Republic of Iraq

Ministry of Higher Education

and Scientific Research

University of Misan

College of Science

Department of Chemistry

Study The Drug Delivery System of Clove Oil and it's Role in some Biological Activities

A Thesis Submitted to

The College of Science / University of Misan as Partial Fulfillment of the Requirements for the Master Degree of Science in Chemistry

By

Mariam Kadhim Taher

B.Sc. Chemistry / Misan University (2020)

Supervisors

Asst. Prof. Dr. Israa Qusay Falih

Asst. Prof. Dr. Younus Jasim Abdullah

2024 A.D ***1446 H.D***

بسم الله الرحمن الرحيم

﴿يرفع الله الذين آمنوا منكم والذين أوتوا العلم درجات والله بما تعلمون خبير﴾

صدق الله العلي العظيم

المجادلة (١١)

Supervisor Certification

We are the supervisors of **Ms. Mariam Kadhim Taher**, certify that the thesis (**Study The Drug Delivery System of Clove Oil and it's Role in some Biological Activities**) was done and written under our supervision as a partial fulfillment of the requirements for the Master of degree of Science in Chemistry.

Signature:

Asst. Prof. Dr. Israa Qusay Falih

Chemistry department

College of science/ Misan University

Date: / / 2024

Signature:

Asst. Prof. Dr. Younus Jasim Abdullah

Northern Technical University

Date: / / 2024

Head of Chemistry department Recommendation

According to the recommendation of supervisors, this thesis if forwarded to the examination committee for approval.

Signature:

Asst. Prof. Dr. Usama Ali Muhsen

Head of Chemistry Department

Date: / / 2024

Dedication

To The Sun and Moon Which Lighting my Life by Pave the Way to my Success…

My Father & My Mother

To Those who have Supported me and are Waiting for my Success…

My little family my wonderful husband "hamza" and my son "al-hassan"

To Those who have Given me their Time and Knowledge…

My Supervisors

Maryam

Acknowledgements

Firstly, very much thanks for Allah the most merciful for his blessings, who gave me health, strength, and facilitated the ways for me to accomplish this work, and the prayer and peace of Allah be upon our Master and prophet Muhammad and his divine good family.

I would like to express my sincere gratitude to my supervisors**. Assistant Professor Dr. Israa Qusay Falih** and **Assistant Professor Dr. Younus Jasim Abdullah** for highly inspiring guidance, patience, motivation, enthusiasm and immense knowledge, continuous support for completing this thesis

I also extend my thanks and gratitude to the Deanship of the College of Science and the Headship of the Chemistry Department

I would like to express my sincere thanks to **Assistant Professor Dr. Ali Taha Saleh** for help to complete my work.

All thanks to the who was with me and helped me in every problem, **Dr. Adel Karim**

Finally, I extend my thanks and gratitude to everyone who provided me with assistance and gave me advice, guidance, or invitations in the back of my mind.

Also, I will never forget the outstanding efforts and continuous support from my family throughout my studies.

Maryam

Contents

List of Figures

List of Table

List of Abbreviations

Abbreviations	Key
AIDS	Acquired Immune Deficiency Syndrome
CEO	Clove Essential Oil
CNE	Clove Nanoemulsion
CNG	Clove Nanogel
CNS	Central Nervous System
CMC	Carboxymethyl cellulose
°C	Celsius degree
DMSO	Dimethyl Sulfoxide
DPPH	Diphenyl-1-picrylhydrazyl
DLS	Dynamic Light Scattering
DDS	Drug delivery systems
ELISA	Enzyme-linked immunosorbent assay
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared
M_1	Glycerin percentage 0
M_2	Glycerin percentage 8
M_3	Glycerin percentage 20
M_4	Glycerin percentage 30
HER2	Human epidermal growth factor receptor-2
IBD	Inflammatory bowel disease
IL-1	Interleukin 1
IL-1RA	Interleukin-1 receptor antagonist
KDa	Kilo Dalton
LC	liquid crystal
LPS	Lipopolysaccharide
MTT	Methyl thiazolyl tetrazolium

MHA	Muller Hinton Agar
µg/ml	Microgram per milliliter
NMR	Nuclear magnetic resonance
NIU	Noninfectious uveitis
PPM	Part per million
PBS	Phosphate Buffered Saline
PAMPs	pathogen-associated molecular patterns
PsA	psoriatic arthritis
$PSLC_S$	precursors systems for liquid crystals
PEBBLE	Probes Encapsu-lated by Biologicall Localized Embedding
SDS	Sodium dodecyl sulfate
SiRNA	Small interfering RNA
SGF	Simulated gastric fluid
SIF	Simulated Intestinal Fluid
T h-1	T-helper -1
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor-α
λ_{max}	wavelength maximum

SUMMARY

Herbal materials have many biomedical applications, and nanogel-based herbal materials have high drug loading capacity. This study endeavors to a drug release system modelling was designed using a nanoemulsion-based gel of cloves oil. Clove oil was mixed with water in the presence of Tween-20 as a polysorbate-type nonionic surfactant, and then carboxy methylcellulose was added with different ratios of glycerin as a cross-linking agent to prepared nanogel formulations of clove essential oil (CEO). Our investigation focused on various aspects which includ in-vitro release, cytotoxicity, antioxidant effect, hemocompatibility, anti-inflammatory, and antibacterial activities. The characterization of the clove Nanogel (CNG) was confirmed using of techniques such as Ultraviolet spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), While nano nature of the CNG were identified through “Dynamic Light Scattering” (DLS), and “Field Emission Scanning Electron Microscope” (FESEM). The results indicated that CNG were highly pure and crystalline, exhibiting agglomerated and spherical particles. Additionally, UV-Vis maximum absorption wavelength 284 nm, while the DLS measurements for CNG were 276 nm, and poly disparity index were value 0.279. CNG release study at pH 1.2, 8.2 and 7 for 96 hours. The study also assessed the antioxidant activity of CNG by using the DPPH assay, which were showed a maximum inhibition of 65% at a concentration of 100µg/mL in clove nanoemulsion CNE, whereas CNG at the same concentration exhibited 59.2% of inhibition. The calculated IC50 values of CNG and CNE were 6.58 and 3.25 µg/mL respectively, as well as the hemocompatibility on erythrocytes and cell-cytotoxic properties through the MTT assay. CNG displayed a good biocompatibility with red blood cells (RBCs) in the hemolytic activity experiment, with no observed hemolytic reaction at

doses 7.5 to 120 µg/ml. Furthermore, the CNG exhibited antibacterial activity against *S.aureus* and *E.coli*. It also results showed that all concentrations of the clove nanogel have no effects on the cytokines secretions by macrophage cells line. This means that glove nanogel has no any inflammatory effects and not acting an antigen. Finally, the CNG displayed good inhibition processes in terms of antibacterial activity, antioxidant activity, and showed minimal cytotoxicity.

CHAPTER ONE

Introduction

1.1 Introduction

Despite the notable systematic progress achieved by therapeutic manufacturing, many individuals frequently resort to natural remedies and custom plants as cures. Clove essential oil (CEO) is a highly concentrated oil derived from the flower blossoms of the Syzgium aromaticum L. plant, which is a member of the Myrtaceae family. It is recommended for use in treating a variety of illnesses. Extracts from CEO have been shown to possess several biological actions, including antioxidant, anticoagulant, antiparasitic, antibacterial, antiviral, and antifungal properties [1, 2].

The considerable volatility, instability, and low solubility in water of this substance impose limitations on its processing, usage, and storage. Developing nanostructures for essential oils shows promise in enhancing their efficacy [3, 4]. Among the various Nano formulations, Nanoemulsions hold more significance. Because of its lower incidence of adverse effects and higher bioavailability, as well as its simpler manufacturing procedures [5-7].

Nanoemulsions are clear dispersals composed of two phases, oil, and water, which are alleviated by surfactants and co-surfactants. These systems exhibit stability during assembly or anointing procedures because their droplets are small, measuring fewer than 200 nm [8, 9]. Topical Nanoemulsion delivery systems have demonstrated positive attributes, such as improved permeability without causing of skin irritation [10, 11]. Nevertheless, Nanoemulsions enhance the efficacy of EOs by improving their topical and physical administration as well as their thermal stability [12, 13]. The potential for enhancing matter compounds before their commercialization or therapeutic application makes this method all the more attractive. Fewer adverse effects, more effectiveness and selectivity, protection from thermal or photodegradation, and active component

release control are all examples of such enhancements [14]. Immediate progress in nanoscience and nanotechnology, together with the advances achieved in pharmacological research at the last few decades, is necessary for the application of nanoscale materials, which have been utilized by the cosmetics industry. Modern scientific knowledge has the potential to radically improve and perhaps eliminate some of the most vexing problems with formulation preparation. Besides making active ingredients more soluble and stabilizing, nanostructures have the potential to lengthen the duration of a formulation's effects and enable the effective combination of active substances with varying degrees of hydrophilicity and lipophilicity. Another use of this technique is to direct drug delivery to certain organs or tissues [15].

Advances in nanotechnology have piqued the attention of the pharmaceutical industry, which stands to gain benefits such as customized release mechanisms and the possibility of creating novel formulations that were previously impossible [16]. Several nanotechnological strategies, such as polymeric nanoparticles, solid lipid nanoparticles (SLNs), liquid crystal (LC) systems, and precursors systems for liquid crystals (PSLCs), liposomes, and microemulsions, have attempted to break this barrier; they allow substances with different properties to be used in the same formulation, and may even change a substance’s behavior in a biological environment [17]. These technical advancements have transformed the delivery of drugs. The novel drug delivery techniques not only increase the strength of the active chemicals in a formulation, but they can also reinstate other ingredients that were removed because they were deemed unnecessary.

1.2 Drug Delivery Systems (DDS)

Formulating and storing drug molecules into appropriate forms, such as tablets or solutions, for administration is the job of drug delivery systems. By reducing the amount of medication that accumulates in areas other than the intended target, they increase the therapeutic effectiveness of the medications administered [18]. Not confined to the oral route of administration, there are several more ways that drugs may be administered into the body [19]. The delivery routes include buccal, sublingual, transdermal, subcutaneous, anal, and transvaginal, as well as nasal and ophthalmic [20]. Moreover, intravesical. The drug's physiochemical qualities and the changes it causes in the body's systems when ingested are attributed to the drug's components. Increased systemic circulation and management of the drug's pharmacological action have made DDS a valuable tool in the treatment of illnesses and enhancement of health during the last several decades. With the development of pharmacokinetics and pharmacology came the realization that the rate of drug release was a key factor in the efficacy of treatments, which led to the idea of controlled release [21]. Due to its substantial benefits over traditional medications, the controlled-release formulation of a medicine has garnered a lot of attention since its approval in the 1950s. It has an interval between doses and a set duration during which the medication is released. Another benefit is that regulated medication delivery systems may last for days or even years since they are not impacted by physiological factors. The medicine may be released at a constant or variable pace, and the delivery can be controlled spatially as well [22]. Furthermore, it reduces the toxicity of medicine while enhancing its solubility, accumulation at specific locations, efficacy, pharmacological activity, pharmacokinetic properties, patient acceptance, and adherence

Taken together, several studies regarding the release mechanisms of drugs in nanocarriers have been conducted. Diffusion, solvent, chemical reaction, and stimuli-controlled release are a few mechanisms that can represent the release of drugs in nanocarriers [23]. as shown in Fig. (1-1)

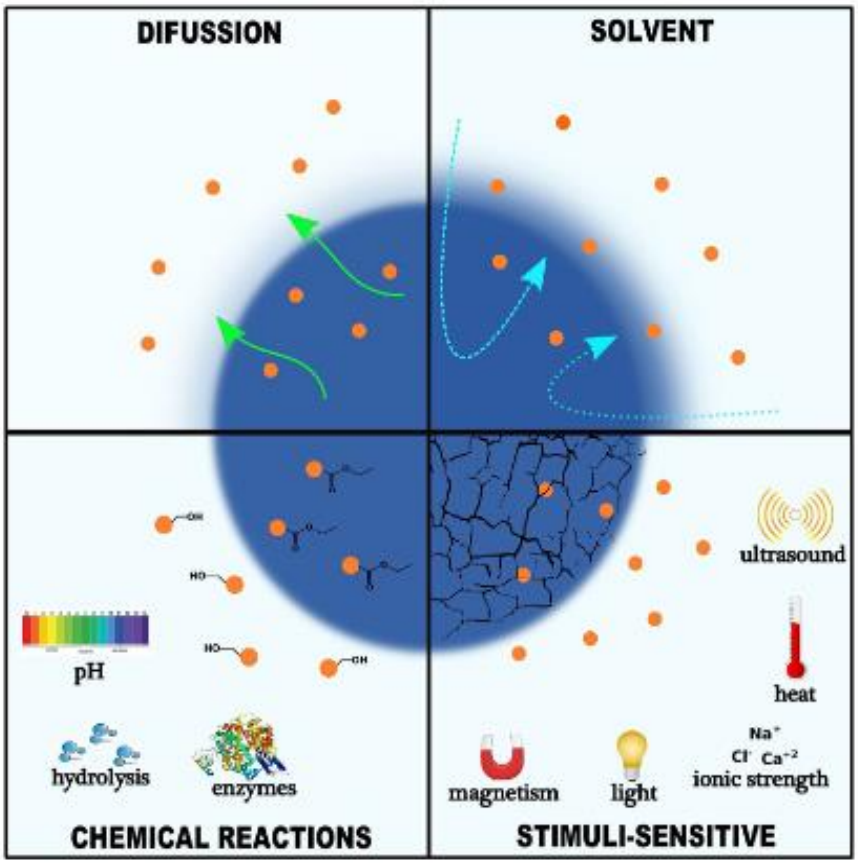


Figure (1-1): Mechanisms for controlled drug release using different nanocarriers [24].

1.3 Recent drug delivery systems

Extensive efforts have been dedicated to developing systems for drug delivery that use inorganic, organic, or hybrid nanoparticles to transport medications with a specific focus on chemotherapy. Several aspects of drug delivery systems (DDS) had been improved, which included the toxicity, prolonged release, efficacy, targeted distribution to particular sites, permeability, and solubility. Comparing the conventional dosage forms, can significantly improve the effectiveness of therapeutic substances [25,26]. The Current drug delivery systems are recognized as the latest breakthroughs and innovative comprehension of pharmaceutical pharmacokinetics and pharmacologic behavior, which is essential in formulating an optimal approach for drug administration. These drug delivery systems (DDS) operate as carriers, enabling the transportation of materials to the intended site of action and ensuring that the concentration of medication remains within the therapeutic range for a prolonged duration. The adoption of the delivery system is determined by the success of the invention in both medical and commercial aspects. Early involvement of patients in the development process would facilitate the identification of concerns and optimize the device's use. Advanced and less detrimental delivery systems are currently being explored. Figure (1-2) displays the many methods used for delivering medicine [27].

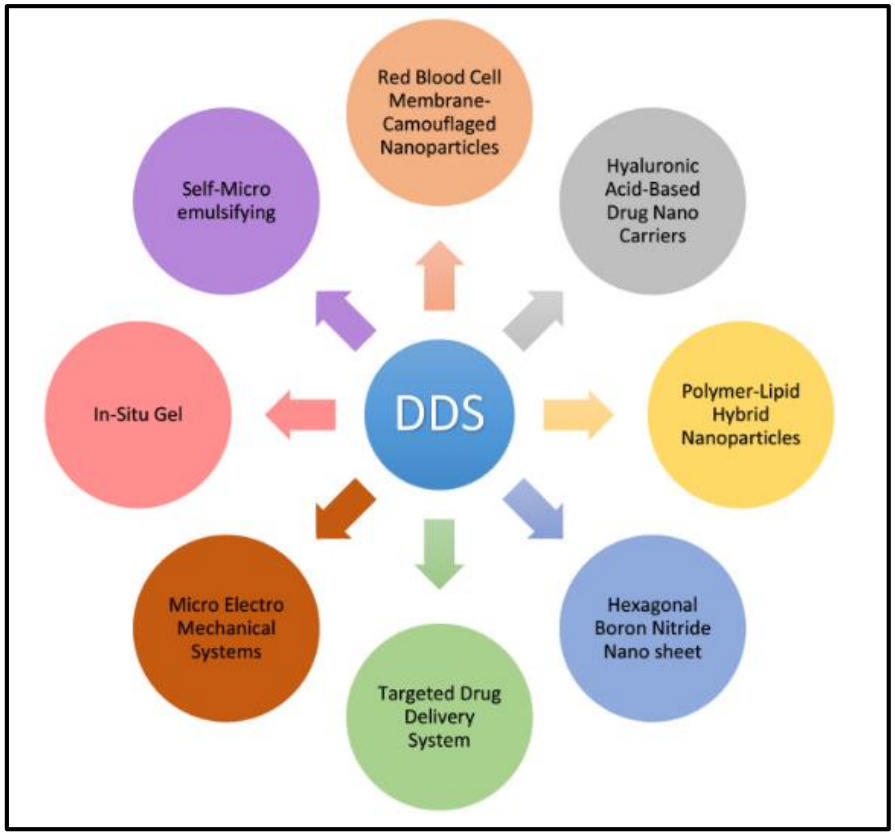


Figure (1-2): Several types of recent drug delivery systems [27].

1.4 In-situ gel drug delivery system

Modifying the medication's pharmacokinetic properties and tissue distribution is the primary objective of every drug delivery system [28]. The past 60 years had seen considerable investment in the research and development of dependable and regulated medication delivery systems. Among the most cutting-edge methods of drug delivery, in-situ gel medicine administration has recently gained prominence. The in-situ gel drug delivery system helps prolong and manage the release of pharmaceuticals, increases patient comfort and compliance, and has the unique ability to convert from Sol to Gel [29]. Oral, nasal, injectable, vaginal, rectal ocular, intravenously, and parenteral routes have all been utilized in various studies to change solution-form formulations into gel-form before they enter the body. Physiological conditions that accomplish

this transformation include changes in pH, modulation of temperature, and solvent exchange. Several polymeric techniques had been developed for the delivery of drugs [30]. A sol-gel transition occurs when these polymers are exposed to physiological stimuli. Drug delivery methods that employ in situ gels may be made from a variety of synthetic and natural polymers. The four recognized mechanisms that result in the creation of in-situ gel biomaterials include changes in temperature and pH, changes in the physical characteristics of the biomaterials such as solvent exchange and swelling, biochemical alterations such as enzymatic and chemical reactions, and photo-polymerization [31]. The drug loading and release characteristics, synthesis ease, controllable swelling, and viscoelasticity, passive and active focusing, and the ability to create nanogel carriers that can react to biological stimuli are all appealing features of polymer-based nanogel formulations that make them ideal for drug delivery. Because of their novel properties and little toxicity, nanogels are a promising candidate for vascular medication targeting [32]. Nanogels and other nano-carriers with three-dimensional (3D) cross-linked structures have recently emerged as promising candidates for the targeted delivery of a variety of therapeutic and diagnostic agents, including chemotherapeutic medicines, chemicals based on nucleic acids and proteins, photodynamic treatment, and dye tracing. Nanogels contains on ample amount of the target substance due to their great loading capacity and exceptional stability in living organisms. To encapsulate tiny molecules, oligonucleotides, or proteins, one may create nanogels out of natural or synthetic polymers with porous architectures ranging in size from 20 to 250 nm [33].

1.5 Nanotechnology

Nanotechnology is a recent development in scientific research. This field was founded by American physicist Richard P. Feynman at Caltech when he presented his lecture titled "There is plenty of room at the bottom," in 1959, he suggested Scaling down to the nanoscale was the way of the future for technology and advancement [34]. The science of nanotechnology deals with understanding and manipulating materials at the nanoscale size, which has dimensions between 1 and 100 nanometers. They have high surface/volume ratios and smaller than their bulk materials [35]. As the particle size decreases, the number of constituent atoms surrounding the surface of the particles increases. Highly reactive particles with unique chemical, optical, physical, and electronic properties develop as a result. Nanostructures is a fast-growing field of technology that has great promise for the chemical, medical, engineering, and food-processing sectors [36-38]. The fast advancement of "nanotechnologies" indicates that nanoscale manufacturing will soon be used in almost all domains of research and technology. There is a clear need to differentiate between nanotechnology and nanoscience. Nanoscience is the study of structures and molecules on the sizes of nanometers ranging from 1 to 100 nm, and the technology that utilizes it in practical applications such as electronics, Nano-medicine, etc. is termed nanotechnology [39]. Nanoscience is an interdisciplinary field of research that focuses on the atomic, molecular, and subatomic levels of matter. Nanotechnology refers to the concentered research and development that is done for the purpose of understanding, manipulating, and measuring materials at the atomic, molecular, and supermolecular levels. The term "nanotechnology" refers to materials, systems, and processes that measured at a size of one hundred nanometers (nm) or less [40].

1.6 Nanoemulsion

Nanoemulsions, which are emulsions with submicron-sized particles, are now being extensively studied as carriers for drugs in order to enhance the delivery of medicinal agents. Nanoemulsions are a stable and uniform mixture of two liquids that do not normally mix together, such as water and oil. This is achieved by using certain surfactants to create a single phase. The diameters of nanoemulsion droplets generally vary from 20 to 200 nanometers. The size and surface characteristics of nanoemulsion droplets significantly influence on biological behavior of the formulation. Nanoemulsions provide significant potential for the advancement of cosmetics, diagnostics, pharmacological treatments, and biotechnologies [41-42]. Nanoemulsions have several benefits, including Nanoemulsions possessing much more surface area and free energy compared to macroemulsions, making them very efficient as a means of transportation. Nanoemulsions do not exhibit the issues of intrinsic creaming, flocculation, coalescence, and sedimentation that are often seen in macroemulsions. Nanoemulsions may be prepared many formulas which includ foams, creams, liquids, and sprays. These nanoemulsions are safe and non-irritating, making them suitable for application on the skin and mucous membranes, making them appropriate for therapeutic applications in humans and animals. It may serve as a replacement for liposomes and vesicles. It enhances the bioavailability of medications [43-44].

A key use of nanoemulsion is to conceal the unpleasant flavor of greasy liquids. Nanoemulsion may provide protection for medications that are vulnerable to hydrolysis and oxidation. Currently, nanoemulsions are used for precise administration of several anticancer medicines, photosensitizers, or therapeutic substances. Nanoemulsion may potentially provide an extended duration of the medications [45].

1.7 Nanogel

Nanogel-based materials have a high drug loading capacity, biocompatibility, and biodegradability which are the key points to design a drug delivery system effectively [46]. Nanogels, often referred to as advanced drug delivery systems, are now the focus of study due to their many benefits such as, adjustable size, reactivity to stimuli, prolonged drug release via in situ gelling processes, and stability. Nanogels have shown superiority in simplifying the delivery method and overcoming the limitations of traditional systems[47].

The emergence of nanotechnology has led to a need for the development of nanogel systems, which have shown their ability to administer medications in a precise, continuous, and targeted way. Due to the rapid development of polymer sciences, it is currently necessary to create intelligent nano-systems that can be helpful for both therapy and clinical trial advancement. The drug loading is significantly high and can be accomplished without the need for chemical reactions. This is a crucial aspect in maintaining the effectiveness of the medicine.

The ability to access the tiniest capillary vessels, plea to their minuscule size, and to infiltrate the tissues via either the paracellular or transcellular routes. It exhibits a high level of compatibility with living organisms and is capable of being broken down naturally over time [48]. The figure (1-3) presents a model illustrating the release of drugs from nanogels. The "intelligent nanogels" maintain the drug molecule in its whole state until it is taken up by the target cell. The use of different cross-linking agents in this drug delivery system, which is responsive to external stimuli, is crucial in reducing unintended side effects and ensuring regulated release at the desired location of action [49-50].

