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Antibacterial Activities of *Rosmarinus officinalis* Extract against *Enterococcus faecalis* and *Enterococcus faecium*

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ABSTRACT

Hospital-acquired infections are caused by multidrug-resistant *Enterococcus* (*E.*) species, especially *E. faecalis* and *E. faecium*, which are zoonotic bacteria and pose a significant public health challenge. This study aimed to identify multidrug-resistant *Enterococcus* isolates and evaluate the antibacterial activity of *Rosmarinus officinalis* (*R. officinalis*) extract against *E. faecalis* and *E.* faecium. Fifty urine and stool samples were collected from hospitalized patients. Four multidrug-resistant strains—*E. faecalis* 6E, 7B, 10D, and *E. faecium* 15E—were identified by polymerase chain reaction (PCR) with universal primers (8F and 1492R) targeting the 16S rDNA gene to confirm their identity as *Enterococcus* species. The antibacterial efficacy of *R. officinalis* extract was assessed using disk diffusion and minimum inhibitory concentration (MIC) determination. High-Performance Liquid Chromatography (HPLC) analysis was conducted to identify phenolic bioactive compounds in *R. officinalis*. The extract demonstrated significant antibacterial activity, with MICs of 25 mg/mL and 30 mg/mL for *E. faecalis* and *E. faecium*, respectively. HPLC revealed phenolic compounds such as rosmarinic acid, caffeic acid, and ferulic acid, likely contributing to the antimicrobial properties. The *R. officinalis* extract could be a promising natural antimicrobial agent against multidrug-resistant *Enterococcus* species. Compounds such as rosmarinic acid, caffeic acid, ferulic acid, apigenin-7-glucoside, syringic acid, and p-hydroxybenzoic acid show potential as safer, environmentally friendly alternatives to traditional antibiotics, aiding in the fight against rising antibiotic resistance.

Keywords: Antibiotic resistance, *Enterococcus faecalis*, *Enterococcus faecium*, Hospital-acquired infection, Natural antibacterial agent, PCR-based identification, *Rosmarinus officinalis*

INTRODUCTION

Enterococci are Gram-positive commensal bacteria that form an integral part of the intestinal flora in humans and animals. *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) are commensal organisms of the gastrointestinal tract, which are found in humans and domesticated species (Fisher and Phillips 2009). However, in recent years, *Enterococcus* species have been recognized more and more as opportunistic pathogens, especially in health care settings, because of their natural and acquired resistance to several different antibiotics, including vancomycin (Ostrowsky et al., 2001). Another severe yet poorly studied route of human colonization and infection is the zoonotic transmission of bacteria between animal and human hosts.

Prolific empirical studies have witnessed the occurrence of *E. faecalis* and *E. faecium* in different animal reservoirs, such as pigs, cattle, and poultry, as the main reservoirs, highlighting interspecies transmission (Shahveh et al., 2023). There is human contamination by ingestion of meat contaminated or direct body contact with livestock on the farm. Further, high prevalence of antimicrobials among food-producing animals has been linked with the emergence and spread of antimicrobial-resistant strains with similar phenotypes as those seen in the medical environment (Hammerum, 2012).

Regarding pig and poultry populations, it has been demonstrated that isolates of *E. faecium* contain vancomycin-resistance genes, especially van A, which suggests the direct connection between aquatic reservoirs and nosocomial human infections (García-Migura et al., 2014). Additionally, these strains have remarkable genetic similarity to clinical isolates, casting concern that there is clonal spread through food or the environment. Animals kept as companion pets, such as dogs and cats, have been accused of harboring resistant *enterococci*. A landmark study included by Damborg et al. (2009) in Denmark indicated that canines are often carriers of *E. faecium* clonal complexes, such as CC17M, which are linked to most human hospital infections. In addition, Damborg et al. (2009) have observed an incidence where a dog and its owner shared an indistinguishable strain, which effectively gave strong evidence that there was zoonotic

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exchange (Damborg et al., 2009). All these evidences support the paradigm of One Health that focuses on the interconnectedness of human, animal, and environmental well-being.

The highly mobile nature of antibiotic resistance genes of *Enterococcus* species, transferable between animals and human within divers' ecological settings, underscores the critical need for multi-sectoral and action plans, however *Enterococcus* is considered harmless to humans (Fisher and Phillips, 2009). Over the past decade, *Enterococcus* species have been widely utilized as probiotics and starter cultures in the food industry (Hanchi et al., 2018). However, *enterococci* have recently emerged as significant nosocomial pathogens, associated with severe infections and high mortality rates among hospitalized patients (El Zowalaty et al., 2023).

