Formulation of a multifunctional nanocomposite hydrogel based on natural polysaccharides, biogenic copper nanoparticles and essential oils

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ABSTRACT

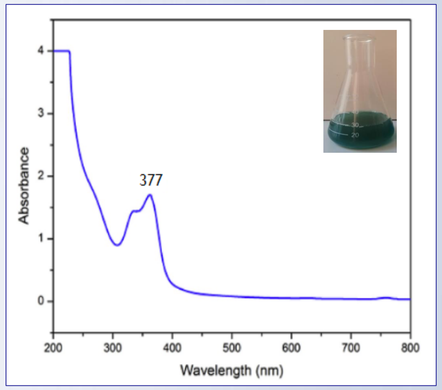
Background: Copper nanoparticles are of tremendous interest due to their strong antibacterial properties, thereby having potential to enhance not only the physical and biochemical characteristics of hydrogels, but also their antimicrobial activity.
Objective: the goal of this study was to develop a multifunctional hydrogel embedded with copper nanoparticles that were manufactured in an eco-friendly manner.
Material and Methods: Copper nanoparticles were biologically synthesized using Cinnamon extract which was mixed with the $CuSO_4$ (pentahydrate copper sulphate) solution (0.1 M) in a 1:5 ratio. The CuNPs suspension was primarily characterized by UV–Visible spectroscopy, the spectrums being recorded from 200 to 800 nm. Xanthan gum, a natural polysaccharides, was used as thickening agent in the formulation of the following hydrogels: control gel, with $CuSO_4$ alone), G1, with biogenic CuNPs and G2 with CuNPs and essential oils (oregano, cinnamon, clove, eucalyptus, thyme, and lavender). The Kirby-Bauer diffusimetric method was used to assess comparatively the antimicrobial activity against Gram-positive, Gram-negative bacteria and fungal species for the formulated gels.
Results: The presence of the copper nanoparticles was confirmed by the presence of a surface plasmon resonance (SPR) peak recorded at 334-355 nm. The lowest antimicrobial activity was observed in the case of control hydrogel. Hydrogel G1 showed significantly better antimicrobial activity, especially on Staphylococcus epidermidis and Candida albicans. Hydrogel G2, embedded with biogenic CuNPs and essential oils, presented a higher antimicrobial activity against both Gram-positive and negative bacteria. It also displayed antibiotic activity against MRSA ATCC 33591, a methicillin-resistant Staphylococcus aureus strain with public health significance.
Conclusions: When coupled with the specified essential oil blend, copper sulfate demonstrated strong synergistic antibacterial action. Altogether, this work assessed and validated the in vitro antibacterial activity of a polysaccharides-based composite hydrogel comprising biologically synthesized copper nanoparticles and essential oils with potential applications in both human and veterinary medicine.
KEYWORDS: antimicrobial; copper; essential oils; hydrogels; nanomaterials; nanoparticles;

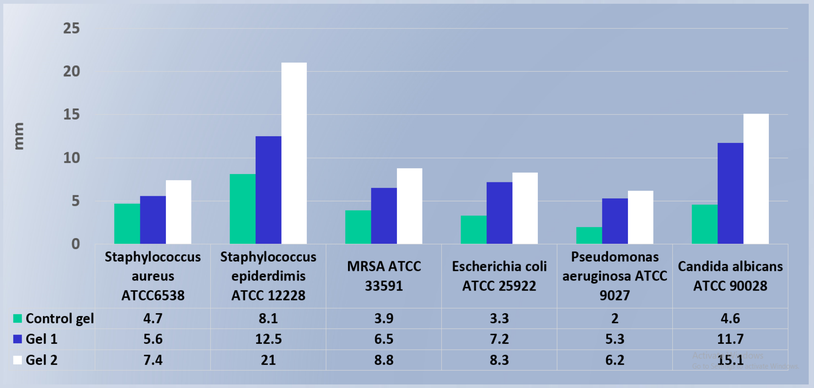
RESULTS and DISCUSSIONS

- The presence of the copper nanoparticles was confirmed by the presence of a surface plasmon resonance (SPR) peak recorded at 334-355 nm.
- The control hydrogel exhibited the lowest antimicrobial activity while hydrogel G1 showed significantly better antimicrobial activity, especially on Staphylococcus epidermidis and Candida albicans.
- Hydrogel G2, embedded with biogenic CuNPs and essential oils, presented a higher antimicrobial activity against both Gram-positive and negative bacteria. It also displayed antibiotic activity against MRSA ATCC 33591, a methicillin-resistant Staphylococcus aureus strain with public health significance.

