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Comparison between different techniques for extracting rosemary extract: solvent, extraction method, particle size, plant to solvent ratio

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ABSTRACT

Rosemary is a plant from the *Lamiaceae* family with antioxidant properties, Therefore, the purpose of this study was to extract rosemary plant extract using different solvents, methods (maceration, hot extraction, Soxhlet, percolation and sonication), particle size (300, 500 and 800 µm) and plant to solvent ratios (1:100, 2:100, 3:100, 4:100 & 5:100) were. In this experimental study, the amount of phenolic and flavonoid compounds was measured by aluminum chloride colorimetric method and the antioxidant activity of plant extracts was evaluated by DPPH method. To extract these compounds, different solvents and methods were used and compared and analyzed and Data analysis was performed using SPSS software and ANOVA test. The best solvents for the extraction of phenolic, flavonoid and antioxidant compounds were water-methanol (20:80). The amount of total phenolic compounds using this solvent was 7.172 (mg/g) and flavonoid compounds were 28.157 (mg/g) and the antioxidant activity to inhibit free radicals was 87.2586 (mg/lit). The best method for extracting phenolic compounds was Maceration method with a rate of 7.481 (mg/g) and for flavonoid compounds with a rate of 47.85 (mg/g) and 73.524 (mg/lit) to inhibit free radicals.The results show that in order to achieve the maximum extraction of total phenolic compounds, flavonoids and antioxidants in rosemary extract, use plant powder with a particle size of 300 µm, water solvent: methanol (80:20), plant to solvent ratio 1:100 (g/ml) and the use of maceration method should be used as optimal operating conditions.

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1. Introduction

As consumers are concerned about the negative impact of synthetic chemicals in food, there is a need to find "products with natural ingredients". Therefore, the tendency to use natural extracts as additional options for synthetic additives increases because (a) their synergy with other preservation methods, (b) they are considered safe, and (c) their specific properties as antioxidants. It is anti-diabetic, anti-mutagenic, anti-toxic and anti-bacterial (1). In general, plants are rich in compounds with antioxidant properties such as vitamins (AND And C), glutathione, enzymes and phenolic compounds (2). Several spice extracts have shown their properties to prevent the oxidation of unsaturated triacylglycerols (3). Especially, natural extracts from the mint family (*Lamiaceae*) (thyme, sage and rosemary) have been reported in several studies for antioxidant activity (1, 4).

Phenolic compounds are classified into simple phenols, phenolic acids, hydroxycinnamic derivatives, tannins, anthocyanins, and flavonoids, which are usually found in fruits and vegetables, leaves, nuts, seeds, roots, and other plant parts. These substances have significant benefits in the fields of food, chemistry, pharmaceuticals and medicine due to a wide range of favorable biological effects including antioxidant properties (5). Phenolic compounds with antioxidant and antiradical properties can play an important role in preserving food products and maintaining human health. Flavonoids and other phenolic compounds are widely distributed in plants, and the various biological activities of these compounds, including their antioxidant, antimicrobial, and anti-inflammatory properties, have been reported in many studies (6).

Antioxidants are compounds that by absorbing free radicals and preventing the continuation of oxidation, prevent spoilage and color change and rancidity of fats. Especially antioxidants that have a phenolic ring base containing the group OH have an important role in preventing the oxidation of fats (7). The health properties of antioxidants and their role in disease prevention are the main reasons for their high use. In fact, antioxidants prevent the oxidation process, which is one of the causes of diseases such as cancer, and therefore have their effects on human health (8). Synthetic antioxidants such as butylated hydroxytoluene (BHT[1]), butylated hydroxyanisole (THERE WERE[2]), hydroxyquinone tert-butylate (TBHQ[3]) are (9). Consumption of synthetic antioxidants in laboratory animals has resulted in cancer and liver problems (7, 10). Recently, there has been a tendency to use natural substances such as flavonoids and tocopherols as non-toxic antioxidants in the food system (8, 11).

Rosemary plant with scientific name *Rosemary officinalis* and is from the mint family. Its Persian name is "mountain wreath" and it is a bushy and evergreen plant. This plant is native to the Mediterranean region and is widely cultivated in temperate climates. In Iran, it is cultivated in most areas. In recent years, there has been an increasing interest in the use of natural antioxidants such as tocopherol and flavonoids for food preservation. In the meantime, rosemary extracts have been considered as a natural antioxidant source in the food industry. Rosemary extracts are available in both water-soluble and oil-soluble products (12). In the world, rosemary extracts are added to meat as a food additive and flavoring to prevent fat spoilage (13). Antioxidant effect of rosemary plant on phenolic diterpenes such as methylcarnosate[4], Cornwall[5]Carnosic acid[6] and phenolic acids such as caffeic acid[7] and rosmarinic acid[8] It is related (10). The antioxidant activity of rosemary extracts has been evaluated using different solvents. One of the most important aspects of rosemary's antioxidant activity is the relationship between diterpenes and radical scavenging activity (3). Several extraction conditions have been reported in studies and solid-liquid extraction is the most common technique for the isolation of plant antioxidant compounds. However, conventional solid-liquid extraction methods such as hot water bath, maceration, Soxhlet extraction, and percolation, which have been used for several decades, are very time-consuming and require relatively large amounts of solvent in addition to extract performance. As a result, antioxidant activities of plant materials are less (14).