The *Endococcus* species work as opportunistic pathogens, and they target people with low immune defenses, people exposed to extensive-spectrum medicines such as cephalosporins, fluoroquinolones, or carbapenems, or people who have endured lengthy stays in the medical facility, such antibiotics used may interfere with the normal colon microbiome and hence provide an optimal ecological niche to the more resistant *Enterococcus* strains especially the *E. faecium* and *E. faecalis* strains thus aiding colonization and establishment of an invasive infection (Sangiorgio et al., 2024).

Enterococcus faecalis and *E. faecium* are the predominant causes of enterococcal UTI, accounting for approximately 95 percent of human infections, alongside *Escherichia coli* (*E. coli*). In Saudi Arabia, common bacteria that cause UTIs include *E. coli*, *Enterococcus* spp., and *Klebsiella* spp. (Codelia-Anjum et al., 2023). Antibiotic resistance is the capacity of bacteria to endure and grow despite exposure to antimicrobial drugs that were previously effective in eliminating them (Dehbanipour et al., 2016; WHO, 2019; Motse et al., 2019).

One of the main virulence traits of *Enterococcus* species is their capacity to develop biofilms, which aid in their attachment to both living tissues and non-living surfaces, thereby promoting persistent colonization and infection.

Biofilms are known as ensembles of microorganisms that are embedded in a water-ample mass constituted of extracellular polymeric substances such as proteins, sugars, and nucleic acids (Flemming and Wingender, 2010). The biofilm structure protects bacteria against host immune defenses, including phagocytosis, as well as antibiotic treatment, thereby making infections more difficult to treat and eliminate (Ghazvinian et al., 2024).

Synthetic bactericides are extensively used to combat bacterial pathogens; however, their application comes with drawbacks, including negative impacts on human health and the environment, as well as the development of bacterial resistance (Jess et al., 2014; Serwecińska, 2020). In recent years, there has been increasing attention toward natural compounds obtained from medicinal plants. These substances, such as essential oils, phenolics, saponins, flavonoids, steroids, and alkaloids, have exhibited notable antimicrobial activity against pathogenic microorganisms (El-Hefny et al., 2017; Gutiérrez-Morales et al., 2017).

Among plant-based antimicrobials, extracts from *Rosmarinus officinalis* (*R. officinalis*) are promising due to their bioactive compounds, including flavonoids, terpenoids, and phenolic acids. Studies have highlighted the strong antimicrobial properties of *R. officinalis* against Gram-positive and Gram-negative bacteria (Mena et al., 2016).

Rosmarinus officinalis L., commonly known as rosemary, is a perennial woody plant native to the Mediterranean region, now cultivated globally for its aromatic and medicinal properties. Rosemary leaves contain bioactive constituents such as 1,8-cineole, camphor, and α-pinene, which contribute to its pharmacological activity (Bozin et al., 2007; Celiktas et al., 2007). Extracts from rosemary and other members of the Lamiaceae family are known for their antioxidant, antifungal, insecticidal, and antibacterial properties. These biological effects are largely attributed to phenolic compounds, which may vary depending on the extraction method and plant part used (Lešnik et al., 2021).

Brito-Júnior et al. (2012) demonstrated the bactericidal effects of hydroalcoholic extracts of *R. officinalis* in endodontic applications, specifically against *E. faecalis*. Similarly, Milyuhina et al. (2021) reported the antibacterial efficacy of *R. officinalis* against *E. faecalis*, *Staphylococcus aureus* (*S. aureus*), and *E. coli*. The present study aimed to isolate and molecularly identify *Enterococcus* species from urine and stool clinical samples and assess the antibacterial properties of *R. officinalis* extract as a potential alternative to conventional antimicrobial agents.

MATERIALS AND METHODS

Ethical approval

The present study followed the principles expressed in the Declaration of Helsinki as recommended by the Research Ethical Committee, Faculty of Medicine, Zagazig University, Egypt.

