

RESEARCH ARTICLE

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# Essential Oils: A Synergistic Approach to Enhance Donor Skin Disinfection and Anti-biofilm Activity

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## Abstract

In blood donations, chlorhexidine gluconate (CHG) and isopropyl alcohol (IPA) are routinely used for skin disinfection. Despite this measure, contamination of platelet concentrates with biofilm-forming *Staphylococcus epidermidis* from donor skin remains a concern in transfusion medicine. Essential oils (EOs) not only possess antibacterial abilities but have possible synergistic effects with traditional disinfectants. This study evaluated the anti-biofilm activity of EOs from four Saudi Arabian plants (*Clitoria ternatea*, *Aerva lanata*, *Lavandula dentata*, and *Plumbago zeylanica*) as potential synergistic enhancers for skin disinfection. EO composition was evaluated by gas chromatography-mass spectrometry (GC-MS), and safe EO concentrations were determined using the rabbit skin irritation test. In anti-biofilm activity assays, *S. epidermidis* biofilms were treated with EOs alone or in conjunction with CHG or CHG-IPA and log-reduction of bacteria was calculated. Synergistic effects were evaluated through the coefficient of drug interaction. EO concentrations up to 20% were found to be non-irritating. Most EOs exhibited some anti-biofilm activity; *P. zeylanica* EO exerted the strongest effect, and also displayed suggestive of synergistic action with CHG and improved CHG-IPA performance. GC-MS identified plumbagin (79%) as the main oxygenated terpenoid component in *P. zeylanica* EO, and plumbagin individual activity demonstrated to be almost equivalent to the activity of *P. zeylanica*. The effectiveness of CHG-IPA against biofilms was greatly improved by combination with *P. zeylanica* EO or plumbagin. These results add to the evidence that natural compounds could enhance skin disinfection agents and help lower the risk of bacterial contamination in blood products.

**Keywords:** Biofilm, *Staphylococcus epidermidis*, Platelets, Skin Disinfection, Essential Oil

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## INTRODUCTION

At present, the main transfusion-associated infection risk from bacterial contamination of platelet concentrates (PCs).<sup>1</sup> PCs are made in an additive, pH-neutral solution containing around 25 g/L glucose, and are maintained under continuous agitation at 22-28 °C.<sup>2,3</sup> Blood centers in Canada, the United States, Germany, China, and New Zealand have shown the most frequently-identified aerobic contaminant of PCs to be coagulase-negative *Staphylococcus epidermidis*.<sup>4-7</sup> A normal human commensal that prefers moist niches such as the antecubital area, *S. epidermidis* is most likely introduced by the venipuncture during blood product collection.<sup>2,8,9</sup> A number of precautions have been implemented to help PC contamination, including screening donors for illness symptoms, skin disinfection prior to venipuncture, diversion of the initial 30-40 mL drawn, and screening PCs for bacteria.<sup>2,10</sup> Despite these measures, bacterial contamination and missed detections continue to occur, and therefore the risk of potentially fatal *S. epidermidis*-associated transfusion reactions.<sup>11,12</sup>

As an effective, broad-spectrum cationic disinfectant, chlorhexidine has become widely adopted by blood centers for donor skin disinfection over the past decade,<sup>13,14</sup> particularly in the form of the one-step ChlorPrep kits, which combine 2% chlorhexidine gluconate (CHG) with 70% isopropyl alcohol (IPA). Used in tandem, these disinfectants reduce the rate of PC contamination, but do not fully eliminate it.<sup>2,13,15</sup> Notably, we previously demonstrated that where planktonic *S. epidermidis* cells are vulnerable to CHG-IPA, cell aggregates encased in a self-produced matrix, termed biofilms, are markedly more resistant.<sup>16</sup>

Plant essential oils (EOs) have been widely demonstrated to possess antimicrobial activity, especially those with oxygenated terpenoid components, such as alcohols and phenolic oxygenated terpenoid.<sup>17-19</sup> Some EOs are also reported to have synergistic action with CHG, enhancing its penetration into skin and its antibacterial activity.<sup>8,20,21</sup> Among plants native to Saudi Arabia, *Clitoria ternatea* (butterfly pea), *Aerva lanata* (mountain knotgrass), *Lavandula dentata* (French lavender), and *Plumbago zeylanica* (Ceylon leadwort) are notable medicinal plants

known to possess antibacterial and/or anti-biofilm properties.<sup>22-25</sup> In this study, EOs were extracted from these four plants and investigated for their antimicrobial efficacy and their synergy with CHG for potential enhancement of skin disinfection practices to improve the purity and safety of PCs. We hypothesized that EOs with elevated concentrations of oxygenated terpenoids would synergistically enhance the anti-biofilm activity of CHG-based disinfectants.

