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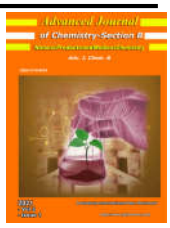

Original Research Article

Determination of Total Phenolic Compounds and Antioxidant Capacity of *Rosmarinus officinalis* L. via Microwave-Assisted Extraction

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ABSTRACT

Due to the toxicity of synthetic antioxidants, their application has been limited or even banned in certain countries. The extraction of phenolic compounds and flavonoids from plant matrices is carried out utilizing a variety of solvents. The aim of this study is to determine the antioxidant activity and total phenolic and flavonoid composition of *Rosmarinus officinalis* L., often referred to as rosemary. The study also examines the potential application of rosemary as a natural antioxidant in the food industry. The extraction technique in this study included maceration and microwave-assisted extraction. Maceration was chosen as the traditional extraction technique, while microwave-assisted extraction was used to reduce the extraction time and solvent volume. In both the traditional and microwave-assisted extraction methods, methanol was employed as a solvent. The total phenolic compounds, total flavonoids, antioxidant activity, metal chelating ability, and beta-carotene and lycopene levels of the samples were determined. TPC yielded 40 and 43 mg/g, TFC yielded 12.4 and 20 mg/g, FRAP yielded 37 and 49 mg/g, and MCC yielded 133 and 134 mg/g, respectively, for conventional and microwave-assisted extraction methods. In comparison to the conventional technique, the microwave-assisted extraction method resulted in greater quantities of bioactive compounds. Additionally, rosemary's beta-carotene and lycopene contents were determined to be 8652 and 7849 mg/g dried plant, respectively. Microwave-assisted extraction was found to be more successful, quicker, and less solvent-intensive than the conventional method. Additionally, rosemary is suggested in the food sector as a natural antioxidant instead of a synthetic antioxidant to prevent health-damaging consequences.

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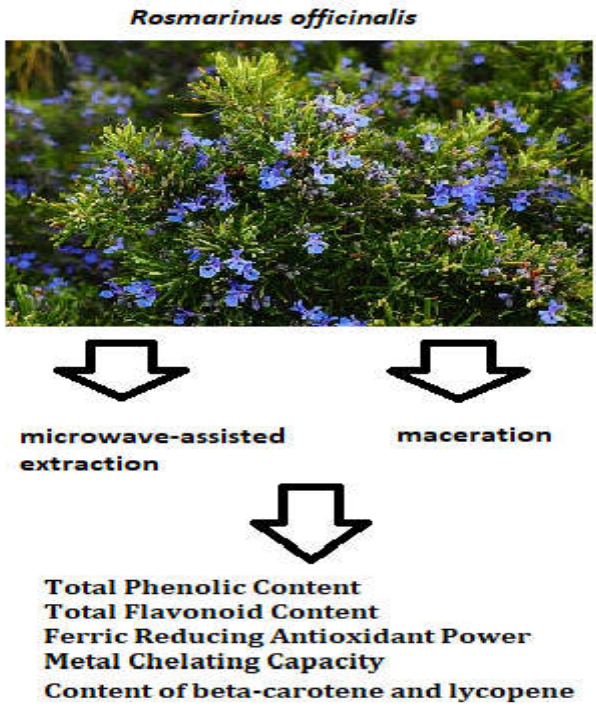
GRAPHICAL ABSTRACT



Introduction

Accumulating evidence suggests a strong connection between the intake of medicinal herbs in the management and cure of different health issues, including cancer, cardiovascular and neurological disorders. Phenolic substances present in therapeutic plants and foods have long been known to significantly decrease the detrimental effects of chemically reactive species on human physiological systems [1, 2]. Protecting against oxidative stress-related diseases such as cancer, atherosclerosis, cardiovascular, and neurological problems are among the phytochemical activities of phenolic compounds. Polyphenolic substances act as scavengers of free radicals by regulating the endogenous antioxidant defense mechanism [3]. Antioxidants may be found in natural or synthetic forms. Natural antioxidants include phenolic compounds like tocopherols, flavonoids, and phenolic acids, as well as nitrogen compounds such alkaloids, chlorophyll derivatives, amino acids, and amines [4]. Unfortunately, due to the relatively cheap cost and bland taste of synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, and tert-butylhydroquinone, they have found widespread use in the food and beverage sector. Foods are treated with synthetic preservatives to protect them from contamination during manufacturing, packing, shipping, and storage. However, it has been observed that long-term excessive consumption of the chemical residues of such synthetic preservatives might cause toxicity, if not carcinogenicity [5]. Thus, in certain nations, such as China, Japan, Canada, and Europe, the use of synthetic antioxidants has been restricted. Butylated hydroxyanisole was recently removed from the list of chemicals “generally regarded as safe” (GRAS). Natural agents are regarded as safe substitutes for synthetic ones due to their numerous positive qualities. However, the application of natural antioxidants in the food

