NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY POLITEHNICA BUCHAREST

Faculty of Chemical Engineering and Biotechnologies

Department of Science and Engineering of Oxide Materials and Nanomaterials

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PhD Thesis - Summary

Polymeric antimicrobial systems used in the treatment of skin conditions

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PART I. State-of-the-Art Review

CHAPTER 1. Skin Health: Physiology, Microbiome and Therapy

1.1. Introduction

The skin is a multifunctional organ, essential for survival, whose activities far exceed the role of a simple barrier that separates the internal tissues from the external environment. It contributes to thermoregulation, maintenance of hydro-electrolytic balance, homeostasis, sensory perception, and immunological protection. Therefore, the skin can be considered a key element of systemic health. Structurally, it is a vascularized connective tissue membrane covering the entire body, fusing with mucous and semi-mucous membranes at the level of natural cavities.

1.2. Structure of the skin: structure, functions, and interactions

Structurally, the skin has three layers: the hypodermis, dermis and epidermis. Yet each layer has its own cellular composition and physiological role, they function in an integrated manner to preserve skin integrity and systemic homeostasis [1].

The hypodermis, located below the dermis, represents the deepest compartment of the skin. It is composed of lobules of adipose tissue, sensory nerve endings, vascular networks, and skin outgrowths such as hair follicles.

The epidermis and dermis are joined by the basement membrane, which constitutes a structural and functional interface between the two. The dermis comprises papillary and reticular regions of connective tissue, which grade into one another without a clear separation. The innermost **epidermal stratum** is the stratum basale. The basal lamina pulls it away from the dermis, and hemidesmosomes securely attach it to it [2,3].

Besides its physical role, the skin additionally performs physiological, sensory, and immunological functions, thus contributing to maintaining homeostasis.

1.3. The skin microbiota and its role in maintaining the local immunological balance

Bacteria are important components of the skin microbiota, having the most common genera represented by *Staphylococcus*, *Corynebacterium*, *Propionibacterium* (recently renamed *Cutibacterium*), and *Micrococcus*. They compete with pathogens for nutrients and space, produce antimicrobial substances that inhibit growth of pathogens, and may produce compounds that affect other microorganisms like *Malassezia* fungi found in lipid-rich areas, or viruses and bacteriophages, which can impact the composition of bacterial communities via mechanisms of gene regulation and horizontal transfer. Thus, most skin-associated bacteria are classified into four main phyla: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* [4–6].

1.4. Skin infections: bacterial, viral, fungal, parasitic - manifestations and complications

Skin infections represent a heterogeneous group of conditions caused by pathogens that colonize or invade the skin, affecting different layers of the skin and its appendages. They are classified, depending on etiology, into bacterial, viral, fungal and parasitic infections. Although in many cases they are self-limiting, they can become severe or systemic if not treated properly. Clinical manifestations and complications are strongly influenced by the causative agent, the immunological terrain of the patient, and the location of the infection [7–9]. Bacterial skin infections are among the most frequent and are mainly caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. Complications can include abscess, lymphangitis, thrombophlebitis and, in severe cases, septicemia [7,10–12]. Viral skin infections are caused by viruses among the most common being varicella-zoster virus, HPV viruses, herpes simplex or poxviruses [13,14]. Fungal infections of the skin, known as cutaneous mycoses, are caused by dermatophytes (*Trichophyton*, *Microsporum*, *Epidermophyton*), yeasts (*Candida* spp.) and opportunistic fungi [15,16]. Parasitic skin infections include scabies (caused by *Sarcoptes scabies* mites) and pediculosis (lice infestation) [17].

Skin infections represent a broad field with multiple etiologies and varied clinical manifestations. These kinds of conditions can be prevented and treated through a combination of therapeutic, hygienic, and educational measures, and knowing both the types and their mode of transmission contributes significantly to maintaining skin health and avoiding systemic complications.

1.5. Treatment of skin infections: topical drugs, antibiotics, antifungals, emerging therapies

Topical medications are the first choice for the treatment of superficial and localized skin infections. They are presented as creams, ointments, lotions and gels formulated with antibiotics, antifungals, antivirals or anti-inflammatory agents. For example, mupirocin and fusidic acid are often utilized in the treatment of impetigo and staphylococcal folliculitis[18]. In contrast, for fungal infections are recommended clotrimazole, miconazole or terbinafine. Topical preparations have the advantage of direct application to the lesion, limiting systemic effects and reducing the risk of microbial resistance [16,19–21].

Systemic antibiotics are indicated in moderate or severe forms of bacterial skin infections, such as cellulitis, erysipelas, recurrent abscesses, or extensive infections in immunocompromised patients [22–24].

In recent years, emerging therapies have earned increasing importance in dermatology. These involve the use of topical probiotics, prebiotics and postbiotics to

support the skin microbiome and prevent flora imbalance that can lead to recurrent infections [4,25]. Also, photodynamic therapy, therapy with synthetic antimicrobial peptides or specific bacteriophages are innovative directions in the treatment of infections with resistant microorganisms to conventional antimicrobials [26,27]. Nanotechnology also provides new vehicles for effective drug delivery through the skin, increasing treatment efficiency and reducing toxicity [28,29].

1.6. Issues in treating skin infections: complications and nosocomial pathogens

Although skin antibiotic treatments (topical or systemic) are generally effective and well tolerated, they can be associated with several local or systemic complications, particularly in relation to long-term, inappropriate use or in the presence of individual contributing factors, such as immunosuppression, polymedication or imbalance of the skin microbiome [30,31]. One of the most common complications of topical antibiotics is skin irritation or allergic contact dermatitis, especially with substances such as neomycin, bacitracin or mupirocin [32–34]. Another significant aspect is the appearance of skin dysbiosis is the imbalance of the normal skin flora, which can occur following the uncontrolled use of antimicrobials and which favors colonization with opportunistic pathogens such as *C. albicans, S. aureus* MRSA or *P. aeruginosa* [35–37].

As for nosocomial skin infections, they occur frequently during prolonged hospitalizations, especially in intensive care units or burn units. The skin of patients with extensive burns is profoundly affected from a structural and immunological point of view, completely losing its barrier role, which facilitates rapid colonization and invasion by bacteria, fungi or viruses from the hospital environment.

CHAPTER 2. Natural Compounds and Plant Extracts with Antimicrobial Activity

2.1. Generalities

Infections induced by microorganisms resistant to antibiotics are a global concern. For this reason, research has increasingly turned to natural sources, especially bioactive plant compounds, which have demonstrated effective antimicrobial properties against a wide range of bacteria, fungi and even viruses. These compounds offer a promising alternative or complement to classical treatments, especially due to the multiple mechanisms by which they act and the lower risk of generating resistance. Among the most studied categories of natural substances with antimicrobial action are volatile phenols, terpenes,

flavonoids, tannins, phenolic acids and alkaloids. They can be isolated from aromatic plants such as thyme (isolated from *Thymus vulgaris*), oregano (isolated from *Origanum vulgare*), cloves (isolated from *Syzygium aromaticum*), but also from coniferous trees, berries, or seeds [107–112].

2.2. Antimicrobial mechanisms of action of natural compounds

The efficacy of natural compounds against microorganisms is not only due to the presence of an active substance, but mainly to the complex way in which they interfere with the functioning of microbial cells. Unlike many classical antibiotics that have a single cellular target (such as inhibiting cell wall synthesis or blocking ribosomes), the active compounds from plant extracts simultaneously intervene in several processes essential for microbial survival, which significantly reduces the chances of resistance [44]. Recent studies confirm the major potential of natural compounds and plant extracts in combating microbial infections, both as direct agents and as adjuvants. They act through multiple mechanisms, which makes them effective against resistant bacteria and makes it difficult for them to adapt to these agents [45].

2.3. Natural compounds that potentiate the activity of antibiotics against resistant strains

In the context where infections with multiresistant bacteria are becoming more and more frequent, researchers are seeking for strategies to use natural compounds not only as antimicrobial agents, but also as adjuvants that can potentiate the effect of existing antibiotics, partially reversing resistance and reducing the required antibiotic doses.

An example is sophoraflavanone G, a flavonoid isolated from the roots of the genus Sophora, which was tested against 27 MRSA strains and produced complete inhibition at extremely low concentrations (3–6 μg/mL) [46]. Compounds derived from chili pepper and ginger have received increased attention for antibiotic potentiation. A study published in 2019 demonstrated that capsaicin, dihydrocapsaicin, 6-gingerol and 6-shogaol can inhibit MRSA strains expressing efflux pumps (TetK, MsrA, NorA), the MIC values being in the range of 8 and 256 mg/L [47].

2.4. Pharmaceutical forms and supplements containing natural compounds and their presence in the global market

In the last two decades, the global pressure generated by infections produced by resistant bacteria has determined not only the acceleration of research in the field of antibiotics, but also the re-evaluation of some natural compounds known for centuries. Some of these compounds have moved beyond the experimental stage and are currently

incorporated into licensed, widely available pharmaceutical products. In a context where the regulatory authorities (FDA, EMA) impose strict standards, only substances with a clearly demonstrated safety and efficacy profile can be formulated into products intended for human or veterinary use.

Among the most widely used natural compounds in antimicrobial treatments are thymol and carvacrol, two main constituents of thyme and oregano essential oils. Thymol is recognized by the FDA as "Generally Recognized as Safe" (GRAS) and *Thymus vulgaris* extracts are documented in the European Pharmacopoeia with clear specifications regarding purity and origin. It is used as an active ingredient in numerous pharmaceutical products, especially for oral hygiene and upper respiratory tract conditions [48,49]. The best-known application is in the formulation of Listerine® antiseptic mouthwash, where thymol is associated with menthol, eucalyptol and methyl salicylate. This combination is [[50].

CHAPTER 3. Systems Utilized in Pharmaceutical Formulations for Active Substances Delivery

Drug-delivery systems designate the set of technologies that transport and release active substances in the body, both by choosing the route of administration (e.g., oral tablets, injectable vaccines) and by designing the carrier (e.g., micelle, nanoparticle) that protects the drug and facilitates its crossing through physiological barriers to the therapeutic target. The field has evolved rapidly in recent decades, but many therapies remain limited by adverse effects driven by nonspecific distribution in healthy tissues, particularly in oncology, neurodegenerative and infectious diseases. The current priority is to increase specificity and therapeutic index through targeted targeting and fine control of exposure [51,52].

3.1. Cyclodextrins

Cyclodextrins are cyclic oligosaccharides composed by the joining of several glucopyranose subunits at the α-(1,4) positions. Cyclodextrins are of three types: α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin, constituted of six, seven, respectively eight units of α-glucopyranose linked by bonds 1-4.

Cyclodextrins are commonly used in numerous industrial processes, technologies and analytical methods due to their ability to form complexes with hydrophobic substances. Another important aspect is represented by the low cytotoxicity which make them suitable for diverse pharmaceutical applications, cosmetic and food industries [53].