## MATERIALS AND METHODS

### Bacterial strain

*Staphylococcus epidermidis* strain ATCC 35984 was used in anti-biofilm activity assays on account of its documented ability to form strong biofilms. Bacteria were stored at -80 °C in brain heart infusion broth enriched with 15% glycerol until required.<sup>26</sup>

### Ethical approval statement

This study was approved by the Ethics Review Committee (ERC), College of Medicine, Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia (Approval No. 23R/442/07, dated 12 November 2024).

### EO extraction and analysis

Four plants native to Saudi Arabia (specifically Jazan Province) were selected for EO extraction: *Clitoria ternatea*, *Aerva lanata*, *Lavandula dentata*, and *Plumbago zeylanica*. EOs were extracted by hydro-distillation from aerial parts,<sup>27</sup> and their respective constituents were identified by means of gas chromatography-mass spectrometry (GC-MS) at King Saud University's Chemistry College Mass Spectrometry Facility. This analysis utilized an Agilent 7820A gas chromatograph with a Stabilwax column (Restek; 30 m, 0.25 mm film thickness, and 0.25 mm internal diameter) and a 5975 series mass selective detector. One microliter of diluted oil (1 µL oil in 40 µL ethyl acetate) was used as input. Helium carrier gas was delivered at a steady rate of 1.8 mL/min. The initial oven temperature of 40 °C was ramped up to 220 °C at 10 °C/min, then held at that temperature for two minutes. For the first three minutes of each run, the GC-MS detector was deactivated to avoid solvent signals.

EO components were identified by comparison to the Wiley 275 database.<sup>27</sup>

### Reagents

Stock solutions of EOs, plumbagin (99%, Fisher Scientific), and disinfectants were formulated in 8% Tween-80 (Sigma-Aldrich). EOs were diluted to concentrations of 10%-50%, plumbagin from 4.5%-9%, CHG (Sigma-Aldrich) to 2%, and IPA (Fisher Scientific) to 70%. The neutralizing solution containing 0.5% lecithin (Fisher Scientific) and 4% Tween-20 (Fisher Scientific) in distilled water was prepared and validated according to the American Society for Testing and Materials protocol (ASTM E1054-08).<sup>28,29</sup>

### Rabbit skin test

Skin irritation tests were conducted in accordance with the US Environmental Protection Agency guideline (OPPTS; 870.2500).<sup>30</sup> Each oil was evaluated on three rabbits, each weighing roughly 1-2 kg. On the first day, the rabbits received anesthesia (1 mL/kg of 66% urethane), and a 3 × 7 cm region on their dorsal surface was shaved and sanitized. On the subsequent day, two circular areas within the sanitized surface were designated: one to receive the non-irritant control (10 µL of Tween-80 solution) and the other the test oil (10 µL). Irritation was evaluated at 30 sec, 10 min, 1 hr, and 4 hrs after application.

### Anti-biofilm activity assays

Anti-biofilm activity was determined according to the procedure of Alabdullatif et al. with some modification.<sup>19,27</sup> Briefly, *S. epidermidis* ATCC 35984 was streaked on trypticase soy agar plates (Watin Biolife, KSA) and incubated at 37 °C overnight. The next day, one to three colonies were inoculated into 3 mL of Mueller-Hinton broth (Watin Biolife, KSA) and the suspension was incubated at 37 °C with agitation at 260 rpm for 17-18 hrs. The resultant cultures were adjusted to an optical density of 0.1 at 600<sub>nm</sub>, which is equivalent to ~1.0 × 10<sup>7</sup> cells/mL. For biofilm production, 0.1 mL of adjusted culture was introduced into each well of a 96-well plate (Corning, Inc.) and incubated at 37 °C for 24 hours. Planktonic cells were then removed, and each well

was carefully washed three times with 0.1 mL of 0.9% saline at pH 7.4.