Sample collection

After obtaining informed verbal/written consent for participation, clinical urine and stool samples were collected from the Central Laboratory at the Faculty of Medicine, Zagazig University Hospitals, Egypt, from February 2021 to March 2022. 50 Samples were transported aseptically and processed within two hours according to Murray et al. (2007) in the Bacteriology Laboratory, Faculty of Science, Zagazig University

Isolation and identification of *Enterococcus* species

The presumptive isolates were subcultured by spreading clinical material on Bile Esculin Agar (BEA) and Brain Heart Infusion (BHI) Agar plates (Oxoid Ltd., Basingstoke, Hampshire, UK). The plates were inoculated and incubated at 37°C for 24 to 48 h in an aerobic atmosphere. Visible bacterial colonies were observed and subcultured to purity after incubation. All media were kept at 2-8°C until further use, according to the manufacturer's instructions. Within 24 to 48 h, depending on species and load, clear primary isolates were evident. Once suspected colonies with typical morphology were selected, Gram's staining was performed (ISO 7218, 2013) and monitored through a series of biochemical tests as catalase, oxidase, haemolysis on blood agar, growth in 6.5% NaCl, temperature tolerance at low (10°C) and high (45°C) temperature, activity of Arginine Decarboxylase (ADH), the bile esculin hydrolysis, sugars fermentation tests (glucose, lactose, sucrose, among others), and hippurate hydrolysis following standard procedures (MacFaddin, 2000).

Molecular identification

Biochemically confirmed isolates were used to extract DNA with QIAamp DNA Mini kit (QIAGEN, GmbH, Hilden, Germany, Catalogue no.51304) according to the manufacturer's instructions. Amplification of the 16S rDNA region with primers 8F and 1492R (Bru et al., 2008) was applied to identify *Enterococcus* spp. in the extracted DNA.

Preparation of rosemary extract

Dried mature seeds of *R. officinalis* L. were obtained from the Agriculture Research Center, Giza, Egypt. Then, the plant was grown from seed to full growth, and the leaves were dried for use. Dried *R. officinalis* L. leaves were powdered and extracted with 80 percent ethanol for 48 hours with continuous shaking. The extract was filtered using a 0.45 µm disposable syringe bacterial filter (Zhejiang Aijiren Technologies Co. Ltd, China) and concentrated using a rotary evaporator (China) at 40°C. The final product (21 mL) was stored at 4°C, then phytochemical screening was applied according to the protocols of Farnsworth (1966) with modifications.

High-performance liquid chromatography analysis

The HPLC analysis for phenolic compounds was conducted using an Agilent 1100 series system with an Eclipse XDB-C18 column (The Agilent Technologies 1100 series liquid chromatograph is designed and manufactured by Agilent Technologies, a multinational corporation headquartered in Santa Clara, California, United States). The mobile phase comprised acetonitrile (Solvent A) and 2% acetic acid (Solvent B) with a gradient elution. Peaks were identified based on retention times and UV spectra compared with standards.

Antimicrobial activity

The antibacterial activity of the *R. officinalis* extract (100 mg/ml) was evaluated against *E. faecali*s using the disc diffusion method. Zone diameters of inhibition were measured after 24 hours of incubation at 37°C. Levofloxacin served as the positive control. Extract concentrations ranging from 10% to 40% were tested using the disc diffusion method. The minimum inhibitory concentration (MIC) was defined as the lowest concentration inhibiting visible growth.

Statistical analysis

Each experiment was carried out three times, and the results were presented as mean standard deviation. One-way ANOVA was used to determine statistical significance ($p < 0.05$) as described by Kumar et al. (2012).

RESULTS

Screening and isolation of *Enterococcus* spp.

Urine and stool samples were collected from 50 patients admitted to Zagazig University Hospitals, Egypt. *Enterococcus* species were identified from 19 samples (38%), of which 11 (57.9%) were classified as *E. faecalis* and eight (42.1%) as *E. faecium*. The initial identification was accomplished by Gram staining, which illustrated Gram-positive cocci or coccobacilli in pairs and short chains. Biochemical confirmation indicated that all isolates were catalase-negative and capable of growing in nutrient broth containing 6.5% NaCl (Oxoid, Basingstoke, Hampshire, UK), a hallmark characteristic of *Enterococcus* species.

Molecular identification of multidrug-resistant bacterial isolates

Out of the 19 biochemically confirmed isolates, four isolates were selected for molecular examination. The four isolates comprised three *E. faecalis* and one *E. faecium* isolates; they were chosen as they exhibited multidrug resistance to different antibiotics, such as ampicillin, Nitrofurantoin, penicillin, tetracycline, ciprofloxacin, and levofloxacin. All

four isolates underwent molecular identification via the 16S rDNA gene amplification (Weisburg et al., 1991). The polymerase chain reaction (PCR) amplification was performed using universal primers 8F and 1492R, yielding a 1500 bp fragment corresponding to the 16S rDNA gene (Figure 1).