The literature reports β-cyclodextrin complexes with essential oils extracted from different parts of plants, such as *Eugenia caryophyllata*, *Lippia berlandieri* [54], *Thymus catharinae* [55], *Cinnamomum verum* [217], *Satureja montana* [57], *Origanum vulgare*, *Coriandrum sativum* [58], *Lippia gracilis* [59]and *Mentha × villosa* [60], *Cymbopogon flexuosus*, *Pelargonium graveolens*, *Eucalyptus citriadora* [58] and *Aloysia citriodora* [61].

3.2. Polymeric gels

Polymers are macromolecules constituted by repeating units of monomers with a similar structure, and synthetic polymers have a fundamental role in the discovery and development of pharmaceuticals. Traditionally, their research and applications have concentrated on their use as excipients and inactive carriers of substances with pharmacological activity, but recent advances have also highlighted their alternative use as pharmacologically active agents (polymeric drugs), with applications in sequestration, antimicrobial, antiviral, anticancer and anti-inflammatory activity. Natural polymers, obtained from plants, animals or micro-organisms, have significant advantages over synthetic ones, being cheaper, non-toxic and abundantly available in nature [62,63].

3.2.1. Alginic acid and alginates

Alginates are natural anionic polysaccharides extracted mainly from the pore cells of brown algae (Laminaria, Macrocystis, Ascophyllum, and Sargassum) [64,65].

One of the characteristics of alginates is their capacity to form three-dimensional gels through an ionic cross-linking process when exposed to divalent ions, the most used being calcium ions (Ca^{2+}). This phenomenon, called the "egg-box model", consists in the cross-linking of Ca^{2+} ions with the G-blocks of the alginic acid chains, resulting in a stable gel network [66]. Alginate gels are used in the management of chronic skin lesions such as pressure ulcers and burns, due to the property of maintaining a moist environment and absorb exudate, alginate gels boost healing and lower the potential for infection. In addition, the gradual release of antimicrobial or anti-inflammatory agents in the affected area contributes to high therapeutic efficiency [67,68].

3.2.2. Chitosan

Chitosan is a natural polymer obtained by deacetylation of chitin, a biopolymer presents in the shell of crustaceans, insects or in the cell walls of fungi. Through this chemical modification, chitin becomes soluble in slightly acidic environments, and the resulting polymer acquires biological and physicochemical properties that recommend it for numerous applications, mostly in the biomedical and pharmaceutical fields [69].

One of the most valuable forms of chitosan formulation is the gel. Chitosan gels are 3D networks capable of absorbing considerable amounts of water or biological fluids and preserving a semi-solid structure. They can be obtained by physical (by adjusting pH or

temperature) or chemical (by adding crosslinking agents like glutaraldehyde or genipin) crosslinking processes. Unlike synthetic gels, chitosan-based gels exhibit biocompatibility, biodegradability and intrinsic antibacterial activity, making them suitable for applications in dermatology, ophthalmology, dentistry or transdermal drug delivery [69–72].

3.2.3. Polyacrylic acid (Carbopol)

Carbopol is a cross-linked polyacrylic acid polymer capable of forming clear, stable gels with a controllable viscosity, being used both for its rheological characteristics and for its property to be compatible with many active substances [73].

The gels of Carbopol can be prepared by dispersing the powder directly in water and then neutralizing it with an alkaline agent such as triethanolamine or sodium hydroxide. The process is sensitive to the speed of mixing, the type of water used (deionized being preferred) and the nature of the ingredients added afterwards. Depending on the concentration used (usually between 0.2% and 1.5%), the gels obtained can have a consistency from fluid to semi-solid, being adaptable for various therapeutic applications [74].

3.3. Approaches in polymeric gels characterization

The characterization methods of polymer gels are crucial for assessing their chemical composition as well as their physical properties, as well as for understanding their behavior when they are stimulated from the outside by different factors. Thus, their characterization can be carried out both structurally, chemically, mechanically, rheologically and functionally [75].

Characterization of hydrogels can be done by various methods, including physicochemical techniques such as FTIR, NMR, UV–Vis spectrophotometry, swelling studies, pH and conductivity measurements, and elemental analysis (XPS, EDX); structural and morphological analyzes by SEM, TEM, CLSM and AFM; mechanical and rheological evaluations by rheometry, compression/tension tests and DMA; thermal analyzes using TGA, DSC and DTA; as well as biological and pharmacological evaluations including biocompatibility (MTT, Alamar Blue, Live-Dead), antimicrobial activity (MIC, MBEC, zone of inhibition assays), controlled release studies (dialysis, Franz diffusion cells) and cytotoxicity/inflammatory assays (ELISA, ROS).

PART II. Original Contributions

CHAPTER 4. General and Specific Objectives of the Research

Infections remain a major cause of morbidity, mortality and costs for the healthcare systems, and the rise of antimicrobial resistance significantly amplifies this burden. Globally, in 2019, bacterial resistance was primarily responsible for ~1.27 million deaths and associated with ~4.95 million deaths, exceeding the burden of priority infectious diseases such as HIV/AIDS or malaria. In Europe, the World Health Organization estimated 133,000 deaths attributed to antimicrobial resistance and 541,000 associated with it. These data validate the need for research into new strategies for the prevention and treatment of infections, including the development of formulations with local action and a superior safety profile. Beyond the rise of antimicrobial resistance, the clinical burden of severe infectious syndromes also remains very high [76,77].

In this context, the choice of a theme focused on the treatment of infections is both scientifically and societally justified: *(i)* the clinical and economic burden is high and increasing in the absence of effective interventions; *(ii)* advances in prevention are not sufficient without better therapeutic alternatives, including locally administered strategies that can achieve concentrations at the site of infection with minimal systemic toxicity; *(iii)* alignment with the global targets and policies and the One Health agenda ensures impact and relevance of outcomes.

Skin health depends on the integrity and balance of its microbiome. Imbalances favor the establishment of infections, delay healing, and increase the risk of recurrence. At the same time, the alarming increase in antibiotic resistance reduces the effectiveness of conventional topical therapies, and their prolonged use can affect the commensal flora and barrier integrity. In this context, it becomes essential to identify effective antimicrobial alternatives, "microbiome-friendly," and well tolerated locally.

Natural plant extracts are valuable resources of antimicrobial and antibiofilm agents with high biocompatibility and acceptability profiles. However, many plant-based active compounds, especially volatile ones, are limited by volatility, oxidative instability, low solubility, and irritant potential, leading to rapid release ("burst"), fluctuating exposure, and inconsistent efficacy in vivo. To overcome these constraints, it is necessary to integrate them into delivery systems capable of ensuring protection, solubilization, and controlled release at the skin level.

Topical matrix polymers, such as chitosan, alginate, or polyacrylic acid/Carbopol gels, offer a versatile platform for topical administration: they are biocompatible, can support the loading of complex phytochemical mixtures, prolong skin surface residence, and modulate release kinetics. In addition, the formation of inclusion complexes with

cyclodextrins for volatile compounds improves solubility and stability, reduces volatilization losses, and allows a gradual release, prolonging the therapeutic effect and potentially reducing local irritation.

Based on promising initial data obtained through antimicrobial screening in undergraduate and dissertation work on clove essential oil and inclusion complexes with β-cyclodextrin, as well as *Verbena officinalis* and *Aloysia triphylla* extracts and considering the clinical and technological needs presented above, the theme of the work focused on the development of polymeric antimicrobial systems loaded with plant extracts for the management of skin infections, as well as on the encapsulation of volatile natural compounds in systems that ensure sustained release, like β-cyclodextrin.

The present study aims to combine antimicrobial and antibiofilm efficacy with a skin-friendly safety profile and respect for skin microbial ecology, offering a relevant alternative in a therapeutic landscape marked by antibiotic resistance and the limitations of conventional forms.

In line with the general goal, three objectives were followed:

1. To obtain *β*-cyclodextrin- clove essential oil complexes, characterize them and evaluate their biological properties.
2. To develop, optimize, and evaluate the biological activity of new antimicrobial gel formulations with polyacrylic acid (a synthetic polymer), *β*-cyclodextrin- clove essential oil complex and essential oils (tea tree and rosemary)
3. To develop new antimicrobial gels based on both, natural and synthetic polymers (sodium alginate, chitosan and polyacrylic acid), using *β*-cyclodextrin- clove essential oil complex and plant extracts (*Verbena officinalis* tincture, *Aloysia triphylla* tincture and bay essential oil).

The first objective was to obtain and characterize β-cyclodextrin–clove essential oil inclusion complexes. Clove essential oil was extracted from clove buds by steam distillation, and its chemical profile was examined by GC–MS. The β-cyclodextrin inclusion complexes with the extracted clove essential oil, as well as with two of its main constituents—eugenol and were prepared using four encapsulation methods: kneading, co-precipitation, lyophilization, and co-precipitation–lyophilization. Further, the complexes were characterized physiochemically and morphologically by UV–Vis spectrophotometry, chromatographic analysis (GC–MS and HS–GC–MS), FTIR, DSC, Job's method, dynamic light scattering (DLS), and SEM. Antimicrobial activity was assessed through qualitative and quantitative assays, followed by a semiquantitative evaluation of adhesion to an inert substrate and of the complexes' effects on the adhesion capacity of pathogenic microbial strains.

The second objective was to develop new gel formulations containing polyacrylic acid (Carbopol 940), a β-cyclodextrin–clove essential oil inclusion complex, rosemary and tea tree essential oils. Tea tree and rosemary essential oils were characterized by GC–MS.

Gel formulations were prepared by varying the concentrations of Carbopol 940 and the essential oils to identify an optimal, stable composition. Macroscopic characteristics and pH were assessed at the time of preparation and after 60 days at 2 °C and 40 °C. The spreading area was also measured to show the differences in consistency. Rheological behavior was analyzed using the Bingham, Casson, Ostwald–de Waele, and Herschel–Bulkley models, and their microstructure was examined by SEM. Antimicrobial activity was performed through qualitative and quantitative assays, followed by a semiquantitative evaluation of adhesion to an inert substrate. Additionally, cytotoxicity was evaluated in the human colorectal adenocarcinoma cell line HT-29 (ATCC HTB-38).

The third objective was to develop new gel formulations with both natural and synthetic polymers, using a β-cyclodextrin–clove essential oil inclusion complex and plant extracts (*Verbena officinalis* tincture, *Aloysia triphylla* tincture, and bay essential oil). Initially, bay essential oil was characterized by GC–MS. Gel formulations based on sodium alginate, medium- and high-molecular-weight chitosan, and polyacrylic acid were prepared. Macroscopic characteristics were assessed immediately after preparation and after 90 days at room temperature and 2–8 °C. Physicochemical characterization was initiated with the analysis of the rheological behavior, followed by thermal analysis (TG–DTA), SEM, and FTIR. Antimicrobial activity was also performed through qualitative, quantitative assays and by the semiquantitative evaluation of adhesion to an inert substrate, as well as the cytotoxicity evaluation on HaCaT human keratinocyte cells by MTT assay, assessment of LDH release and NO production.