Eos were evaluated for their effects against *S. epidermidis* biofilms either alone, with CHG, or with CHG-IPA. Concentrations of 10% and 20% were tested for each EO, and 4.5% and 9% for plumbagin. To mimic traditional skin-disinfection methods, the biofilms were subjected to 0.1 mL of treatment for one minute, followed by 0.2 mL of the neutralizing solution for five minutes. After removal of the neutralizing solution, each well was carefully washed three times with 0.2 mL of 0.9% saline. Finally, in accordance with our prior protocol, 0.2 mL of fresh 0.9% saline was dispensed into each well and biofilm cells were extracted from the plate through 30 minutes of sonication at 60 Hz with an ultrasonic cleaner (Model 5510; Branson Ultrasonics Corp.). The obtained solutions were serially diluted with 0.9% saline, plated on trypticase soy agar, incubated overnight at 37 °C, and colonies counted the next morning.

### Determination of drug interaction

Drug-drug interactions between each EO and CHG were assessed in terms of the coefficient of drug interaction (CDI), calculated with the formula  $CDI = AB / (A \times B)$ , where AB denotes the ratio of bacterial counts (CFU/mL) in the combination treatment (EO + CHG) to counts in the control treatment, and A (EO) and B (CHG) the respective counts themselves. Interactions were classified based on the CDI value as antagonistic (CDI > 1), additive (CD = 1), or synergistic (CDI < 1).<sup>31,32</sup> An antagonistic interaction occurs when the effect of a combination treatment is less than the cumulative effects of the individual treatments, whereas in a synergistic interaction, the combination is more effective than the sum of the individual treatments.<sup>31,33</sup>

### Statistical analysis

Viable cell counts were log<sub>10</sub> transformed in order to determine the logarithmic difference in viable cells between treated and untreated (control) cultures (i.e. log reduction). Multiple treatment comparisons to detect differences were performed in the Statistical Analysis System (SAS Institute, Inc.) and used mixed-model analysis, with the results considered significant for P-values

**Table 1.** Rabbit skin test results for the four EOs

Plant	EOs concentrations							
	50%	40%	30%	25%	20%	15%	12.50%	10%
<i>C. ternatea</i>	+++	+++	+--	-+-	---	---	---	---
<i>A. lanata</i>	+++	-++	--+	---	---	---	---	---
<i>L. dentata</i>	+++	+--	---	---	---	---	---	---
<i>P. zeylanica</i>	+++	+++	--+	-+-	---	---	---	---

+, Irritation; -, no irritation (n = 3)

**Table 2.** Efficacy of EOs in reducing *S. epidermidis* (CFU/mL) when administered alone or in combination with 2% CHG

EO concen., %	EOs CFU/mL Log reduction $\pm$ SD*	P-value †	EO plus CHG CFU/mL Log reduction $\pm$ SD*	P-value ‡	CDI	Result
<b><i>C. ternatea</i></b>						
Control, 0%	0.00 $\pm$ 0.11		1.60 $\pm$ 0.19			
10%	0.19 $\pm$ 0.10	0.09	1.72 $\pm$ 0.24	0.53	1.15	Anta
20%	0.29 $\pm$ 0.10	0.27	1.80 $\pm$ 0.39	0.77	1.2	Anta
<b><i>A. lanata</i></b>						
Control, 0%	0.00 $\pm$ 0.18		1.18 $\pm$ 0.16			
10%	-0.19 $\pm$ 0.12	0.23	0.99 $\pm$ 0.07	0.18	1.01	Anta
20%	0.25 $\pm$ 0.24	0.07	1.31 $\pm$ 0.16	0.06	1.32	Anta
<b><i>L. dentata</i></b>						
Control, 0%	0.00 $\pm$ 0.14		1.31 $\pm$ 0.08			
10%	0.65 $\pm$ 0.26	0.03	1.65 $\pm$ 0.26	0.14	2.04	Anta
20%	1.24 $\pm$ 0.11	0.04	2.30 $\pm$ 0.10	0.04	1.78	Anta
<b><i>P. zeylanica</i></b>						
Control, 0%	0.00 $\pm$ 0.15		1.67 $\pm$ 0.20			
10%	0.54 $\pm$ 0.15	0.01	2.46 $\pm$ 0.51	0.01	0.56	Syn
20%	1.21 $\pm$ 0.10	<0.01	2.99 $\pm$ 0.08	<0.01	0.78	Syn

\* Mean log<sub>10</sub> reduction (CFU/mL)  $\pm$ SD; n = 3. † The P-value is shown for each EO concentration compared with controls.

