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Original Article

Antibacterial and Antibiofilm Activity of *Hypericum Perforatum* L. Extract on Bacteria Isolated from Used Cosmetic Tools

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Abstract
Cosmetic products and shared cosmetic tools can serve as reservoirs for pathogenic microbes, increasing the risk of cross-contamination and infection. This study evaluated the antibacterial and antibiofilm activities of *Hypericum perforatum* L. extracts against bacteria isolated from cosmetic tools used in beauty salons in Istanbul, Turkey. A total of 40 tools were examined, and bacterial identification was performed using selective media. Antibiotic resistance profiles were determined using the Kirby–Bauer disk diffusion method, and the multiple antibiotic resistance index (MARI) was calculated. Biofilm formation was assessed using the crystal violet assay. Bacterial growth was detected in 77.5% of samples. Isolates included Gram-positive cocci (32.25%), Gram-negative bacilli (22.59%), and Gram-positive bacilli (45.16%). MRSA was identified in 25.80% of isolates, and 70.97% showed alpha-hemolysis. The highest antibiotic resistance was observed against cefotaxime (41.93%), while susceptibility was noted for ciprofloxacin, tetracycline, amikacin, and gentamicin. The pharmaceutical-grade *H. perforatum* extract showed antibacterial activity (51.61%) and antibiofilm inhibition (60.23%). The market extract exhibited lower antibacterial activity (35.48%) but higher antibiofilm activity (87.55%). These results demonstrate that antibacterial effects on planktonic cells and antibiofilm activity are distinct properties, as stronger growth inhibition did not necessarily correspond to greater biofilm inhibition. The findings suggest that *H. perforatum* extracts may help control bacterial growth and biofilm formation on cosmetic tools.
Keywords. Antibacterial Activity, Antibiofilm, *Hypericum Perforatum*, Cosmetic Tools, Antibiotic Resistance.

Introduction

Makeup tools and cosmetics are among the most widely used and shared products in recent years, frequently handled by multiple individuals. These tools, including brushes and sponges that come into direct contact with cosmetic products, are considered primary vectors for microbial cross-contamination [1]. Products such as foundation sponges, blushes, eyeshadows, and lipstick brushes can transmit microorganisms due to repeated use without cleaning, direct contact with facial flora, inadequate hygiene practices, improper storage, and communal use [2]. Such contamination poses not only personal and public health risks but also contributes to the development of skin infections [2].

Cosmetic products and applicators provide ideal conditions for microbial growth, increasing the risk of bacterial, fungal, and viral infections [3]. Factors contributing to contamination include the presence of nutrients in cosmetics, such as humectants and minerals, as well as the lack of clearly indicated production and expiration dates, which complicates monitoring of preservative efficacy over time [2]. Consequently, there is a need for strict quality control and hygiene practices in the cosmetics industry [4].

As cosmetics are applied to sensitive areas such as the face, eyes, and lips, pathogenic microbes present on contaminated products and tools can cause skin infections, conjunctivitis, and other health complications [5]. Items like brushes, sponges, and lipstick applicators may harbor bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis*, *Bacillus subtilis, Pseudomonas aeruginosa*, *Escherichia coli, Enterococcus*, and *Klebsiella species* [4,7]. These organisms can lead to abscesses, impetigo, dermatitis, and even systemic infections, especially in individuals with weakened immunity [8,9].

The ability of bacteria to form biofilms represents a major adaptive mechanism that enhances resistance to environmental stress, antibiotics, disinfectants, and biocides [8]. Biofilms are structured communities of bacteria embedded in an extracellular matrix composed of proteins, DNA, and polysaccharides, which develop preferentially on nutrient-rich and moisture-retaining surfaces such as cosmetic tools [10,11]. Common biofilm-forming pathogens include *S. aureus, S. epidermidis, B. subtilis*, and *P. aeruginosa,* frequently isolated from cosmetic products and applicators [10]. Biofilm formation promotes the rapid spread of antibiotic resistance and diminishes the effectiveness of routine cleaning, posing additional public health risks [8]. *Hypericum perforatum* L., a member of the *Hypericaceae* family, exhibits antibacterial, antiviral,

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antitumoral, antibiofilm, antioxidant, anti-inflammatory, and analgesic activities [13,14]. Its bioactive compounds, including flavonoids, terpenoids, and phenolic acids, can inhibit bacterial growth by disrupting cell morphology, reducing membrane permeability, and interfering with quorum sensing, thereby controlling virulence factor production [14,15].