1- Butylated hydroxytoluene (BHT)
2- Butylated hydroxyanisole (THERE WERE)
3- Tert-butylate hydroxyquinone (TBHQ)
4- Methyl carnosate
5- carnosol
6- carnosic acid
7- Caffeic acid
8- Rosmarinic acid

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Del Bano et al. (2003) investigated the distribution of polyphenolic compounds in different parts of the rosemary plant. DMSO used for extraction and concluded that the concentration of phenolic compounds such as carnosic acid, carnosol and rosmarinic acid in the leaves of this plant is higher than in the stem of the plant. They also stated that the concentration of polyphenols is higher during the growth stages of the plant (15).

Wellwood and Carroll (2004) compared the antioxidant activity of rosemary extracts using three extraction solvents including petroleum ether, dichloromethane and ethanol and a mixture of dichloromethane and ethanol. In this study, ethanol was found to be the most suitable solvent (16). Yesil Seliktas et al. (2007) found the total phenolic compounds of some rosemary extract samples to be 12-20 per mg of extract, they used methanol solvent to extract the extract (17). According to the research of Jamshidi et al. (2010), the amount of total phenolic compounds of rosemary extract is 59.14 mg per gram, for which they used methanol solvent (18). Virakudi et al. (2010) investigated the antibacterial effects and phenolic compounds of rosemary extract and expressed the values as 56.66 mg/g, for which they used ethanol solvent (19). Each plant material has its own unique properties in terms of phenolic compounds. Therefore, it is important to study the optimal extraction method and provide a better understanding of the potential application of different techniques in the effective processing and use of solvents. Also, the technological parameters affecting the industrial processes used for extraction such as preliminary preparation, particle size of the extracted substance, solvent type, solvent composition, solid to solvent ratio, extraction temperature and pressure, extraction time and pH have (20) Achieving optimal operating conditions for an extraction method is essential for commercial applications of the process. Therefore, in this study, the effect of type of solvent, extraction method, size of plant particles and the ratio of plant to solvent used on the antioxidant properties and amount of phenolic and flavonoid compounds of rosemary plant have been investigated.

2- Materials and methods

2-1 Preparation of the plant

rosemary*Rosemary officinalis*) was collected manually from Kadir mountain area in the heights of Noor city and was confirmed by the experts of agricultural Jihad of the city. To increase the extraction efficiency, solid materials are pre-processed before entering the extraction system. This processing includes grinding and crushing. In this research, the rosemary plant, including leaves, was kept in a dry and shaded environment away from sunlight. After drying the leaves of the plant, the grinding step was carried out with a size smaller than 2 mm for extraction.

2-2 Chemicals

All the chemicals and solvents used in this research are from Merck, Germany and Ma'arif DPPH (Diphenylpicrylhydrazyl) was obtained from Sigma Company.

2-3 Choosing the best solvent for extracting phenolic, flavonoid and antioxidant compounds

0.2 grams of the sample passed through a 500 micron sieve was carefully weighed and poured into closed containers. Then, on each glass, 10 ml of each solvent: water (100 percent), water: methanol (50:50), water: methanol (20:80), water: ethyl acetate (50:50), chloroform: water. (50:50), ethanol: water (50:50), ethanol (100 percent), hexane (100 percent), acetone (100 percent), methanol (100 percent), acetone: water (50:50), acetone: Water (70:30) was poured. After 72 hours, the extracts were brought to 10 ml by filter paper and then each with its respective solvent. Extraction was done three times with each of the solvents and each extract was analyzed three times and the amount of phenolic, flavonoid and antioxidant compounds of each extract was measured by the mentioned methods (21).

2-4 Determining the best extraction method to extract phenolic, flavonoid and antioxidant compounds

After testing different solvents on extracts, it was found that 80% methanol solvent is the best solvent for extracting phenolic compounds. In the next step, to determine the best extraction method, maceration, hot extraction, percolation, continuous extraction using Soxhlet and sonication methods were compared with each other. Extraction was done three

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times with each of the methods and each extract was analyzed three times.

2-4-1 maceration method (cold)

1 gram of plant powder with a particle size of 300 micrometers was carefully weighed and 10 milliliters of 80% methanol was poured on it, the mixture was placed on a shaker for 3 days and after the relevant time, the extracts were filtered through filter paper and in a flask. 10 ml was made up to volume with 80% methanol solvent (22).

