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# Exploring the Potent Antioxidant and Antibacterial Properties of *Rosmarinus Officinalis* L. Leaf Extract: Health-Promoting Benefits of Rosemary Leaf Extract

*Jummana M Makhammra[1], Rezq Basheer Salimia[2] & Hatem A Hejaz[1,3*]*

[1]Department of Pharmacy, College of Pharmacy& Medical Sciences, Hebron University- Palestine, 21919078@students.hebron.edu. [2]College of Agriculture, Hebron University – Palestine, basheer@hebron.edu. [1,3*]Department of Pharmacy, Faculty of Pharmacy, Arab American University, P.O Box 240 Jenin, 13 Zababdeh, Jenin– Palestine.

*Corresponding author: hatem.hijaz@aaup.edu

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## ABSTRACT

*Rosmarinus officinalis* L. belongs to the Lamiaceae family. Over time, *Rosmarinus officinalis* has been employed for its potential contributions to health. This study aimed to investigate the antioxidant and antibacterial properties of *R. officinalis* leaves. *Rosmarinus officinalis* leaves were collected from various locations in the southern region of Palestine, subjected to air-drying, grinding into powder, and then extracted. The methanol extracts obtained were subsequently analyzed using Gas chromatography-mass spectrometry (GC-MS). The sections were evaluated for their antioxidant activities through assays measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH) and [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) radical scavenging and their antibacterial activities were examined by disk diffusion. The analysis successfully separated and identified various volatile compounds and semi-volatile phytochemicals. Eucalyptol (1,8-cineole) and camphor were the primary components in all analyzed *Rosmarinus officinalis* samples. The analyzed samples' extracts demonstrated scavenging abilities ranging from 73.25% to 88.82% and 73.25%-76.36%, using the ABTS and DPPH assays, respectively. In antibacterial investigations, inhibitory zones were observed against *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*. These findings regarding *Rosmarinus officinalis* 's antioxidant and antibacterial properties suggest its potential as a promising antioxidant and potential antibacterial solution.

**Keywords:** Antioxidant; DPPH; ABTS; Antibacterial; Rosmarinus Officinalis; Rosemary.

## INTRODUCTION

Various natural antioxidants can be found in medicinal plants, harnessed globally to treat a broad spectrum of illnesses. Due to the positive benefits of natural antioxidants in treating and preventing conditions such as cancer, diabetes, atherosclerosis, heart disease, nephrotoxicity, hepatotoxicity, cognitive loss, and vision loss, there is much interest in discovering natural plant antioxidants. According to studies on medicinal herbs, most have substantial antioxidant activity [1-2]. Traditional medicine is considered part of the popular culture and religious beliefs in Palestine, and Palestinians commonly use traditional medicine.; this may be due to political conflict and the high cost of traditional medicines. Traditional remedies were empirically applied for decades in Saudi Arabia and throughout Asia to treat various diseases. Therefore, medicinal herbs are frequently used in conventional medicine and are commonly used as routine treatments and home remedies [3-4].

Flavonoids, alkaloids, tannins, and terpenoids are phytochemicals that are present in medicinal plants and have antibacterial and antioxidant activities. Rosemary extracts from dried rosemary leaves are of great interest to the food and drug industries because they have several health benefits, including antioxidant, antibacterial, anti-inflammatory, and anti-cancer properties [5-6]. The medicinal value of rosemary is due to the high bioactive compounds present in the plant, such as triterpenes, tricyclic diterpenes, phenolic acids, and essential oils [7, 8]. Rosemary essential oils comprise 1,8-cineole (eucalyptol), α-pinene, verbenone, camphor, and borneol, but their compositions vary considerably [9]. Rosemary is high in antioxidants, particularly carnosic acid, which

has been proven to be a powerful antioxidant. The principal antioxidant components in rosemary are phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, epiand iso-rosmanol, rosmadial and methyl carnosate. Infectious illnesses are the leading cause of morbidity and mortality worldwide. According to the World Health Organization (WHO), 55 million people died globally in 2011 due to infectious diseases [10-12].