Given the widespread use and sharing of cosmetic tools, it is crucial to investigate the antibiotic resistance profiles and biofilm-forming abilities of bacteria isolated from these products. This study aims to evaluate the antibacterial and antibiofilm activity of *H. perforatum* L. extract against bacterial isolates from commonly used cosmetic tools, while also raising public awareness of the potential risks associated with pathogenic microbes in makeup applicators [16,17].

Methods

Sample Collection and Bacterial Isolation

Forty cosmetic tools, including foundation sponges, blush brushes, eyeshadow brushes, and lipstick brushes, were collected from beauty salons in Istanbul, Turkiye, using sterile techniques and transported to the laboratory under aseptic conditions. Samples were immediately processed to minimize contamination. Bacteria were isolated by inoculating the samples onto selective and differential media, including MacConkey agar, Eosin Methylene Blue agar, Mannitol Salt Agar, and 5% blood agar, followed by incubation at 37°C for 24–48 h. Distinct colonies were selected based on morphology and subcultured to obtain pure isolates for further identification and analysis.

Biofilm Formation Assay and Controls

The biofilm-forming capacity of bacterial isolates obtained from make-up tools was evaluated using the crystal violet staining method as described by [16]. *Staphylococcus aureus* ATCC 25923 was used as a positive control for biofilm formation, while sterile distilled water served as the negative control.

Antibacterial Activity Assay

Two *Hypericum perforatum* L. extracts, a pharmaceutical-grade and a traditionally prepared extract, were tested for antibacterial and antibiofilm activities. Bacterial isolates from make-up tools were subcultured on Nutrient Agar and incubated at 37°C for 24–48 h. Single colonies were suspended in Mueller–Hinton Broth and adjusted to 0.5 McFarland. Antibacterial activity was assessed using the agar-well diffusion method, with 50 µL of each extract added to 6 mm wells on inoculated Mueller–Hinton Agar. Gentamicin (0.20 mg/mL) and Ampicillin (0.20 mg/mL) were used as positive controls for Gram-negative and Gram-positive bacteria, respectively, and sterile distilled water as a negative control. Plates were incubated at 37°C for 24 h, and inhibition zones were measured according to CLSI guidelines.

Antibiofilm Activity Assay

For antibiofilm assays, bacterial isolates were cultured on Nutrient Agar and incubated at 37°C for 24–48 h. Fresh colonies were suspended in Mueller–Hinton Broth and adjusted to a 0.5 McFarland standard. The antibiofilm activity of *Hypericum perforatum* L. extracts (HE1 and HE2) was evaluated using the crystal violet staining method [17]. Briefly, 180 µL of bacterial suspension and 20 µL of the tested extract were added to each well of sterile 96-well microplates. Wells containing bacterial suspension without extract served as positive controls, while broth alone served as a negative control. After incubation at 37°C for 24 h, wells were gently washed with PBS to remove planktonic cells, fixed with methanol, and stained with 1% crystal violet. The bound dye was solubilized using 95% ethanol, and absorbance was measured at 570 nm using a microplate reader. Biofilm inhibition (%) was calculated relative to untreated control wells.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) of the extracts were determined using the broth microdilution method [18]. Bacterial suspensions were diluted to obtain a final inoculum of 5×10^5 CFU/mL. Sterile U-bottom 96-well microplates were used, with serial two-fold dilutions of the extracts prepared in Mueller–Hinton Broth. Ceftriaxone was used as a positive control and tested using serial two-fold dilutions in parallel with the extracts, while sterile distilled water was used as a negative control. Plates were incubated at 37°C for 24 h, and MIC values were recorded as the lowest concentration showing no visible growth.

