



Evaluation of the Antibacterial Activity of Green Propolis Extract and Meadowsweet Extract Against *Staphylococcus aureus* Bacteria: Importance in Wound Care Compounding Preparations



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INTRODUCTION

Wound healing is a complex and dynamic physiological process that involves a series of biochemical and cellular events that help to restore tissue integrity after the skin barrier has been broken. Wound healing is divided into multiple steps including hemostasis, inflammation, proliferation, and remodeling.^{1,2} Immediately after injury, hemostasis minimizes blood loss by initiating a coagulation cascade to promote platelet aggregation and clot formation.³ Hemostasis is followed by the inflammatory phase, which serves to establish an immune

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ABSTRACT

The use of natural products in compounded wound care formulas is an exciting avenue to pursue for compounding pharmacists since these natural products may contain compounds that promote healing on their own. The use of these natural extracts as an alternative therapy for wound care may also provide several benefits, such as decreased inflammation, infection, side effects, and treatment costs. Thus far, several studies have demonstrated antimicrobial activity for various natural product extracts, including green propolis and meadowsweet. The antimicrobial properties of these extracts make them particularly interesting for wound care because the healing process is significantly delayed by bacterial infection and colonization at the site of injury. Therefore, to further investigate the antimicrobial properties of green propolis and meadowsweet extracts, we performed minimum inhibitory concentration and minimum bactericidal concentration assays against *Staphylococcus aureus*, a microorganism known to cause wound infections. The antimicrobial activity of green propolis and meadowsweet extracts was tested *in vitro* against a standard strain of *Staphylococcus aureus* in brain heart infusion broth and Mueller-Hinton agar plates. Green propolis extract demonstrated antimicrobial activity against *Staphylococcus aureus* with a minimum inhibitory concentration of 1.25 mg/mL and a minimum bactericidal concentration of 1.25 mg/mL. In contrast, meadowsweet extract failed to inhibit *Staphylococcus aureus* growth at the highest concentration tested (30 mg/mL). Green propolis was more effective than meadowsweet extract at inhibiting the growth of *Staphylococcus aureus*, suggesting that the addition of green propolis extract in wound care formulas might be more beneficial for the treatment of wounds. Therefore, we propose that green propolis extract is a promising natural product for wound care formulations.

barrier against invading pathogens including bacteria and other microorganisms.² Subsequently, the proliferative phase, which typically starts at day 3 and lasts about 2 weeks, involves fibroblast migration and the deposit of newly synthesized extracellular matrix to replace the provisional network of fibrin and fibronectin established in the early phases of wound repair.² This phase is also accompanied by the formation of granulation tissue, which involves creating new tiny blood vessels and connective tissue.² Finally, the remodeling phase, which can last 1 to 2 years or longer,⁴

involves remodeling of the acute wound to develop new epithelium and mature scar tissue.²

The inflammatory phase begins immediately after injury and neutrophils are among the first immune cells to reach the wound, usually between 24 to 36 hours after injury. Neutrophil migration into the lesion is favored by vasodilation and increased vascular permeability, which occur as part of the inflammatory process. These neutrophils produce free radicals which help destroy bacteria.² However, prolonged and abundant neutrophil infiltration at the site of injury may induce chronic inflammation causing a delay in the healing process.⁵ Therefore, bacterial colonization and chronic inflammation should be avoided to prevent delays in the healing process to reduce patient recovery time and additional medical expense.

