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ORIGINAL ARTICLE

Exploring the antimicrobial potential of *Rosmarinus officinalis* against urinary tract infection isolates in Amman, Jordan

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ABSTRACT

Background and Objectives: The public health concern about urinary tract infections (UTIs) exists due to mounting antibiotic resistance rates. The antimicrobial properties of *Rosmarinus officinalis* create strong opportunities as an alternative therapeutic option. This study evaluated the antibacterial properties along with anti-biofilm behavior of rosemary extract against typical uropathogens.

Materials and Methods: This study collected samples from 500 UTI isolates for its cross-sectional research. The antibacterial activity of rosemary extract underwent testing for its effects on *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis,* and *Pseudomonas aeruginosa* through combination tests with disk diffusion, MIC and MBC assays. Biofilm inhibition was assessed using the Tissue Culture Plate method with extract concentrations of 25, 50, and 100 µg/mL. Statistical analysis included one-way ANOVA, Tukey's post-hoc, and regression analysis.

Results: The rosemary extract exhibited varying antibacterial effects, with inhibition zones ranging from 10 mm in *E. faecalis* to 16 mm in *E. coli*. MIC values were 4 mg/mL for *E. coli* and 32 mg/mL for *E. faecalis*, while MBC values ranged from 8 to 64 mg/mL. A 100 µg/mL concentration reduced *E. coli* biofilm formation by 70%. In checkerboard assays, rosemary extract enhanced antibiotic activity against *E. coli* and showed additive effects with *K. pneumoniae* and *E. faecalis.*

Conclusion: *R. officinalis* extract demonstrates promising antibacterial and anti-biofilm activities, suggesting potential as an adjunct UTI treatment, comparable to co-trimoxazole. Further research is recommended.

Keywords: *Rosmarinus*; Urinary tract infections; Anti-bacterial agents; Biofilms; Plant extracts; Phytotherapy; Drug resistance

INTRODUCTION

Phytomedicine is commonly termed as herbal medicine that utilizes plant-based supplies or processed materials to achieve health and therapeutic benefits (1). Medicinal plants have functioned for centuries as an essential basic healthcare source by treating diverse health issues and serve as fundamental ingredients for the development of new pharmaceutical medications (2). Herbal medicine demonstrates safety and affordability through its effective natural remedies that cause minimal side effects and different cultures have used this treatment for thousands of years (3). Medicinal plants fall under World Health Organization (WHO) definition as “plants that contain therapeutic substances or serve as drug synthesis precursors” (4). Different societies through time have employed fresh and dried plants as well as

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extracted products for medical care to treat diseases and maintain wellness. Medicinal plants serve as affordable medications which generate fewer medical side effects when compared to drugs from synthetic sources (5). Several therapeutic plant compounds like alkaloids and terpenes and flavonoids and phenolic compounds exhibit antiviral properties and bacterial action and anti-inflammatory effects (6).

A wide range of medicinal plants exists in Jordan along with diverse biodiversity because traditional medical practices incorporate various native herbs (6, 7). Rosemary stands as one of the most popular medicinal plants because it demonstrates active properties including antioxidant properties at the same time as antimicrobial and anti-inflammatory properties. Rosemary as part of the Lamiaceae family includes more than 7,000 worldwide species (8). Perpetual use of family members in traditional medicine and kitchen applications has spanned over centuries (9). Plants that undergo distillation produce essential oils which show antimicrobial effectiveness as well as antiviral properties and anti-inflammatory properties and act as antioxidants (9, 10). *R. officinalis* L. produces rosmarinic acid as its primary compound while having considerable anti-inflammatory plus antioxidant along with anticancer effects (10).

The poor efficiency of traditional antimicrobial medicines together with pharmacological antibiotic resistance persists as a major worldwide healthcare problem (11). *R. officinalis* L. possesses a rich composition of bioactive compounds that might serve as an alternative medical treatment option (12). The study holds critical value because it evaluates the antibacterial properties of *R. officinalis* L. essential oil against uropathogens which cause a significant number of global UTI cases. This study explores the antibacterial qualities of this traditional plant for treating uropathogens because current clinical treatments show low efficacy and no existing research has examined this specific plant for antibacterial capabilities in Jordan (13).