CHAPTER 5. β-Cyclodextrin Inclusion Complexes with Antimicrobial Activity

The aim of this study was to obtain and characterize the inclusion complexes of β-cyclodextrin with clove essential oil (ECEO) and two of its main constituents, eugenol and eucalyptol. Clove essential oil was extracted from clove buds by steam distillation and chemically profiled by GC–MS. Inclusion complexes were prepared using four encapsulation methods: kneading, co-precipitation, lyophilization, and co-precipitation–lyophilization. Physicochemical and morphological characterization was performed by UV–Vis spectrophotometry, GC–MS, HS–GC–MS, FTIR, DSC, Job's method, dynamic light scattering (DLS), and SEM. Antimicrobial activity was investigated through qualitative and quantitative assays, followed by semi-quantitative evaluations of adhesion to inert substrates and the effects of the complexes on the adhesion capacity of pathogenic strains, including biofilm formation.

Materials and Methods

Extraction of Eugenia caryophyllata Essential Oil

The dried clove inflorescences were purchased from Solaris Plant S.R.L. Volatile oil extraction was performed by steam water extraction using a standard Neo-Clevenger apparatus [18].

Preparation of β-Cyclodextrin Inclusion Complexes

The encapsulation of Eugenia caryophyllata essential oil (eugenol and eucalyptol) in β-cyclodextrin was carried out by four methods: kneading, co-precipitation, lyophilisation, and by a combined method of co-precipitation followed by lyophilisation.

Results and Discussions

Evaluation of Physicochemical Characterisation of Inclusion Complexes

The ECEO chemical composition, as well as the compounds identified in the β-cyclodextrin encapsulated ECEO by the GC-MS and HS-GC-MS methods, are pre-sented in Table 1 and Table 2, respectively.

Table 1. The chemical composition of ECEO and its *β*-cyclodextrin complexes obtained by various methods resulting from GC-MS analysis.

Compounds	RT (min.) [1]	RI [2]	UV [3]	K [4]	L [5]	C-L [6]	C [7]
			Relative Area %				
Eugenol	12.84	1358	90.67 ± 1.15	94.71 ± 1.37	93.81 ± 1.33	96.30 ± 1.69	90.99 ± 1.49
Vanillin	13.29	1391	n.d.	0.20 ± 0.01	0.30 ± 0.02	0.26 ± 0.01	1.90 ± 0.04
(E)-*β*-Caryophyllene	13.63	1417	3.98 ± 0.09	0.33 ± 0.03	0.63 ± 0.03	0.50 ± 0.07	2.01 ± 0.04
Humulene	14.03	1449	0.41 ± 0.03	0.06 ± 0.00	0.10 ± 0.00	0.08 ± 0.00	0.27 ± 0.01
Eugenyl acetate	14.84	1513	4.77 ± 0.15	4.66 ± 0.20	5.16 ± 0.13	2.81 ± 0.04	4.68 ± 0.05
Caryophyllene oxide	15.66	1582	0.17 ± 0.02	0.04 ± 0.00	n.d.	0.05 ± 0.00	0.14 ± 0.00
Identified compounds	-	-	100	100	100	100	100

1 retention time; 2 Kovats indices; 3 Eugenia caryophyllata essential oil (ECEO); 4 K: complexes obtained by kneading; 5 L: complexes obtained by lyophilisation; 6 C-L: complexes obtained by co-precipitation followed by lyophilisation; 7 C: complexes obtained by co-precipitation; n.d.= not detected.

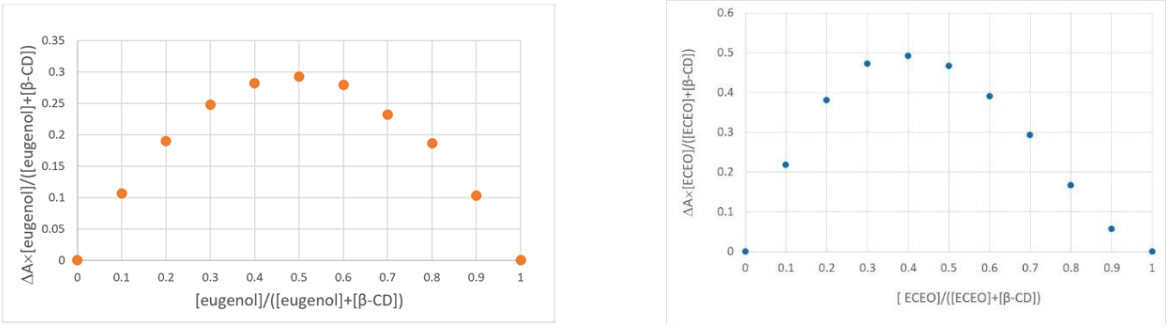
Table 2. The chemical composition of ECEO and its *β*-cyclodextrin complexes obtained by various methods resulting from HS-GC-MS analysis.

Compounds	RT (min.) [1]	RI [2]	Relative Area (%)				
			ECEO [3]	K [4]	L [5]	C-L [6]	C [7]
p-Cymene	7.49	1012	0.10 ± 0.00	0.70 ± 0.03	0.84 ± 0.04	1.57 ± 0.04	1.32 ± 0.07
Limonene	7.56	1016	0.30 ± 0.01	0.73 ± 0.03	0.75 ± 0.02	2.55 ± 0.12	1.29 ± 0.06
Eucalyptol	7.64	1020	0.36 ± 0.03	5.10 ± 0.17	2.45 ± 0.20	6.63 ± 0.20	4.00 ± 0.38
Eugenol	12.72	1349	86.46 ± 0.89	91.44 ± 1.91	92.63 ± 1.84	85.57 ± 1.40	88.75 ± 1.75
Vanillin	13.27	1390	n.d.	n.d.	n.d.	n.d.	0.32 ± 0.03
(E)-*β*-Caryophyllene	13.58	1415	10.96 ± 0.30	0.37 ± 0.02	0.26 ± 0.00	0.17 ± 0.00	0.30 ± 0.03
Humulene	14.01	1455	0.85 ± 0.03	n.d.	n.d.	n.d.	n.d.
Caryophyllene oxide±	14.79	1509	0.98 ± 0.04	1.67 ± 0.05	3.07 ± 0.20	3.52 ± 0.20	4.03 ± 0.29
Identified compounds	7.49	1012	0.10 ± 0.00	0.70 ± 0.03	0.84 ± 0.04	1.57 ± 0.04	1.32 ± 0.07

1 retention time; 2 Kovats indices; 3 Eugenia caryophyllata essential oil (ECEO); 4 K: complexes obtained by kneading; 5 L: complexes obtained by lyophilisation; 6 C-L: complexes obtained by co-precipitation followed by lyophilisation; 7 C: complexes obtained by co-precipitation; n.d.= not detected.

Stoichiometry of Inclusion Complexes

The Job plots for the eugenol–*β*-CD and ECEO–*β*-CD systems are shown in Figure 1a and Figure 1b, respectively. The shape of the curve in Figure 1a indicates 1:1 stoichiometry between the host and guest molecules, since the maximum of the graph is located at a value of 0.5 for the eugenol mole fraction. On the contrary, the equilibrium between ECEO and *β*-CD shows a stoichiometry of 1:2, with the maximum of Job's plot being at 0.33 for the essential oil mole fraction



(a) (b)

Figure 1. (a) Job's plot for eugenol–β-CD inclusion complex. (b) Job's plot for ECEO–β-CD inclusion complex

Entrapment Efficiency

The results obtained for encapsulation efficiency are presented in Table 3.

Table 3. The entrapment efficiency (EE) of eugenol, eucalyptol, and ECEO in *β*-cyclodextrin by different preparation methods.

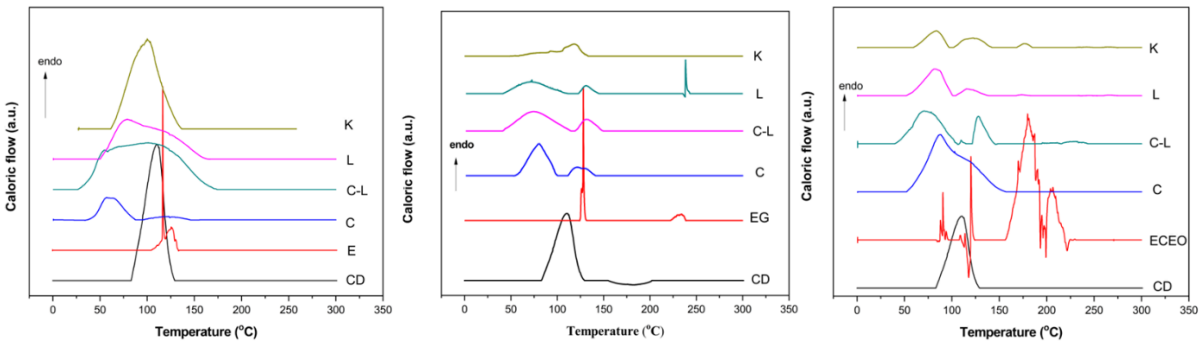
Complexation Method	EE (%)		
	Eucalyptol	**Eugenol**	**ECEO**
K	90.06 ± 3.3	99.38 ± 5.2	99.40 ± 1.9
C	48.68 ± 2.8	55.01 ± 4.7	69.26 ± 2.4
L	n.d.	77.66 ± 4.9	79.02 ± 2.0
C-L	95.62 ± 3.7	53.96 ± 4.4	60.48 ± 1.9

For eucalyptol, the highest encapsulation efficiency was achieved by the C–L method (95.62 ± 3.7%), followed closely by kneading (90.06 ± 3.3%). The co-precipitation method resulted in a significantly lower efficiency (48.68 ± 2.8%). In the case of eugenol, the kneading method provided the best encapsulation efficiency at 99.38 ± 5.2%, followed by lyophilisation (77.66 ± 4.9%). The co-precipitation and C–L methods yielded considerably

lower efficiencies of 55.01 ± 4.7% and 53.96 ± 4.4%, respectively. For ECEO, kneading again achieved the highest efficiency at 99.40 ± 1.9%, while lyophilisation reached 79.02 ± 2.0%. Co-precipitation (69.26 ± 2.4%) and C–L (60.48 ± 1.9%) methods resulted in lower encapsulation efficiencies.

Differential Scanning Calorimetry

The DSC curves obtained for the eucalyptol–*β*-cyclodextrin, eugenol–*β*-cyclodextrin, and ECEO–*β* cyclodextrin complexes are shown in Figure 2A–C.



