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Research Article

Improved Antibacterial Activity of Honey, Propolis and Beeswax-Sodium Carboxymethyl Cellulose Composite Hydrogel

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ABSTRACT

Honey, propolis, and beeswax are natural products that have been used in alternative medicine and traditional practices. This study aimed to develop an antibiotic hydrogel containing propolis, honey, and beeswax and evaluation of its antibacterial potentials. Fourier transform infrared (FTIR) analysis was employed to characterise the NaCMC-H-P-BW hydrogel. Besides, the antibacterial activity of composite hydrogel was determined by using agar well diffusion assay against *Staphylococcus aureus* and *Escherichia coli*, and the zone of inhibition was measured in terms of millimeters. A topical cream containing 1% silver sulfadiazine was used as a positive control and distilled water will be used as the negative control. Fourier transform infrared spectroscopy (FTIR) analysis suggested the formation of strong intermolecular interactions as hydrogen, oxygen, and carbon attractions between polymer and propolis, honey and beeswax. The antibacterial properties showed the inhibition activity against *Staphylococcus aureus* (23.10±4.60 mm) and *E. coli* (22.95±4.76 mm). Moreover, the inhibition activity observed for the positive control (silver sulfadiazine) was comparatively less compared to NaCMC-H-P-BW hydrogel *Staphylococcus aureus* (8.25±0.95) and *Escherichia coli* (9.30±1.53). These studies suggest that the topical formulation could be used as an antibacterial topical apitherapeutic product.

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INTRODUCTION

Several natural products have been used in wound care since they contain, antimicrobial properties, and anti-inflammatory substances [1]. Honey, propolis, and beeswax present and promotes wound healing with minimal scarring [2]. However, to control chronic wounds, several biomaterials have been developed for clinical applications. Several studies have investigated the properties of gels formed from blends of Na-CMC with natural products, frequently for biomedical applications such as wound healing [3].

In addition, CMC wound dressings are recognized for being flexible, capable of absorbing exudate, low-cost, biocompatibility, biodegradability, low toxicity, non-immunogenicity, promoting angiogenesis, autolytic debridement good film-forming and activate macrophages, and increase cytokine levels in wounds [3]. In recent times, Lately, Park et al. prepared chestnut honey impregnated CMC hydrogel for diabetic ulcer wound healing [4]. In this study, they used CMC for the increase of moisture and viscosity of the hydrogels Also, in the same *in vitro* studies the materials, however, exhibited vital antibacterial activity towards *Staphylococcus aureus* and *Escherichia coli*. Some topical antibiotics and antiseptics are not recommended in infected chronic wound therapy [5]. A rich source of many substances exhibiting antibacterial activity is apitherapy. In this study, we investigated three bee-derived products from Algeria, Sahara honey, propolis, and beeswex. Euphorbia honey is a well-known natural material from Algeria, considered to has been demonstrated to have positive effects on wound healing [6]. However, it is difficult to apply honey directly to the wound bed, because, with time, it flows out of the wound bed and causes inconvenience for the patient. Consequently, the incorporation of honey in a hydrogel system seems to be more beneficial and applicable [7]. Based on the positive healing

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properties of honey, alone or in combination with other products, we hypothesized that a new dressing based on honey, propolis, and bee wax could be used to improve the regeneration process of wounds.

MATERIALS AND METHODS

Honey, Propolis, and Beeswax Samples

All the Bee products samples who were selected in the southern Algerian region during the year 2020.

Topical Formulation Preparation of NaCMC/HPW Hydrogel

The composition of NaCMC-H-P-BW hydrogel (10 g Na CMC, 80 g honey (H), 5 g propolis (P), and 5 g beeswax (BW) added to 100 mL water). A topical cream containing 1% silver sulfadiazine (SSD) (FLAMAZOLE®1%) was used as a positive control and distilled water will be used as the negative control.

FTIR Spectroscopy Measurements of the NaCMC-H-P-BW Mixture

Chemical characterization of scaffolds was performed using a Fourier Transform Infrared (FTIR) Spectrometer. The FTIR spectra of samples were founded at the scanning range of 4000−550 cm−1.