‡ The P - value is shown for each EO plus CHG compared with CHG alone.

SD = standard deviation; Anta = antagonistic; Syn = synergistic

below 0.05. A minimum of three independent biological experiments were performed for each treatment, each with two technical replicates.

## RESULTS

### Irritation of rabbit skin by EOs

A positive (+) reaction was determined to be present if irritation was observed at any assessment time point, and a negative (-) reaction if no irritation was detected after four hours of exposure. The control solution induced no signs of irritation at any time point. EOs tested at 20% concentration also produced no evident signs of irritation (Table 1). However, skin irritation was observed for all EOs at higher concentrations;

specifically, *C. ternatea* and *P. zeylanica* induced irritation at 25%, *A. lanata* at 30%, and *L. dentata* at 40%. As a result, the anti-biofilm assays were conducted with EO concentrations of 10% and 20%, well below the levels shown to produce significant irritation.

### Anti-biofilm activity of EOs

EOs were further evaluated for their ability to reduce preformed bacterial biofilms upon 60 seconds of exposure. Neither distilled water nor the control solution, 8% Tween-80, produced any substantial change in viable bacterial counts; moreover, the combination of control solution with either CHG or CHG-IPA did not affect the anti-biofilm properties of the latter

**Table 3.** Oxygenated terpenoids percentage and most abundant EO oxygenated terpenoid

Plant	Total percentage of oxygenated terpenoids, %	Most abundant oxygenated terpenoid compound (%)
<i>C. ternatea</i>	27	stigmasterol (41)
<i>A. lanata</i>	34	lupeol (38)
<i>L. dentata</i>	56	1,8-cineole (63)
<i>P. zeylanica</i>	76	plumbagin (79)

(data not shown). The tested EOs demonstrated varying degrees of anti-biofilm activity. The *P. zeylanica* EO exhibited the most potent effect, with significant log reductions of  $0.54 \pm 0.15$  CFU/mL at 10% concentration ( $P = 0.01$ ) and  $1.21 \pm 0.10$  CFU/mL at 20% concentration ( $P < 0.01$ ). The *L. dentata* EO also showed considerable activity, with log reductions of  $0.65 \pm 0.26$  CFU/mL at 10% concentration ( $P = 0.03$ ) and  $1.24 \pm 0.11$  CFU/mL at 20% concentration ( $P = 0.04$ ). Meanwhile, *C. ternatea* and *A. lanata* EOs displayed lesser, non-significant anti-biofilm effects. Specifically, the *C. ternatea* EO achieved log reductions of  $0.19 \pm 0.10$  CFU/mL at 10% and  $0.29 \pm 0.10$  CFU/mL at 20% ( $P = 0.09$  and  $P = 0.27$ , respectively), while the *A. lanata* EO showed minimal anti-biofilm activity ( $P = 0.23$  and  $P = 0.07$  at 10% and 20%, respectively).

#### Interaction of EOs with CHG

Compared to the 8% Tween-80 control solution, CHG by itself significantly lowered bacterial concentration ( $P < 0.0001$ ), achieving an estimated 2 log reduction (Figure 1A). Similarly, compared to each EO alone, combined treatment with CHG produced a significant increase in anti-biofilm activity ( $P < 0.0001$ ). The CDI was calculated to determine the nature of these interactions. Interestingly, the results for *P. zeylanica* EO were suggestive of a synergistic interaction with CHG at the tested levels, as evidenced by CDI values of 0.56 at 10% and 0.78 at 20% concentration. However, it should be noted that these interactions may be concentration-dependent and specific to the ratios tested in this study. The other EOs exhibited antagonistic effects when combined with CHG (*C. ternatea*, CDI of 1.15 and 1.2; *A. lanata*, CDI of 1.01 and 1.2; *L. dentata*, CDI of 2.04 and 1.78) (Table 2).

#### *P. zeylanica* EO is abundant in oxygenated terpenoids, mostly as plumbagin

The determination of EO constituents through GC-MS analysis revealed *P. zeylanica* to harbor the highest percentage of oxygenated terpenoids (76%). Second was *L. dentata*, with 56% oxygenated terpenoids, followed by *A. lanata* at 34%. The lowest oxygenated terpenoid content was observed for *C. ternatea*, at 27% (Table 3).