(A) (B) (C)

Figure 2. Differential Scanning Calorimetry curves of the eucalyptol–β-cyclodextrin (A), eugenol–β-cyclodextrin (B), and ECEO–β-cyclodextrin (C) complexes. CD: β-cyclodextrin; E: eucalyptol; EG: eugenol; K: encapsulation by kneading; L: encapsulation by lyophilisation; C-L: encapsulation by co-precipitation–lyophilisation; C: encapsulation by co-precipitation; ECEO: Eugenia caryophyllata essential oil.

The DSC analysis of β-cyclodextrin revealed a broad endothermic peak in the temperature range of 75–125 °C, characteristic of the loss of hydration water from the cyclodextrin molecule. For eucalyptol, the DSC curve displayed a broad peak between 116 and 130 °C, with two maxima at 116.72 °C and 125.27 °C, which are lower than the values reported in the literature (176 °C). Eugenol exhibited two distinct endothermic peaks, one at 127.7 °C and another at 232.7 °C. The peak at 127.7 °C aligns with findings from a 2022 study, indicating thermal oxidation of eugenol, while the peak at 232.7 °C corresponds to its boiling point [78].

SEM Analysis of the Inclusion Complexes

SEM images of ECEO–β-cyclodextrin complexes are presented in Figure 3.

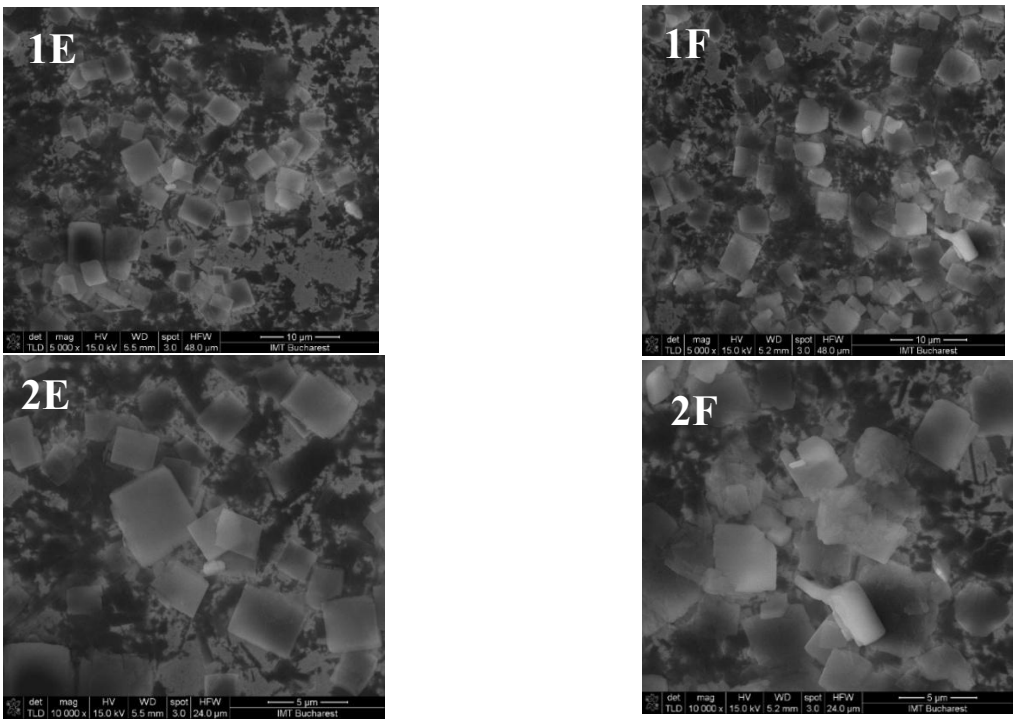
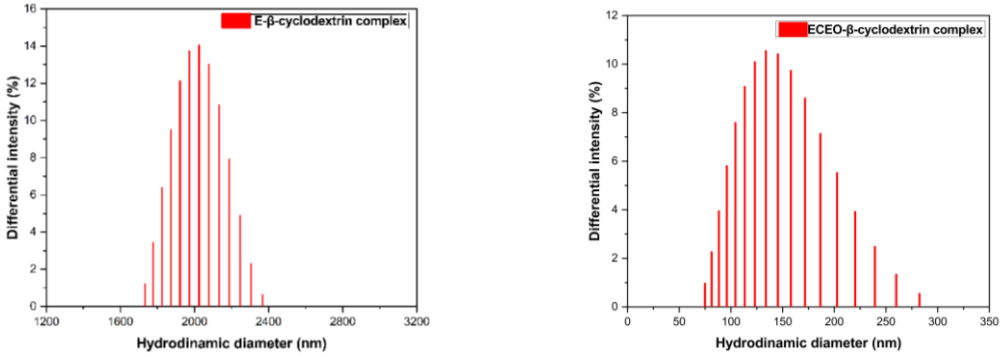


Figure 3. SEM images of ECEO–β-cyclodextrin complexes obtained by lyophilization (E) and kneading (F) at magnifications of ×5000 (1) and ×10,000 (2).

The β-cyclodextrin complexes with eugenol exhibit parallelepiped particle morphology, distinct from the complexes with ECEO, while the eucalyptol complexes show the formation of conglomerates.

Particles Size Distribution

The particle size distribution of the eugenol–β-cyclodextrin and ECEO–β-cyclodextrin complexes via the kneading method are presented in Figure 4.



(A) (B)

Figure 4. Particle size distribution of the eugenol–β-cyclodextrin and ECEO–β-cyclodextrin complexes

Dynamic light scattering analysis of the ECEO–β-cyclodextrin complex revealed nanostructured assemblies with a hydrodynamic diameter of 186.4 nm and a polydispersity index (PI) of 0.298. The diffusion coefficient was recorded at 2.127 × 10−8 cm2/s, while the estimated molecular weight was 2.209 × 109 g/mol.

For the β-cyclodextrin–eucalyptol complex, DLS analysis indicated a markedly larger hydrodynamic diameter of 2929.0 nm. The system exhibited a PI value of –2.651, falling outside the accepted range for DLS interpretation. The diffusion coefficient was notably lower, at 1.354 × 10−9 cm2/s.

FTIR Analysis

The IR spectra of ECEO- β-Cyclodextrin complex is presented in Figure 5.

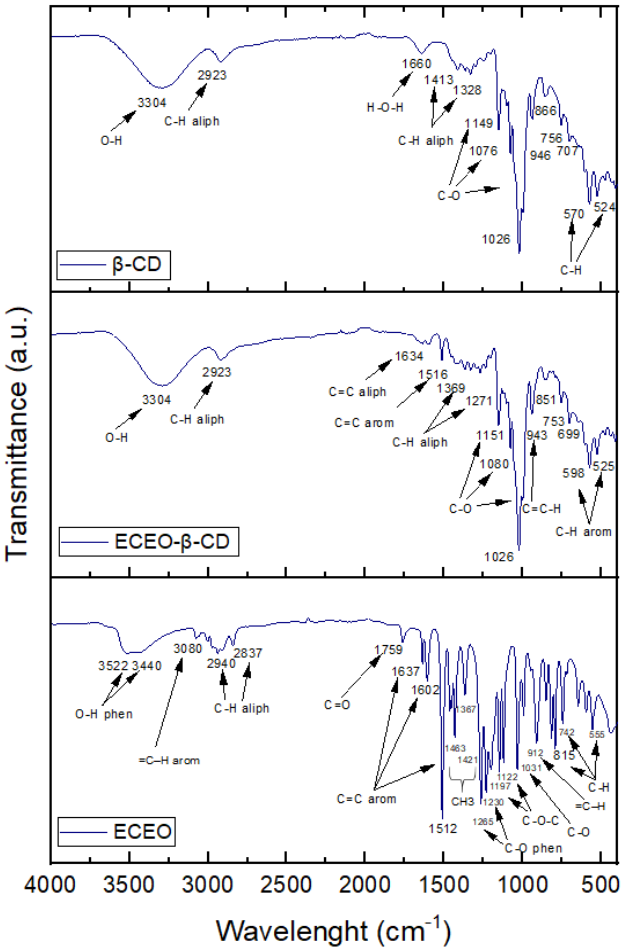


Figure 5. FTIR spectrum of the β-Cyclodextrin, ECEO- β-Cyclodextrin complex and ECEO. *Legend*: Eugenya cariophyllata essential oil= ECEO; β-Cyclodextrin= β-CD; β-Cyclodextrin complex with Eugenya cariophyllata essential oil = ECEO- β-CD;

The FTIR analysis confirms the successful inclusion of ECEO, EG, and E into the cyclodextrin cavity, stabilised by hydrogen bonding and hydrophobic effects. The cumulative spectral changes confirm the encapsulation of ECEO constituents, particularly

eugenol, within the hydrophobic cavity of β-cyclodextrin, contributing to the stabilisation of the volatile components' bioactivity [79,80].

Quantitative Assessment of the Antimicrobial Activity and Semiquantitative Assessment of the Microbial Adherence to the Inert Substratum

The MIC and MBEC results are presented in Figure 6 (A) and (B).

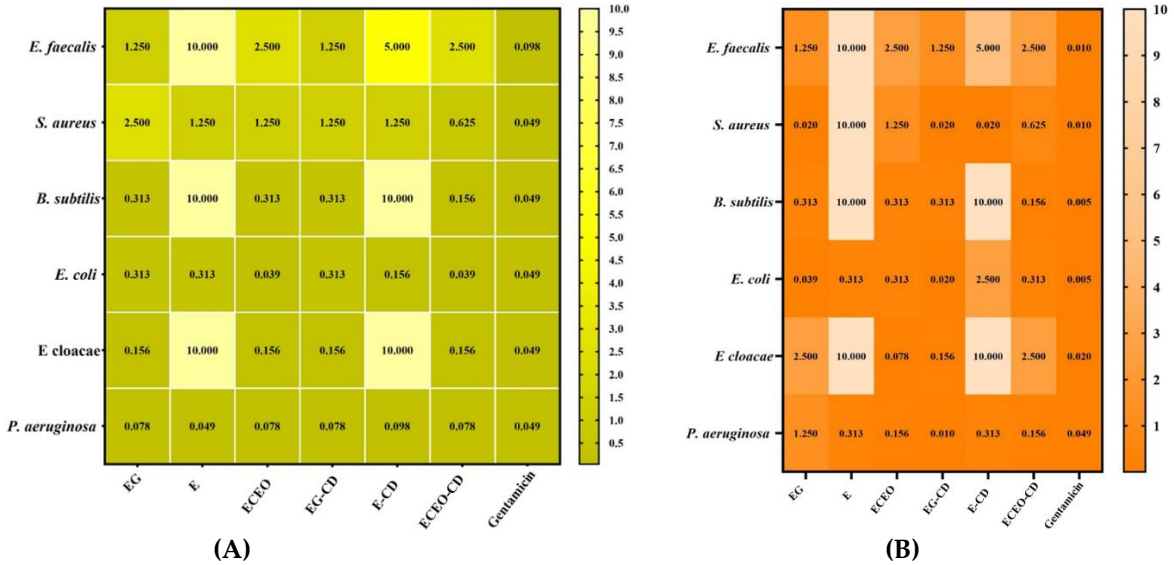


Figure 6. Heatmap of bioactive components and complexes' MIC values (mg/mL) (A) and MBEC values (mg/mL). Legend: Eugenol- EG; Eucalyptol- E; Eugenia caryophyllata essential oil- ECEO; β-cyclodextrin complex with EG- EG-CD; β-cyclodextrin complex with E- E-CD; β-cyclodextrin complex with ECEO- ECEO-CD.