industry is still extremely limited due to their relatively high costs. There are increasing reports on "green" preservation agents known as food-grade antioxidants that are derived from natural sources. Numerous studies have recommended that antioxidants derived from rosemary, such as rosmarinic acid, should be substituted with synthetic antioxidants. Recently, rosemary antioxidants were proven to be more effective than tert-butylhydroquinone at stabilizing oils at high temperatures during the frying process of potatoes [6]. Additionally, recent research on this subject proposes the use of antioxidants derived from rosemary as food additives to improve stability in the presence of oxidative stress [7] [8] [9]. In addition to their antioxidant benefits, research has revealed that phenolic compounds have substantial antibacterial activity [10]. Therefore, natural antioxidants may prove to be a viable substitute for synthetic antioxidants in the food business [11]. Carotenoids are also secondary plant metabolites that are often seen in conjunction with phenolic substances. Carotenoids' absorption has been found to be influenced by phenolic compounds [12]. Carotenoids have been linked to a decreased risk of developing a number of chronic illnesses, including age-related macular degeneration and some malignancies [13]. The primary components of rosemary, rosmarinic acid, carnosol, and carnosic acid, have been implicated in the antioxidant, anti-inflammatory, and anticarcinogenic effects. Additionally, many rosemary constituents have been shown to have anti-cancer properties. Rosmarinic acid is a phenolic molecule with many uses ranging from food preservation to cosmetics. It is also a pharmaceutically active molecule [14]. On the other hand, rosemary's bioactive components may work synergistically with a number of other health-promoting properties. Numerous plant extraction methods may be classified into two broad categories: conventional and innovative (modern). Maceration is an example of a conventional or classical extraction method. On the other hand, new or so-called modern extraction techniques include microwave-assisted extraction (MAE). The advantage of maceration is that the unlimited quantity of sample can be extracted which in turn produces significantly higher amount of extract. However, limitations of conventional methods include lengthy extraction times and excessive solvent use. Thus, assessing plant phenolic components and antioxidant activity requires a reliable, simple, quick, and environmentally acceptable approach [15]. This study examined rosemary's total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), metal chelation capacity (MCC), and beta-carotene and lycopene levels. These characteristics were proposed to be associated with the ability of the biomolecules of rosemary to protect food from oxidative degeneration and its contribution to health benefits. Traditional and MAE methods were used in their measurements, with the goal of developing a more efficient procedure that needs less time and solvent.

Materials and Methods

Chemicals and materials

Merck (Germany) supplied aluminum chloride (≥98.0 %), sodium acetate (≥99.0 %), potassium ferricyanide (≥99.0 %), iron chloride (III) (≥98.0 %), 1,10-phenanthroline monohydrate (≥99.5 %), potassium hydrogen phosphate (≥99.99 %), sodium hydroxide (≥99.0 %), sodium carbonate (≥99.9 %), hydrogen phosphate (≥98.0 %), trichloroacetic acid (≥98.0 %), iron sulfate monohydrate (86.0-89.0 %), and Folin-phenol Ciocalteu's reagent (2N). Sigma-Aldrich (Germany), IsoLab (Eschau, Germany), and Carlo Erba (Sabadell, Spain) supplied the quercetin (>95 %), gallic acid (≥99.0 %), and *n*-hexane (99.0 %), respectively. To evaporate solvent for sample preparation, a rotary evaporator (Buchi,

Rotavapor R-100) was utilized in combination with a heating bath (B-100) and vacuum bath (V-100); the extraction was done in a microwave oven (Mars Express). A UV-VIS spectrophotometer was used to perform UV spectroscopy measurements (Isolab, Germany).

