**CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE**

**Faculty of Agrobiology Food and Natural Resources**

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Antimicrobial and antifungal activity of encapsulated Cannabis extracts against microbes causing skin diseases

**MASTER'S THESIS**

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**Declaration**

I hereby declare that I have done this thesis entitled "**Antimicrobial and antifungal activity of encapsulated Cannabis extracts against microbes causing skin diseases**" independently. All texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references according to the citation rules of the FAFNR.

In Prague April 2022

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**Bashir Mohamed Maelin**

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## Abstract

Skin is the first defenses barrier against physical and chemical agents as well as microbial pathogens. The most common bacterial skin pathogens are *Staphylococcus aureus* and group A *β-hemolytic streptococci. Impetigo, folliculitis*, and boils are common types of diseases caused by bacteria. Several genera of fungi (*Dermatophytes)* are responsible for diseases of the skin. Universally recognized causing agents of these diseases are *Trichophyton, Microsporum* and *Epidermophyton.* Despite the undeniable global benefits of antibiotics, the overuse or misuse of antibiotics has been linked to antimicrobial resistance (AMR) which is one of the most serious global public health threats in this century rendering treatment and effectivity of the antibiotics. The development of this antimicrobial resistance has revealed the need for new effective and safe antimicrobial compounds for skin diseases. The study evaluates *in vitro* antimicrobial activities of cannabis sativa extracts and chitosan encapsulated cannabis extracts. Both non-capsulated and encapsulated cannabis extract of Chocolope strain were active against the microbial strains tested both bacteria and fungus, the most sensitive bacteria to all tested antimicrobial agents was *S. epidermidis 4069.*The results of the experiment verified that cannabis extracts and chitosan encapsulated cannabis extract have antimicrobial activity against microorganisms causing skin diseases. The minimal inhibitory concentration (MIC) of non-encapsulated cannabis extracts ranging 8- 16 µg /ml and minimum bactericidal concentration 16-32 µg /ml and chitosan encapsulated cannabis has MIC ranging 0.5mg/ml – 1mg/ml. In comparison with chloramphenicol the minimal inhibitory concentration (MIC) of all tested strains ranged 2-8 µg /ml and minimal bactericidal concentration (MBC) ranged 4-16 µg /ml. Against *T. rubrum 4934* the minimum inhibitory concentration (MIC) of cannabis extracts was 128 µg /ml the minimum fungicidal concentration was 256 µg /ml. In conclusion, cannabis extracts could be suitable for the treatment of skin diseases caused by the microbials tested

**Key words**: encapsulation, cannabis extract, skin, antimicrobial activity, resistance

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# 1. Introduction

Our skin is home to millions of bacteria, fungi and viruses that compose the skin microbiota. Like those in our gut, skin microorganisms have essential roles in the protection against invading pathogens. In circumstances where the barrier is broken or when the balance between commensals and pathogens is disturbed, skin disease or even systemic disease can result **(Byrd et al., 2018).** Human skin sites can be categorized by their physiological characteristics: - sebaceous gland-rich (SGR), apocrine gland-rich (AGR), and gland-poor (GP) skin regions, creating a diverse environment for microbiota. Thus, the antimicrobial barrier of the skin cannot be considered uniform. The regulation and maintenance of the antimicrobial and permeability skin barriers are closely connected **(Kapitány et al., 2021).** In assigning health priorities, skin diseases are sometimes thought of, in planning terms, as small-time players in the global burden of diseases compared with diseases that cause significant mortality, such as HIV/AIDS, community-acquired pneumonias, and tuberculosis. However, based on the Global Burden of Disease Project, skin diseases were the 4th leading cause of non-fatal morbidity worldwide in 2010 and 2013 **(Hay et al., 2014***).* They can be caused by different kinds of germs and are dominated by bacterial and superficial fungal infections. The most common bacteria responsible for skin diseases are Staphylococcus aureus and group A *β-haemolytic streptococci.* Fungi (dermatophytes) are *Epidermophyton* (E) *Microsporum*(M) and *Trichophyton* (T).The prevalence of skin fungal infections is expected to reach 20 -25% of the world's population, and its incidence continues to increase *(***Sang Ha Kim et al., 2015***).*The treatment depends on the type of infection and its seriousness.  Some infections will go away on their own. Bacterial infections are often treated with topical antibiotics applied directly to the skin or with oral antibiotics *(***Klahn, 2020***).*The mode of treatment for dermatophytes depends on the extent and location of the infection, topical therapy is used for most dermatophyte infections. Cure rates are higher and treatment courses are shorter with topical fungicidal allylamines than with fungistatic azoles *(**Dowd, 2007).** Although the discovery of antibiotics is undoubtedly one of the most important scientific achievements in modern medicine and has saved millions of lives since their discovery. Antibiotic resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. To overcome these problems with current treatments for skin infections, alternative strategies are being researched and developed with the aim to provide better treatment options for skin infections. It has been demonstrated that several *Cannabis sativa* preparations have a long history of medical applications and  showed

potent antimicrobial activity against pathogenic Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *fungus* (**Schofs et al., 2021**). Despite the potential pharmacological use of cannabis preparations, they re characterized with hydrophobic in nature, poor solubility and low bioavailability that limit their suitability as pharmacological agents. To address these limitations the current study of researches sought to design nanomaterials with different compositions and biological properties have been extensively investigated for drug and gene delivery applications. An effective approach for achieving efficient drug delivery would be to rationally develop nanomaterials based on the understanding of their interactions with the biological environment, target cell-surface receptors. Therefore, one of the most promising candidates for this type of biomedical device is chitosan (CS) -a biopolymer characterized by excellent biocompatibility with skin cells *(***Esposito et al., 2016***).*For that reason, the aim of this diploma thesis is to examine the antimicrobial and antifungal activity of encapsulated cannabis extracts of selected fungi and bacteria causing skin diseases.

## 2. Scientific hypothesis and objectives of the work

Encapsulated extracts of *Cannabis sativa* are more effective against to human skin pathogens compare to non-encapsulated extracts.

**Aim:** The aim of the work was to evaluate the antimicrobial activity of encapsulated cannabis extracts prepared by various methods against selected fungi and bacteria causing skin diseases and compare *Cannabis sativa* extracts in form of non-encapsulated extracts (e.g., chitosan, silica, lipids).

## 3. Literature Review

### 3.1. Microbial infections of the skin

The skin is the largest organ of the human body, with an average surface area of 30 $m^2$ in adults. It provides a remarkably good barrier against microbial infections. The skin normally provides a barrier to infection, however when it is penetrated by microorganisms, infection can develop **(Skowron et al., 2021).**The most common bacterial infections include impetigo, erysipelas, cellulitis, ecthyma, furuncles, carbuncles, and subcutaneous abscesses and the most fungal skin infections include dermatophyte or ringworm infections, candidosis, and pityriasis versicolor. These infections cause a significant morbidity and have to be diagnosed and treated promptly. Although they may affect in all regions, based on the Global Burden of Disease Project they are likely to be underestimated due to a variety of factories, but socioeconomic factors contribute greatly to the epidemiology of skin disease. This underscores the need for strengthening of a global dermatologic research infrastructure towards finer granularity of dermatologic disease burden in both resource-poor and resource-rich regions *(Seth et al., 2017).*

### 3.2. Common superficial skin infections

### 3.2.1. *The Dermatophytes*

*Dermatophytes* are a group of filamentous fungi and encompass the seven genera of *Trichophyton, Microsporum, Epidermophyton, Nannizzia, Arthroderma, Lophophyton* and *Paraphyton (Taghipour et al., 2021).*

In total there are approximately 40 different species of *dermatophytes* (**Havlickova et al., 2009**).Among the seven genera ,*Trichophyton, Microsporum and Epidermophyton* species are the three most common causative agents in clinical trials *(Liu et al., 2021).*The geographic distribution of dermatophytes varies from country to country and from continent to continent and over time within a region (**Ngwogu et Otokunefor, 2007).** Among the factors that increase predisposition to dermatophytosis are environmental factors, such as humidity and temperature, pathogens, virulence, traits ,and hosts, immunological and health status, genetics, occupation, hygiene, contact with animals, and socioeconomic factors *(Mechanisms et al., 2021).*based on the host factor they are divided in to *zoophilic, anthropophilic, and geophilic* classes. *D*ermatophytes thrive at surface temperatures of 25–28°C and infection of human skin is supported by warm and humid conditions. For these reasons, superficial fungal infections are

relatively common in tropical countries and are exacerbated by the wearing of occlusive clothing (**Havlickova et al., 2009**).They can be spread by direct contact from other people, animals and soil and indirectly from fomites (e.g., upholstery, hairbrushes, hats) (**Dowd, 2007**). Symptoms of dermatophyte infection may range from mild to severe because of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors *(***Ngwogu et Otokunefor,** *2007).*Clinical manifestations include circular, erythematous, and scaly lesions on the skin, while nail infections (*tinea unguium* or *onychomycosis*) lead to discoloration, thickening, desquamation, and separation from the nail bed (**Mechanisms et al., 2021**).*Trichophyton interdigitale* and *Trichophyton rubrum* are anthropophilic species that are most frequently isolated from tinea unguium, tinea pedis, and tinea corporis worldwide (***Kano et al., 2021***). Genetic and biochemical mechanisms of antifungal resistance have been reported in dermatophytes, including point mutations, alteration in drug target sites, and increased efflux-mediated activity to the currently available drugs (**Mechanisms et al., 2021**).

