FORMULATION AND EVALUATION OF ANTIBACTERIAL PHYTOTHERAPEUTIC GELS CONTAINING METAL IONS AND ESSENTIAL OILS

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Abstract

The purpose of this study was to develop topical phytotherapeutic gels containing essential oils alone or in combination with copper ions and to assess their antibacterial activity in vitro. Following the formulation of the gels, the organoleptic properties, physicochemical properties, and in vitro antibacterial activity against Escherichia coli and Staphylococcus aureus were evaluated. Copper sulfate demonstrated strong synergistic antibacterial activity when combined with the selected essential oil blend. This preliminary study assessed and confirmed the synergistic antimicrobial properties of sulfate copper and essential oils in phytotherapeutic formulations with topical applications.

Key words: phytotherapeutic gel; essential oils; metal ions; copper sulfate

Introduction

Due to the rising concerns regarding multidrug-resistant pathogens, the need for novel therapeutics has grown considerably, the emphasis being now placed on the development of innovative and effective alternatives to conventional antimicrobials. From ancient times the people have used it extensively natural herbal products as medicines against various diseases. According to a study conducted by Mohanty et al., 2019, almost 25% of the main pharmaceutical products and their derivatives available today are obtained from natural resources, therefore phytotherapy can be a good starting point for the development of products with antimicrobial activity (Bhinge et al., 2017; Morteza-Semnani et al., 2021). More recent research has shown that the effectiveness of phytotherapy and essential oils could be greatly improved, even against antibiotic-resistant pathogens, by its association with metal ions (Low et al., 2017; Prasad et al., 2021). The most frequently investigated metal ions as possible antibacterial agents are silver, copper, zinc, and iron (Holloway et al., 2012; Morrill et al., 2013) have been investigated in recent years. The antimicrobial properties of copper are widely recognized and it has been used successfully as biocide and antifungal. Cooper may bind to protein molecules leading to DNA denaturation and inactivation of bacterial enzymes.

In spite of their great potential in the development of novel antimicrobial agents, the incorporation of metal ions in topic formulations may be challenging because even in small concentrations, the oxidation processes in fatty molecules may be enhanced, causing visible degradation of the product (color, texture and odor modifications). Thereby, the quality, effectiveness, consumer appeal, and shelf-life of formulations may be affected by metal ion interactions with the components. The purpose of this study was to develop topical phytotherapeutic gels containing essential oils alone or in combination with copper ions and to assess their antibacterial activity in vitro.

MATERIAL AND METHOD

The polymer used to make the gels, xanthan gum, was dispersed in the aqueous plant extract (at 75°C), cinnamon or oregano under stirring. When the polymer was dissolved, the mixture was removed from the heat source. The essential oils (oregano, cinnamon, clove, eucalypt, thyme, lavender) 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. For all formulations, certain characteristics were assessed by visual observation, such as physical appearance, color, texture, phase separation, and homogeneity. Homogeneity and

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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 analyzed to evaluate the texture and homogeneity of the formulations. Immediate skin sensation (including stiffness, oily sensation) was also assessed. The spread ability of the product 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. The standard weight applied to the top plate was 25 g. Each formulation was tested three times. The pH was determined using a ph-meter. One gram of each formulation was dissolved in 25 ml of deionized water. The PH-meter was calibrated with standard buffers (pH 4, 7 and 10) before each use.

The qualitative assessment of the antimicrobial activity of the hydrogels was 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.

RESULTS AND DISCUSSIONS

Hydrogels are three-dimensional network structures that may absorb large amounts of water and have numerous applications in fields such as biomedicine and pharmacology. Hydrogels adhere to the skin and allow an efficient release of active substances. They also spread easily in a thin layer and can be easily removed by washing. However, for a proper formulation of this type of product, a number of practical aspects must be taken into consideration.