Antibacterial Activity

The clinical reference strains included *Staphylococcus aureus* and *Escherichia coli*. These reference strains were used to study the antibacterial efficacy of NaCMC-H-P-BW as they are well-known biofilm producers in burns and wounds.

Determination of Antibacterial Activity

Agar well-diffusion bioassay technique described earlier Ahmed et al., [8] was used to evaluate the antibacterial efficacy of Na CMC-H-P-BW. Briefly, agar plates (90 mm) were containing 20 mL of nutrient agar at 37°C for 24 h and adjusted by diluting fresh cultures to a turbidity equivalent to 0.5McFarland scale (approximately 2 × 108 colony-forming unit/ mL). An 8 mm diameter well was cut into the agar and 100 µL of was aliquoted into the well. A topical cream containing 1% SSD was used as a positive control and distilled water will be used as the negative control.

The plates were placed in an incubator at 37°C for 24 hours. After incubation, the diameters of zones of inhibition of Na CMC-H-P-BW and silver sulfadiazine(1%) were measured. Each experiment was performed in triplicates.

RESULTS

A visual inspection of the FTIR spectra (Fig. 1). between 1300 and 1000 cm−1 represent C–O stretching and C–OH bending originated from ethers alcohols, esters, and carboxylic acids, which are functions present mainly in phenolic acids and flavonoids [9-10].