Extensively studied plant species display effective antimicrobial characteristics against a broad spectrum of gram-positive and gram-negative bacteria. Many types of bacteria, including *E. coli*, *Staphylococcus aureus*, *Candida albicans*, and *Saccharomyces cerevisiae*, can be efficiently inhibited by rosemary extracts. Rosemary inhibits the growth of bacteria that cause illness and delays the growth of bacteria. Antimicrobial action is mainly caused by α-pinene, bornyl acetate, camphor, and 1,8-cineole (Eucalyptol) in medicinal plants [13-14].

Traditional herbal treatments have been utilized for generations to cure a variety of ailments in Palestine. These treatments are widely used, but their safety and effectiveness have not been properly examined, besides the various side effects of conventional medications and the resistance of antibacterial agent issues, and to ascertain whether traditional herbal remedies have the potential to provide new therapeutic agents, the biological studies of the remedies are needed [3, 15-17]. As a result, there is a heightened focus on discovering naturally occurring antioxidant and antimicrobial compounds suitable for both dietary and medicinal applications. In Palestine, the research on the effects of *Rosmarinus officinalis* L. on overall health and therapeutic properties is limited, and there has yet to be an investigation into the availability of antioxidants and antibacterial agents. Therefore, the aim is to comprehensively assess rosemary leaves' antioxidants, phytochemicals, antimicrobial efficacy, and biological potential. *Rosmarinus officinalis* L. was chosen for this study due to its established medicinal reputation among Palestinians and its frequent utilization. There needs to be more information on the antioxidant or antibacterial activities of methanol extracts from *Rosmarinus officinalis* leaves grown in Palestine [15]. Thus, the current research seeks to isolate and identify the chemical composition of these extracts while evaluating their potential as antioxidants and antibacterial agents.

## MATERIALS AND METHODS

**Study area:** This research was conducted at the Department of Pharmacy Laboratory, College of Pharmacy & Medical Sciences, University of Hebron, Palestine, between January 2021 and January 2022.

**Collection of Data and Samples:** During 2021 (from February to July 2021), a total of five samples of rosemary leaves were collected from different sites situated in the southern West Bank region, specifically in Hebron, Palestine. These sites encompass Raqah, Khilt Al-Adrah, Umm Lasfah village, Hebron City, and Bani Naim. The harvested *R. officinalis* leaves underwent a shade drying process at ambient temperature. Subsequently, the desiccated samples were carefully placed within airtight paper bags, shielded from exposure to light.

**Antioxidant activity:** The measurement of antioxidant capacity was conducted using 2,2'-Diphenyl-1-picrylhydrazyl stable radical (DPPH) and [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS), employing the methodology outlined similarly as described by Dowek *et al.* [18].

**Extract preparation:** Ten milliliters of 80 % methanol were used to extract one gram of each rosemary sample over 24 hours at a temperature of 25 ℃ within a shaking incubator (IKA, Baden-Württemberg-Germany). Subsequently, the filtered extracts were employed for further analysis.

**2.6 [2, 2'-Diphenyl-1-picrylhydrazyl] (DPPH) Assay***:* To assess the electron-donating capability of rosemary leaves, purple-hued methanol of 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) solution was decolorized, and this process was measured *via* a UV-visible spectrophotometer (Shimadzu, Tokyo-Japan). The samples were diluted at a ratio of 1:10, and 30 μL of the extract was introduced to 2 mL of DPPH solution in a cuvette. Subsequently, all cuvettes were mixed and kept in darkness at RT for an hour. Following this incubation, the

absorbance was measured using a spectrophotometer at 517 nm. The scavenging action against radicals was determined as a %age of DPPH discolored using the following equation: [18]

