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ORIGINAL ARTICLE / ÖZGÜN MAKALE

Antimicrobial activity studies of encapsulated hemp seed oil

Enkapsüle edilmiş kenevir tohumu yağının antimikrobiyal aktivite çalışmaları

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Abstract: In this study, it was aimed to evaluate to what extent the oil obtained from the seeds of the C. sativa plant affects the antimicrobial activity of liposome and liposome-gel formulations that will increase the stabilization, absorption and dispersion effect.

Material and Methods: Hemp Seed Oil was used as an antimicrobial agent in our study. C. albicans was investigated as a microorganism. Eight different formulations were prepared from cannabis seed oil and the effect was examined by the spread plate method.

Results: The most effective results were observed in the liposomal formulation containing Carbopol® Ultrez 30 compared to the formulation applications and control groups of Cannabis Seed Oil. In a smear sowing study with C. albicans, it was determined that the growth of Cannabis Seed Oil formulations and control groups was limited during one-week examinations.

Conclusions: It was determined that the prepared Cannabis Seed Oil formulations had a highly inhibitory effect on the proliferation of C. albicans used in the study. Considering these results, it will lay the groundwork for studies that benefit from Cannabis Seed Oil and C. albicans.

Keywords: Antimicrobial activity, Candida albicans, Hemp seed oil, Liposome, Liposome-gel

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Öz

Amaç: Bu çalışmada Cannabis sativa bitkisinin tohumlarından elde edilen yağın stabilizasyon, emilim ve dağılım etkisini arttıracak lipozom ve lipozom- jel formülasyonlarının antimikrobiyal etkinliğini ne ölçüde etkilediğini değerlendirmek amaçlanmıştır.
Gereç ve Yöntem: Çalışmamda antimiktobiyal etken olarak Kenevir Tohumu Yağı kullanıldı. Mikroorganizma olarak Candida albicans araştırıldı. Kenevir tohumu yağından 8 farklı formülasyon hazırlanarak yayma plak yöntemiyle etki incelendi.
Bulgular: Kenevir Tohumu Yağının formülasyon uygulamaları ve kontrol gruplarına göre en etkili sonuçlar, Carbopol® Ultrez 30 içeren lipozomal formülasyonda gözlendi. C. albicans ile yapılan yayma ekim çalışmasında, Kenevir Tohumu Yağını formülasyonları ve kontrol gruplarının bir hafta süresince yapılan incelemelerde çoğalmasının sınırlandığı belirlenmiştir.
Sonuç: Hazırlanan Kenevir Tohumu Yağı formülasyonları, çalışmada kullanılan C. albicans'a çoğalmasını büyük oranda önleyici etkisi olduğu tespit edildi. Ulaşılan bu sonuçlara bakıldığı zaman, Kenevir Tohmu Yağının ve C. albicans' ın faydalanıldığı çalışmalara zemin hazırlayacaktır.

Anahtar Kelimeler: Antimikrobiyal Aktivite, Kenevir Tohumu Yağı, Candida albicans, Lipozom, Lipozom- jel

INTRODUCTION

C. sativa is a plant with a long history of medicinal use, dating back to ancient times. The plant is a member of the Cannabaceae family and is cultivated on a wide scale around the world (1). Its medicinal use is known to have begun long before modern times. Its mention in Chinese sources dating back to before the Common Era indicates that hemp has been used for centuries. Its integration into Western medicine occurred in the early 19th century. The plant has been used in the treatment of various diseases such as pain, glaucoma, oedema, inflammation, dysmenorrhea, malaria, nausea, and depression (2). Hemp (*C. sativa*) is defined as an annual, herbaceous, dioecious plant. Its growth potential is considerable, with the capacity to increase in height from half a metre to six metres. Each node on the stem produces leaves consisting of 3-11 leaflets. The arrangement of the leaves is such that they are positioned opposite each other on the lower part of the stem and alternately on the upper part (3).