Qualitative screening for phytochemicals

The phytochemical analysis of *R. officinalis* extract indicated the presence of several bioactive compounds, including alkaloids, saponins, tannins, glycosides, terpenoids, and steroids (Table 1, Figure 2).

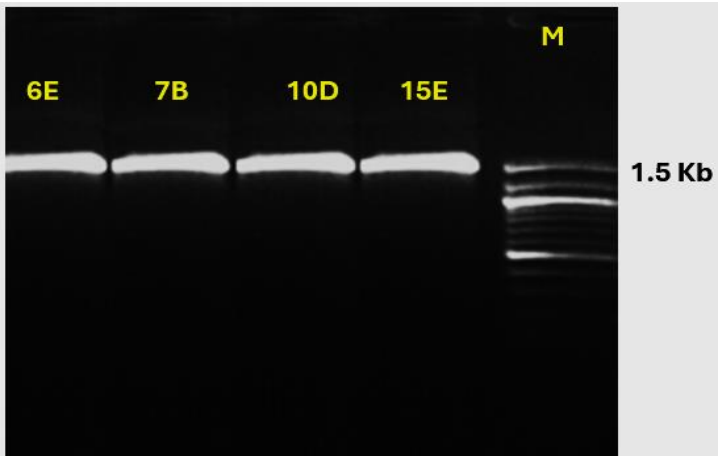

Figure 1. The PCR amplification of 16S rDNA for four bacterial isolates. 1500bp lane 1-4. M: 100 bp DNA ladder. Source: Authors of the present study

Table 1. Phytochemical screening of the aqueous *Rosmarinus officinalis* extract

Aqueous extract of *Rosmarinus officinalis*	**Result** (+/-)
Alkaloids	+
Flavonoids	-
Glycosides	+
Terpenoids	+
Saponins	+
Steroids	+
Tannins	+

-: Absent, +: Present

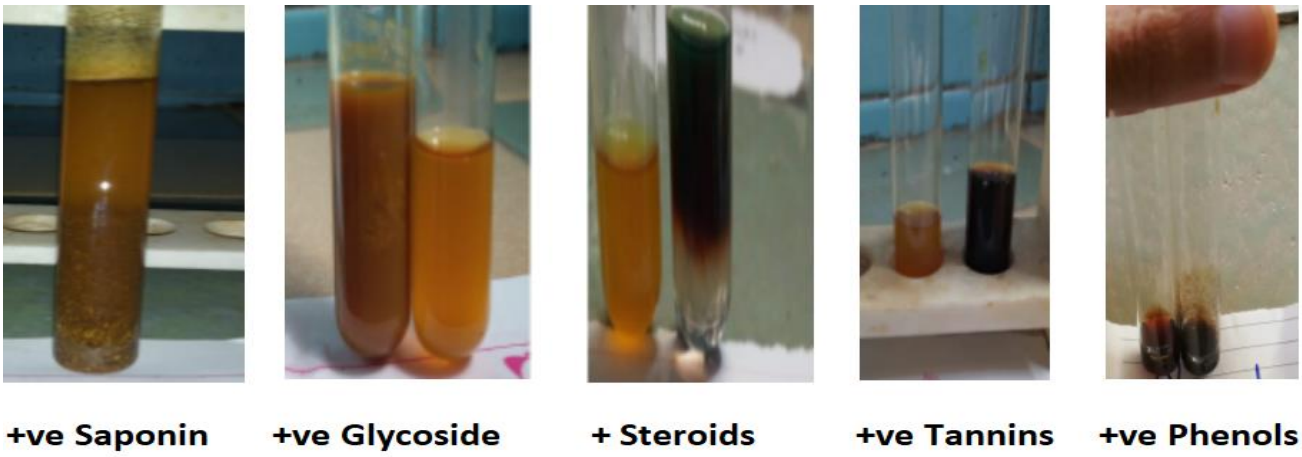

Figure 2. Qualitative phytochemical analysis of *Rosmarinus officinalis* extract. +ve: Compound detected. Source: Authors of the present study

High-Performance Liquid Chromatography analysis of *Rosmarinus officinalis* extract

The *R. officinalis* extract HPLC profiling revealed the presence of several polyphenols, such as rosmarinic acid rosmarinic acid, caffeic acid, ferulic acid, apigenin-7-glucoside, sinapic acid, protocatechuic acid, syringic acid, p-hydroxybenzoic acid, apigenin, gentisic acid, catechin, and kaempferol (Table 2, Figure 3). These compounds were detected, which justified the antibacterial effect in further tests.