In the *P. zeylanica* EO, the most abundant oxygenated terpenoid compound was plumbagin, which constituted 79% of the oil. In *L. dentata*, 1,8-cineole predominated at 63%. In *A. lanata*, lupeol was the dominant oxygenated terpenoid at 38%, and in *C. ternatea*, the leading compound was stigmasterol at 41%.

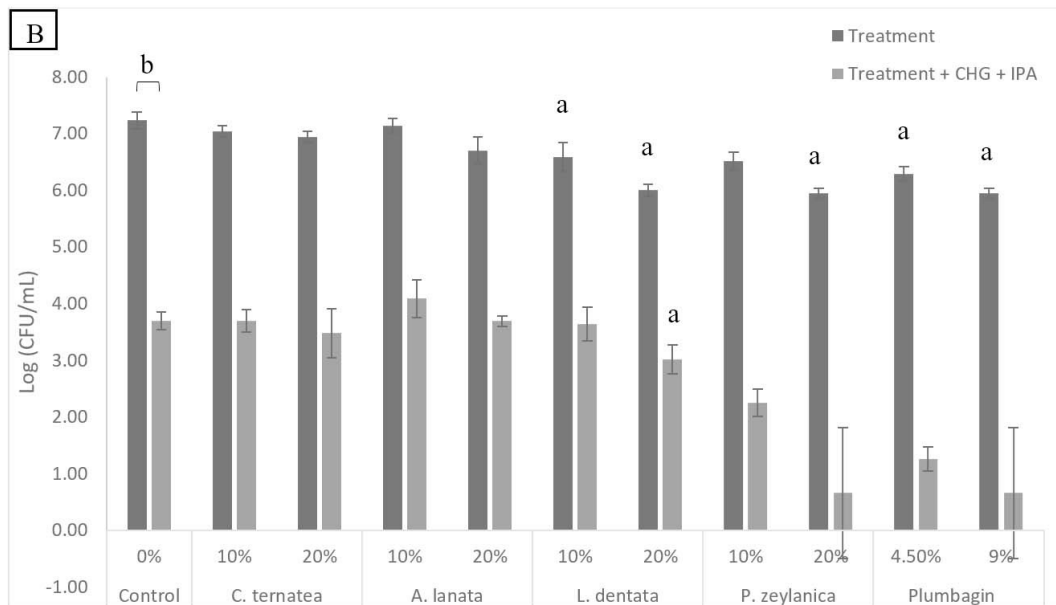
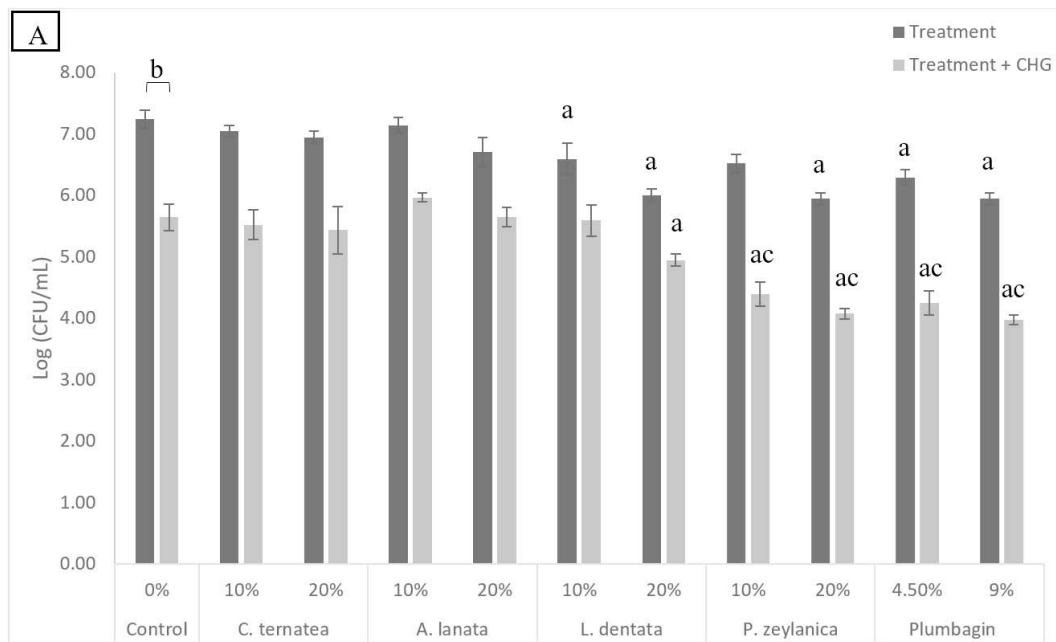
#### Anti-biofilm activity of *P. zeylanica* EO and plumbagin with CHG-IPA