**DPPH Scavenging (%) = [1-(At/Ab)] *100**

***At:*** *sample absorbance, and* ***Ab***: *control absorbance*

**2.7 [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) Assay:** The ABTS solution was prepared by combining 3 mL of ABTS stock solution (stock solution concentration; 18 mg of ABTS/ 5 mL water) with 3 mL of potassium persulfate solution (formed by dissolving 10.5 mg of $K_2S_2O_8$ in 15.9 mL of water). This mixture was then placed in darkness for 24 hours at room temperature (RT). The ABTS working solution was created by diluting the ABTS solution with distilled water until the final absorbance at 734 nm reached 0.7000 ± 0.02. In micro cuvettes, the samples were cut at a ratio of 1:10, and 15 µL of the precise solutions were combined with 1 mL of the ABTS working solution. For the control, 15 µL of 80% methanol was mixed. The cuvettes were thoroughly mixed and allowed to sit at RT for an hour in darkness. After this incubation period, the absorbance of the extracted sample was determined at 734 nm using a UV spectrophotometer, and methanol was used as a control.

**Antimicrobial activity Reagents:** The Central Public Health Laboratory, Ministry of Health, Ramallah, Palestine, generously provided Muller-Hinton agar and nutrient broth.

**Extract preparation:** Two distinct approaches were employed to extract rosemary plants, utilizing varying methanol concentrations (80% and 90%). In the initial method, 5 grams of powdered plant material were measured and mixed with 50 mL of methanol at room temperature, undergoing stirring for 24 hours. The second extraction method used a reflux apparatus, weighing 5 grams of powdered plant material and 50 mL of methanol, followed by a 24-hour heating process. Both extraction techniques were applied to methanol concentrations (80 % and 90 %).

**Muller Hinton agar (MHA) preparation:** Thirty-eight grams of Mueller Hinton agar powder was mixed into 1000 mL of distilled water. The mixture was briefly heated until all components were fully dissolved. Sterilization was achieved through autoclaving at 121°C for 15 minutes. Afterward, the solution was allowed to cool down to 45–50 °C. Subsequently, the liquid was carefully poured into a sterile petri dish, and the medium was patiently waited to solidify.

**Nutrient broth preparation:** Thirteen grams of Mueller-Hinton agar powder were suspended in 1000 mL distilled water. The medium was heated to dissolve it entirely, dispensed into tubes and flasks as desired, and sterilized by autoclaving at 15 lbs. pressure (121 C) for 15 minutes. A colony of each bacterium (*Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa*) using the wire loop and mixed with broth, then incubated at 37 °C for 24 hours. Microbial strains of *Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa* were obtained from Princess Alia Governmental Hospital, Hebron.

**Procedure:** The assessment of antibacterial activity followed the methodology outlined by Jahiman *et al.* [19]. Antibacterial efficacy was evaluated through the disk diffusion assay technique, involving three distinct bacterial strains: Staphylococcus *aureus*, *E. coli*, and *Pseudomonas aeruginosa*. Petri dishes were loaded with Mueller-Hinton agar. Bacterial cultures were initiated using the primary colony, followed by injection into the nutrient broth, and then incubated at 37 ºC for 24 hours. Subsequently, five disks were evenly distributed onto the media surface of each plate. A 50 µL portion of rosemary extract was applied to each disk. All dishes were subsequently incubated at 37 °C for 24 hours. The diameter of the inhibition zone was measured and recorded as the result.

## RESULTS

### Antioxidant activity

The antioxidant activity of rosemary leaves was examined by the DPPH method.

The different rosemary samples' diluted methanol extract (1:10) showed antioxidant activities. The average percentage of scavenging capacity is shown in **Figure 1.** Leaf extracts from Bani Naim (76.36%). Umm, Lasfah village (75.99%) samples exhibited the highest levels of antioxidant capacity, followed by Raqaa (75.86%) and Khilt Al-Adrah (74.58%) samples. The Hebron sample has the lowest level of antioxidant capacity (73.25%). The antioxidant activity of rosemary leaves was also examined by the ABTS method. The different rosemary samples' diluted methanolic extracts (1:10) showed antioxidant activities. The average percentage of scavenging capacity is shown in **Figure 2.** The rate of scavenging of diluted methanol extracts of leaves from Umm Lasfah village (88.82%) exhibited the highest levels of antioxidant capacity, followed by Bani Naim (79.67%), Raqaa (75.89%), Khilt Al-Adrah (75.58%), and Hebron (73.91%) samples.