To reduce medical costs associated with delayed wound healing, various methods and experimental procedures have been explored including the pharmacological evaluation of phytochemical and natural products, such as *Plantago lanceolata* L.⁶ and *Atropa belladonna* L.^{7,8} water extracts, flavonoid oligoglycosides from *Ophioglossum vulgatum* L.,⁹ and *Ageratina pichinchensis*¹⁰ extracts. Phytotherapy is in fact commonly used for wound treatment in many regions across the world,¹¹⁻¹³ with about 65% to 80% of the world's population in developing countries dependent on plants for primary healthcare.¹⁴ Brazilian green propolis is a resin produced by honeybees (*Apis mellifera*) that has been used for a long time in traditional medicine for its antimicrobial and anti-inflammatory properties in the treatment of dermal lesions such as burns, wounds, and ulcers.¹⁵ The efficacy of propolis in promoting wound healing is related to the antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties of its chemical constituents including flavonoids, phenolic substances, fatty acids, alcohols, amino acids, vitamins, minerals, and cinammic acid derivatives such as artepillin and baccharin.¹⁶⁻¹⁸ Meadowsweet extract is another natural product used for wound treatment, which is derived from *Filipendula ulmaria* L. flowers. Meadowsweet extract also has antimicrobial and anti-inflammatory properties due in part to the presence of salicylates and flavonoids.¹⁹⁻²¹

Given the antimicrobial and anti-inflammatory properties of green propolis and meadowsweet extracts, these natural products may be useful for treating dermal lesions by preventing bacterial colonization and chronic inflammation at the site of injury. To investigate this, we evaluated the antiseptic properties of these natural extracts in culture by assessing their ability to inhibit the growth of *Staphylococcus aureus* (*S. aureus*) in microdilution plates. *S. aureus* is one of the most commonly found microorganisms in burns and other skin lesions. By evaluating the antimicrobial activity of these extracts on *S. aureus*, we aimed to provide scientific evidence that these natural extracts can be incorporated into the base of topical dosage forms used for wound care in pharmaceutical compounding.

MATERIALS AND METHODS

MATERIALS

Green propolis extract (Lot 18990114) was obtained from a Brazilian manufacturer in Ribeirão Preto, SP (Brazil) and meadowsweet extract (Lot FIU1503L1-BGBIO) was acquired from a French manufacturer in Saint Beuzire (France).

MICROBIAL STRAIN

S. aureus (ATCC 25923) was obtained from American Type Culture Collection (Manassas, Virginia). The strain was inoculated in Brain Heart Infusion (BHI) broth (RenyLab, Brazil) and incubated at 37°C for 24 hours. The inoculum was then spread onto Mueller-Hinton agar plates (RenyLab, Brazil) and incubated at 37°C for 24 hours, to ensure the media was not contaminated. Prior to each experiment, three isolated *S. aureus* colonies were selected from the agar plates and transferred to test tubes containing 2 mL of sterile 0.85% NaCl solution. The suspensions were then adjusted to achieve a turbidity corresponding to the 0.5 MacFarland standard, which is approximately 1.5×10^8 CFU/mL. The bacterial suspensions were then diluted with media to 1.5×10^4 CFU/mL. To test the antimicrobial activity of green propolis and meadowsweet extract, we determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these extracts on the growth of *S. aureus*.

MINIMUM INHIBITORY CONCENTRATION

The MIC was determined in 96-well microdilution plates. Green propolis and meadowsweet extract were separately combined to 1% Tween 80 (Lot 142234) in sterile purified water to form a 1% w/v (10 mg/mL) green propolis extract solution and a 6% w/v (60 mg/mL) meadowsweet extract solution. An ultrasonic bath was used to improve the solubilisation of these extracts in water. Tween 80 was used as a wetting agent to improve the incorporation of these extracts into the water and bacteria culture medium.

To set up the MIC assay, 100 µL of 2X concentrated BHI medium was added to all wells. Then, 100 µL of the meadowsweet extract (columns 1 through 3) and green propolis extract (columns 4 through 6) solutions were added to the first row of the plate to obtain a final concentration of 3% w/v (30 mg/mL) meadowsweet extract and 0.5% w/v (5 mg/mL) green propolis. A series of two-fold dilutions was performed for each extract by transferring 100 µL of the contents of the first well to the subsequent well in the next row and so forth until the last row H was filled. Finally, the 100 µL of content in each well of row H was removed and discarded to ensure the total volume of each well was the same. The concentration of the extract solution in each row is listed in Table 1. 100 µL of BHI broth with 1% Tween 80 was added to the wells in column 7 as a vehicle control, and 50 µL of chlorhexidine solution 200 mg/mL (Lot SMAART/CHG/2012/028 3) and 50 µL of 2X concentrated BHI medium was added to the wells in column 8 as a positive control for

TABLE 1. Concentration of Green Propolis and Meadowsweet Extracts in Minimum Inhibitory Concentration Assay.