Table 1. Physico-chemical characteristics of the gel formulations

Formulation	Physical appearance	Colour	Texture	Phase separation	Homogenity	Immediate skin sensation	pH	Spred ability (mm)
Control gel	Semi Transparent	Intese Blue	Smooth	no	yes	Moisturizing No signs of coarse particles	6.78 ± 0.08	48 ± 0.11
G1	Transparent	Light yellow	Smooth	no	yes	Refreshing, Cooling No signs coarse particles	6.26± 0.06	55. 91 ± 0.08
G2	Semi transparent	Green-blue	Smooth	no	yes	Moisturizing Refreshing Cooling No signs coarse particles	6.08 ± 0.04	59.52 ± 0.10





METHODS

Green synthesis of copper nanoparticles

- Copper nanoparticles were synthesized by biologic route (green synthesis), using cinnamon extract as both plant extract acts both as reducing agent as well as capping agent.
- The cinnamon extract was obtained through ultrasound assisted extraction, which is a rapid and effective extraction technique that uses ultrasound to generate rapid movement of solvents, resulting in a higher mass transfer speed as well as acceleration of extraction (Figure 1)

Formulation of the gels

- Gel synthesis: The polymer used to make the gels, xanthan gum, was dispersed in the aqueous plant extract (at 75°C) under stirring. Control gel contained only sulphate copper, G1 contained copper nanoparticles in the gel basis, while G2 gel contained both essential oils and copper nanoparticles. The essential oils (oregano, cinnamon, clove, eucalypt, thyme, lavender) and copper nanoparticles were added slowly to the gel basis, with continuous stirring, to ensure proper encapsulation. The final weight of the gel was adjusted to 50 g with distilled water.

Physico-chemical evaluation

- Visual observation: for all formulations, certain characteristics such as physical appearance, colour, texture, phase separation, and homogeneity were assessed. Homogeneity and texture were tested by pressing a small amount of gel between the thumb and forefinger. The consistency of the formulations and the presence of coarse particles were analysed to evaluate the texture of the formulations. Immediate skin sensation (including stiffness, oily sensation) was also assessed.
- Spread ability of the gels: was determined by measuring the spreading diameter of 1 g of gel sample between two horizontal glass plates (10 cm × 20 cm) after one minute, using a standard weight of 25 g applied to the top plate.
- Ph: One gram of each formulation was dissolved in 25 ml of deionized water and the ph was determined using a ph-meter determined using a ph-meter calibrated with standard buffers (pH 4, 7 and 10) before each use.

In vitro antibacterial activity

- Qualitative assessment of the antimicrobial activity: performed in triplicate, by the diffusimetric technique. For this, 50 ul of hydrogel was distributed in Petri dishes with Muller Hinton culture medium previously sown with various standardized microbial species: Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC 12228, MRSA ATCC 43300, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027, Candida albicans ATCC 90028. Evaluation of the antimicrobial effect was performed by measuring the area of inhibition created from the edge of the hydrogel spot to the edge of the microbial culture.

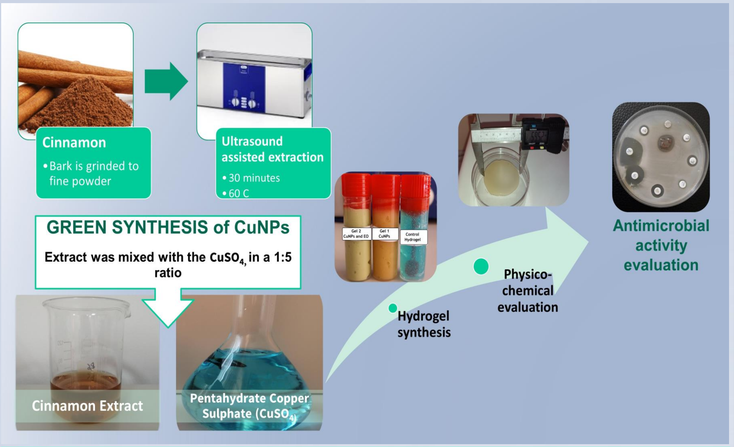


Figure 1. Formulation of a multifunctional nanocomposite hydrogel based on natural polysaccharides, biogenic copper nanoparticles and essential oils

CONCLUSIONS

- The physico-chemical structure of the hydrogels G1, G2 and G-control allowed the release of the compounds and the evaluation of their antimicrobial effect against all the microbial strains tested.
- The antimicrobial activity was different depending on the composition of the hydrogels and the microbial type. Gram-positive bacterial strains showed the best sensitivity to the tested hydrogels.
- In vitro, hydrogels were active against methicillin-susceptible and methicillin- resistant strains of Staphylococcus aureus. The yeast Candida albicans had a sensitivity profile similar to Gram-positive strains.
- The most active hydrogel formula was G2, thus when coupled with the specified essential oil blend, copper nanoparticles demonstrated strong synergistic antibacterial action.
- This work assessed and validated the in vitro antibacterial activity of a polysaccharides-based composite hydrogel comprising biologically synthesized copper nanoparticles and essential oils with potential applications in both human and veterinary medicine

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