2-4-2 warm extraction method

1 gram of plant powder was carefully weighed and 10 milliliters of 80% methanol was poured on it, the mixture was shaken well and then placed in a bain-marie at 40 degrees Celsius for 4 hours. After 4 hours, the extracts were made up to volume with 80% methanol solvent in a 10 ml volumetric flask with filter paper.

2-4-3 Percolation method

1 gram of plant powder was carefully weighed and poured into a decanter with cotton at the end. Then 80% methanol solvent was poured on it regularly and emptied until this work continued until when 80% methanol was poured, the solution inside the decanter became colorless. Then, Arlene containing the collected solution was rotary distilled until its volume was reduced to 10 ml. Then the extracts were brought up to volume in 10 ml volumetric flasks with 80% methanol solvent (22).

2-4-4 continuous extraction method (Soxhlet)

1 gram of plant leaf powder was carefully weighed and poured into a 50 ml cartridge and placed in a Soxhlet apparatus. 50 ml was placed. 80% methanol solvent was poured into the round bottom flask and Soxhlet was carried out for 8 hours. Then the balloon containing the collected solution was distilled by rotary until its volume was reduced to 10 ml. Then the extracts were brought up to volume in 10 ml volumetric flasks with 80% methanol solvent (23).

2-4-5 Sonication method

1 gram of plant powder was carefully weighed and 10 ml of 80% methanol was poured on it. It was placed in the sonicator for 3 hours. After the relevant time, the extracts were made up to volume by filter paper and in a 10 ml volumetric flask with 80% methanol solvent (24).

2-5 Determining the best particle size of plant powder for the extraction of phenolic compounds and flavonoid and antioxidant

After choosing the best solvent and extraction method, 1 gram of plant powder that was passed through sieves with particle size of 300, 500 and 800 microns was extracted using 80% methanol and hot method. Three extracts were extracted from each and each extract was analyzed three times.

6-2 The effect of plant to solvent ratio in the extraction of total phenolic, flavonoid and antioxidant compounds

In the next step, to determine the effect of the ratio of plant to solvent on the amount of extracted phenolic, flavonoid and antioxidant compounds, weights of 1, 2, 3, 4, 5 grams were removed from the plant and after passing through a sieve with 300 micrometer holes, hot extraction with 80% methanol solvent was made and it was brought to a volume of 10 milliliters, and as a result, the ratios of 1:100, 2:100, 3:100, 4:100 and 5:100 were obtained from the plant to the solvent, and as in the previous steps, three times from each One of them was extracted and each extract was analyzed three times.

2-7 Investigation of antioxidant properties

2-7-1 Evaluation of antioxidant activity with diphenylpicrylhydrazyl test (DPPH)

The ability to inhibit free radical by the method DPPH Done. For this purpose, 1 mM solution DPPH It was prepared in methanol solvent. Then 250 microliters of it was mixed with 750 microliters of the extract and stirred vigorously. The test tubes were placed in a dark place for 20 minutes. After this period, the amount of absorbance at the wavelength of 515 nm was read with a spectrophotometer. Finally, the percentage of inhibition of radicals DPPH It was calculated by the extract with the following formula:

$100 \times A_{blank} (A_{blank} - A_{sample})$ = Percentage of free radical inhibition

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where in A_{blank} AndA_{sample} The absorption in the control sample and the absorption in the extract sample are respectively (22).

2-7-2 Measurement of total flavonoid content of extracts

The amount of total flavonoids was measured by aluminum chloride colorimetric method. In this method, 0.5 ml of extract solution with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate، 2.8 ml of distilled water was mixed. After keeping the samples at room temperature for 30 minutes, the absorbance of the mixture was read at a wavelength of 415 nm. Quercetin was used to draw the standard curve (25).

2-7-3 Method of measuring the amount of phenolic compounds

400 microliters of the extract was poured into a test tube with a lid and after adding 3 milliliters of Folin Ciocaltio reagent (diluted with water at a ratio of 10:1), it was placed in a bain-marie at a temperature of 22 degrees Celsius for 5 minutes. Then 3 milliliters of 6% sodium bicarbonate solution was added to it and it was again placed in a bain-marie with a temperature of 22 degrees Celsius for 90 minutes. After 90 minutes, the absorbance of the sample was measured at a wavelength of 765 nm against a water blank. It should be mentioned that the blank was prepared like the sample, with the difference that instead of the extract, 400 microliters of distilled water was poured into the test tube. This method was also performed on each of the standard solutions of chlorogenic acid with concentrations of 0.03, 0.05, 0.1, 0.3, 0.5 mg/ml and the calibration curve of the concentration was drawn against absorption (26):

$(9999/0R^2 =$ ‹0362/0x + 0619/3y =)

2-8 Statistical analysis

In this research, a completely random design was used to perform all the tests with at least 3 repetitions, and the data was analyzed using one-way analysis of variance. (ANOVA)And with the help of software SPSS (Version 16) was done, and if the difference between the average data was significant, Duncan's test was used at the 95% confidence level. Software for drawing diagrams Excel (2013) Was used.

