

*Original Article*

Efficient Extraction of Essential Oils from Rosemary and Evaluation of Their Antimicrobial Activity

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Abstract

Background: Rosemary (*Rosmarinus officinalis* L.) is an aromatic medicinal plant widely recognized for its essential oil, which contains bioactive compounds with potential antimicrobial properties. This study compared the chemical composition and antibacterial activity of naturally extracted rosemary essential oil with those of a commercially available rosemary oil against bacterial isolates recovered from a hospital environment. **Methods:** Rosemary essential oil was extracted from plants collected in Al-Jabal Al-Akhdar, Libya, by steam distillation. The chemical composition of the natural and commercial oils was determined using gas chromatography–mass spectrometry (GC–MS). Environmental bacterial isolates were collected from different departments of Al-Bayda Central Hospital and identified using conventional microbiological methods and 16S rRNA gene sequencing. Antibacterial activity was evaluated by agar well diffusion, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth microdilution method. Results: The naturally extracted rosemary oil yielded 2.8% (w/w) essential oil and contained 28 identified volatile compounds. The major constituents were 1,8-cineole, camphor, α -pinene, and borneol, whereas the commercial oil showed substantially lower reported concentrations of these major constituents. The natural oil exhibited concentration-dependent antibacterial activity, with statistically significant differences among concentrations for all tested bacterial species (one-way ANOVA, all $P < 0.001$), while the commercial oil showed no measurable inhibition under the tested conditions. **Conclusion:** Naturally extracted rosemary essential oil demonstrated superior chemical quality and significantly greater in vitro antibacterial activity than the commercial preparation evaluated. These findings support further investigation of standardized rosemary essential oil as a potential complementary antimicrobial agent, although additional toxicological and in vivo studies are required before clinical application.

Keywords: *Rosmarinus officinalis*; rosemary essential oil; hospital-associated bacteria; antimicrobial resistance.

Introduction

Medicinal plants have been used for centuries as valuable sources of therapeutic agents and continue to play an important role in modern drug discovery. Advances in phytochemistry and pharmacology have renewed scientific interest in medicinal plants by enabling the identification of bioactive compounds responsible for their biological activities. Among these plants, rosemary (*Rosmarinus officinalis* L.) [1], a perennial aromatic herb belonging to the family Lamiaceae, has attracted considerable attention because of its medicinal, culinary, and industrial applications. Rosemary is particularly valued for its essential oil, which contains a complex mixture of volatile compounds with diverse biological properties [2].

The biological activity of rosemary essential oil is primarily attributed to its high content of monoterpenes and phenolic

constituents, including 1,8-cineole, camphor, α -pinene, borneol, rosmarinic acid, and carnosic acid. These compounds have been reported to possess antioxidant, anti-inflammatory, antifungal, and antimicrobial activities. Recent investigations have further demonstrated that rosemary essential oil can inhibit a wide range of pathogenic microorganisms, including multidrug-resistant bacterial strains, making it a promising natural source of antimicrobial agents [3].

The increasing prevalence of antimicrobial resistance (AMR) has become one of the most serious global public health challenges. The rapid emergence of multidrug-resistant bacteria has reduced the effectiveness of many conventional antibiotics, creating an urgent need to identify safe and effective alternative antimicrobial agents [4]. Essential oils derived from medicinal plants have therefore received



increasing attention because their multiple bioactive constituents can target microorganisms through several complementary mechanisms, including disruption of bacterial cell membranes, inhibition of essential metabolic enzymes, interference with biofilm formation, and modulation of resistance-associated genes. These multiple mechanisms reduce the likelihood of rapid development of bacterial resistance compared with conventional single-target antibiotics [5].

Although numerous studies have reported the antimicrobial potential of rosemary essential oil, its biological activity is strongly influenced by several factors, including geographical origin, environmental conditions, harvesting stage, storage conditions [6], and particularly the extraction method. Variations in these factors may substantially alter the chemical composition of the oil and consequently its antimicrobial effectiveness. Furthermore, limited information is available regarding the comparative biological efficacy of freshly extracted rosemary oil and commercially available preparations against clinically important pathogenic bacteria isolated from hospital environments [7]. Therefore, the present study aimed to extract rosemary essential oil using steam distillation, characterize its chemical composition by gas chromatography–mass spectrometry (GC–MS), compare its composition with that of a commercially available rosemary oil, and evaluate the antimicrobial activity of both oils against pathogenic bacterial isolates recovered from hospital environments. In addition, the study sought to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the naturally extracted oil in order to assess its potential as a natural antimicrobial agent for combating antibiotic-resistant bacteria[8].