Plant material

In 2020, samples of *Rosmarinus officinalis* were harvested in the Gaziantep area. Prof. Dr. Semsettin Civelek identified the plant species. The leaves of the plant specimens were removed and air-dried at room temperature in a shaded area, then pulverized in a high-speed home blender.

Plant Extraction

Conventional extraction (Maceration)

10 g of plant material was ground into powders by a blender and put in a round-bottom flask with 75 mL methanol. A magnetic stirrer was used to constantly stir the flask at room temperature for 24 hours. Following that, the samples were filtered via Whatman filter paper (102 Medium, 125 mm, S-H Labware). This operation was carried out three times. Subsequently, all extracts were mixed and evaporated at 40 ^{o}C under a pressure of 150 mbar using a rotary evaporator. Hexane was used to defeat the extract after evaporation. The extract was then air dried before being fully dried in a vacuum oven (Nuve 180). 0.1 g of the extract was dissolved in 8 mL methanol and used as the starting material for the biochemical tests.

Microwave-assisted extraction

Closed-system MAE was applied in this study. MAE was carried out utilizing a microwave device with a closed vessel. The dried sample (0.1 g) was put in a 70 mL polytetrafluoroethylene tank and extracted with 8 mL of solvent under various conditions (70 % methanol in water). The system's temperature was set at 60 ^{o}C, 70 ^{o}C, and 85 ^{o}C. Three different microwave powers were utilized (200, 300, and 400 W). The ramp time was set at ten minutes. The hold time was eight, fourteen, and twenty minutes. After extraction, the vessels were allowed to cool and then centrifuged for 10 minutes at 3000 rpm. Biochemical analyses were performed on the supernatant. Figure 1 illustrates the processes associated with biochemical tests.

Determination of Total Phenolic Content

Folin–Ciocalteu test is a well-established technique for determining TPC. Initially developed for the study of proteins, this technique was subsequently used for the determination of phenolic components in foods and plant extracts. This test is based on the reduction of the Folin–Ciocalteu reagent in alkaline circumstances by phenolic substances [16]. The total phenolic content (TPC) of the extracts was measured using the Folin-Ciocalteu reagent [17]. Fig 1a illustrates the method for performing the total phenolic content test. The absorbances were determined using a UV-Vis spectrophotometer at 760 nm. The findings are presented as gallic acid equivalents on a dry plant basis (mg GAE/g dw), using a calibration curve produced with a genuine reference chemical (5, 10, 20, 50, 100, and 200 ppm).

Determination of Total Flavonoid Content

The total flavonoid content was measured using a colorimetric aluminum chloride technique. Fig 1b illustrates the method used. The absorbances were determined at 420 nm. The findings are presented as quercetin equivalents on a dry plant basis (mg QE/g dw), using a calibration curve produced with a genuine reference compound (5, 10, 20, 50, 100, and 200 ppm).