### 3.2.1.1. *The trichophyton spp.*

A genus of trichophyton is morphologically characterized by the development of both smooth -walled macro and microconidia. The macroconidia originate laterally in the hyphae or in short particles of thin or thick walls and are club -shaped or fusiform, with a size that varies from 4–8 to 8–50 μm. The microconidia are abundant, spherical, pyriform, or irregularly shaped, with sizes varying from 2–3 to 2–4 μm. *Trichophyton rubrum* appears to be the most common causative agent of dermatophytosis, followed by *Trichophyton interdigitale* (**Mechanisms et al., 2021***).* Involving any area of the skin, but the most frequently affected sites are the feet (tinea pedis), groin (tinea cruris), scalp (tinea capitis), and nails (*tinea unguium*) (**Si Hyun Kim et al., 2016**). Local socio-economic conditions and cultural practices can also influence the prevalence of a particular infection in a given area. For example, tinea pedis (athlete's foot) is more prevalent in developed countries than in emerging economies and is likely to be caused by the anthropophilic germ *T. rubrum*. In poorer countries, scalp infections (tinea capitis) caused by *T. soudanense* or *M. audouinii* are more prevalent (**Havlickova et al., 2009).**

### 3.2.1.1.1. *Trichophyton interdigitale*

*Trichophyton interdigitale* is a species of Trichophyton mentagrophytes complex, it is one of the three common fungi which cause ringworm in companion animals. It is also the second most commonly isolated fungus causing so-called tinea infections in humans, and the most common or one of the most common fungi that causes zoonotic skin disease *(***Surdu et al., 2014)** . Although identification and delineation of dermatophytes of this species remain difficult, particularly because of phenotypic variation within and between isolates. *T. interdigitale* can show wide variability in its phenotypic features, including the presence or absence of ornamental bodies (spiral hyphae) and the number and size of macroconidia and microconidia **(Dias AL. 2011***).*Conventionally, is identified based on its macro and microscopic features, and sometimes, for its physiological characteristics (hair perforation and urease activity), particularly in the case of atypical isolates *(*Frías-De-león et al., 2020*).*

### 3.2.1.1.2. *Trichophyton concentricum*

*Tinea imbricata* is a recurrent chronic dermatophytosis caused by Trichophyton concentricum. Itis easily transmitted to equipment used together, such as towels, spray, shoes, clothing **(Selviana et al., 2019).**This mycosis is characterised by widespread, annular, concentric, squamous lesions, often accompanied by pruritus **(Veraldi et al., 2015).** Patients may be infected at any age, although infants and young children are most frequently affected. Inherited autosomal-recessive susceptibility may play a part in determining the epidemiology of tinea imbricata. Patients with this disease often have a negative delayed-type hypersensitivity to T. concentricum cytoplasmic antigen and T-lymphocyte hypo reactivity **(Patel et Asia, 2017***).*

### 3.2.1.1.3. *Trichophyton rubrum*

As an obligate human pathogenic fungus *(***Blechert et al., 2020***).* Trichophyton rubrum is the most commonly observed dermatophyte isolated from humans in European countries **(Jousson et al., 2004**). The global predominance of T. rubrum suggests that this species has a significantly higher capacity of transmission than other *anthropophilic dermatophyte (***Jiang et al., 2021**). Also, it's the most common dermatophyte associated with onychomycosis and the most common cause worldwide for *tinea pedis,* nail infection, *tinea cruris* and *tinea corporis (***Havlickova et al., 2009***).*Onychomycosis is difficult to cure, impacting the patient's quality of life by resulting in walking difficulties and poor nail appearance, and can be a source of secondary infection or spread to other family members **(Sugiura et al., 2021***). Trichophyton*

*rubrum* is the most recurrently described in resistance to standard treatments, followed by T. interdigitale (**Mechanisms et al., 2021**).

### 3.2.1.2. Epidermophyton spp.

*Epidermophyton floccosum* is so far, the only representative species of genera *epidermophyton,* is an anthropophilic dermatophyte that causes tinea pedis, tinea unguium, tinea corporis, tinea cruris, and tinea manuum in humans. It possesses uniqueness in ecology traits and rarely causing hair infections (**Liu et al., 2021**). *Epidermophyton floccosum* is widely distributed in the tropics and subtropics (**Kitisin et Luplertlop, 2015**). Since the knowledge of the factors mediating invasion of *E.floccosum* to host tissues is little known, the protein and carbohydrates of host tissue are the main sources of carbon, nitrogen, phosphorus, and sulphur for dermatophytes. Moreover, it has been indicated that the enzymes secreted by dermatophytes can act as antigens and induce various degrees of inflammatory responses (**Khedmati et al., 2020**). Although infections by dermatophytes are usually restricted to the superficial epidermis, these fungi can be invasive and cause highly severe infections in immune-deficient patients, leading to the development of *Dermatophytic granulomas* (**Kitisin et Luplertlop, 2015**).

### 3.2.1.3. Genus Microsporum

The *genus microsporum* characterized by fusiform or spindle shaped microconidia, often with thick, rough, or spiny walls and of the dermatophyte genus Microsporum are among the first fungi reported from humans and has been regarded as principally zoophilic (***Gräser et al., 2000***). However, the natural habitat some of the Microsporum spp. is soil (the geophilic spp.),and some are isolated from both soil and animals (geophilic and zoophilic) (**Ramos et al., 2020**).The species occurring on human have received considerable attention from clinicians and dermatologists who initially used clinical and later cultural and morphological features for species recognition (***Gräser et al., 2000***).Species of *microsporum* (*Microsporum canis, Microsporum audouinii,* and *Microsporum ferrugineum*),cannot be consistently differentiated into distinct subclades through molecular analysis (**Ramos et al., 2020**).Most of the *microsporum spp.* are widely distributed in the world while some have restricted geographic distributions (**Caffara et Scagliarini, 1999**) . *Microsporum audouinii* and *Microsporum canis* have been reported to be the main causative agents in western and Mediterranean Europe (**Ungo-kore et al., 2021**).

### 3.2.1.3.1. *Microsporum canis*

*Microsporum canis* is a worldwide diffused *zoophilic dermatophyte* which causes clinical conditions. Macroconidia of *M. Canis* are typically spindle-shaped with 5-15 cells (figure 1.) and often characterised by multifocal alopecia, scaling, and circular lesions in many animal species, including humans **(Aneke et al., 2018).** *Microsporum canis* belongs to the group of dermatophyte that have ability to invade the stratum corneum of the epidermis and keratinized tissues derived from it *(Pasquetti et al., 2017).* Therefore, the use of phenotypic and ecological data is required in their identification. *Microsporum Canis* produces a yellow-greenish fluorescence in hair, and it is associated with small ectothrix spores. Its colonies are white to buff in colour with a characteristic yellow to orange-brown reverse. The head is the most common site of infection, with areas of *alopecia* around the nose, eyes, and ears, but the infection can become generalized *(Pasquetti et al., 2017).* The transmission of *M.canis* occurs through direct contact with diseased or subclinically infected animals, mainly cats, via arthrospores. Human-to-human infection has been frequently recorded in small, self-limited outbreaks (**Zheng et al., 2020**).In adults, unusual clinical presentations of *M. canis* infection have been described such as severe and inflammatory tinea barbae and very atypical *tinea faciei*, and *M. canis* infection in adults has often been associated with immunosuppressive states and a large variety of oral and topical antifungal protocols are available for treating M. canis infection. However, the efficacy of these drugs and treatment protocols is variable, with treatment failure up to 40% of patients possibly due to resistance phenomena. Currently, the application of molecular methods to *microsporum canis* diagnostics is becoming more common since they allow final diagnosis to be obtained with the improved sensitivity, permitting the initiation of appropriate treatment and infection control **(Brillowska-Dabrowska et al., 2013).**In humans, topical antifungals are recommended for the treatment of tinea capitis though once monotherapy is discontinued relapses may occur **(Aneke et al., 2018).**
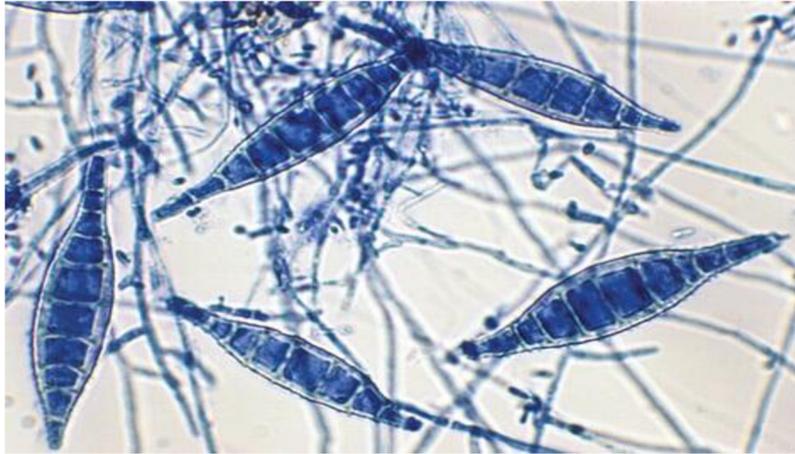
**Figure.1 :** *Microsporum canis* (: https://www.adelaide.edu.au/mycology/mould-identification-a-virtual-self-assessment)

### 3.2.2. Yeast Skin Infection

Candida species are important components of the microflora of the human skin, oral and vaginal mucosa, and gastrointestinal tract. They cause mild superficial and serious infections in humans**.** *Candidiasis* is an acute or subacute fungal infection caused by fungi that belongs to candida genus, with Candida albicans being the most frequent causative agent (**Nurdin et al., 2021***)*. *Candida species* can grow as either yeast cells or filamentous forms, with mixtures of the two phases generally seen in tissue infections. Interdigital candidiasis between the fingers and toes is a common manifestation of candidal skin infection and may develop after maceration of the skin of the fingers. *Candida spp.*, like all fungi, prefer a dark. In some cases, superficial C. albicans infections may be particularly severe, persistent, and recalcitrant to treatment, producing the uncommon disorder known as chronic mucocutaneous candidiasis. This condition consists of persistent and recurrent infections of the mucous membranes, skin, and nails, along with a variety of other manifestations (**Kaufman et al., 2019**)