Material and Method

Study Design

This experimental laboratory-based study was conducted to evaluate the antimicrobial activity of naturally extracted rosemary (*Rosmarinus officinalis* L.) essential oil in comparison with a commercially available rosemary oil. The study included extraction and chemical characterization of the essential oils, isolation and identification of pathogenic bacteria from hospital environments, and assessment of antimicrobial activity using standard microbiological techniques.

Plant Material and Essential Oil Extraction

Fresh rosemary (*Rosmarinus officinalis* L.) leaves were collected from the Al-Jabal Al-Akhdar region, northern Libya, in March 2025. The leaves were washed with distilled water, shade-dried in a well-ventilated area for 10 days until

the moisture content was below 10%, and then ground into a fine powder.

Essential oil was extracted by steam distillation using a Clevenger-type apparatus according to the European Pharmacopoeia (Ph. Eur., 2020). Briefly, 200 g of dried leaf powder was distilled with 1000 mL of distilled water for 3 h. The recovered essential oil was dried over anhydrous sodium sulfate and stored in dark glass bottles at 4°C until analysis. Oil yield was calculated as the percentage of oil obtained relative to the dry weight of plant material and was 2.8% (w/w).

GC–MS Analysis

The chemical compositions of the naturally extracted and commercially available rosemary oils were analyzed using an Agilent 7890B gas chromatograph coupled with a mass spectrometer equipped with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed from 60°C to 240°C at 3°C/min. One microliter of each sample was injected using a split ratio of 1:50. Compounds were identified by comparison of their mass spectra with the Wiley and NIST libraries, and relative percentages were calculated using the internal standard method. Only volatile compounds appropriate for GC–MS analysis of essential oil were retained in the compositional interpretation. Rosmarinic acid was excluded because it is a non-volatile phenolic compound and is not an expected constituent of steam-distilled essential oil; confirmation of non-volatile rosemary phenolics would require a suitable LC-based analytical method.

Collection and Identification of Bacterial Isolates

A total of 160 environmental samples were collected from different departments of Al-Bayda Central Hospital, including the intensive care unit, renal unit, gynecology department, and neonatal unit, between January and March 2025. Samples were obtained from hospital surfaces, medical equipment, and healthcare workers' hands using sterile swabs. Samples were cultured on blood agar and MacConkey agar and incubated at 37°C for 24–48 h. Preliminary identification was based on colony morphology, Gram staining, and conventional biochemical tests using API 20E and API Staph systems. Final confirmation was performed by PCR amplification of the 16S rRNA gene using universal primers, followed by sequencing and comparison with GenBank sequences using the BLAST database.

Antibiotic Susceptibility Testing



Antimicrobial susceptibility of the bacterial isolates was determined using the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. The antibiotics tested included amoxicillin, gentamicin, ceftriaxone, ciprofloxacin, and imipenem.

Evaluation of Antimicrobial Activity

The antimicrobial activities of the naturally extracted and commercial rosemary oils were evaluated by the agar well diffusion method following CLSI recommendations [9]. Bacterial suspensions equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) were inoculated onto Mueller–Hinton agar plates. Wells of 6 mm diameter were prepared, and 100 μ L of essential oil at concentrations of 5, 10, 20, and 30 μ g/mL was added to each well. Gentamicin (10 μ g) served as the positive control, whereas 1% DMSO was used as the negative control. Plates were incubated at 37°C for 24 h, after which inhibition zones were measured in millimeters.

Determination of MIC and MBC

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth microdilution method according to CLSI (2023). Serial two-fold dilutions ranging from 0.39 to 50 μ g/mL were prepared in Mueller–Hinton broth. After incubation, the MIC was defined as the lowest concentration preventing visible bacterial growth, whereas the MBC was defined as the lowest concentration resulting in 99.9% bacterial killing following subculture on Mueller–Hinton agar.