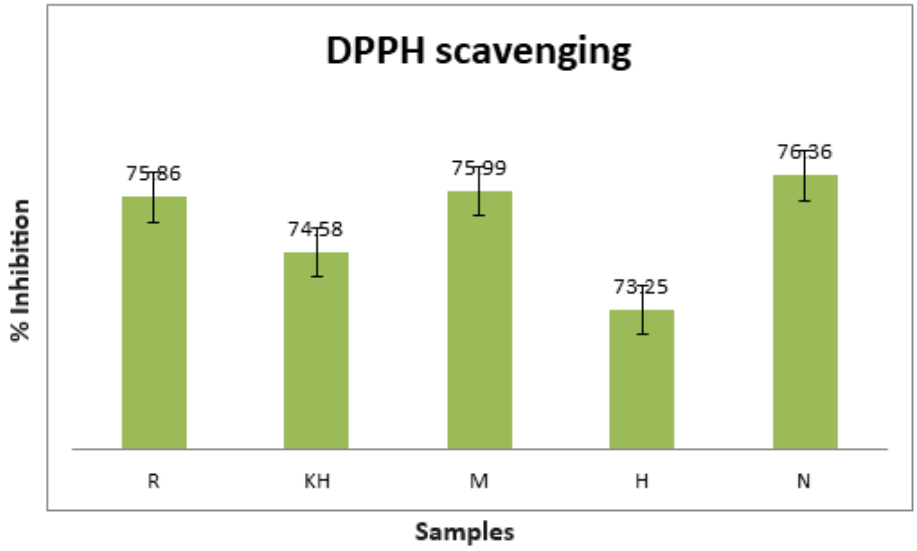


**Figure (1):** Antioxidant capacity (%) of the methanolic extracts of five rosemary samples, assayed by the DPPH free radical scavenging assay, n = 3. **(R):** Raqaa, **(KH):** Khilt Al-Adrah; **(M):** Umm Lasfah village; **(H):** Hebron; **(N):** Bani Naim samples.

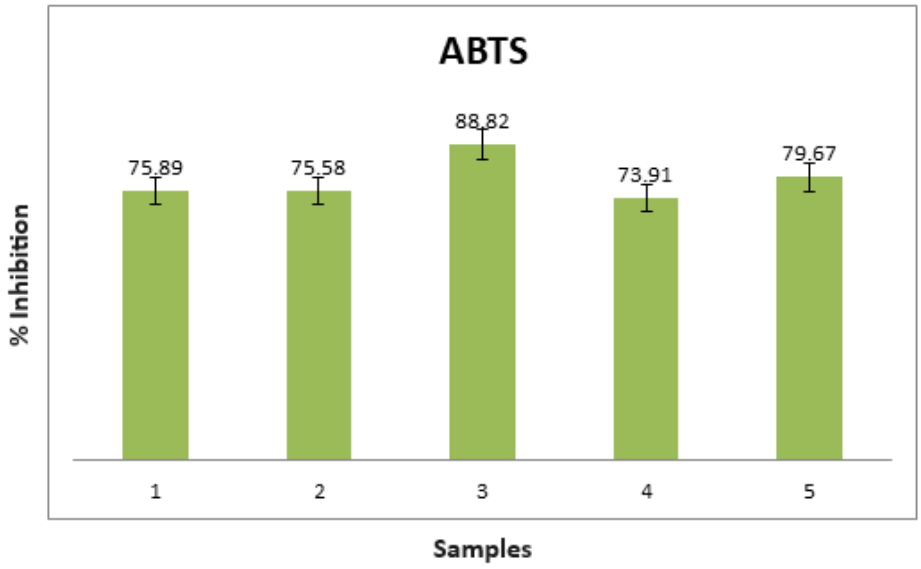


**Figure (2):** Antioxidant capacity percentage of the methanolic extracts of five rosemary samples, assayed by the ABTS free radical scavenging assay, n = 3. **(1):** Raqaa; **(2):** Khilt Al-Adrah, **(3):** Umm Lasfah village; **(4):** Hebron; **(5):** Bani Naim samples.