WELLS	GREEN PROPOLIS EXTRACT (MG/ML)	MEADOWSWEET EXTRACT (MG/ML)
Row A	5	30
Row B	2.5	15
Row C	1.25	7.5
Row D	0.625	3.75
Row E	0.312	1.87
Row F	0.156	0.94
Row G	0.078	0.47
Row H	0.039	0.23

antimicrobial activity. To ensure the BHI media was not contaminated with bacteria, 100 μ L of BHI broth alone was added to the wells in column 9 as a negative growth control. After the media and appropriate solutions were added to their respective wells, 10 μ L of the bacterial suspension (1.5×10^4 CFU/mL) was added to each well from column 1 to 8 and incubated at 37°C for 48 hours. To measure cell viability, 10 μ L of tetrazolium bromide was added to each well at the end of the 48 hours and left to incubate an additional hour at 37°C. At the end of the incubation period with tetrazolium bromide, the MIC was evaluated qualitatively for green propolis and meadowsweet solutions by determining the lowest extract concentration where the media was yellow without purple precipitates (bacteria numbers below detection limit).

MINIMUM BACTERICIDAL CONCENTRATION

The MBC was determined by measuring the amount of *S. aureus* colonies observed after plating on Mueller-Hinton agar plates 10 μ L of 1X, 2X, and 4X the MIC of the green propolis and meadowsweet solutions for 24 hours at 37°C. The MBC is defined as the lowest concentration at which less than three colony forming units (CFU) are observed. Therefore, concentrations at which three CFU were observed was not considered inhibitory for bacterial growth, while concentrations at which no growth or less than 3 CFU were observed, was considered bactericidal. Each experiment was performed in triplicate.

RESULTS

The results from the *in vitro* antimicrobial activity assays revealed that green propolis extract effectively inhibited *S. aureus* growth, since no precipitate or color change was detected in the samples treated with at least 1.25 mg/mL of green propolis extract (Figure 1A). Therefore, the MIC for the green propolis extract solution was 1.25 mg/mL. We also observed an absence of bacterial growth on Mueller-Hinton agar plates at 1X MIC concentration, suggesting that the MBC is also 1.25 mg/mL (Table 2). Conversely,

the meadowsweet extract solutions did not exhibit significant antibacterial activity against *S. aureus* at the concentrations tested, as we detected bacterial growth in all samples containing meadowsweet extract (Table 2). We confirmed these findings by performing an MBC assay, which showed that even at the highest concentration tested, the meadowsweet extract failed to inhibit bacterial growth (Figure 1B). As expected, purple precipitate was observed in the BHI + 1% Tween 80 control sample, suggesting that bacterial growth was not inhibited by the 1% Tween 80 vehicle. In contrast, we did not detect any precipitate in the BHI media only control or chlorhexidine antiseptic control samples. Collectively, these data suggest that green propolis extract is more effective than meadowsweet extract in inhibiting *S. aureus* growth in culture.

DISCUSSION

To effectively aid in the wound healing process, it is important that the natural products used in topical formulations exhibit antimicrobial activity because bacteria at the site of injury can promote inflammation and significantly delay the healing process.² In this study, we chose to evaluate the effects of green propolis and meadowsweet extracts on the growth of *S. aureus*, a clinically important bacteria responsible for wound infections, to provide evidence for the addition of these natural products in topical formulations for wound care. Our results showed that while green propolis extract effectively inhibited the growth of *S. aureus* in culture, meadowsweet extract demonstrated no significant antimicrobial activity against *S. aureus*. In fact, meadowsweet extract failed to inhibit the growth of *S. aureus* even at the highest concentration tested (30 mg/mL). Similarly, Panzaru-Woods et al showed that meadowsweet at 150 mg/mL did not have antimicrobial activity against the 34 microorganisms they tested, which included *S. aureus* and other bacteria and fungi.²² However, other studies have suggested that meadowsweet extract exhibits some antimicrobial and anti-inflammatory activities.^{20,21} Therefore, additional studies will need to be performed to further evaluate the effects of meadowsweet extract on bacterial growth and wound healing.

