Antimicrobial Effects of Rosmarinus Officinalis; in-vitro Study

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Abstract

Aim: The plant Rosmarinus officinalis (RO) is a member of the Lamiaceae family and is more commonly known as rosemary. This study's primary objective is to compare the antimicrobial effect of RO extract at different concentrations on various microorganisms.

Methods: Strains of S. aureus ATCC 29213, K. pneumoniae ATCC 13883, and E. coli ATCC 25922 obtained from the national reference center were inoculated into liquid Müller Hinton broth (Oxoid, UK) and incubated at 37 °C' for 24 hours. To assess the antimicrobial efficacy of the RO extract, dilutions of 0.0625%, 0.125%, 0.25%, 0.5%, 1%, 2%, 3%, and 4% were prepared.

Results: The MIC value for RO extract was 2% for S. aureus ATCC 29213 and K. pneumoniae ATCC 13883 and 3% for E. coli ATCC 25283. A statistically significant difference was found between the three groups, including 0.0625% and 2% of RO, in terms of the growth rates of microorganisms ($p<0.001$). There was no statistically significant difference between the concentrations of 3% and 4% ($p=1.00$).

Conclusions: The antimicrobial effect potential of RO has been demonstrated in the literature and in-vitro in this study. In addition, we believe it can be used as a prophylactic or as an alternative to antimicrobial agents in the topical or systemic treatment of SSIs due to its various effects, topical, oral, and systemic use, and low cost. Thus, it is anticipated that the costs of treatment will be reduced. To determine the efficacious dose and implement it in clinical practice, experimental and clinical studies are necessary.

Keywords: *S. aureus ATCC 29213, K. pneumoniae ATCC 13883, E. coli ATCC 25922, Rosmarinus officinalis, surgical site infection, antimicrobial.*

1. Introduction

The plant *Rosmarinus officinalis* (*RO*) is a member of the Lamiaceae family and is more commonly known as rosemary[1]. RO is a herbal product used for years in food and cosmetic/pharmaceutical applications[2,3]. Numerous studies have been published in the literature that demonstrate antioxidant, anti-inflammatory, anti-ulcer, cardiovasculoprotective, neuroprotective, hepatoprotective, antineoplastic, and antimicrobial effects[3-6]. Although *RO* extract contains many biomolecules, the specific effects of these biomolecules have rarely been demonstrated due to their synergistic effects[7]. According to scientific studies, the 1,8-cineole molecule has a bacteriostatic effect[8,9]. Surgical infections are usually polymicrobial. Therefore, microbial synergy may increase the net pathogenic effect and the severity of infection. The most commonly isolated microorganisms in surgical infections are *E. coli*, *Staphylococcus aureus*, *Klebsiella spp.*, *Pseudomonas aeruginosa, Bacteroides fragilis*, and *Peptostreptococcus spp.*[10]. Since it was predicted that it could be used in the prevention of surgical infections due to its antimicrobial effect potential and low cost, it was planned to investigate the effect of RO on the most frequently isolated microorganisms in surgical site infections in this study. This study investigated the effects of *RO*

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extract on the three most frequently isolated bacterial agents in surgical infections.

This study's primary objective is to compare the antimicrobial effect of *RO* extract at different concentrations on various microorganisms.

2. Materials and methods

Strains of *S. aureus ATCC 29213*, *K. pneumoniae ATCC 13883*, and *E. coli ATCC 25922* obtained from the national reference center were inoculated into liquid Müller Hinton broth (Oxoid, UK) and incubated at 37 °C' for 24 hours.

To assess the antimicrobial efficacy of the *RO* extract, dilutions of 0.0625%, 0.125%, 0.25%, 0.5%, 1%, 2%, 3%, and 4% were prepared (*RO* doses mg/dL). A 0.5 McFarland turbidity standard suspension (final measurement concentration of 1.5x108 CFU/mL) and 10 µl were added to the wells for each strain. Each procedure underwent three repetitions. Microwell plates were incubated for 24 hours on a microplate incubator shaker at 37°C. After incubation, the wells were measured with an Epoch spectrophotometer (BioTek Inst. Inc., Vermont, USA) at a wavelength of 600 nm (OD600). Wells containing neither antimicrobials nor plant extracts were growth controls, while wells containing only Mueller-Hinton broth were negative controls. The percentage of viable cells for growth control was normalized to 100%[11].

Broth microdilution method with Mueller Hinton broth (Oxoid, UK) using 96-well microplates in accordance with CLSI guidelines to determine minimum inhibitory concentration (MIC) values against *S. aureus ATCC 29213*, *K. pneumoniae ATCC1 3883*, and *E. coli ATCC 25922.* was used. To ascertain the minimal bactericidal concentration (MBC), 10 µl samples from each well that did not exhibit visible growth (viability) after 24 hours were seeded on Mueller-Hinton agar and examined for viable organisms. After 24 hours, it was incubated at 37°C to observe any colony growth[12].