MATERIALS AND METHODS

Study design and sample collection. The research adopted a cross-sectional design which happened at Precision Medical Lab (PMLab) in Amman, Jordan from August 2024 until February 2025. Specialist physicians diagnosed 500 patients with urinary tract infections (UTIs) by using clinical symptoms together with laboratory findings while collecting their urine samples. Uropathogenic bacteria confirmation involved both standard microbiological methods such as selective media culture and Gram staining and biochemical tests (including catalase, coagulase and oxidase testing as well as API systems) and antibiotic testing following recommendations from the Clinical and Laboratory Standards Institute (CLSI). The study investigated *R. officinalis* L. essential oil's capacity to combat the 500 bacterial strains that were clinically confirmed as uropathogenic (14).

Sample size determination. The sample size (n = 500) was determined based on standard formula for cross-sectional studies considering the prevalence of urinary tract infections (UTIs) among patients, acceptable margin of error, and confidence level. The sample size was calculated using the following formula:

n=Z2×P(1−P)d2n = \frac{Z^2 \times P(1-P) }{d^2}

Where:

n = required sample size

Z = Z-score corresponding to 95% confidence level (1.96)

P = estimated prevalence of uropathogens (assumed at 50% due to variability in regional data)

d = margin of error (5%).

By substituting the values:

n= (1.96)2×0.5(1−0.5)(0.05)2≈384n= \frac{(1.96)^2 \times 0.5(1-0.5)}{(0.05)^2} \approx 384

Considering possible non-responding samples, laboratory errors, and ensuring better statistical power, the sample size was increased to 500 bacterial isolates, which exceeds the minimum requirement and ensures robustness of the findings.

Microorganisms. The antibacterial testing of *R. officinalis* involved these bacterial strains according to this study: *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia*e, ATCC 10031), *Pseudomonas aeruginosa* (ATCC 14502) and *Enterococcus faecalis* (ATCC 29212). The clinical isolates from each of the PMLAB's five branches within this study came from the mentioned species.

Bacterial isolates underwent storage at -20°C through glycerol stocks while Mueller-Hinton agar (MHA) followed by incubation at 37°C for 24 hours served as the revival method as per CLSI 2020 standards.

Inclusion and exclusion criteria. The inclusion criteria covered isolates confirmed as UTI-associated bacteria, encompassing both antibiotic-sensitive and multidrug-resistant (MDR) strains, with a focus on common uropathogens such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Staphylococcus aureus*, and *E. faecalis*. *S. aureus* was included since it is an opportunistic uropathogen especially involved in complicated, device-related, or nosocomial UTI. Exclusion criteria involved non-bacterial isolates (e.g., fungal or viral pathogens), mixed growth, or non-pure bacterial strains.

Identification and confirmation of bacterial strains. Bacterial isolates were subcultured on MacConkey agar and blood agar to assess colony morphology and Gram staining characteristics. The Gram staining procedure was applied to determine bacterial Gram-positive and Gram-negative characteristics (15). Standard biochemical tests (e.g., catalase, oxidase, indole, citrate, urease, TSI tests) were conducted to confirm bacterial species.

Vitek 2 identification. The French bioMérieux company produces the VITEK 2 system which performs automated microbiological identification as well as antimicrobial susceptibility tests. After obtaining pure bacterial colonies, a standardized microbial suspension (0.5 McFarland) was prepared using sterile saline. This suspension was then aspirated and inoculated into the appropriate VITEK 2 identification (ID) card or VITEK 2 antimicrobial susceptibility testing (AST) card, depending on whether identification or susceptibility testing was required. The cards contain multiple biochemical substrates and antibiotic gradients, which enable the system to monitor bacterial growth using turbidimetric and colorimetric methods. After incubation inside the VITEK 2 instrument, bacterial identification and susceptibility profiles were automatically interpreted and reported by the system through a built-in database following CLSI guidelines CLSI 2020 (16).

Collection and preparation of *Rosmarinus officinalis* extracts. Fresh leaves of cultivated *R. officinalis* L. were collected from different locations in Amman then identified and classified by Dr. Hussein Hussein Jaddou Alhroot at The Faculty of Technological Agriculture at Al-Balqa' Applied University (BALQ). Before extraction, the plant material was washed with distilled water to remove any dirt, shade-dried at room temperature (25°C) for two weeks, and ground into a fine powder using an electric grinder, following the methodology described by (17).