Two subspecies of the *C. sativa* species have been identified. These are identified as *C. sativa L. Subsp. sativa* and *C. sativa* L. Subsp. indica (4). *C. sativa L. subsp.* sativa is cultivated for fibre or seed production and has low levels of the main psychoactive component of cannabis, Δ-9 tetrahydrocannabinol (THC). In contrast, *C. sativa* L. subsp. indica has a relatively higher THC content and is primarily cultivated for this purpose. *C. sativa L.* subsp. sativa plants are taller than *C. sativa* L. subsp. indica and, when cultivated outdoors, possess broader, darker green leaves that mature earlier and are denser (5). Cannabis has been demonstrated to exhibit a high level of productivity in the synthesis of secondary metabolic compounds. The

plant contains a plethora of components, numbering over 500. A plethora of alkenes, in addition to nitrogen compounds, flavonoids, and other complex compounds, have been identified in cannabis. Terpenes are abundant and contribute to the plant's characteristic odour (6).

The plant also contains resin, in addition to a negligible quantity of essential oil. The active chemical drug content is species-specific and is referred to as cannabinoids. The two phytocannabinoids that have been identified as the principal agents responsible for the effects of the plant are cannabidiol (CBD) and delta-9 tetrahydrocannabinol (Δ9-THC) (7). The effects of cannabis on the central nervous system are primarily attributable to the presence of Δ9-THC. The most significant components of the plant that are involved in cannabinoid production are the epidermal glandular organs, which exhibit variations in size, shape, and population density depending on their anatomical location (8).

The antimicrobial properties of *C. sativa* have been documented. It has long been established that hemp contains cannabinoids, whose potential for combating antibiotic resistance has yet to be investigated. Cannabidiol, cannabichromene, cannabigerol, Δ9-hydrocannabinol and cannabinol have demonstrated significant activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strains (9). Cannabidiol has been identified as an effective component of hemp oil against Gram-positive bacteria and yeasts (10). *C. sativa* chemotypes cultivated at northern latitudes have been shown to exhibit a heightened cannabidiol/tetrahydrocannabinol ratio, resulting in augmented antimicrobial potency (11). In 1998, the Canadian government implemented regulatory measures pertaining to the cultivation and commercialisation of hemp, with the stipulation that the content of delta-9-tetrahydrocannabinol (Δ9-THC) in industrial hemp must be less than 0.3%, and that the content of Δ9-THC in hemp products must be less than 10 micrograms per gram (12). The European Union, in a law drafted in 1989, permitted the cultivation of hemp plants with a Δ9-THC content below 0.3%. While 60% of global hemp production occurs in Europe, France is the largest producer of hemp seeds (13).

Hemp seed oil

Hemp seeds are 4-5 mm in diameter, spherical in shape, and grey in colour (14). Hemp Seed Oil, obtained by cold pressing the seeds, is a stable oil that is greenish-brown in colour, has a slightly spicy taste, and dries quickly. Its shelf life is limited and it rapidly deteriorates when exposed to air (2). Despite the challenges in distinguishing industrial hemp seeds and plants from cannabis, regions where hemp is cultivated for fibre production can be readily identified. The planting configuration for industrial hemp seeds is characterised by a high density, with a narrow spacing between plants, with the objective of promoting fibre yield and restricting leaf growth. In contrast, hemp cultivated for seed production is planted at a greater distance, allowing for a more expansive growth environment (8). Hemp seed contains approximately 35% fixed oil and resin. It is notable that the seed does not contain alkaloids. The fruit is rich in beta-carotene, vitamins, and minerals. Hemp seed contains 20-25% protein, 20-30% carbohydrates, 25-35% fat, 10-15% insoluble fibre, and is rich in minerals (especially phosphorus, potassium, magnesium, sulfur, and calcium). Hemp seed oil is characterised by a high polyunsaturated fatty acid content, ranging from 72% to 83%. The oil is characterised by a high content of essential fatty acids, including linoleic (ω-6) and linolenic acids. The predominant fatty acid in hemp seed oil is linoleic acid, comprising between 50% and 70% of the total fatty acid composition. The α-Linolenic acid (ALA) content of the oil is approximately 15–34% (15).