Table 2. Polyphenolic compounds of *Rosmarinus officinalis*

Compound	Concentration (µg/g)
Gallic	1.45
Protocatechuic	99.73
p-hydroxybenzoic	65.46
Gentisic	19.33
Catechin	12.47
Chlorogenic	ND
Caffeic	2442.60
Syringic	69.44
Vanillic	3.03
Ferulic	469.38
Sinapic	253.44
Rutin	ND
p-coumaric	2.24
Apigenin-7-glucoside	320.43
Rosmarinic	5280.57
Cinnamic	ND
Qurecetin	12.46
Apigenin	50.42
Kaempferol	6.95
Chrysin	2.39

ND: Not detected

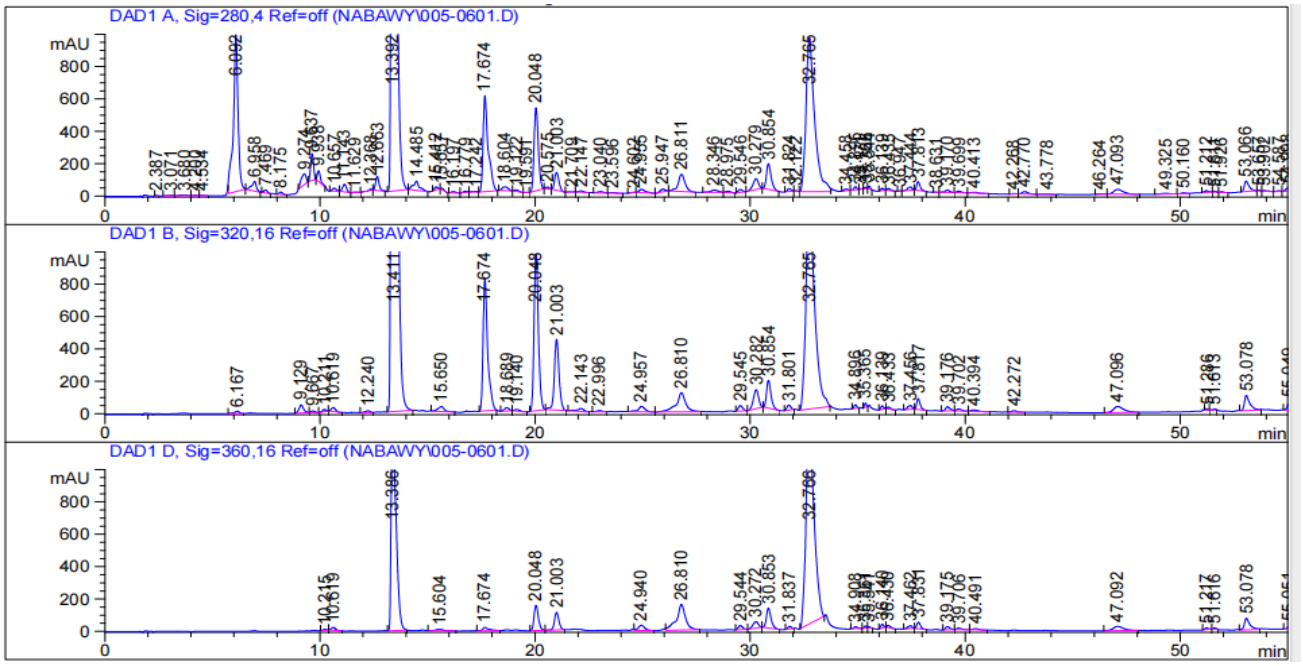

Figure 3. The high-performance liquid chromatography of aqueous extract of *Rosmarinus officinalis.* Source: Authors of the present study

Antibacterial activities of the *Rosmarinus officinalis* plant extract against *Enterococcus faecium* and *Enterococcus faecalis*

The antibacterial activity of *R. officinalis* extract was assessed against the most resistant isolates, *E. faecium* and *E. faecalis*. The *R. officinalis* extract exhibited a better antimicrobial capability than the reference antibiotic, and the differences in the mean zones of inhibition were found to be significant. The mean zones of inhibitions for *E. faecium* were 35 mm +/- 0.15 compared to 33 mm +/- 0.10 ($p < 0.05$) and 26 mm +/- 0.15 compared to 19 mm +/- 0.15 ($p < 0.05$) for *E. faecalis*. These results pointed to the fact that *R. officinalis* has distinctive antibacterial activity at low doses and

can be used as a promising natural alternative for managing infections caused by drug-resistant *Enterococcus* strains (Table 3). In *E. faecium*, the inhibition measure was 35 mm with 25 mg/mL MIC (25%). In *E. faecalis*, the zone of inhibition was 26mm with 30 mg/mL (30%). Such zones of inhibition, especially the big zone in *E. faecium*, demonstrated a high antimicrobial activity of the *R. officinalis* extract against the multidrug-resistant *enterococcus* strain. The current findings proved that the extract can be effective at relatively low concentrations, which makes it a promising antimicrobial agent because of its natural nature.