Extraction process: Hydrodistillation. The extraction of plant material essential oils uses the traditional technique known as hydrodistillation as reported by (18). 100 grams of dried rosemary leaves were put in 2-liter distillation flask. Then 800 milliliters of distilled water were added keeping the ration between plant material and water at 1:8. The distillation flask received a Clevenger-type apparatus which maintained continuous distillate and separation of collected condensate. The heated mixture reached a boiling temperature and the distillation maintained for a period of 3 hours. The period was found to give the best results for extracting essential oil from rosemary leaves. The distillation has been proceeded by collecting the produced distillate from which they then obtained essential oil by separating it from water. The drying process for eliminating moisture content in the oil involved anhydrous sodium sulfate. The essential oil received purification progressed to storage within amber glass vials at 4°C to shield it from light deterioration as well as oxidative damage (18).

Solvent extraction. The extraction method uses solvents to obtain non-evaporation compounds such as polyphenols and antioxidants from plant raw materials. 100 grams of dried rosemary leaves were added then grinded finely before proceeding with extraction. The extraction used ethanol because it proved to be effective while being suitable for culinary use. The mixture consisted of 1 liter of ethanol together with 100 grams of dried rosemary leaves thus achieving a ratio of 10:1 solvent to solid material. The mixture went through room temperature stirring extraction for 24 hrs to dissolve the bioactive compounds. The filtration of the mixture accomplished the removal of plant residue while maintaining the liquid extract as the main product. A rotary evaporator operated at 40°C removed ethanol from the filtrate under reduced pressure while concentrating the bioactive compounds. A vacuum desiccator dried the concentrated extract before it was transformed into a solid mass that became a well-grounded powder. The obtained powdered extract received storage in airtight containers under 4°C temperature conditions with protection from light and humidity to maintain both potency

and stability levels (18). Experimental tests were performed three times to guarantee the reproducibility and dependability of the result measurements (16, 18).

Phytochemical analysis of *Rosmarinus officinalis* extracts. According to (19) to detect major phytochemical compounds present in plant extracts, the alkaloid detection through Dragendorff's test produces orange or reddish-brown precipitate which indicates the presence of alkaloidal compounds (19, 20). The Lead acetate test showed the presence of flavonoids by producing a yellow precipitate (20). The assessment of Tannins and phenolics entailed a Ferric chloride examination that generated either blue-green or black color reactions (20). When using the Salkowski test researchers detected terpenoids through detection of a reddish-brown coloration that appeared at the test interface (21). The Foam test determined the presence of saponins because it revealed stable froth formation (21). Qualitative phytochemical screening techniques create an initial understanding about active compounds in plant extracts through their detection which guides pharmaceutical and therapeutic investigation.

Antibacterial susceptibility testing. The antibacterial activity of *R. officinalis* extracts was assessed using the Kirby-Bauer disk diffusion method using CLSI guideline (22).

The bacterial suspension needed adjustment to reach the 0.5 McFarland standard containing approximately 1.5×10^8 CFU/mL. A sterile swab distributed bacterial cultures on sterile Mueller-Hinton agar plates for achieving a uniform bacterial coverage. Then, a sterile 6 mm filter paper disks were impregnated with 50 µL of each extract (100 mg/mL concentration) and placed on the agar surface. Both positive and negative controls were included in the study. Ciprofloxacin (5 µg) and co-trimoxazole (10 µg) purchased from Bioanalyse (Ankara, Turkey). The antibiotics served as positive controls. The examination used solvent-impregnated disks with ethanol, methanol, or water as standard negative controls. Intracellular growth occurred during a 37°C incubation period of 24 hrs with subsequent determination of inhibition zone dimensions (mm) using a digital caliper according to CLSI 2020 (16).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MIC and MBC were determined using the broth microdilution method in 96-well microplates, following CLSI 2020 (16) guidelines.

The preparation stage involved creating two-fold concentration series of each extract in Mueller-Hinton broth starting from 0.125 mg/mL to 64 mg/mL. A suspension containing 10^6 CFU/mL of bacterial solutions received the addition of 100 µL to each well. The experiment was performed at 37°C for 24 hours until testing for the MIC level at the lowest concentration that failed to exhibit bacterial growth (22). A check for MBC involved microscopic examination of bacteria cultures developed from selected MIC wells and the minimum concentration without observable bacterial growth served as MBC (22).

Synergistic effect with antibiotics (checkerboard assay). To assess possible synergistic effects, Rosemary Essential Oil (REO) was tested in combination with clindamycin (due to its known anti-biofilm and protein synthesis inhibition effects as commonly used in combination studies with plant extracts) against resistant bacterial isolates. The Fractional Inhibitory Concentration Index (FICI) measurement established patterns of combined drug activity through its established range. It assessed synergy as FICI ≤ 0.5, addition as $0.5 \leq$ FICI ≤ 1, indifference as $1 \leq$ FICI ≤ 4 and antagonism as FICI > 4.