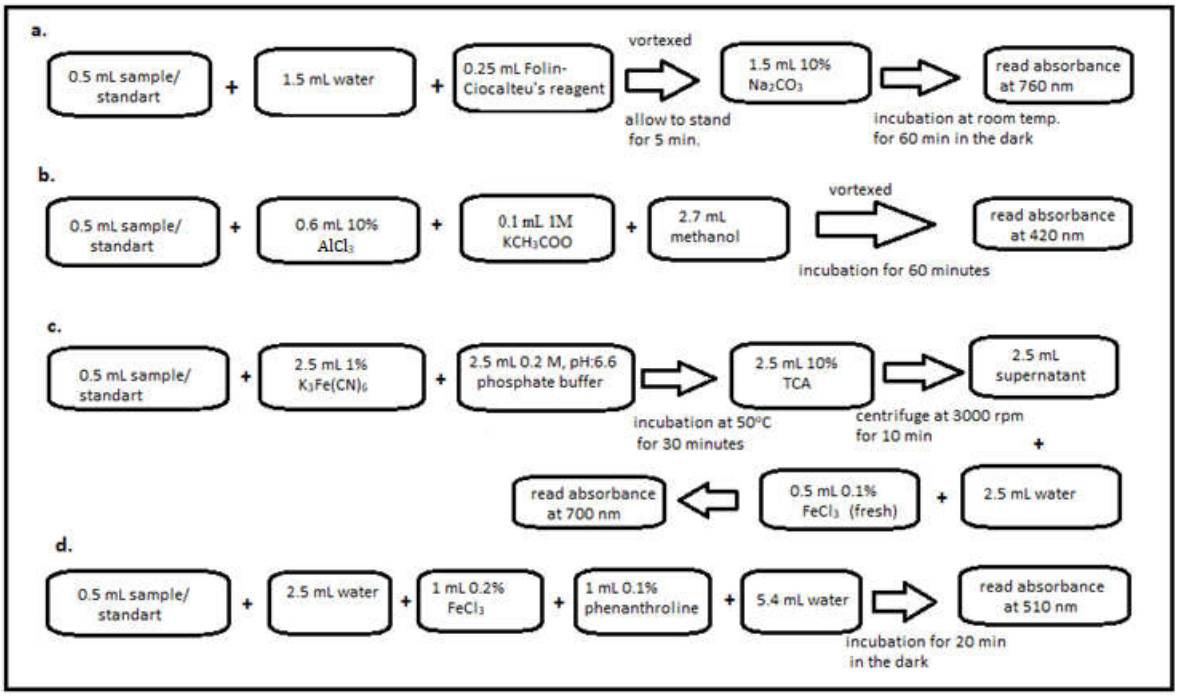


Fig. 1. The experimental processes of a. total phenolic content, b. total flavonoid content, c. ferric reducing antioxidant power, d. metal-chelating assays used in this study.

Ferric Reducing Antioxidant Power Assay

On the basis of their antioxidant properties, the reducing power of methanolic rosemary extracts was evaluated by their ability to form colorful complexes with potassium ferricyanide. Prussian blue is the final result, which is measured spectrophotometrically and shows the antioxidants' reducing potency. Prussian blue may be produced in two distinct ways with the same result. Antioxidants may either reduce the ferricyanide in the solution to ferrocyanide, which then binds the free Fe3+ in the solution to create Prussian blue, or they may reduce the iron(III) to iron(II), which then binds the ferricyanide in the solution to create Prussian blue [18]. Fig 1c illustrates the experimental method. The findings are given in terms of quercetin equivalents per dry weight of the plant (mg QE/g dw). The calibration curve was constructed using quercetin concentrations ranging from 5-200 ppm. The absorbances were determined at a wavelength of 700 nm.

Metal Chelating Capacity (Phenanthroline Method)

The phenanthroline assay was used to determine the reducing capacity of plant extracts according to the method of Szydlowska-Czerniaka [19]. Herein, the samples were first mixed with ferric chloride. The antioxidant molecules in the plant extracts reduce the Fe3+ to Fe2+. Then a 1,10-phenanthroline solution was added. The reduced Fe2+ forms a complex that has an orange-red color. Fe3+ does not form this complex with 1,10-phenanthroline solution [20]. The absorbance of the orange-red solution was measured at 510 nm against a blank control [21]. Fig. 1d illustrates the method used. The calibration curve was constructed using values ranging from 50 to 800 ppm. FeSO4, as the standard, forms a complex with 1,10-phenanthroline and is measured at the same wave length. The calibration curve, therefore, was generated. The findings are presented as FeSO4 equivalents (mg FeSO4/gdw) on a dry plant basis.

Content of beta-carotene and lycopene

The dried extract was agitated for 1 minute with 10 mL of acetone-hexane solution (4:6) and filtered with whatman no:4 filter paper. The absorbances were determined at 453 nm, 505 nm, and 663 nm. The following equations were used to determine the quantities of beta-carotene and lycopene: Beta carotene is 0.216 A663 – 0.304 A505 + 0.452 A453, while lycopene is -0.0458 A663 + 0.372 A505 – 0.0806 A453. Beta carotene and lycopene concentrations are shown as mg/100 mg.

Statistical Analyzes

Each test was performed in triplicate. The mean and standard deviation of each test were computed. To compare these results statistically, the one-way ANOVA and Tukey's post hoc test for multiple comparisons were employed. A difference that was statistically significant was determined as $p<0.05$.