### 3.2.3. Common bacterial skin diseases

Bacterial skin and soft-tissue infections (SSTIs) are the most common type of infectious skin disease and encompass an array of conditions that may be classified by the skin layers and structures they affect. The clinical presentation of infectious skin diseases varies based on the type of pathogen involved, and the underlying medical condition of the patient (**Dawson et al., 2012).** The common causative bacterial pathogens of superficial infections include the *staphylococci*, particularly *Staphylococcus aureus* and coagulase-negative species (CoNS), and Streptococcus, such as Streptococcus pyogenes (group A B-hemolytic streptococci) (**Jacobs et**

**al., 2007)**.Common bacterial skin infections include cellulitis, erysipelas, impetigo, folliculitis, and furuncles and carbuncles (*Stulberg et al., 2002)*.Impetigo occurs most frequently on the exposed parts of the body: the face, hand, neck, and extremities. Cellulitis is a suppurative inflammation particularly involving the subcutaneous tissue. In the past, the most common site of involvement of erysipelas was the face, but the lower limbs now predominate. The face, arm, and upper thigh are the other most affected sites (**Matz et al., 2005)**.Folliculitis is an inflammation of the hair follicles (**Stulberg et al., 2002)**.Abscesses are localized skin infections, including carbuncles and furuncles, commonly called boils. These are subcutaneous infections or more extensive infections of the hair follicle, and are also usually caused by *S. aureus* (**Jacobs et al., 2007)**.Skin infections with *Streptococcus pyogenes* are linked to acute rheumatic fever or rheumatic heart disease and acute post-streptococcal glomerulonephritis, often leading to significant morbidity (**Nepal et al., 2018)**. Only a trained and updated dermatologist will recognize the unusual forms and rare variants of these diseases (*Matz et al., 2005)*.Effective treatment of infectious skin disease requires timely identification or estimation of the offending pathogen, and selection of a treatment that is effective against the pathogen and is administered via the optimal route and dosing schedule (**Dawson et al., 2012)**. Bacterial skin infections are treated with oral or topical antibiotics depending on the strain causing the infection *(***Stulberg et al., 2002***)*.

### 3.2.3.1. *Genus Staphylococcus*

*Staphylococcus* are Gram-positive, non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation with diameters of $0.5 - 1.5$ μm and characterised by individual cocci, which divide in more than one plane to form grape-like clusters. Staphylococcus organisms can be coagulase-positive or coagulase-negative *(Harris et al., 2002)* . Coagulase is an enzyme produced by many bacteria that allows the conversion of prothrombin to staphylo thrombin, which in turn activates the protease activity of thrombin. Coagulase-positive microorganisms tend to be considered pathogens and are traditionally associated with S. aureus, including methicillin-resistant and methicillin-susceptible strains *(***Heldt Manica et Cohen, 2017***)*.To date, there are 32 species and eight sub-species in the genus Staphylococcus, many of which preferentially colonise the human body (**Harris et al., 2002).** Staphylococci are ubiquitous in the environment. Natural populations are associated with skin, skin glands and mucous membranes of warm-blooded animals *(Wieser et Busse, 2000)*.However, *Staphylococcus aureus* and *Staphylococcus epidermidis* are the two most characterised and studied strains *(Harris et al., 2002)* .The bacterial community of the sick skin

is reduced in diversity compared with the bacterial communities on healthy skin and this increases disease severity. In contrast, fungal communities are richer and more diverse on the skin of patients with atopic dermatitis, although distribution of the most common species is similar in patients and controls **(Edslev et al., 2020***)*.

### 3.2.3.1.1. *Staphylococcus aureus*

*Staphylococcus aureus* occurs naturally on the skin and mucus membranes of healthy individuals and is a common cause of *pneumonia,* skin infections, and systemic infections in humans and other animals **(Lalouckova et al., 2021).**The skin and mucous membrane are excellent barriers against local tissue invasion by S. aureus. However, if these is breached due to trauma or surgery, S. aureus can enter the underlying tissue, creating  local abscess under this   favourable conditions can cause a number of local infections, **(Chmielowiec-Korzeniowska et al., 2020***)*. *S. aureus* causes diseases through production of toxins or through direct invasion and destruction tissue. Some strains have developed resistance to several b-lactam antibiotics used in hospitals. Methicillin-resistant S. aureus (MRSA) is a major opportunistic pathogen that causes both nosocomial and community-acquired infections (community-associated MRSA, CA-MRSA) (**Chakraborty et al., 2018***)*.

### 3.2.3.1.2. *Staphylococcus lugdunensis*

*S. lugdunensis* is a coagulase-negative, Gram-positive bacterium that can be isolated as a component of normal skin flora in humans *(***Heldt Manica et Cohen, 2017***)*.However, although S. lugdunensis does not produce secreted coagulase, it can sometimes be mistaken for *S. aureus* at clinical microbiology laboratories **(Taha et al., 2019).***Staphylococcus lugdunensis* has previously been considered as a non-pathogenic organism. But , *S. lugdunensis* can result in significant skin and soft tissue infection *(***Heldt Manica et Cohen, 2017***)*.These infections commonly affect the middle-aged to elderly patient populations, with greater prevalence in females. Approximately half of all patients affected have some form of concurrent comorbidity, either in the form of chronic immunosuppressive therapy, diabetes mellitus, or a history of trauma to the site (**Parthasarathy et al., 2020***)*.The back was the most common location for the *S. lugdunensis* infection, followed by the digits. All of the sites of infection clinically presented as cellulitis (figure 2 .) *(***Heldt Manica et Cohen, 2017***)*.

*S. lugdunensis*, like other coagulase-negative *staphylococcus* is able to produce a biofilm that makes infections become significantly more difficult to treat even with the high susceptibility of *S. lugdunensis* to most antibiotic treatments (**Parthasarathy et al., 2020**).*S. lugdunensis*

remains remarkably susceptible to most antibiotics, unlike many other CoNS such as S. epidermidis *(Taha et al., 2019).*



**Figure 2:** Closer view of Staphylococcus lugdunensis cutaneous infection of the back of a 70-year-old woman; the infection occurred at a presumed bite site(*Heldt Manica et Cohen, 2017*).

### 3.2.3.1.3. *Staphylococcus epidermidis*

*Staphylococcus epidermidis* is coagulase-negative staphylococci (CoNS) in contrast with *S. aureus* and have been commonly isolated in blood cultures **(Asai et al., 2021).**As part of the human epithelial microflora, S. epidermidis usually has a benign relationship with its host*(* **Michael Otto, 2009***).S. epidermidis* can form biofilms on the surface of implanted materials **(Takahashi et al., 2021***). S. epidermidis* produces exopolymers, namely poly-γ-glutamic acid (PGA) poly-N-acetyl-β-(1-6)-glucosamine (PNAG) and Polysaccharide intercellular adhesin (PIA), that protect from important mechanisms of innate host defense and represents the most common source of infections on indwelling medical devices. This likely stems from the fact that *S. epidermidis* is a permanent and ubiquitous colonizer of human skin, and the resulting high probability of device contamination during insertion ( **Michael Otto, 2009***).*In accordance with this general notion, *S. epidermidis* shows significant, genome-wide adaptation to the biofilm mode of growth including down-regulation of basic cell processes such as nucleic acid, protein and cell wall biosynthesis. The thick film protects the bacterial cells from antibiotics and host defenses, and thus eradicating biofilms by drug administration is difficult (**Takahashi et al., 2021**).  As part of the human epithelial microflora, *S. epidermidis* usually has a benign relationship with its host. Furthermore, it has been proposed that *S. epidermidis* may have a probiotic function by preventing colonization of more pathogenic bacteria such as S. aureus.

However, there is no clear evidence indicating that *S. epidermidis* secretes factors that impact colonization of other microorganisms *in vivo* ( **Michael Otto, 2009***).*

### 3.2.3.2. Genus Streptococcus

The *genus Streptococcus* consists of Gram-positive, spherical or ovoid cells that are typically arranged in chains or pairs. These cocci are facultatively anaerobic, non-sporing, , homofermentative, and have complex nutritional requirements **(Fischetti et Ryan, 2008)**

It is one of the most important human pathogens, which is catalase-negative, coccus-shaped organisms that can be also commensal , (**Janda, 2014 and Li et al., 2021**).Although many distinct taxa have been recognized in the streptococci, their classification and nomenclature have caused considerable confusion over years one of the first characteristics to be recognized and used for distinguishing between isolates was the ability of certain clinically important streptococci to cause complete (β-) haemolysis around colonies grown in blood containing culture media **(Fischetti et Ryan, 2008***).*Species of the *genus Streptococcus* are extensively studied with respect to their cell wall polysaccharide (CWPS) structure and antigenicity owing to their pathogenesis towards both humans and animals **(Lavelle et al., 2021)**..Pathogens and some commensals of Streptococcus show a surprising capacity for adaptation to new hosts and resistance to antibiotics and immune responses. Some species being highly virulent and responsible for major diseases: *S. pyogenes, S. agalactiae and S. pneumoniae* are particularly notable as causes of serious acute infections in man. As a result, they have caused the spread of infection and significantly increased mortality rates all over the world, leading to huge health and economic loss (**Gao et al., 2014**).

### 3.2.3.2.1 Streptococcus pyogenes

Group A *Streptococcus* (GAS), infection induces a wide spectrum of symptoms, from superficial skin and throat infections to life-threatening streptococcal toxic shock syndrome and necrotizing soft tissue infections. The mortality rate of the streptococcal toxic shock syndrome may exceed 50% in spite of aggressive treatment *(***Tsao et al., 2021***).Streptococcus pyogenes* is a strict human pathogen responsible for a wider variety of human diseases than perhaps any other microorganism. The bacterium can survive and persist within the human host for a long time as it is observed in up to 40% of the population who are considered as carriers **(Menschner et al., 2020***).*Biofilm is one of the important virulence factors that is responsible for the severity and progression of the *Streptococcus pyogenes* diseases. The increasing occurrence of antibiotic

resistance in GAS infection may be associated with many significant outbreaks (**Tsao et al., 2021**).

