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Phytochemical, Antibacterial and Antioxidant Study of *Rosmarinus officinalis* from the Region of Khenchela, Algeria

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Abstract

Rosmarinus officinalis is a medicinal plant. It was analyzed for its phytochemical constitution, antibacterial activity and antioxidant activity. For this purpose, we use the Rosmary of Khirane province of Khenchela (Algeria). The tests of phytochemical screening showed that the plant contain flavonoids, tannin saponins, mucilage, and polyphenols. Conversely, alkaloids and coumarin was absent. The reported extraction of phenolic compounds was 9.3%, while the methanolic extract was found to contain 129.55 mg of polyphenols and 92.5 mg of flavonoids, indicating a rich content of these compounds in the extract .The extracting rosemary essential oil yielded 11 ml, which corresponds to a 2% yield. The antibacterial activity was determined against E. coli and staphylococcus aureus, The results reveal differential sensitivity patterns between the two bacterial strains. Both exhibited resistance to the negative control (distilled water) while showing pronounced susceptibility to the essential oil (17 mm inhibition zone for Staphylococcus and 19 mm for Escherichia coli) and antibiotic. Notably, the methanolic extract's effectiveness varied between the strains, being inactive against Staphylococcus but mildly effective against E. coli. The results showed that the highest mean inhibition zone was observed in the methanolic extracts. Our findings demonstrate significant antioxidant activity of rosemary, with a value of 0.45 mg/ml.

Keywords: Antibacterial, antioxidant, essential oil, phytochemical compounds, *Rosmarinus officinalis*

Introduction

Medicinal plants are pivotal to pharmacological research and drug development. Their significance extends beyond the direct use of plant constituents as therapeutic agents, they also serve as essential raw materials for drug synthesis and as models for creating pharmacologically active compounds (Chaachouay and Zidane, 2024).

In Algeria, the natural medicinal flora is notably abundant, comprising over 3,000 species utilized in traditional medicine (Djahra, 2023). This flora, which includes species from several botanical families and has 15% endemism, remains largely underexplored both phytochemically and pharmacologically (Bouzid *et al*., 2017). Furthermore, aromatic plants hold significant potential due to the progressive discovery of applications for their essential oils (EOs) in healthcare and other economically relevant fields. Their diverse uses, particularly their medicinal properties such as anti-inflammatory, antiseptic, antiviral, antifungal, antibacterial, and antioxidant effects, have driven increasing demand in global markets (Laiche and Mecheri, 2023).

One notable example of such an aromatic plant is rosemary (*Rosmarinus officinalis L.*). Originating from the Mediterranean regions, this medicinal herb is cultivated worldwide from seeds or cuttings during the spring season. It thrives best in warm, moderately dry climates (Meziane *et al*., 2024). According to Pappachan *et al*. (2023), rosemary is found in Morocco, Spain, Portugal, Turkey, Tunisia, Algeria, and India. Moreover, it thrives both in the wild and in cultivation, especially in the Mediterranean basin of Algeria, where it is exceptionally popular. Its flowers bloom year-round, attracting a variety of insects (Larit and León, 2023).

Extracting EOs is a complex and delicate process aimed at capturing the plant's most volatile and fragile compounds without compromising their quality (Cimino *et al*., 2021). Rosemary essential oil stands out for its renowned refreshing scent and therapeutic benefits. Rich in pinene it promotes mental clarity, memory enhancement, and stress relief (Pant *et al*., 2019). Possessing antioxidant and antimicrobial properties, it's prized in skincare and hair **care** (González-Minero *et al*., 2020). In light of its beneficial attributes, our study focuses on the phytochemical compounds of the rosemary plant from the Khirane region in Khenchela Province, exploring their efficacy against bacteria and antioxidant properties. It comprises two main components: a phytochemical analysis and investigations into its antibacterial and antioxidant activities.

Materials and methods

The description of the study area

The study area is located in the east of Algeria, and more specifically in the Wilaya of Khenchela, Khirane's region wich is provence of Khenchela situated in the South East of Constantinois and at the opposite of the Mount of Aurès between 34°6'36"and 35°41'21"latitude North, between 6°4'12" and 7°35 '56"east longitud (Fig.1), it extends over an area of 9715.6km^2 (Khabtane, 2010). , It occupies a geographical position between the Atlas chain and the highlands, which gives it a character of agro-pastoral area. Khirane is part of the high plains of eastern Algeria, it is located in the north of the Wilaya of Khenchela. The climate of this forest massif is characterized by a long, dry and hot summer season and an increasing number of years with less rainfall. Rainfall is generally low and irregular, resides in an arid area (Hani et al., 2020). The experimental was conducted in the spring of 2024.