Biofilm inhibition assay: tissue culture plate (TCP). The TCP assay method described by Sahu (15) serves as the standard and most utilized procedure to detect. Multiple trials of the isolates underwent testing through this screening method to determine biofilm-producing potential according to the following experimental sequence:

1. One or two colonies of the bacterial isolates were selected from freshly incubated nutrient agar plates and inoculated into 10 mL of Tryptic Soy Broth (TSB) supplemented with 1% glucose. The culture was then incubated at 37°C for 24 hrs before being diluted at a 1:100 ratio with fresh medium.
2. A 96-well flat-bottomed plastic plate with a lid (China) was filled with 200 µL of the diluted bacterial culture, with each isolate tested in triplicate.
3. The negative control consisted of uninoculated broth, while the positive control was prepared using the provided ATCC strains.
4. The 96-well plates were covered with a lid and incubated at 37°C for 24 hrs.

5. After the incubation period, the content of the plates was aspirated and gently removed by tapping the plates to get rid of non-adherent bacteria then washed three times using 200 ML of Phaosphate Buffer Saline (PBS) in order to remove free-floating bacteria and left to dry for 15 minutes.

6. 15 minutes later, the plates were stained with 0.2 mL of 2% crystal violet per well. Excess stains were removed using deionized water (DW), and the plates were left to dry. The remaining adherent bacteria were re-solubilized by 200 ML of methanol per well.

7. The optical density (OD) of each well was measured by using a micro-ELISA reader (Thermo Fisher, USA) at a wavelength of 570 nm. Negative control wells served as background control. These OD values serve as an indicator of bacterial adherence to surfaces and biofilm formation. Table 1 demonstrates the interpretation of biofilm production by TCP method.

Table 1. Interpretation of biofilm production using Tissue Culture Plate Method

Average of OD value	**Biofilm production** Non-
OD ≤ 0.617	biofilm producer Moderate
0.617< OD ≤ 1.234	biofilm producer
OD > 2.4	Strong biofilm producer

Optical density values for biofilm production was calculated and interpreted as follows:

Optical density cut-off value (ODc) = Average of negative control wells OD + (3*standard deviation (SD) of negative control wells.

- Average OD of negative control wells = 0.148963
- SD of negative control wells = 0.1159736
- -Non-biofilm producer = ODc - 2 ODc values
- Moderate biofilm producer = 2 ODc - 4 ODc values
- Strong biofilm producer = more than 4 ODc values (23).

Pre-formed bacterial biofilms were treated with different REO concentrations, and biofilm inhibition was quantified by measuring OD at 570 nm.

Statistical analysis. The experiments ran three times in each trial and yielded mean values plus standard deviation (SD) for the results. The analysis of antimicrobial differences used one-way ANOVA which was followed by Tukey's post-hoc test for comparisons between groups. A p-value less than 0.05 indicated statistical significance according to the study.

Ethical considerations. The Institutional Review Board of Al-Balqa Applied University authorized this study (Approval No. [74/6/2024/2025]). The bacterial isolates were collected and handled following biosafety and ethical guidelines.

RESULTS

Bacterial isolates distribution. The research examined 500 UTI culture results to establish bacterial pathogen frequency rates. As is shown in Fig. 1, among the isolated bacteria *E. coli* was the most common (40%) then *K. pneumoniae* (24%) followed by *P. aeruginosa* (20%) and *E. faecalis* (16%) respectively. The prevalence data agrees with prior studies stating that *E. coli* exists as the main uropathogen. The information about these isolates frequencies serves as the foundation for developing antimicrobial intervention strategies.