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Culture Media and Chemicals

All culture media and solutions, including Nutrient Agar, Mueller–Hinton Agar and Broth, Tryptic Soy Agar, MacConkey Agar, Eosin Methylene Blue Agar, Mannitol Salt Agar (with and without oxacillin), Plate Count Agar, Brain Heart Infusion Broth, phosphate-buffered saline (PBS), physiological saline, crystal violet, ethanol, glycerol, and blood agar, were prepared according to manufacturer instructions or standard protocols and sterilized by autoclaving at 121°C. Media formulations and applications followed established microbiological procedures and relevant references.

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean values, and statistical analyses were conducted to evaluate differences in antibacterial, antibiofilm, and antibiotic resistance results. Statistical significance was assessed at the predefined confidence level.
The Multiple Antibiotic Resistance Index (MARI) was calculated using the formula:
MARI = a / b
where a is the number of antibiotics to which the isolate was resistant, and b is the total number of antibiotics tested.

Results

Microbial Profile of Tested Cosmetic Products

Microbiological analysis revealed variable bacterial contamination among cosmetic tools (Table 1). The highest counts were detected in several blush brushes and foundation sponge samples, whereas lipstick and eyeshadow brushes showed comparatively lower contamination levels.

Table 1. Total aerobic mesophilic bacterial counts in cosmetic tools (CFU/mL)

Sample Code	Tool Type	Total Bacterial Count (CFU/mL)
B1	Blush brush	TNTC
B2	Blush brush	56 × 10^3
B3	Blush brush	4 × 10^3
F1	Foundation sponge	141 × 10^3
L1	Lipstick brush	2 × 10^3
S1	Eyeshadow brush	2 × 10^3

TNTC: Too Numerous to Count; CFU: Colony Forming Unit; mL: milliliter.

Bacterial counts were grouped by tool type, showing clear differences in contamination levels among the cosmetic instruments (Figure 1).

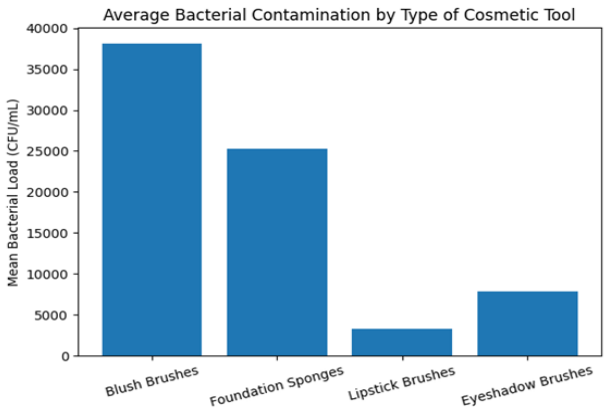

Figure 1. Mean aerobic mesophilic bacterial load (CFU/mL) according to cosmetic tool type

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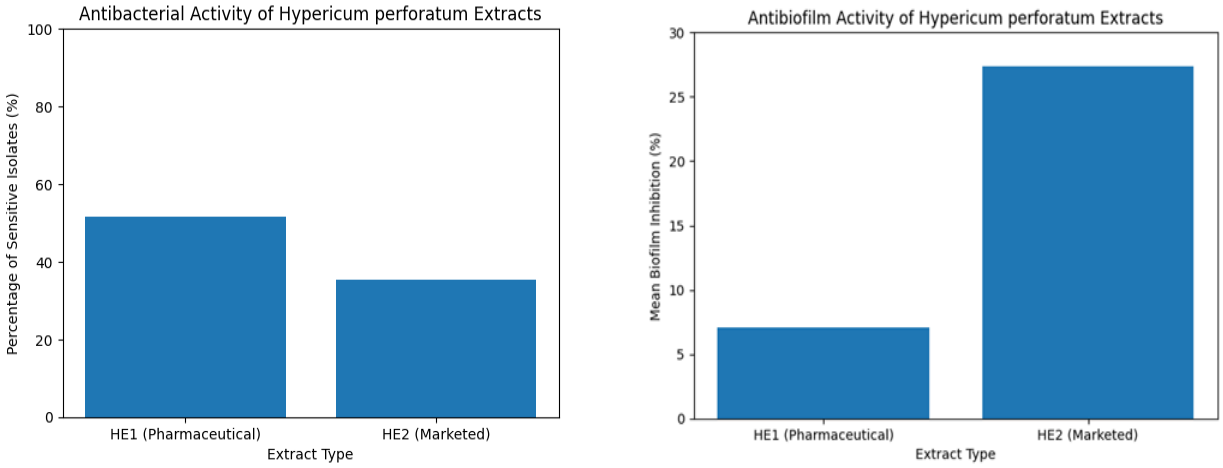
Antibacterial and Antibiofilm Activity of Hypericum perforatum L. Extracts