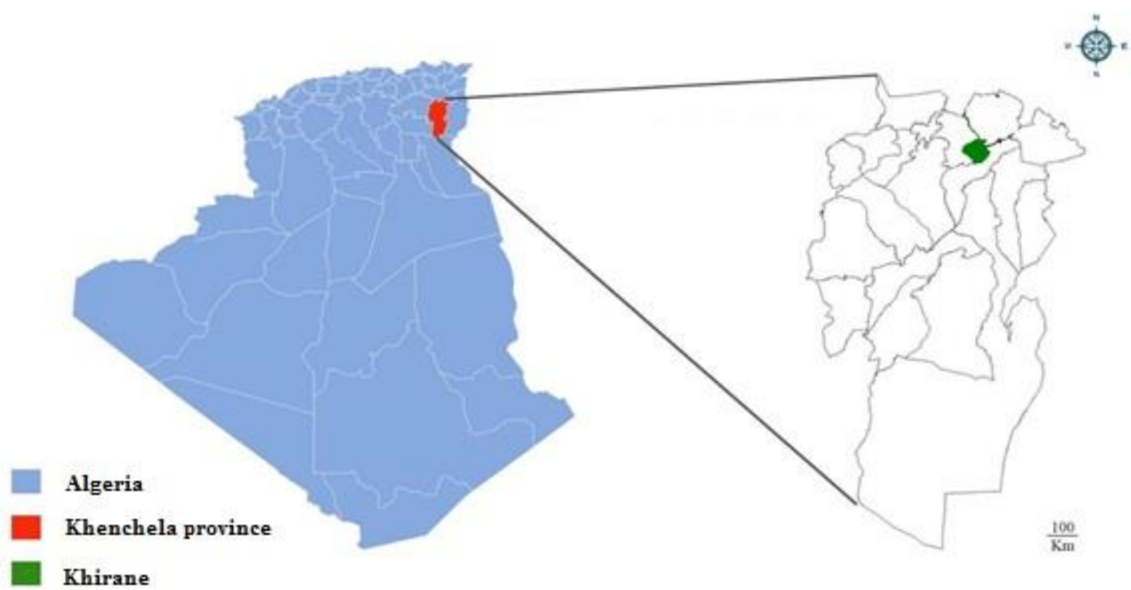

Figure 1. Map of study area

Description of the Plant

Rosmarinus officinalis is a medicinal plant belonging to the family Lamiaceae. It is an evergreen, perennial bush, the height is 1-2 meters long (Okwu et al., 2006). The stamens are filamentous (Ali et al., 1990). It is an evergreen perennial shrub, best known for its strongly aromatic, needle-like evergreen leaves. The flowers are purplish white and strongly two-lipped, and have two long-exserted (protruding) stamens. The fruit consists of four dry nutlets. It is found especially the mediterranean dishes and it has naturally occurred antioxidant and derives its name from its refreshing effects (Mohsenzadeh et al., 2007) and decorative plant gardens or even as a fence, having many culinary and medical uses.

Table 1. Scientific classification of *Rosmarinus officinalis* (Miliauskas et al., 2004)

Kingdom	Plantae
Class	Equisetopsida
Subclass	Magnoliidae
Order	Lamiales
Family	Lamiaceae
Genus	Rosmarinus

Rosmarinus officinalis showed good adaptability in cold and dry areas cultivated in all countries as an ornamental and medicinal plant, aromatic and seasoning plant used in cosmetic and perfume industries and also used as a flavoring agent. Rosemary oil has an effective antimicrobial agent inhibiting the growth of molds and many types of bacteria. Rosemary extract relaxes smooth muscles and has choleretic, hepatoprotective and antitumorigenic activity.

Figure 2. Rosemary plant during harvesting and drying

Plant material collection and preparation of powder

Fresh arial parts of the *Rosmarinus officinalis* were collected in May 2024 from the Khirane region of Khenchela Province. The plant materials were taxonomically identified and authenticated by the Department of biology, Abbes Laghrour University. The plant materials were shade dried in a well-ventilated area for 11 days until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using a blender into fine powder.