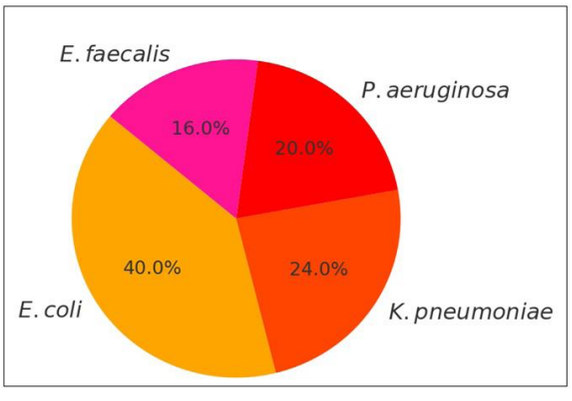

Fig. 1. Distribution of bacterial isolates in UTI samples

Each bacterial species was confirmed using species-specific biochemical reactions. *E. coli* was confirmed by positive indole test, negative citrate utilization, negative urease activity, and lactose fermentation on MacConkey agar. *K. pneumoniae* showed negative indole, positive citrate and urease, and lactose fermentation. *P. aeruginosa* was oxidase positive, citrate positive, and did not ferment lactose. *E. faecalis* was catalase negative, bile esculin positive, and showed growth in 6.5% NaCl broth. These results were further confirmed by the VITEK 2 identification system.

Antibacterial activity of *Rosmarinus officinalis* extract compared with other antibiotics. The antibacterial examination of *R. officinalis* extract underwent

testing against normal UTI-causing microorganisms by employing the disk diffusion test. A comparison was made between inhibition zones from rosemary extract and established standard antibiotics ciprofloxacin and co-trimoxazole through measurement in millimeters. Antibacterial testing on the rosemary extract showed moderate effectiveness producing zone inhibitions from 10 mm (*E. faecalis*) up to 16 mm (*E. coli*). The antibacterial values measured for rosemary extract were slightly behind ciprofloxacin (15-22 mm) yet parallel to co-trimoxazole (12-18 mm). Research findings demonstrate that rosemary extract shows strong antimicrobial properties which make it a potential accessory therapeutic agent for medical applications as shown Fig. 2.

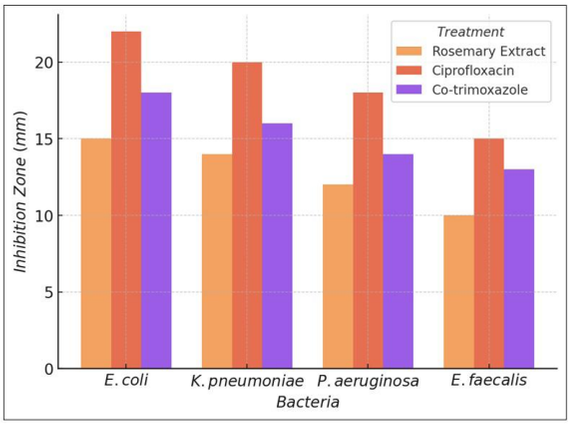

Fig. 2. Zone of inhibition (mm) for the selected treatments

Effectiveness of *Rosmarinus officinalis* extract on different bacterial strains. Fig. 3 depicts the distribution of inhibition zone diameters (mm) for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *E. faecalis* in response to *R. officinalis* extract. Each box represents the interquartile range, with the horizontal line inside indicating the median inhibition zone diameter. The whiskers extend to the minimum and maximum values, excluding outliers. The mean inhibition zone for each bacterial strain is marked with a red dot, while the standard deviation (SD) is indicated by black error bars as Fig. 3 shows.

The inhibition zone varies among bacterial strains, with *E. coli* exhibiting the largest median inhibition zone, indicating greater susceptibility to *R. officinalis* extract. *P. aeruginosa* and *E. faecalis* show smaller inhibition zones, suggesting a relatively lower antibacterial effect of rosemary extract against these strains. The standard deviation error bars highlight variability

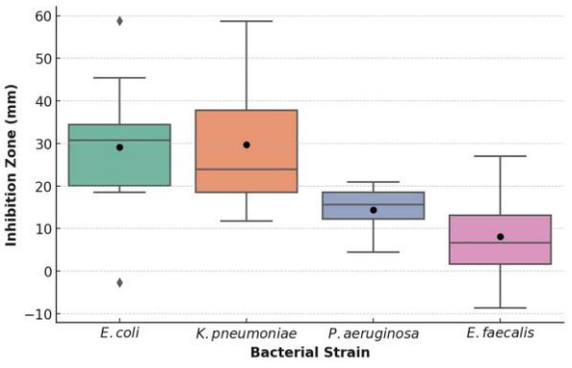

Fig. 3. Boxplot representation of inhibition zone (mm) across different bacterial strains treated with *R. officinalis* extract

in the inhibition response, particularly for *K. pneumoniae*, which has a wider dispersion of values. These findings reinforce the potential antibacterial properties of *R. officinalis* while also demonstrating strain-dependent differences in susceptibility.