Microorganisms used in the study were divided into three groups. Groups;

Group 1: *S. Aureus ATCC 29213*

Group 2: *E. Coli ATCC 25922*

Group 3: *K. Pneumoniae ATCC 13883*

2.1. Preparation of Rosmarinus officinalis extract: *RO* extract was prepared by the methodology described by Roohbakhsh et al. in their study[13]. Above ground parts of *RO* were obtained from Yalova Atatürk Horticultural Center. The shade-dried and powdered aerial portions of *RO* (150 g) were extracted at room temperature with a 70% hydroethanol solution. The selected solvent-plant ratio was 1:10. Using a rotary evaporator; the solution was filtered and concentrated under reduced pressure at 38-40 °C to produce an extract. The extract was then completely dried using a lyophilizer. Concentrations of 2% and 4% were obtained by diluting with isotonic.

2.2. Statistical analysis: Descriptive statistics were used to identify continuous variables. Mean+standard deviation values were given for parameters suitable for normal distribution, and median (minimum-maximum) values were given for parameters unsuitable for normal distribution. The conformity of continuous variables to the normal distribution was examined using the Shapiro-Wilks test. The Kruskal-Wallis test was used to analyze the difference between more than two independent group continuous variables that did not conform to the normal distribution. Significant results were analyzed with the double Post Hoc comparison Bonferroni corrected Mann Whitney U test. The statistical significance level was determined as 0.05. MedCalc Statistical Software version 12.77 was used for analyses.

3. Results

The MIC value for *RO* extract was 2% for *S. aureus ATCC 29213* and *K. pneumoniae ATCC 13883* and 3% for *E. coli ATCC 25283*. When the MBC value was examined, no MBC values were found in the examined range. The MIC values and dose-response curve are displayed in Table 1 and Graphic 1, respectively.

A statistically significant difference was found between the three groups, including 0.0625% and 2% of *RO,* in terms of the growth rates of microorganisms (**p<0.001**). There was no statistically significant difference between the concentrations of 3% and 4% (p=1.00). Table 2 displays the growth rates of microorganisms in each of the three groups, while Table 3 provides pairwise comparisons between the groups.

Figure 1

The dose-response curve for *Rosmarinus officinalis* extracts (%) against S. *aureus*, *E. Coli*, and *K. pneumoniae* after 24 h.

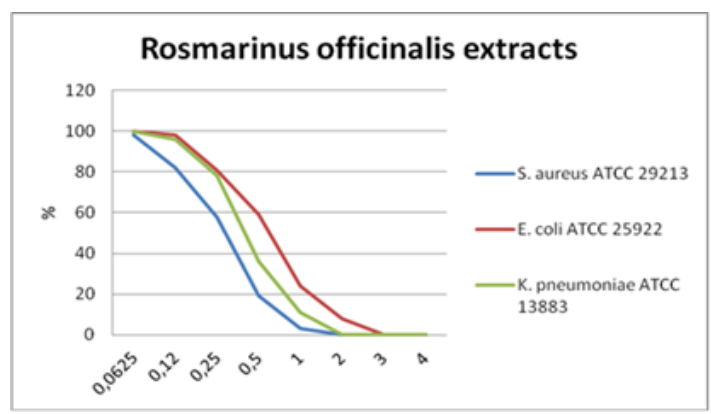

Table 1

MIC values of *Rosmarinus officinalis*

RO (%)	0.0625	0.12	0.25	0.5	1	2	3	4
Group 1	+	+	+	+	+	-*	-	-
Group 2	+	+	+	+	+	+	-*	-
Group 3	+	+	+	+	+	-*	-	-

*: MIC values, RO: Rosmarinus officinalis extracts

Group 1: S. Aureus ATCC 29213, Group 2: E. Coli ATCC 25922, Group 3: K. Pneumoniae ATCC 13883

Table 2

Reproduction percentages between groups

	0.0625	0.120	0.250	0.500	1.000	2.000	3.000	4.000
Group 1 (n=10)								
Mean+Sd	97.6±1.6	81.6±4.4	58.2±4.9	19.1±3.9	2.8±1.5	0±0	0±0	0±0
Med (min-max)	98 (94-99)	82 (76-88)	59 (50-5)	18.5 (14-26)	2.5 (1-5)	0 (0-0)	0 (0-0)	0 (0-0)
Group 2 (n=10)								
Mean+Sd	99.5±0.7	97.6±2.2	80.6±3.6	58.3±4.2	23.8±5.7	8.4±2.2	0±0	0±0
Med (min-max)	100 (98-100)	97.5 (94-100)	81.5 (76-85)	58.5 (51-65)	21.5 (17-34)	8.5 (5-12)	0 (0-0)	0 (0-0)
Group 3 (n=10)								
Mean+Sd	99.6±0.5	95.7±3.9	78.2±7.5	36.4±3.3	10.9±3.2	0±0	0±0	0±0
Med (min-max)	100 (99-100)	96.5 (85-99)	80.5 (60-85)	36 (31-42)	10.5 (7-18)	0 (0-0)	0 (0-0)	0 (0-0)
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	1.00	1.00

Group 1: S. Aureus ATCC 29213, Group 2: E. Coli ATCC 25922, Group 3: K. Pneumoniae ATCC 13883
Sd: Standard deviation, Med: Median