The active and auxiliary ingredients play an important role in the formulation process. These ingredients have a decisive influence on the physicochemical properties of the formula, in order to obtain the desired therapeutic effect. The release and absorption of active substances from the hydrogel depends on a number of factors such as the nature of the polymer used, the physicochemical properties of the incorporated ingredients.

The hydrogels in this study were obtained from colloidal macromolecules which have the property of combining with water or other hydrophilic solvents by absorption. For the formulation of gel basis, we selected excipients without irritating action, suitable for the place of application, respectively the mammary gland. In this regard, we used xanthan gum as both gelling and thickening agent. Due to its well-known antimicrobial and antiviral activity, copper sulfate pentahydrate (CuSO4 5H2O) (Figure 1) was selected as one of the key active components in the elaborated topical formulations. In a gel formulation, isopropyl myristate was utilized as an emollient to offer a softening or soothing effect on the skin.

Glycerin was added to the gel composition and served as a humectant, enhancing the hydration of the stratum corneum. Cinnamon and turmeric extracts were used as solvents, to disperse the water-soluble ingredients. The essential oils used for the formulation of the topical gels were selected based on their antibacterial, anti-inflammatory proprieties and synergistic potential (Neculai-Valeanu et al., 2021). Cosgard, an easy-to-use and approved preservative for use in organic cosmetics ensured the efficient preservation of the topic formulations.

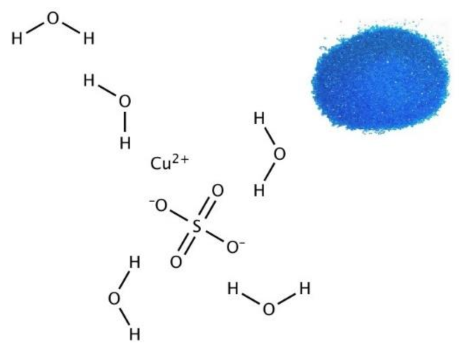


Figure 1 **Copper (II) sulfate pentahydrate**

The polymer was hydrated with distilled water before the dispersion in the cinnamon extract, alongside the other active ingredients. Three different gels were formulated in this study.

R1 and R2 contained only copper sulfate, respectively essential oils incorporated in the gel basis, while R4-13 gel contained both essential oils and sulfate copper. Table 1 shows the organoleptic characteristics of the evaluated topical gels, including physical appearance, color, texture, phase separation, homogeneity, and initial skin sensation. The gels had a visual appealing appearance and smooth texture, and they were all uniform with no evidence of phase separation. The copper sulfate gave the formulation a green-blue color (Figure 2). R1 gel, containing only copper sulfate incorporated in the polymer matrix presented a light blue color.

The spread ability plays an essential role in the effectiveness of topical therapy, which depends on the operator spreading the formulation in a uniform layer to administer a standard dose. In our study, the spread ability of the developed gels varied between 48 mm and 59.52 mm (Table 1). The gel containing only essential oils presented the highest spread ability, 59.52 mm (Figure 3).

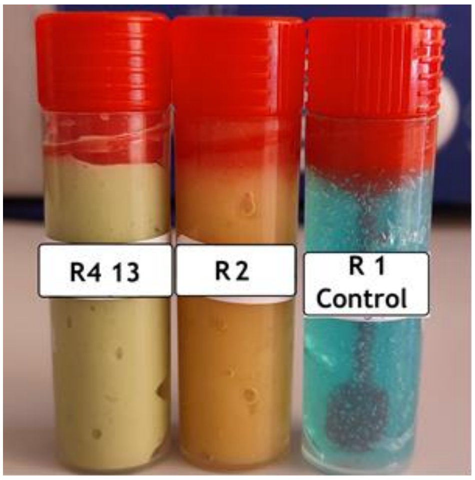


Figure 2. **Visual aspect of formulated topical gels**

Table 1

Physico-chemical characteristics of the gel formulations

Formulation	Physical appearance	Color	Texture	Phase separation	Homogenity	Immediate skin sensation	pH	Spredability (mm)
R1	Semi Transparent	Intese Blue	Smooth	no	yes	Moisturizing No signs of coarse particles	6.78 ± 0.08	48 ± 0.11
R2	Transparent	Light yellow	Smooth	no	yes	Refreshing, Cooling No signs coarse particles	6.26± 0.06	55. 91 ± 0.08
R413	Semi transparent	Green-blue	Smooth	no	yes	Moisturizing Refreshing Cooling No signs coarse particles	6.08 ± 0.04	59.52 ± 0.10

The in vitro antibacterial activity of the three gels was assessed by comparing the inhibition zone (in mm) of each product (Figure 3).