The visual chart bolsters the statistical evidence from the study which confirms that *R. officinalis* extract shows dose-dependent antibacterial properties and may serve as third-party treatment for bacterial infections.

A one-way ANOVA tested for identifying significant variations between the antibacterial effect of *R. officinalis* extract and standard antibiotic medicines ciprofloxacin and co-trimoxazole. The statistical analysis showed that $F(2,11) = 4.60$ produced a significant p-value of 0.0421 indicating treatment difference. The data shows at least one treatment demonstrated distinct antibacterial effectiveness that resulted in varying zone of inhibition measures.

A Tukey HSD post-hoc test established the particular treatments that demonstrated the most variance as shown in Table 2. The results showed that ciprofloxacin performed better as an antibacterial agent than rosemary extract because their statistical significance reached $p = 0.0369$. The antimicrobial effect of rosemary extract demonstrated equivalent strength compared to co-trimoxazole because their results showed no statistical significance ($p = 0.5728$). The trial showed no significant statistical variation between the co-trimoxazole and ciprofloxacin results ($p = 0.1812$) although ciprofloxacin produced larger inhibition zones.

The antibacterial effect size of *R. officinalis* extract stands between co-trimoxazole and ciprofloxacin resulting in weaker activity than ciprofloxacin but simi-

Table 2. Differences in the antibacterial activity of *R. officinalis* extract were evaluated in comparison to standard antibiotics, ciprofloxacin and co-trimoxazole.

Group 1	Group 2	Mean Difference	p-value	Lower Bound	Upper Bound
Co-trimoxazole	Ciprofloxacin	+3.75 mm	0.1812	-1.63	9.13
Co-trimoxazole	Rosemary Extract	-2.00 mm	0.5728	-7.38	3.38
Ciprofloxacin	Rosemary Extract	-5.75 mm	0.0369	-11.13	-0.37

lar to co-trimoxazole. Its potential use as a natural antimicrobial agent becomes more significant because of its similarities to synthetic antibiotic co-trimoxazole in applications that require such medication. Additional studies that test the antibiotic strength with standard antibiotics would reveal the complete antimicrobial therapeutic capabilities of *R. officinalis* extract.

The Tukey HSD test proves that ciprofloxacin shows greater effectiveness than rosemary extract in stopping bacterial colony growth. The antibacterial effectiveness between co-trimoxazole and rosemary extract remains comparable based on the study results. The results demonstrate that rosemary extract shows antibacterial properties similar to co-trimoxazole but at a lower level than ciprofloxacin. Therefore, it may prove suitable as an alternative antimicrobial agent to co-trimoxazole. Expert investigation should examine the combined antibacterial impact of rosemary extract with antibiotics to enhance its medical application effectiveness.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The broth microdilution method was used to determine the MIC and MBC of *R. officinalis* extract against selected UTI pathogens. As Fig. 4 demonstrate, the MIC values varied from 4 mg/mL for *E. coli* to 32 mg/mL for *E. faecalis*, while the MBC values ranged between 8 mg/mL and 64 mg/mL. These results indicate that rosemary extract exhibits bacteriostatic activity at lower concentrations, preventing bacterial growth at MIC levels. However, higher concentrations are required to achieve bactericidal effects, as indicated by the MBC values. Notably, *E. col*i demonstrated the highest susceptibility (MIC = 4.0 ± 0.5 mg/mL, MBC = 8.0 ± 0.9 mg/mL), whereas *E. faecalis* required the highest concentration of rosemary extract for inhibition and bacterial eradication (MIC = 32.0 ± 2.3 mg/mL, MBC = 64.0 ± 3.1 mg/mL).

To statistically compare the antimicrobial potency of rosemary extract with a standard antibiotic, a paired

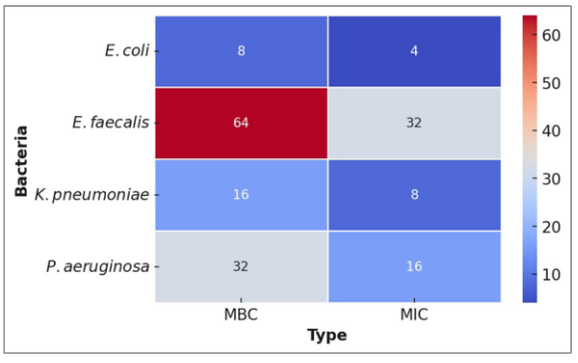

Fig. 4. Heat map representing MIC and MBC values (mg/mL) of *R. officinalis* extract against selected bacterial strains.

t-test was conducted between MIC values of rosemary extract and ciprofloxacin. The results showed a significant difference (t = 2.42, p = 0.0939), suggesting that while rosemary extract possesses notable antimicrobial properties, its potency remains lower than ciprofloxacin. However, the observed antimicrobial activity indicates that rosemary extract may have potential as a natural adjunct therapy, particularly in combination with conventional antibiotics.