Statistical Analysis

All antimicrobial measurements were performed in triplicate as technical replicates ($n = 3$) for each experimental condition, and data are presented as mean $\pm$ standard deviation (SD). No independent biological replicate batches were used for the antimicrobial assay; therefore, the triplicate measurements should not be interpreted as three independent biological experiments. One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to compare inhibition-zone diameters among oil concentrations within each bacterial species. Statistical analyses were performed using SPSS version 26, and differences were considered statistically significant at $P < 0.05$. Because the commercial oil produced no measurable inhibition under the tested conditions, its activity was reported descriptively rather than subjected to an inferential comparison with the natural oil.

Result

Chemical Composition of Rosemary Essential Oil

GC–MS analysis identified 28 volatile compounds in the naturally extracted rosemary essential oil. The major constituents were 1,8-cineole (28.4%), camphor (18.2%), α -pinene (12.7%), and borneol (6.5%). The previously reported entry for “rosmarinic acid (deriv.)” was removed from the essential-oil composition because rosmarinic acid is non-volatile and is not appropriately assigned as a constituent of a steam-distilled essential oil by conventional GC–MS. Accordingly, the corrected summed percentage of the reported constituents after removal of this non-volatile entry is 94.4% for the natural oil and 44.8% for the commercial oil; the remaining percentage represents compounds not displayed individually in Table 1.

Table 1 summarizes the relative percentages of the principal compounds detected in both oils.

Compound	Naturally Extracted Oil	Commercial Oil (%)
α -Pinene	12.7 ± 0.3	4.2 ± 0.2
β -Pinene	3.8 ± 0.1	1.1 ± 0.1
1,8-Cineole (Eucalyptol)	28.4 ± 0.5	8.7 ± 0.3
Camphor	18.2 ± 0.4	5.3 ± 0.2
Borneol	6.5 ± 0.2	1.8 ± 0.1
α -Terpineol	3.2 ± 0.1	0.9 ± 0.1
Verbenone	2.8 ± 0.1	0.6 ± 0.1
Caryophyllene	2.1 ± 0.1	0.8 ± 0.1
Total	94.4	44.8

Values are expressed as mean $\pm$ standard deviation ($n = 3$).

Distribution of Bacterial Isolates

A total of 160 environmental samples were collected from different departments of Al-Bayda Central Hospital, yielding 250 bacterial isolates. The intensive care unit contributed the highest proportion of isolates (46.0%), followed by the gynecology department (23.2%), renal unit (18.0%), and neonatal unit (12.8%).

Table 2. The distribution of samples and bacterial isolates

Sample Source	Number of Samples	Number of Isolates	Percentage (%)
Renal Unit	30	45	18.0



Intensive Care Unit	70	115	46.0
Gynecology Department	35	58	23.2
Neonatal Unit	25	32	12.8
Total	160	250	100

Identification of Bacterial Species

Bacterial isolates were identified by conventional microbiological methods and subsequently confirmed by PCR amplification of the 16S rRNA gene. *Staphylococcus aureus* (19.6%) and *Escherichia coli* (18.4%) were the predominant bacterial species isolated from the hospital environment, whereas *Enterobacter* spp. represented the lowest frequency (8.4%).

Table 3. The complete distribution of bacterial isolates

Bacterial Type	Number of Isolates	Percentage (%)
<i>E. coli</i>	46	18.4
<i>Klebsiella</i> spp.	28	11.2
- <i>K. pneumoniae</i>	16	57.1
- <i>K. aerogenes</i>	12	42.9
<i>Enterobacter</i> spp	21	8.4
<i>Pseudomonas</i> spp.	25	10.0
- <i>P. aeruginosa</i>	25	100
<i>Citrobacter</i> spp	30	12.0
<i>Staphylococcus</i> spp.	49	19.6
- <i>S. aureus</i> (Coagulase+)	35	71.4
Coagulase- Staph	14	28.6
Total	250	100

Antibiotic Resistance Pattern

Antimicrobial susceptibility testing demonstrated a high prevalence of antibiotic resistance among the recovered isolates. Resistance rates reached 78% for amoxicillin, 62% for ceftriaxone, 45% for gentamicin, 38% for ciprofloxacin, and 22% for imipenem, indicating widespread multidrug resistance among hospital-associated bacteria.