Minimum Inhibitory Concentration of the *Rosmarinus officinalis* extract against *Enterococcus faecium and Enterococcus faecalis*

The effects of different concentrations of the *R. officinalis* plant extract were reported in Table 5. It was found that the inhibitory effects of the plant extract ranged from 25 to 30 mg/ml (25% and 30% MIC) with inhibition zones of 35 and 26 mm against *E. faecium and E. faecalis*, respectively (Table 5, Figure 4).

Table 3. Antibacterial activity of *Rosmarinus officinalis* extract and streptomycin against *Enterococcus faecium* and *Enterococcus faecalis*

Treatments	**Inhibition zones (mm)**	
	Enterococcus faecium	***Enterococcus faecalis***
Rosmarinus officinalis extract	35	26
Streptomycin	33	19

Table 4. Minimum inhibitory concentration of the *Rosmarinus officinalis* extract against *Enterococcus faecium* and *Enterococcus faecalis*

Treatment	**Concentration**	**Inhibition zone (mm)**			
		Enterococcus faecium	**MIC**	*Enterococcus faecalis*	**MIC**
***Rosmarinus officinalis* extract**	10%	10	25%	6	30%
	15%	15		8	
	20%	29		17	
	25%	35		23	
	30%	35		26	
	35%	35		26	
	40%	25		16	

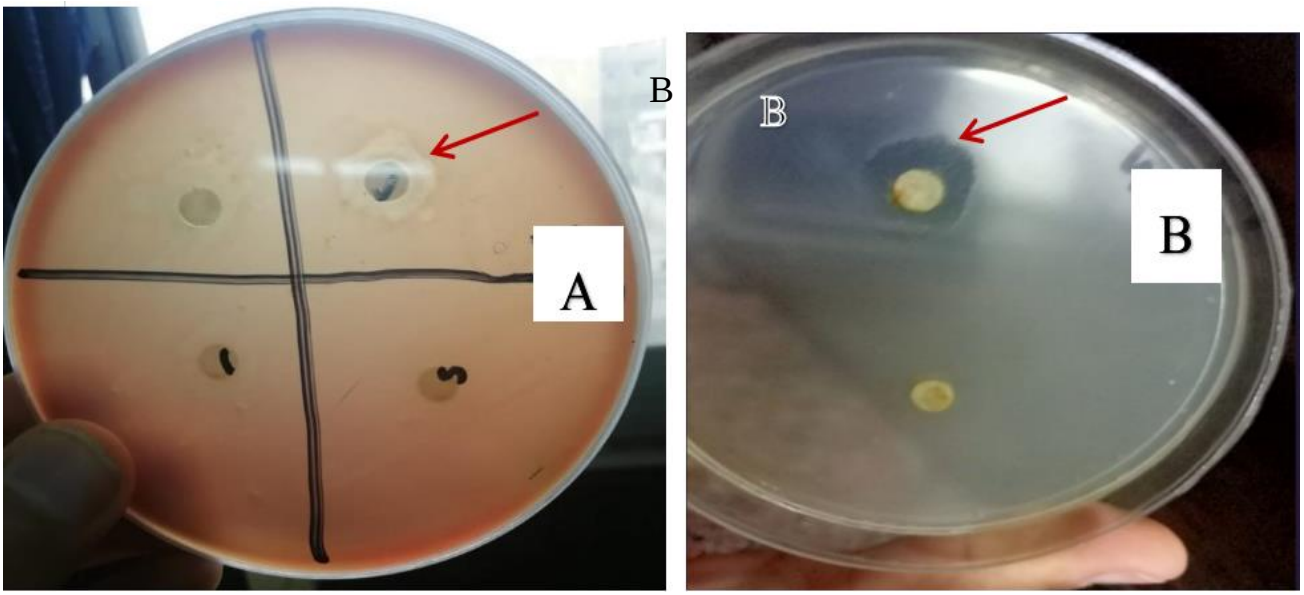

Figure 4. Minimum inhibitory concentration of the *Rosmarinus officinalis* extract against *Enterococcus* species. **A**: *Enterococcus faecium*, **B**: *Enterococcus faecalis.* Source: Authors of the present study