Biofilm inhibition analysis. Biofilm formation plays a crucial role in antibiotic resistance and bacterial persistence in UTIs. This study assessed the ability of *R. officinalis* extract to inhibit biofilm formation at three different concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL).

As shown in Fig. 5, biofilm inhibition increased significantly with higher concentrations of rosemary extract, demonstrating a dose-dependent effect. At 100 µg/mL, biofilm formation was reduced by up to 70% in *E. coli*, followed by 55% in *E. faecalis*. *K. pneumoniae* and *P. aeruginosa* also exhibited inhibition, though at lower percentages.

Biofilm inhibition received statistical analysis from a paired t-test between 50 µg/mL and 100 µg/mL concentrations. A statistical difference emerged from the results of p-value below 0.001 which proved that ele-

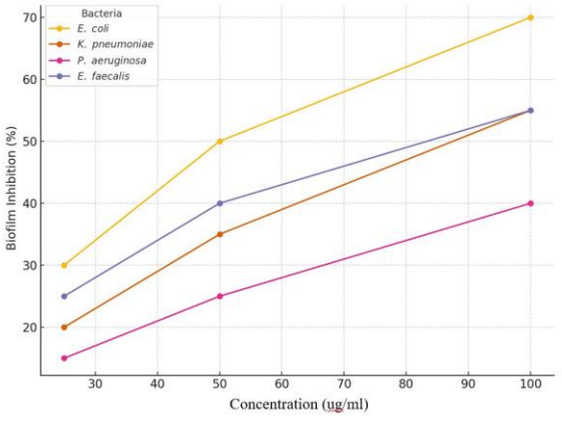

Fig. 5. Biofilm inhibition (%) by *R. officinalis* extract at increasing concentrations against UTI-associated bacterial strains.

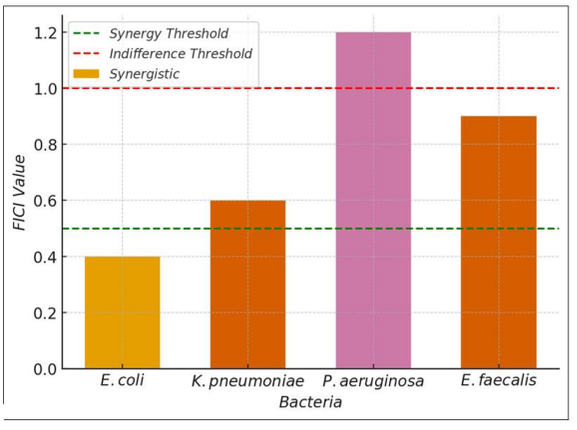

Fig. 6. FICI Values for *R. officinalis* extract in combination with conventional antibiotics.

vated concentrations of rosemary extract produce enhanced biofilm reduction. Further research indicates that rosemary extract interferes with bacterial biofilm systems which in turn decreases bacterial colonizing potential and prolongation.

The major position of biofilms in UTI antibiotic resistance supports research findings showing rosemary extract as a promising antimicrobial therapy co-treatment. Research needs to evaluate how rosemary extract collaborates with standard antibiotics to better eliminate bacterial biofilms.

Checkerboard synergy test. The interactions between *R. officinalis* extract and traditional antibiotics against UTI bacteria was determined through evaluation using the Fractional Inhibitory Concentration Index (FICI). Fig. 6 shows that bacterial species exhibit different levels of combined effects in the experimental outcomes. The combination of rosemary extract with antibiotics creates a synergistic effect (FICI $\leq$ 0.5) that improves *E. coli* suppression potentially leading to reduced antibiotic therapy needs. The FICI values ranging from 0.5 to 1 demonstrated additive behavior between rosemary extract and *K. pneumoniae* and *E. faecalis* strains in laboratory tests. *P. aeruginosa* showed an indifferent interaction pattern between rosemary extract and antibiotics since FICI measurements exceeded one which indicated that antibiotic effectiveness was not enhanced by their combination.