Figure 3: *Rosmarinus officinalis* after grinding

Preparation of crude extracts for phytochemical, antioxidant and antibacterial assays.

Phytochemical analysis

highlight the presence or absence of certain secondary metabolites, a phytochemical screening was conducted using established techniques (Harborne, 1998). For tannins, 5g of rosemary powder were boiled in 100 ml of distilled water and filtered after 15 minutes. An additional 100 ml of water was added, and 5 ml of the 5% infusion were treated with 1 ml of 1% aqueous $FeCl_3$ solution, where a greenish or blackish-blue color indicated tannins (Auwal et al., 2014; Tan et al., 2013).

For saponins, 1g of rosemary powder was boiled with 100 ml of distilled water, filtered, and diluted to 100 ml. A 1 ml aliquot of the 1% Then, 2 mg of sodium carbonate in methanol was added to 1 ml of the filtrate, and absorbance was measured after 4 minutes (Hayat, 2020). Alkaloids were detected by macerating 10g of powder in 50 ml of decoction was diluted to 10 ml with water, and foam formation indicated saponins (Hiai and Nakajima, 1976). **For flavonoids,** 3g of rosemary powder were boiled with 80 ml of water, filtered, and cooled. 1/10 diluted H2SO4, then filtering (Harborne, 1998) 3q. **For mucilages**, 1 ml of a 10% decoction was mixed with 5 ml of absolute ethanol, and a flocculent precipitate indicated mucilages (Oh and Kim, 2022). Coumarins were identified by adding 1g of powder to a test tube, covering it with NaOH-soaked paper, water bathing, adding 0.5 ml of 10% NH_4OH, and observing fluorescence under UV light (Harborne, 1998**). For total phenolic compounds**, 1 ml of 1/10 Folin reagent was added to 200 µl of the sample, followed by 600 µl of Na2CO3 after 5 minutes. Absorbance was measured at 765 nm after 2 hours, and polyphenol concentration was calculated using a standard curve with gallic acid (Wagner et al., 1984).

To extract essential oil using steam distillation, the plant material is placed in a glass column with its lower and upper sections connected to a water flask and a condenser, respectively. Steam from the flask moves through the plant material, collecting the essential oil, and then travels to the condenser, where it is cooled and condensed. Once condensed, the oil is separated from the water by decantation. The process of extracting essential oil by hydrodistillation follows the same principles as steam distillation. The key difference is that, in hydrodistillation, the plant material is directly placed in the water flask, and the mixture is then boiled. The resulting steam-oil mixture is directed to the condenser for condensation, and the oil is subsequently separated by decantation (Chen et al, 2021).

To prepare the methanolic extract, 50g of dry powder was mixed with 200 ml of a methanolic solution (150 ml of CH_3OH + 50 ml of distilled water), covered, and stirred for 24 hours; this process was repeated three times to obtain extracts from three separate batches (three macerations). The mixtures were then filtered and evaporated at 40°C using a rotary evaporator (Ogunlana et al., 2008).

For the antibacterial study, the activity of plant extracts was assessed using the agar diffusion method with sterile disks against Staphylococcus aureus ATCC 25923 (Gram-positive) and Escherichia coli ATCC 25922 (Gram-negative), obtained from the Microbiology Laboratory, Khenchela University, Algeria, and maintained at 4°C on nutrient agar slants. Mueller-Hinton agar (40g in 1 liter of distilled water) was prepared, and bacterial samples were swabbed into sterilized saline tubes, mixed, and drained. Petri dishes were divided into sections for negative control (distilled water), positive control (oxacillin), methanolic extract, and essential oil, with sterile 6 mm discs placed in each section. The solutions were applied to the discs, dried, and incubated at 37°C for 24 hours (Al Laham and Al Fadel, 2014).

For the antioxidant study, a DPPH solution (5 mg DPPH in 100 ml methanol) was prepared, and 1 ml of this solution was combined with 1 ml of methanolic rosemary extract, shaken and incubated for 30 minutes. Controls included a negative control (1 ml methanolic DPPH + 1 ml methanol) and a blank (2 ml methanol). Readings were taken at 517 nm, with ascorbic acid as a positive control (Adris et al., 2019). The antioxidant capacity was quantified by IC50, the concentration required to reduce free radicals by 50%

(Rodríguez-Rojo et al., 2012).