3- Results and Discussion

1-3% of phenolic, flavonoid and antioxidant compounds in rosemary plant using different solvents

The results of using different solvents to extract phenolic, flavonoid and antioxidant compounds in rosemary plant are reported in table (1) and also shown as a diagram in figure (1). From 13 solvents: methanol (100%), ethanol (100%), acetone (100%), hexane (100%), water:methanol (50:50), water:methanol (80:20), water:ethanol (50:50), water:ethanol (80:20), water:acetone (50:50), water:acetone (70:30), water:ethyl acetate (50:50), chloroform:water (50:50) and pure water were used to extract phenolic, flavonoid and antioxidant compounds of rosemary extract. The highest and lowest total phenolic content in water:methanol (80:20) extract, respectively mg/g 7/172 and chloroform: water in proportion mg/g 0.72 was observed. Also, water:methanol hydroalcoholic extract (80:20) had the highest and chloroform:water hydroalcoholic extract (50:50) had the lowest flavonoid content and free radical inhibition percentage. According to the obtained results, it was observed that there is a significant difference ($0.05 < p$) in the amount of total phenol compounds, flavonoids, and antioxidants were found between all the used solvents.

Table 1. Results of determining the best solvent for extracting phenolic compounds, flavonoids and inhibiting the free radicals of Rosemary extract*

Solvent	Total phenolic (Mg/g of extract)	Flavonoid (Mg/g of extract)	DPPH (Mg/lit of extract)
Water	1.343± 0.001^{i**}	13.426± 0.005^{i}	13.425± 0.005^{l}
Methanol (100%)	2.843± 0.026^{g}	16.236± 0.003^{g}	42.684± 0.004^{g}
Ethanol (100%)	2.434± 0.003^{h}	15.853± 0.003^{h}	38.575± 0.004^{h}

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Acetone (100%)	0.81± 0.033[l]	13.125± 0.003[k]	23.534± 0.008[i]
Hexane (100%)	0.915± 0.002[k]	13.284± 0.003[j]	15.287± 0.004[j]
Water: Methanol (50:50)	6.26± 0.025[b]	24.360± 0.004[c]	70.237± 0.003[b]
Water: Methanol (20:80)	7.173± 0.001[a]	28.155± 0.004[a]	87.236± 0.003[a]
Water: Ethanol (50:50)	5.15± 0.026[d]	21.114± 0.003[d]	66.285± 0.002[c]
Water: Ethanol (20:80)	5.367± 0.003[c]	25.141± 0.003[b]	59.241± 0.006[d]
Water: Acetone (50:50)	4.525± 0.003[f]	18.355± 0.004[f]	44.440± 0.004[f]
Water: Acetone (30:70)	4.732± 0.003[It is]	19.575± 0.002[It is]	54.353± 0.003[It is]
Water: Ethyl Acetate (50:50)	1.133± 0.003[j]	11.856± 0.003[l]	13.856± 0.003[k]
Water: Chloroform (50:50)	0.722± 0.003[m]	9.254± 0.004[m]	11.565± 0.006[m]

* Data are reported in two replications in terms of (Mean ± SD value).

** The similar small letters (a-m) in each column indicate a significant no difference ($p<0.05$) based on the Duncan test between the data.

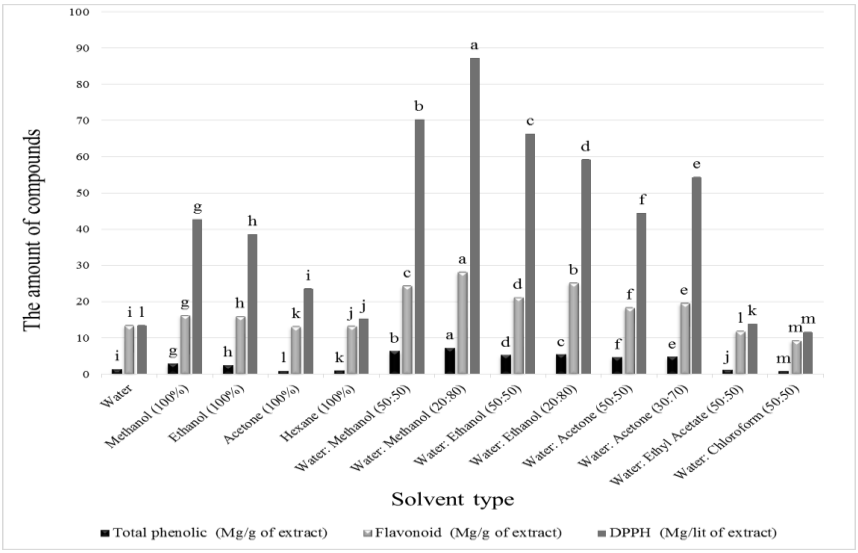

Figure 1. Results of determining the best solvent for extracting phenolic compounds, flavonoids and inhibiting the free radicals of Rosemary extract