The two primary proteins found in hemp seeds are edestin and albumin. Both of these

high-quality storage proteins are easily digestible and contain all essential amino acids in nutritionally significant amounts. Moreover, hemp seeds have been shown to contain high levels of arginine (16). Hemp seeds have a long history of utilisation as a foodstuff or for the extraction of oil and the addition of flavour to food during the cooking process. The seeds are also used in the soap, cosmetics, linseed, varnish, and oil paint industries. Hemp seeds have been shown to be rich in unsaturated fats and protein. A 100 g portion of seeds has been shown to provide 63% of the recommended daily value for protein (17). Hemp seed flour provides a sufficient protein intake for individuals following a vegetarian diet. In addition to its nutritional value, hemp seed has a long history in folk medicine and as an animal feed, with traditional uses including the management of cholesterol and hypertension. The utilisation of hemp seeds in traditional Eastern medicine has a long and extensive history, spanning millennia, with the seeds being employed to treat a wide range of diseases. Hemp seed oil is characterised by a superior flavour profile when compared with other vegetable oils, and it possesses numerous advantages as a food source. Recent clinical trials have determined that hemp oil is a functional food (18). A study has shown that natural essential oils exhibit high antibacterial activity. This indicates that the antimicrobial potential of plant oils, such as hemp seed oil, can be enhanced through encapsulation (19).

Microorganisms

Candida albicans

The genus Candida belongs to the family Cryptooccaceae. The most commonly known species of the genus Candida are *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis* (20). It is important to note that certain species are an integral part of the normal flora, and only 10% are known to cause infections in humans (21). These organisms have the capacity to cause infections in various anatomical sites, including the blood, mucous membranes, skin, nails, and multiple systemic areas. In the context of systemic infections, *Candida spp*. has been observed to predominantly trigger acute suppurative inflammation, at times giving rise to abscesses comprising polymorphonuclear and mononuclear cells (9). In patients with neutropenia, suppurative reactions are superseded by coagulative necrosis. *Candida spp*. have also been observed to invade blood vessels, resulting in infarction (22). All Candida species form single chains or small, round or oval budding yeast cells (23). One of the most significant human fungal pathogens and a causative agent of life-threatening systemic infections is *C. albicans* (24).

C. albicans is the most common cause of candidiasis, an acute, subacute, or chronic infection affecting any part of the body. This organism is typically present as part of the flora on the skin, in the mouth, vaginal area, and gastrointestinal tract (25). The yeast *C. albicans* has been observed to undergo rapid growth and complete maturation within a period of three days. The colony is characterised by a creamy pigmentation, a paste-like consistency, and a smooth appearance. Colonies are green on CHROMagar Candida. Under routine light microscopy, yeast cells manifest as either round or oval in shape. Media such as SDA and blood agar are commonly utilised in laboratories for the examination of Candida species. Colonies cultivated on SDA media manifest as cream-coloured and doughy in appearance. The distinguishing characteristic of *C. albicans*, when evaluated through the germ tube test, sets it apart from other Candida species. The test involves the incubation of microorganisms in serum at a temperature of 37 °C for a period of 2 hours (26). Subsequent to the process of incubation, the formation of germination tubes within the serum serves to distinguish *C. albicans* from other species (27). It is estimated that there

are over 200 species of Candida. *C. albicans* has been demonstrated to be the causative agent of a greater number of diseases in comparison to other species. It has been identified as the most significant causative agent of fungal infections in the human digestive system, vascular system, vagina, and esophagus (28).