Results and dissection

Phytochemical study

Based on phytochemical screening method for (*Rosmarinus officinalis*), the results tested positively for tannins, mucilage, saponins, and flavonoids indicating their presence in the plant. Conversely, alkaloids and coumarin yielded negative results, indicating their absence These results emphasizing the plant's potential health benefits.

.All results obtained from phytochemical screening on the *Rosmarinus officinalis* are recorded in (Table 1).

Table 2. Chemical composition of rosemary

Composition	Results
Alkaloids	-
Tannins	+
Coumarin	-
Mucilage	+
Saponins	+
Flavonoids	+

(+) Presence (-) **Absence**

Results of the methanolic extract

The search results provide valuable information on the extraction of phenolic compounds from *Rosmarinus officinalis* (Rosemary) using different solvents and extraction methods. Specifically, the results indicate that the yield of methanolic extracts of rosemary can vary significantly depending on the extraction conditions.

The reported extraction yield of 9.3% for a methanolic extract of rosemary is within the range observed in the literature. For example, the search results mention that ethanol-water mixtures (59-70% ethanol) and acetone (80%) have been found to be effective for the simultaneous extraction of the key antioxidant compounds in rosemary, such as carnosic acid, carnosol, and rosmarinic acid, with yields around 90% (Santana-Méridas et al., 2014). In comparison, methanol-based extracts were found to have lower yields of carnosic acid due to its transformation to carnosol.

Furthermore, the search results highlight that the extraction method can significantly impact the yield and composition of the rosemary extracts. Conventional solvent extraction (CSE) methods typically require longer extraction times (30-180 minutes) compared to novel techniques like ultrasound-assisted extraction (UAE), which can achieve similar or higher yields in a much shorter time (10-12 minutes) (Psarrou et al., 2020). The enhanced efficiency of UAE is attributed to the improved mass transfer and cell disruption caused by the ultrasonic cavitation effects.

Additionally, the search results indicate that factors such as temperature, solvent-to-solid ratio, and the presence of water in the solvent can all influence the extraction yield and the relative proportions of the key phenolic compounds (Psarrou et al., 2020). For example, higher temperatures (up to 50°C) can increase the extraction rate, but may also lead to increased oxidation of carnosic acid to carnosol.

Table 3. Yield of the methanolic extract

The amount of extract from 150g	***Rosmarinus officinalis***
Yield (g)	10.78
Yield **%**	9.33

Results of polyphenol and flavonoid assays

The results of the polyphenol and flavonoid assays are summarized in Table 3. The methanolic extract was found to contain 129.55 mg of polyphenols and 92.5 mg of flavonoids, indicating a rich content of these compounds in the extract. Specifically, the polyphenol content was higher than the flavonoid content in the methanolic extract.

In the course of phytochemical experiments focusing on chemical compounds, it was discovered that the powdered extract of rosemary also contains flavonoids. However, upon conducting an assay on the methanolic extract, it was noted that the quantity of flavonoids present was lower compared to the amount of polyphenols.

Comparing the total polyphenol content of *Rosmarinus officinalis* with previous studies, it was found to be consistent with the findings of Erkan *et al*. (2008) who reported a content of 162 mg GAE/g and Saini *et al*. (2020) with a value of 127 ± 3 mg GAE/g. The total polyphenol content in this study was calculated to be 136.66 ± 7.41 mg GAE/g. On the other hand, Saini *et al*. (2020) reported a flavonoid content of 37.13 ± 6.04 mg rutin/g, indicating a lower concentration of flavonoids compared to polyphenols in the methanolic extract. Variation in the chemical composition of polyphenolic extracts have been attributed to many factors, including a biotic stress,genetic heritance and the phenological stages of the plants (Jordan et al., 2013).

Table 4. Flavonoid and polyphenol content of *Rosmarinus officinalis*

Content (mg GAE/g)	***Rosmarinus officinalis***
Total polyphenol content	129.55
Flavonoid content	92.5

Antibacterial study

Rosemary essential oil extract

Table 4 shows that extracting rosemary essential oil from 3 kg of plant material yielded 11 ml, which corresponds to a 2% yield.

Table 5. Yield of Rosemary essential oil extraction

The quantity of extract from 3 kg	**Rosemary essential oil extract**
Yield (%)	2%
Yield (ml)	11 ml

Results of antibacterial tests

Tables 5 and 6 present comparative analyses of antimicrobial efficacy against *Staphylococcus* and *Escherichia coli*, respectively. For *Staphylococcus* (Table 5), both distilled water and the methanolic extract showed no inhibition (0 mm), while the essential oil demonstrated significant effectiveness with a 17 mm inhibition zone. The antibiotic proved most potent, producing a 32 mm inhibition zone.