Solvent extraction is the most common technique for the isolation of plant antioxidant compounds. However, the extract yield, polyphenolic content and antioxidant activities obtained from plant materials are highly dependent on the nature of the solvent and the extraction method due to the presence of different antioxidant compounds with diverse chemical properties and different polarities that may be soluble or insoluble in a pure solvent. has (27, 28). Polar solvents are often used to extract polyphenols from a plant matrix. The most suitable solvents (hot or cold) are aqueous mixtures containing ethanol, methanol, acetone and ethyl acetate (27). Sultana et al. (2009) reported that aqueous, ethanolic and methanolic extracts of the bark of bitter olive trees (*Azadirachta indica*), acacia tree (*Acacia nilotica*), jumbo tree (*Eugenia jambolana*), Hallila plant (*Terminalia arjuna*), leaves and roots of Moringa oleifera (*Moringa oleifera*), Fig fruit (*Religious fig tree*) and aloe vera leaves (*Aloe barbadensis*) showed better antioxidant activities and phenolic content compared to pure methanol and ethanol (27), and their results are consistent with the results of the present study. In this regard, 50% aqueous

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solvent extracts of black tea showed a significant amount of total polyphenols and antioxidant activity in 2, 8 and 18 hours compared to the pure type of solvent (29). Comparing the flavonoid content with the phenolic content, methanol aqueous solvent also extracted the highest flavonoid from rosemary plant extract. Similar to this result, a study was conducted by Anukovara et al. (2011) which showed that the flavonoid content of Acalyfa plant (*A. Wilkesiana*) compared to the phenolic content, the methanol solvent extracted the highest flavonoid, also in their study it was shown that the antioxidant activities strongly depend on the solvent used in the extract extraction. (29). The present study shows that the amount of flavonoids, phenols and the amount of antioxidant activity depends on the type of solvent used for extraction, and the best type of solvent used in this study was methanol aqueous solvent with a ratio of (20:80) for all three factors.

The results of this article, with the method DPPHIt showed that the methanolic extract is 42.685 mg/ml and the water:methanol solvent (80:20) has the highest antioxidant content, and the lowest amount of antioxidant compounds was related to the chloroform:water (50:50) solvent extract. Flavonoids are polyphenolic compounds that are mainly found in plants and appear as strong antioxidant and anti-radical compounds. Research has shown that increasing the level of flavonoids in the human diet can lead to the reduction of some diseases in humans (30). Stakovic et al. (2011) aqueous and methanolic extracts of different parts of mountain syzab plant (*Tecurium Montanum*) in terms of phenolic and flavonoid content and in this study they concluded that the phenolic and flavonoid content of the aqueous extract is higher than the methanolic extract (31). The amount of antioxidant activity of different phenolic compounds is different according to their chemical structure. In addition, during the extraction process, other compounds that have high solubility in alcoholic solutions enter the extract along with phenolic compounds. In the field of extraction of phenolic compounds, the ethanol extract of rosemary showed effective effects on stopping the proliferation of cancer cells in breast cancer and leukemia, as well as anti-inflammatory activity (25). The reason for the low content of phenolic substances in water extracts can also be the increase in the activity of enzymes (polyphenol oxidase), which causes the destruction of phenolic substances, while these enzymes are inactive in the alcoholic environment. The use of pure ethanol as a solvent reduces the extraction efficiency of polyphenols, due to a number of hydroxyl groups (such as flavonoids, especially those containing sugar in the molecule), which are hydrophilic and generally more soluble in water-ethanol solutions than in alcoholic solutions. are pure (32).

2-3% of phenolic compounds, flavonoids and inhibition of free radicals in rosemary plant with different extraction methods

Phenolic, flavonoid compounds and inhibition of free radicals in rosemary plant with different extraction methods, incl Maceration methods، Hot extraction method, percolation method, continuous method (Soxhlet) and sonication were performed. The results obtained from the extraction of phenolic compounds, flavonoids and inhibition of free radicals in Rosemary plant using different extraction methods presented in table (2) shows that the maceration and hot extraction methods are superior to other methods, so that the maceration method showed a significant difference in all three measured factors with other extraction methods (05 /0< p). But in order to extract total phenolic compounds, using the temperature of the maceration method increases the extraction (Chart No. 2).

Table 2. Results of determining the best methods for extracting phenolic compounds, flavonoids and inhibiting the free radicals of Rosemary extract*

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Methods	Total phenolic (Mg/g of extract)	Flavonoid (Mg/g of extract)	DPPH (Mg/lit of extract)
Maceration	7.486± 0.006[a**]	47.852± 0.003[a]	73.524± 0.003[a]
Hot extraction	6.151± 0.003[b]	32.753± 0.006[b]	66.828± 0.004[b]
Percolation	3.33± 0.11[d]	28.57± 0.043[c]	44.160± 0.004[It is]
Soxhlet extraction	3.176± 0.003[d]	22.246± 0.024[d]	47.114± 0.003[d]
Sonication	5.525± 0.002[c]	17.623± 0.033[It is]	50.748± 0.004[c]

* Data are reported in two replications in terms of (Mean ± SD value).