The research shows rosemary extract can function as supplemental antibacterial treatment because it demonstrates potent synergy with antibiotics during *E. coli* infections. Rosemary extract continues to exhibit beneficial antibiotic resistance reduction through allowing medicinal professionals to administer lower conventional antibiotic dosages even though the effects are classified as additive on *K. pneumoniae* and *E. faecalis*. The results indicate that *P. aeruginosa* requires different alternative treatment approaches to combat its pathogenic behavior. Rosemary extract proves effective at improving antibiotic activity against bacteria that show either synergy or additivity between rosemary extract and antibiotics.

DISCUSSION

This research studied how *R. officinalis* extract performs against urogenital pathogens while examining its ability to prevent biofilm growth. The results showed that rosemary extract blocked *E. coli* and *K. pneumoniae* and showed bacterial inhibition zones comparable to co-trimoxazole. The research demonstrated a direct correlation between rosemary extract concentration and biofilm development inhibition thus indicating potential success in treating infections associated with biofilms.

Studies conducted before having validated that *R. officinalis* possesses antimicrobial properties. The research by (22) found that rosemary essential oil showed antibacterial functions against *E. coli* clinical strains using MIC readings between 18.0 to 20.0 µL/mL. The investigators from (15) discovered that *R. officinalis* ethanol extracts blocked the multiplication of drug-resistant *Salmonella* spp. and *S. aureus* clinical isolates in their experiments. The results from previous studies confirm those of this work by

demonstrating that rosemary extract demonstrates antibacterial properties against numerous microbial strains.

These results are consistent with recent literature highlighting the antimicrobial properties of *R. officinalis.* For instance, a study by (23) demonstrated that rosemary extract effectively inhibited multidrug-resistant clinical isolates and meat-borne pathogens, indicating its potential as a source of antimicrobial agents. Similarly, research by (24) reported that rosemary essential oil exhibited significant anti-biofilm activity against various pathogens, including *S. aureus* and *P. aeruginosa*. The observed antibacterial efficacy of rosemary extract can be attributed to its rich composition of bioactive compounds such as genkwanin, camphor, endo-borneol, and hydroxyhydrocaffeic acid, which have been identified as major constituents responsible for antimicrobial activity. These compounds may disrupt bacterial cell membranes, inhibit essential enzymes, or interfere with nutrient uptake, leading to bacterial growth inhibition.

Research shows that the antibacterial effects of *R. officinalis* L. result from its high content of phenolic acids together with flavonoids and essential oils. The antibacterial effects of carnosic acid, rosmarinic acid, and eucalyptol have been shown to act against pathogenic bacteria, including *S. aureus, E. coli, P. aeruginosa*, and others (24). Several studies have focused on rosemary extract's ability to break bacterial cell membranes, inhibit biofilm development, and control microbial enzyme behavior, making it a promising natural antibacterial agent (25).

Biofilm development decreased significantly as the amount of rosemary extract used in the experiments increased. The research determined that *R. officinalis* extract functioned as a multidirectional biofilm inhibitor against *Candida albicans, S. aureus, E. faecalis, Streptococcus mutans,* and *P. aeruginosa*. Bioactive compounds such as phenolics and flavonoids from rosemary extract demonstrate anti-biofilm properties because they both disrupt biofilm structure and block microbial adhesion (26).

The effectiveness of rosemary extract as an antibacterial agent shows diversity across different bacterial species according to various scientific research findings. In the present study rosemary extract showed potency against *P. aeruginosa* yet (27) discovered that clinical *E. coli* strains exhibited reduced sensitivity toward rosemary oil with MICs at 18.0 to 20.0 µL/mL. Various strains of bacteria and extraction methods along with different compositions of rosemary extract might explain these observed differences in effectiveness.

The findings of this study suggest that *R. officinalis* extract could serve as a natural alternative or adjunct to conventional antibiotics in treating urinary tract infections, especially those involving biofilm-forming pathogens. However, further research is necessary to elucidate the exact mechanisms of action, optimize extraction methods, and assess the clinical efficacy and safety of rosemary-based treatments.

CONCLUSION

In conclusion, this study demonstrates that *R. officinalis* extract possesses significant antibacterial and antibiofilm activities against uropathogenic bacteria, particularly *E. coli* and *K. pneumoniae*. The extract's efficacy, comparable to that of standard antibiotics like co-trimoxazole, underscores its potential as a natural therapeutic agent in managing urinary tract infections. Further investigations, including clinical trials, are warranted to validate these findings and explore the practical applications of rosemary extract in antimicrobial therapy.

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