DISCUSSION

Hospital-acquired infections, yet another term referred to as nosocomial infections, are either localized or systemic infections that occur as a result of exposure to healthcare settings comprising infectious agents. These infections usually do not exist or are under incubation at the moment of the admission of a patient, but happen during the hospital stay as exposure to pathogenic microorganisms occurs. The most common offenders are *S. aureus*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *E. coli*, and *Enterococcus* spp., which are readily transmissible and can manifest themselves systemically. The patients who are more susceptible to developing these infections are hospitalized patients with weakened immune systems, surgical wounds, or invasive devices (Magill et al., 2018; CDC, 2023).

Hospital-acquired infections are a major global concern, affecting both developed and developing countries. They present significant challenges to public health by increasing patient morbidity and mortality, making them a serious healthcare issue (Chakraborty and Mukherjee, 2016; Zavaryani et al., 2020). *Enterococcus faecalis* and *E. faecium* are the most clinically significant species associated with various hospital-acquired infections (Brinkwirth et al., 2021). *Enterococci* cause many types of infections, such as urinary tract infections, bloodstream infections, surgical site infections, and endocarditis (Arias and Murray, 2012; WHO, 2019; Miller et al., 2020). All the present isolates were catalase-negative, distinguishing *Enterococcus* from other Gram-positive cocci like *Staphylococcus*, which is catalase-positive, consistent with the findings of PraxiLabs (2024).

Identification of the bacterial isolates was done at the molecular level by sequencing of the 16S ribosomal RNA (rDNA) gene. The PCR-based molecular characterization technique is an accurate and acceptable method of determining *Enterococcus* species, especially by amplification of a specific species gene. To give an example, the D-alanine: D-alanine (*ddl* gene) ligase has been used to differentiate *E. faecalis*, whereas the superoxide dismutase A (*sodA* gene) is aimed at identifying *E. faecium* (Ke et al., 1999; Kariyama et al., 2000; Jackson et al., 2004). These specific-species genes are highly specific and are often applied in clinical and microbiological research to verify *Enterococcus* species.

The use of multilocus sequence typing and virulence gene profiling further aided in understanding the genetic diversity and pathogenic potential of clinical isolates (Lebreton et al., 2017). Resistance mechanisms primarily involve mutations in antibiotic targets, altered membrane permeability, activation of alternative metabolic pathways, and enzymatic degradation of antibiotics (Arsène et al., 2022).

The present study reported that MICs of *R. officinalis* extract against *E. faecalis* and *E. faecium* were 25 mg/ml and 30 mg/ml, respectively. These results were consistent with those of Silva et al. (2019), who demonstrated the antibacterial efficacy of *R. officinalis* extracts against different bacterial strains, even at lower concentrations MICs of 25 and 30 mg/mL against *E. faecalis* and *E. faecium*.

Moreover, the same result is consistent with the findings of Nazzaro et al. (2017), Nieto et al. (2018), and Salehi et al. (2021), who found that *R. officinalis* showed remarkable antibacterial activity against both Gram-positive and Gram-negative bacteria. Disruption of the membrane, inhibition of metabolic enzymes, and antioxidant activity were some of the mechanisms that were brought out in these studies, contributing to the antimicrobial action.

Historically, *R. officinalis* has been employed to treat wounds, infections, and respiratory disorders (Jedid et al., 2022). These applications are largely attributed to the phytochemicals in rosemary extracts, which significantly enhance their antibacterial activity (Gonelimali et al., 2018; Oliver-Méndez et al., 2022). Numerous studies have investigated the extraction and characterization of bioactive compounds from *R. officinalis*, most of which identify the leaves as having the highest concentration of secondary metabolites, which are key to the plant's antimicrobial and antioxidant properties (Nieto et al., 2018; Lešnik et al., 2021).

Modarresi-Chahardehi et al. (2012) reported that organic solvent extracts from the aerial parts of *R. officinalis* exhibited bactericidal effects against *Enterococcus* species. A study by Silva et al. (2019) evaluated ethanolic extracts of *R. officinalis* against *E. faecalis* and *E. faecium*, applied 12 hours before bacterial inoculation, had the most significant reduction in bacterial growth, further supporting its potential as a natural antimicrobial agent. This was consistent with the present findings, which showed that organic solvent extracts were more effective than aqueous extracts in resisting bacterial growth.