The MIC values ranged from 0.039 mg/mL to 10.000 mg/mL. The lowest MIC values, indicating the highest antimicrobial efficacy, were observed for ECEO and its *β*-cyclodextrin inclusion complex, both exhibiting a MIC of 0.039 mg/mL against the *Escherichia coli* strain. These findings suggest superior antimicrobial activity compared to gentamicin, for which a MIC value of 0.049 mg/mL was recorded. Among the bacterial strains tested, *Pseudomonas aeruginosa* exhibited the highest sensitivity to ECEO, eugenol, and eucalyptol, as well as to their respective inclusion complexes.

The MBEC values ranged from 0.010 mg/mL to 10 mg/mL, with the lowest concentration recorded for the eugenol–β-cyclodextrin complex against the Pseudomonas aeruginosa strain, the value also lower than that obtained for gentamicin. This value was even lower than that obtained for gentamicin. Overall, the encapsulated compounds exhibited superior biofilm eradication efficacy compared to their free counterparts. As expected, eucalyptol and its complex were the least effective samples in eliminating bacterial biofilms, with MBEC values reaching up to 10 mg/mL.

Conclusions

The encapsulation of ECEO in β-cyclodextrin (β-CD) inclusion complexes has demonstrated significant potential for stabilizing and controlling the release of its volatile bioactive components, primarily eugenol. GC-MS and HS-GC-MS analyses con-firmed that eugenol is the major constituent of ECEO, and its encapsulation was most efficiently achieved using the kneading method. Other compounds, such as eugenyl acetate, eucalyptol, and p-cymene, were also encapsulated—likely due to their favorable polarity and size compatibility with the β-CD cavity.

Overall, the study underscores the potential of β-CD inclusion complexes as a promising strategy to enhance the stability and antimicrobial activity of volatile oils, such as ECEO. These complexes have the potential to modulate the release of natural products, thereby improving their efficacy and stability in pharmaceutical, food, and cosmetic applications. Future studies could further investigate the antimicrobial properties of these complexes, particularly their ability to inhibit the formation of biofilms by multi-resistant strains.

CHAPTER 6. New Carbopol Gels with Cyclodextrin Complex and Volatile Oils

This study focused on the development and comprehensive evaluation of hydrogels formulated with a cyclodextrin complex containing clove essential oil (CO), as well as hydrogels incorporating free volatile oils such as tea tree and rosemary, known for their antimicrobial properties. The research aimed to assess the pharmaco-technical properties of these hydrogels, including their stability, texture, ease of application, and rheological behavior, which is crucial for understanding the flow and deformation characteristics of the gels. By combining these natural antimicrobial agents with cyclodextrin, the study sought to enhance the stability and effectiveness of the volatile oils, leading to innovative topical formulations with improved therapeutic potential. The composition of tea tree and rosemary essential oils was determined by GC-MS. For the obtained gels rheological behavior, SEM analysis, antimicrobial activity and cytotoxicity were evaluated.

Materials and Methods

For the formulation of gels with antimicrobial action, two levels of variation of the composition were chosen: the concentration of hydrophilic polymer (Carbopol 940, Sigma-Aldrich, Darmstadt, Germany) in the gel base and the concentration of volatile oils in the final formula (Table 4).

Table 4. Gels composition.

Components	Formula A	Formula B	Formula C	Formula D	Formula E	Formula F
Carbopol 940	0.8 g	1 g	1.2 g	1 g	1 g	1 g
Glycerin	5 g	5 g	5 g	5 g	5 g	5 g
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Cyclodextrin complex with clove essential oil	1 g	1 g	1 g	1 g	1 g	1 g
Usnic acid	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
Tea tree essential oil	1.5 g	1.5 g	1.5 g	1 g	2 g	2.5 g
Rosemary essential oil	1.5 g	1.5 g	1.5 g	1 g	2 g	2.5 g
Purified water	until 100g	until 100g	until 100g	until 100g	until 100g	until 100g

Results and discussions

GC-MS analysis

GC-MS analysis showed that the most abundant constituent of the Melaleuca alternifolia volatile oil is terpinene-4-ol (36.32%), followed by γ-terpinene (18.85%) and α-terpinene (10.85%). A study from 2019 [81]confirmed that these three components are the major constituents of the tea tree essential oil (TTO), taking into account small variations of percentages.

For Rosmarinus officinalis, the most abundant component of the volatile oil is eucalyptol (49.70%), followed by camphor (14.12%), α-pinene (11.83%), and β-caryophyllene (5.80%). The literature data confirms the results obtained. Studies [82,83] conducted on volatile oil hydrodistilled from different parts of Rosmarinus officinalis L. also showed that the major component of the oil is eucalyptol, followed by α-pinene and camphor. Furthermore, significant amounts of borneol, camphene, and verbenone were found.

Gels characteristics

The characteristics of the gel samples are presented in Table 5.

Table 5. Gels composition.

Characteristic	Formula A	Formula B	Formula C	Formula D	Formula E	Formula F
Initial macroscopic characteristics	appearance: homogenous; color: yellowish; smell: specific	appearance: homogenous; color: yellowish; smell: specific	appearance: homogenous; color: yellowish; smell: specific	appearance: homogenous; color: yellowish; smell: specific	appearance: homogenous; color: yellowish; smell: specific	appearance: homogenous; color: yellowish; smell: specific
Macroscopic characteristics after 60 days	appearance: homogenous; color: yellowish smell: specific	appearance: homogenous; color: yellowish; smell: specific	appearance: homogenous; color: yellowish; smell: specific	appearance: homogenous; color: yellowish smell: specific	appearance: homogenous; color: yellowish; smell: specific	appearance: homogenous; color: yellowish; smell: specific
Initial pH	6.50	6.00	6.70	6.70	6.60	6.40

pH after 60 days	6.40	6.60	6.60	6.70	6.50	6.30

Rheological data

For each carbopol gel, an upward rheogram was obtained by plotting the shear stress versus shear rate (Figure 7).

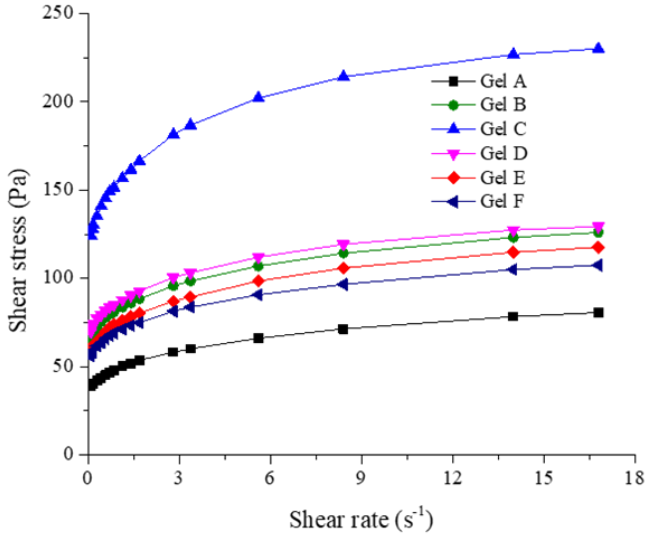


Figure 7. The upward rheograms for carbopol gels were analyzed at 33°C.

As can be seen from Figure 2, all formulations presented similar shapes of flow profiles with a shear stress increase for the shear rate increase, indicating a non-Newtonian behavior.

Scanning electron microscopy

The SEM images detailed these gels' microarchitecture, highlighting the gel matrix's uniformity and homogeneity. This uniform distribution of encapsulated compounds is essential for maintaining the stability of the active ingredients, as it prevents their premature release and degradation. Additionally, the fine, interconnected network observed in the gel structure contributes to its desirable texture, ensuring a smooth and consistent application on the skin. This structural integrity also enhances the gel's ability to retain its shape and resist deformation under stress, which is critical for maintaining its rheological properties over time.

Antimicrobial Activity

The data obtained demonstrated that both gel formulations exhibited antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and yeasts from *Candida*

spp., cultured from standardized ATCC strains or isolated from biological samples (Table 7). Although the antimicrobial activities of the two formulations (B and E) were generally comparable, specific differences were evident. The MICs ranged from 31.250 to 125.000 mg/mL for bacterial strains and from 3.907 to 62.500 mg/mL for yeasts. Both formulations showed similar activity, but formulation E exhibited superior efficacy against *Candida tropicalis* 5770, with a MIC as low as 3.907 mg/mL, compared to formulation B, which had a MIC of 15.625 mg/mL. This enhanced activity in formulation E is likely due to its higher content of TTO and RO, known for their potent antimicrobial properties (Figures 5 and 6). Despite the differences, the similar antimicrobial activity observed against the other microbial strains may be attributed to the identical concentration of Carbopol 940 in both formulations, as well as the similar inclusion of the cyclodextrin complex. These factors could result in comparable release kinetics of the bioactive compounds, thus leading to similar levels of antimicrobial effectiveness [84]. These results encouraged us to continue testing the gels using a larger number of bacterial strains isolated from the clinic.

However, by evaluating microbial adherence to an inert substrate, neither formulation significantly affected the Staphylococcus aureus 14 clinical strain. Additionally, higher concentrations of both products were required to inhibit microbial adherence in the cases of Enterococcus faecalis ATCC 29212 and Pseudomonas aeruginosa ATCC 27853, indicating that while the gels are effective in inhibiting microbial growth, their impact on microbial adherence may be limited and strain-specific.

Cytotoxic activity

The impact of pharmaceutical formulations B, E, and the gel base (GB) on cell viability and proliferation of the Human Colorectal Adenocarcinoma Cell Line (ATCC HTB-38) was assessed using the MTT assay (Figure 8).

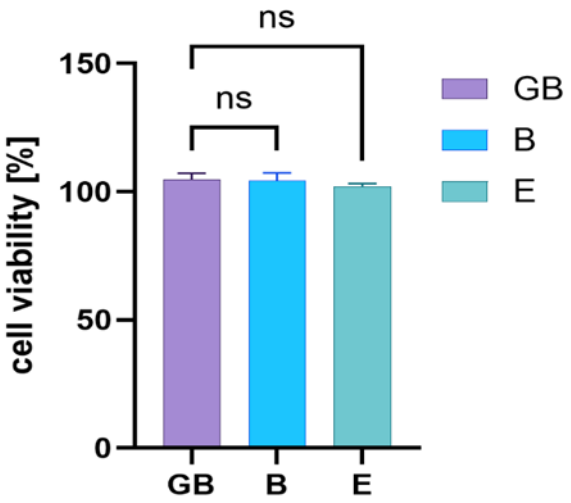


Figure 8. The gels' effect on the viability of the antitumoral cells. Legend: GB = gel base; B = formula B; E = formula E. The gels' influence was statistically analyzed using two-way ANOVA

followed by Dunnett's multiple comparisons tests, which did not reveal statistically significant correlations.