## Antibacterial activity

The initial screening of the different concentrations of methanol extracts of *R. officinalis* against various types of organisms was performed. The antibacterial activity of 50 µL of *R. officinalis* leaf extracts was examined using the disc diffusion method on gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The results of the zones of inhibition are summarized in **Table 1.**

**Table (1):** The antibacterial activity results of *R. officinalis* leaf extracts.

| Sample | Average zone of inhibition (cm) 80% Methanol (RT) | | | | | |
|---|---|---|---|---|---|---|
| | **R** | **KH** | **M** | **H** | **N** | **Control** |
| *S. aureus* | 1.7 | 1.6 | 1.8 | 1.7 | 2.2 | * |
| *E. coli* | 0.9 | 1 | 1.1 | 1.2 | 1.2 | * |
| *P. aeruginosa* | 1.2 | 1.4 | 1.6 | 1.6 | 1.6 | * |
| | **Average zone of inhibition (cm) 80% Methanol (Reflux)** | | | | | |
| **Sample** | **R** | **KH** | **M** | **H** | **N** | **Control** |
| *S. aureus* | 1.4 | 1.3 | 1.2 | 1.2 | 1.5 | * |
| *E. coli* | 1.4 | 1.4 | * | * | 0.6 | * |
| *P. aeruginosa* | * | 1.2 | 1.8 | 0.8 | 1.5 | * |
| | **Average zone of inhibition (cm) 90% Methanol (RT)** | | | | | |
| **Sample** | **R** | **KH** | **M** | **H** | **N** | **Control** |
| *S. aureus* | 0.5 | 0.4 | 0.4 | 0.1 | 0.1 | 1.1 |
| *E. coli* | 1.2 | 2 | 1.5 | * | * | * |
| *P. aeruginosa* | 1.4 | 1.6 | 1.2 | 1.6 | 1.2 | 0.8 |
| | **Average zone of inhibition (cm) 90% Methanol (Reflux)** | | | | | |
| **Sample** | **R** | **KH** | **M** | **H** | **N** | **Control** |
| *S. aureus* | 0.9 | 0.5 | 0.4 | 0.7 | 1.4 | 1.1 |
| *E. coli* | * | 1.4 | * | 1 | 1.2 | No effect |
| *P. aeruginosa* | 1.2 | 1 | 0.4 | 0.8 | 0.8 | 0.8 |

(**R**): Raqaa; (**KH**): Khilt Al-Adrah; (**M**): Umm Lasfah village; (**H**): Hebron; (**N**): Bani Naim samples. *** = *No effect.***

## DISCUSSION

The study's findings show that rosemary leaves can be a promising source of antibacterial and antioxidant properties. It is clear that 50 µL of *R. Officinalis* exhibits notable antibacterial activities against gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*). For the 80 % methanolic extracts at room temperature and reflux extraction methods, the observed activities in gram-positive bacteria were higher than in gram-negative bacteria. Conversely, no action on the *P. aeruginosa* strain was shown by the Raqaa sample, and both Hebron and Umm Lasfah extracts (methanol extractions of 80% and reflux method) were inactive against the *E. coli* strain. It was also observed that the antibacterial activity of 90 % methanol extract at room temperature was high against the *P. aeruginosa* strain. Conversely, no action was shown by Hebron, Bani Naim, Raqaa, and Umm Lasfah extract samples on *E. coli* in both methanolic extraction methods (90% at room temperature and Reflux). *R. officinalis* has been shown in multiple scientific research to possess antibacterial action against *S.*

*aureus*, *P. aeruginosa*, and *E. coli* [20]. Furthermore, *R. officinalis* obtained in Tunisia has been shown by Jardak *et al.* [21] to have potent antibacterial properties against *S. aureus* and *S. epidermidis*. Furthermore, *R. officinalis* have antibacterial action against *Bacillus subtilis*, *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*, according to a study by Wang *et al.* [22]. In addition, *R. officinalis* has been shown by Al-Maharik *et al.* to have high antibacterial action against *S. aureus*, *E. coli*, *K. pneumonia*, and *P. vulgaris* [15].