Results

Total phenolic content (TPC): The amount of total phenolics in rosemary extract generated using MAE (43 mg/g) was found to be somewhat more than that obtained using the traditional technique (40 mg/g) (Fig 2). On the other hand, the MAE-acquired phenolic compounds were significantly ($p<0.05$) poorer (24 mg/g) at 85 °C for 20 minutes than the TPC value recorded at 60 °C after 20 minutes, suggesting that the total phenolic components are annihilated at high temperatures. On the contrary, this phenomenon was not seen at the same temperature for 8 or 14 minutes, indicating that the rate of phenolic degradation is also dependent on the duration of the process.

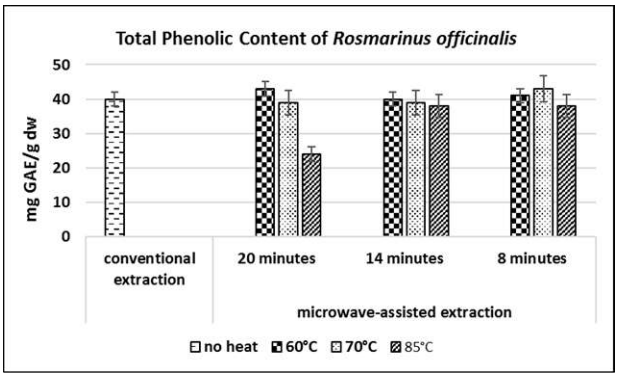


Fig. 2. Total *Rosmarinus officinalis* phenolic content. All results are shown in milligrams of GAE per gram of dry plant material.

Total Flavonoid Content: Flavonoids are a class of chemicals that exist naturally in plants and are involved in secondary metabolism. They are regarded as a sign of a fruit's or medicinal plant's quality [22]. The concentration (20 mg/g) of flavonoids produced in this study by MAE (85 °C, 8 min) was found to be substantially greater ($p<0.05$) than the maceration value (12.4 mg/g) (Fig 3).

Ferric Reducing Antioxidant Power Assay: The FRAP technique quantifies an antioxidant's ability to act as a reducing agent. In the FRAP assay, potassium ferricyanide was employed as the ferric reagent. The antioxidant capacity of rosemary extract obtained by MW-assisted extraction was found to be significantly greater ($p<0.05$) than that produced using the conventional methodology. On the other hand, using the MW-assisted extraction technique, lower FRAP values were achieved at 60 °C and 85 °C for 20 minutes. The lower result obtained after 20 minutes at 60 °C might be a result of inadequate temperature. The decreased result at 85 °C after 20 minutes may be a result of the breakdown of the antioxidant molecules. This indicates that the molecules responsible for FRAP in rosemary are highly temperature sensitive.

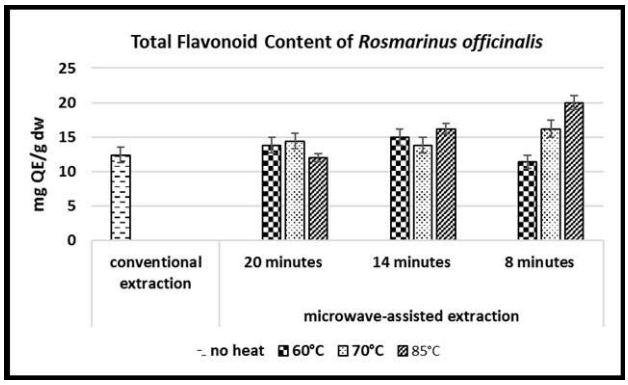


Fig. 3 Total *Rosmarinus officinalis* flavonoid content. All results are shown in milligrams of QE per gram of dry plant material.

As a result, the highest concentration was reached by extracting at 70 °C for 20 minutes. However, after 14 minutes of extraction, rosemary extract had significantly greater antioxidant profiles at 60 °C and 85 °C of extraction temperature.

Furthermore, the maximum degree of antioxidant power was seen at 85 °C after 8 minutes of extraction, indicating that the antioxidant molecules contributing to the FRAP assay are extremely sensitive to extraction time and temperature (Fig 4). As a result, the optimal microwave-assisted extraction conditions for the FRAP test were found to be 8-minutes of extraction time and 85 ^{o}C.