Nanogels met many, if not all of the key basic requirements of a versatile nanocarrier delivery vehicle, as shown in Figure (1-4)

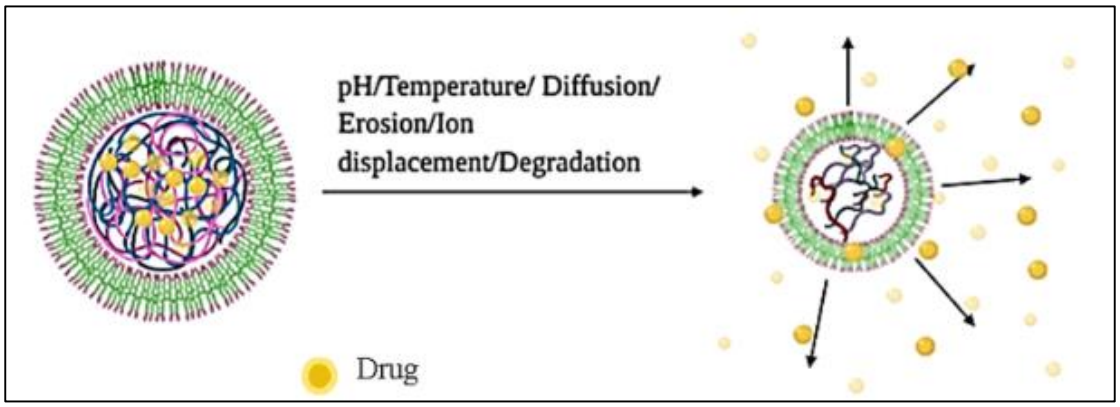


Figure (1-3): Drug release mechanisms involved in Nanogels [44]**.**

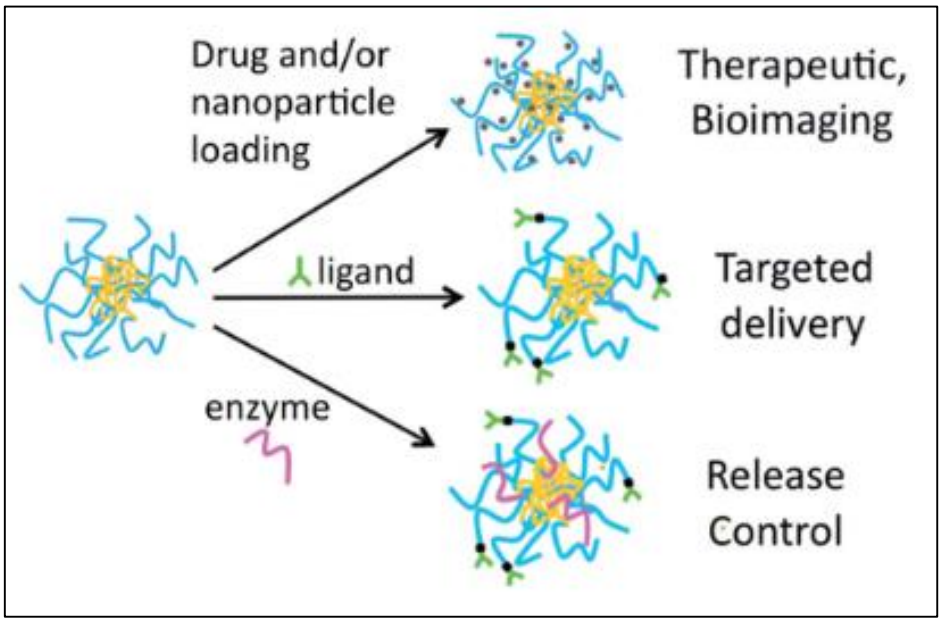


Figure (1-4): Example of nanogel with a lysozyme core and dextran shell prepared by the Maillard reaction followed by heating [51]**.**

1.8 Biomedical applications of herbal materials

Several studies have shown the biological uses of herbs and herbal products. Despite their ability to limit bacterial growth, antibiotics and other chemical antimicrobial agents may have adverse effects such as the formation of reactive oxygen species (ROS) [52]. Clove, portulaca, cinnamon, ginger, thyme, mint, fennel, chamomile, burdock, eucalyptus, primrose, garlic, and lemon balm are some of the herbal ingredients that might kill bacteria [53]. The intended cell death may be prevented by using herbal medications, which function as direct antioxidants by inhibiting the creation of ROS. The essential oil or extract of these herbal remedies often contains phenolic chemicals, which show that they are powerful antimicrobials and antioxidants. Because of their lack of toxicity and side effects, herbal medications are often chosen over synthetic antibacterial drugs. Additionally, herbal remedies have a cheaper price tag and a great antibacterial potential. Phytopharmaceutical uses have skyrocketed in recent years, although in large part to the widespread belief that natural cures are safer than prescription drugs [54]. Because of this, studies in the future may concentrate on developing pharmaceutical products and improving treatments by characterizing the active ingredient and studying the effects of herb-herb combos. Biomaterials with strong antiviral activity are being considered for use in the fight against the COVID-19 virus. Drug delivery (COVID-19), cancer treatment, infection, and speeding up the healing process are all areas that might benefit from these materials [55]. In figure (1-5) we can see the materials' potential uses in biomedicine.

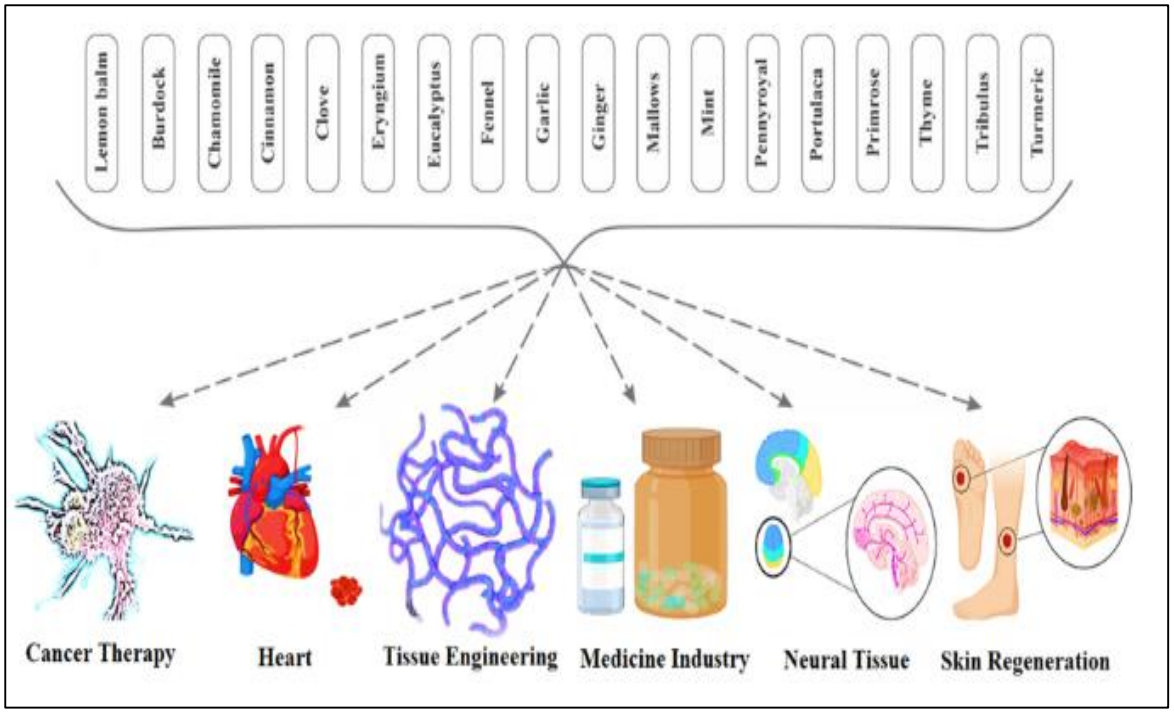


Figure (1-5): The biomedical applications of herbal materials [56].

1.9 Clove Essential Oil

In recent years, essential oils have gained massive acceptance due to their applications in several pharmaceutical facets and food products because of their potential to inhibit the growth and propagation of a wide range of microorganisms such as bacteria, fungi and viruses ,Cloves essential oil (CEO) is an essential oil isolated from flower buds of *Syzygium aromaticum L. (Family: Myrtaceae),* advocated in the treatment of various diseases [57].

Figure (1-6): Illustrates the plant Cloves and Cloves Oil

CEO has been shown to possess many pharmacological bioactivities, such as anti-inflammatory, analgesic, antibacterial, antifungal, anti-allergic, anti-carcinogenic, and anti-mutagenic activities [58]. CEO contains a variety of phytochemicals, including eugenol, acetyleugenol, isoeugenol, methyleugenol, carvacrol, thymol, cinnamaldehyde, eugenyl acetate, β-caryophyllene, 2-heptanone, methyl salicylate, α-humulene, gallic acid, ellagic acid, and oleanolic acid. Eugenol, which constitutes 88.58% of CEO, has notable antioxidant and insecticidal activities [59].

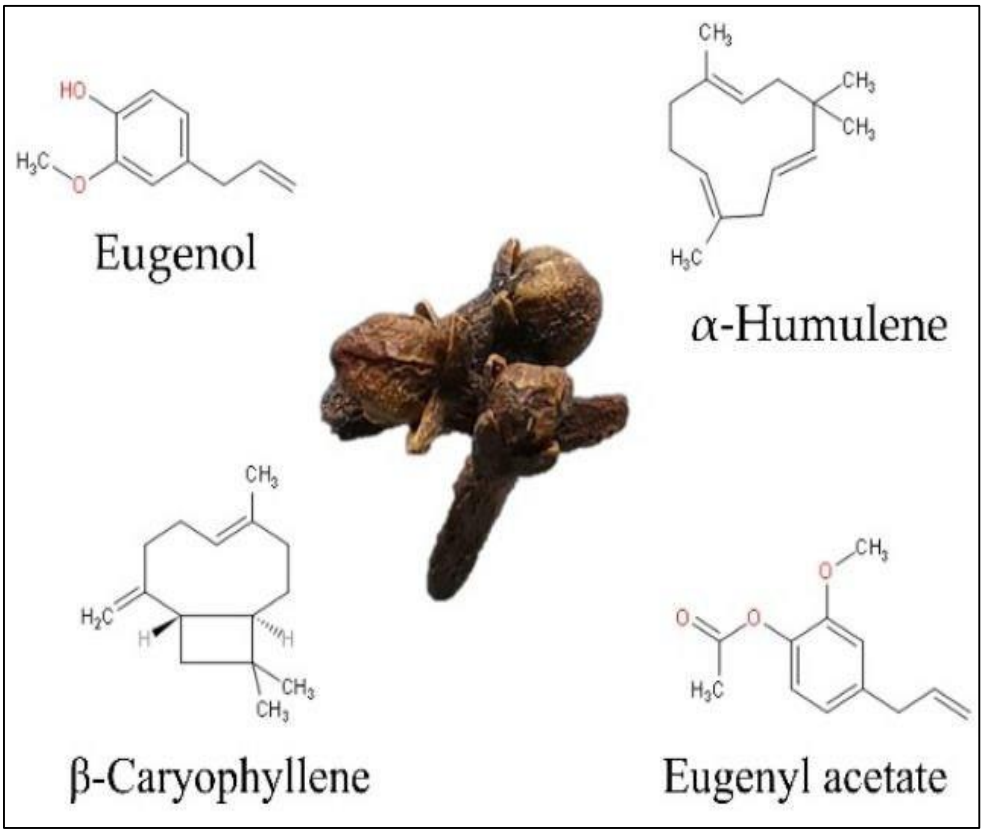


Figure (1-7): Chemical structure of main compounds of clove (*S. aromaticum* L.) essential oil [60]

1.10 Cytokines

Cytokines are small, soluble proteins (6-70 kDa) that are released by several types of cells, including lymphocytes, macrophages, natural killer (NK) cells, mast cells, and stromal cells. Cytokines are essential components of the immune reaction and mediate important interactions within the immunity system's communications of connections. They are responsible for dynamically regulating the development, proliferation, and reactivity of immune cells, and are significant factors in determining overall health [61]. Single cytokine can be released by various types of cells and affect on multiple types of cells, resulting in various biological effects. The variation in cytokine levels in different biological fluids, such as serum, blood, and saliva, can provide important information about the diagnosis, stage, and prognosis of different diseases. Excessive or aberrant synthesis of cytokines, as shown in a cytokine storm, may result in organ failure and mortality. One example is the unfavourable outlook for severe cases of Corona Virus Disease 2019 (COVID-19) that caused by excessive production or cytokine storm syndrome [62]. Therefore, the quantities of cytokines are considered a crucial signal for assessing clinical conditions. Precise measurement of cytokines provides valuable clinical information for monitoring patients' immune status and adjusting therapies for various diseases, such as asthma, atherosclerosis, cancer, depression, cardiovascular disease, acquired immune deficiencies (AIDS), kidney injury, sepsis, rheumatic arthritis, and other chronic diseases [63].

1.11 Classification of Cytokines

Cytokines are classified into several categories based on their cellular sources and their role in the immune response

1.11.1 Based on their cellular source:

Type 1 cytokines, which are generated by (CD4)+ T-helper 1 (Th1) cells, consist of IL-2, IL-12, IFN-γ, and TNF-β. The CD4+ T h 2 cells generate type 2 cytokines, such as IL-4, IL-5, IL-6, IL-10, and IL-13 [64].

1.11.2 Depending on Their role in the immune response :

According to their role in the immune response, cytokines are classified as pro-inflammatory and anti-inflammatory. Pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-12, TNF-α, and interferons, promote inflammatory responses and tend to activate immune cells. On the other hand, anti-inflammatory cytokines such as IL-4, IL-6, IL-10, IL-11, IL-13, IL-1 receptor antagonist (IL-1RA), and TGF-β, have the ability to prevent inflammation and reduce the activity of immune cells. Certain cytokines, such as IL-6, exhibit both pro-inflammatory and anti-inflammatory characteristics [64].

1.11.1.1 Pro-Inflammatory Cytokines

Cytokines play a major role in controlling the inflammatory response, initiating an severe phase reply to protect the body from irritation, injury, and infection. The process starts with the release of pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8, IL-12, IFN-γ, and TNF-α, either since the same cell or from different cells. The main role of these cytokines is to convey information to nearby tissues about the existence of infection or injury. In addition, these cytokines have the ability to enter the circulation, stimulating immune cells and leading to significant alterations in the body's functioning, such as fever and the acute-phase response [65]. Pro-inflammatory cytokines have immunological qualities that may benefit the host by helping to protect against bacterial invasion and other microorganisms in the immediate environment, as well as the natural bacteria present on the skin and in the digestive system. It is important to acknowledge that an excessive pro-inflammatory response can cause ongoing inflammation and disrupt the pathways that regulate biological equilibrium, resulting in detrimental health conditions such as cancer, heart failure, diabetes, gastrointestinal diseases, Parkinson's disease, and age-related diseases [66] .

1.11.1.2 Anti-Inflammatory Cytokines

The anti-inflammatory cytokines, such as IL-1 receptor antagonists, IL-4, IL-6, IL-10, IL-11, IL-13, and TGF-β, are a set of molecules that control the immune system and inhibit excessive inflammation induced by pro-inflammatory cytokines [67]. IL-1 is an influential anti-inflammatory cytokine with immunoregulatory capabilities, capable of inhibiting the production of other pro-inflammatory cytokines, IL-1 has an anti-inflammatory effect on eosinophils, basophils, and mast cells, hence playing a crucial role in the control and regulation of allergies and asthma, Physiologically, these cytokines function to limit the deleterious effects of prolonged or excessive pro-inflammatory reactions. The effectiveness of these anti-inflammatory cytokines has been shown in the treatment of several clinical conditions defined by excessive inflammation. Pharmaceutical drugs that have anti-inflammatory properties may be used to treat illnesses that are related to inflammation [68]

1.11.2.1 Interleukin 1 (IL-1)

IL-1 α and IL-1 β are considered the original members of the IL-1 family. The cytokines of the interleukin-1 (IL-1) family consist of 11 proteins (IL-1F1 to IL-1F11) that are encoded by 11 separate genes [69]. The primary role of IL-1-type cytokines is to regulate proinflammatory responses triggered by pathogen-associated molecular patterns (PAMPs), which are substances produced by bacteria or viruses, or damage- or danger-associated molecular patterns (DAMPs), which are released from injured cells [70]. Therefore, they play a crucial role in regulating innate immune responses, and their effects are carefully controlled [71]. Macrophages and monocytes, which are part of the innate immune system, are the primary producers of IL-1α and IL-1β. However, other cell types

such as epithelial cells, endothelial cells, and fibroblasts are also capable of producing IL-1α and IL-1β. IL-1α is mostly attached to the cell membrane and transmits signals by autocrine or juxtacrine pathways, whereas IL-1β is released through an unusual protein secretion route and may function in a paracrine manner or affect the whole system [72]. The inflammatory activity of IL-1 cytokines is controlled by other members of the same family, such as receptor antagonists, soluble receptors, and anti-inflammatory cytokines [73].

1.11.2.2 Tumor necrosis factor-α (TNF-α)

Tumor necrosis factor alpha (TNF-α) is a cytokine that has pleiotropic effects on various cell types. It has been identified as a major regulator of inflammatory responses and is known to be involved in the pathogenesis of some inflammatory and autoimmune diseases [74]. From a physiological standpoint, TNF-α plays a vital role in maintaining a healthy immune response. TNF-α has the ability to stimulate the immune system for regulation. However, when TNF-α is produced inappropriately or excessively, it may be detrimental and could result in illness. Rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriatic arthritis (PsA), psoriasis (PS) , and noninfectious uveitis (NIU) are caused by the aberrant release of TNF-α. Therefore, TNF-α may be considered a crucial element in the pathological progression of these conditions [75].

TNF-α inhibitors have been effectively developed and utilized in the clinical treatment of autoimmune diseases like Crohn's disease (CD) and rheumatoid arthritis (RA) due to the involvement of TNF-α in the development of these diseases. Several TNF-α inhibitors, including etanercept, infliximab, adalimumab, golimumab, and certolizumab, have been approved for clinical use [76].

1.12 Antioxidant Activity

A chemical that prevents other molecules from becoming oxidized is called an antioxidant. In oxidation, a material gives up an electron or a hydrogen atom to an oxidizing agent. A free radical may be a byproduct of an oxidation process. Then, these radicals may set off a chain reaction, which, if it happens within a cell, can destroy or severely harm that cell. By neutralizing free radical intermediates and preventing further oxidative reactions, antioxidants stop these cascades of events [77]. One way they do this by oxidizing themselves. Thiol, ascorbic acid, and polyphenols are examples of antioxidants that are reducing agents. Numerous definitions have been putted forward for the term "antioxidant," including substances that, when present in low concentrations relative to an oxidizable substrate, considerably delay or prevent the oxidation of that substrate, or substances that, when present in small quantities, can prevent or greatly retard the oxidation of easily oxidizable materials [78]. Because of their protective functions against oxidative degradation in food items and in the body against oxidative stress-mediated pathological processes, antioxidants are attracting increasing attention from food scientists, health professionals, and the general public. Preservatives for food and nutraceuticals/pharmaceuticals are two areas seeing a surge in demand for natural and powerful antioxidants [79]. Cloves oil has the antioxidant compounds eugenol, eugenyl acetate,-caryophyllene, and-humulene, which protect cells from free radical oxidation. Diseases such as cancer, arteriosclerosis, Alzheimer’s disease, and Parkinson’s disease are related to the presence of ROScompounds. Cloves oil has shown scavenging activity on radicals and inhibition of lipid peroxidation [80]

1.13 Antibacterial activity

Bacteria, or germs are microscopic, single-celled organisms, including Cocci, bacilli, and spirals. The gather together and take multiple forms, such as knots or rosaries, so they are called streptococci, or in the form of clusters, so they are called staphylococci. The cytoplasm, which contains ribosomes, the nucleoid, and the cell membrane make up the primary components of bacteria, which are broad sorts of organisms. Cocci, bacilli, and spirochetes are the three main categories of bacteria according to their overall shape [81]. Antibiotics primarily include penicillin, cephalosporins, aminoglycosides, macrolides, and quinolones, which combat bacterial illnesses by halting the development of harmful microorganisms and eliminating their root causes [82]. But medication resistance among bacterial infections has emerged due to the abuse of antibiotics. When bacteria come into touch with medications on a regular basis, their sensitivity to those drugs diminishes or vanishes altogether. This process is known as bacterial drug resistance [83]. In the battle against the increasing drug resistance caused by the rise of multidrug and extensively drug-resistant bacterial phenotypes, natural products may be a valuable source of novel and highly potent antibacterial medicines [84].

1.14 Literature review

Previous studies focused on many applications that use cloves in various ways to obtain the optimal benefit.

The *in vitro* release tests demonstrated cumulative releases of native clove oil (NC) and CON of 76% and 42%, respectively at pH 7.4. The cellular toxicity of CON was significantly reduced by four fold compared to NC at a concentration of 60 µg/mL when tested on Caco2 cells. Similarly, haemolytic activity on red blood cells revealed less than 10% haemolysis signifying the compatibility of CON for its nutraceutical applications [85].

Penetration of the drugs encapsulated has been studyed in emulsions is enhanced when the droplet size is in nanometric range (less than 500 nm) Reducing the droplet size below 500 nm produces higher penetration through different skin layers and absorption of drug ingredients with higher particle uptake by enhancing the mechanism of passive transport. Among the olive oil nanoemulsions, F1 was considered as optimized nanoemulsion formulation with droplet size value of 490 nm, while for clove oil nanoemulsion F7 was optimized formulation with droplet size value of 222nm. Due to the droplet sizes below 500 nm, both these nanoemulsions are expected to face no barrier in passage through biological barriers [86].

In other study, an amphiphilic polymer-based nano-green coating (CMC-CYST-EUG) based on carboxymethyl cellulose (CMC) has been prepared, EUG was grafted into CMC by thiolene click reaction of Cysteamine hydrochloride (CYST) to reduce the volatility of EUG. When exposed to the same environment, the antibacterial duration of CMC-CYSTEUG increased by 7.8 times compared with pure EUG. Liquid holding capacity of CMC-CYST-EUG on pakchoi leaf surface increased

by 81% compared with EUG solution. It shows that CMC-CYST-EUG has good adhesion to fruits and vegetables and can prevent the loss of EUG. Strawberry treated with CMC-CYST-EUG can be stored for 7 days. Within the concentration range of 0.5–0.25 mg/mL, the antifeedant rate of CMC-CYST-EUG to pests was significantly higher than that of EUG. Importantly, cytotoxicity experiments indicate that low quantities of CMC-CYST-EUG are deemed safe. This artificial substance offers a method for creating and applying volatile active ingredients [87].

a reaschers had explored the anti-infammatory efect (paw edema test) and the anti-nociceptive efect (hot plate and formalin test) of nanoemulsion-based gels containing the essential oils in the animal model. First, nanoemulsions containing essential oils of clove and cinnamon were made, with droplet sizes of 12±3 and 28±6 nm, respectively. The nanoemulsions were subsequently gelifed by adding carboxymethylcellulose (3.5% w/v). Ultimately, ATR-FTIR analysis was utilised to characterise the nanogels, which were then applied topically as a pre-treatment prior to inducing inflammation or pain in both acute and long-term analgesic experimental investigations.The formalin and paw edoema results demonstrated the significant anti-nociceptive and anti-inflammatory effects of the nanogel formulations.The produced nanogels may be used as analgesic medications to reduce inflammation and discomfort associated with illnesses; research like this led to the development of cinnamon-NG and clove-NG as a topical delivery mechanism. This research has led to the conclusion that cinnamon-NG and other nanoemulsion-based gel formulations may have use as anti-nociceps [88].

Utilizing nanoparticles to specifically target cancer cells Cancer is a very complex illness, and brain cancer is particularly problematic to identify and treat due to the obstacles in delivering imaging and therapeutic substances via the blood-brain barrier and into the brain. Several researchers have discovered that nanoparticles show potential for transporting these substances into the brain. Apolipoprotein E has been proposed as a mediator for drug transportation across the blood-brain barrier. Loperamide, a substance that can not pass across the blood-brain barrier but has pain-relieving effects when injected directly into the brain, was encapsulated in nanoparticles made of human serum albumin and connected to apolipoprotein E. When mice had been given this compound via their veins, it caused a reduction in pain sensitivity as seen in the tailflick test. The effectiveness of this medication delivery method relies on the identification of lipoprotein receptors. Kopelman and his colleagues developed Probes Encapsulated by Biologically Localized Embedding (PEBBLE) with the purpose of carrying diverse agents on their surface and executing different activities [89].

Nanoparticulate methods for medication delivery to the brain One potential method for transporting medications to the brain is via the use of nanoparticles. Poly (butylcyanoacrylate) nanoparticles are currently the only nanoparticles that have been effectively used for delivering medications to the brain in living organisms.The first drug to be transported to the brain using nanoparticles was hexapeptidedalargin, which is an analogue of Leu-enkephalin with opioid activity. Nanoparticles and nanoformulations have already been successfully used as drug delivery systems. Nanoparticulate drug delivery systems have even greater potential for various applications [90].

1.15 Aims of the study

1) Design of a drug release system model using a nanoemulsion-based gel of cloves oil

2) Preparation and characterization of Clove Nano-emulsion gel (CNG) by using Fourier Transform Infrared Spectroscopy (FTIR), Nuclear magnetic resonance (^{1}H-NMR), Dynamic light scattering (DLS), Field emission scanning electronic microscope (FESEM).

3) Study Biological activities of Clove Nano-emulsion gel (CNG):-

- Study Drug release of Clove Nano-emulsion gel (CNG) in SGF, SIF and Buffer
- Study Anti-inflammatory effects of Clove Nano-emulsion gel:

  Induction of inflammation in the cell line using inducers LPS and demonstrate the anti-inflammatory activities of Nano-emulsion gel in the cell line through the management of the IL-6, and TNF alpha using the technique ELISA.
- Study antioxidants properties by DPPH assay.
- Study Antibacterial properties.
- Study Hemolysis and MTT effect of Clove Nano-emulsion gel

CHAPTER TWO

Materials and Methods

2. Materials and Methods

2.1 Instruments

The instruments used in the present study and their models, companies and Country are listed in Table (2-1).