### 3.2.4. *Genus Pseudomonas*

Bacteria of the genus pseudomonas are G- microorganisms possessing unique adaptive capabilities allowing them to colonize almost all terrestrial and aquatic habitats. Some of them are pathogenic to humans, animals, and plants. They differ in the adaption to a wide range of temperatures, pH values, and salinity, (**Bel'kova et al., 2018**).They are motile by means of one or more polar flagella and have a very strict aerobic respiratory metabolism with may cause skin infections under certain circumstances, i.e. in immunocompromised subjects (**Weckesser et al., 2007**).Representatives of the *genus Pseudomonas* may not always be reliably identified at the species level based on ribosomal phylogeny (**Bel'kova et al., 2018**).

### 3.3. Treatment for some bacterial and fungal skin diseases
### 3.3.1. Antifungal agents for skin diseases

Dermatophytic infections of the skin and its appendages are a common occurrence, yet their treatment is challenging in pregnant women, children, and the elderly age group (**Bhat et al., 2020**).  In the recent past, there has been an increased incidence and unresponsiveness to various antifungal agents. Treatment of dermatophytosis involves the use of an antifungal drug in either a topical or oral application form or a combination of both**.** The latter is often applied in case of persistent onychomycosis (**Smijs et Pavel, 2011**).There are many topical agents for treating several less severe forms of tinea. Theazole derivatives, such as clotrimazole, miconazole, econazole, and oxiconazole, are the generally used (**Brescini et al., 2021**)**.** Itraconazole is an orally active triazole antifungal with lipophilic properties, is used as a broad-spectrum antifungal agent against dermatophytes, yeasts, and moulds  and  is an effective, safe antifungal agent used, especially in adults (**Bhat et al., 2020**).Dermatophytes are often tolerant or resistant to these drugs (regardless of the mode of administration), bringing therapeutic failure (**Pereira et al., 2021**).Topical antifungals are preferred in the paediatric age group as they have a thin skin which enables better penetration of the topically applied creams and the high turnover rate which allows rapid elimination of the fungus (**Gandhi et Vasani, 2020**).Two topical agents (E. finaconazole and luliconazole) recently have been approved for treatment of onychomycosis. These two topical agents have certainly increased treatment options for onychomycosis in the

daily practice of dermatology (**Kawai, 2019**).Systemic antifungal therapy which was usually reserved for the treatment of superficial dermatophytosis of the scalp, nails, palms, and soles, now, needs to be administered more often in nonresponsive cases of tinea cruris/corporis/ faciei (**Gandhi et Vasani, 2020**).

### 3.3.2. Antibacterial agents for skin diseases

Antibiotics can effectively treat the disease caused by bacterial infections .However, the antibacterial efficacy of antibiotics is gradually weakening due to the emergence of multidrug-resistant bacteria (**Yan et al., 2021**).There are numerous reports indicating that this overall increase is primarily due to a dramatic increase in the number of infections caused by community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA), an organism that often presents with skin and soft tissue infections in the paediatric population (**Duong et al., 2010**). It is crucial that dermatologists to be good stewards for limiting unnecessary use of antibiotics and educating a patient population on risks of overuse and resistance, so that it is important to re-evaluate the effectiveness of adjunctive antibiotic (**Goettsche et al., 2019**).The antibiotics are classified into three categories based on their main therapeutic indications. This classification was aided using the anatomical therapeutic chemical classification.The three groups were as follows antibiotics used primarily for treatment of skin and soft tissue infections (b-lactamase-resistant penicillins and lincosamides;, respectively);antibiotics used primarily for UTI (pivmeccillinam, sulfonamides and trimethoprim, flouroquinolones, and nitrofurantoin derivatives; respectively); and those used primarily for RTI (antibacterial glycopeptides and methenamine;, respectively) (**Daryapeyma et al., 2016**). Amoxicillin is a useful agent for treating strepto- coccal infections, whereas amoxicillin–clavulanate is a useful agent for treating methicillin-susceptible staphylococcal infections because the addition of the B-lactamase inhibitor, clavulanate, inhibits staphylococcal B-lactamase. These agents diffuse readily into most body tissues and fluids. Cefpodoxime proxetilis active against methicillin-susceptible staphylococci, including B-lactamase–producing strains and *S. pyogenes.* Cefprozil is active against Gram-positive bacteria including methicillin-susceptible S. aureus, including B-lactamase producing strains, as well as S. pyogenes (**Jacobs et al., 2007**).

### 3.4. Antimicrobial resistance (AMR) in dermatology

It is estimated that we share this world with 1 trillion different microbial species. Most of which are still unknown to us. Some are essential for our survival, but small fraction can cause diseases

and are commonly referred to us pathogens (**Locey et Lennon, 2016**).The discovery of antibiotics is undoubtedly saved millions of lives since their discovery. But their misuse has resulted in the emergence of AMR, which is a significant threat to global human health (**Karas et al., 2020**). Reservoirs for resistance may be present in healthy human and animal populations (**Okeke et al., 2005**). Several published reports indicated that AMR has reached an alarming stage (**Sweileh, 2021**). Antimicrobials resistance genes (ARG) can enter the environment from several anthropogenic point sources, including pharmaceutical factories, hospitals, wastewater treatment plants, animal husbandry operations, and agricultural activities (**Bueno et al., 2021**).Initially, microbial resistant to multiple drugs were found mostly in hospitals, where antimicrobial agents are used most extensively, but resistance is currently found almost as frequently in the community (**Okeke et al., 2005**).The treatment of S. aureus infections is becoming increasingly more complicated and tough, due to the emergence of various types of antibiotic resistance (**Chakraborty et al., 2018**).Optimising the use of antimicrobial agents is a key element in the global response to the antimicrobial resistance (AMR) crisis (**Pauwels et al., 2021**).Antimicrobial agents can be divided into groups based on the mechanism of antimicrobial activity (**Salleh et al., 2017**).Bacteria may be inherently resistant to an antimicrobial. This passive resistance is a consequence of general adaptive processes that are not necessary linked to a given class of antimicrobials. An example of natural resistance is *Pseudomonas aeruginosa*, whose low membrane permeability is likely to be main reason for its innate resistance to many antimicrobials. The active resistance, the major mechanism of antimicrobial resistance, is the result of a specific evolutionary pressure to develop a counterattack mechanism against an antimicrobial so that bacterial populations previously sensitive to antimicrobials become resistant. These type of resistance results from changes in the bacterial genome (**Bockstael et Van Aerschot, 2009**). In case of antifungal resistance as with other microorganisms, antifungal resistance is a broad concept that can be divided into clinical and microbiological resistance (**Garcia-Effron, 2021**). Resistance is also correlated with antifungal mis use because patients often fail to finish the full course of treatment. Thus, the inadequate use or dosage of drugs contributes to the failure in eliminating the disease agent completely, encouraging growth of the most resistant strains, which may lead to hard-to-treat fungal infections (**Martinez-Rossi et al., 2008**). Intrinsic microbiological resistance is the innate ability of a fungal species to resist the activity of a particular antifungal drug due to its inborn functional or structural features (e.g., absence of the drug target, inaccessibility of the drug into the cell). This resistance is exhibited by all strains of the same species of a fungus and is not related to exposure to the antifungal (**Garcia-Effron, 2021**) . Various biochemical

mechanisms contribute to the phenotype of drug resistance in fungi. The most frequent ones involve a decrease in drug uptake, structural alterations in the target site and an increase in drug efflux or in intracellular target levels (**Martinez-Rossi et al., 2008**).

### 3.5. Composition and bioactive Compounds in *Cannabis sativa*

The variety of chemical compounds found in plant material has been inspiring scientists for years and contributes to the fact that active compounds contained in plant materials are used in many industries (**Zagórska-Dziok et al., 2021**). *Cannabis sativa* L. has beneficial impact on human health because of its wide variability of bioactive compounds present in it (**Palmieri et al., 2021**).To the date, it has been reported a total number of 565 natural constituents, 120 of which correspond to the cannabinoid class, some of them with opposing effects. Cannabinoids comprise either a C21 or C22 terpeno-phenolic skeleton differing in their state of cyclization and oxidation pattern (**Klahn, 2020**). They have been detected in different parts of the plant; however, they largely accumulate in the secretory cavity of the glandular trichomes of the female flower(**Schofs et al., 2021**) . The most known cannabinoids include$\Delta$9-tetrahydrocannabinol ($\Delta$9-THC)tetrahydrocannabinolic acid (THCA), Cannabielsoin (CBE) cannabino*l* (CBN), cannabidiol (CBD) Cannabigerol (CBG) , Cannabicyclol (CBL) and cannabidiolic acid (CBDA) (figure 3.) (**Pourseyed Lazarjani et al., 2020**).They have various biological effect depending on their concentration in the plant *(Yang et al., 2020)* . The rest of cannabis phytochemicals include primary metabolites such as amino acids, fatty acids, and steroids or secondary metabolites as terpenoids, flavonoids, stilbenoids, lignans, and alkaloids among others.

**Terpenes,** which are found abundantly in *Cannabis sativa* and other essential oil producing plants have shown strong inhibition against *S.aureus* and E.coli (**Zengin et Baysal, 201**4).

**Flavonoids**' have been known to share properties with terpenes and cannabinoids and have been shown to present anti-inflammatory, anti-cancer and neuroprotective properties.