The zone of inhibition can be defined as the clear region surrounding the hydrogel drop. The antimicrobial agent's potency is known to be proportional to the size of the inhibition zone. The results obtained from the conducted study are shown in figure 4.

It may be observed that the lowest antimicrobial activity was reported in the R1 hydrogel containing only metal ions (cooper sulfate). *Staphylococcus epidermidis* (8.2 mm) compared to other Gram-positive bacterial species: *Staphylococcus aureus* (4.6 mm), MRSA (3.8 mm) and Gram-negative bacteria: *Escherichia coli* (3.2 mm) and *Pseudomonas aeruginosa* (2 mm). The antimicrobial effect on *Candida albicans* yeast (4.6 mm) was similar to that for Gram-positive bacteria.

R2 hydrogel showed significantly better antimicrobial activity compared to the results of inhibition activity of the R1 hydrogel. This inhibitory effect was due to the active compounds from the essential oils of oregano, cinnamon, clove, eucalyptus, thyme, lavender.

The most sensitive microbial species were *Staphylococcus epidermidis* (12.7 mm) and *Candida albicans* (12 mm).

The extended effect of the combination of essential oils is known, and this aspect can be noticed by the zones of inhibition created against *Escherichia coli* (7.4 mm) and MRSA (6.4 mm).

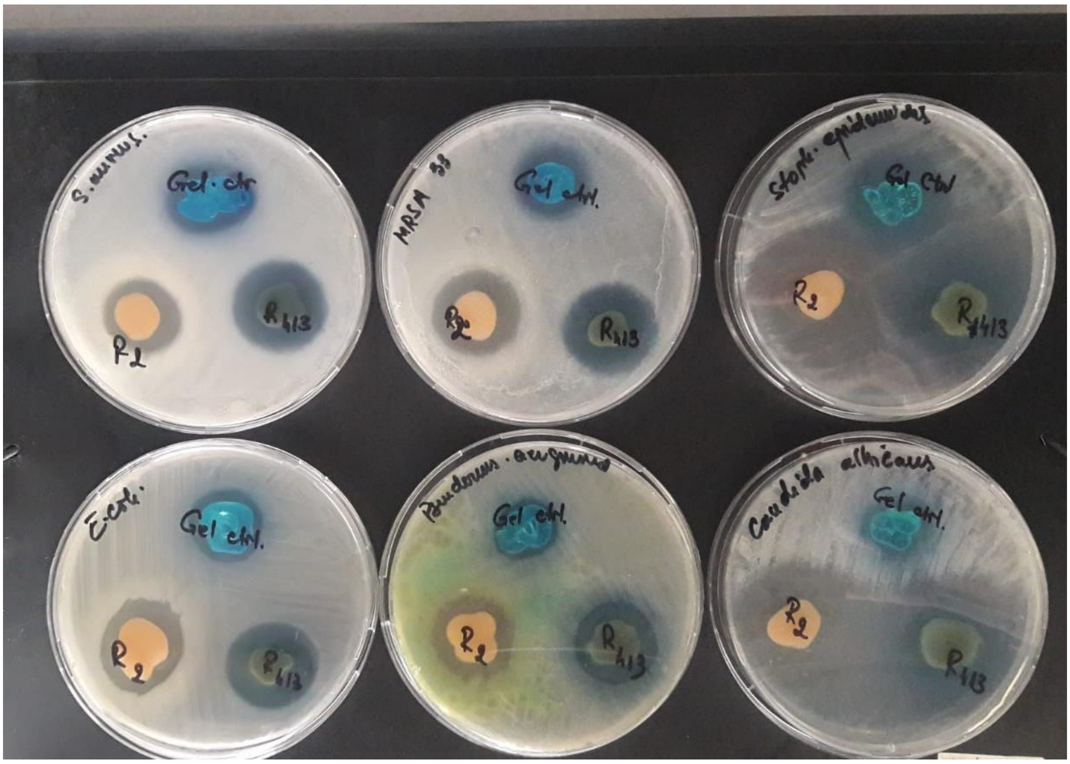
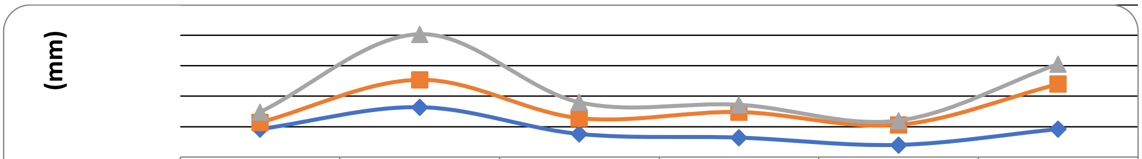