Table (2-1): Instruments used in study and their models, companies, Country

Ser	Devices	Company	Country
1	UV-Vis spectrophotometer, UV-1800	Shimadzu	Japan
2	FT-IR spectrophotometer, FTIR—8400S	Shimadzu	Japan
3	pH meter, pH 7110	Inolab	Germany
4	Incubator	Faithful	China
5	Incubator CO2	Genex Lab	USA
6	Dynamic light scattering (DLS)	Brookhaven	USA
7	Centrifuge	Hettich-D-78532	Germany
8	Field emission scanning electronic microscope (FESEM), 5 KV	TESCAN	French
9	Rotary	Heidolph	USA
10	Micro ELISA Reader	BioTek	USA
11	Overhead Stirrer	Joanlab	China
12	Hotplate Magnetic Stirrer	medilab	Korea
13	Vortex mixer	Joanlab	China

2.2 Chemicals

Table (2-2): All materials and Chemicals used, with their company and Country

Ser	Chemicals	Company	Country
1	Dimethyl Sulfoxide (DMSO)	Sigma Aldrich	UK
2	2,2-diphenyl-1 picrylhydrazyl (DPPH)	Sigma Aldrich	UK
3	Sodium Hydroxide	ChemLab.	UK
4	Sodium Decyl Sulfate (SDS)	Sigma Aldrich.	UK
5	Mueller Hinton Agar	HiMedia	India
6	Phosphate Buffered Saline(PBS)	Sigma-Aldrich.	UK
7	Methanol	ChemLab.	UK
8	Sodium Chloride	Thomas beaker	India
9	Hydrochloric acid	Applichem	USA
10	Tween 20	Loba Chemie	India
11	Carboxy methyl Cellulose	ShengHuangchm	China
12	Glycerin	Thomas Baker	India
13	Lipopolysaccharide (LPS)	Solarbio	China
14	Ethyl Acetate	Srlchem	India
15	Magnesium Sulphate	CDH	India
16	Mono Potassium Phosphate	TM Media	India
17	MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide	Roth	Germmany
18	RPMI 1640 medium w/L-glutamine, 25mM HEPES (powder)	US Biological life science	USA

2.3 Assay Kit

The assay kits used in this study include:

Table (2.3) list of ELISA assay kits used the present study

No.	Kits	Company	Country
1	ELISA Kit (Interleukin 1-β)	Elabscience	USA
2	ELISA kit (Tumor Necrosis Factor ALPHA (TNF-α))	Elabscience	USA

2.4 Methods

2.4.1 Extraction of Cloves Oil

The Steam distillation (SD) methods are the most extensively used for extracting cloves oil, because These are easy to operate, so the Steam hydro distillation is select as a method to isolate clove oil. Was performed according to the previous study with a few modifications and as shown in Figure (2.1) [91]. The oil was extracted from dried clove flowers by Steam distillation. 100 gm of cloves powder plant were taken and this quantity was divided into batches, where each batch contained 20 gm of cloves and 200 ml of distilled water and placed in the device for three hours the time started to be counted when first drop of distillate comes out. The oil was extracted from the water by mixing it with diethyl ether and placing it in a separating funnel with vigorous shaking during the separation stage. Each batch was repeated three times, and each time the separating funnel contained 50 ml of clove extract and 50 ml of diethyl ether and was shaken vigorously for a minute 5 Then ,After that, the diethyl ether was evaporated using a rotary evaporator to obtain only oil [92].

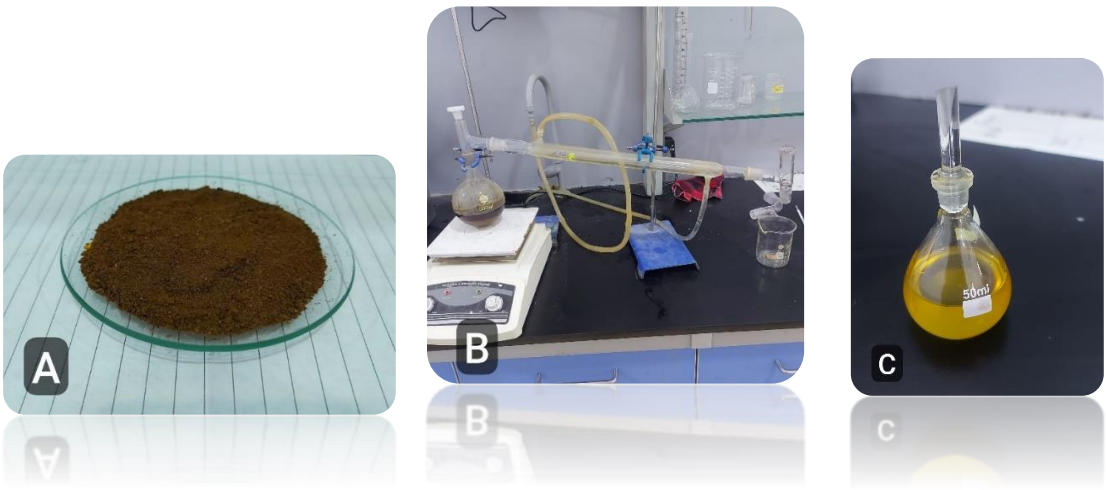


Figure (2-1): (A) Dry Powder of Cloves plant (B) The Steam distillation method. (C) Cloves oil .

2.4.2 Preparation of Clove Nanoemulsions-gels

The beaker holding the Tween-20 solution was stirred at 2000 rpm using an overhead mixer (AC 100-24v, 50-60 Hz, Overhead, China) while clove essential oils (2.5 ml) were added. To make the nanoemulsion, distilled water was added slowly until the volume was reached, and then the mixture was agitated at 2000 rpm for 40 minutes. Then, the nanogels were made by adding carboxymethyl cellulose (CMC 3.5 gm) to the nanoemulsion and mixing it at 180 rpm for 4 hours. The cross-linking agent used was glycerin (0, 8, 20, and 30 ml). The names of the produced gels were shortened to Clove-NG. At room temperature, all ingredients were combined [93].

Table (2-4): Composition (w/w %) of clove Nanoemulsion gel (CNG) (M1-M2) prepared using Glycerin, Tween 20, CMC, and water.

Code	Clove oil	Tween 20	Glycerin	CMC	Water
M1	2.5 ml	10 ml	0	3.5 gm	84 ml
M2	2.5 ml	10 ml	8 ml	3.5 gm	76 ml
M3	2.5 ml	10 ml	20 ml	3.5 gm	64 ml
M4	2.5 ml	10 ml	30 ml	3.5 gm	54 ml

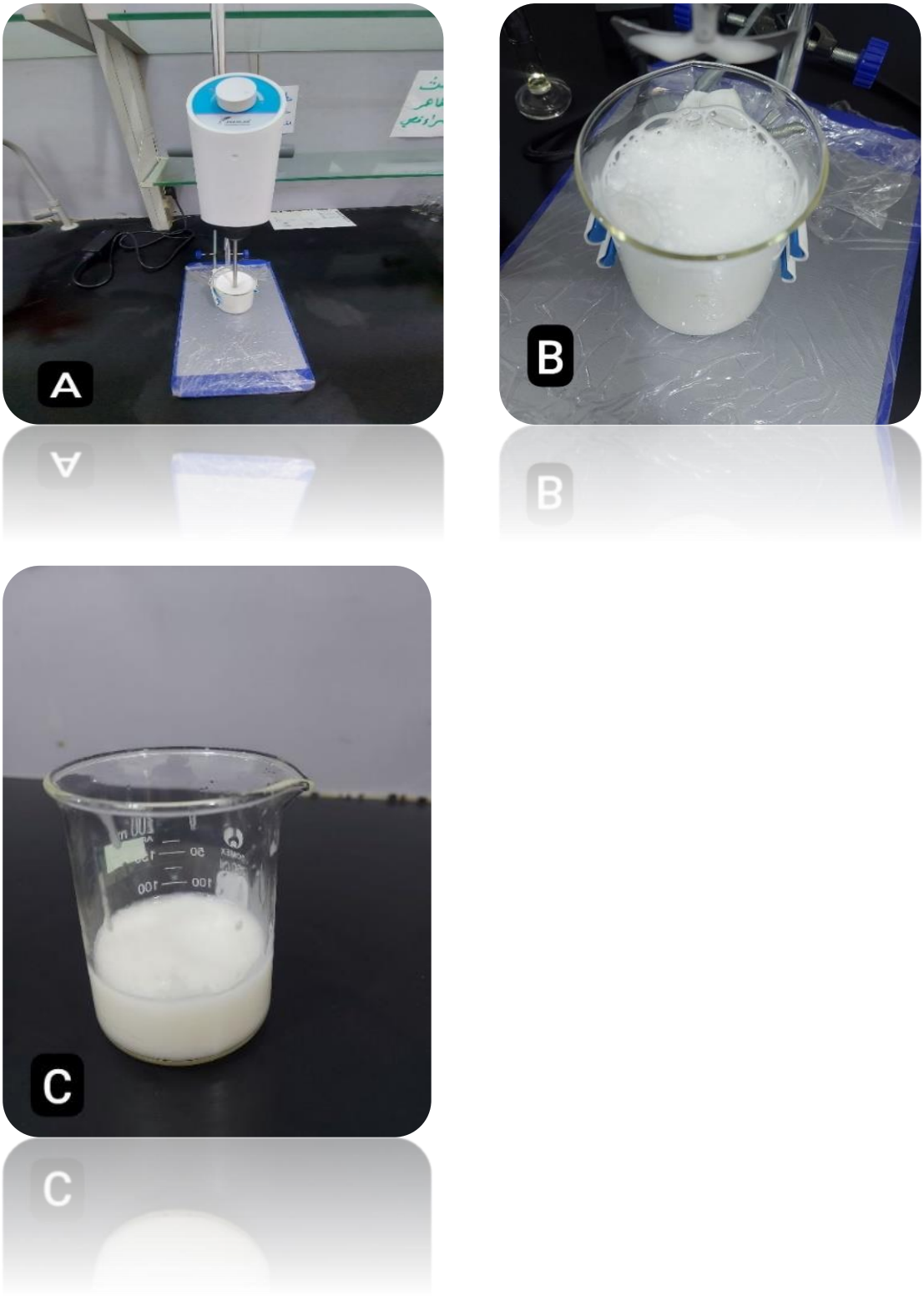


Figure (2-2): (A) During the preparation of the Clove Nanoemulsion gel (CNG) (B) Clove Nanoemulsion (CNE) (C) Clove Nanogel (CNG).

2.4.3 Preparation of Simulated Intestinal Fluid (SIF)

The simulated Intestinal Fluid (SIF) was prepared by dissolving 6.8 g of potassium phosphate (monobasic) in 250 ml of distilled water, then mixed with 190 ml (0.2 M), of sodium hydroxide solution and 400 ml of distilled water .Then the pH was adjusted using sodium hydroxide, and the pH was (pH= 8.2) [94].

2.4.4 Preparation of Simulated gastric fluid (SGF)

Simulated gastric fluid (SGF) was prepared by dissolving (2 g) of sodium chloride in 500 ml of distilled water, then adding 7 ml of concentrated hydrochloric acid to the solution and diluted to 1000 ml of distilled water, to adjust (pH=1.2) [94].

2.4.5 Determination of the standard calibration curves of the Clove Nanoemulsion (CNE) in SGF, SIF and Buffer.

The standard calibration curves of the Clove Nanoemulsion (CNE) in SGF, SIF and Buffer Solution were determined according to Beer-Lambert law using ultraviolet spectroscopy at λ max absorption 284 nm. The absorbance was plotted against the different concentration of (CNE) in SGF,SIF and Buffer.

2.5 Characterization of Clove Nanogel (CNG)

2.5.1 UV-Vis Analysis

Sample Preparation After centrifugation operation was finished, the pellets was collected, the superannuated was ejected, distilled water was added to the pellets. Forming a stock solution, for further analysis.

Experiment Method Analysis of the UV-Vis spectrophotometer was done using stock solution. These analyses allowed for the observation and identification its λmax of Clove Nanogel (CNG). Water was first used as a blank reference. Nearly 4 ml of (CNG) solution were combined and placed inside the spectrophotometer., light in the 265-320 nm range was generated in a UV-Vis spectrophotometer, note that it was measured λmax manually [95].

2.5.2 Fourier Transform Infrared Spectroscopy (FT-IR).

The FTIR spectra of CMC powder, clove oil, nanoemulsion (NE), and nanogel (NG) were obtained using a Shimadzu spectrum FT-IR Spectrometer (Japan). The spectra were recoded between 400 and 4000 cm-1 to determine the functional groups contained in the chemical compounds.

2.5.3 Field Emission Scanning Electronic Microscope (FESEM)

A specimen container covered with cabon was used to attach the spray-and freeze-dried (CNG). Using a 30 kV accelerating voltage in high vacuum mode with a (TESCAN MIRA3) French detector FESEM, a FESM Quanta 200 scanning electron microscope was used to capture their morphologies. The size and shape of the acquired (CNG) were then determined [85].

2.5.4 Dynamic Light Scattering (DLS) Analysis

The average droplet size and polydispersity index (PI) of Clove Nanogel (CNG) were determined using a dynamic light scattering analyzer (Brookhaven, USA) at a temperature of 25°C. To conduct these measurements, the CNG sample was diluted with deionized water and then filtered using 0.45 µm membrane filters. The measurement was conducted with a fixed scattering angle of 15° degrees. A high-sensitivity device detected the scattering signals [96].

2.5.5 Nuclear Magnetic Resonance Spectrometer

^{1}H-NMR spectra of the clove Nanogel was measured at the Department of Chemistry, College of Education for Pure Sciences - University of Basra, Iraq using a Bruker 400-DRX spectrometer (Germany). D_2O was used as solvent and TMS as internal standard.

2.6 Biological Evaluation of Synthesized Clove Nanogel (CNG)

2.6.1 Hemolytic activity assay

To determine the effect of Clove Nanogel (CNG) and clove oil on red blood cells (RBCs), the hemolysis test was used. Donors were healthy adults about 20 and 30 years old, and they provided fresh red blood cells. For 10 minutes at 1500 rpm, the blood sample was centrifuged to prepare it. After that, the pellet was washed three times with PBS to remove any remaining debris. The red blood cells that were extracted were mixed with 10% v/v PBS. The samples were mixed with RBC suspension at a ratio of 1:1, and then incubated at 37 °C for 60 minutes. The quantities of CNG and cloves oil used were 7.5, 15, 30, 60, and 120 µg mL−1. Then, for 5 minutes, each sample was spun in a centrifuge at 5000 rpm. Lastly, a 96-

well plate was used to transfer 100µL of the sample supernatant. A plate reader was used to determine the supernatants' absorbance values at 540 nm. A 0.1% SDS in PBS solution served as the positive control, while a PBS solution served as the negative control. How much hemolysis was present in red blood cells was determined using the following formula [97].

$$\boldsymbol{H}\% = \frac{\boldsymbol{Sample\ Absorbance - Nengative\ Control\ Absorbance}}{\boldsymbol{Positive\ Control\ Absorbance - Negativee\ Control\ Absorbance}} \times 100\%$$

The symbol H for Hemolysis

2.6.2 Antioxidants Evaluation

The DPPH method was used to determine the efficacy of Clove Nanoemulsion (CNE) and Clove Nanogel (CNG) as antioxidants. With ascorbic acid acting as the positive control The CNE and CNG solution (1 mg/mL) was diluted with methanol to produce final concentrations of 100, 200, 300, and 400 µg/ml. 300 µl of methanol DPPH solute was added to CNE and CNG. Following incubation of the combination at ambient temperature for a duration of 30 minutes in the absence of light, its absorbance at a wavelength of 517 nm was quantified using a UV VIS-1800 spectrophotometer manufactured by Shimadzu in Japan [98]. The absorption bands shift from the violet end to the yellow end, as seen in figure (2-3). The scavenging activity was computed using the following formula :-

$$\text{Scavenging activity } \% = \frac{(\boldsymbol{Absorbance\ C\ ontrol - Absorbance\ Sample})}{(\boldsymbol{Absorbance\ Control})} \times 100\%$$

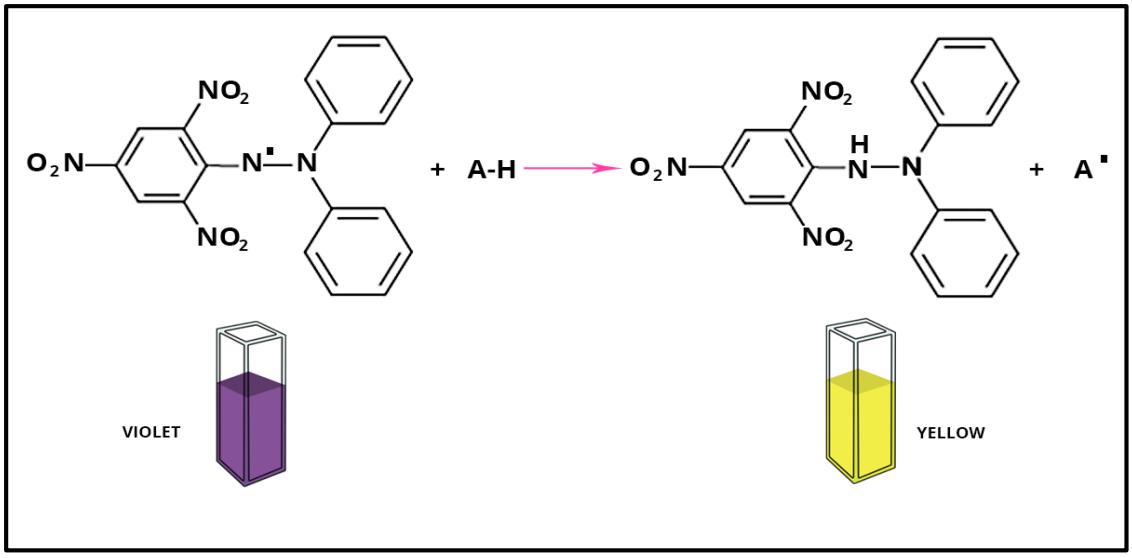


Figure (2-3): Reaction DPPH with AH [99].

2.6.3 Anti-Bacterial Activity:

This study aimed to determine the antibacterial activity of clove oil (CEO) and clove nanogel (CNG) against bacterial, two types of standard bacterial strains were used, *Staphylococcus aureus* (ATCC25923) and *Escherichia coli* (ATCC25923) as positive and negative gram stains respectively which were provided by the American Type Culture Collection (ATCC). The Agar-Cup Well Diffusion Method was employed to assess the antimicrobial properties. These investigations were conducted against two types of bacteria, *Staphyloccocus aureus* and *Escherichia coli*, which are known to be harmful to humans and animals. These bacterial strains were incubated for 24 hours at 37°C in nutrient agar. The pharmacological and therapeutic significance of these bacterial strains was taken into consideration during their selection. The bacterial species were grown on Mueller-Hinton agar. After solidification, a sterile borer was used to drill 5 mm holes, and 0.25 ml of test strains were added. After adding solutions of clove oil and CNG (250, 500, and 1000 µl/ml) to each well, the plates were incubated for 24 hours at 37°C. A blank well was carried by adding solvent alone (DMSO) to act as a negative control. After

an incubation period under 37 °C for 24 h, was measured growth inhibition zones for all sample investigated [100]

2.6.4 In vitro release assay

In this study, we examined the slow release of Clove Oil (CEO) from Nanogel (CNG) encapsulated in SGF, SIF, and buffer over a period of 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 24, 48, 72, and 96 hours. We used UV-Vis spectroscopy at 284 nm to measure the amount of clove oil released from the NG. The following equations were used to compute the percentage of clove oil release, and a standard curve was used to quantify the concentration of released clove oil [85].

$$\text{Amount of clove oil released} = \frac{\text{Concentration x Dissolution bath volume x dilution factor}}{1000}$$

$$\text{Percentage of clove oil release} = \frac{\text{amt of clove oil released}}{\text{weight of nanogel}} \quad x\ 100$$

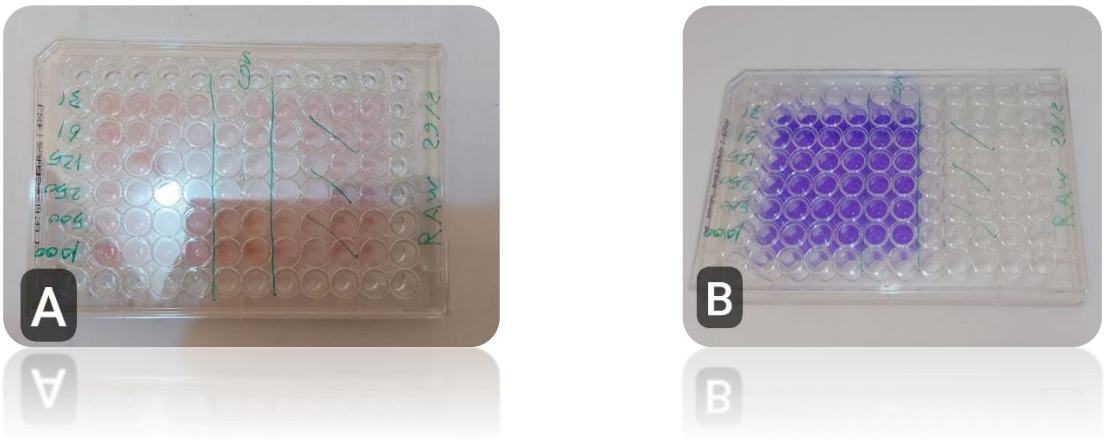
2.6.5 MTT Assay

The MTT test is often used to measure viable cell numbers on 96-well plates, which allow for fairly high throughput, without the need for complex cell counting. So far, testing the cytotoxicity of different doses of many medicines has been the most well-known use. The RAW 264.7 macrophage cell line, which was generated from a male mouse tumor, was used to assess the impact of Clove Nanogel on cell proliferation in vitro. The tissue culture laboratory at the College of Medicine at the University of Babylon generously provided us with this cell line. Growing the cells in RPMI 1640 media was the main method [101]. The cells were cultured in a humidified incubator with 5% CO_2 at 37 °C after plating them in 96-well plates with 100 ml of RPMI 1640. The cells were treated with different doses of CNG (31-1000 µg/mL) and a medium control for 24 hours at 37°C to assess the cytotoxic effects of the compound. After the exposure time, a

fresh 100 ml of medium containing 5 mg/mL of MTT solution was added to each well as a post-treatment measure. A microtiter plate ELISA reader was used to measure the absorbance at 570 nm after incubating the formazan crystal of the MTT reduction for four hours in the dark. The crystal was dissolved in DMSO. Every experiment was conducted four times. The ability of the cells to convert the yellow MTT dye into a blue formaldehyde crystal was used to measure their viability. A reduction in absorbance relative to the control suggests cell death or inhibition of proliferation, while an increase in absorbance relative to the control indicates cell growth. To determine the proportion of cell viability or inhibition, the following formula was used.

$$\textit{\% viability } \frac{\textit{Absorbance Sample} - \textit{Absorbance Blank}}{\textit{Absorbance Control} - \textit{Absorbance Blank}} \times \textit{100\%}$$

% Inhibition =100 - % Viability



Figure(2-4): (A) the plate befor incubated and add dye MTT,(B) after incubated , add dye MTT and DMSO.

2.6.6 Anti-inflammatory effect studies

To Estimate the anti-inflammatory effects of the CNG in vitro was used RAW 264.7 a macrophage cell line that was established from a tumor in a male mouse. This cell line was kindly supplied from tissue culture laboratory/ Collage of medicine/ University of Babylonl. The cells were essentially grown in RPMI 1640 medium. Cells were plated into 24-well dishes with 100 ml of RPMI 1640 and kept at 37 °C in a humidified, 5% CO_2 incubator, in plate was used different concentrations 250, 500, and 1000 µg/ml of the CNG with or without (80 ng/ml) of LPS and incubated for 48h , tests were performed in triplicates, the positive control was LPS in medium and the negative control was medium only solution, After incubation the levels of (IL-1) and (TNF-α) was measured in cell lines with and without LPS using ELISA method , if the data is analyzed using SPSS and a test is performed T-Test [102].

2.6.6.1 Test principle

This ELISA kit uses the sandwich-ELISA principle in which the plate is precoated with an antibody specific to the human cytokine of interest. Samples or standards are added to the plate and combined with the antibody. The biotinylated detection antibody and avidin-HRP conjugate are added to the plate changing the color to blue. The enzyme-substrate reaction is terminated by addition of stop solution and the color turns yellow. The optical density is measured at wavelength of 450 nm. The concentration of cytokine of interest is measured by comparing the OD of sample to the standard curve .