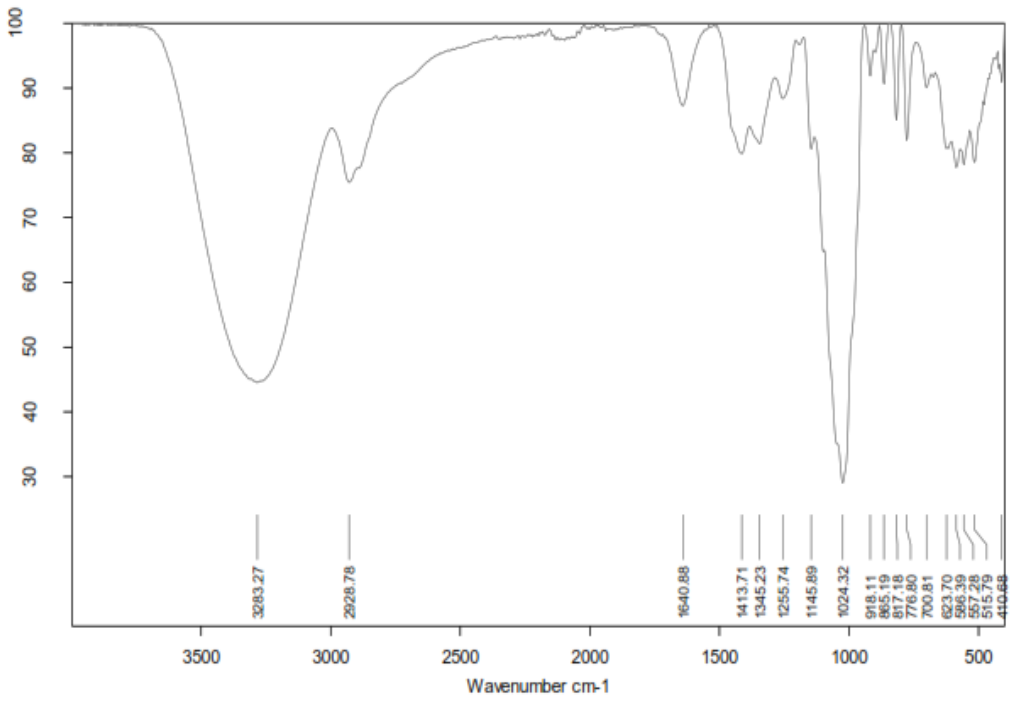

Figure 1: Fourier Transform Infrared (FTIR) spectrum of NaCMC-H-P-BW Hydrogel

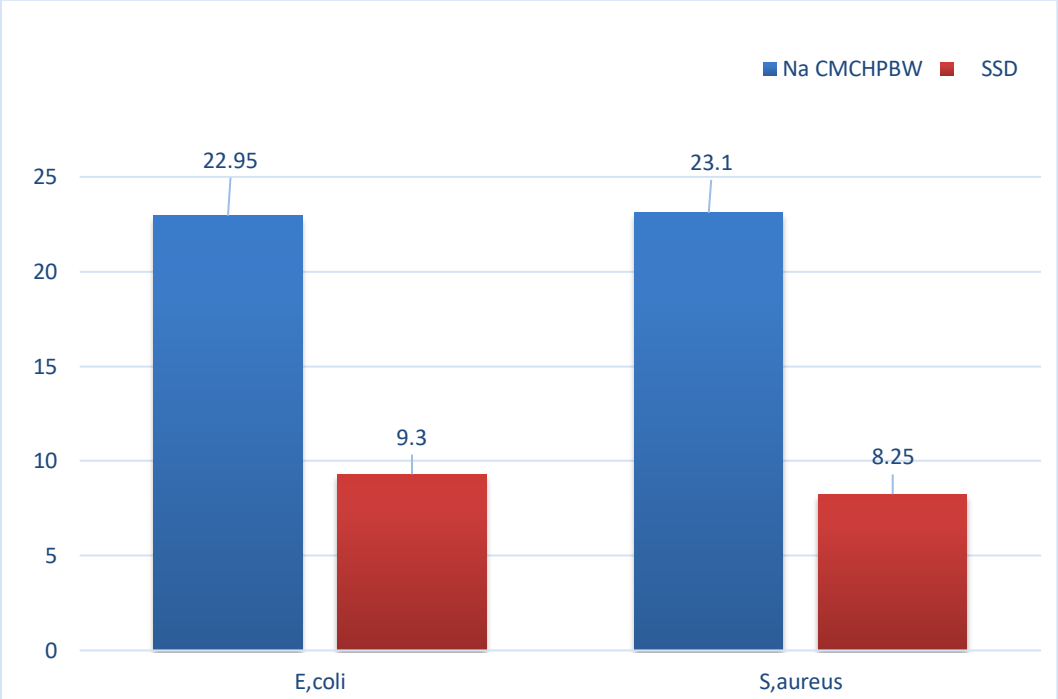

Figure 2: Effect of NaCMC-H-P-BW and SSD on the growth of pathogenic bacteria by agar well diffusion method.

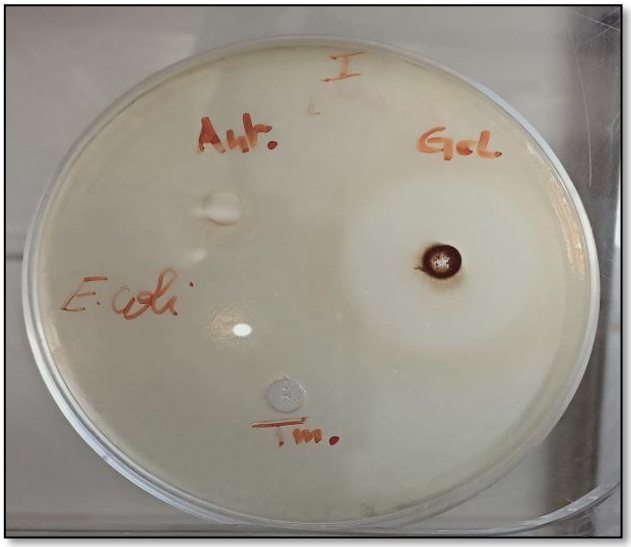

Figure 3a: Effect of NaCMC-H-P-BW and SSD on the growth of *Escherichia coli* by agar well diffusion.

Figure 3b: Effect of NaCMC-H-P-BW and SSD on the growth of *Staphylococcus aureus* by agar well diffusion method.

The region between 1700−1600 cm−1 shows the vibrational modes from carbohydrates [11] and the amide I band [12] water [13] and amino acids [14].