The essential oil components added to GB did not modify HTB-38 tumoral cell proliferation in the 1% concentration gels tested. On the other part, values close to 100% indicate that most cells survived the treatment with the respective gels, and their cytotoxicity is relatively low at the 1% concentration, where no statistically significant changes are observed.

Other types of complex BCD-based also proved to be non-toxic [85]. Toxicity studies on a curcumin-loaded hydrogel with formulation materials based on 2-hydroxypropyl-β-CD, sodium alginate, and chitosan designed for wound dressing materials with antimicrobial activity, tested on NCTC clone 929 cells and normal human dermal fibroblasts, showed the hydrogel was non-toxic.

A doxorubicin-loaded hydrogel containing β-CD and agarose did not exhibit toxicity toward HEK-293 (Human Embryonic Kidney) and HeLa cells [86].

Otherwise, the majority of the inclusion complexes of LCEO (*Litsea cubeba* essential oil) into β-CD (β-cyclodextrin) exhibited antitumor activity against the tumor (HT-29 and HeLa) and non-tumor (Vero) cell lines by MTT assay [87].

Conclusions

The composition of the designed gels influences the flow parameters, which is more evident for the carbopol concentration variation. All hydrogels exhibited non-Newtonian pseudoplastic behavior with yield stress. The rheological evaluation is paramount for a complex physico-chemical evaluation of semisolid dosage forms, providing valuable information concerning their fabrication technology, quality and stability control during preservation, and therapeutical activity. This study developed and evaluated hydrogels containing a cyclodextrin complex with CO alongside TTO and RO, focusing on their pharmaco-technical and rheological properties. The results demonstrate that the concentration of Carbopol 940 is the primary factor influencing the gels' rheological behavior, with a higher concentration leading to increased yield stress, consistency index, and viscosity. Formula B, containing 1% Carbopol, 1.5% TTO, and 1.5% RO, exhibited optimal stability and rheological properties, making it suitable for practical application. In contrast, Formula E, with the same Carbopol concentration but higher amounts of TTO (2%) and RO (2%), displayed superior antimicrobial efficacy, particularly against *Candida tropicalis*. This enhanced activity is attributed to the increased levels of free volatile oils. The study underscores the importance of balancing the polymer and volatile oil concentrations to optimize the hydrogels' physical properties and antimicrobial effectiveness, highlighting their potential for medical and cosmetic applications. Based on this study, the proof of concept was laid down and further studies will be done for a complete characterization considering the desired pharmaceutical and therapeutic applications.

The current focus on topical applications could be expanded to explore other therapeutic areas, such as mucosal drug delivery, ophthalmic formulations, or vaginal/rectal delivery. Hydrogels are ideal candidates for these applications due to their ability to adhere to mucosal surfaces and provide localized drug delivery with minimal systemic absorption. Research into biodegradable hydrogels for tissue engineering or regenerative medicine could also be an important future direction, exploring how these formulations can serve as scaffolds for cell growth or as delivery vehicles for growth factors and stem cells in tissue repair.

CHAPTER 7. Gel Formulations with Cyclodextrin Complex and Volatile Oils

This study aimed to develop and characterise novel hydrogels based on natural bioactive compounds for topical antimicrobial applications. Four gel systems were formulated using different polymers, namely polyacrylic acid (Carbopol 940, CBP-G), chitosan with high and medium molecular weights (CTH-G and CTM-G), and sodium alginate (ALG-G), incorporating tinctures of *Verbena officinalis* and *Aloysia triphylla*, *Laurus nobilis* essential oil, and a *β*-cyclodextrin–clove essential oil complex.

Materials and methods

The objective of this study was to develop and pharmaco-technically characterise, especially in terms of rheology, volatile oil-based hydrogels. For the formulation of gels with antimicrobial action, the variation in the polymer was considered to select the optimal gel formulation for topical use.

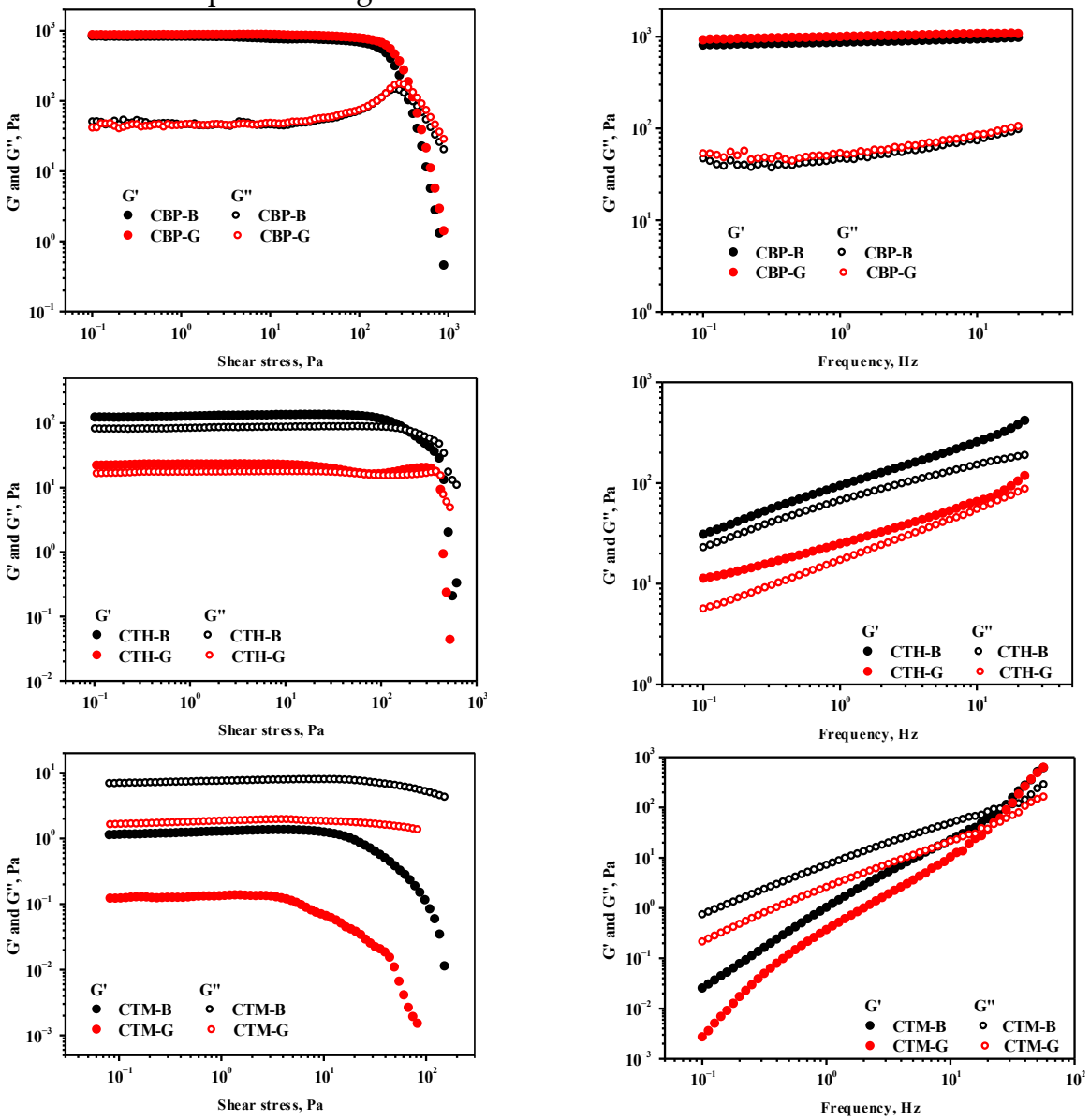
The gels' compositions are presented in Table 6. The gel bases are considered as the formulation without bioactive compounds (VAT, ATT, *Laurus nobilis* essential oil, and *β*-CD-ECEO complex).

Table 6. Gel compositions.

Formulation	Polymer	Active Ingredients			Plasticizer	Gelling Agent	Solvent
		Tinctures	Essential Oil	Other			
	2%	2%	1%	0.50%	5%	1%	Until 100%
CBP-G	Carbopol 940	*Verbena officinalis* (VAT) *Aloysia triphylla* (ATT)	*Laurus nobilis*	*β*-CD-ECEO complex	Glycerin	NA	Purified water
CTH-G	Chitosan HMW						
CTM-G	Chitosan MMW						
ALG-G	Sodium alginate					$CaCl_2$	

Rheological Data

The rheological behaviour of the new gels and their bases was analysed, and the obtained data are depicted in Figure 9.



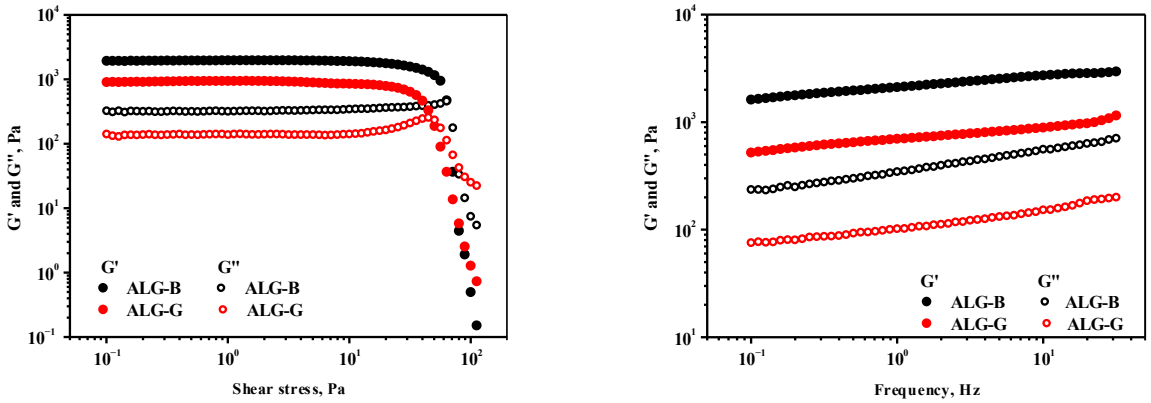


Figure 9. Variation in rheological moduli with shear stress ((A). CBP; (C). CTH; (E). CTM; (G). ALG) and frequency ((B). CBP; (D). CTH; (F). CTM; (H). ALG). *Legend:* CTH-G—chitosan high-molecular-weight gel; CTM-G—medium-molecular-weight gel; ALG-G—sodium alginate gel; CBP-G—Carbopol 940 gel; CTH-B—chitosan high-molecular-weight gel base; CTM-B—medium-molecular-weight gel base; ALG-B—sodium alginate gel base; CBP-B—Carbopol 940 gel base.