MATERIAL AND METHODS

Encapsulation of samples

Encapsulation can be defined as the process of producing particles at the millimetre, micrometre or nanometre scale by incorporating an active substance into a different structure. The nomenclature employed for these particles varies depending on their size, with the terms "nanocapsules," "microparticles," and "macroparticles" being used to describe them (29). In general, the active substance is referred to as the inner phase, while the structure forming the capsule is called the matrix, outer phase, or shell. The substance in question is typically a liquid, although it may also manifest as a gas or a solid (30). The process of microencapsulation facilitates the precise modulation of the rate of release of active substances. It is evident that microcapsules can manifest in a variety of forms. As Tomsone (2020) demonstrate, the diversity of microcapsule shape is a key factor in their ability to protect the material and enhance release efficiency (31). The development of encapsulation and controlled release systems has been a recent focus in order to protect the active structure from unwanted interactions (30). The encapsulation process is employed to enhance the stability of the encapsulated active substance, thereby serving as an effective protective agent against deleterious environmental factors (32). Furthermore, encapsulation confers a number of advantages, including the masking of undesirable tastes and odours, the preservation of volatile components, the control of the adjustable properties of the active ingredient (e.g. particle size, structure, solubility in oil or water, colour), and the facilitation of storage (33). Coating materials are utilised in the encapsulation process with a view to increasing the stability of bioactive substances. The properties of the coating materials utilised in the encapsulation process have a significant impact on the size, structure, and shape of the capsule particles. It is imperative that coating materials are utilised in a manner that ensures the stability and release of the product is controlled against the external environment during the production, storage, and consumption stages (34). In the selection of the type of encapsulation and coating material, particular attention should be paid to the physicochemical properties of the active ingredient, processing and storage conditions, capsule size, and density. The initial stage in the encapsulation process is the selection of the most suitable coating material. The selection of method and coating material is contingent upon the specific coating material or method that is to be employed. The selection of polymers, whether by natural or artificial means, is made in different variations according to the substance to be coated and the desired qualities in microcapsules. Protein and carbohydrate polymers are frequently employed in the encapsulation process (35). A plethora of methodologies are employed for the encapsulation of the active ingredient. The methods employed in this process encompass coacervation, the Wurster Method, spray drying, freeze drying, and the preparation of microcapsules through emulsion. The most common types of encapsulation are spray drying and freeze drying. The term "liposomal encapsulation" refers to the process of entrapping bioactive substances within liposomes. This is achieved by binding the bioactive substances to the phospholipid or aqueous portion of the liposome membrane, depending on the structure of the bioactive substances in question. (36).

Lipozoms

Liposomes are a subject of significant interest in the field of drug delivery systems due to their potential as a new generation of such systems. Liposomes are defined as spherical vesicles ranging in size from 30 nm to several micrometers. The composition of these

particles includes sterols and non-toxic phospholipids. Liposomes are characterised by the presence of both hydrophilic and hydrophobic groups within their structure. The general structure of liposomes is shown in Figure 1 below. A comparison of the structure and content of the subject in question with cell membranes reveals a certain degree of similarity. Phospholipids containing glycerol represent a significant component in the composition of liposomes (37). The classification of liposomes is based

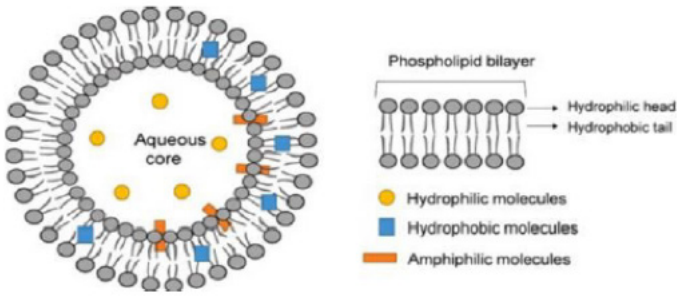

Figure 1. Illustration of the general structure of liposomes (38)

All methods employed for the production of liposomes are predicated on four fundamental steps. The procedure entails the following steps: firstly, the separation of lipids by drying organic solvents; secondly, the dispersion of lipids in an aqueous medium; thirdly, the purification of the obtained liposomes; and finally, the analysis of the resulting substance (39). A variety of methodologies are employed in the preparation of liposomes. The following methods are regarded as conventional liposome preparation techniques: thin film hydration, detergent removal, solvent injection, reverse phase evaporation, and emulsion. The thin film hydration method is one of the most commonly used in R&D. It is evident that traditional methodologies are accompanied by a number of disadvantages, including the necessity for substantial quantities of organic solvents, which can result in the toxicity of the resulting liposomes. Furthermore, all of these methodologies are multi-step processes, and involve downstream steps such as sonication or extrusion to obtain liposomes of the desired size with good homogeneity. The limitations of these methodologies pertain to their inability to ensure reproducibility and their non-GMP nature. The aforementioned disadvantages hinder the industrial-scale production of liposomes under GMP conditions (40). The synthesis of small unilamellar vesicles (SUVs) can be accomplished through a variety of methodologies, including sonication, French pressure cell, and microfluidization. LUVs are obtained through a variety of methods, including freeze-thaw cycles. The acquisition of MLVs is facilitated by thin lipid film hydration and dialysis methodologies (37).