**Myrcene** and other aromatic compound found in cannabis also shows ant-inflammatory properties and is thought to help preventing cancer *(Stolzenberg et al., 2016).* There are hundreds of varieties of medical cannabis available in the market and these can be obtained in the forms of dried flowers, cannabis extracts, and infused oil. In fact, there have been no systematic studies investigating the relationship between the chemical constituents of deferent medical cannabis varieties and their corresponding combined therapeutic effects and receptor activities. A very large group of them have strong antioxidant properties. They show anti-

inflammatory, cytotoxic, but also antibacterial or antifungal properties. In addition to antioxidant activity, plant extracts contain a number of active substances that have protective, regenerative, and anti-inflammatory properties **(Zagórska-Dziok et al., 2021).** Research suggests that there may be some therapeutic benefit from the various cannabinoids contained within the cannabis plant. Regular cannabis use for medicinal purposes is a relatively recent regimen and has been used for its multiple curative properties such as diuretic , anti-emetic, anti-epileptic ,anti-inflammatory,anti-parasitic,antipyretic,antibacteria, anti-tumour properties **(Shakil et al., 2021)** . Another interesting of the current study in therapeutics is that cannabis most likely has the ability to improve sleep quality, decrease sleep disturbances, and decrease sleep onset latency **(Kuhathasan et al.2019).**The basal gangliais not only a site for dopamine receptors but also cannabinoid receptors and there is at least a potential role for the endocannabinoid system to control voluntary movement in Parkinson's diseases **(Fernández-Ruiz et al., 2015**). A number of studies have demonstrated some efficacy with cannabinoids for the use of bladder symptoms in various conditions, particularly multiple sclerosis which is often associated with urinary incontinence **(Tyagi et al., 2010**). Glaucoma which is the second causing of blindness in the world which is an irreversible eye diseases characterized an increased intraocular pressure inside the eye ,it is now known that the intraocular pressure can be lowered by use of cannabinoids *(**Cairns et al., 2016***).
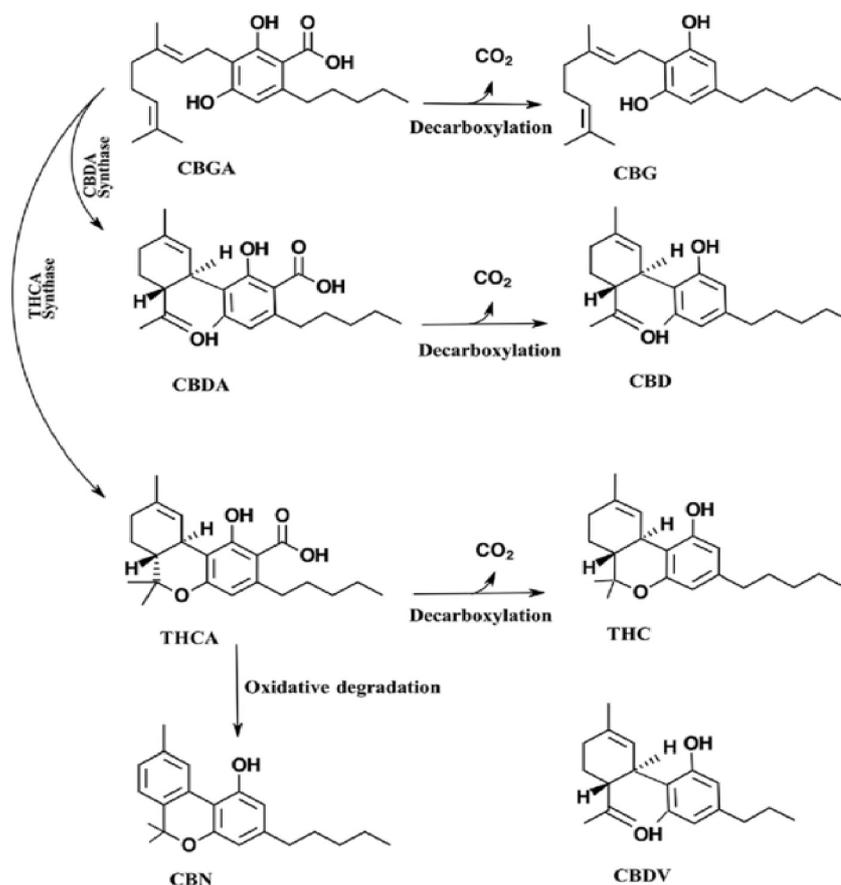
**Figure 3 :** The most common cannabinoids and their conversion pathway by decarboxylation because of heat or aging. CBGA can convert to CBDA and THCA by CBDA synthase and THCA synthase, respectively. CBGA: cannabigerolic acid, CBG: cannabigerol, CBDA: cannabidiolic acid, CBD: cannabidiol, THCA: tetrahydrocannabinolic acid, THC: tetrahyrocannabinol, CBN: cannabinol **(Pourseyed Lazarjani et al., 2020)**.


## 3.6. The Antimicrobial Properties of *Cannabis sativa*

As highlighted above A post-antibiotic world is fast becoming a reality, given the rapid emergence of pathogens that are resistant to current drugs *(**Karas et al., 2020***)*. So that therapeutic products derived from plants are gaining from popularity since long time and this is primarily due to their ability to overcome the side effects of allopathic forms of medicine *(**Tandon et Mathur, 2017***)*.*Cannabis sativa* plants were found to possesses modest action have been suggested. Generally, phytochemicals can act harming the bacterial membrane, inhibiting the formation of bacterial biofilm or through the suppression of virulence factors such as enzymes and toxin **(Frassinetti et al., 2020)**. Its bioactivity is largely due to a class of compounds known as cannabinoids. *(**Karas et al., 2020***)*. Two particularly effective antimicrobial cannabinoids are *Cannabinochromene* ( CBC) and *Cannabinogerol* (CBG) with

CBG being more potent antimicrobial and CBC being better antifungal agent **(Palali et van Ours, 2017***)*. Essential oils extracted from five fiber varieties of C. sativa had antimicrobial activity with the degree of antimicrobial activity varying between cultivars *(***Ali et al., 2012***).*The most abundant compounds in each oil sample were α-pinene, myrcene, *trans*-β-ocimene, α-terpinolene, *trans*-caryophyllene and α-humulene *(***Karas et al., 2020***)*.


### 3.7. Nanoparticles and Their usage as delivery systems

Nanomaterials are the nanometre sized falls with ranges from 1-100 nm and these materials showed enhanced unique properties (**Sujatha et al., 2018***)*. They are composed of primary and agglomerated particles that can vary in size, shape, charge, crystallinity, chemical composition and other characteristics, and this variety will increase even further in the future. All these characteristics have been suggested to affect the toxicity of nanomaterials, but not all existing and emerging types of nanomaterials can be tested separately in studies to evaluate their safety *(***Braakhuis et al., 2014***)*. The nanoparticles offer several advantages over other conventional drug delivery systems and have been used as a physical approach to modify and advance the pharmacokinetics and pharmacodynamics possessions of various types of drug molecules *(***PATIL et al., 202***1)*. In addition to the drug delivery nanoparticles are highly used as a antimicrobial agents like antibacterial and antifungal activities against different types of disease causing pathogens (**Sujatha et al., 2018**). For example, zinc oxide nano-particles (ZnO-NPs) were found to inhibit *Staphylococcus aureus*, and silver nano-particles (Ag- NPs) exhibit concentration-dependent antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Therefore, one of the most promising candidates for this type of biomedical devices is chitosan (CS)—a biopolymer characterized by excellent biocompatibility with skin cells. It can be obtained from various natural sources such as wastes from food industry, insect exoskeletons, or *mushrooms* and *fungi* (**Radwan-Pragłowska et al., 2021**).Chitosan (CS) is a linear polysaccharide polymer composed of β-(1–4)-linked N-acetyl-D-glucosamine. Chitosan contains three types of reactive functional groups: amino group at the C-2, secondary hydroxyl group at the C-3 and primary hydroxyl group at the C-6. The amino group at the C-2 of chitosan as shown in (Figure 5.) and can be modified to improve its physicochemical properties to suit various applications. Chemical and biological functions, and the chemical modification of chitosan is mainly carried out on amino group at the C-2 and primary hydroxyl group at the C-6.It has been explored as a drug carrier due to its biocompatible properties and have been demonstrated the use of chitosan to coat nanoparticles made of other materials would help in reducing their impact on the body and also increase their bioavailability *(***Ramawat et**

**Mérillon, 2015).** It is known that chitosan's antimicrobial activity is influenced by a number of factors that act in an orderly and independent fashion and are reviewed in (Figure 4.). The most prevalent proposed antibacterial activity of chitosan is by binding to the negatively charged bacterial cell wall causing disruption of the cell, thus altering the membrane permeability, followed by attachment to DNA causing inhibition of DNA replication and subsequently cell death **(Atay, 2020).**
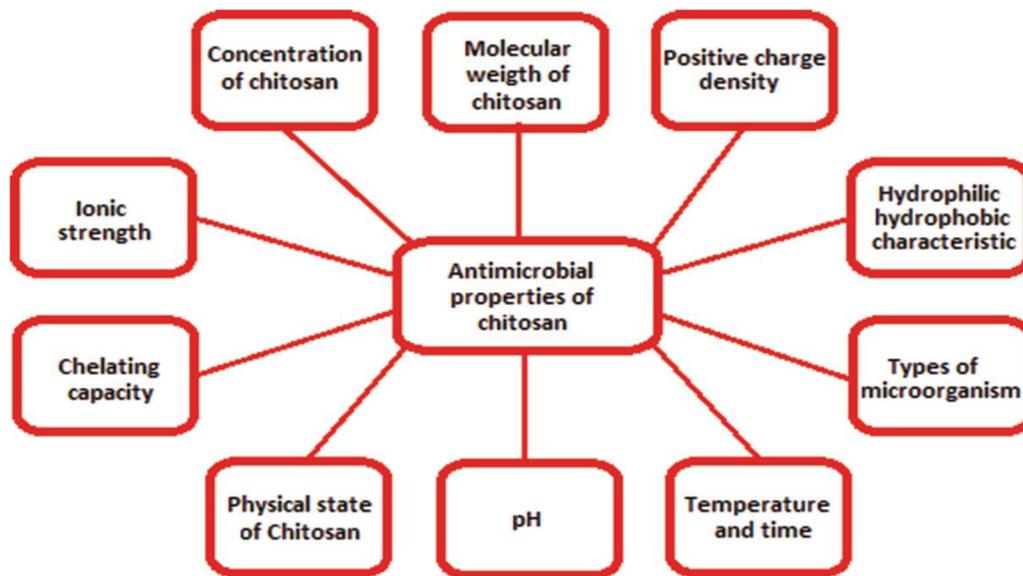