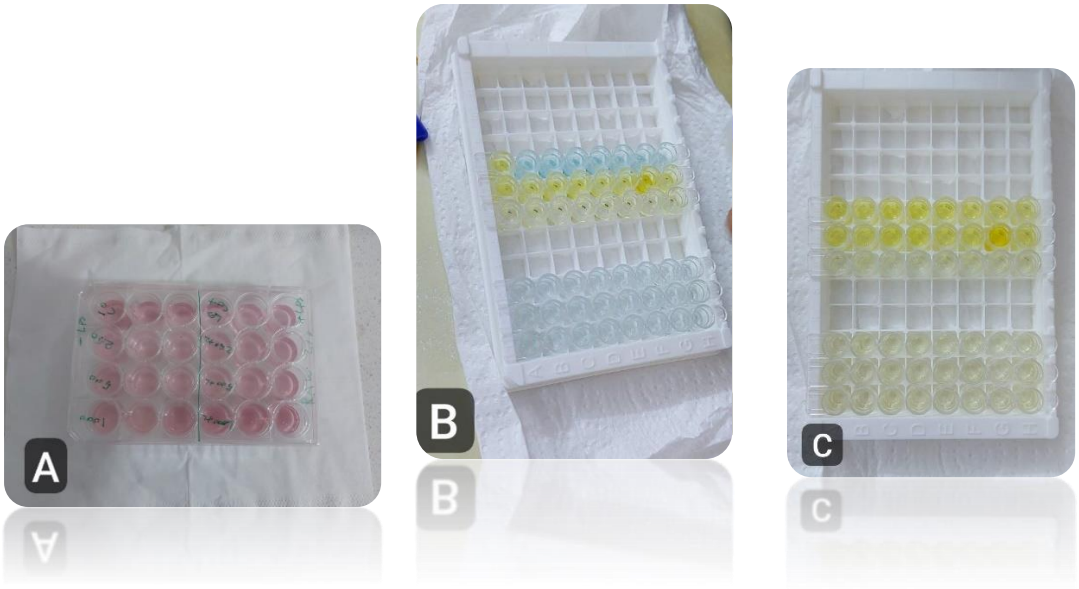


Figure (2-5): (A) the plate befor incubater, (B) after incubater and during add stop solution, (C) after the reaction termination stage.

CHAPTER THREE

Results and Discussion

3. Results and Discussion

3.1 Percentage of Oil Isolated from Cloves

The weight of the isolated oil which was obtained from the method (2.4.1) was calculated and the percentages of the oil were calculated according to the following equation.

$$\textbf{Percentage of oil} = \frac{(\textbf{Oil weight})}{(\textbf{sample weight})} \times 100\%$$

The findings demonstrated that the clove plant produced a good 10% of volatile oil. According to Amelia et al. [103], clove buds from Java and Manado had essential oil contents of roughly 4.99% and 4.58%, respectively, when subjected to the steam distillation method—a lower percentage than what was found in the current study. Conversely, Guan et al. [104] discovered that Chinese clove buds had a comparable essential oil content (10.1% by weight of steam distillation and 11.5% by weight of water distillation). According to Kapadia et al. [105], clove buds (using a microwave-assisted extraction process) contain between 11.93% and 13.11% percent essential oil by weight. The yields of 1.87% [106], 7.05% [107], 11.35% [108], and 15.40% [108] are reported by other sources. Clove bud origin, variety, storage conditions, and sample preparation all affect variations in essential oil production. The isolation process's and as well as the technique used.

Table (3-1): the physical properties of clove oil obtained.

Physical properties	
The appearance	Transparent, yellowish liquid
Density	1.07 kg/cm^3
Boiling Temperature	251 $^{\circ}$C

3.2 Characterization of the Clove Nanogel (CNG)

3.2.1 Determination wavelength maximum (λ_{max}) of Clove Nanogel (CNG) by UV-visible spectrophotometer.

To further verify the results, the formation and stability of the synthesized Clove Nanogel were determined by measuring the absorption spectrum in the 265-320 nm wavelength range. As shown in Figure (3-1), the maximum absorption peaks at 284 nm, that was attributed to electronic transfers ($\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$) due to the presence of electronic doublets in both (tween - 20) and the active groups as phenol in the eugenol compound that forms 87% of cloves oil. A study done by [85] agree with of this study. The position and shape of the absorption peaks of nanoparticles are strongly related to particle size and morphology. A sharp, single absorption peak corresponds to spherical nanoparticles [109].

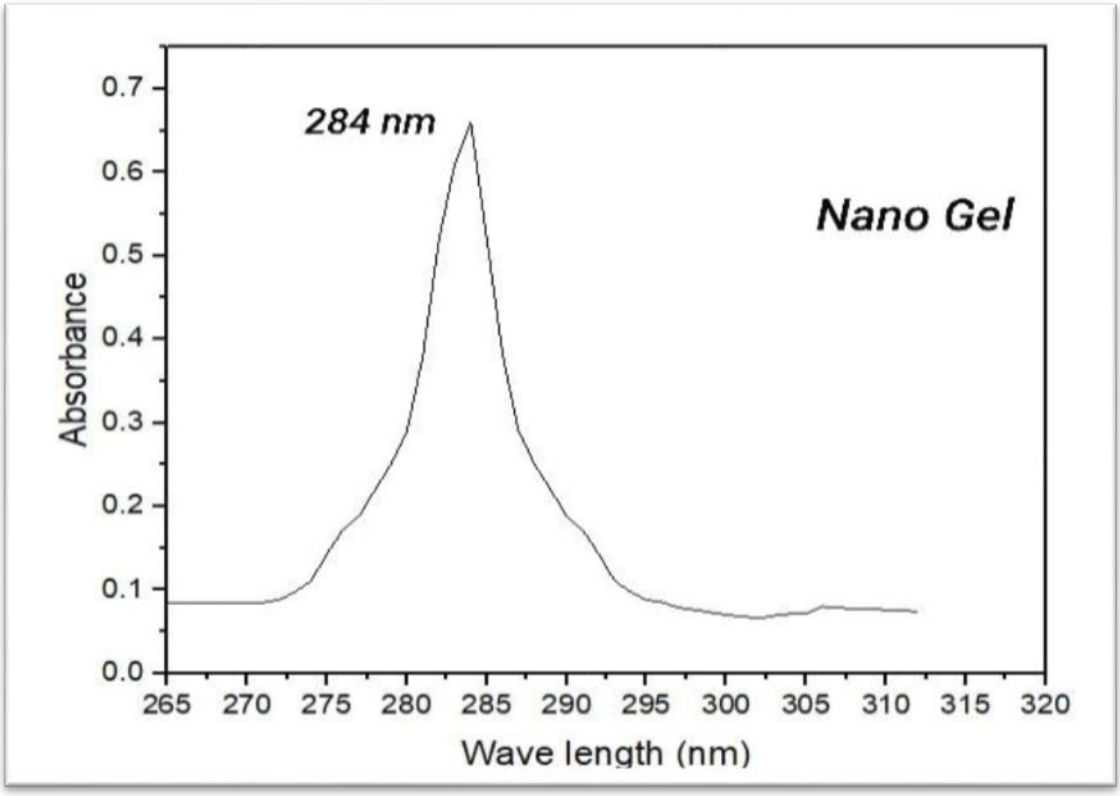


Figure (3-1): Absorption spectrum of Clove Nanogel**.**

3.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

Figure (3-2) shows the FTIR spectra of the samples in terms of transmittance rate (%). In the cloves oil spectrum, the peak at 2924 cm^{-1} is attributed to O-H stretching in the phenol group for eugenol component in the oil [110]. The appearance of weak to moderate absorption bands between 2951.19-2856.67 cm^{-1} due to the amplitude vibration of the aliphatic bond C-H is attributed methylen group CH_2 and methyl group CH_3 in Eugenol and Eugenyl acetate components. The peak at 1684.2 cm^{-1} is attributed to functional group C=O stretching in Eugenyl acetate, and the peak at 1631.83 cm^{-1} is attributed to C=C stretching in allyl group in Eugenol, and peak at 1514.17 cm^{-1} is attributed to C=C in aromatic ring. A very strong absorption band was observed at 885.36 cm^{-1} is attributed to C-O band of methoxy group.

FTIR spectrum of CMC showed the broad bands at 3333 cm^{-1} can be attributed to the stretching of the hydroxyl group O-H, the peak which indicated the presence of hydroxyl groups in carboxymethyl cellulose (polysaccharide). The peaks at 2915 cm^{-1} corresponded to the (-CH) groups stretching vibration in caboxymethyl cellulose [111]. The strong band at 1586 cm-1 related to C=O Carbonyl group. In addition, the FTIR absorption bands at 1412/1322 cm^{-1} was assigned to O-H bending vibration in polysaccharide. The peak at 1054 cm^{-1} is attributed to C-O stretching.

FTIR spectrum of clove Nanoemulsion, the broadband at 3458 cm^{-1} is attributed to O-H stretching vibration due to hydrogen bonding. The peak at 2855 cm^{-1} is attributed to C-H stretching, and the peak at 1750 and 1644 cm^{-1} exhibited C=O stretching, and the peak at 1163 cm^{-1} is attributed to C-O stretching.

Moreover, in the spectrum of clove-NG (1) without glycerin showed broad bands between 3416.05-3257.88 cm^{-1} is attributed to O-H stretching vibration due to hydrogen bonding, the peak at 1635.69 cm^{-1} attributed exhibited C=O stretching, and the sharp and strong peak at 1084 cm^{-1} is attributed to C-O stretching.

The spectrum of clove-NG (2) with glycerin the peak at 3306.10 cm^{-1} is attributed to O-H stretching vibration due to hydrogen bonding, the peak at 2924.18 and 2881.75 cm^{-1} are attributed to C-H stretching. The peak at 1649.19 cm^{-1} exhibited C=O stretching, representing the overlap carbonyl group in Clove EO with tween molecules. The sharp and strong peak at 1041 cm^{-1} is attributed to C-O stretching.

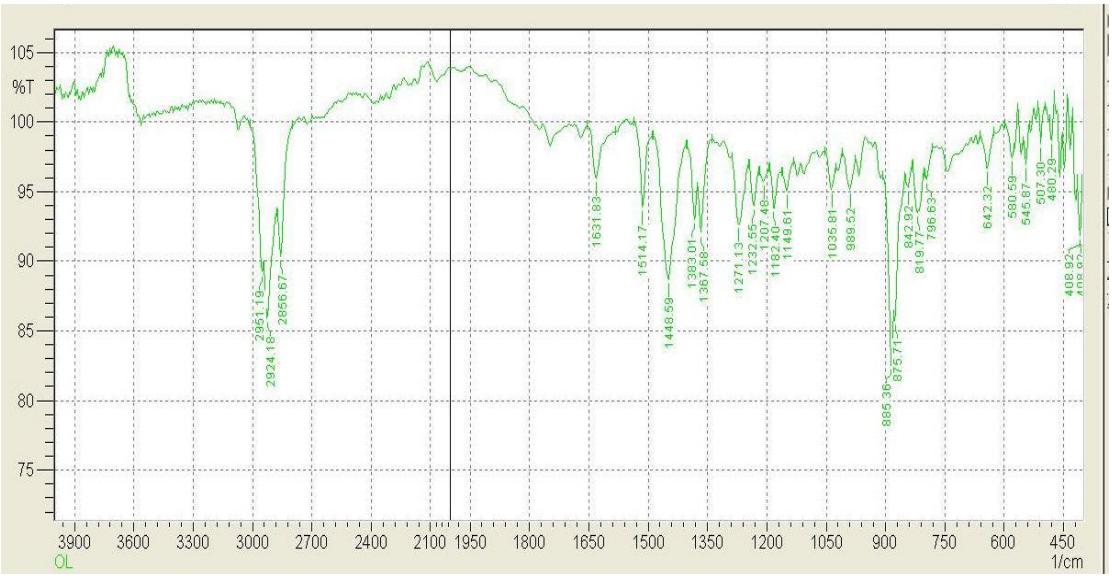


Figure (3-2): FTIR spectra of the clove oil.

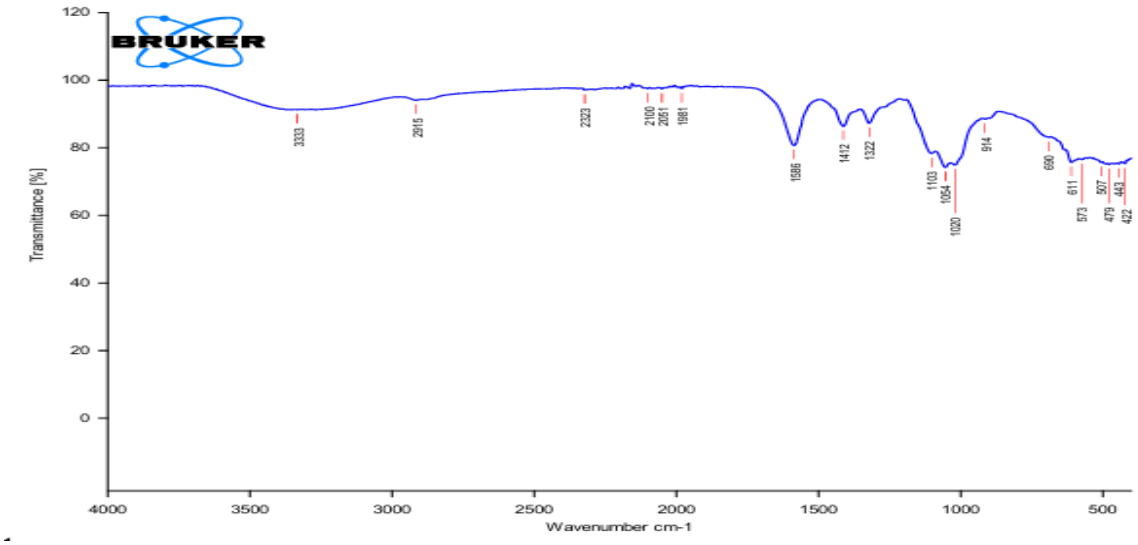


Figure (3-3) FTIR spectra of the CMC .

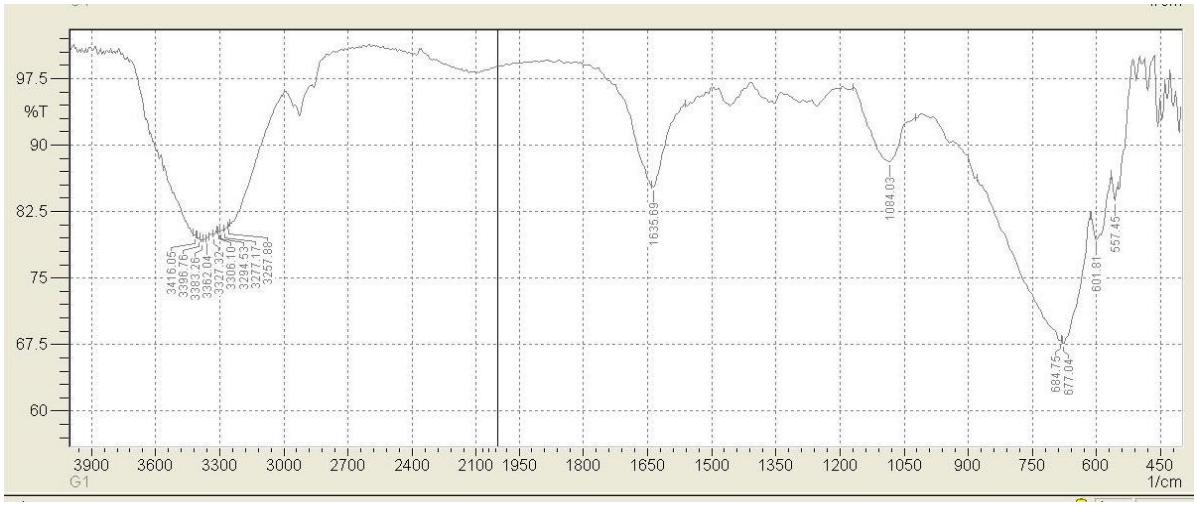


Figure (3-4): FTIR spectra of the Nnaogel

1 .

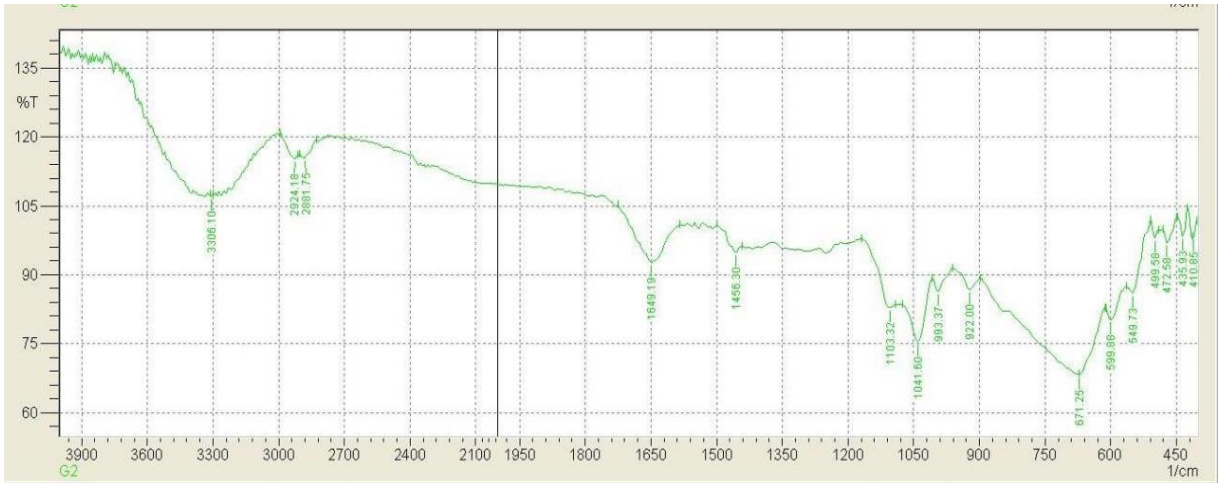


Figure (3-5) : FTIR spectra of the Nanogel 2

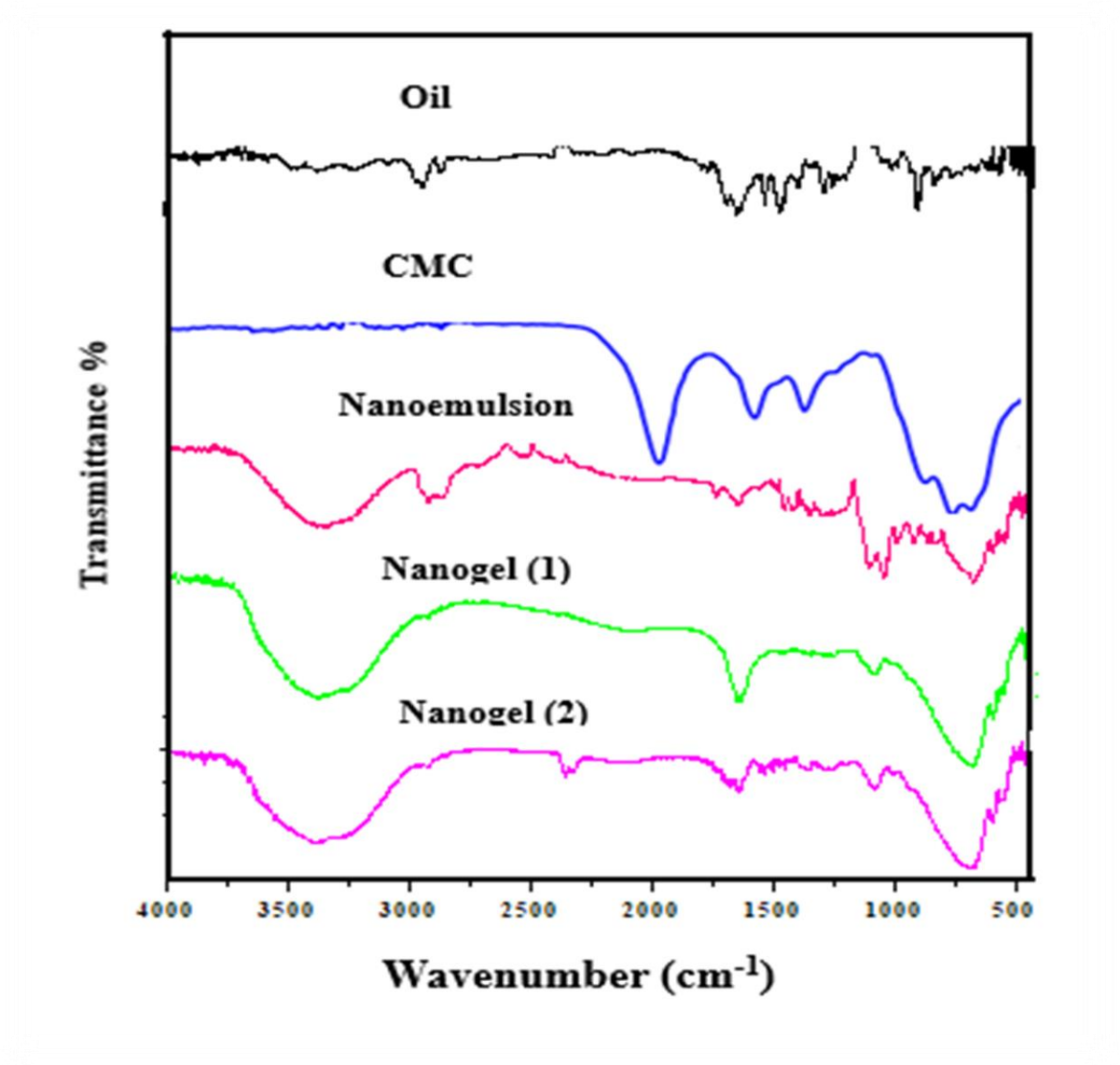


Figure (3-6): FTIR spectra of the sample (Oil, CMC, Nanoemulsion, Nanogel 1, Nanogel 2)

Table (3-2) Infrared spectrum results for clove EO, (CMC) carboxymethyl cellulose, Nanoemulsion, Nanogel with glycerin, and Nanogel without glycerin: its absorption bands, and their functional group.

	Functional group	**Bond**	**Bond shape**	**Bond Frequency cm^{-1}**
Oil	Phenolic	O-H stretching	B	3612
	C-H sympatric of aliphatic CH3, CH=CH	C-H stretching, CH_3 CH_2, stretching, CH=CH stretching	m-sh	2951.19, 2924.18, 2856.67
	C=O stretch Carbonyl group	C=O	m-sh	1684.2
	(alkene) Allyl group	C=C	m-sh	1631.83
	C=C aromatic	C=C	S-sh	1514.17
	C-H	C-H bending	Sh	1448.59
	O-H	O-H bending	m-sh	1383.84
	C-O (methoxy group)	C-O stretching	Sh	885.36
CMC	Polysaccharide	O-H stretching	B	3333
	(-CH)groups stretching	(-CH) stretching	m-sh	2915
	C=O stretch Carbonyl group	C=O	m-sh	1586
	O-H at polysaccharide	O-H bending	m-sh	1412
		O-H bending	w-sh	1322
	C-O vibration of carbonC2	C-O	w	1054
Emulsion	hydroxyl group O-H	O-H stretching		3458
	C-H sympatric of aliphatic CH3,	C-H stretching		2855
	C=O stretch Carbonyl group at tween20 ,eugenal acetate	C=O	w-sh	1750, 1644
	C-O (methoxy group)	C-O stretching	S-Sh	1163

Nanogel with glycerin	O-H,	O-H stretching	B	3306.10
	C-H sympatric of aliphatic CH_2	C-H stretching	m-sh	2924,2881
	C=O stretch ester group	C=O	m-Sh	1649
	O-H,	O-H bending	m-sh	1456.30
	C-O	C-O stretching	m-sh	1041
Nanogel without glycerin	Alcohol, Carboxy methyl cellulose, clove oil	O-H stretching	B	3416-3257.44
	carbonyl group in Clove with tween molecules	C=O stretching	m-Sh	1635
	C-O	C-O Stretching	m-Sh	1084

3.2.3 ^{1}H-NMR spectra of Clove Nanogel (CNG)

In Figure (3-7), shows the ^{1}H NMR spectra of Clove-NG. Figure 4 shows the proton signals obtained from the ^{1}H NMR spectra of CMC: Hydrogen, water, and hydrogen ions have concentrations of 4.114 ppm, 4.697 ppm, 4.103 ppm, and 3.284-3.911 ppm, respectively, according to reference [112]. Peaks at 2.36 ppm, 3.94 ppm, and 3.381 ppm (H8, H9, and H10) were seen in the ^{1}H NMR spectra of CMC-Glycerin, indicating the existence of the ester signal (Hou et al., 2018). Notably absent from the CMC-Glycerin-EUG ^{1}H NMR spectra were any vinyl peaks at 5.7-6.0 ppm for clove-NG. Nevertheless, a distinct signal appeared between 1.9 and 2.2 ppm, suggesting that the vinyl double bond was reduced to a single bond while being mixed.