** The similar small letters (a-e) in each column indicate a significant no difference ($p<0.05$) based on the Duncan test between the data.

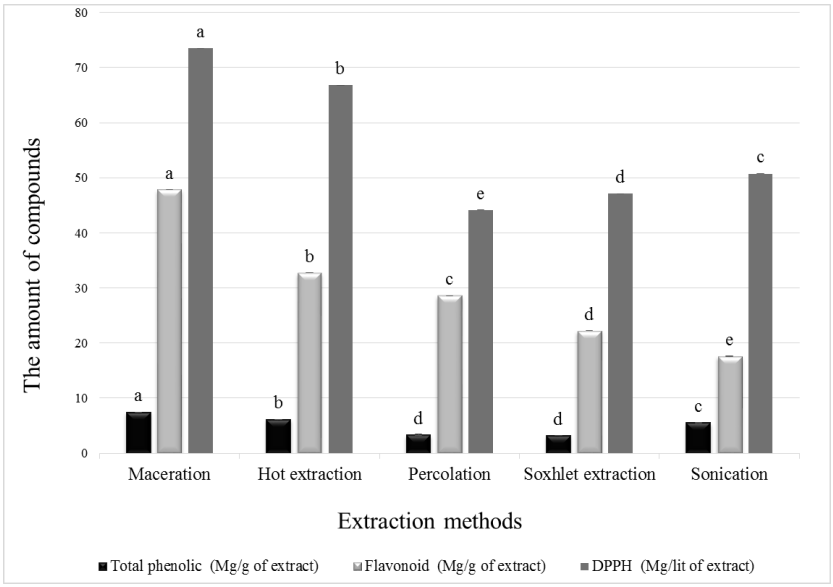

Figure 2. Results of determining the best methods for extracting phenolic compounds, flavonoids and inhibiting the free radicals of Rosemary extract

The process of extracting phenolic compounds is a main factor in the antioxidant properties of the extract, but temperature, solvent extraction power, time and method adopted for extraction significantly affect the composition of the extract. Plants contain many compounds that have different structures. The extraction of these compounds depends on several factors, the most important of which are the solvent and the extraction method. The choice of solvent and extraction method depends on the different parts of a plant and its ingredients. The key role of phenolic compounds as radical scavengers has been reported in several papers (33). The method of using the Soxhlet device showed the least efficiency in extraction, and this shows that in the Soxhlet method, high temperature leads to the destruction of some compounds. The comparison of the results of this research with other research shows that although for the extraction of chicuric acid[9] and caffeic acid, using a stirrer and sonication method at room temperature is suitable (17). In a study, nine extraction methods were used for total saponins, and the best method reported in that study was the percolation method (34), which is not consistent with the present study.

9 -Cichoric acid

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3-3% of phenolic, flavonoid and antioxidant compounds in rosemary plant extract with different particle sizes

The average results of determining the best particle size of rosemary plant powder for extracting phenolic, flavonoid and antioxidant compounds in rosemary plant extract are reported in table (3) and the results are also shown as a graph in figure (3). The results of the test of different particle sizes in the extraction of compounds show that with the reduction of the particle size, the extraction efficiency increases, so that the use of plant powder with a particle size of 300 micrometers had the best efficiency in the extraction of phenolic compounds, flavonoids and inhibition of free radicals in rosemary plant. >0.05 p), which can be caused by more breaking of the wall The plant cells were more finely divided and of course, the solvent penetrated more into the plant.

Table 3. Results of determining the best particle size for extracting phenolic compounds, flavonoids and inhibiting the free radicals of Rosemary extract*

Particle size (Micrometer)	Total phenolic (Mg/g of extract)	Flavonoid (Mg/g of extract)	DPPH (Mg/lit of extract)
300	7.217± 0.006^{a**}	4.894± 0.006^{a}	8.596± 0.006^{a}
500	5.288± 0.006^{b}	3.250± 0.006^{b}	7.123± 0.010^{b}
800	4.462± 0.006^{c}	1.478± 0.004^{c}	5.016± 0.032^{c}

* Data are reported in two replications in terms of (Mean ± SD value).

** The similar small letters (a-c) in each column indicate a significant no difference ($p<0.05$) based on the Duncan test between the data.