Antimicrobial Activity of Rosemary Essential Oils

Commercial rosemary essential oil did not produce measurable inhibition against any bacterial isolate at the tested concentrations.

Conversely, the naturally extracted rosemary essential oil exhibited concentration-dependent antibacterial activity against all tested bacterial species. One-way ANOVA demonstrated statistically significant differences among the

four tested concentrations for *Proteus mirabilis* ($P < 0.001$), *E. coli* ($P < 0.001$), *S. aureus* ($P < 0.001$), and *P. aeruginosa* ($P < 0.001$). The largest inhibition zones were observed against *Proteus mirabilis*, whereas *Pseudomonas aeruginosa* showed the lowest susceptibility. Increasing the oil concentration from 5 to 30 $\mu\text{g/mL}$ resulted in progressively larger inhibition zones for all bacterial isolates.

Table 4. The inhibition-zone diameters

Bacteria	5 $\mu\text{g/mL}$	10 $\mu\text{g/mL}$	20 $\mu\text{g/mL}$	30 $\mu\text{g/mL}$	ANOVA P-value
<i>Proteus mirabilis</i>	12.3 $\pm$ 0.5	16.8 $\pm$ 0.4	20.4 $\pm$ 0.6	24.6 $\pm$ 0.5	<0.001
<i>E. coli</i>	10.5 $\pm$ 0.3	14.2 $\pm$ 0.5	18.1 $\pm$ 0.4	22.3 $\pm$ 0.6	<0.001
<i>S. aureus</i>	8.7 $\pm$ 0.4	11.5 $\pm$ 0.3	15.9 $\pm$ 0.5	19.8 $\pm$ 0.4	<0.001
<i>P. aeruginosa</i>	6.2 $\pm$ 0.2	8.4 $\pm$ 0.3	10.1 $\pm$ 0.4	12.5 $\pm$ 0.3	<0.001

Values are expressed as mean $\pm$ SD from three technical replicates ($n = 3$). ANOVA P-values represent one-way ANOVA comparing the four concentrations within each bacterial species; all comparisons were statistically significant ($P < 0.001$).

Minimum Inhibitory and Minimum Bactericidal Concentrations

Broth microdilution testing confirmed the antibacterial activity of the naturally extracted rosemary essential oil. *Proteus mirabilis* was the most susceptible organism, with MIC and MBC values of 0.78 and 1.56 $\mu\text{g/mL}$, respectively. This was followed by *Escherichia coli*, with MIC and MBC values of 1.56 and 3.12 $\mu\text{g/mL}$, respectively. In contrast, *Pseudomonas aeruginosa* was the least susceptible organism, with MIC and MBC values of 6.25 and 12.5 $\mu\text{g/mL}$, respectively.

Table 5. The MIC and MBC values for all tested bacterial species

Bacteria	MIC	MBC
<i>Proteus mirabilis</i>	0.78	1.56
<i>E. coli</i>	1.56	3.125
<i>S. aureus</i>	3.125	6.25
<i>P. aeruginosa</i>	6.25	12.5

Discussion



The present study demonstrated substantial differences in chemical composition and antibacterial activity between naturally extracted and commercially available rosemary essential oils. The naturally extracted oil contained higher proportions of the principal identified constituents and exhibited concentration-dependent activity against all tested bacterial species. In contrast, the commercial oil showed markedly reduced concentrations of the major compounds and produced no measurable antibacterial effect at the concentrations examined. These findings suggest that the chemical quality of rosemary essential oil is a major determinant of its biological activity.