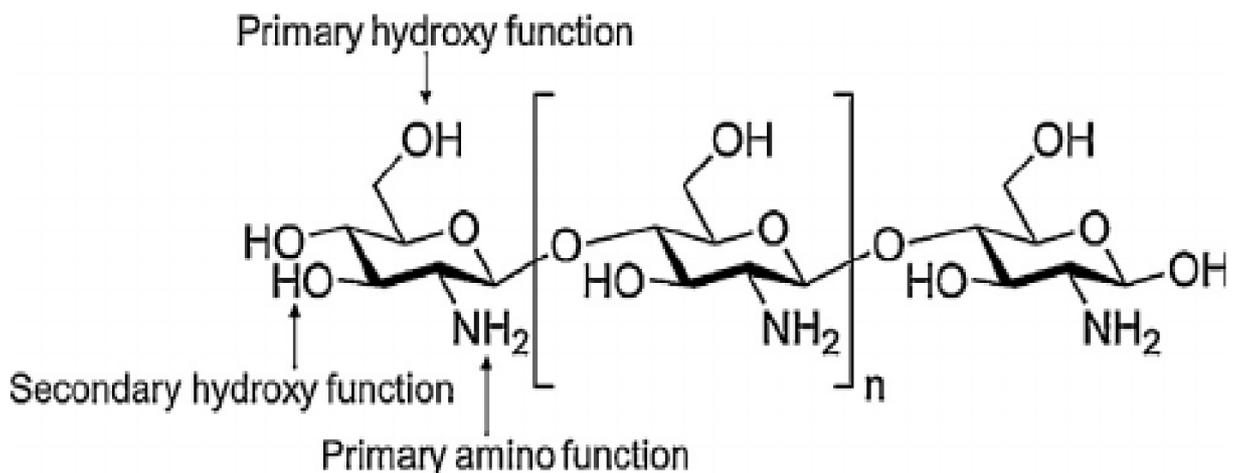Figure 4-Factors affecting antibacterial property  (Atay, 2020)



Figure5. Functional-groups-in-chitosan *https://www.researchgate.net/figure/Functional-groups-in-chitosan-that-can-be-modified_fig3_284921454*(Ramawat et Mérillon, 2015)

# 4. Materials and Methods

Ethanol extract was prepared from *Cannabis sativa* strain *Chocolope* and subsequently encapsulated in chitosan. Antimicrobial and antifungal activity of non-capsulated and encapsulated extract was tested against three bacterial strains and one dermatophyte by modified broth dilution method.

## 4.1. Equipment, Chemicals, and reagents used
### 4.1.1. Equipments

Den-1b McFarland densitometer (Biosan , CZ).

IKA® C-MAG HS hotplate stirrers (IKA, DEU )

Bandelin SONOREX Digitech Ultrasonic baths (BANDELIN electronic GmbH & Co, DEU)

GFL Shakers, Vortexers

Multiscan Ascent Microplate photometer (Winooski, USA)

Universal 320 R Centrifuge (Hettich , DEU)

Analytical balance (Precisa, CH)

Laminar flow cabin (Faster S.r.I, ITA)

Lyophilizer (Gregor instruments, s. r. o., CZ.)

Vacuum evaporator (Hei-VAP Advantage, Heidolph, DEU)

### 4.1.2. Chemicals

Terbinafine hydrocholoride (Sigma - Aldrich, CZ).

Chloramphenicol (Sigma – Aldrich, CZ).

Chitosan (Sigma - Aldrich, CZ)

Muller-Hinton Broth (Oxoid, UK).

Muller -Hinton Agar (Oxoid, UK).

Sabouraud Dextrose Agar (Oxoid, UK).

Sodium Hydroxide beads A.G. (Penta s.r.o, CZ.)

RPMI 1640 Medium (Sigma-Aldrich, CZ).

MOPS Ultra-pure (USA)

Sodium Triphosphate Penta basic (TPP) (Sigma Aldrich, CZ)

Ethanol (Penta Chemicals, CZ)

Acetic acid (Sigma -Aldrich, CZ)

## 4.2. Preparation of cannabis extracts by ethanol

The extract was prepared from six dried inflorescences of *Cannabis sativa Chocolope* strain. The strain was cultivated at the Departments of Food science, Faculty of Agrobiology, Food and Natural Resources Czech University of Life Sciences in Prague at 2020. Cannabis inflorescences was mixed with 80% ethanol in ratio 1:9 (w/w) and shaken for 48 hours and then evaporated under vacuum in warm bath 40 °C then the extract was placed into – 20 °C in refrigerator.

## 4.3. Preparation of chitosan nanoparticles

1 g of chitosan powder was added 200 mL of distilled water with 1% of acetic acid,then the bottle was put inside sonic bath for 15 minutes. The pH was adjusted to 5.1 by adding 10 M NaOH. After that 133 ml of sodium tripolyphosphate (TPP) in concentration 0,25% in distilled water was added to the solution. The final ratio of chitosan: TPP was 3: 1.  Chitosan nanoparticles formed spontaneously upon magnetic stirring **(Figure 6.)**. Nanoparticles were then purified by centrifugation twice at 9000 rpm for about 6 minutes at temperature 22°C followed by successive washing with distilled water for resuspending the nanoparticles were frozen in freezer then dried with freeze-drying for 72 hours, chitosan nanoparticles were stored in 4°C container until further use.

Figure 6. speed magnetic stirring for preparation of chitosan-based nano particles (sources: author)

## 4.4. Encapsulation of cannabis extract into chitosan

In the same procedure as chitosan-nanoparticles (CS-NPs) 1 g of chitosan powder was added to 200 mL of distilled water with 1% of acetic acid, then the bottle was put inside sonic bath for 15 minutes. Eventually 100 mg ethanolic extract of Chocolope was dissolved with 1 mL 96% of ethanol, and added to the chitosan solution, the pH was again adjusted to 5.1 by adding 10 M NaOH. After that 133 ml of sodium tripolyphosphate (TPP) in concentration 0,25% in distilled water was added to the solution. The final ratio of chitosan: TPP was 3: 1. Chitosan nanoparticles formed spontaneously upon magnetic stirring. Nanoparticles were then purified by centrifugation twice at 9000 rpm for about 6 minutes at temperature 22°C followed by successive washing with distilled water for resuspending the nanoparticles were frozen in freezer then dried with freeze-drying for 72 hours, encapsulated cannabis with chitosan nanoparticles were stored in 4°C container until further use.



Figure 7. Centrifugation of chitosan and Cannabis extract nanoparticles (source: author)

## 4.5. Microorganisms tested

The antimicrobial activity of *Cannabis sativa* extracts encapsulated, and non-capsulated and clean chitosan and chitosan nano particles was tested against three bacterial strains *Staphylococcus epidermis* (CCM 50, and CCM 4418), and *Staphylococcus saprophyticus* (CCM 2727) and *Staphylococcus lugdunensis* (CCM 4069) and one fungal strain *Trichophyton rubrum (*CCM 4934) causing skin diseases All the microbial strains were purchased from Czech Collection of microorganisms (CCM). Antibiotics chloramphenicol and terbinafine were used as positive control for bacteria and fungi respectively. All samples were tested in triplicate.

## 4.6. Preparations of agar and medium for testing antimicrobial agents

Mueller Hinton broth (MHB) adjusted to pH 6.5 by acetic acid and MH agar were used for antibacterial susceptibility testing. For fungi RPMI 1640 medium with the addition of MOPS and chloramphenicol and adjusted to pH 7 with NaOH and SDA agar was used. All media were sterilized in autoclave 121 ℃ for 15 minutes.

## 4.7. Preparation of inoculum for microbials

A 0.5 McFarland bacterial inoculum was prepared using a densitometer (Figure 8.) by diluting a bacterial suspension cultured in MHB broth at 37 ° C for 24 hrs. The finished inoculum was used in tests. Two weeks old inoculum of the fungus was prepared in the same manner of bacteria but RPMI medium by suspending fungal colonies, and the inoculum was agitated for 15 s with a Vortex mixer The resulting suspension was adjusted to 0.5 McFarland using a densitometer.



Figure 8. McFarland standard.DEN-1B Densitometer (suspension turbidity detector)

## 4.8. Determination of antifungal and antimicrobial activity of non-encapsulated cannabis extract by broth microdilution method

The minimum inhibitory concentration (MIC) which is defined as the lowest concentration of antimicrobial that will inhibit the visible growth of micro-organisms of non-encapsulated cannabis extract of Chocolope was determined by the broth microdilution method. The tests were performed in 96-well plates using RPMI 1640 medium as growth medium for fungus strain. The stock solution of cannabis extract was by two-fold serial dilution dosed to the 96-well plate to give ratio of tested concentrations ranging from 1024 to 8 μg / ml. After that the each well was inoculated by 100 μl of the prepared inoculum. The microtiter plates were then incubated in the temperature 25 ° C for 5 days. The same method to evaluate susceptibility of bacteria. In this case MHB was as growth medium, the ratio of the tested concentrations was 512 to 4, μg / ml, the inoculum was added by 96 pin multi-blot replicator. The bacteria were cultivated for 24 hrs incubation at 37 °C, the MIC was determined as the lowest concentration that completely inhibited the growth of the bacteria by Multiscan Ascent Microplate photometer.