The thin lipid hydration method involves the formation of a thin lipid film in a spherical vial by means of solvent removal. The formation of heterogeneous liposomes occurs as a result of the addition and subsequent agitation of the dispersion medium (41). Sonication is the most prevalent method for preparing SUVs. In this method, MLVs are sonicated under a passive atmosphere using a bath-type sonicator or a probe sonicator (42). The drawbacks of this method are manifold, including but not limited to: the extremely low encapsulation rate, the potential degradation of the phospholipids and components to be encapsulated, the elimination of large molecules, metal contamination from the probe tip, and the inability to obtain uniform liposomes (43). There are two categories of sonication: probe and bath-type. In the bath-type sonication method, the liposome dispersion is placed in a cylinder, which is then placed in a bath sonicator. In this method,

the temperature of the lipid dispersion can be controlled with greater ease than in sonications performed using a direct tip. In probe sonication, the tip of the sonicator is immersed directly into the liposome dispersion. This method requires a significant amount of energy

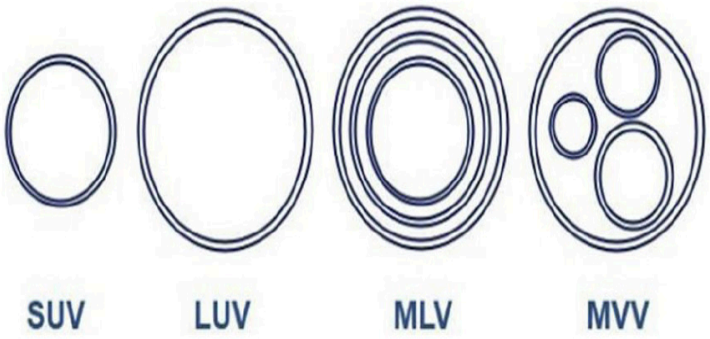

Figure 2. Types of liposomes according to size and lamellarity (45)

on various criteria, including their structure, size, and composition. The formation of multi-lamellar vesicles (MLV) and unilamellar vesicles (Small Unilamellar Vesicle = SUV, Large Unilamellar Vesicle = LUV) is contingent upon the preparation method employed. The classification of these liposomes is based on their composition and includes the following categories: traditional, pH-sensitive, cationic, fusogenic, immunoliposomes, and long-circulating liposomes (36).

Research conducted on the subject of liposome production has focused on physical, biological, and chemical characterisation. Chemical characterisation is the process of examining the concentration of structural elements. The biological characterisation of substances can be categorised into three distinct domains: sterility, pyrogenicity, and animal toxicity (37). Conversely, physical characterisation is contingent on variables such as size, vesicle shape, surface morphology and charge, lamellarity (number of multilayers), phase behaviour, and encapsulation efficiency (46). Liposomes have found widespread application in the cosmetic and pharmaceutical industries. The utilisation of liposomes has been demonstrated to engender favourable outcomes, including a reduction in adverse effects, targeted delivery, and a slow and prolonged drug release. Moreover, research is underway to explore the potential of these materials in the development of delivery systems capable of encapsulating unstable compounds and preserving their functionality, with particular relevance to the food and agricultural sectors. The increased utilisation of liposomes as drug delivery systems and in commercial applications can be attributed to their low biodegradability, drug capture capabilities, and ability to simplify drug delivery to tumour tissues (47). Ozen et al. conducted a study investigating ways to increase the anti-biofilm activity of vegetable oils using liposomal encapsulation. The study shows that hemp seed oil can acquire strong antimicrobial properties using the same technique (48). A study conducted by Ethemoglu et al. demonstrated that liposomal encapsulation of resveratrol increases its bioavailability, indicating that biologically active oils such as hemp seed oil can be made more effective through encapsulation (49).