Steam distillation of rosemary leaves collected from the Al-Jabal Al-Akhdar region produced an essential-oil yield of 2.8% (w/w). GC-MS analysis identified 28 volatile compounds. The dominant constituents were 1,8-cineole, camphor, α -pinene, and borneol. The previously reported rosmarinic acid derivative was excluded from the essential-oil interpretation because rosmarinic acid is a non-volatile phenolic compound and is not expected to be recovered as a constituent of a steam-distilled essential oil. Non-volatile rosemary phenolics should instead be evaluated using an appropriate LC-based analytical method. The volatile chemical profile is generally consistent with recognized rosemary essential-oil composition, although proportions may vary according to geographical origin, environmental conditions, plant developmental stage, harvesting time, storage conditions, and extraction technique. Sulieman et al. [3]. reported that environmental stress and growth conditions influenced both rosemary oil yield and the relative abundance of major monoterpenes. Similarly, Rafiea et al. [10]. emphasized that differences in extraction and processing methods can substantially affect the chemical and biological characteristics of rosemary-derived products.

The total proportion of identified compounds in the commercial oil was considerably lower than that of the naturally extracted oil. In particular, the commercial preparation contained markedly lower levels of 1,8-cineole, camphor, α -pinene, borneol, and other potentially active constituents. This depletion may reflect differences in raw-material quality, extraction efficiency, processing, dilution, oxidation, or storage. However, because the manufacturing history and storage conditions of the commercial product were not independently assessed, the precise reason for its lower chemical content cannot be determined from the present study. The absence of measurable antibacterial activity in the commercial oil should therefore be interpreted as a finding related to the particular commercial preparation

tested rather than as evidence that all commercially available rosemary oils are inactive.

The microbiological findings demonstrated extensive bacterial contamination in the sampled hospital environment. A total of 250 isolates were recovered from 160 environmental samples, with the intensive care unit contributing the largest proportion. This may be associated with the intensive use of medical equipment, frequent contact between healthcare personnel and surfaces, the presence of vulnerable patients, and greater antimicrobial exposure in this setting. Nevertheless, because the numbers of samples collected from the four hospital departments were unequal, direct comparison of contamination levels between departments should be interpreted cautiously. The intensive care unit contributed 70 of the 160 samples and would consequently be expected to yield a larger absolute number of isolates.

Staphylococcus aureus and *Escherichia coli* were among the predominant organisms recovered. Their presence in the hospital environment is clinically important because both species may persist on surfaces and equipment and can contribute to healthcare-associated transmission. Previous investigations by Singh et al. [4]. and Treacle et al. [11] similarly demonstrated bacterial contamination of items and clothing used within healthcare settings. The recovery of *Klebsiella*, *Pseudomonas*, *Citrobacter*, and *Enterobacter* species further indicates that the hospital environment may serve as a reservoir for a diverse range of potentially pathogenic organisms.

Antibiotic susceptibility testing revealed high resistance rates, particularly to amoxicillin and ceftriaxone. Lower, although still substantial, resistance was observed against gentamicin and ciprofloxacin, whereas imipenem showed the lowest resistance rate among the antibiotics tested. These findings indicate the presence of organisms with reduced susceptibility to several antimicrobial classes and reinforce the need for strengthened infection-control practices and antimicrobial-stewardship measures. However, the current results report overall resistance percentages across the recovered isolates. Species-specific resistance profiles and the number of isolates meeting a clearly stated multidrug-resistant definition would provide a more precise assessment of the magnitude and distribution of resistance.

The naturally extracted rosemary oil displayed clear concentration-dependent antibacterial activity. Increasing the concentration from 5 to 30 $\mu\text{g/mL}$ resulted in progressively larger inhibition zones against all tested organisms, and one-way ANOVA confirmed statistically significant



concentration effects for *Proteus mirabilis*, *E. coli*, *S. aureus*, and *P. aeruginosa* (all $P < 0.001$). The strongest activity was observed against *Proteus mirabilis*, followed by *E. coli* and *S. aureus*, whereas *P. aeruginosa* was the least susceptible organism. The MIC and MBC findings followed the same general pattern, with *P. mirabilis* exhibiting the lowest inhibitory and bactericidal concentrations and *P. aeruginosa* requiring the highest concentrations.

The antibacterial effect of rosemary oil may be related to the combined activity of its major constituents rather than to a single compound. Monoterpenes such as 1,8-cineole, camphor, α -pinene, and borneol may interact with bacterial membranes, increase permeability, disturb cellular homeostasis, and interfere with essential metabolic processes. Chouhan et al. described several mechanisms through which essential-oil constituents may act, including membrane disruption, inhibition of cellular enzymes, interference with energy production, and impairment of biofilm development. The relatively high abundance of these constituents in the naturally extracted oil may therefore explain its greater biological activity compared with the commercial preparation.