Two *Hypericum perforatum* L. extracts (pharmaceutical HE1 and locally marketed HE2) were tested against bacteria isolated from cosmetic tools. In agar diffusion assays, HE1 showed antibacterial activity against a greater proportion of isolates (51.6%) compared with HE2 (35.5%), as shown in Figure 2 (a). The antibacterial activity was further examined using the broth microdilution method. Both *Hypericum perforatum* extracts exhibited inhibitory effects against a limited number of isolates, while several strains showed no detectable inhibition within the tested concentration range, indicating variable susceptibility among bacteria. The observed MIC responses were strain-dependent and supported the agar diffusion findings, but did not demonstrate uniform activity against all isolates. These results suggest that the antibacterial effect of the extracts varies according to bacterial strain and does not necessarily reflect their antibiofilm performance. Biofilm inhibition showed a strain-dependent pattern. HE2 was active against more isolates and achieved higher biofilm inhibition percentages than HE1. HE1 demonstrated a mean biofilm inhibition of 60.23%, whereas HE2 reached 87.55%. Overall, the pharmaceutical extract demonstrated superior antibacterial activity against planktonic bacteria, whereas the locally marketed extract showed stronger antibiofilm effects, as shown in Figure 2 (b).



(**a**) (**b**)

Figure 2. (a) Antibacterial Activity of Hypericum perforatum Extracts (Comparison); (b) Antibiofilm Activity of Hypericum perforatum Extracts (Comparison).

Antibiotic Resistance Profiles of Cosmetic-Derived Isolates

Antibiotic susceptibility testing revealed heterogeneous resistance patterns among bacterial isolates recovered from cosmetic tools. All isolates were sensitive to ciprofloxacin, tetracycline, amikacin, and gentamicin, indicating the continued effectiveness of these agents. However, varying levels of resistance were observed toward β-lactam antibiotics, particularly cefotaxime, which showed the highest resistance rate among the tested antibiotics. Resistance was also noted against amoxicillin-clavulanic acid, cephalothin, and cefazolin. Variations in inhibition zone diameters among isolates are illustrated in Figure 3 (a). Several isolates exhibited multidrug resistance, with the highest antibiotic resistance index recorded for bacteria isolated from eyeshadow and lipstick brushes. Figure 3 (b) illustrates the resistance percentages of bacterial isolates to the tested antibiotics. The overall MARI value was 0.364, exceeding the critical threshold of 0.2, suggesting exposure to high-risk contamination sources with frequent antibiotic exposure.