Notwithstanding the numerous advantages of liposomes, their low physical stability imposes limitations on their potential benefits. A promising approach to minimise this limitation involves the provision of mechanical support for the lipid membrane. The incorporation of a polymeric structural framework into the liposomal core has been demonstrated to enhance physical stability, thereby increasing the stability of the

liposome. Examples of polymeric materials employed for this purpose include poly(lactic-co-glycolic) acid (PLGA), poly(acrylic acid) (PAA), Carbopol®, and alginate. The synthetic polymer poly(ethylene glycol) (PEG) has been identified as a material with considerable potential for use in this application. PEG hydrogels are extensively utilised in biomedical applications, including tissue engineering and drug delivery, due to their capacity to exhibit adjustable physical and chemical properties. PEG-diacrylate (PEG-DA) hydrogel has been demonstrated to function as an effective scaffold within liposomes (50). Hydrogel-liposome formulations, designated as lipobeads, are regarded as a novel category of nanocapsule. These formulations combine the biocompatible surface properties of liposomes with the mechanical stability and higher loading capacity of the polymer network. This combination serves to expand the potential of nanocapsules for pharmaceutical applications, biomimetic sensory systems, and controlled release fields (51).

Carbopol® Ultrez 21 Polymer and Carbopol® Ultrez 30 Polymer are frequently utilised in the context of hydrogel liposome formulations. Carbopol® Ultrez 30 Polymer is a white powder, cross-linked polyacrylic acid homopolymer (52). It has been demonstrated to exhibit good electrolyte tolerance and a wide pH range. Carbopol® Ultrez 30 Polymer has been demonstrated to achieve the desired rheological and aesthetic properties in the presence of electrolytes or acidic reagents, which are required for the formulation (53). Its high electrolyte tolerance and wide pH compatibility make it ideal for formulation with water-soluble salts and acidic components such as alpha hydroxy acids and salicylic acid at low pH ranges. Carbopol® Ultrez 21 Polymer is a polymer that has found application in a variety of personal care products. The polymer exhibits rapid and facile wetness when applied in isolation. The substance exhibits elevated viscosity, attributable to its diminished flow characteristics. This polymer demonstrates a broad pH range (54). HELIOGEL™ is a gelling agent with emulsifying properties. It contains Helianthus annuus seed oil, Sodium Acrylate Copolymer, Hydrogenated Polyisobutene, Phospholipids, and Polyglyceryl-10 Stearate. The composition of the substance under scrutiny includes sunflower phospholipids, as evidenced by Pozo-Antonio (2016) (55).

METHODS

Preparation of hemp seed oil

Hemp Seed Oil has been procured commercially. The production of hemp seed oil has been undertaken using the cold-press method. The oil is manufactured by GENÇAY BAHARAT.

Preparation of hemp seed oil-loaded liposome and liposome-gel formulations

Initially, 3 g of soy lecithin and 3 g of cholesterol were introduced into a balloon flask, where they were dissolved in the specified solvent. Subsequently, the solvent contained within the prepared solution was subjected to evaporation via a rotary evaporator, after which the solution was subjected to a series of processes. The duration of this process was approximately 30 minutes. The resulting product was then stored in a refrigerator. The product was designated

Figure 3. Preparation and appearance of Carbopol® Ultrez 21

HB. The following day, HB was treated separately with hemp oils at concentrations of 1%, 2%, and 5%. Liposomes loaded with hemp seed oil at varying concentrations were obtained. The production of liposomes involved a series of experimental procedures, including sonication in an ultrasonic water bath, stirring, and the utilisation of a homogeniser. In order to obtain the gel, 0.5 g of Carbopol® Ultrez 21 was weighed and mixed with approximately 80 mL of water using a mechanical mixer. Subsequently, a formulation comprising HB, Carbopol® Ultrez 21, and 5% hemp seed oil was prepared. In the second experiment, 0.5 g of Carbopol® Ultrez 30 was weighed and processed in 80 mL of water using a mechanical mixer. The homogeniser was utilised in the process. The formulation under consideration comprised HB, Carbopol® Ultrez 30, and 5% hemp seed oil.

RESULTS

The positive control group was designated as HB. In addition, the antimicrobial efficacy of Carbopol® Ultrez 21 and Carbopol® Ultrez 30 was examined. Five different formulations were prepared. These are: 1. HB+ 1% Hemp Seed Oil (A), 2. HB+ 2% Hemp Seed Oil (B), 3. HB+ 5% Hemp Seed Oil (C), 4. HB+ Carbopol® Ultrez 21+5% Hemp Seed Oil (D), 5. HB+ Carbopol® Ultrez 30+5% Hemp Seed Oil (E). The preparation step for Carbopol® Ultrez 21 is shown in Figure 3 above.