Conversely, our data showed that green propolis extract exhibits antimicrobial activity against *S. aureus*, suggesting that green propolis might indeed be effective in treating wounds by preventing bacterial colonization and infection at the site of injury. These findings are consistent with other published studies evaluating the antimicrobial properties of propolis^{23,24} and provide additional evidence for the use of propolis in the treatment of dermal lesions.^{25,26} For example, Oliveira et al demonstrated that polyvinyl alcohol hydrogels with propolis extract was effective in preventing *S. aureus* growth and acted as a barrier to prevent bacterial penetration and colonization.²⁵ Similarly, Berreta et al demonstrated that a topical formulation of standardized propolis in poloxamer gels was effective at treating wounds and inhibiting the growth of several microorganisms, including *S. aureus*.²⁶ Furthermore, other studies evaluating the biological properties of propolis in different experi-

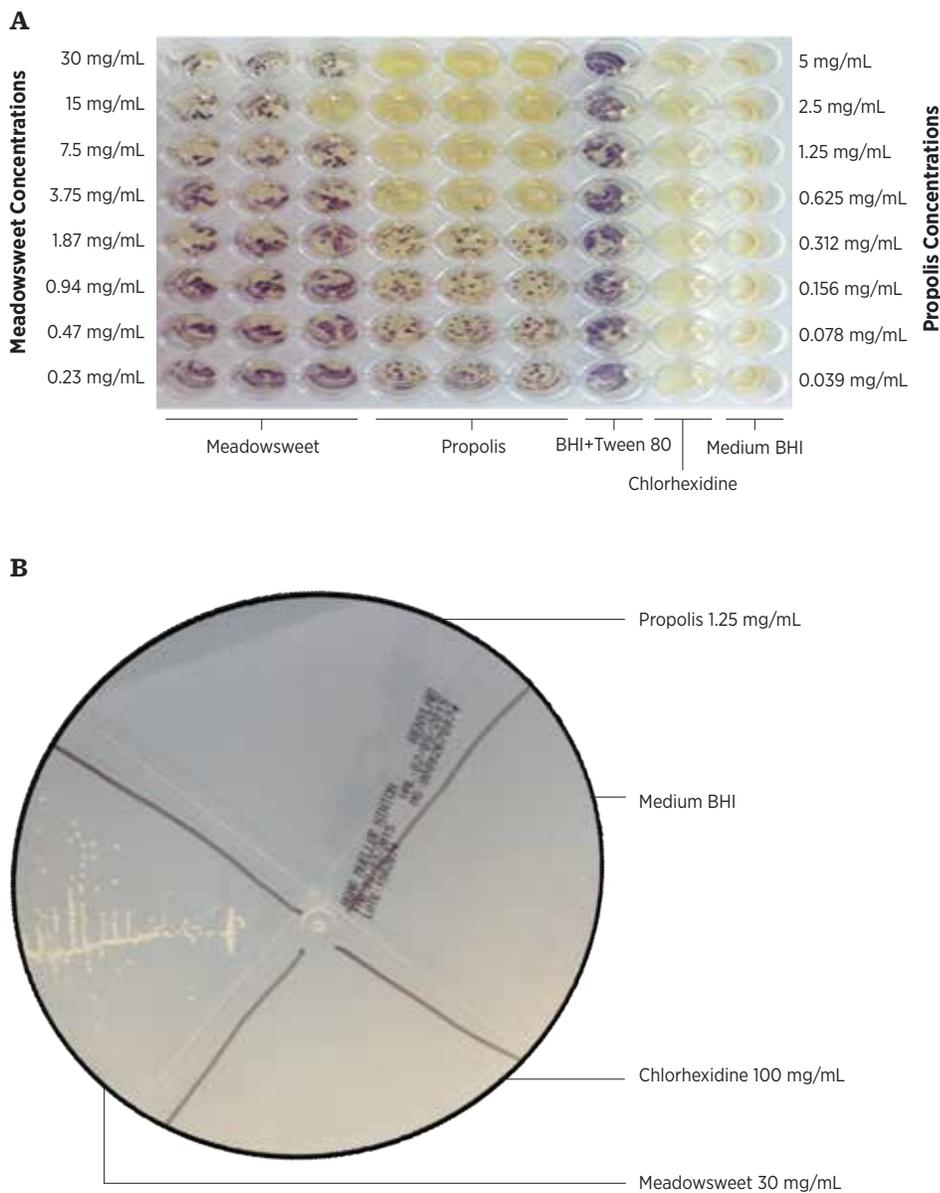
mental settings and dosage forms support the benefits of using propolis in treating wounds (Table 3).²⁵⁻²⁸ Therefore, the addition of propolis in topical compounded formulas might be beneficial for wound care.