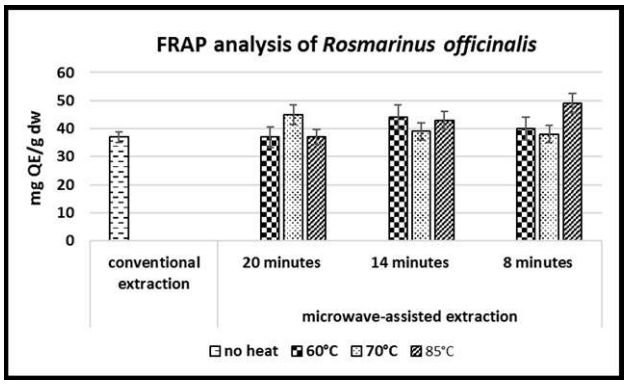


Fig. 4 *Rosmarinus officinalis* FRAP values. All results are shown in milligrams of QE per gram of dry plant material.

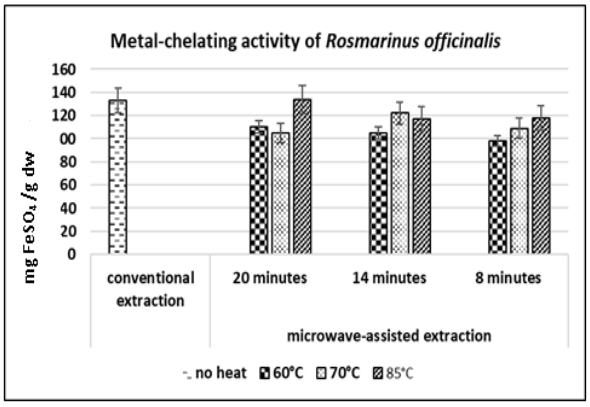


Fig. 5 *Rosmarinus officinalis* metal-chelating activity. All results are shown as equivalants of milligram $FeSO_4$ per gram of dry plant material.

Metal chelating assay: In this study, no significant changes in MCA values were established between MAE (98-134 mg/g) and conventional (133 mg/g) extraction methods (Figure 5). The results obtained for metal chelating activity were shown to be undependent to the time and temperature conditions used in the MAE technique.

Content of beta-carotene and lycopene: The beta-carotene and lycopene concentrations of rosemary were determined to be 8652 and 7849 mg/g dry weight, respectively.

Discussions

Phenolic compounds are strong antioxidants that have gained great attention in recent years due to their potential benefits for human health in the defense against degenerative illnesses. A phenolic compound is defined structurally as having an aromatic ring with one or more hydroxyl groups. It has been reported that phenolic compounds are found in both unbound and bound forms in plants [23]. The bonded phenolic compounds are ester-attached to cell wall components such as xylans, pectin, and lignin, or are covalently connected to sugars in the form of depsides or simple glycosides [23].

Soxhlet extraction, ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and rapid solvent

extraction are all new extraction techniques that have been developed recently. The benefits and drawbacks of various extraction techniques are determined by the physical and chemical variables present during the processes. The principal disadvantages of soxhlet extraction are the time and solvent requirements, as well as the possibility of light and heat deterioration of the extract. On the other hand, supercritical fluid extraction is an expensive process that requires highly skilled staff to configure the equipment and monitor the extraction. Additionally, supercritical carbon dioxide is inefficient for extracting highly polar phenolic acids, whereas ultrasound-assisted extraction has a number of drawbacks, including a significant volume of solvent needed and the possibility of extract destruction by light and oxygen [24].

Unlike the other methods described before, MAE may be used to extract the bound phenolic acids. Thus, MAE provides superiority for the investigated factors, including TPC, TFC, FRAP, and MCC. Additionally, MAE offers fast and cost-effective procedures, quick extraction times, the flexibility to perform several extractions at once, and reduced solvent needs. Two different systems of MAE have been developed: open-system and closed-system. The closed-system of MAE, therefore, offers fast and efficient extraction with less solvent consumption. However, limited amount of sample (max. 1.0 g) can be extracted in the closed-system of MAE which is the main disadvantage [25].