Figure 3. **Antibacterial evaluation of the formulated gels**



	Staphylococcus aureus ATCC 6538	Staphylococcus epidermidis ATCC 12228	MRSA ATCC 33591	Escherichia coli ATCC 25922	Pseudomonas aeruginosa ATCC 9027	Candida albicans ATCC 90028
R1 x̄	4.6	8.2	3.8	3.2	2	4.6
R2 x̄	5.7	12.7	6.4	7.4	5.3	12
R413 x̄	7.4	20.2	9	8.6	6	15.3

Figure 4. **Mean arithmetic value obtained from three tests**

Hydrogel R4-13, formed by combining compounds from hydrogels R1 and R2, cumulated their inhibitory effects, and the results were confirmed by the increasing diameter of the inhibition zones formed around all tested microbial species. *Staphylococcus epidermidis* (20.2 mm) and *Candida albicans* (15.3 mm) remain the most sensitive microbial species. The antimicrobial action developed against MRSA ATCC 33591 (9 mm), a strain of methicillin-resistant *Staphylococcus aureus* with important public health implications, is noted. Also, the antimicrobial effect obtained against *Escherichia coli* (7.4 mm) and *Pseudomonas aeruginosa* (6 mm) is very important, as it is known that Gram-negative bacteria have a different cell wall structure than Gram-positive bacteria, structure that makes them more resistant and gives them a different response to various antimicrobial agents.

The minimal evaluation of the antimicrobial activity of these hydrogels, compared to the three types of microorganisms (Gram positive bacteria, Gram negative bacteria and yeasts) shows the potential of the selected active compounds and the results encourage further testing and explanation of the mechanisms by which this antimicrobial effect is created, compared to antibiotic-resistant strains.

The antimicrobial potential of these hydrogels must be transferred, tested and confirmed *in vivo*, so that this drug formula becomes a therapeutic option.

CONLUSIONS

1. The physico-chemical structure of the hydrogels R1, R2 and R 3-14 allowed the release of the compounds and the evaluation of their antimicrobial effect against all the microbial strains tested.
2. The antimicrobial activity was different depending on the composition of the hydrogels and the microbial type.
3. Gram-positive bacterial strains showed the best sensitivity to the tested hydrogels.
4. *In vitro*, hydrogels were equally active against methicillin-susceptible and methicillin-resistant strains of *Staphylococcus aureus.*
5. The yeast Candida albicans had a sensitivity profile similar to Gram-positive strains.
6. The most active hydrogel formula was R 3-14 which incorporated copper sulfate and the essential oils of oregano, cinnamon, clove, eucalyptus, thyme, lavender.

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