To combat many ailments whose pathophysiology is connected to oxidative stress and bacterial infection, rosemary could be used as a natural source of antioxidants in the food and pharmaceutical industries. It also has an antibacterial effect. Our bodies produce free radicals and reactive nitrogen species due to various endogenous systems, environmental exposure, or pathological situations. When free radicals outnumber the body's ability to manage them, the condition is known as "oxidative stress." Oxidative stress plays a central role in the pathogenesis of diverse chronic diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer [23]. Applying an external source of antioxidants can assist in coping with this oxidative stress. Herbs are a good source of naturally occurring antioxidants, mainly phenolic compounds with hydroxyl groups that may readily donate an atom of hydrogen to a free radical. The herb rosemary is a rich source of lipid-soluble antioxidants, particularly carnosic acid, which has been proven to be a powerful antioxidant. It is more efficient than synthetic antioxidants like butylated hydroxyanisole and butylated hydroxytoluene [24]. Saito *et al.* found that the volatile constituents of rosemary, particularly 1,8-cineole (eucalyptol), exert an antioxidant effect [25]. Rosemary extracts are used as a natural antioxidant, extending the shelf life of perishable foods. Rosemary extract (E392) has received approval from the European Union as a safe and reliable natural antioxidant for food preservation [26].

Investigation of the diluted 1:10 methanol extracts of the rosemary extract showed a remarkable scavenging capacity for both DPPH and ABTS free radical scavenging assays. The two types of radical scavenging activity assays showed an approximately similar percentage for each sample. Some studies have shown that rosemary extract has potent antioxidant effects. Our rosemary leaves from our pieces showed antioxidant activity, with a range of percentages of scavenging activity from 73.25% to 75.99% for DPPH. As stated by Aktaş and Malayoğlu, the antioxidant activity (DPPH radical scavenging activity) of rosemary extract (94%) was higher than the current study results [27]. In another study, antioxidant activity was increased in all tested rosemary (72–85% inactivation of DPPH); these ratios are consistent with the current study [28]. According to Martínez *et al.*, the capacity of rosemary extract to scavenge the DPPH free radical was recorded at 81.8% [29].

Additionally, the ABTS assays were higher than 80%, consistent with our study, especially in the Umm Lasfah village sample (88.82%). It has been noted that the solvent employed in extraction can impact the extract's antioxidant activity, depending on the phenolic content. Liu *et al.* discovered that methanol extracts of rosemary have more phenolic and flavonoid contents than hexane extracts [30]. The importance of the antioxidant action of methanol extracts of rosemary is probably due to their richness in phenolic compounds. The compounds associated with the antioxidant activity of rosemary are the phenolic diterpenes (carnosol, romano, 7-methyl-epirosmanol, isorosmanol, and carnosic acid) and the phenolic acids (rosmarinic and caffeic acids). The qualitative phytochemical analysis indicated that rosemary leaves contained terpenoids and phenolic acid in all the samples tested, which are responsible for their antioxidant activity. The total phenol contents in plants depend on plant species, plant tissue, developmental stage, and environmental factors such as temperature, water stress, and light conditions. These results suggested that the rosemary plant could be of great industrial importance and can support the development of natural additives with the potential for application in food technology. Plant-based antioxidants are becoming increasingly crucial in nutrition (food preservation and stability) and preventive medicine [31-33].