The total phenolic content, total flavonoid content, total antioxidant capacity, beta-carotene, and lycopene levels of *Rosmarinus officinalis* were determined in this research utilizing conventional and microwave-assisted extraction techniques. In this study, total phenolics were significantly destroyed in a microwave oven at 85 ^{o}C for 20 minutes. However, at 85 ^{o}C, the phenolic compounds were not significantly reduced after 8 and 14 minutes. This indicates that a high temperature and prolonged extraction process are ineffective for extracting phenolic chemicals. The literature reports a broad range of TPC values for rosemary (from 1.4 [26] to 127 mg/g [27]), which may be attributed to the solvent type, extraction method, duration, and temperature. In comparison to our research (40 mg/g), higher TPC values were reported utilizing maceration and different solvents such as water (98 mg/g [28] and acetone (65 mg/g [29]. Additionally, extraction methods and circumstances such as static magnetic field and ultrasound aided extraction have been found to result in more successful extraction. Using a static magnetic field, high total phenolic content was obtained (121.2 mg/g) at 70 ^{o}C in 1 hour of extraction time using water as the solvent. The significantly higher concentration of the total phenolic components in that study resulted from the irrigation with water-treated with a static magnetic field (SMF). The high phenolic composition may be attributed to the fact that the magnetic field induces cell metabolism and mitosis of meristematic cells. Moreover, the synthesis of new proteins could also underly the growth stimulation observed in SMF plants [28]. For the same conditions, maceration yielded less total phenolic content in rosemary (98 mg/g) [28]. On the contrary, a significantly lower amount of total phenolics was obtained in 48 hours of extraction time using methanol as a solvent [26]. Different solvents were also used to obtain total phenolics in rosemary. 60 % ethanol and 60 % acetone as solvents were used, yielding 60 mg/g, and 65 mg/g total phenolics using maceration for 70 minutes of extraction time with 4 hours of pretreatment. In the same conditions, ultrasound-assisted extraction yielded 73.4 mg/g and 77.5 mg/g using acetone and ethanol, respectively [29]. Sequential maceration was also used to extract rosemary in 24 hours of extraction time, yielding 26 mg/g total phenolics [30]. On the other hand, using ethanol as solvent in 72 hours of maceration

yielded 30 mg/g [27], and 49 mg/ml [31]. In another study, electromagnetic induction heating-assisted extraction yielded 127 mg/g total phenolics using water as solvent [27]. The heat reflux method, however, yielded 107 mg/g total phenolics in 6 hours of extraction time at 100 ^{o}C [27]. In another study, modified maceration with heat yielded 46.5 mg/g total phenolics in 30 minutes of extraction time at 70 ^{o}C [32]. In another study, rosemary leaves were allowed to stand for 4 hours with ethanol as the solvent, then placed into the percolator for 3 days. The total phenolic content of rosemary sample extracted via this procedure, called as percolation, was reported as 219 mg/ml [31].

In this study, to extract flavonoids 8 minutes was found to be sufficient time at 85 ^{o}C. However, regardless of temperature, the extraction efficiency was poor after 14 and 20 minutes of extraction time. To extract total flavonoids in rosemary, a variety of extraction methods using various solvents and extraction time were reported. Maceration, for instance, yielded 12 mg/g total flavonoids using ethanol, while 15 mg/g using acetone as solvent [29]. On the other hand, in another study, maceration was reported to yield 3 mg/g total flavonoids using ethanol as a solvent [27]. After 30 minutes of stirring in a 70 ^{o}C water bath, rosemary extract yielded 11.9 mg/g of total flavonoids [32]. However, maceration in closed bottles was reported to yield total flavanoids of 1 mg/g [26]. Ultrasound-assisted extraction, on the other hand, yielded 16 mg/g total flavonoids using acetone, and 15 mg/g using ethanol as solvent [29]. Electromagnetic induction heating assisted extraction was shown to yield 14 mg/g using water, while heat reflux yielded 5 mg/g using water as solvent [27].

The ferric reducing antioxidant power technique quantifies an antioxidant's capacity to act as a reducing agent. In the FRAP test, potassium ferricyanide was employed as the ferric reagent. A limited number of studies on the FRAP of rosemary have been reported. A sequential maceration technique was reported to yield a high FRAP value (95 mg/g) [30], while the low value of FRAP was obtained via maceration with heat (11 mg/g) [32]. Our study yielded 37 mg/g FRAP value using maceration, and 49 mg/g using MAE. The high FRAP value in the literature may result from the sequential (hexane/ethyl acetate/methanol) maceration technique [30].