The CBP-B and CBP-G samples show a similar behaviour, where the elastic modulus (G′) and the viscous modulus (G″) have a well-defined viscoelastic region, after which we observe a decrease in the moduli. This decrease represents the irreversible deformation of the CBP-B and CBP-G structures and the appearance of the yield stress point. The improved moduli and critical point in the CBP-G variant are consistent with observations from Carbopol–polymer composite systems, where additives such as glycerin and hyaluronic acid enhance structural integrity [88,89]. Rheological analysis of CTH-B and CTH-G systems showed that although G′ is slightly larger than G″, it is still very close in value to G″, indicating that both materials are near a transition between solid and liquid behaviour. The observed trend can be attributed to weak network interactions and structural rearrangements that reduce the difference between the moduli. In addition, the CTH-B sample consistently displays higher G′ and G″ values than CTH-G over the entire range of the shear stress and frequencies studied, indicating a stronger structural network. A dominant fluid character with detectable LVER but no yield stress points was observed for the CTM-B and CTM-G systems in the sweep amplitude tests (Figure 1E). Moreover, both CTM-based samples showed a predominantly viscous character (G″ > G′) at low frequencies and a more elastic behaviour (G′ > G″) at high frequencies. This phenomenon suggests that CTM-B and CTM-G flow like viscoelastic liquids, but when applying high frequencies, they bounce like viscoelastic solids. The ALG-B and ALG-G samples exhibit a short LVER where G′ is larger than G″, indicating solid-like behaviour. By increasing shear stress, the two rheological moduli tend to decrease, marking the yield stress point. The found yielding points were about 65 Pa for ALG-B and 45 Pa for ALG-G.

Thermogravimetric Differential Thermal Analysis (TG-DTA)

The TG-DTA curves obtained for both the hydrogel formulations, and their corresponding bases (Figure 10) showed a consistent two-step thermal degradation pattern, characteristic of multicomponent hydrogel systems. In all cases, the exothermic events observed in the DTA profiles corresponded with the mass loss steps indicated by the TG data.

The first degradation stage, between approximately 100 and 250 °C, is attributed primarily to the evaporation of free and bound water, as well as the evaporation of low-molecular-weight components such as glycerin, ethanol residues from the tinctures, and essential oil constituents. The second degradation stage, extending from ~230 °C to 500 °C, corresponds to the thermal decomposition of the primary polymeric matrix (Carbopol, chitosan, or alginate), as well as the breakdown of *β*-cyclodextrin complexes and thermolabile organic compounds.

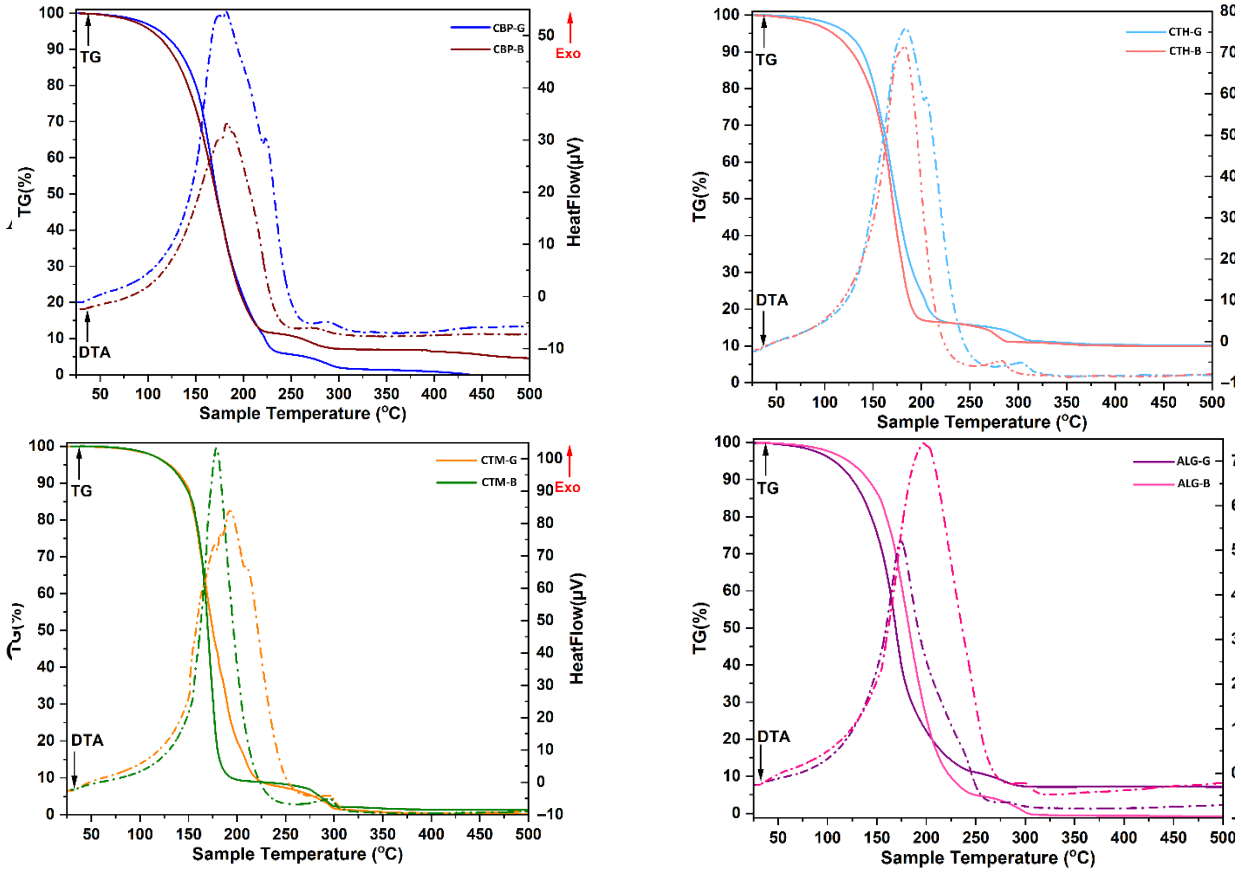


Figure 10. The TG-DTA curves of the gels. (A) CBP-G—Carbopol 940 gel and CBP-B—Carbopol 940 gel base; (B) CTH-G—chitosan high-molecular-weight gel and CTH-B—chitosan high-molecular-weight gel base; (C) CTM-G—medium-molecular-weight gel and CTM-B—chitosan medium-molecular-weight gel base; (D) ALG-G—sodium alginate gel and ALG-B—sodium alginate gel base.

Scanning Electron Microscopy (SEM) Analysis

These SEM images highlight the microarchitecture of the gels, emphasising the uniformity and homogeneity of the gel matrix. The uniform distribution of the active compounds is essential for maintaining the stability of the active ingredients, as it prevents their premature release and degradation, while the hydrogel network contributes to obtaining a texture that allows easy and consistent application on the skin. In addition to these, the gel must keep both its shape and resistance to deformation, key aspects when it comes to maintaining its rheological properties over time and the effective delivery of active ingredients, while the appropriate texture promotes ease of application.

FTIR Analysis Results

The FTIR analysis indicates the successful incorporation of plant-derived bioactive compounds, essential oils, and the inclusion complex into the gel matrices. Also, the FTIR analysis demonstrates that interactions between the polymer and bioactive compounds are dependent on the nature of the polymeric matrix. The Carbopol and HMW chitosan matrices exhibited more pronounced physical interactions with the incorporated compounds, while the MMW chitosan and alginate matrices demonstrated limited potential for interaction. All around, predominantly in all gel formulations, was the physical encapsulation of the active compounds.

Analysis of Antimicrobial Activity

The minimum inhibitory concentrations (MICs) determined by quantitative testing ranged from 0.58 to 75 mg/mL. According to the qualitative screening results, the chitosan-based gel showed the most potent antimicrobial activity against all tested strains, especially against *S. aureus* and *E. coli* (MIC = 2.34 mg/mL). For the Carbopol gel, the most effective inhibition was observed against *S. epidermidis* (2.73 mg/mL), like the alginate gel, but at a higher concentration (9.38 mg/mL). *Laurus nobilis* essential oil showed notable antimicrobial activity on *C. albicans* (1.25 mg/mL), with slightly weaker effects on the other strains (2.50 mg/mL). No antimicrobial activity was recorded for the Carbopol and alginate bases.

Following the assessment of microbial adhesion on an inert substrate, MBEC values ranged from 0.58 to 75.0 mg/mL. As expected, the best results were obtained for the chitosan-based gel, with the lowest MBEC values recorded for *S. aureus* and *E. coli* strains, consistent with previous MIC data. The alginate gel showed the lowest antibiofilm efficacy, with MBEC values ranging from 37.5 to 75.0 mg/mL.

The demonstrated antimicrobial activity of the chitosan-based gel is due not only to the active components in its composition, but also to the presence of chitosan itself. Chitosan adds significant intrinsic antimicrobial activity to the gel due to its cationic properties. Protonation of amine groups in an acidic environment confers its polycationic character, facilitating electrostatic interaction with the cell wall, resulting in disruption of integrity and bacterial lysis.

Cell Viability Assessment

The MTT assay revealed differential effects of the pharmaceutical formulations on HaCaT cell viability after 24 h of treatment. Formulations with high-molecular-weight chitosan (CTH-G and CTH-B) and medium-molecular-weight chitosan gels (CTM-G and CTM-B) induced a significant increase in cell viability compared to the control, suggesting a potential proliferative effect, which may be attributed to the presence of chitosan. Conversely, formulations containing alginate exhibited a dose-dependent reduction in viability, with a statistically significant decrease (30% compared to untreated cells) observed at the 5 mg/mL and 10 mg/mL concentrations. These results indicate that higher concentrations of alginate-based gels may exert cytotoxic effects on keratinocytes.

NO production, measured as an indicator of inflammatory response, remained unchanged for many formulations and concentrations. However, a significant increase in nitric oxide levels was detected only at the highest concentration (10 mg/mL) of formulation CG. This may suggest a mild pro-inflammatory effect associated with this specific condition.

Conclusions

This study presents a comparative evaluation of topical antimicrobial gels incorporating plant-derived bioactive products—*Laurus nobilis* essential oil, tinctures of *Verbena officinalis* and *Aloysia triphylla*, and a *β*-cyclodextrin–clove essential oil inclusion complex—formulated within four distinct polymer matrices: Carbopol 940, sodium alginate, and chitosan of high and medium molecular weight.