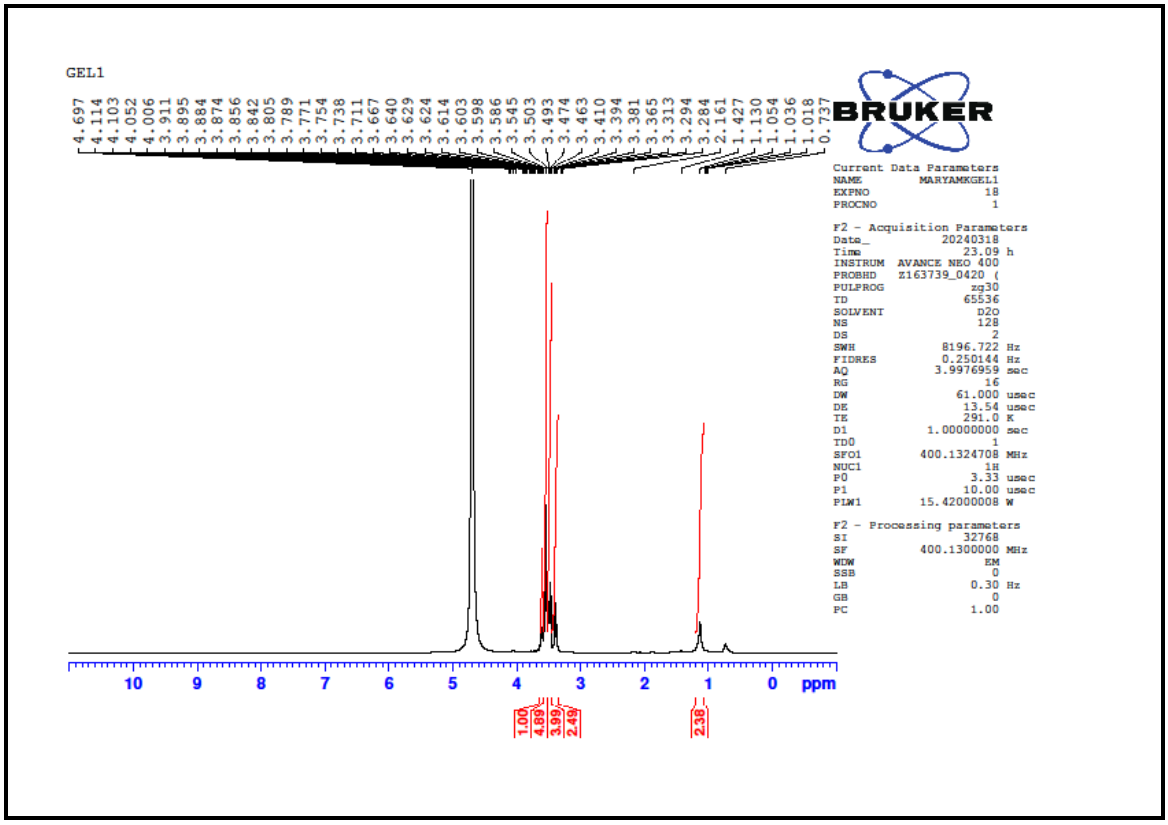


Figure (3-7): ^{1}H-NMR spectra of the Clove Nanogel

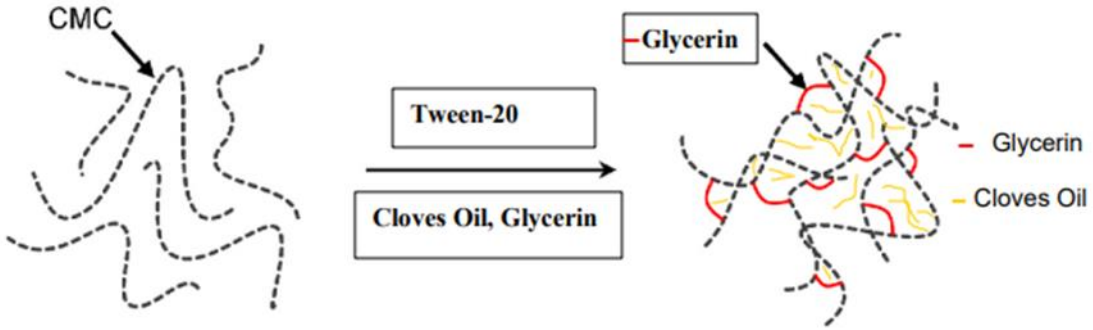


Figure (3-8): Suggestion scheme of the prepared Cloves Nanogel

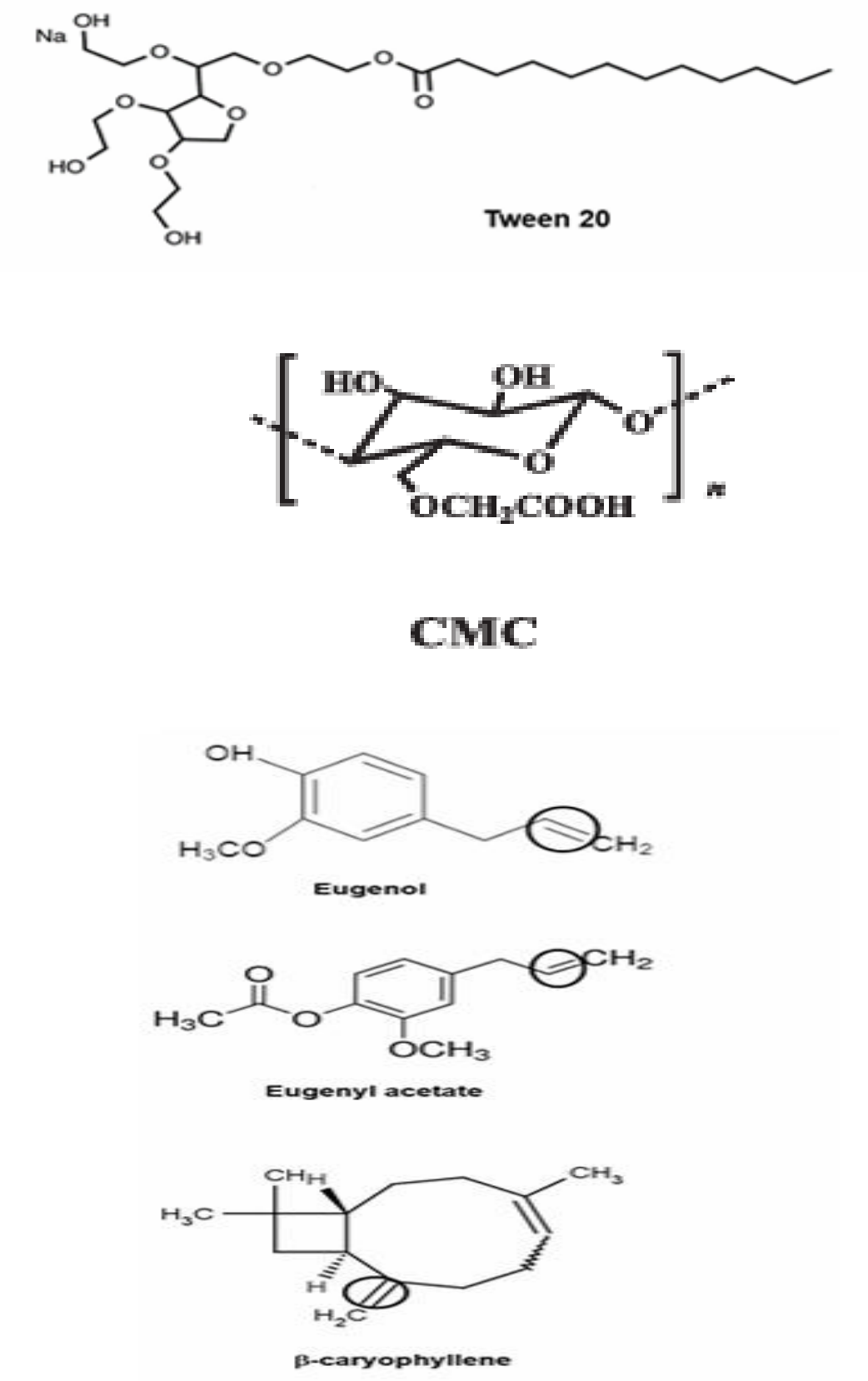


Figure (3-9): Chemical composition of the materials used in preparation the Nanogel.

3.2.4 Field Emission Scanning Electronic Microscope Analysis (FESEM).

Clove Nanogel without glycerin FESEM micrographs verified the debris to be densely packed, erratically shaped agglomerates. The nucleation-and boom-floor-free strength-based sample generation methods are hindered by the molecular attractions of specific-scale forces that cause elemental crystals to aggregate [113]. As seen in Figures (3 – 10). As a result, surface-loss power is reduced. Aggregation is the result of crystal growth inside the aggregates, which occurs in the presence of a persistent residual supersaturation. The agglomerated particle then combines with additional particles to form secondary debris, which grows in size over time.

Clove Nanogel with glycerin FSEM images also revealed surface lamellar structure creation of varied intensities, suggesting the possibility of microporous-like structures. Little patchy structures and a few big lamellar structures that are only partially hydrated are the primary byproducts of the sample's hydration process.as seen in figures (3-11).

Figure (3-10). The FESEM analysis of Clove Nanogel without glycerin at (500nm, 200nm, 100nm, 1µm) scales.

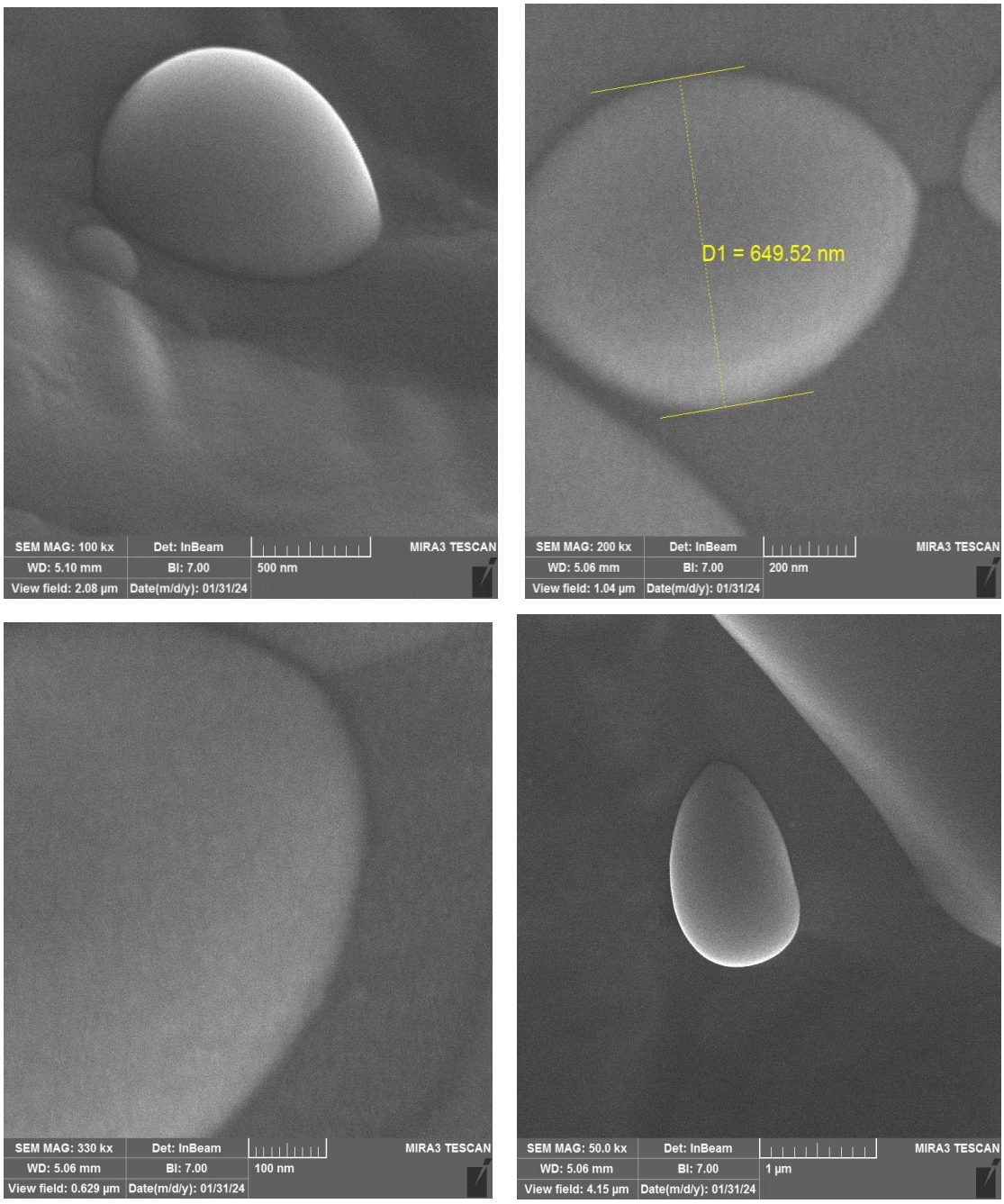


Figure (3-11): Clove Nanogel with glycerin at (500nm, 200nm, 100nm, 1µm) scale.

3.2.5 Dynamic Light Scattering (DLS) Analysis

calculated Clove Nanogel (CNG) hydrodynamic diameters and polydispersity indices (PDIs) using density-functional theory (DLS) modeling. Figure (3-12) shows that the usual hydrodynamic widths of the clove Nanogel are measured to be 276 nm, as seen in the DLS size distribution histogram. DLS uses Brownian motion as a basis for measuring the diameter of solution-bound particles. The average clove nanogel diameters were bigger than those found with FESEM analysis [114]. This is because DLS analysis takes into account the hydration layer of water molecules when measuring particle size. A polydispersity index (PDI) value of 0.279 indicated good stability and monodispersity, settled on the best formulation (CNG) according to PDI values and particle size. Particle size and intensity are affected by the surfactants' physicochemical and steric structures as well [115]. In the 276 nm range, a large peak with a wide dispersion of particles was seen in the CNG produced with Tween 20. The probable reason for the larger average sizes in DLS compared to SEM pictures is that SEM only reveals the CNG on the surface of the agglomerates. However, DLS takes a three-dimensional view of the particles, which may include a bimolecular covering. Previous documentation has documented the disparity between the average sizes of SEM and DLS, which is associated with method-specific details.

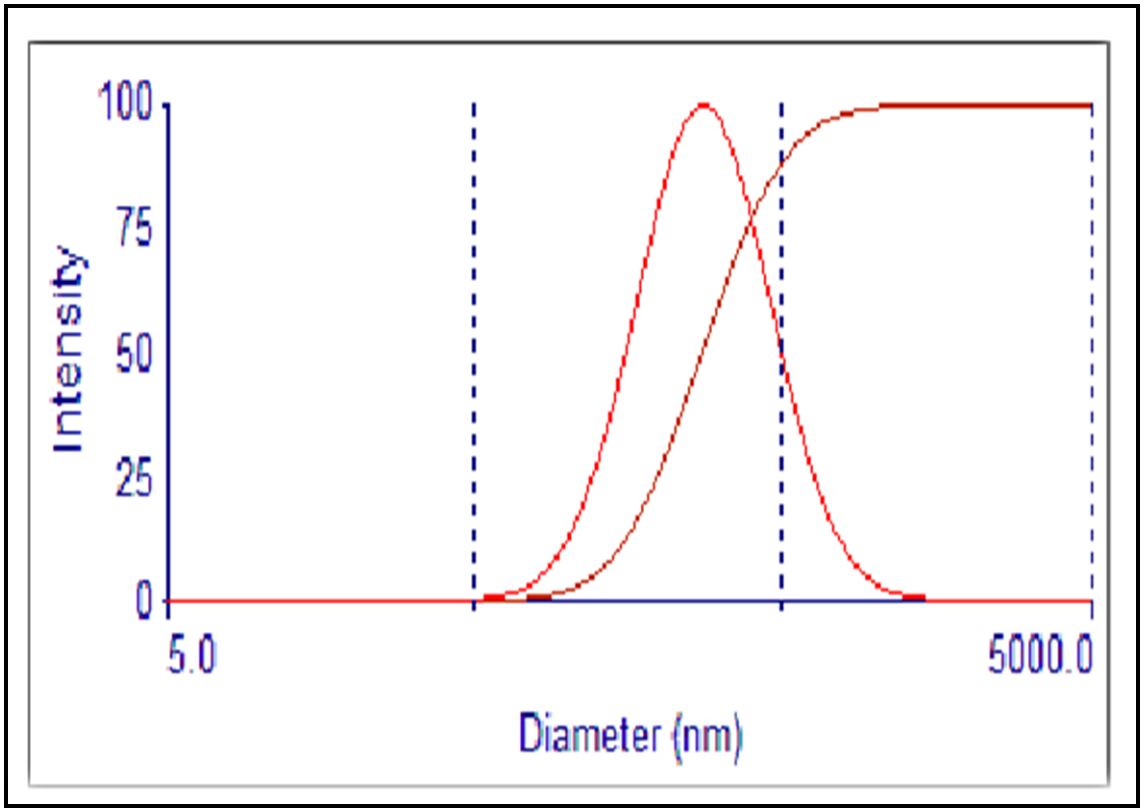


Figure (3-12): Size distribution by intensity of the clove Nanogel.

3.3 Application for Clove Oil and Clove Nanogel (CNG)

3.3.1 Hemolysis assay

Isolated volatile oil and Clove Nanogel's toxicity was assessed using red blood cells since the procedure is cheap, simple, and yields findings quickly. At doses ranging from 7.5,15,30,60 and 120 µg/mL, Figure (3–13) shows that both clove Nanogel and clove oil had less hemolytic activity in comparison to the control. While cloves oil shown a lower proportion of hemolysis activity at lower doses, nanogel exhibited a greater percentage at concentrations of 7.5 and 15 µg/mL. and they was lower than cloves oil; this agrees with a research [85], which found that the rate of red blood cell breakdown is dependent on three variables: concentration, incubation time, and temperature. The covalent interaction between harmful chemicals and the free radical of the protein (SH) causes hemolysis

by destroying the red blood cell membrane [116]. One sure sign of a substance's biological incompatibility is hemolysis, which may happen as a consequence of direct or indirect injury to the RBC membrane. Finding out how a biomaterial reacts when exposed to blood is one way to measure its hemolytic potential [117]. Hemolysis occurs when the red blood cells release hemoglobin due to cell rupture. The results show that the nanogel greatly decreases the cytotoxicity of clove oil, as confirmed by both the cytotoxicity and hemolysis data.

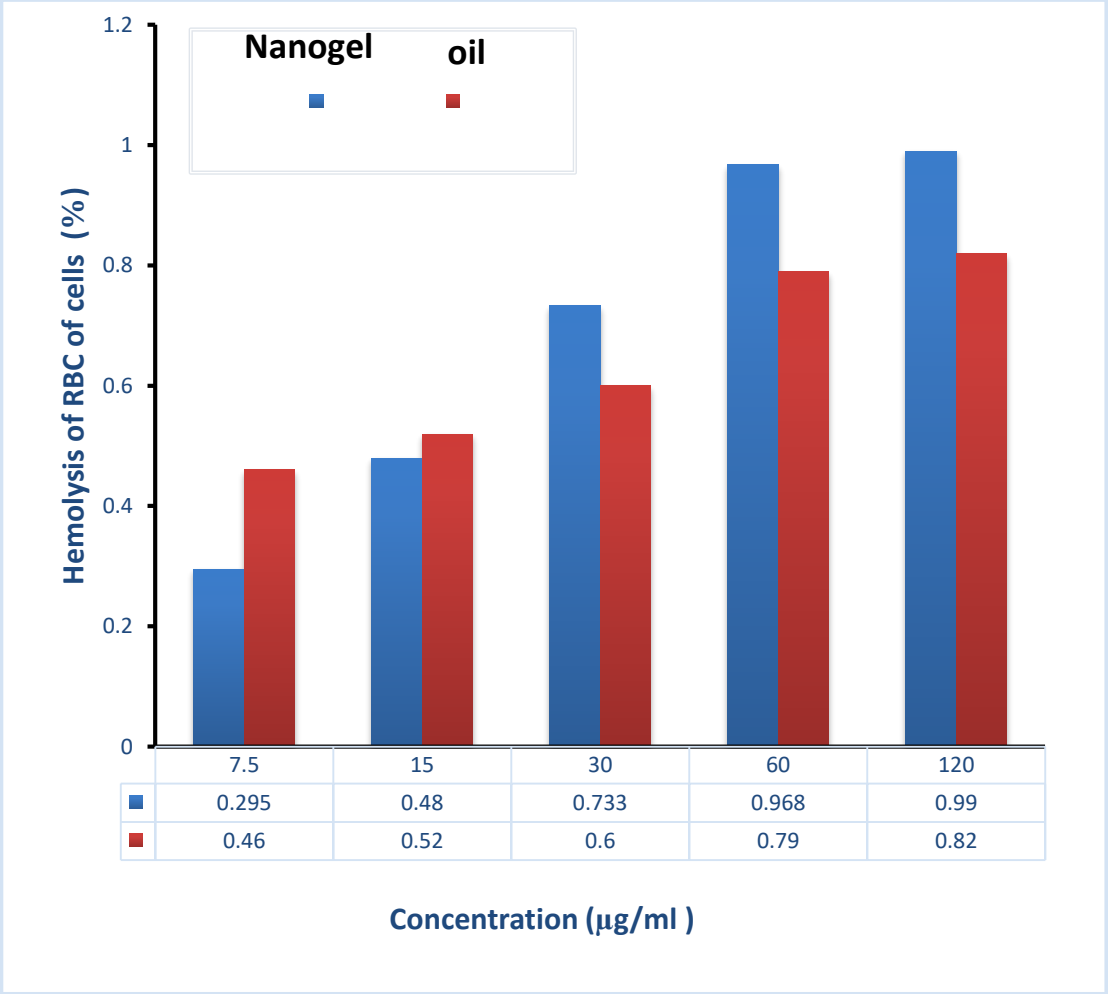


Figure (3-13): Hemolysis activity of the Clove oil and Clove Nanogel.

3.3.2 Cytotoxicity assay

Evaluating the cytotoxic activity of clove nanogel (CNG) is a crucial factor in assessing the cellular level safety properties of CNG. Also, the *vitro* cytotoxicity of CNG differs depending on their size and the kind of cells being tested [118,119]. Therefore, a cell viability experiment was conducted to examine the in vitro toxicity of CNG on RAW 264.7 cell lines using MTT. According to Figure (3-14), CNG demonstrated higher cell viability compared to the control. Not even at the highest possible dose of 1000 µg/mL is there any indication of cell toxicity. Higher absorbance levels, in comparison to the control, indicate cell multiplication, while lower values suggest cell death or inhibition. Consequently, the positive results suggest that there is no cellular toxicity at any quantity, and the Clove nanogel that was created does not harm cells.