The combination of CHG and IPA substantially reduced bacterial concentrations, achieving a 3-log decrease, significantly greater than when using CHG alone ( $P < 0.0001$ ) (Figure 1B). Likewise, addition of CHG-IPA to either EOs or plumbagin resulted in a significant enhancement of bacterial reduction ( $P < 0.0001$ ), with decreases reaching up to 5.5 logs. Both *P. zeylanica* and plumbagin exhibited synergistic interactions with CHG and the most significant enhancements of anti-biofilm activity ( $P < 0.05$ ). However, no significant difference was observed for *P. zeylanica* and plumbagin when used in conjunction with CHG versus CHG-IPA ( $P > 0.05$ ) (Figure 1A and 1B).

While the anti-biofilm activity of *P. zeylanica* closely mirrored that of pure plumbagin, it is important to note that the EO's total efficacy may not be exclusively restricted to this dominant compound. The presence of other minor oxygenated terpenoids and trace constituents could contribute to the overall potency or stability of the EO's antimicrobial action.

## DISCUSSION

The safety of blood products is a universal priority of blood centers. Bacterial contamination of PCs nonetheless remains a persistent challenge. Such contamination is frequently associated with



**Figure 1.** (A) Shows the anti-biofilm activity of 10% and 20% EOs, as well as 4.5% and 9% plumbagin, was tested alone and in combination with 2% CHG. (B) Shows the anti-biofilm activity of 10% and 20% EOs, as well as 4.5% and 9% plumbagin, was tested alone and in combination with CHG-IPA. EOs and plumbagin experiments were conducted in three independent replicates (n = 3). (a) represents P < 0.05, and (b) represents P < 0.0001 were used in the mixed-model analysis that was conducted for multiple comparisons. As determined by the coefficient of drug interactions, (c) represents that synergy was observed when combined with CHG. Treatments = Control or EO; Control = 8% Tween 80

skin flora such as *S. epidermidis*.<sup>2,10</sup> Many bacteria, including *S. epidermidis*, live in biofilms: populations of microorganisms surrounded by a matrix. *S. epidermidis* biofilms have been observed between squamous epithelial cells, as deep as five cells (10-20  $\mu\text{m}$  below the surface).<sup>34,35</sup> CHG and IPA are often used in combination as a skin disinfectant; interestingly, Taha and colleagues found that while this combination can decrease the number of viable *S. epidermidis* biofilms, it does not totally eradicate them.<sup>16</sup> Karpanen and his coworkers further reported that even after as much as 30 minutes of contact, 2% CHG penetrates less than 300  $\mu\text{m}$  into the skin; however, bacteria can live at up to 1500  $\mu\text{m}$  deep in hair follicles.<sup>35,36</sup> These findings suggest a possible explanation for the recurrent bacterial contamination of PCs and blood products in spite of skin disinfection procedures.

Plant EOs are of considerable interest for their disinfecting properties and potential to enhance transdermal medication delivery.<sup>22-25,37</sup> In addition, many EOs are known to have anti-biofilm properties; for example, eucalyptus oil can reduce viable biofilms of *S. aureus*, *Escherichia coli*, and *S. epidermidis*, and can even boost the effectiveness of chemical disinfectants such as CHG against biofilms.<sup>21,38</sup> However, eucalyptus oil is also reported to cause skin irritation at concentrations as low as 1%, limiting its practical application.<sup>39</sup> Identifying EOs that possess antibacterial activity alongside minimal skin irritation is critical for establishing better disinfectant methods.