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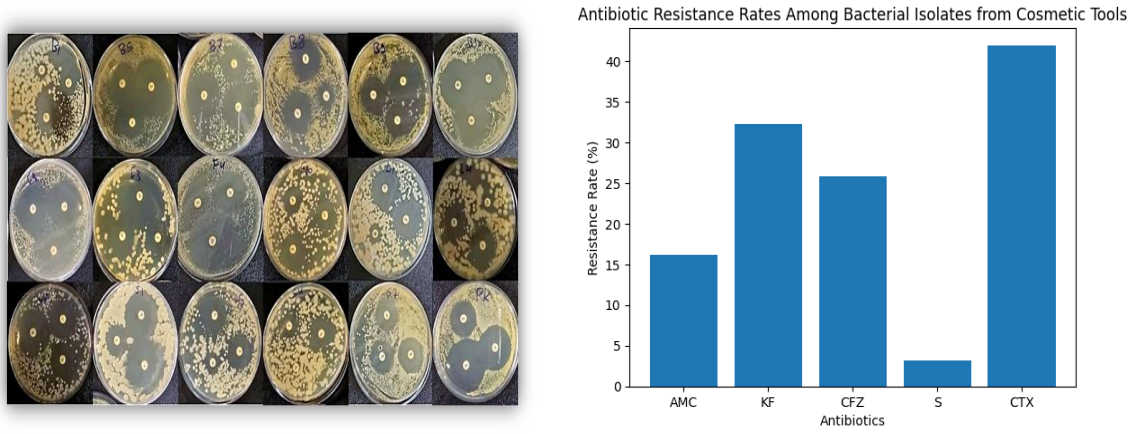

(**a**) (**b**)

Figure 3. (a) Inhibition zones (mm) of different antibiotics against bacterial isolates from cosmetic tools; (b) Antibiotic resistance rates (%) among bacterial isolates from cosmetic tools.

Discussion

The present study revealed a high contamination rate (77.5%) in cosmetic tools collected from beauty salons, consistent with previously reported high microbial loads in cosmetic products and applicators [12,21,22]. Variations among studies may be related to differences in product type, frequency of use, and hygiene practices. The predominance of *Staphylococcus, Streptococcus, and Bacillus* species agrees with earlier findings identifying these organisms as common contaminants of makeup sponges and brushes [23,12]. The porous and moisture-retaining nature of these tools facilitates microbial persistence, biofilm formation, and cross-contamination. The detection of MRSA supports concerns that beauty salons may serve as reservoirs for resistant pathogens [6],[22].

Antibiotic susceptibility testing demonstrated considerable resistance, with an overall MARI value of 0.364, exceeding the high-risk threshold of 0.2 and indicating exposure to antibiotic-rich environments [23],[24]. The presence of β-lactam resistance and multidrug-resistant isolates aligns with reports linking cosmetic tools to the dissemination of resistant bacteria [20]. Moreover, 41.93% of isolates formed biofilms, a trait known to enhance tolerance to disinfectants and antibiotics and complicate eradication from surfaces [6].

The evaluation of *Hypericum perforatum* L. extract showed notable antibacterial and antibiofilm activity, particularly against Gram-positive bacteria, consistent with earlier studies demonstrating the sensitivity of *Staphylococcus spp.* and other Gram-positive pathogens to hyperforin- and phenolic-rich extracts [26,27]. Reduced activity against Gram-negative bacteria may be attributed to outer membrane barriers [28,29]. The observed variation between extracts highlights the importance of extraction methods and standardization [29].

Overall, cosmetic tools in beauty salons represent potential vectors for pathogenic and antibiotic-resistant bacteria. The high resistance indices and biofilm formation capacity emphasize the need for strict sterilization protocols and hygiene monitoring from a public health perspective. The antimicrobial and antibiofilm properties of *H. perforatum* suggest its potential as a supportive natural agent in decontamination strategies, though further applied and in vivo studies are required.

Conclusion

Cosmetic tools were shown to be reservoirs of antibiotic-resistant and biofilm-forming bacteria, posing an overlooked public health risk and facilitating the spread of opportunistic pathogens. *Hypericum perforatum* extracts demonstrated notable antibacterial and strong antibiofilm activity, with biofilm inhibition in some cases exceeding direct antibacterial effects, indicating their potential to disrupt microbial persistence on cosmetic surfaces.

The high resistance profiles of the isolates highlight the need for alternative antimicrobial approaches that do not promote resistance. Plant-derived agents such as *H. perforatum*, combined with improved cosmetic hygiene practices, may help reduce infection risks associated with contaminated cosmetic products and warrant further applied research.

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Conflicts of Interest
The authors declare no conflicts of interest.

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