## 4.9. Determination of antifungal and antimicrobial activity of nanoparticles by broth Macrodilution method

The minimum inhibitory concentration (MIC) of 5, 10, and 20 mg of chitosan, chitosan nanoparticles and encapsulated cannabis with chitosan was determined by a macrodilution method using 10 ml tubes. Powder form of these were added to MHB as growth medium for bacterial strains with 0.25% acetic acid adjusted to pH 6.5, the ratio of the tested concentrations to each tube was 0,5, and 1 mg/ml. After that each tube was inoculated by 0,1 ml of prepared inoculum. The bacteria were then cultivated for 24 hrs incubation at 37 °C. The same method to evaluate susceptibility of fungus. The tests were performed using RPMI 1640 medium as growth medium for fungus strain, the ratio of the tested concentrations to each tube was 1 mg/ml and 2 mg/ml. After that each tube was inoculated by 0,1 ml of prepared inoculum. The tubes were then incubated in the temperature 25 ° C for 5 days. Besides that, in the same method to evaluate the susceptibility of microbials, the minimum inhibitory concentration ((MIC) of chloramphenicol was tested using 10 ml tubes. The stock solution of chloramphenicol was by two-fold serial dilution dosed to tubes using MHB as growth medium with 0.25% acetic acid adjusted to pH 6.5 for bacteria to give ratio of tested concentrations ranging from 128 to 1 μg / ml. After that each tube was inoculated by 0,1 ml of

prepared inoculum except the clean control. The bacteria were then cultivated for 24 hrs incubation at 37 °C. the MIC was determined as the lowest concentration that completely inhibited the growth of the bacteria by turbidity detector. For antimycotic terbinafine in the same steps as chloramphenicol but RPMI 1640 medium as growth medium for fungal strain to give ratio of tested concentrations ranging from 4 μg / ml to 0,03125 μg / ml. After that each tube was inoculated by 0,1 ml of prepared inoculum of fungus except the clean control. The tubes were then incubated in the temperature 25 ° C for 5 days.

## 4.10. Determination of minimum bactericidal concentration and minimum fungicidal concentration of tubes from the MIC test that showed no growth

The minimum bactericidal concentration (MBC) is defined to be the minimum concentration of antimicrobial capable of inactivating more than 99.9% of the bacteria present. After determining the MIC, an aliquot of 5 μl sample was withdrawn from each clear tube and plated onto MHA agar plates. The agar plates were then incubated at 35 °C for 24 hrs. The reduction was expressed as the proportion of the inoculum - colony forming unit (CFU) which is a measure of viable colonogenic cell numbers in CFU/mL) introduced. The minimum fungicidal concentration (MFC) which is defined as the lowest concentration of antifungals that results in total inhibition of visible growth against fungal strain was also determined as follows: After determining the MIC, an aliquot of 5 μl sample was withdrawn from each clear tube and plated onto a RPMI-1640 agar plate buffered with SDA and Inoculated plates were the incubated at 25 ° C for 5 days.

## 5. Results

**Table 1**. Antibacterial activity of cannabis extracts and with chitosan against selected bacteria causing skin diseases

| Bacteria strains | CCNB | | ECNB | | CS-NPS | CCS | | CLP | |
|---|---|---|---|---|---|---|---|---|---|
| | MIC µg/ml | MBC µg/ml | MIC mg/ml | MBC mg/ml | MIC mg/ml | MIC mg/ml | MBC mg/ml | MIC µg/ml | MBC µg/ml |
| *S. epidermids 50* | 8 | 16 | 0,5 | 1 | NI | NI | - | 8 | 16 |
| *S. epidermis 4418* | 8 | 16 | 0,5 | 1 | NI | NI | - | 2 | 4 |
| *S. saprophyticus 2727* | 8 | 16 | 1 | - | NI | NI | - | 8 | 16 |
| *S. lugdnensis 4069* | 16 | 32 | 1 | - | NI | NI | - | 4 | 8 |

CCNB- clean Cannabis, ECNB- encapsulated cannabis, CS-NPs- chitosan nanoparticles, CCS-clean chitosan, CLP- chloramphenicol, NI- No inhibition.

The results in (**table 1.**) showed the most sensitive bacteria to all tested antimicrobial agents was *S. Epidermidis 4069*, and the most resistant bacteria was *S. lugdnensis 4069*. The minimal inhibitory concentration (MIC) of chloramphenicol of all tested strains ranged between 2-8ug/ml and minimal bactericidal (MBC) ranged between 4-16 µg/ml. Clean cannabis extracts had comparative antimicrobial activities against all three bacterial strains tested with minimum inhibitory concentration (MIC) ranged between 8- 16 µg/ml and minimum bactericidal concentration 16-32 µg/ml. The minimum inhibitory concentration encapsulated cannabis was 0,5mg/ml and 1 mg/ml. Results also showed that clean chitosan and chitosan nanoparticles didn't inhibit the growth of various bacteria tested.

**Table 2.** Antifungal activity of cannabis extracts and with chitosan against selected fungal strain.

| Fungal strain | CCNB | | ECNB | | CS-NPS | | CCS | | TRB | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MIC µg/ml | MFC µg/ml | MIC mg/ml | MFC mg/ml | MIC mg/ml | MFC mg/ml | MIC mg/ml | MFC mg/ml | MIC µg/ml | MFC µg/ml |
| *Trichophyton rubrum* 4934 | 128 | 256 | 1 | 2 | 2 | - | Ni | - | 0,03125 | 0.0625 |

CCNB – clean cannabis, ECNB -encapsulated Cannabis, CS-NPs- Chitosan nanoparticles, CCS-Clean chitosan, TRB – terbinafine, NI – No inhibition

The results in **(table 2.)** showed the minimum inhibitory concentration (MIC) of clean cannabis, encapsulated cannabis, clean chitosan, chitosan nanoparticles and terbinafine against *Trichophyton rubrum* at 25 ℃ at 10 days incubation. MIC of *T. rubrum.* for terbinafine was 128 µg/ml and Minimum fungicidal concentration was 256 µg/ml. The minimum inhibitory concentration of clean cannabis against *T. rubrum* was 128 µg/ml and the minimum fungicidal concentration was 256 µg/ml. When MIC of clean cannabis extract against T. rubrum, is 128 µg/ml to compare this value with encapsulated in Chitosan NP. The inhibiting concentration of nanoparticles with cannabis extract was 1 mg/mL against T. rubrum so that 1 mg contains at least 0,128 mg of extract. Based on the result preparing two repeats 100 mg of extract in 1 mL of ethanol so that the minimal encapsulation was 51,2 mg from 100 mg = 51,2 % efficiency. The minimum inhibitory concentration (MBC) of encapsulated cannabis was 1 mg/ml and minimum fungicidal concentration 2 mg/ml. Results also showed that clean chitosan had some affect against fungal strain tested with minimum inhibitory concentration 2 mg/ml and chitosan nanoparticles didn't inhibit the growth of *T. rubrum* .

## 6. Discussions

The aim of this work was to evaluate the antimicrobial activity of encapsulated cannabis extracts prepared by various methods against selected fungi and bacteria causing skin diseases and compare Cannabis sativa extracts in form of non-encapsulated extracts. According to established hypotheses in case that encapsulated extracts of Cannabis sativa are more effective against human skin pathogens comparing with to non-encapsulated extracts. However, their effectiveness varied. The extracts of clean cannabis showed the best results against all strains tested with a MIC in the range of 8 to 16 µg / ml. As stated *Karas et al., (2020) Ali et al., (2012)* essential oils extracted from five fiber varieties of C. sativa had antimicrobial activity with the degree of antimicrobial activity varying between cultivars the same author published that C. sativa chemotypes grown in northern latitudes are reported to have a higher ratio of cannabidiol to tetrahydrocannabinol resulting in stronger antimicrobial activity. Another referred articles as reported by **Klingeren and Ten Ham** many pharmacological properties of among CBD's minimum inhibitory concentrations (MICs) for both purified extracted CBD and THC ranged 1-5 µg/ml for genus staphylococcus  this result mostly aligns with this diploma thesis the non-capsulated cannabis extract acted against all the bacterial strains of the genus staphylococcus  exhibited with the minimum inhibitory concentration of 8 µg / ml except *S. lugdunensis* with MIC 16 µg / ml. Similarly,*Sarmadyan et* al. investigated the antimicrobial properties of cannabis against common hospital-associated bacterial strains in disc diffusion experiments he found that cannabis extract exerted the greatest antimicrobial effects on *S. aureus* 25923, in concentration of 25 µg/ml, with an inhibition zone of 14 mm, followed by MRSA, of 50 µg/ml  with values of 10 mm. Method by **Chakraborty et al. (2018)** stated clinical and non-clinical isolates of Methicillin-Resistant S. aureus (MRSA) were tested and showed that Crude ethanol cannabis extracted inhibited. However, when comparing the results from other published literatures, there was a certain difference in the research of individual authors in the studies, the authors very often dealt only with non-capsulated cannabis extracts. Antibacterial and  antifungal activity of different extracts (aque-ous  and  acetone extracts) of C. sativa leaves   was   tested   by Lone   and Lone (2012) and they confirmed that  both extracts showed inhibition against P. aeruginosa 5 µg/ml. *Isahq et al. (2015)* evaluated the antimicrobial activity of cannabis leaves, stems and seeds of six multidrug -resistant bacterial strains which one of them was S. aureus and five fungal strains which included candida genera, and all extracted revealed a range of antimicrobial activity and the stated that methanol and N-butanol of cannabis extracts showed prominent activities against S. aureus (18.00 ± 0.41) mm

and (14.00 ± 0.49) mm respectively and other different articles confirmed that the whole Cannabis plant and leaves extracts, EOs, seed oils as well as isolated components such as cannabinoids have shown antimicrobial effects against pathogenic bacteria and fungus. Turner and El Sohly and their research group published an expanded study on the antibacterial and antimycotic activities of further CBC-type and CBG-type cannabinoid derivatives the results of their studies showed the highest activity of all tested CBC-type cannabinoids against S. aureus with a MIC of 1.56 µg/mL even superior compared to Streptomycin. In 2008, Appendino et al. reported a more focused structure–activity relationship study on the anti-staphylococcal activity of different naturally occurring cannabinoids and synthetic derivatives against a broad range of various multi-drug resistant strains of S. aureus, including EMRSA- one of the main epidemic methicillin-resistant strain.