The mechanism of action of antioxidant action has been extensively discussed in several articles. For instance, it was found by Höulihan *et al.* [34] and Wu *et al.* [35] that rosemary's abundance in isoprenoid quinones, which function as chelators of reactive oxygen species (ROS) and chain terminators of free radicals, is responsible for its antioxidant qualities. Furthermore, according to Gordon [35], the phenolic chemicals found in commercial rosemary extracts function as the main antioxidants when they react with hydroxyl and lipid radicals to produce stable products. Later, Fang and Wada [37] noted that these substances might function as metal ion chelators (Fe+2) in a basic way, lowering the ratio at which oxygen-derived reactive species arise. Löliger [38] claims that carnosic acid and carnosol are effective peroxyl radical scavengers. This fact clarifies the findings of Chen *et al.* [39], who established that both substances had a greater effect on membrane lipid peroxidation than chemical antioxidants like propyl gallate, BHA, and BHT have [40]. According to Inatani *et al.* [41], rosmanol exhibited an antioxidant capacity in linoleic acid and lard that was four times more than that of BRT and BRA (synthetic antioxidants). Additionally, using the TBA and ferric thiocyanate techniques, this study reported the antioxidant activity of carnosol and rosmanol. They presented the relationship between an antioxidant's chemical structure and activity. Studies on the pro- and antioxidant characteristics of rosemary were conducted by Aruoma *et al.* [42]. Ninety percent of the properties of antioxidants are attributed to two primary constituents: carnosic acid and carnosol. Both lower cytochrome C, scavenge hydroxyl radicals and suppress lipid peroxidation in liposomal and microsomal systems. They are also effective scavengers of CCl3O2 (peroxyl radicals). In particular, carnosic acid scavenges H2O2, but it may also function as an enzyme system substrate. It is also found that fruiting stages affect the antioxidant properties as an increase in polyphenol concentration during the fruiting stage, such as rosmarinic acid, hesperidin, and carnosol, is directly linked to an increase in the extract's antioxidant capacity. Scientific studies by Cui *et al.* [43] and Kontogianni *et al.* [44], who identify lactone carnosol as the primary component causing this activity, corroborate this claim. The literature has also mentioned hesperidin and rosmarinic acid as significant free radical scavengers [45, 46].

The worldwide prevalence of bacterial infections is a significant public health concern. Today's widespread use of synthetic antibiotics has increased the majority of resistant strains and side effects. The antimicrobial activities of medicinal plants are based on their bioactive phytochemicals known as secondary metabolites, which include terpenoids, alkaloids, flavonoids, tannins, and glycosides [47]. The phenolic compounds damage the cell membrane and block the cell's functional characteristics, eventually leaking the inner materials of the cell [48]. The present study investigated the antibacterial activities of the rosemary plant. However, using the disc-diffusion method to calculate the inhibitory zone diameters, various concentrations of methanol extracts were tested for their antibacterial activities against three bacterial species, including gram-positive *Staphylococcus aureus* and gram-negative *Pseudomonas aeruginosa* and *Escherichia coli*. Methanol extracts showed suitable antimicrobial activities against all microorganisms tested. The current study results indicated that the rosemary extracts showed antibacterial activities, consistent with other studies that demonstrated an effect against gram-positive bacteria (*S. aureus*) [49].

Additionally, the extracts impacted gram-negative bacteria *(E. coli* and *P. aeruginosa*). Rosemary extracts contain bioactive chemicals, such as phenolic compounds, that inhibit the growth of both gram-negative and gram-positive bacteria. The major compounds responsible for the antibacterial actions include 1,8-cineole (eucalyptol), camphor, bornyl acetate, and α-pinene. As stated by another study, the presence of α-pinene as a significant component improves antimicrobial activity compared to *S. aureus* [49-51]. Some of the samples are inactive against some strains of bacteria, as shown in **Table 1** The lack of an inhibition zone does not always imply that compounds are inactive. For instance, non-polar compounds might not diffuse into the culture medium.