The analysis of *R. officinalis* extract in the present study was consistent with a study by Salehi et al. (2021) and Bejenaru et al. (2024), which confirmed the presence of different phenolic compounds such as rosmarinic acid, caffeic acid, ferulic acid, apigenin-7-glucoside, sinapic acid, protocatechuic acid, syringic acid, p-hydroxybenzoic acid, apigenin, gentisic acid, and catechins. The present study hypothesized that the primary reason for the excellent antibacterial activity of rosemary extract was its natural composition, which included a variety of phenolic compounds. These compounds, which are fat-soluble and volatile, yielded higher concentrations when extracted with ethanol, enhancing their antimicrobial and antioxidant effects.

The mechanism of action of this antimicrobial activity of these polyphenols seems to be in the disruption of the bacterial plasma membrane due to the concentration of hydroxyl groups. This aggregation changed membrane hydrophobicity and charge surfaces and initiated segregation of lipid, localized rupture, pore formation, and leakage. The present mechanism was consistent with the former results of Borges et al. (2013) and Álvarez-Martínez et al. (2021).

Compounds such as Protocatechuic Acid (PCA) are effective against a wide spectrum of both Gram-positive and Gram-negative bacteria, such as *S. aureus* and *E. coli*. Protocatechuic acid significantly reduces biofilm formation in *P. aeruginosa* (Fifere et al., 2022) by disrupting bacterial cell walls, inhibiting biofilm formation, and interfering with bacterial enzymatic systems. Caffeic acid disrupts bacterial membranes, generating oxidative stress and inhibiting key enzymes involved in energy metabolism and cell wall synthesis, leading to bacterial death (Gül and Günay, 2021).

The mechanisms of action are also more or less the same, as ferulic acid and sinapic acid have antibacterial activity, which disrupts bacterial cell membranes, producing reactive oxygen species (ROS), and hindering important enzymatic processes needed by the bacteria to survive (Sánchez-Maldonado et al., 2011; Li et al., 2018; Huang et al., 2020; Hussain and Reigosa, 2021). Apigenin-7-glucoside also inhibits biofilm formation and is effective against both Gram-positive and Gram-negative bacteria, including *S. aureus*, *E. coli*, and *Salmonella enterica*.

Finally, rosmarinic acid has been shown to disrupt bacterial cell membranes, generate ROS, and interfere with bacterial quorum sensing, reducing bacterial pathogenicity (Fernández et al., 2018). Due to the presence of these bioactive compounds, rosemary extract demonstrated significant antibacterial activities against *Enterococcus* species. Thus, it can serve as a safer and more environmentally friendly alternative to traditional antibiotics against bacteria that have developed increasing resistance.

CONCLUSION

The present study highlighted the significant antimicrobial activity of *Rosmarinus officinalis* extract against *Enterococcus faecalis* and *Enterococcus faecium in vitro*, demonstrating its potential to inhibit bacterial growth effectively. The current findings suggested that plant-derived extracts could serve as a viable and eco-friendly alternative to conventional chemical antibiotics in managing bacterial infections, particularly those caused by antibiotic-resistant strains. Future studies should focus on evaluating the efficacy of rosemary extract in clinical settings to assess its potential in reducing the incidence of nosocomial bacterial infections.

DECLARATIONS

Ethical considerations

The present study was originally written by the authors and has not been published elsewhere. The authors checked the text of the article for plagiarism index and confirmed that the manuscript is written based on their original scientific results

Competing interests

The authors declare that there are no conflicts of interest regarding the publication of the present study. No financial, institutional, or personal relationships have influenced the interpretation or presentation of the data discussed in this article.

Availability of data and materials

The data to support this study finding is available upon reasonable request to the corresponding author

Authors' contributions

Eman Selema was responsible for the overall conceptualization of the study, supervised the research process, and performed the final revision of the manuscript. Eman Y. T. Elarinya conducted the literature review and contributed to the initial drafting of the manuscript. Nabawy Elnabawy contributed to data analysis and the interpretation of findings. Ahmed F. El-Sayed contributed to the development of the methodology and verification of references. Heba A. Ahmed assisted in data collection and the preparation of tables and figures. Safaa A. M. Abdel-Karim provided a critical review of the manuscript and handled formatting for submission. All authors have read and approved the final edition of the manuscript.

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