In the case of *E. coli* (Table 6), a more nuanced response was observed. While distilled water again showed no effect (0 mm), the methanolic extract exhibited mild antimicrobial activity with an 8 mm inhibition zone. The essential oil demonstrated strong efficacy, creating a 19 mm inhibition zone, nearly matching the antibiotic's performance (21 mm zone).

These results reveal differential sensitivity patterns between the two bacterial strains. Both exhibited resistance to the negative control (distilled water) while showing pronounced susceptibility to the essential oil and antibiotic. Notably, the methanolic extract's effectiveness varied between the strains, being inactive against *Staphylococcus* but mildly effective against *E. coli*.

Table 6. Average inhibition zones of Staphylococcus

Zones of Inhibition	Sensibilité	Diameter (mm)
Distilled Water	None	0 (negative)
Methanolic Extract	None	0 (negative)
Essential Oil	Very sensitive	17
Antibiotic	Extremely sensitive	32

Figure 4. Test photograph on the *staphylococcus* strain.

Our findings align with those reported by Celiktas et al. (2012) regarding the methanolic extract's lack of antibacterial activity against *Staphylococcus*. However, our observation of mild activity against *E. coli* suggests potential strain-specific effects. The essential oil's robust antimicrobial action against both strains corroborates its potential as a natural antimicrobial agent, as also noted by Elyemni et al. (2022).

Table 7. Average inhibition zones of *E. Coli*

Zones of Inhibition	Sensitivity	Diameter (mm)
Distilled Water	None	0
Methanolic Extract	Sensitive	8
Essential Oil	Very sensitive	19

Antibiotic	Extremely sensitive	21

Figure 5. Test photograph on *E.coli* strain. .

Several studies (Vaara, 1992; Myali et al., 2020) suggests that *Rosmarinus officinalis* leaf extract demonstrates multiple mechanisms of action and exhibits enhanced biological effectiveness against gram-positive bacteria. The varying sensitivity between bacterial types can be attributed to structural differences. In gram-negative bacteria, the presence of an outer membrane surrounding the cell wall, along with a periplasmic space containing enzymes that can degrade external substances, likely explains their reduced susceptibility to the extract. These protective features in gram-negative bacteria may account for the observed differences in antimicrobial efficacy. Research conducted by Santoyo et al., (2005) revealed that the essential oil extracted from *Rosmarinus officinalis* (rosemary) demonstrates effective antibacterial properties. Their findings showed that this essential oil successfully inhibited the growth of *Staphylococcus aureus*, a gram-positive bacterium. Moreover, the study also found that the same essential oil was capable of inhibiting *Escherichia coli*, a gram-negative bacterium. These results highlight the broad-spectrum antimicrobial potential of rosemary essential oil, as it proved effective against both gram-positive and gram-negative bacterial strains.

Antioxidant study

Our findings demonstrate significant antioxidant activity in the methanol extract of rosemary, with a value of 0.45 mg/ml for free radical inhibition.