It is well established that the Fenton reaction and breakdown of lipid hydroperoxides into more reactive peroxyl and alkoxyl radicals enhance lipid oxidation. The Fenton reaction transforms ferrous ions into extremely reactive •OH radicals, which contribute significantly to oxidative damage [33].

$Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}+OH^{-}+OH^{\cdot}$ Fenton reaction

The hydroxy radicals produced by this process destroy proteins, carbohydrates, lipids, and nucleic acids, causing cell damage. Cu^{+}, $Ti^{3+,}$ $Cr^{2+,}$ and Co^{2+} ions, as well as their lower oxidation state complexes, react similarly to Fe2+ with H_2O_2 and are referred to as "Fenton-like" reagents. As powerful metal chelators, antioxidants can easily neutralize prooxidant metal ions, thereby inhibiting metal ion-induced lipooxidation. Phenolic compounds serve as metal chelators and radical scavengers in iron-driven Fenton reactions. By reducing reactive oxygen and nitrogen species (ROS/RNS), such as •OH, O2•, NO•, and OONO- polyphenols prevent damage to biomolecules and the generation of more reactive ROS. Typically, rosmarinic acid (RA) in rosemary is known to be intercalated in Fenton reaction as following [34].

$RA–Fe(III) + H_2O_2 \rightleftharpoons RA – Fe(II) + HOO\cdot + H^{+}$

$RA– Fe(II) + H_2O_2 \rightleftharpoons RA – Fe (III) + OH^{-} + \cdot OH$

$Fe^{3+} + RA \rightleftharpoons RA – Fe(III)$ (chelate)

$RA – Fe(III) + RA \rightleftharpoons RA – Fe(II) + [RA]_{ox}$

As previously stated, phenolic compounds inhibit the formation of further damaging

radicals such as peroxyl and alkoxyl by metals. The antioxidant property of metal chelators is determined when a complex formed between the antioxidant and the metal such that metal ions are unable to function as an activator of lipid oxidation. Polyphenols have the ability to chelate transition metal ions due to their numerous OH groups and carbonyl moiety [33]. According to the structure and properties of rosmarinic acid, rosemary's primary phenolic component, the adjacent pair OH groups may bind ferric iron ions to create a Fe^{2+}-rosmarinic acid complex. The proposed structure of rosmarinic acid-iron complex is given below. Taking into consider the stearic effect and because of the –C=O and –COOH groups are non-planar, those groups cannot form a stable ring with Fe2+. Generally, iron has 6 coordination centers, however in this complex, the number of coordination centers must be 2 for iron(II) [35].

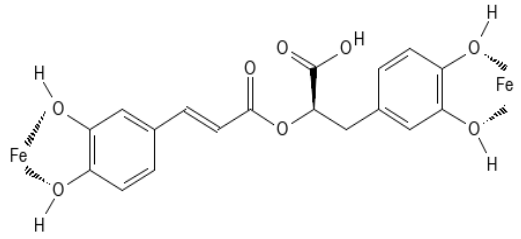


Fig. 6. The proposed structure of rosmarinic acid-iron complex.

As a result, the ability of metals to chelate is often employed as a proxy for antioxidant activity [36]. Beta-carotene is the most prevalent and long-lasting natural pigment in the world. It is present in plants, fungus, and algae. Beta-carotene may be transformed in the human body to vitamin A, where it exhibits anti-oxidant, anticancer, and anticardiovascular effects. As a consequence, beta-carotene is widely used in the food, medical, and nutrition industries [37]. Carotenoid lycopene, on the other hand, is a possible cancer-fighting medication that may help prevent or slow the spread of the disease [38]. The significant beta-carotene and lycopene contents of rosemary were identified in this work.

Conclusion

In this study, TPC, TFC, FRAP, MCC, beta carotene, and lycopene concentrations, extraction duration, and solvent volume were compared between conventional and microwave-assisted extraction methods for rosemary. In the extraction of *Rosmarinus officinalis*, the microwave-assisted extraction technique was shown to be superior to the traditional approach. Briefly, microwave-assisted extraction of *Rosmarinus officinalis* offers several benefits, including increased efficacy, reduced extraction time, and reduced solvent use. Additionally, rosemary is recommended for use in the food industry as a natural antioxidant rather than a synthetic one to avoid health-damaging effects.

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