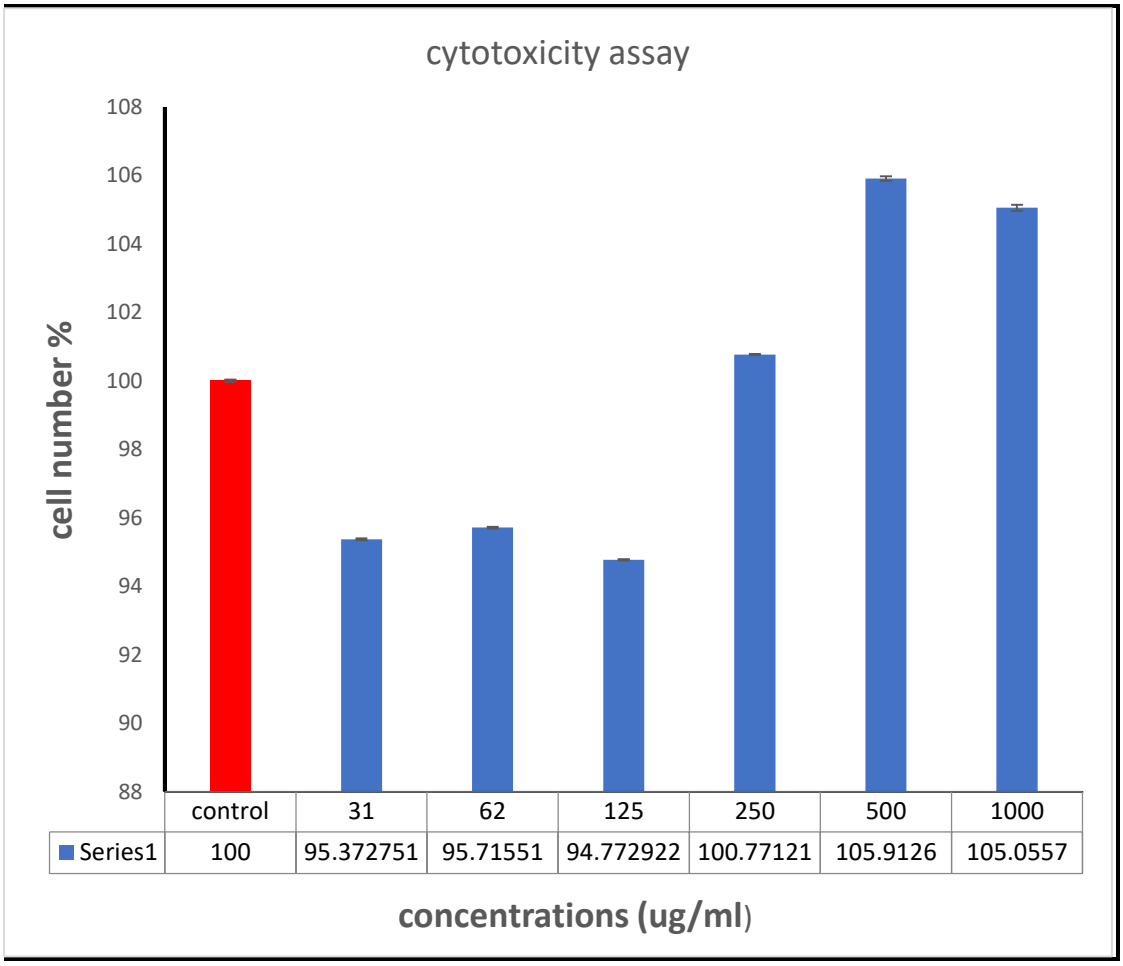


Figure (3-14): The cell toxicity of the clove Nanogel**.**

3.3.3 Antioxidant properties of Clove Nanogel (CNG) and Clove Nanoemulsion (CNE)

The ability of CNE and CNG to remove free radicals was evaluated using the DPPH assay. Nanomaterials have been extensively used to evaluate the antioxidant capacity by using free radicals like DPPH [120]. The DPPH solution was initially deep violet, but it became light yellow with the addition of CNE and CNG, with ascorbic acid serving as the standard reference. Based on Figure (3-15), it can be shown that the CNE and CNG exhibited significant antioxidant activity when administered at doses ranging from 100-400 µg/mL. Clove Nanoemulsion (CNE) reduced the DPPH radical formation in a dose-dependent manner. The CNE showed inhibition of 65% at a concentration of 100µg/mL, whereas CNG at the same concentration exhibited 59.2% of inhibition. The calculated IC50 values of CNG and CNE were 6.58 and 3.25 µg/mL respectively.

It is well-established that nanoencapsulation may boost the antioxidant capacity of natural compounds, as supported by references [121, 122]. The explanation for this is, also linked to antioxidant chemicals in clove oil, such as eugenol, eugenol acetate, ß-caryophyllene, and a-humulene. These compounds help protect cells from oxidation caused by free radicals. Clove oil has shown the ability to scavenge radicals and reduce lipid peroxidation [123, 124]. The presence of the hydroxyl group on the aromatic ring of eugenol is accountable for its antioxidant procedure [125]. Phenolic chemicals can transport electrons or hydrogen atoms and neutralize them, inhibiting the oxidative process [126]. As seen in Figure (3-16).

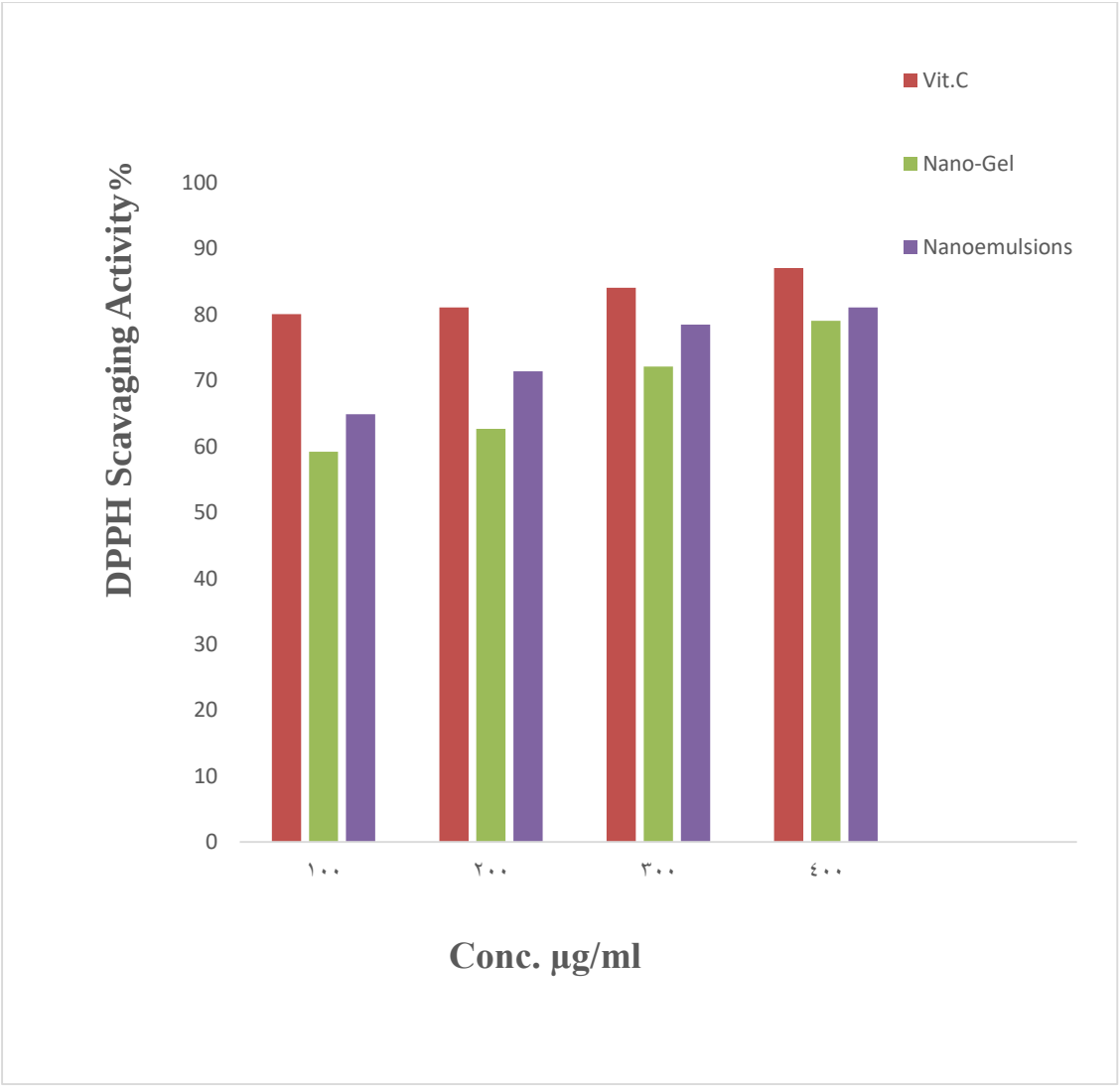


Figure (3-15): Antioxidant activities of CNE and CNG.

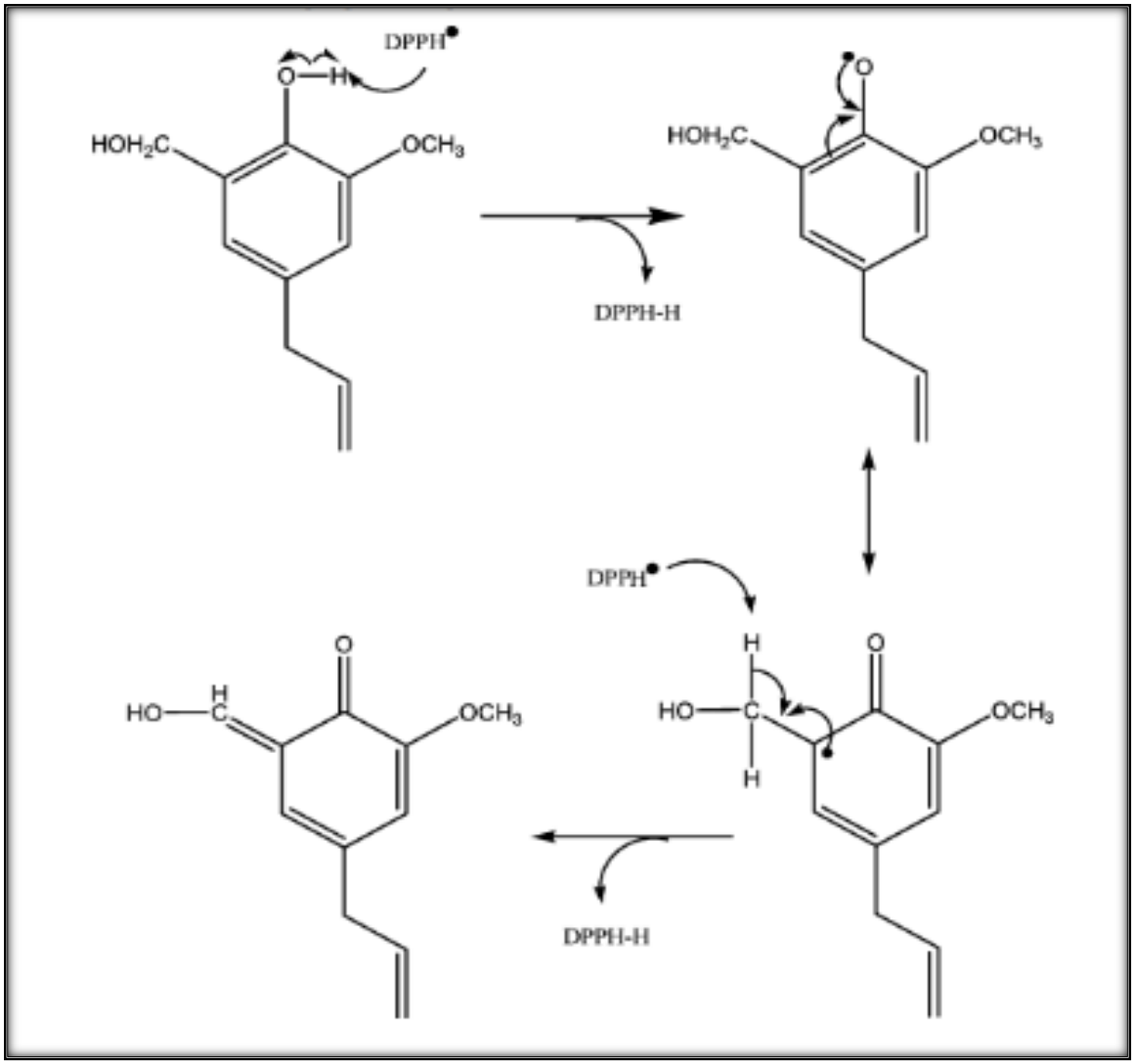


Figure (3-16): Suggested mechanism Potential Contribution of the Hydroxymethyl Group to DPPH Radical Scavenging Activity [127].

3.3.4 Determination of the Calibration Curve of Clove Nanoemulsion (CNE) by UV-visible Spectrophotometer

The calibration curve of CNE was determined by using a series of standard solutions (0.5, 1, 1.5, 2, 2.5, and 3 ppm) of CNE in SGF, SIF and Buffer, at λ max = 284nm as shown in Fig. (3-17), (3- 18) and (3-19) respectively.

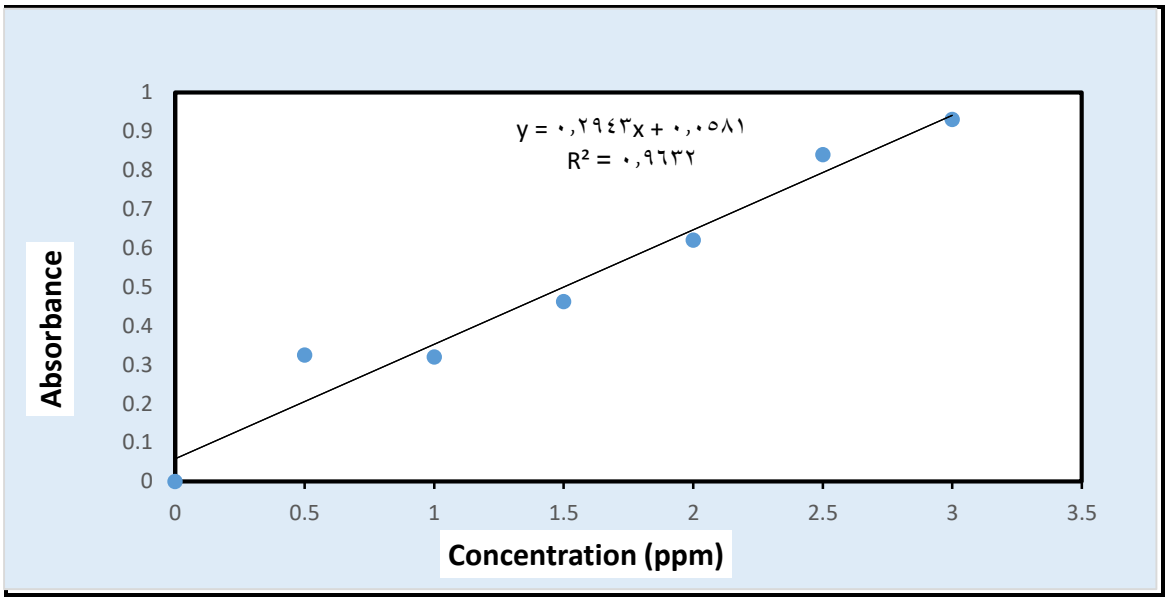


Figure (3-17): Calibration curve of CNE in SGF at 284 nm**.**

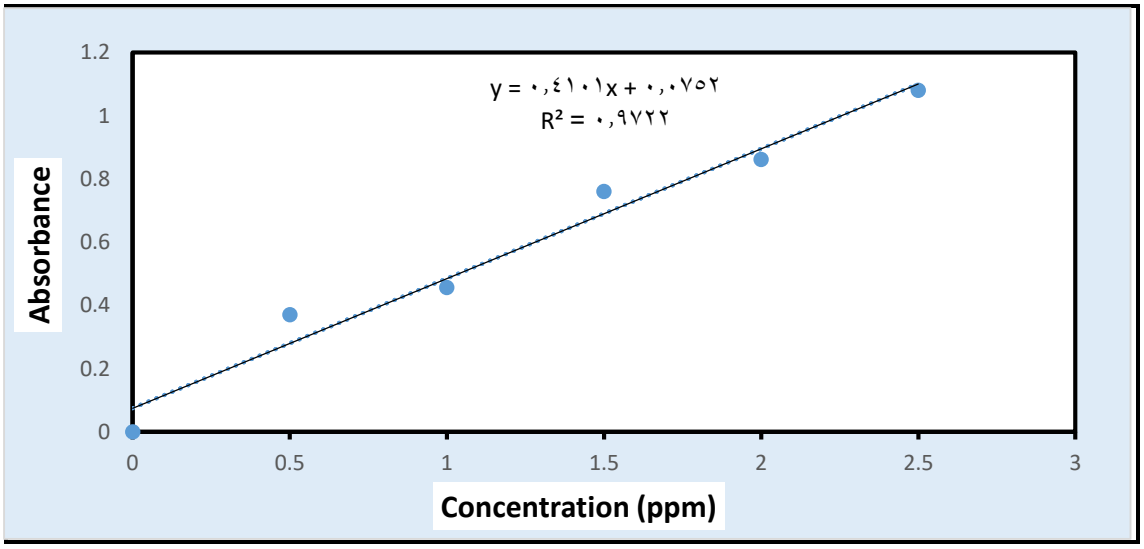


Figure (3-18): Calibration curve of CNE in SIF at 284 nm.

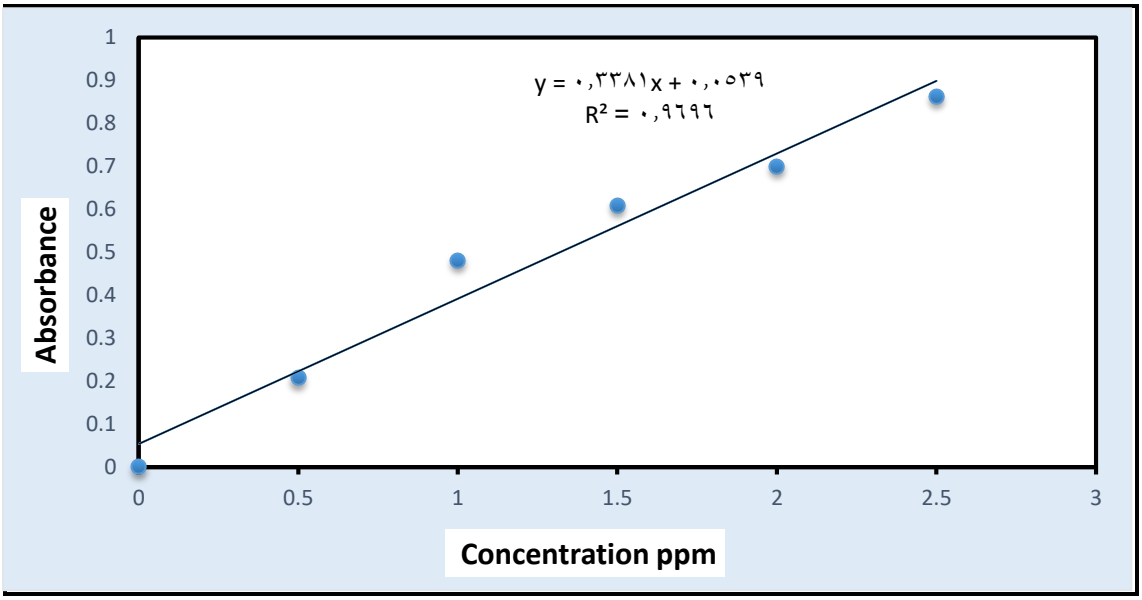


Figure (3-19): Calibration curve of CNE in Buffer at 284 nm

3.3.5 In vitro drug release of clove oil from Nanogel (M1, M2, M3 and M4) in SGF, SIF, and Buffer, for 1-12 h, 24h, 48h, and 96 h.

Based on the method described in (2.4.2), cloven Nanogel (CNG) was synthesized and characterized using a mixture of CMC and glycerin as a cross-linking agent. The presence of cloves between the chains played a crucial role in the preparation of clove Nanogel. This study involves measuring the release of Clove EOs in pH solutions various. The results discuss the impact of glycerin, a commonly used and safe plasticizer, on water flexibility, permeability, and stiffness reduction [128]. The concentration of glycerin is typically associated with the matrix basis, such as polysaccharides or proteins. The release is expeditious when glycerin is not used and takes place during the first few hours of submersion in various pH settings. Furthermore, the discharge has become more limited as the glycerin ratio has increased with time. As seen in figure (3-20), (3-21), (3-22), and (3-23)

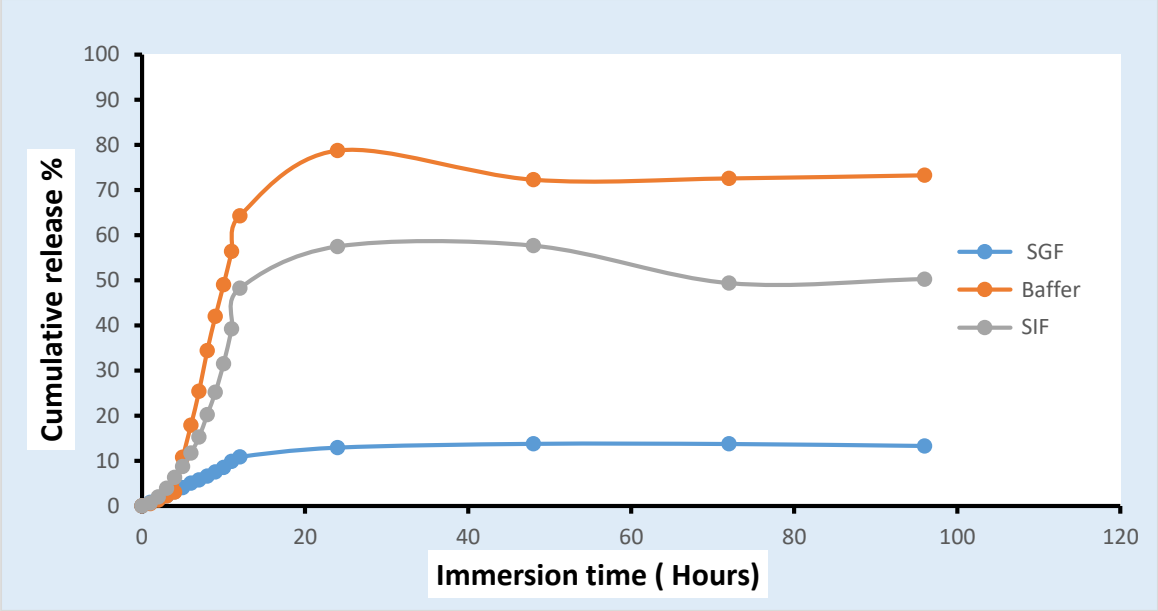


Figure (3-20): In vitro release cloves oil from NG (M1) in SGF, SIF, and Buffer

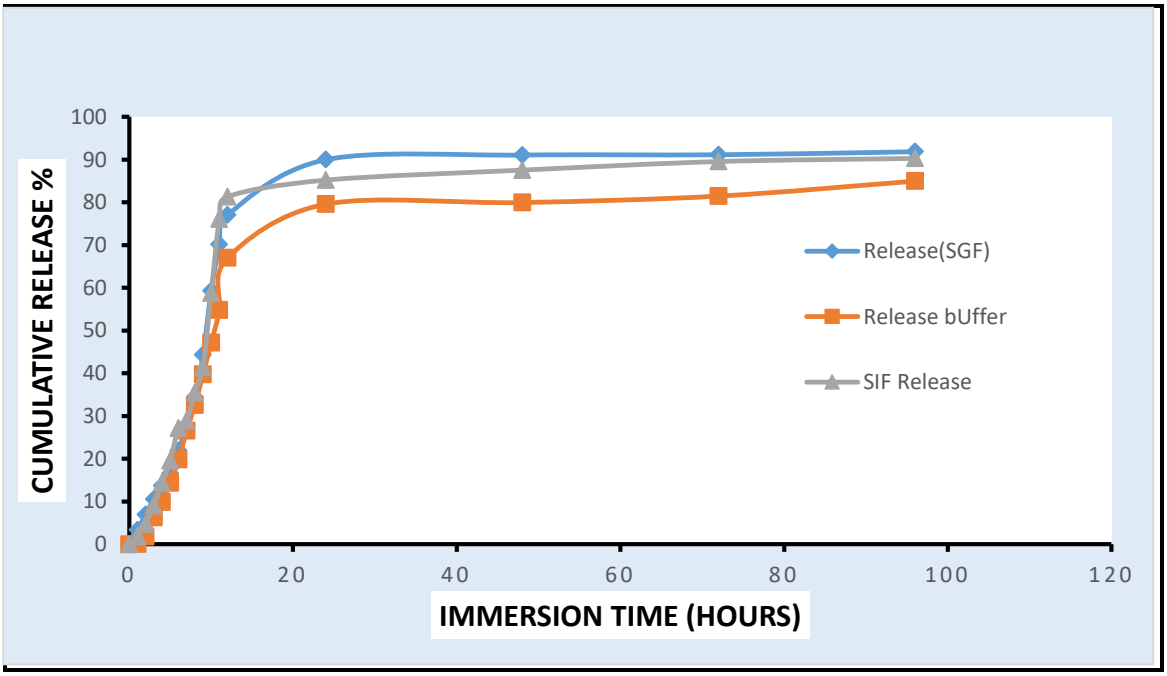


Figure (3-21): In vitro release cloves oil from NG (M2) in SGF, SIF and Buffer.