This study examined EOs extracted from four plants native to Saudi Arabia, *C. ternatea*, *A. lanata*, *L. dentata*, and *P. zeylanica*, for their potential as anti-biofilm disinfectant agents. Initial assessments in a rabbit model demonstrated concentrations of up to 20% to be generally non-irritating, aligning with the “generally regarded as safe” classification of EOs and their oxygenated terpenoids by the US Food and Drug Administration.<sup>40</sup> This low irritancy profile is very important for their possible use in disinfecting skin, especially in the context of sensitive operations like blood donation.<sup>19</sup>

Our findings align with existing research and the broad recognition of natural compounds, EOs, and plant extracts as having potent antimicrobial activities.<sup>22-25</sup> In particular, the significant anti-biofilm activity of *P. zeylanica* EO

observed in this work is consistent with reports that other EOs, such as eucalyptus oil, can reduce viable *S. epidermidis* biofilms and enhance the activity of chemical disinfectants.<sup>8,20,21</sup> Likewise, our observation that higher oxygenated terpenoid content corresponds to greater activity against *S. epidermidis* biofilms corroborates previous studies.<sup>17-19,41</sup> Finally, we also identified plumbagin as the predominant oxygenated terpenoid in *P. zeylanica* and observed it to have a synergistic anti-biofilm effect when combined with CHG, confirming a prior report.<sup>42</sup> Taken together, these results highlight the potential of *P. zeylanica* and its active constituent plumbagin as valuable adjuncts to disinfection protocols aimed at minimizing bacterial contamination in blood products.

The complex chemical profile of EOs suggests that their biological activity often results from a delicate balance between major and minor constituents. In this study, the antagonistic interactions observed with *C. ternatea*, *A. lanata*, and *L. dentata* when combined with CHG highlight how minor components may interfere with or modulate the disinfectant’s efficacy. Conversely, the synergy observed in *P. zeylanica* may be facilitated by minor compounds acting in concert with plumbagin to enhance membrane permeability or inhibit biofilm matrix integrity, a phenomenon requiring further investigation.

While the findings of this study are promising, several limitations must be acknowledged. First, the *in vitro* nature of the anti-biofilm assays may not fully reflect the complexities of the human physiological environment. Similarly, the rabbit skin irritation model, while useful for initial safety screening, cannot be directly extrapolated to human skin responses. This is due to significant interspecies differences in skin structure, permeability, and immune response; consequently, human safety cannot be assumed based on these animal results alone. Most notably, this study relied exclusively on a single reference strain, *S. epidermidis* ATCC 35984. Although this is a robust model for studying biofilm-forming bacteria, it does not represent the clinical heterogeneity or the varied antimicrobial resistance profiles found in diverse clinical isolates. Consequently, these results should not be broadly extrapolated to all clinical settings. Future research should incorporate a diverse panel of clinically

isolated strains, including multidrug-resistant isolates, to enhance the translational relevance and confirm the broad-spectrum efficacy of these EO-disinfectant combinations. Furthermore, while the CDI values indicate a positive interaction between *P. zeylanica* or plumbagin with CHG, these findings are based on single-concentration pairings rather than extensive dose response or ratio-dependent validation. Consequently, the observed effects should be viewed as suggestive of synergy within the specific parameters of this assay, and further research is required to determine the optimal ratios and concentration-dependent thresholds of these interactions.

Future studies should interrogate the synergistic interaction of *P. zeylanica* or plumbagin with CHG, utilizing checkerboard assays and dose-response modeling to fully characterize the concentration-dependent nature of this interaction across a broader range of ratios. In addition, it should be determined whether *P. zeylanica* EO or plumbagin might enhance the transdermal penetration of CHG-IPA in a blood donation scenario, as was previously reported for linalool, the main component of *Lavandula multifida* EO.<sup>19,43</sup> Finally, rigorous in vivo studies in appropriate animal models must be conducted to validate the current in vitro findings, optimize formulations, and evaluate their safety and long-term effectiveness in the clinical setting. Given that CHG can cause skin reactions, further research on the allergenic potential of its combinations is warranted.

## CONCLUSION

This study successfully identified *P. zeylanica* EO and its principal constituent, plumbagin, as highly promising and non-irritating agents that synergistically boost the anti-biofilm activity of CHG. This synergy provides a novel approach to overcome the failure of traditional disinfectants to eradicate deep resistant *S. epidermidis* biofilms and offering a viable pathway to significantly reduce bacterial contamination in platelet concentrates. Future research should focus on elucidating the molecular mechanisms of this powerful plumbagin-CHG synergy and determining if plumbagin enhances the transdermal penetration of CHG-IPA in vivo.

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## FUNDING

None.

## DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

## ETHICS STATEMENT

This study was approved by the Ethics Review Committee (ERC), College of Medicine, Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia (Approval No. 23R/442/07, dated 12 November 2024).

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