Table 3

Pairwise Post Hoc comparisons between groups

Group	0.0625	0.120	0.250	0.500	1.000	2.000
1-2	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
1-3	0.001	0.003	0.002	0.033	0.031	1.00
2-3	1.000	1.000	1.00	0.033	0.038	<0.001

Group 1: S. Aureus ATCC 29213, Group 2: E. Coli ATCC 25922, Group 3: K. Pneumoniae ATCC 13883

4. Discussion

Surgical site infection (SSI) is an important health problem that increases morbidity, mortality, and treatment costs[14]. It has been reported that the incidence of SSI in surgical patients is between 2% and 5%[15]. It is the most prevalent and expensive of all hospital-acquired infections and accounts for twenty percent[16]. Microorganisms in the cutaneous flora are frequently isolated in SSIs, and antimicrobial agents cefazolin, cefuroxime, cefoxitin, cefotetan, ertapenem, and vancomycin are frequently used for prophylaxis in many kinds of surgical applications[17]. SSIs have numerous intrinsic and extrinsic risk factors. Few of these risk factors are within the control of the surgeon. SSI prevention strategies are multimodal. A high level of adherence to these prevention strategies is crucial for success. As SSIs are the most prevalent and expensive hospital-acquired infections, preventing and reducing treatment costs is crucial. It is stated in the literature that 60% of SSIs can be prevented through the use of evidence-based measures[15]. Precautions are, therefore, the most essential and cost-effective method. Superficial SSIs that cannot be prevented can be treated topically, whereas deeper infections require debridement and antimicrobial treatment[18]. The use of topical and local antibiotics will continue to evolve in the context of SSIs[15]. For this reason, it is thought that *RO*, which has the potential for antimicrobial effects and has a very low cost, may be promising in treating SSIs. The clinical and experimental studies literature reveals that *RO* can be administered topically, orally, and systemically (intraperitoneally)[1,13,19]. For this reason, it can be predicted that the cost and efficacy of treatment will increase if it is used alone or as a supplement to conventional antibiotic therapy for both superficial and deep-seated SSI. In our study, *RO* was found to be antimicrobial against SSI-common pathogens such as *S. aureus, K. pneumoniae*, and *E. coli*. Among these three microorganisms, it was observed that the highest antimicrobial effect at different concentrations was against *S. aureus, K. Pneumoniae*, and *E. coli*, respectively. Bowbe et al.[8] reported that the MIC value of *RO* against *S. aureus* was 0.7 mg/mL. In our study, the minimum inhibitory concentration (MIC) against S. aureus was determined to be 20 mg/mL (2% concentration). It was hypothesized that the difference was due to using different strains in the studies. This significant dose difference in MICs indicates that resistant strains may require high concentrations for antimicrobial efficacy. According to Dhouibi et al.[20], *RO* has antimicrobial activity against diverse microorganisms. Although MIC values were not given in this study, it was reported that the antimicrobial activity against *S. aureus* was higher than that against *E. coli* when inhibition zone diameters were considered. In line with the results of this study, we discovered that the antimicrobial activity against *S. aureus* was greater than that against *E. coli*. According to Luca et al.[3], the MIC of *RO* against Methicillin-resistant *S. aureus* (*MRSA*) is 62.5 mg/mL. Based on these findings, the antimicrobial effective dose of *RO* varies with the virulence of microorganisms, and further research is required to determine the optimal effective dose. According to Ielciu et al.[21], the MIC value of *RO* was greater for *E. coli* than for *S. aureus.* In accordance with our findings, this study demonstrates that the antimicrobial effect potential of *RO* against *S. aureus* is greater than that against *E. coli*. In addition, Ielciu et al.[21] reported that even though the diameter of the *MRSA* inhibition zone was less than that of Methicillin-resistant *S. aureus* (*MSSA*), there was no difference in MIC values. This study's findings also disclose the need for more certainty regarding the optimal dose.

5. Conclusions

SSI increases morbidity, mortality, and treatment costs significantly. Consequently, prevention and treatment are crucial. A significant proportion of SSIs can be prevented with evidence-based

measures. However, topical use in superficial cases and debridement and systemic antibiotheraphy in deep SSI cases are required to treat unpreventable SSI. In this instance, the use of antimicrobial agents significantly increases treatment costs. In terms of SSIs, the application of topical and local antibiotics will continue to evolve. The antimicrobial effect potential of *RO* has been demonstrated in the literature and in-vitro in this study. In addition, we believe it can be used as a prophylactic or as an alternative to antimicrobial agents in the topical or systemic treatment of SSIs due to its various effects, topical, oral, and systemic use, and low cost. Thus, it is anticipated that the costs of treatment will be reduced. To determine the efficacious dose and implement it in clinical practice, experimental and clinical studies are necessary.

Statement of ethics

This study is an in vitro study and ethics committee approval is not required. Informed consent is not required for IVD studies involving samples that are non-identifiable (i.e., are labeled with identifiers or accompanied by the patient's non identifiable clinical information), as well as for studies in which the samples are not identifiable but are coded.

Conflict of interest statement

The authors declare that they have no financial conflict of interest with regard to the content of this report.

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