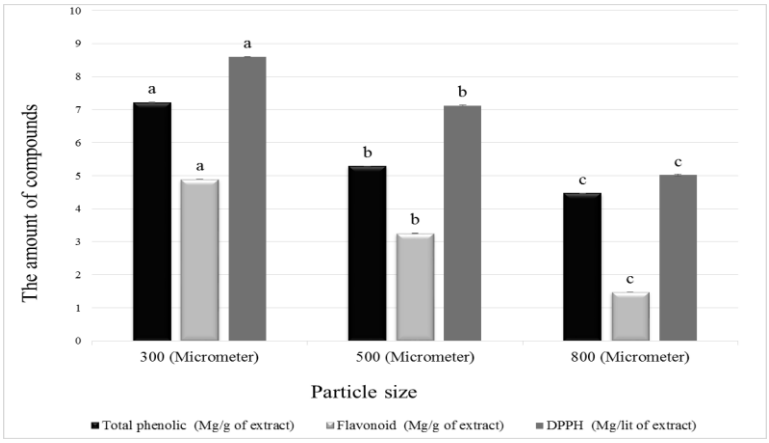

Figure 3. Results of determining the best particle size for extracting phenolic compounds, flavonoids and inhibiting the free radicals of Rosemary extract

Particle size is probably the most important factor related to the raw material that affects the extraction of phenolic compounds. Reducing the size of the particles causes an increase in the surface of plant material in contact with the solvent and as a result the speed of mass transfer (35). Therefore, the reduction of particle size has also been shown as a positive factor for improving the extraction of polyphenol compounds (36). In the study of Pinello et al. (2007) they investigated the size of coffee particles (125, 250, 500 and 1000 micrometers) to check the extraction rate of polyphenol compounds, and their results showed a direct relationship between the reduction of particles and the increase of compound extraction (37), which results They are consistent with the present study.

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Therefore, it can be said that one of The parameters influencing the industrial processes used for extraction are the particle size of the material. Probably, when the particle size decreases, the available surface area for the extraction solvent and enzyme attack increases, and as a result, an increase in the extraction efficiency is observed (37). The positive effect of particle size reduction was previously reported in other studies on phenolic extraction, these results have also been very positive in other plant materials such as black grape pomace and black cohosh (36, 38). However, the positive effects of particle size reduction are not always obvious, because particle size reduction can block solvent access to all solid surface areas and release phenolic compounds (39). In addition, grinding, which is usually used to obtain finer particles, may cause rupture of plant cells and, as a result, release some phenolic compounds located inside the cells (35). Grinding rosemary to a particle size in the range of 0.2-0.8 mm, depending on the solvent and extracted compound, increased the yield of total phenol, rosmarinic acid and carnosic acid by 2 to 10 times (14). According to the results and reports, the particle size distribution was found to have a significant effect on the extraction of phenolic compounds, and the extraction rate of these compounds and antioxidant properties increased when smaller particle sizes were used.

3-4% of phenolic, flavonoid and antioxidant compounds in rosemary plant extract with different ratios of plant: solvent

The results obtained from this experiment in Table (4) show that the best ratio of plant to solvent for extracting phenolic, flavonoid and antioxidant compounds in rosemary plant extract, which is 1, 2, 3, 4, 5 grams of this plant's powder is equal to 10 Received milliliters ie Ratios of 1:100, 2:100, 3:100, 4:100 and 5:100 were obtained from plant to solvent, the ratio of 1:100 according to the results seen in table (4) and figure (4) caused optimal extraction of these compounds. and with other ratios of solid to solvent, there was a significant difference in all three factors (05/0> p). As seen in the results, with the increase in mass to solvent ratio, the amount of extraction decreased significantly (<0.05). p). Of course, it should be noted that these results were obtained by keeping the optimal conditions determined in the previous stages of this research with the maximum amount of extraction, i.e. solvent type (80% methanol), extraction method (maceration) and particle size (300 micrometers).

Table 4. Results of determining the best plant to solvent ratio for extracting phenolic compounds, flavonoids and inhibiting the free radicals of Rosemary extract*

Plant to solvent ratio Methanol (80%)	Total phenolic (Mg/g of extract)	Flavonoid (Mg/g of extract)	DPPH (Mg/lit of extract)
1:100 (g/ml)	7.358± 0.006[a**]	5.988± 0.007[a]	9.115± 0.007[a]
2:100 (g/ml)	6.183± 0.006[b]	5.680± 0.009[b]	7.907± 0.012[b]
3:100 (g/ml)	5.478± 0.049[c]	3.668± 0.008[c]	7.648± 0.009[c]
4:100 (g/ml)	5.145± 0.005[d]	3.660± 0.01[c]	5.269± 0.008[d]
5:100 (g/ml)	3.689± 0.006[It is]	2.142± 0.011[d]	2.845± 0.012[It is]

* Data are reported in two replications in terms of (Mean ± SD value).

** The similar small letters (a-e) in each column indicate a significant no difference (p<0.05) based on the Duncan test between the data.