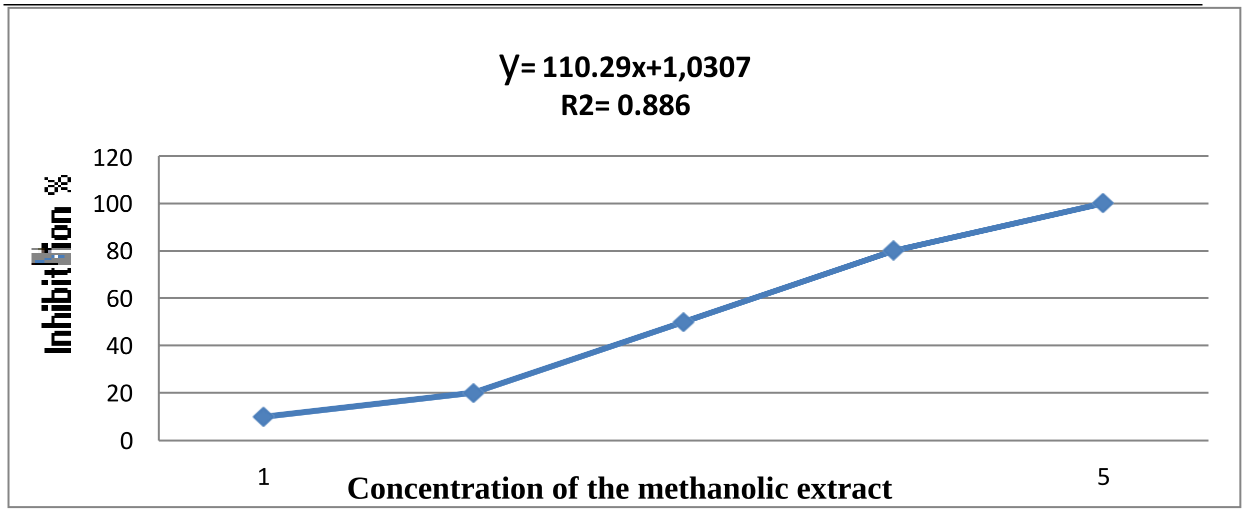

Figure 6. Percentage of DPPH inhibition as a function of EM concentrations.

This low IC50 value indicates high antioxidant efficacy, as it represents the concentration needed to neutralize 50% of free radicals. Furthermore, our findings on the antioxidant activity of rosemary essential oils corroborate previous studies. We observed striking similarities in both free radical scavenging capacity and chemical composition to rosemary oil derived from plants cultivated in Tunisia (Kadri et al., 2011). These results collectively highlight rosemary's significant potential as a natural source of antioxidants, with consistent properties across different extraction methods and geographical locations. According to Rašković et al, (2014) found that essential oil from *Rosmarinus officinalis* (rosemary) sourced in Serbia demonstrated strong antioxidant properties. Its effectiveness, measured by an IC50 value of 77.6 µL/mL, was notable when compared to the potent antioxidant vitamin E (α-tocopherol), which had an IC50 of 25.3 µg/mL. Hussain et al., (2010) examined *R. officinalis* essential oil from Pakistan. They observed even more powerful antioxidant activity, with an IC50 of 20.9 ± 0.9 µg/mL. This outperformed 1,8-cineole, the primary constituent of *R. officinalis essential* oil, which had an IC50 of 45.7 ±

1.5 µg/mL.

Carnosic acid and carnosol, found in rosemary, offer benefits beyond their antioxidant properties, which are mainly seen in the liver (Rašković et al.,2014). These compounds demonstrate anti-inflammatory effects and have the ability to influence lipid and glucose metabolism. Due to their impact on metabolic processes, they show promise in the treatment of diabetes mellitus and its related health issues. This combination of antioxidant, anti-inflammatory, and metabolic regulatory effects makes these compounds particularly interesting for medical research and potential therapeutic applications (Akrout et al., 2010).

Conclusion

The data obtained from this study demonstrated that the plant extracts from *Rosmarinus officinalis* possess considerable amounts of phytochemical compounds, antioxidant properties and antibacterial activity. The plant material was collected from Khyrene Sector of Khenchela (Algeria).The analyses of phytochemical screening showed that the plant contain flavonoids, tannin, saponins, mucilage, and polyphenols. However, alkaloids and coumarin was absent. The reported extraction of phenolic compounds was 9.3%, while the methanolic extract was found to contain 129.55 mg of polyphenols and 92.5 mg of flavonoids, indicating a rich content of these compounds in the extract. This study also shows that *Rosmarinus officinalis* extracting essential oil yielded 11 ml, which corresponds to a 2% yield. The antibacterial activity of, methanolic extract and essential oil of rosemary was determined against E. coli and staphylococcus auras, the results reveal differential sensitivity patterns between the two bacterial strains. Both exhibited resistance to the negative control (distilled water) while showing pronounced susceptibility to the essential oil (17 mm inhibition zone for Staphylococcus and 19 mm for Escherichia coli) and antibiotic (32 mm inhibition zone for Staphylococcus and 21 mm for Escherichia coli). Notably, the methanolic extract's effectiveness varied between the strains, being inactive against Staphylococcus but mildly effective against E. coli. The results showed that the highest mean inhibition zone was observed in the methanolic extracts. Our findings demonstrate significant antioxidant activity of rosemary, with a value of 0.45 mg/ml. Thus, the researcher suggests future research to do an advanced Analyses to perform test on their biological activities and pharmacological properties.

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