The results showed that Cannabis extracts both encapsulated and non-capsulated possesses antifungal activity against T. rubrum presenting MICs and MFCs 1mg/ml, 128 µg/ml and 2mg/ml, 256 µg/ml respectively. When MIC of clean cannabis extract against T. rubrum, is 128 µg/ml to compare this value with encapsulated in Chitosan NP. The inhibiting concentration of nanoparticles with cannabis extract was 1 mg/mL against T. rubrum. S. So these 1 mg contains at least 0,128 mg of extract (because in this concentration it worked as a clean agent against T. rubrum) then we have 0,128 mg in 1 mg of nanoparticles in, the experiment we prepared about 1600 mg (1.6 g) of nanoparticles in two repeats during preparation we put inside 100 mg of extract in 1 mL of ethanol so that the minimal encapsulation was 51,2 mg from 100 mg = 51,2 % efficiency. There is fragmentary evidence in the literatures that cannabis compounds have efficacy against some fungus. (Mahmud et al., 2021).Chitosan and Chitosan nanoparticles was also evaluated in this study in vitro with the purpose to test their antibacterial and antifungal activity. The antibacterial effect against all of the tested bacteria didn't show inhibition for chitosan- nano particles (Cs-NPs) and clean chitosan (CCs,) although it needs further research the reason for inactivity could be the different sensitivity of bacteria to chitosan or an error in diluting the concentration. From most of the referred articles and studies the bacterial effectiveness on gram-positive or gram-negative bacteria is however, somewhat controversial. Chitosan and its derivatives show antibacterial activity against fungi, gram-positive bacteria and gram-negative bacteria. The frequent and prolonged use of some fungicide compounds are responsible for strain resistance representing a potential risk for the environment and human health but chitosan, as a natural compound, does not present until now such problems as stated by Sajomsang et al., (2012).It is believed that chitosan's cationic nature and high MW helps in its antifungal action, since it interferes with negatively charged residues

of macromolecules on the fungal cell surface, thus causing changes in cell membrane permeability (Di Piero and Garda, 2008.Coqueiro and Di Piero, 2011). It can, also, prevent DNA transcription to RNA (Li, et al.,2008). Some authors (Li, et al., 2008) mentioned that antifungal activity of chitosan is MW dependent and that the smaller the molecular weight is, the stronger will be the antifungal activity. The same author stated the inhibitory action of chitosan is directly proportional to the concentration, because at higher concentrations of chitosan, fungi will produce higher. Some authors also studied chitosan antifungal activity on dermatophytes. Balicka Ramisz et al. (2005) obtained a MIC value of 1.1 mg/mL for M. Canis. This is the same MIC value obtained in this thesis for low MW chitosan for the mentioned fungus. Goy et al. (2009). The bacterial effectiveness on gram-positive or gram-negative bacteria is however, somewhat controversial. Some authors have stated that chitosan generally showed stronger effects for gram-positive bacteria whereas some other articles have been demonstrated that hydrophilicity ingram-negative bacteria is significantly higher than in gram-positive bacteria, making them most sensitive to chitosan. These findings are confirmed by several in vitro experiments in which gram-negative bacteria appear to be very sensitive to chitosan, exhibiting increased morphological changes on treatment when compared to gram-positives (Goy et al., 2009). For chitosan the results showed that CCs has antifungal activity against T. rubrum presenting MICs 1 mg/ml and MFC 2mg/ml where Cs-NPs didn't show any affect to the tested concentrations(I Lopes et al., 2017) summarized The MICs of high, medium and low MW chitosans towards T. rubrum and M. canis were determined. The MICs values were the same for HMW (1.3 mg/mL) and MMW (1.6 mg/mL) chitosan for both T. rubrum and M. canis. With LMW chitosan, significant differences were observed for both fungi, where T. rubrum required twice as much chitosan to be inhibited when compared to M. canis. So, the chitosan that showed the best inhibitory effect for M. canis was the LMW chitosan (1.1 mg/mL) whereas high HMW chitosan was the chitosan with the best inhibitory effect towards T. rubrum. Besides MICs determination, MFCs were also determined for T. rubrum and M. canis MFC values of 1.3 mg/mL for HMW chitosan for both fungi, 1.6 mg/mL for MMH chitosan for both fungi and 2.2 mg/mL for LMW chitosan for T. rubrum and 1.1 mg/mL for LMW chitosan for M. canis were found to be the same as the MIC values for both fungi. He added also that he believed that chitosan's cationic nature and high molecular mass helps in its antifungal action, since it interferes with negatively charged residues of macromolecules on the fungal cell surface, thus causing changes in cell membrane permeability .Balicka-Ramisz et al. obtained a MIC value of 1.1 mg/mL for M. canis using chitosan with a DD degree greater than 80% and a MW in the range of $4.0 \times 10^5$ to $5.0 \times 10^5$, corroborating the MIC value obtained in this study

for LMW chitosan for the mentioned fungus. These authors also determined the MIC of chitosan for another dermatophyte fungus, T. mentagrophytes and reached the value of 2.2 mg/mL.

## 7. Conclusion

It has been hypothesized that encapsulated extracts of Cannabis sativa are more effective against to human skin pathogens comparing to non-encapsulated extracts. Moreover, according to my best knowledge and investigations this is the first study with encapsulated cannabis against microbials causing skin diseases, generally these findings can further be tested so far, and based on these results, it´s possible to conclude that Cannabis extract has an antifungal action against T. rubrum. Another conclusion we can draw from this study is that cannabis extract is a more efficient antifungal and anti-microbial than other antimicrobial agent used in this study. It can therefore be stated that the hypothesis need still investigation, but the objectives of the work was fulfilled. The results of the MICs and MBCs of cannabis sativa in both cases indicated that non-encapsulated extract had an inhibitory effect on all tested bacterial, and fungal strains although the inhibitory effect of clean cannabis is higher than the one with capsulated. Regarding the effect of chitosan of the tested, dermatophytes and bacteria, chitosan showed a better effect on the dermatophytes which was the opposite of what was found in the bacterial strains and chitosan substances have an in vitro inhibitory effect to T. rubrum.

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## 9. List of the abbreviations used in the thesis

| | |
|---|---|
| **AD** | Atopic dermatitis |
| **Ag- NPs** | Silver nanoparticles |
| **ARG** | Antimicrobials resistance genes |
| **AMR** | Antimicrobial resistance |
| **ATB** | Antibiotics |
| **CWPS** | Cell wall polysaccharide |
| **CLSI** | Committee of Clinical Laboratory Standards Institute |
| **CS** | Chitosan (CS) |
| **CoNS** | Coagulase-negative species |
| **CA-MRSA** | Community-Associated Methicillin-Resistant *Staphylococcus aureus* |
| **CBDA** | Cannabidiolic acid |
| **CBE** | Cannabielsoin |
| **CBN** | Cannabinol |
| **CBD** | Cannabidiol |
| **CBG** | Cannabigerol |
| **CBL** | Cannabicyclol |
| **CFU** | Colony-forming unit |
| **CS-NPs** | Chitosan nanoparticles |
| **CZ** | Czech Republic |
| **CCM** | Czech Collection of microorganisms |
| **DD** | Degree of deacetylation |
| **DNA** | Deoxyribonucleic acid |
| **EOs,** | Essential oils |

| | |
|---|---|
| **GAS** | Group A *Streptococcus* |
| **HIV /AIDS** | Human immunodeficiency virus) (acquired immunodeficiency syndrome |
| **HMW** | High molecular Weight |
| **LMW** | Low molecular weight |
| **MMW** | Medium molecular weight |
| **MRSA** | Methicillin-resistant *Staphylococcus aureus* |
| **MBC** | Minimum bactericidal concentration |
| **MFC** | Minimum fungicidal concentration |
| **MIC** | Minimal inhibitory concentration |
| **MHB** | Mueller Hinton broth |
| **PGA** | Poly-$\gamma$-glutamic acid |
| **PH** | Power of hydrogen |
| **PNAG** | Poly-N-acetyl-$\beta$-(1-6)-glucosamine |
| **PIA** | Polysaccharide intercellular adhesin |
| **RPM** | Revolutions per minute |
| **RPMI** | Roswell Park Memorial Institute |
| **RTI** | Respiratory tract infections |
| **SSTIs** | Skin and soft-tissue infections |
| **SDA** | Sabouraud Dextrose Agar |
| **SGR** | Sebaceous gland-rich |
| **NaOH** | Sodium hydroxide |
| **THCA** | Tetrahydrocannabinolic acid |
| **TPP** | Tripolyphosphate |
| **$\Delta$9-THC** | $\Delta$9-tetrahydrocannabinol |

| UK | United Kingdom |
|---|---|
| UTI | Urinary tract infections |
| ZnO –NPs | Zinc oxide nanoparticles |

## 10.List of tables

## 11.List of figures