Analysis methods

Physical Analysis of Formulations

The five formulations prepared (A, B, C, D, E), the positive control group (HB), Carbopol® Ultrez 21, and Carbopol® Ultrez 30 were subjected to physical analysis. In the physical analysis, viscosity, temperature, and pH values were examined and compared.

Determination of the antimicrobial effects of hemp seed oil-loaded liposomes and liposome-gel formulations

Sabouraud Dextrose Agar medium was prepared and sterilised in an autoclave at 121°C for 15 minutes. Subsequent to its removal from the autoclave, the medium was cooled in a water bath. The pH level was measured. The prepared medium was then transferred into eight Petri dishes, where it was left to solidify.

C. albicans strains were cultivated in 100 ml of Sabouraud dextrose agar (SDA). The samples were then subjected to an incubation process at a temperature of 23°C for a duration of one day. The strains were then prepared for placement within the tube. The tube was labelled "C. albicans," and 2 ml of liquid medium was transferred to the labelled tube. The McFarland 0.5 Standard solution tube was then placed adjacent to the tube containing the medium. Physiological serum was added to the medium until the turbidities were equal, and the mixture was then agitated using a vortex. The solutions were then subjected to an incubation process at a temperature of 23 ± 2°C for a duration of 15 minutes.

Diffusion Plate Test Application

Eight Petri dishes containing SDA were prepared for *C. albicans*. The nomenclature and composition of the formulation were inscribed on the Petri dishes. A sterile cotton swab was used to streak the edge of the tube with a *C. albicans* suspension adjusted to the 0.5 McFarland standard. The swab was then streaked across the entire surface of the agar. The Petri dishes were then permitted to dry at room temperature. Subsequent to the drying of the agar, the prepared formulations, the control group, Carbopol® Ultrez 21, and Carbopol® Ultrez 30 were applied over the surface with a swab stick. The Petri dishes were then subjected to an incubation process at a temperature of 23 ± 2°C.

Diffusion Plate Test Evaluation

Commencing from the day on which the formulation was prepared, the petri dishes were subject to daily inspection in order to identify any alterations. The days on which colony formation was observed were meticulously recorded.

Table 1. Physical analysis values of formulations

formulations	temperature (^{0}C)	pH	Viscosity (cP)
HB	25,6	5, 083	5200
U 21	25,5	5, 509	39700
U 30	25,3	4, 672	40300
A	24,3	4,990	73800
B	24,2	4,986	81300
C	25,2	5, 064	60700
D	24,2	5,037	49600
E	24,3	4, 693	47200

(A: HB+ %1 KTY, B: HB+ %2 KTY, C: HB+ %5 KTY, D: HB+ U 21+ %5 KTY, E: HB+ U 30+ %5 KTY)

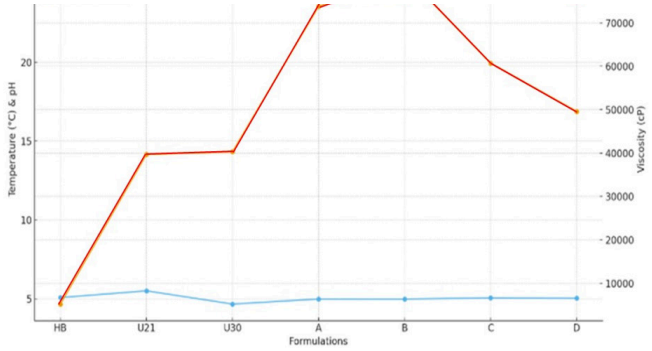

Figure 4. Physical analysis values of formulations

Physical analysis results of formulations

The study encompassed the execution of five distinct formulations, the incorporation of a positive control group, and the physical analysis of U21 and U30. The ensuing analysis yielded a comparison of the viscosity, temperature, and pH values of the formulations, as demonstrated in the subsequent table 1.

The viscosity of hemp seed oil in different formulations was found to be highest in the C formulation, while the lowest viscosity was observed in the control group HB. With regard to pH, U 30 was found to be the most acidic, followed by the E formulation. The rationale behind the necessity for an acidic pH level is to impede the proliferation of microorganisms, with a particular emphasis on *C. albicans*. The graph related to the physical analysis of the formulation is shown in Figure 6 above.