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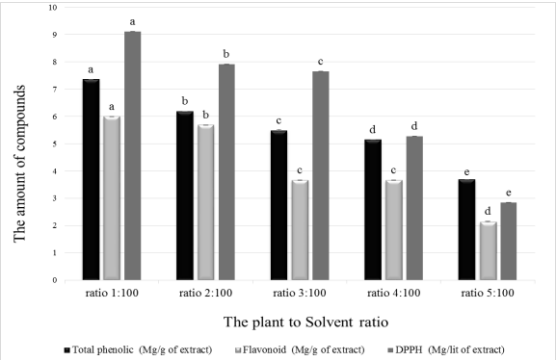

Figure 4. Results of determining the best plant to solvent ratio for extracting phenolic compounds, flavonoids and inhibiting the free radicals of Rosemary extract

Extraction is a very important step in the isolation and identification of phenolic compounds. As a result, many researchers have studied the effect of different extraction conditions on the extraction performance of phenolic compounds from natural sources (32). With increasing solid-solvent ratio, the amount of total phenols, flavonoids and antioxidant capacity decreased significantly (>0.05). p). The solid/liquid ratio is an important operating parameter in terms of process profitability, which greatly affects the operating costs of extraction plants. Increasing the amount of solvent creates favorable interactions that occur between biomass and solvent due to the increase in contact between the two substances, thus increasing the extraction until reaching equilibrium, in addition, at this stage, solvent saturation may also occur and limit the amount of more extractable solutes (40). In addition, a high solid/liquid ratio (lower solid content) increases the concentration gradient, and this is directly proportional to the diffusion rate, and as stated in Fick's law, this increases the solvent extraction ability (41). In this regard, the study of Ozturk et al. (2018) showed that increasing the solid/liquid ratio of (mg/ml(1:5 to 1:10 amount of total polyphenol compounds)TPC) increased the extracts for most solvents, they also stated that although the ratio of (mg/ml) 1:20 generally leads to TPC higher for all components studied, less concentrated polyphenols are obtained and the use of higher amounts of solvent may not be economically viable; Hence the ratio (mg/ml) chose 1:10 as the optimal ratio to increase the extraction efficiency of polyphenols as extraction solvents (40). A higher ratio of solvent to solid accelerates the mass transfer phenomena due to the greater concentration difference between the solid matrix and the upper phase of the solvent. As a result, extraction is done faster, and the concentration of phenolic compounds in the extract is higher (35). Yang and Zhang (2008) concluded that the optimal ratio for the recovery of quercetin and rutin from a Chinese medicinal plant (ml/g) is 40:1, while a higher ratio did not provide higher yields (42). In general, researchers try to use a low solvent-to-solid ratio to minimize process cost. Ratio (ml/g) 20:1 was proved to extract antioxidant phenolic compounds from oregano (43) and sage (44).

4 - Conclusion

rosemary*Rosemary officinalis*) is a potential source rich in polyphenols. Parameters affecting the case processes

Used for extracting phenolic compounds such as solvent type, solvent composition, solid to solvent ratio, particle size of the extracted material. Therefore, it is necessary to estimate the optimal operating conditions for extraction. In this study, in order to achieve the maximum extraction of total phenolic, flavonoid and antioxidant compounds in rosemary plant extract, plant powder with a particle size of 300 micrometers, water solvent: methanol

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(80:20), plant to solvent ratio (g/ml) 100: 1 and using the maceration method should be used as the optimal operating conditions.

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Scientific Research

Comparison between different techniques for extracting rosemary extract: solvent, extraction method, particle size, plant to solvent ratio

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ABSTRACT

Rosemary is a plant from the *Lamiaceae* family with antioxidant properties, Therefore, the purpose of this study was to extract rosemary plant extract using different solvents, methods (maceration, hot extraction, Soxhlet, percolation and sonication), particle size (300, 500 and 800 µm) and plant to solvent ratios (1:100, 2:100, 3:100, 4:100 & 5:100) were. In this experimental study, the amount of phenolic and flavonoid compounds was measured by aluminum chloride colorimetric method and the antioxidant activity of plant extracts was evaluated by DPPH method. To extract these compounds, different solvents and methods were used and compared and analyzed and Data analysis was performed using SPSS software and ANOVA test. The best solvents for the extraction of phenolic, flavonoid and antioxidant compounds were water-methanol (20:80). The amount of total phenolic compounds using this solvent was 7.172 (mg/g) and flavonoid compounds were 28.157 (mg/g) and the antioxidant activity to inhibit free radicals was 87.2586 (mg/lit). The best method for extracting phenolic compounds was Maceration method with a rate of 7.481 (mg/g) and for flavonoid compounds with a rate of 47.85 (mg/g) and 73.524 (mg/lit) to inhibit free radicals.The results show that in order to achieve the maximum extraction of total phenolic compounds, flavonoids and antioxidants in rosemary extract, use plant powder with a particle size of 300 µm, water solvent: methanol (80:20), plant to solvent ratio 1:100 (g/ml) and the use of maceration method should be used as optimal operating conditions.

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