Preliminary evidence for the use of propolis to promote wound healing came from a clinical study by Gregory et al comparing a propolis skin cream to silver sulfadiazine (SSD) lotion for the treatment of superficial second degree burns affecting less than 20% of the total body surface area.²⁹ This clinical study revealed that patients treated with propolis cream displayed less inflammation and more rapid cicatrization than those treated with SSD lotion.²⁹ However, based on post-treatment cultures taken from samples collected on day 5 and 8 after the injury occurred, they could not detect a significant change between the number of bacterial colonies grown on agar plates from propolis skin cream and SSD-treated patients,²⁹ suggesting that the beneficial effects of propolis skin cream over SSD might in fact be due to its anti-inflammatory, antioxidant, immunomodulatory, or other properties. Therefore, the addition of propolis extract to topical formulations for wound care might be beneficial to patients and aid in the healing process by decreasing inflammation at the site of injury through its antimicrobial and anti-inflammatory properties.

CONCLUSION

Based on this study and others, there is evidence to support the use of green propolis extract in the treatment of dermal lesions, including burns and wounds. Our results demonstrate that green propolis extract is more effective than meadowsweet extract at inhibiting the growth of *S. aureus* in culture and, thus, may potentially be used to prevent infection and bacterial colonization of the wound to promote healing. In addition to its antimicrobial properties, propolis has anti-inflammatory, antioxidant, and immunomodulatory properties that render it more effective at treating burns. After evaluating our results and the data described in the literature, we propose

FIGURE 1. Qualitative assay to measure the Minimum Inhibitory Concentration (A) and Minimum Bactericidal Concentration (B) assay for meadowsweet and green propolis extract solutions.



that green propolis extract is a natural product with potential benefit to compounding pharmacists since its addition to topical formulas for wound care might confer added antimicrobial and anti-inflammatory benefits for the treatment of dermal lesions.

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TABLE 2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Green Propolis and Meadowsweet Extracts (n=3).

MICROORGANISM	GREEN PROPOLIS EXTRACT		MEADOWSWEET EXTRACT	
	Minimum Inhibitory Concentration (mg/mL)	Minimum Bactericidal Concentration (mg/mL)	Minimum Inhibitory Concentration (mg/mL)	Minimum Bactericidal Concentration (mg/mL)
<i>Staphylococcus aureus</i>	1.25	1.25	*	*

* No antimicrobial activity was detected.

TABLE 3. Brief Summary of the Studies Investigating the Biological Properties of Propolis in Different Dosage Forms in the Treatment of Wounds and Burns.

DOSAGE FORM	USE	IN VIVO/IN VITRO	AUTHORS
Gel	Wound	<i>In vivo</i>	Berreta et al (2012) ²⁶
Solution	Wounds	<i>In vivo</i>	Kucharzewski et al (2013) ²⁷
Film	Burn Healing	<i>In vivo</i>	de Almeida et al (2013) ²⁸
Gel	Burn Wound	<i>In vitro</i>	Oliveira et al (2015) ²⁵

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