Diffusion plate test results for encapsulated hemp seed oil

The diffusion plate method was utilised to assess the antimicrobial activity. The Petri dishes were monitored on a daily basis, and any alterations in the agar were meticulously documented. The results of the antimicrobial activity are shown in the following table 2. The positive control group Petri dish exhibited signs of growth from the third day onwards. As demonstrated in Table 4.2, formulation E exhibited a more pronounced effect on *C. albicans* than the other formulations, with colony formation only occurring on the final day of the week.

Table 2. Antimicrobial effect results of formulation

DAYS	KG (HB)	A	B	C	D	E	U 21	U 30
Day 1	-	-	-	-	-	-	-	-
Day 2	-	-	-	-	-	-	-	-
Day 3	+	+	-	-	-	-	-	-
Day 4	+	+	+	-	-	-	+	-
Day 5	+	+	+	+	-	-	+	+
Day 6	+	+	+	+	+	-	+	+
Day 7	+	+	+	+	+	+	+	+

(+: Reproduction observed, -: No reproduction)
(A: HB+ %1 KTY, B: HB+ %2 KTY, C: HB+ %5 KTY, D: HB+ U 21+ %5 KTY, E:HB+ U 30+ %5 KTY)

DISCUSSION

In the study, encapsulated hemp seed oil formulations were examined, with physical analysis and the spread plate method being utilised in the process. All tests conducted demonstrated that the encapsulated hemp seed oil used exhibited a significant inhibitory effect against *C. albicans*. The antimicrobial efficacy of liposomal systems prepared at varying concentrations increased concomitantly with the increase in concentration. However, the antimicrobial effect increased significantly with the incorporation of Carbopol® Ultrez 30 and Carbopol® Ultrez 21 into the formulation. The liposomal formulation of hemp seed oil has been demonstrated to enhance both its stability and the duration of its effects. The spread plate method was selected for the examination of the antimicrobial activity of the formulations. Following a week of studies, growth was observed on day 7 in the petri dish containing the Carbopol® Ultrez 30 formulation in comparison to other formulations. The most efficacious formulation in opposition to *C. albicans* is Carbopol® Ultrez 30. The petri dish containing the formulation with 5% hemp seed oil demonstrated reproduction at the conclusion of the fifth day, while reproduction was observed earlier in the petri dishes containing formulations with 1% and 2% hemp seed oil. No reproduction was observed over a prolonged period in formulations containing Carbopol® Ultrez 30 and Carbopol® Ultrez 21. The formulation containing Carbopol® Ultrez 30 demonstrated the longest period without growth. It was determined that the incorporation of the excipient yielded a more pronounced effect in comparison to the liposome-gel formulations, thereby impeding fungal proliferation. It was determined that the liposomal-gel formulation of hemp seed oil increased the inhibitory effect. As posited by Gunal et al., the liposomal encapsulation of resveratrol has been demonstrated to significantly enhance tissue regeneration. The biological activity of bioactive lipids, such as hemp seed oil, following encapsulation has been identified as a contributing factor to this effect (56). The finding that liposomal carriers provide a significantly higher release rate and bioavailability than conventional formulations may enhance the antimicrobial efficacy of encapsulated hemp seed oil (57). As Appendino et al. (2008) stated in their study, the secondary metabolites of *C. albicans* demonstrated significant activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strains (9). However, due to the absence of these metabolites in hemp seed oil, the necessary research could not be conducted. VanDolah (2019) defined cannabidiol as a component of hemp oil that has been demonstrated to be effective against Gram-positive bacteria and

yeasts (10). The study did not ascertain the identity of the substance responsible for the observed antimicrobial effect.

CONCLUSSION

The present study investigated the effect of liposomal formulations containing hemp seed oil and the addition of excipients to these formulations on the *C. albicans* strain. It was observed that the inhibition levels of liposomal formulations containing hemp seed oil changed proportionally. The antimicrobial effect of the liposomal gel formulation was found to be enhanced by the addition of an excipient to the encapsulated hemp seed oil. In view of the findings obtained, the development of new liposome gel formulations from hemp seed oil will provide further insight into its use in *C. albicans* infections. The findings of this study are expected to be applicable in cosmetics and other fields in the future.

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