The mechanism of antibacterial activity of rosemary is due to the presence of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol, and isorosmanol which work together to give the antibacterial effect. Their interactions with the cell membrane result in modifications to the genetic material and nutrients, changes to electron transport, leakage of cellular components, and changes to fatty acid synthesis. Furthermore, it resulted in an interaction with the protein membrane that led to the structural and functional loss of the membrane [52]. Carnosic acid is more effective than any other main extract component, including rosmarinic acid in killing pathogenic bacteria, according to Vegara *et al.* [53]. On the other hand, there is disagreement in several studies on potential connections between the antibacterial activity of polyphenolic extract and its composition. In this instance, Moreno *et al.* [28] and Ivanovic *et al.* [54] showed that a potential synergy between the carnosic acid diterpene and rosmarinic phenolic acid may be responsible for the efficiency of rosemary. However, according to Bernardes *et al.* [55], there is a direct correlation between these extracts' antibacterial activity and their concentration levels of carnosic acid and carnosol diterpenes. Zaouali *et al.* [51] also reported that α-pinene in rosemary is a key component that boosts antibacterial action when compared to *S. aureus*. According to Bjapai *et al.* who stated that terpenes can disorganize the cell membrane, which in turn promotes lysis, which may explain this impact [49]. Thus, the antibacterial activities of rosemary leaf extracts were probably due to their constituents (eucalyptol, camphor, camphene, and caryophyllene). As mentioned, it has been observed that, depending on the phenolic content, the extraction solvent used can affect the antioxidant activity of the extract. Liu *et al.* [30] found that rosemary methanol extracts have higher levels of flavonoids and phenolic compounds than hexane extracts.

The current research on rosemary leaf extracts revealed that this plant contains secondary chemicals that have antibacterial effects on gram-positive and gram-negative bacteria. These findings demonstrated that these plants have antibacterial effects, and the activities depended upon the presence of the phytochemicals, which are affected by the extraction method. These findings indicated that the phytochemicals present in certain ethnobotanical species, including rosemary, have antibacterial properties, which is the main reason for their use as a traditional remedy in Palestinian folk medicine. More research is needed to identify which exact compound in rosemary is responsible for inhibiting gram-negative and gram-positive bacteria. According to the current study, the screening and evaluation of the phytochemical compounds in the rosemary plant for their antibacterial and antioxidant activities was the first in Palestine. There were some limitations in our study, we did not measure the minimum inhibitory concentration, and the stages of the plant life cycle and seasonal variation were not considered.

## CONCLUSION

The observed antioxidant activities of the methanol extracts of *R. officinalis* L. suggest their potential as a promising source of antioxidants. Moreover, the sections could inhibit pathogenic gram-positive and gram-negative bacterial colony growth. However, rosemary could be used as an antioxidant with antibacterial activity against numerous diseases whose pathogenesis is associated with oxidative stress and bacterial infection as natural sources of antioxidants in the food and pharmaceutical industries.

***Competing Interests:*** The authors declare that there are no conflicts of interest.

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**Significance Statement:** The research finding suggests that rosemary leaves could have the potential as a promising antioxidant and antibacterial source. However, rosemary could be used as an antioxidant with antibacterial activity against numerous diseases whose pathogenesis is associated with oxidative stress and bacterial infection as natural sources of antioxidants in the food and pharmaceutical industries.

**Author's Contribution: Jummana M Makhammra**; Responsible for the initial draft of the manuscript, curated and analyzed data, conducted formal analysis, investigated

the subject matter, and contributed resources to the project. This research was carried out by Jummana M Makhammra (a master's student, at the Department of Pharmacy, College of Pharmacy& Medical Sciences, Hebron University). **Rezq Basheer-Salimia**; Provided logistical assistance, conceptualized and designed the study, and assisted in writing and editing the manuscript. **Hatem A Hejaz**; The supervisor who contributed to the original draft, conducted formal analysis, developed methodology, managed the project, provided oversight, ensured validation, created visualizations, and participated in both reviewing and editing the writing. All authors read and approved the final manuscript.

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