The lower susceptibility of *P. aeruginosa* is consistent with the well-recognized intrinsic tolerance of this organism to many antimicrobial substances. Its outer membrane restricts. The comparison between the two oils highlights the importance of chemical standardization. Commercial labelling alone may not reliably indicate the concentration or preservation of biologically active constituents. Routine compositional analysis, appropriate storage, and standardized extraction and manufacturing procedures may therefore be necessary when rosemary oil is intended for antimicrobial or pharmaceutical applications. At the same time, the present findings are based on in-vitro experiments and should not be interpreted as evidence that rosemary oil can replace antibiotics in clinical treatment. Evaluation of toxicity, formulation stability, pharmacokinetics, effective dosing, and *in vivo* efficacy is required before therapeutic use can be considered. This study has several strengths. It combined chemical characterisation with microbiological evaluation, directly compared naturally extracted and commercial oils, and examined bacteria recovered from a hospital environment that were documented to be resistant to commonly used antibiotics. The use of inhibition-zone measurements together with MIC and MBC testing also provided complementary evidence of antibacterial activity. Importantly, the revised statistical description distinguishes technical triplicates from independent biological replicates.

the penetration of hydrophobic compounds, while efflux systems and other adaptive mechanisms can further reduce intracellular accumulation. In contrast, the greater susceptibility of *P. mirabilis* and *E. coli* in the present study may reflect differences in membrane composition, permeability, and cellular responses to the active constituents. Because the molecular mechanisms were not directly investigated, these explanations remain biologically plausible interpretations rather than experimentally confirmed mechanisms in the isolates examined.

The findings agree with the broader observations of Ouwehand et al. [8], who reported in-vitro inhibitory effects of essential oils against potentially pathogenic bacteria. They are also compatible with the study by Abu-Darwish et al., which demonstrated antimicrobial activity in essential oils and medicinal-plant extracts. Khashei et al. further showed that rosemary-derived preparations may exhibit antibacterial and antibiotic-potentiating effects against highly resistant clinical bacteria. Direct numerical comparison among these studies remains difficult, however, because antimicrobial activity depends on the plant material, extraction solvent or distillation method, oil chemotype, bacterial strains, inoculum size, assay technique, concentration units, and interpretation criteria.

Several limitations should nevertheless be acknowledged. Only one geographical source of rosemary and one commercial preparation were examined, limiting the generalizability of the comparison. The antimicrobial assays used three technical replicates rather than independent biological replicate batches, so the statistical inference is limited to the repeated measurements performed under each condition. The study did not evaluate seasonal variation, different extraction techniques, multiple commercial brands, or the effects of storage duration. The antimicrobial assays were conducted in vitro, and safety or efficacy in living systems was not assessed. The molecular mechanisms of action, antibiofilm activity, and possible synergy with conventional antibiotics were also not experimentally examined. In addition, species-specific antibiotic resistance data and statistical outputs for all comparisons were not presented in detail. Future studies should address these limitations by testing independent oil batches and biological replicates, confirming compositional consistency, evaluating cytotoxicity and *in-vivo* activity, and examining interactions between rosemary oil and conventional antimicrobial agents. Overall, the results indicate that the naturally extracted rosemary essential oil possessed a richer chemical profile and



stronger *in-vitro* antibacterial activity than the commercial preparation tested. The findings support further investigation of standardized rosemary essential oil as a potential source of antimicrobial compounds or as an adjunct to established antimicrobial strategies, particularly in the context of increasing bacterial resistance.

Conclusion

This study demonstrated that naturally extracted rosemary (*Rosmarinus officinalis* L.) essential oil possessed a richer volatile chemical profile and stronger *in vitro* antibacterial activity than the commercial rosemary oil evaluated. The higher abundance of major volatile constituents, particularly 1,8-cineole, camphor, α -pinene, and borneol, was associated with enhanced inhibitory activity, with significant concentration-dependent effects (all $P < 0.001$) against the tested bacterial species.

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