In the present study, using the agar well diffusion method, the diameters of zones of inhibition of *E.coli* and *S.aureus* in confronting NaCMC-H-P-BW were measured. The highest zone of inhibition of the NaCMC-H-P-BW was 23.10±4.60 mm for *Staphylococcus aureus* followed by *E.coli* (22.95±4.76 mm). Moreover, the inhibition activity observed for the positive control (SSD) was comparatively less compared to NaCMC-H-P-BW hydrogel *Staphylococcus aureus* (8.25±0.95) and *E.coli* (9.30±1.53) (Fig. 2 and 3).

DISCUSSION

In recent years, Carboxymethyl cellulose (CMC) hydrogel has been used in drug carriers for wound care due to its ability to absorb a significant amount of water and good biocompatibility while retaining gel integrity for

longer, low cost, and non-toxic and are useful as occlusive dressings [3]. Some cellulosic membranes incorporating natural products have been developed recently.

Since the last decade, Bee products: honey, propolis, pollen, Beeswax, and others have become very popular for many different applications. However, bee products are known as possible sources of natural antibiotics [15]. Several researchers have explored the effects of combining honey with other products of the hive on antimicrobial activity *in vitro.*

In Algeria, Sahara honey (SH) has attracted the attention of the Algerian scientific community for its antimicrobial, anti-inflammatory, and healing properties [16]. Besides, the use of SH has been previously shown in animal experiments [17]. Also, Algerian researchers used a well diffusion assay to demonstrate a synergistic effect of Algerian honey and propolis against *Escherichia coli*, *Staphylococcus aureus* and Pseudomonas aeruginosa [9].

Later in 2019, Bacha et al. prepared -Na CMC hydrogels for bee pollen drug delivery to wounds [18].

Since the last decade, many researchers have invented several new wound dressing materials. Oliveira et al. prepared PVA-Na CMC hydrogels for propolis drug delivery to wounds [19].

Additionally, other researchers support the occurrence of enhanced effects of propolis and honey. Nho et al. developed CMC hydrogels, which were crosslinked with antibacterial Propolis and honey via radiation method for diabetic ulcer [20].

Similarly, Lately, Park et al. prepared chestnut honey impregnated CMC hydrogel for diabetic ulcer wound healing [4]. In this study, they used CMC for the increase of moisture and viscosity of the hydrogels.

About beeswax, a little more has been studied and tested regarding the antimicrobial action of beeswax in synergy with the other products of the hive products.

For example, Honey, beeswax and olive oil mixture (1:1:1, v/v) are useful to inhibit the growth of *S.aureus* and *C. Albicans*, isolated by human patients [21]. Similarly, the antimicrobial activity of propolis and beeswax in synergy (1:1, v/v) has also been investigated to inhibit the growth of *S. aureus* ATCC25923, Staphylococcus epidermidis ATCC12228, B. subtilis ATCC27853 and *C. albicans* NCTC270 [22].

Regarding our study, so far there is no study investigating the effect of Na CMC hydrogels for propolis, honey and beeswax on the growth of *S. aureus* and *E.coli.* In addition, to our knowledge, there is no study investigating the synergism between propolis, honey and beeswax.

The mechanism of honey, beeswax and propolis antimicrobial activity is complex and might be attributed to the synergistic activity between its various potent biological ingredients such as phenolic and flavonoids. Other factors are involved in the antibacterial activity of bee products: nitric-oxide, phagocytic and lymphocytic activity increase, and prostaglandins' reduction [23].

Based on the results for the antibacterial effects, we can state that NaCMC-H-P-BW gel is a good candidate for the prevention and treatment of skin wound healing. Our findings showed that the NaCMC-H-P-BW gel had significant antiseptic effectiveness against *E.coli* and *S.aureus*. and were in agreement with those of previous studies. In conclusion, The present study concludes that honey alone and in combination with various bee products show great promise as natural antibiotic agents against *E.coli* and *S.aureus* could lead to increase in their combined effects and consequently they can be used to treat various skins infections caused by these organisms.

CONCLUSION

Further clinical studies in animals and humans are needed to fully understand the effectiveness of this mixture for the treatment of skin and wound infections.

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