [15,16].

The extracts from a mixture of herbs (black cumin-oregano), were prepared at the optimal extraction conditions that were defined previously (extraction time: 30 min, extraction temperature: 60 °C), using distilled water (solvent extraction), and ultrasonic treatment. The antioxidant activity of these extracts is presented in Figure 3.

![Antioxidant activity](image2)

**Figure 3.** Antioxidant activity of (cumin + oregano) extracts (at 60 °C, using distilled water+ after ultrasonic treatment)
The diagram in Figure 3 shows that the antioxidant activity of extracts prepared from (cumin + oregano) increases when using ultrasonic treatment compared to obtaining these extracts using the solvent extraction method.

4. Conclusion
This study demonstrates that it is essential to optimize the extraction method, temperature, and time to get a high yield of extraction.

Results show that the pH values of extracts increase when using ultrasonic treatment, compared with the pH values when extracts were obtained using the solvent extraction method.

Antioxidant activity of plant extracts increases with increasing extraction temperature and time, so it was revealed that the highest value of antioxidant activity was observed when obtaining extracts from rosemary using ultrasonic treatment according to the following parameters: (60 °C - 30 min), frequency - 22.5 kHz, power - 100 watts.

Rosemary, cumin and oregano extracts provided antioxidant rich material which would be helpful as natural alternatives to replace synthetic antioxidants in edible and medicinal products and can be used in functional foods.

References
[1] Justesen U, Knuthsen P 2001 Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes Food Chem 73 245–250
[2] Proestos Ch, Lytoudi K, Mavromelanidou O K, Zoumpoulakis P and Sinanoglou V J 2013 Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils 2 11–22
[3] Skowyra M, Pablos M P A 2014 Antioxidant properties of extracts from selected plant materials (Caesalpinia Spinosa, Perilla frutescens, Artemisia annua, and Viola wittrockiana) in vitro and in model food systems 2 41-60
[4] Leonova M V, Klemachki U N 2012 Extraction methods for the manufacture of medicinal products from plant materials 64 111-121
[5] Kukhareva L V 1989 Local spicy-aromatic plants, their use, and cultivation techniques 68 48
[6] Nuñez de Gonzalez M T, Hafley B S, Boleman R M, Miller R K, Rhee K S and Keeton J T 2008 Antioxidant Properties of Plum Concentrates and Powder in Precooked Roast Beef to Reduce Lipid Oxidation Meat Science 80 997–1004
[7] Altinier G, Sosa S, Aquino R P, Mencherini T, Loggia R D and Tubaro A 2007 Characterization of Topical Anti-inflammatory Compounds in Rosmarinus officinalis L. Journal of Agricultural and Food Chemistry 55 1718–1723
[8] Okamura N, Haraguchi H, Hashimotok K and Yagi A 1994 Flavonoids in Rosmarinus officinalis Leaves. Phyto chemistry 37 1463–1466
[9] Mohamed Fawzy Ramadan Hassanien, Samir A. Mahgoub, Kahled M. El-Zahar 2013 Soft cheese supplemented with black cumin oil: Impact on foodborne pathogens and quality during storage 50 36–37
[10] Keith Singletary 2010 Oregano: Overview of the Literature on Health Benefits 45 129–138
[11] Pávia Michelon Dalla Nora, Caroline Dellinghausen Borges 2017 Ultrasound pretreatment as an alternative to improve essential oils extraction 47 56–62
[12] Hesham H A Rassem, Abdurahman H Nour, Rosli M Yunus 2016 Techniques for Extraction of Essential Oils From Plants: A Review 16 117–127
[13] Brand-Williams W, Cuvelier M E, Berset C 1995 Use of free radical method to evaluate antioxidant activity 1 25–30
[14] Fung Chun Chong and Lee Suan Chua 2020 Effects of Solvent and pH on Stingless Bee Propolis in Ultrasound-Assisted Extraction 2 308–31
[15] Al-Farsi M A, Lee C Y 2008 Optimization of phenolic and dietary fiber extraction from date seeds Food Chemistry 3 977–985
[16] Wang J, Sun BG, Cao Y, Tian Y, Li XH. 2008. Optimization of ultrasound-assisted extraction of phenolic compounds from wheat bran *Food Chemistry* 2 804–810