This comparative approach enabled systematic insight into how polymer architecture influences rheology behaviour, antimicrobial efficacy, and cytocompatibility. Among them, HMW chitosan-based gels showed the most promising profile, combining potent antimicrobial activity (low MIC and MBEC values against *S. aureus* and *E. coli*) with enhanced HaCaT cell viability and viscoelastic properties suitable for topical application. CBP-based gels exhibited excellent structural integrity, with high yield points (~400 Pa), thermal stability, and minimal cytotoxicity, making them suitable carriers for sensitive bioactive compounds when low cellular interaction is desired. In contrast, alginate-based gels displayed moderate mechanical stability and dose-dependent cytotoxicity at higher concentrations, suggesting some limitations for direct skin contact in undiluted forms.

The incorporation of plant extracts, essential oils, and *β*-cyclodextrin–clove essential oil complexes in gel bases was successfully achieved, as confirmed by FTIR and SEM analysis. The structural analysis supported the sustained release potential and uniform distribution of the compounds within the gel matrices. Thermogravimetric evaluation confirmed the thermal robustness of all systems.

Overall, the findings support the suitability of chitosan- and Carbopol-based gels for formulating stable, biocompatible, and effective topical delivery systems for natural antimicrobials. These systems offer a promising foundation for further development of

dermatological formulations aimed at managing cutaneous infections and promoting wound healing.

CHAPTER 8. General Conclusions and Originality

General Conclusions

The thesis addressed a current clinical and technological problem: the need for "microbiome-friendly" topical alternatives for the control and treatment of skin infections, in the context of increasing antibiotic resistance and the limitations of conventional forms. The proposed strategy: the integration of natural compounds (especially essential oils and plant tinctures) into polymer-based delivery systems and/or β-cyclodextrin inclusion complexes, proved to be feasible, reproducible, and compatible with the requirements of skin application.

The objectives of the thesis were structured in such a way as to address the limitations identified in previous studies and, at the same time, to introduce original elements not previously reported in the specialized literature.

The PhD thesis is organized into major sections, divided into chapters. The first section is a review of recent scientific literature that presents the main topic: the importance of the local microbiota in skin health. The thesis also reviews the main therapeutic approaches to skin infections and the advantages of incorporating plant-derived antimicrobial compounds into polymeric delivery systems. The second part presents the original experimental work, states the objectives and demonstrates their fulfilment through results published in scientific papers. A third section is devoted to General Conclusions, while the fourth section is listing the References.

Chapter 1 establishes the importance of skin health by detailing skin structure, functions, and host–microbe interactions, and explains the microbiota's role in local immune balance, and overviews the types of infections that can occur and their complications. Moreover, Chapter 1 reviews topical drugs, antibiotics, antifungals, emerging therapies and highlights treatment challenges, including nosocomial pathogens.

Chapter 2 summarizes natural compounds and plant extracts with antimicrobial activity: describes mechanisms of action, examines their capacity to potentiate antibiotics against resistant strains, and reviews pharmaceutical forms/supplements and their global market presence.

Chapter 3 underlined the systems used in pharmaceutical formulations for the active substances distribution: presents the role of cyclodextrins in the formation of inclusion complexes to increase solubility, stability, and control the release of compounds

(including volatiles). Chapter 3 also details polymer-based gelled matrices based on sodium alginate, chitosan and polyacrylic acid (Carbopol) as loading and controlled release systems and summarizes approaches in gels characterization.

The second part presented the main stated objective of the thesis, which is divided into four parts (Chapters 4-7): (*i*) to describe general and specific objectives of the research; (*ii*) to obtain β-cyclodextrin- clove essential oil complexes, characterize them and evaluate their biological properties; (*iii*) to develop, optimize, and evaluate the biological activity of new gel formulations with polyacrylic acid (a synthetic polymer), β-cyclodextrin- clove essential oil complex and essential oils with antimicrobial properties (tea tree and rosemary essential oils); (*iiii*) to develop new antimicrobial gels based on both, natural and synthetic polymers (sodium alginate, chitosan and polyacrylic acid), using β-cyclodextrin- clove essential oil complex and plant extracts (*Verbena officinalis* tincture, *Aloysia triphylla* tinctures and bay essential oil).

Chapter 4 summarizes the main objectives of the thesis, providing a brief description of the principal research directions that were addressed.

Chapter 5 presents the comparative evaluation of β-cyclodextrin complexes with eugenol, eucalyptol, and clove essential oil prepared by four methods: co-precipitation, kneading, lyophilization, and a combined method of co-precipitation–lyophilization. All complexes were physico-chemically and morphologically characterized. The encapsulation of the bioactives in the cyclodextrin cavity showed an enhancement of the antimicrobial activity across several microbial strains, demonstrating their potential for use in pharmaceutical formulations.

Chapter 6 reports the optimization of polyacrylic-acid (Carbopol 940) gels incorporating a β-cyclodextrin–clove essential oil inclusion complex and tea tree essential oil by varying the concentrations of Carbopol and the bioactive components. The gels were physicochemically and morphologically characterized. The antimicrobial activity and cytotoxicity of the optimized formulation were evaluated. The obtained results support its use as a topical alternative for the treatment of various infections.

Chapter 7 reports the design and development of novel gel formulations using both natural and synthetic polymers, incorporating the β-cyclodextrin–clove essential oil inclusion complex and plant extracts (*Verbena officinalis* tincture, *Aloysia triphylla* tincture, and bay essential oil). The gels were physicochemically and morphologically characterized. The biological activity (antimicrobial activity and cytotoxicity) demonstrated their potential as candidates for topical treatment of skin infections.

Original Contributions and Perspectives

The novelty of the first study involved the assessment of a combined encapsulation method of eugenol, eucalyptol, and clove essential oil in β-cyclodextrin: co-precipitation-

lyophilization. Moreover, the growth inhibitory effects of the obtained complexes against *Enterobacter cloacae* and *Pseudomonas aeruginosa* microbial strains, as well as their ability to attach to inert substrate, were first reported.

Perspectives: Further investigations regarding the antimicrobial properties of the complexes, focusing on their ability to suppress biofilm formation of multi-resistant bacterial strains, release and stability studies for further pharmaceutical, food, and cosmetic applications.

The novelty of the second study was the development and optimization of new polyacrylic acid hydrogels containing a β-cyclodextrin complex with clove essential oil and other free volatile oils with antimicrobial properties (tea tree and rosemary essential oils). Furthermore, their antimicrobial activity against bacterial and yeast strains collected from biological samples was evaluated in terms of both growth inhibition and biofilm formation inhibition. Notably, the gels exhibited growth inhibition against *Escherichia coli* ESBL (extended-spectrum β-lactamase), a strain capable of inactivating common antibiotics such as cephalosporins and penicillins. The β-cyclodextrin complex with clove essential oil alone was also tested and showed antibacterial activity against all bacterial strains tested.

Perspective: Performing release and stability studies as well as *in vivo* investigations of the antimicrobial properties of the optimal gel formulations.

The novelty of the third study was the development and comparative analysis of gels containing plant-derived bioactive products (bay essential oil, tinctures of *Verbena officinalis* and *Aloysia triphylla*), and a β-cyclodextrin–clove essential oil inclusion complex, formulated within four polymer matrices: Carbopol 940, chitosan of high and medium molecular weight, and sodium alginate. Their antimicrobial activity against several bacterial strains (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231) and low cytotoxicity on HaCaT human keratinocyte cells demonstrated their potential in the treatment of skin infections.

Perspective: Future studies will be directed towards the identification of innovative advanced antimicrobial strategies to address stability issues and expand the applicability of preservative-free drugs in a wide range of pharmaceutical forms.

List of publications

- **Stancu, A.I.**; Mititelu, M.; Ficai, A.; Ditu, L.-M.; Buleandră, M.; Badea, I.A.; Pincu, E.; Stoian, M.C.; Brîncoveanu, O.; Boldeiu, A.; et al. Comparative Evaluation of *β*-Cyclodextrin Inclusion Complexes with Eugenol, Eucalyptol, and Clove Essential Oil: Characterisation and Antimicrobial Activity Assessment for Pharmaceutical Applications. *Pharmaceutics* 2025, *17*. https://doi.org/10.3390/pharmaceutics17070852 (Q1, IF=5.5).
- **Stancu, A.I.**; Oprea, E.; Ditu, L.M.; Ficai, A.; Ilie, C.-I.; Badea, I.A.; Buleandra, M.; Brîncoveanu, O.; Ghica, M.V.; Avram, I.; et al. Development, Optimization, and Evaluation of New Gel Formulations with Cyclodextrin Complexes and Volatile Oils with Antimicrobial Activity. *Gels* **2024**, *10*, 645. https://doi.org/10.3390/gels10100645 (Q1, IF=5.3).
- **Stancu, A.I.**; Ditu, L.M.; Oprea, E.; Ficai, A.; Badea, I.A.; Buleandră, M.; Brîncoveanu, O.; Mirea, A.G.; Voicu, S.N.; Musuc, A.M.; et al. New Antimicrobial Gels Based on Clove Essential Oil–Cyclodextrin Complex and Plant Extracts for Topical Use. *Gels* **2025**, *11*, 653. https://doi.org/10.3390/gels11080653 (Q1, IF=5.3).
- **Stancu, A.-I**.; Geană, E.I.; Ditu, L.-M.; Ficai, A.; Nagoda, E.; Oprea, E. Chemical Composition and Antimicrobial Activity of *Verbena officinalis* and *Aloysia citrodora* Extracts Obtained by Traditional and Laboratory Methods. *U.P.B. Sci. Bull. Ser. B* **2024**, *86*, 111–122.ISSN 1454 – 2331.

Conference attendance during PhD stage

- **Stancu Alina Ionela**, Ficai Anton, Dițu Lia-Mara, Mititelu Magdalena, Oprea Eliza, APPLICATIONS OF CHEMISTRY IN NANOSCIENCES AND BIONANOMATERIALS ENGINEERING, July 16-17, 2020, Bucharest – oral presentation

Patent application

I. Application title: Vaginal suppositories with a β-cyclodextrin inclusion complex of clove essential oil

Application number: RO137321-A0

Filing date: 25.10.2022

Publication date: 30.03.2023 CC BY 4.0 license

Applicant: "CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
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https://0510qpd9a-y-https-www-webofscience-com.z.e-nformation.ro/wos/diidw/full-record/DIIDW:2023383570

II. Application title: Mouthwash containing a β-cyclodextrin inclusion complex with clove essential oil
Application number: RO137277-A0
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Publication date: 28.02.2023
Applicant: "CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
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https://0510qpd9a-y-https-www-webofscience-com.z.e-nformation.ro/wos/diidw/full-record/DIIDW:202330614E

III. Application title: Composition for a multivalent toothpaste containing a β-cyclodextrin inclusion complex with clove essential oil
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Filing date: 25.10.2022
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Applicant: "CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
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https://0510qpd9a-y-https-www-webofscience-com.z.e-nformation.ro/wos/diidw/full-record/DIIDW:202330612X

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