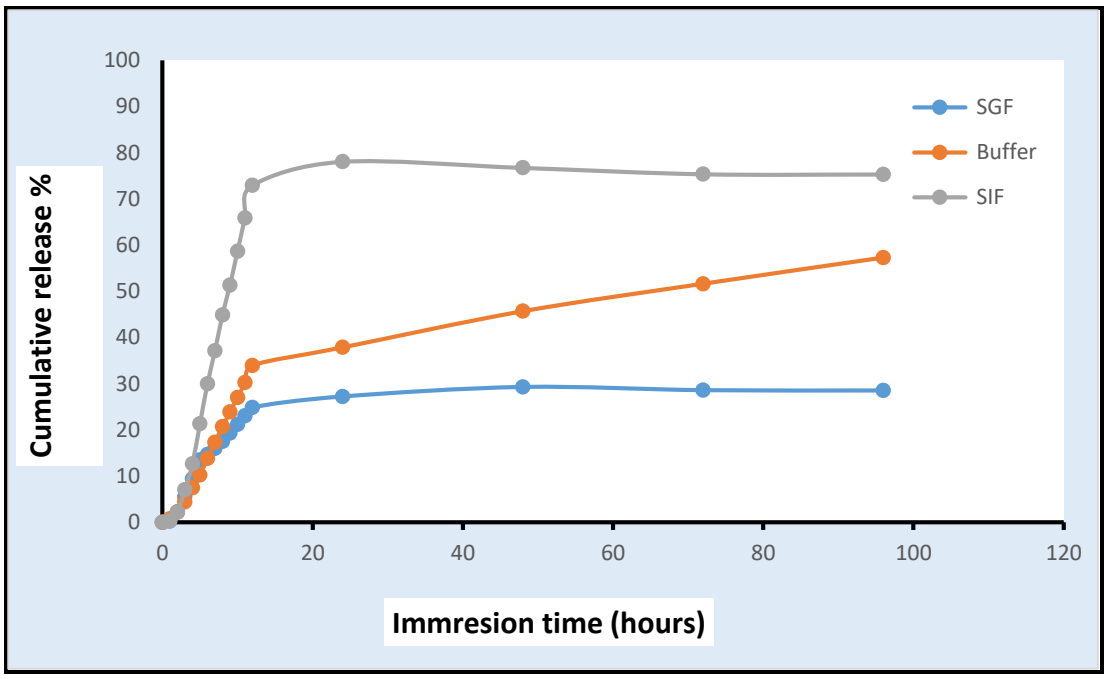


Figure (3-22): In vitro release cloves oil from NG (M3) in SGF, SIF and Buffer.

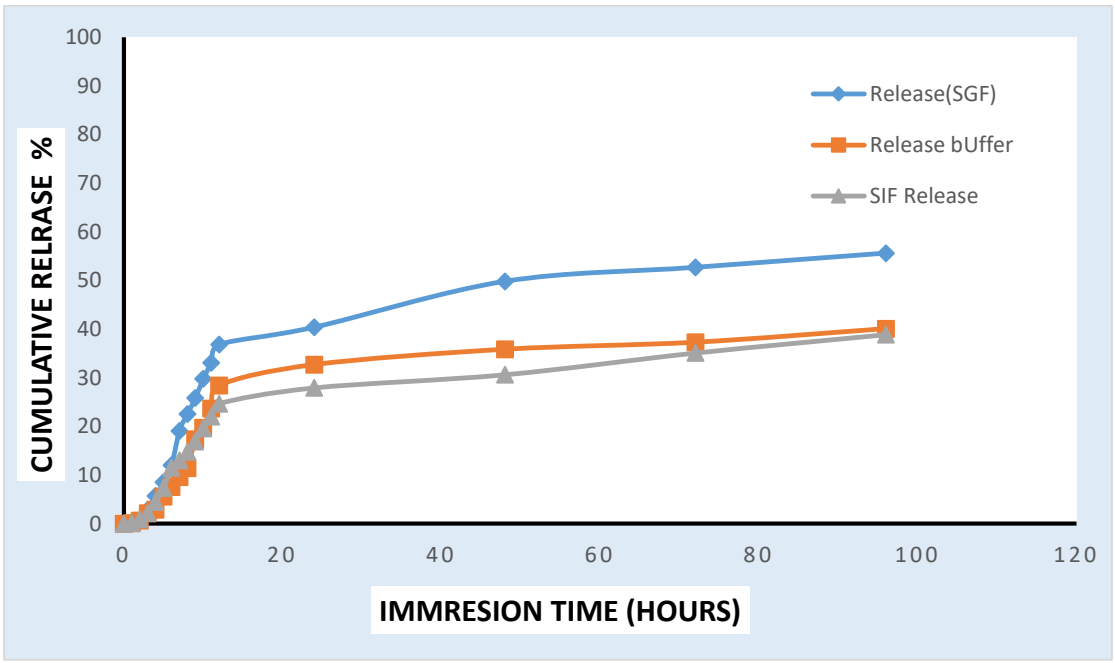


Figure (3-23): In vitro release cloves oil from NG (M4) in SGF, SIF, and Buffer.

3.3.6 Anti-bacterial activity of Cloves Oil (CEO) and Clove Nanogel (CNG)

The antibacterial activity of cloves oil and clove Nanogel was tested by using the agar wells diffusion method as described [129]. Two bacterial species were used in the study: *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive). The results presented in Tables (3-3) and (3-4) showed that Cloves oil and *clove Nanogel* caused different inhibition zones ranging for the cloves oil from the highest (23 mm) against *E. coli* at 1000 µg/ml concentration to lowest (11 mm) inhibition zone on *S. aureus* at 250 µg /ml. Other inhibition zones caused by *clove Nanogel*, ranged from the highest (20 mm) against *E. Coli* at 1000 µg /ml concentration to the lowest inhibition zone (10 mm) on both of *E. coli* and *S. aureus* at 250 µg /ml concentration. From these results, we notice that the effect of oil and gel on inhibiting negative bacteria is greater than their effect on positive bacteria due to the thickness of the cell wall bacteria (*S. aureus*), through the current findings, the insulated volatile oil has been shown to have high anti-bacterial efficacy compared with clove Nanogel.

The anti-bacterial efficacy of this oil can be due to the phenolic compounds that are known for their anti-bacterial efficacy, discuss [130–132] the antibacterial effects of cloves oil and its main components, particularly β-caryophyllene, which is comparable to eugenol. According to previous research, the -OH groups found at the ortho and meta locations in cloves oil basic chemical makeup are responsible for its antibacterial action because they may interact with the bacterial cell membrane [133]. Some researchers think this occurrence might damage the bacterial phospholipid membrane, which in turn can stop the cell from transferring electrons, proteins, or phosphorylating [134,135].

An outstanding antibacterial action against *Bacillus subtilis, S. aureus, Klebsiella pneumonia,* and *Vibrio cholera* was shown by Hemalatha et al., [136] in the methanolic clove extract. In their study, Cui et al. [137] discovered that CEO had positive effects against *E. coli* and *S. aureus.* Additionally, using histological and microbiological techniques, the anticandidal effects of eugenol and carvacrol were investigated and researchers have found that both eugenol and cinnamaldehyde, at a concentration of 2 g/mL, were more efficient than amoxicillin in inhibiting the growth of Helicobacter pylori after 9–12 hours of incubation, respectively [138,139].

Table: (3-3) Anti-bacterial activity of Clove oil (CEO) against bacterial Gram-negative (*E. coli*) and Gram-positive (*S. aureus*)**.**

Concentration µg/ml	**Inhibition zone (mm)**		
	250 µg/ml	**500 µg/ml**	**1000 µg/ml**
E. coli	12	15	23
S. aureus	11	13	20

Table: (3-4) Anti-bacterial activity of (CNG) against bacterial Gram-negative (*E. coli*) and Gram-positive (*S. aureus*)**.**

Concentration µg/ml	**Inhibition zone (mm)**		
	250 µg/ml	**500 µg/ml**	**1000 µg/ml**
E. coli	10	13	20
S. aureus	10	12	18

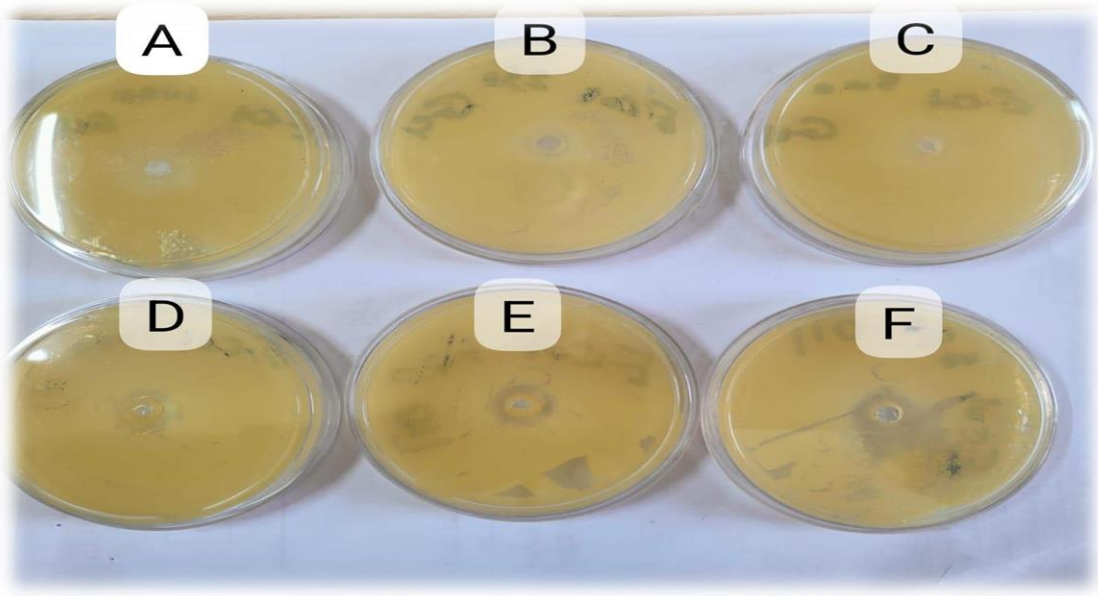


Figure (3-24): Anti-bacterial activity of cloves oil and Clove Nanogel against Gram-negative (Escherichia coli) (A) when (1000 µg/ml) of CNG (B) (500 µg/ml) of CNG (C) (250 µg/ml) of CNG (D) (1000 µg/ml) of CEO (E (500 µg/ml) of CEO (F) (250 µg/ml) of CEO.

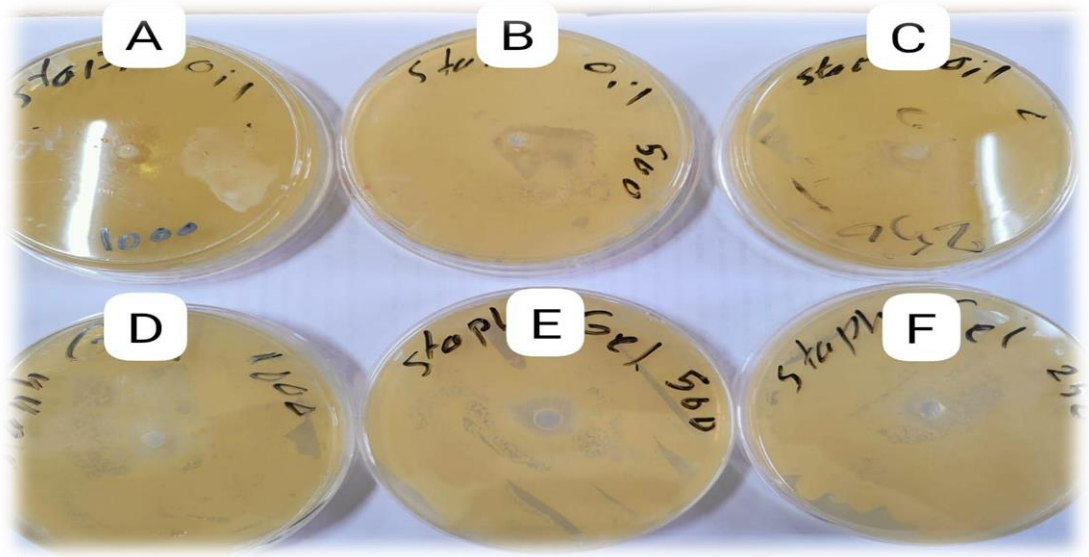


Figure (3-25): Anti-bacterial activity of clove oil and Clove Nanogel against Gram-positive (Staphylococcus aureus) (A) when (1000 µg/ml) of CEO (B) (500 µg/ml) of CEO (C) (250 µg/ml) of CEO (D) (1000 µg/ml) of CNG (E) (500 µg/ml) of CNG (F) (250 µg/ml) of CNG.

3.3.7 Anti-inflammatory Effect of clove Nanogel

Biological properties of clove had been reported, but little is known about its effect on the immune system. The present study considered studying the effects of the Clove nanogel on the secretion of proinflammatory cytokines (IL-1 and TNF-α) using inflammatory macrophage cell line (RAW 264.7) supplied by the tissue culture laboratory/College of Medicine/ University of Babylon. Iraq. Also, an inflammatory antigen composed of 80 ng/ml lipopolysaccharides (LPS) extracted from gram-negative bacterial (*Escherichia coli*) and provided by (Solarbio Comp. China) was used as an inducer for the secretion of cytokines by the cell line after incubation in 24-well dishes with 100 ml of RPMI 1640 medium at 37 °C in a humidified, 5% CO_2 incubator for 48 hours.

The anti-inflammatory effects of different concentrations (250 µg/ml, 500 µg/ml, and 1000 µg/ml) of clove nanogel were tested using four 24-well plates. The first plate was indicated as positive control which contained macrophage cells line and LPS antigen incubated in 100 ml of RPMI 1640 medium at 37 °C in a 5% CO_2 humidified condition for 48 hours, while the second, third, and fourth plates contained the same aforementioned components in addition to the clove nanogel in a concentration of 250, 500, and 1000 µg/ml respectively. Each experiment was repeated three times. Moreover, two 24-well plates were used as comparison between positive control (LPS+ cell line) and negative control (Cell line only) and incubated in the same conditions.

The results found as shown in Table (3-5) that the concentrations of IL-1 and TNF-α were significantly higher in the positive control wells compared to the negative control (P value <0.000 and 0.0012 respectively). The elevated concentrations of IL-1 and TNF-α in the positive control wells are due to the existence of LPS which stimulates the production of cytokines by macrophage cells.

Table (3-5): Concentration of IL-1 and TNF-α in Positive and Negative control of the cell culture.

Cytokines	Positive Control	Negative Control	*P value*
	Mean ± SE	Mean ± SE	
IL-1 pg/ml/10^5 cell	62. 07± 0.15	39.39 ± 0.38	<0.000**
TNF-α pg/ml/10^5 cell	266.03 ± 0.71	32.71 ± 0.43	0.0012**

****Results were significant at 0.01 level. SE= Standard Error**

The results found, as presented in Table (3-6), that adding 250 µg/m of the clove nanogel to the inflammatory macrophage cells incubated with LPS of 80 ng/ml caused a significant decrease in the secretion of the cytokine IL-1 (*P* value <0.000) compared to inflammatory macrophage cells incubated with LPS only (control positive), however, the secretion of TNF-α was non-significantly affected by the 250 µg/ml concentration of the clove nanogel (*P* value =0.560).

Table (3-6): Concentration of IL-1 and TNF-α in macrophage cell culture incubated with LPS and 250 µg/ml of clove nanogel.

Cytokines	Positive Control	Nanogel 250 µg/ml	*P value*
	Mean ± SE	Mean ± SE	
IL-1 pg/ml/10^5 cell	62. 07± 0.15	41.97 ± 0.29	<0.000**
TNF-α pg/ml/10^5 cell	266.03 ± 0.71	236.06 ± 0.95	0.560

****Results were significant at 0.01 level. SE= Standard Error**

Moreover, the results of Table (3-7), revealed that the concentration of the proinflammatory cytokines (IL-1 and TNF-α) were significantly decreased (*P* value <0.000 for both) after the addition of 500 µg/ml of the nanogel to macrophage cell culture incubated with LPS.

Table (3-7): Concentration of IL-1 and TNF-α in macrophage cell culture incubated with LPS and 500 µg/ml of clove nanogel.

Cytokines	Positive Control	Nanogel (500 µg/ml)	*P value*
	Mean ± SE	Mean ± SE	
IL-1 pg/ml/10^5 cell	62. 07± 0.15	41.53 ± 0.05	<0.000**
TNF-α pg/ml/10^5 cell	266.03 ± 0.71	191.50 ± 0.21	<0.000**

****Results were significant at 0.01 level. SE= Standard Error**

In the same context, the results of Table (3-8) showed that the 1000 µg/ml concentration of the nanogel has no significant effects on the secretion of IL-1 and TNF-β by the macrophages incubated with 80 ng/ml of LPS antigen.

Table (3-8): Concentration of IL-1 and TNF-α in macrophage cell culture incubated with LPS and 1000 µg/ml of nanogel

Cytokines	Positive Control	Nanogel 1000 (µg/ml)	P value
	Mean ± SE	Mean ± SE	
IL-1 pg/ml/10^5 cell	62. 07± 0.15	46.96 ± 0.27	0.368
TNF-α pg/ml/10^5 cell	266.03 ± 0.71	203.60 ± 2.32	0.986

SE= Standard Error

To demonstrate the effects of the nanogel on the inflammatory macrophage cells without using inflammatory inducer (LPS), cell culture of the RAW 264.7 macrophage cell line was incubated in a 24-well palter with 100 ml of RPMI 1640 medium in a condition of 37 °C, 5% CO_2 humidified incubator for 48 hours with 250, 500 and 1000 µg/ml concentrations of the nanogel respectively and compared to the negative control. The results showed that all concentrations of the nanogel have no effects on the cytokines secretions by macrophage cell line as presented in tables (3-9), (3-10) and (3-11). This means that nanogel has no inflammatory effects and not acting as an antigen.

Table (3-9): Concentrations of IL-1 and TNF-α in macrophage cell line incubated with 250 µg/ml of nanogel only.

Cytokines	Negative Control	Nanogel (250 µg/ml)	P value
	Mean ± SE	Mean ± SE	
IL-1 pg/ml/10^5 cell	39.39 ± 0.38	32.60 ± 0.45	0.131
TNF-α pg/ml/10^5 cell	32.71 ± 0.43	32.41 ± .49	0.213

SE= Standard Error

Table (3-10): Concentrations of IL-1 and TNF-α in macrophage cell line incubated with 500 µg/ml of nanogel only

Cytokines	Negative Control	Nanogel 500 µg/ml	P value
	Mean ± SE	Mean ± SE	
IL-1 pg/ml/10^5 cell	39.39 ± 0.38	35.16 ± 2.89	0.313
TNF-α pg/ml/10^5 cell	32.71 ± 0.43	31.25± 0.25	0.142

SE= Standard Error

Table (3-11): Concentration of IL-1 and TNF-α in macrophage cell line incubated with 1000 µg/ml of nanogel only

Cytokines	Negative Control	Nanogel 1000 µg/ml	P value
	Mean ± SE	Mean ± SE	
IL-1 pg/ml/10^5 cell	39.39 ± 0.38	41.14 ± 0.60	0.616
TNF-α pg/ml/10^5 cell	32.71 ± 0.43	32.51 ± 0.38	0.294

SE= Standard Error

Discussion:

The results of the tables (3-5) to (3-8) revealed that adding the CNG to a macrophage cell culture stimulated by LPS led to a significant decrease in the production of IL-1 and TNFα.

Similar findings had been reported in other studies. For example [140] discovered that non-cytotoxic amounts of clove have anti-inflammatory and immunomodulatory effects on the production of cytokines by mice macrophages. In a single study, the production of interleukin (IL)-1, IL-6, and IL-10 was successfully decreased at a concentration of 100 g per well [141].

Similarly, CEO at a concentration of 0.011% significantly reduced the increase in the production of multiple proinflammatory biomarkers, including interferon-induced protein 10 (IP-10), vascular cell adhesion molecule-1 (VCAM-1), interferon-inducible T-cell chemoattractant (I-TAC), and monokine induced by c interferon (MIG) [142].

Also, clove oil reduced the release of IL-1β by inhibiting the NLRP3 inflammasome, which in turn reduced the inflammation caused by LPS in RAW264.7 cells [143].

CEO's anti-inflammatory properties stem from its eugenol, which works by blocking the production of prostaglandins and the chemotaxis of neutrophils. Furthermore, it can prevent the NF-kB factor from stimulating the tumor necrosis factor-α (TNF-α) and cyclooxygenase (COX)-2 expression in lipopolysaccharide (LPS) triggered by macrophages. Eugenol has the potential to be an anti-inflammatory drug because studies have demonstrated that it lowers TNF signals and COX-2 expression [144].

The study notice that at concentrations of 250, 500, and 1000, the gel worked to inhibit cytokines stimulated by LPS, and its inhibition at concentrations of 250 and 500 was better than at concentrations of 1000. This means that increasing the concentration does not lead to increased inhibition, The study showed that the optimal concentration of Clove nanogel is 250 µg/m had the best anti -inflammatory activity.

Altogether, our results suggest that the preparation of clove nanogel we used contains intriguing chemicals like eugenol that may be utilized to decrease TNF-α and IL-1. This study is supported by another study [145]. Eugenol inhibits neutrophil chemotaxis and prostalglandin production as part of its anti-inflammatory mechanism of action [146].

Also The results showed that all concentrations of the nanogel have no effects on the cytokines secretions by macrophage cell line as presented in tables (3-8), (3-9) and (3-10). This means that nanogel has no inflammatory effects and not acting as an antigen.

Thus, the study concluded that clove nanogel showed anti-inflammatory effect be candidates for further developing anti-inflammatory drug

CHAPTER FOUR

Conclusion and Recommendation

Conclusion

The objective of the present study was to develop clove-NG as a medication delivery method. The in vitro release of the substance had been examined for 96 hours at pH levels of 1.2, 8.2, and 7. Furthermore, the CNG variant of the gel did not exhibit any harmful effects on cells. Our research shows that the CNG had an antioxidant effect as determined by the DPPH test. The CNG had little hemolysis activity and demonstrates antibacterial efficacy against S. aureus and E. coli. The findings of the anti-inflammatory study indicated that all concentrations of the clove nanogel (CNG) did not have any impact on the release of cytokines by macrophage cell lines. This indicates that clove nanogel does not have any inflammatory effects and does not function as an antigen.

Recommendations

The following items may be suggested based on the findings of this study:

1. Preparation of clove Nanogel using Tween-40 and Tween-80 and compare them in terms of release.
2. Investigating the effect of the CNG on anti-inflammatory cytokines such as IL-10, IL-4 and IL-11 and compare result them with pro-inflammatory IL-1 and TNF-β.
3. Conduct studies to evaluate the long-term safety and efficacy of clove nanogel *in vivo.*
4. The possibility of improving the properties of nanogels to affect drug loading and release to achieve better therapeutic efficacy.
5. In vivo studies with clove nanogel may provide encouraging outcomes when it comes to medicine delivery.

References

References

1. Valizadeh, A., Khaleghi, A. A., Alipanah, H., Zarenezhad, E., & Osanloo, M. (2021). Anticarcinogenic effect of chitosan nanoparticles containing syzygium aromaticum essential oil or eugenol toward breast and skin cancer cell lines. *BioNanoScience*, *11*(3), 678-686.
2. Zarenezhad, E., Abdollahi, A., Esmaeili, F., Satvati, S., & Osanloo, M. (2020). A fast-degradable nano-dressing with potent antibacterial effect. *BioNanoScience*, *10*, 983-990.
3. Osanloo, M., Arish, J., & Sereshti, H. (2020). Developed methods for the preparation of electrospun nanofibers containing plant-derived oil or essential oil: a systematic review. *Polymer Bulletin*, *77*, 6085-6104.
4. Abedinpour, N., Ghanbariasad, A., Taghinezhad, A., & Osanloo, M. (2021). Preparation of nanoemulsions of mentha piperita essential oil and investigation of their cytotoxic effect on human breast cancer lines. *BioNanoScience*, *11*(2), 428-436.
5. Maghbool, M., Khosravi, T., Vojdani, S., Chaijan, M. R., Esmaeili, F., Amani, A., ... & Hashempur, M. H. (2020). The effects of eugenol nanoemulsion on pain caused by arteriovenous fistula cannulation in hemodialysis patients: a randomized double-blinded controlled cross-over trial. *Complementary therapies in medicine*, *52*, 102440.
6. Ghiasi, Z., Esmaeli, F., Aghajani, M., Ghazi-Khansari, M., Faramarzi, M. A., & Amani, A. (2019). Enhancing analgesic and anti-inflammatory effects of capsaicin when loaded into olive oil nanoemulsion: An in vivo study. *International Journal of Pharmaceutics*, *559*, 341-347.
7. Sondari, D., & Tursiloadi, S. (2018, December). The effect of surfactan on formulation and stability of nanoemulsion using extract of Centella Asiatica and Zingiber Officinale. In *AIP conference proceedings* (Vol. 2049, No. 1). AIP Publishing.
8. Solans, C., Izquierdo, P., Nolla, J., Azemar, N., & Garcia-Celma, M. J. (2005). Nano-emulsions. *Current opinion in colloid & interface science*, *10*(3-4), 102-110.
9. Ee, S. L., Duan, X., Liew, J., & Nguyen, Q. D. (2008). Droplet size and stability of nano-emulsions produced by the temperature phase inversion method. *Chemical Engineering Journal*, *140*(1-3), 626-631.

10. Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in colloid and interface science*, *108*, 303-318.
11. Abbasifard, M., Yousefpoor, Y., Amani, A., & Arababadi, M. K. (2021). Topical bee venom nano-emulsion ameliorates serum level of endothelin-1 in collagen-induced rheumatoid arthritis model. *BioNanoScience*, *11*(3), 810-815.
12. Hussain, A., Samad, A., Singh, S. K., Ahsan, M. N., Haque, M. W., Faruk, A., & Ahmed, F. J. (2016). Nanoemulsion gel-based topical delivery of an antifungal drug: in vitro activity and in vivo evaluation. *Drug delivery*, *23*(2), 642-657.
13. Ghanbariasad, A., Azadi, S., Agholi, M., & Osanloo, M. (2021). The nanoemulsion-based nanogel of Artemisia dracunculus essential oil with proper activity against Leishmania tropica and Leishmania major. *Nanomedicine Research Journal*, *6*(1), 89-95.
14. Pan, S. Y., Zhou, S. F., Gao, S. H., Yu, Z. L., Zhang, S. F., Tang, M. K., ... & Ko, K. M. (2013). New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evidence-based complementary and alternative medicine*, *2013*.
15. Bhosle, A., & Baste, N. (2023). Nanotechnology and its therapeutic and cosmetic applications: a review. *International Journal of Pharmaceutical Sciences*, *1*(10), 1-1.
16. MN Iqbal, H., MV Rodriguez, A., Khandia, R., Munjal, A., & Dhama, K. (2016). Recent trends in nanotechnology-based drugs and formulations for targeted therapeutic delivery. *Recent patents on inflammation & allergy drug discovery*, *10*(2), 86-93.
17. Ahmed, H. M., Nabavi, S., & Behzad, S. (2021). Herbal drugs and natural products in the light of nanotechnology and nanomedicine for developing drug formulations. *Mini Reviews in Medicinal Chemistry*, *21*(3), 302-313.
18. Vargason, A. M., Anselmo, A. C., & Mitragotri, S. (2021). The evolution of commercial drug delivery technologies. *Nature biomedical engineering*, *5*(9), 951-967.

19. Alqahtani, M. S., Kazi, M., Alsenaidy, M. A., & Ahmad, M. Z. (2021). Advances in oral drug delivery. *Frontiers in pharmacology*, *12*, 618411.
20. Mahant, S., Sharma, A. K., Gandhi, H., Wadhwa, R., Dua, K., & Kapoor, D. N. (2023). Emerging trends and potential prospects in vaginal drug delivery. *Current Drug Delivery*, *20*(6), 730-751.
21. Verma, R. K., & Garg, S. (2001). Drug delivery technologies and future directions. *Pharm. Technol*, *25*(2), 1-14.
22. Keraliya, R. A., Patel, C., Patel, P., Keraliya, V., Soni, T. G., Patel, R. C., & Patel, M. M. (2012). Osmotic drug delivery system as a part of modified release dosage form. *International Scholarly Research Notices*, *2012*.
23. Ding, C., & Li, Z. (2017). A review of drug release mechanisms from nanocarrier systems. *Materials Science and Engineering: C*, *76*, 1440-1453.
24. Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. D. P., Acosta-Torres, L. S., ... & Shin, H. S. (2018). Nano based drug delivery systems: recent developments and future prospects. *Journal of nanobiotechnology*, *16*, 1-33.
25. Faheem, A. M., & Abdelkader, D. H. (2020). Novel drug delivery systems. In *Engineering drug delivery systems* (pp. 1-16). Woodhead Publishing.
26. Rahman, H. S., Othman, H. H., Hammadi, N. I., Yeap, S. K., Amin, K. M., Abdul Samad, N., & Alitheen, N. B. (2020). Novel drug delivery systems for loading of natural plant extracts and their biomedical applications. *International journal of nanomedicine*, 2439-2483.
27. Ezike, T. C., Okpala, U. S., Onoja, U. L., Nwike, P. C., Ezeako, E. C., Okpara, J. O., ... & Nwanguma, B. C. (2023). Advances in drug delivery systems, challenges and future directions. *Heliyon*.
28. Glassman, P. M., & Muzykantov, V. R. (2019). Pharmacokinetic and pharmacodynamic properties of drug delivery systems. *Journal of Pharmacology and Experimental Therapeutics*, *370*(3), 570-580.
29. Vigani, B., Rossi, S., Sandri, G., Bonferoni, M. C., Caramella, C. M., & Ferrari, F. (2020). Recent advances in the development of in situ

gelling drug delivery systems for non-parenteral administration routes. *Pharmaceutics*, *12*(9), 859.

30. Khule, M. R., & Vyavahare, S. B. (2021). A Review: In-Situ gel drug delivery system. *Int. J. Res. Education and Scientific methods*, *9*(3), 899-909.
31. Bashir, R., Maqbool, M., Ara, I., & Zehravi, M. (2021). An in sight into novel drug delivery system: in situ gels. *CellMed*, *11*(1), 6-1.
32. Eckmann, D. M., Composto, R. J., Tsourkas, A., & Muzykantov, V. R. (2014). Nanogel carrier design for targeted drug delivery. *Journal of materials chemistry B*, *2*(46), 8085-8097.
33. Abdolahinia, E. D., Barati, G., Ranjbar-Navazi, Z., Kadkhoda, J., Islami, M., Hashemzadeh, N., ... & Sharifi, S. (2022). Application of nanogels as drug delivery systems in multicellular spheroid tumor model. *Journal of Drug Delivery Science and Technology*, *68*, 103109.
34. Hulla, J. E., Sahu, S. C., & Hayes, A. W. (2015). Nanotechnology: History and future. Human & experimental toxicology, 34(12), 1318-1321.
35. Singh, A. R., Desu, P. K., Nakkala, R. K., Kondi, V., Devi, S., Alam, M. S., ... & Kesharwani, P. (2021). Nanotechnology-based approaches applied to nutraceuticals. Drug Delivery and Translational Research, 1-15.
36. Shah, S. S., Shaikh, M. N., Khan, M. Y., Alfasane, M. A., Rahman, M. M., & Aziz, M. A. (2021). Present status and future prospects of jute in nanotechnology: A review. *The Chemical Record*, *21*(7), 1631-1665.
37. Rahman, S., & Lu, Y. (2022). Nano-engineering and nano-manufacturing in 2D materials: marvels of nanotechnology. Nanoscale Horizons, 7(8), 849-872.
38. Harish, V., Tewari, D., Gaur, M., Yadav, A. B., Swaroop, S., Bechelany, M., & Barhoum, A. (2022). Review on nanoparticles and nanostructured materials: Bioimaging, biosensing, drug delivery, tissue engineering, antimicrobial, and agro-food applications. Nanomaterials, 12(3), 457.

39. Mansoori, G. A., Mohazzabi, P., McCormack, P., & Jabbari, S. (2007). Nanotechnology in cancer prevention, detection and treatment: bright future lies ahead. World Review of Science, Technology and Sustainable Development, 4(2-3), 226-257.

40. Predicala, B. (2009). Nanotechnology: potential for agriculture. Prairie Swine Centre Inc, University of Saskatchewan, Saskatoon, SK, 123-134.

41. Soni, H., & Sharma, S. (2021). Current update on nanoemulsion: a review. *Sch. Int. J. Anat. Physiol*, *4*(1), 6-13.

42. Jaiswal, M., Dudhe, R., & Sharma, P. K. (2015). Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech*, *5*, 123-127.

43. Chime, S. A., Kenechukwu, F. C., & Attama, A. A. (2014). Nanoemulsions—advances in formulation, characterization and applications in drug delivery. *Application of nanotechnology in drug delivery*, *3*, 77-126.

44. Singh, Y., Meher, J. G., Raval, K., Khan, F. A., Chaurasia, M., Jain, N. K., & Chourasia, M. K. (2017). Nanoemulsion: Concepts, development and applications in drug delivery. *Journal of controlled release*, *252*, 28-49.

45. Moghassemi, S., Dadashzadeh, A., Azevedo, R. B., & Amorim, C. A. (2022). Nanoemulsion applications in photodynamic therapy. *Journal of Controlled Release*, *351*, 164-173.

46. Sultana, F., Imran-Ul-Haque, M., Arafat, M., & Sharmin, S. (2013). An overview of nanogel drug delivery system. *Journal of Applied Pharmaceutical Science*, *3*(8,), S95-S105.

47. Shah, S., Rangaraj, N., Laxmikeshav, K., & Sampathi, S. (2020). Nanogels as drug carriers–Introduction, chemical aspects, release mechanisms and potential applications. *International Journal of Pharmaceutics*, *581*, 119268.

48. Soni, K. S., Desale, S. S., & Bronich, T. K. (2016). Nanogels: An overview of properties, biomedical applications and obstacles to clinical translation. *Journal of Controlled Release*, *240*, 109-126.

49. Anooj, E. S., Charumathy, M., Sharma, V., Vibala, B. V., Gopukumar, S. T., Jainab, S. B., & Vallinayagam, S. (2021). Nanogels: An overview

of properties, biomedical applications, future research trends and developments. *Journal of Molecular Structure*, *1239*, 130446.

50. Yetisgin, A. A., Cetinel, S., Zuvin, M., Kosar, A., & Kutlu, O. (2020). Therapeutic nanoparticles and their targeted delivery applications. *Molecules*, *25*(9), 2193.
51. Eckmann, D. M., Composto, R. J., Tsourkas, A., & Muzykantov, V. R. (2014). Nanogel carrier design for targeted drug delivery. *Journal of materials chemistry B*, *2*(46), 8085-8097.
52. Ansary, J., Forbes-Hernández, T. Y., Gil, E., Cianciosi, D., Zhang, J., Elexpuru-Zabaleta, M., ... & Battino, M. (2020). Potential health benefit of garlic based on human intervention studies: A brief overview. *Antioxidants*, *9*(7), 619.
53. Radünz, M., da Trindade, M. L. M., Camargo, T. M., Radünz, A. L., Borges, C. D., Gandra, E. A., & Helbig, E. (2019). Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (Syzygium aromaticum, L.) essential oil. *Food chemistry*, *276*, 180-186.
54. Percival, S. S., Vanden Heuvel, J. P., Nieves, C. J., Montero, C., Migliaccio, A. J., & Meadors, J. (2012). Bioavailability of herbs and spices in humans as determined by ex vivo inflammatory suppression and DNA strand breaks. *Journal of the American College of Nutrition*, *31*(4), 288-294.
55. Homaeigohar, S., & Boccaccini, A. R. (2020). Antibacterial biohybrid nanofibers for wound dressings. *Acta biomaterialia*, *107*, 25-49.
56. Parham, S., Kharazi, A. Z., Bakhsheshi-Rad, H. R., Nur, H., Ismail, A. F., Sharif, S., ... & Berto, F. (2020). Antioxidant, antimicrobial and antiviral properties of herbal materials. *Antioxidants*, *9*(12), 1309.
57. Anwer, M. K., Jamil, S., Ibnouf, E. O., & Shakeel, F. (2014). Enhanced antibacterial effects of clove essential oil by nanoemulsion. *Journal of oleo science*, *63*(4), 347-354.

58. Kalemba, D. A. A. K., & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Current medicinal chemistry*, *10*(10), 813-829.

59. Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-Nakbi, A. B., Rouabhia, M., Mahdouani, K., & Bakhrouf, A. (2007). The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): a short review. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *21*(6), 501-506.

60. Haro-González, J. N., Castillo-Herrera, G. A., Martínez-Velázquez, M., & Espinosa-Andrews, H. (2021). Clove essential oil (Syzygium aromaticum L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. *Molecules*, *26*(21), 6387.

61. Kulbe, H., Chakravarty, P., Leinster, D. A., Charles, K. A., Kwong, J., Thompson, R. G., ... & Balkwill, F. R. (2012). A dynamic inflammatory cytokine network in the human ovarian cancer microenvironment. *Cancer research*, *72*(1), 66-75.

62. Fajgenbaum, D. C., & June, C. H. (2020). Cytokine storm. *New England Journal of Medicine*, *383*(23), 2255-2273.

63. Liu, C., Chu, D., Kalantar-Zadeh, K., George, J., Young, H. A., & Liu, G. (2021). Cytokines: from clinical significance to quantification. *Advanced Science*, *8*(15), 2004433.

64. Sprague, A. H., & Khalil, R. A. (2009). Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochemical pharmacology*, *78*(6), 539-552.

65. Boshtam, M., Asgary, S., Kouhpayeh, S., Shariati, L., & Khanahmad, H. (2017). Aptamers against pro-and anti-inflammatory cytokines: a review. Inflammation, 40, 340-349.

66. Webb, C. R., Koboziev, I., Furr, K. L., & Grisham, M. B. (2016). Protective and pro-inflammatory roles of intestinal bacteria. *Pathophysiology*, *23*(2), 67-80.

67. Opal, S. M., & DePalo, V. A. (2000). Anti-inflammatory cytokines. Chest, 117(4), 1162-1172.

68. Al-Dabbagh, M., Lapphra, K., Scheifele, D. W., Halperin, S. A., Langley, J. M., Cho, P., ... & Bettinger, J. A. (2013). Elevated inflammatory mediators in adults with oculorespiratory syndrome following influenza immunization: a public health agency of Canada/Canadian Institutes of Health Research influenza research network study. Clinical and Vaccine Immunology, 20(8), 1108-1114.

69. Pizarro, T. T., & Cominelli, F. (2007). Cloning IL-1 and the birth of a new era in cytokine biology. *The Journal of Immunology*, *178*(9), 5411-5412.
70. Dinarello, C. A. (2009). Immunological and inflammatory functions of the interleukin-1 family. *Annual review of immunology*, *27*, 519-550.
71. Weber, A., Wasiliew, P., & Kracht, M. (2010). Interleukin-1 (IL-1) pathway. *Science signaling*, *3*(105), cm1-cm1.

72. Dinarello, C. A. (2005). Blocking IL-1 in systemic inflammation. *The Journal of experimental medicine*, *201*(9), 1355-1359.
73. Migliorini, P., Italiani, P., Pratesi, F., Puxeddu, I., & Boraschi, D. (2020). The IL-1 family cytokines and receptors in autoimmune diseases. *Autoimmunity reviews*, *19*(9), 102617.
74. Bradley, J. (2008). TNF-mediated inflammatory disease. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *214*(2), 149-160.
75. Celis, R., Cuervo, A., Ramírez, J., & Cañete, J. D. (2019). Psoriatic synovitis: singularity and potential clinical implications. *Frontiers in Medicine*, *6*, 14.
76. Jang, D. I., Lee, A. H., Shin, H. Y., Song, H. R., Park, J. H., Kang, T. B., ... & Yang, S. H. (2021). The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *International journal of molecular sciences*, *22*(5), 2719.
77. Shenoy K., & Shirwaiker, A. 2002. Anti-inflammatory and free radical scavenging studies of Hyptis snravolens. Indian drug, 39: 574-577
78. Moharram, H. A., & Youssef, M. M. (2014). Methods for determining the antioxidant activity: a review. *Alexandria Journal of Food Science and Technology*, *11*(1), 31-42.
79. Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of functional foods*, *18*, 757-781.

80. Dahham,S.; Tabana, Y.; Iqbal, M.; Ahamed, M.; Ezzat, M.; Majid, A.A.; Majid, A.A. (2015) The anticancer, antioxidant and antimicrobial properties of the sesquiterpene-Caryophyllene from the essential oil of Aquilaria crassna. Molecules, 20, 11808–11829.
81. Gupta, R., Gupta, N., Gupta, R., & Gupta, N. (2021). Prokaryotic cell structure and function. *Fundamentals of Bacterial Physiology and Metabolism*, 43-79.
82. Li, F., & Xu, Z. K. (2018). Medical microbiology. *People's Health Publishing House: Beijing, China*.
83. Zaman, S. B., Hussain, M. A., Nye, R., Mehta, V., Mamun, K. T., & Hossain, N. (2017). A review on antibiotic resistance: alarm bells are ringing. *Cureus*, *9*(6).
84. Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. *Antibiotics*, *10*(3), 318.
85. Nagaraju, P. G., Sengupta, P., Chicgovinda, P. P., & Rao, P. J. (2021). Nanoencapsulation of clove oil and study of physicochemical properties, cytotoxic, hemolytic, and antioxidant activities. *Journal of Food Process Engineering*, *44*(4), e13645.
86. Gul, U., Khan, M. I., Madni, A., Sohail, M. F., Rehman, M., Rasul, A., & Peltonen, L. (2022). Olive oil and clove oil-based nanoemulsion for topical delivery of terbinafine hydrochloride: in vitro and ex vivo evaluation. *Drug Delivery*, *29*(1), 600-612.
87. Lv, N., Zhao, M., Hao, L., Zhou, X., Chen, H., & Zhou, H. (2022). Eugenol and carboxymethyl cellulose derived nanocoating with insect repellent and long-term antibacterial activity. Industrial Crops and Products, 190, 115902.
88. Esmaeili, F., Zahmatkeshan, M., Yousefpoor, Y., Alipanah, H., Safari, E., & Osanloo, M. (2022). Anti-inflammatory and anti-nociceptive effects of Cinnamon and Clove essential oils nanogels: An in vivo study. BMC Complementary Medicine and Therapies, 22(1), 143.
89. Suri, S. S., Fenniri, H., & Singh, B. (2007). Nanotechnology-based drug delivery systems. *Journal of occupational medicine and toxicology*, *2*, 1-6.

90. Tiwari, G., Tiwari, R., Sriwastawa, B., Bhati, L., Pandey, S., Pandey, P., & Bannerjee, S. K. (2012). Drug delivery systems: An updated review. *International journal of pharmaceutical investigation*, *2*(1), 2.
91. Ratri, P. J., Ayurini, M., Khumaini, K., & Rohbiya, A. (2020). Clove oil extraction by steam distillation and utilization of clove buds waste as potential candidate for eco-friendly packaging. *Jurnal Bahan Alam Terbarukan*, *9*(1), 47-54.
92. Karkosh, A. S. A. (2012). Study of in vitro antibacterial activity of the essential oils of Cloves (Syzygium aromaticum) and the effect of temperature on antibacterial activity. *Euphrates Journal of Agriculture Science*, *4*(1), 15-19.
93. Esmaeili, F., Zahmatkeshan, M., Yousefpoor, Y., Alipanah, H., Safari, E., & Osanloo, M. (2022). Anti-inflammatory and anti-nociceptive effects of Cinnamon and Clove essential oils nanogels: An in vivo study. *BMC Complementary Medicine and Therapies*, *22*(1), 143.
94. Mahdavi, M., Ahmad, M. B., Haron, M. J., Namvar, F., Nadi, B., Rahman, M. Z. A., & Amin, J. (2013). Synthesis, surface modification and characterisation of biocompatible magnetic iron oxide nanoparticles for biomedical applications. *Molecules*, *18*(7), 7533-7548.
95. Souto, U. T. D. C. P., Barbosa, M. F., Dantas, H. V., de Pontes, A. S., da Silva Lyra, W., Diniz, P. H. G. D., ... & da Silva, E. C. (2015). Identification of adulteration in ground roasted coffees using UV–Vis spectroscopy and SPA-LDA. LWT-Food Science and Technology, 63(2), 1037-1041
96. Anwer, M. K., Jamil, S., Ibnouf, E. O., & Shakeel, F. (2014). Enhanced antibacterial effects of clove essential oil by nanoemulsion. *Journal of oleo science*, *63*(4), 347-354.
97. Liaqat, N., Jahan, N., Anwar, T., & Qureshi, H. (2022). Green synthesized silver nanoparticles: Optimization, characterization, antimicrobial activity, and cytotoxicity study by hemolysis assay. Frontiers in Chemistry, 10, 952006.
98. Martins, N., Barros, L., Dueñas, M., Santos-Buelga, C., & Ferreira, I. C. (2015). Characterization of phenolic compounds and antioxidant properties of Glycyrrhiza glabra L. rhizomes and roots. *RSC Advances*, *5*(34), 26991-26997.

99. Khane, Y., Benouis, K., Albukhaty, S., Sulaiman, G. M., Abomughaid, M. M., Al Ali, A., ... & Dizge, N. (2022). Green synthesis of silver nanoparticles using aqueous Citrus limon zest extract: Characterization and evaluation of their antioxidant and antimicrobial properties. Nanomaterials, 12(12), 2013.
100. Lior, H. (2018). Serotyping of Campylobacter jejuni and C. coli by slide agglutination based on heat-labile antigenic factors. In Campylobacter infection in man and animals (pp. 61-76). CRC Press.
101. Albukhaty, S., Naderi-Manesh, H., Tiraihi, T., & Sakhi Jabir, M. (2018). Poly-l-lysine-coated superparamagnetic nanoparticles: a novel method for the transfection of pro-BDNF into neural stem cells. Artificial cells, nanomedicine, and biotechnology, 46(sup3), 125-132.
102. Moutia, M., El Azhary, K., Elouaddari, A., Al Jahid, A., Jamal Eddine, J., Seghrouchni, F., ... & Badou, A. (2016). Capparis Spinosa L. promotes anti-inflammatory response in vitro through the control of cytokine gene expression in human peripheral blood mononuclear cells. *BMC immunology*, *17*, 1-12.
103. Amelia, B., Saepudin, E., Cahyana, A. H., Rahayu, D. U., Sulistyoningrum, A. S., & Haib, J. (2017, July). GC-MS analysis of clove (Syzygium aromaticum) bud essential oil from Java and Manado. In *AIP conference Proceedings* (Vol. 1862, No. 1). AIP Publishing.
104. Guan, W., Li, S., Yan, R., Tang, S., & Quan, C. (2007). Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. *Food Chemistry*, *101*(4), 1558-1564.
105. Kapadiya, S. M., Parikh, J., & Desai, M. A. (2018). A greener approach towards isolating clove oil from buds of Syzygium aromaticum using microwave radiation. *Industrial crops and products*, *112*, 626-632.
106. Silvestri, J. D. F., Paroul, N., Czyewski, E., Lerin, L., Rotava, I., Cansian, R. L., ... & Treichel, H. (2010). Perfil da composição química e atividades antibacteriana e antioxidante do óleo essencial do cravo-da-índia (Eugenia caryophyllata Thunb.). *Revista Ceres*, *57*, 589-594.

107. Sohilait, H. J. (2015). Chemical composition of the essential oils in Eugenia caryophylata, Thunb from Amboina Island. *Science Journal of Chemistry*, *3*(6), 95-99.

108. Kapadiya, S., & Desai, M. A. (2017). Isolation of essential oil from buds of Syzygium aromaticum using hydrodistillation: multi-response optimization and predictive modelling. *International Journal of Advanced Science and Technology*, *6*(1), 405-418.

109. Singh, A., Gautam, P. K., Verma, A., Singh, V., Shivapriya, P. M., Shivalkar, S., ... & Samanta, S. K. (2020). Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review. Biotechnology Reports, 25, e00427.

110. Gul, U., Khan, M. I., Madni, A., Sohail, M. F., Rehman, M., Rasul, A., & Peltonen, L. (2022). Olive oil and clove oil-based nanoemulsion for topical delivery of terbinafine hydrochloride: in vitro and ex vivo evaluation. *Drug Delivery*, *29*(1), 600-612.

111. Hamed, S. B., Hamed, M. B. B., Kassouar, S., & Abi Ayad, S. M. E. A. (2016). Physicochemical analysis of cellulose from microalgae Nannochloropsis gaditana. *African Journal of Biotechnology*, *15*(24), 1201-1206.

112. Kono, H., Oshima, K., Hashimoto, H., Shimizu, Y., & Tajima, K. (2016). NMR characterization of sodium carboxymethyl cellulose: Substituent distribution and mole fraction of monomers in the polymer chains. *Carbohydrate Polymers*, *146*, 1-9.

113. Mayeen, A., Shaji, L.K., Nair, A.K., & Kalarikkal, N. (2018). chapter12: Morphological Characterization of Nanomaterials. 335-364. doi.org/10.1016/B978-0-08-101973-3.00012-2.

114. Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., ... & Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics, 10(2), 57.

115. Lindner, M., Bäumler, M., & Stäbler, A. (2018). Inter-correlation among the hydrophilic–lipophilic balance, surfactant system, viscosity,

particle size, and stability of candelilla wax-based dispersions. *Coatings*, *8*(12), 469.

116. Sicińska, P., Bukowska, B., Michałowicz, J., & Duda, W. (2006). Damage of cell membrane and antioxidative system in human erythrocytes incubated with microcystin-LR in vitro. *Toxicon*, *47*(4), 387-397.

117. Singh, N., Jenkins, G. J., Asadi, R., & Doak, S. H. (2010). Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). Nano reviews, 1(1), 5358.

118. Sohaebuddin, S. K., Thevenot, P. T., Baker, D., Eaton, J. W., & Tang, L. (2010). Nanomaterial cytotoxicity is composition, size, and cell type dependent. Particle and fibre toxicology, 7, 1-17.

119. Kong, B., Seog, J. H., Graham, L. M., & Lee, S. B. (2011). Experimental considerations on the cytotoxicity of nanoparticles. *Nanomedicine*, *6*(5), 929-941.

120. Khalil, I., Yehye, W. A., Etxeberria, A. E., Alhadi, A. A., Dezfooli, S. M., Julkapli, N. B. M., ... & Seyfoddin, A. (2019) Nanoantioxidants: Recent trends in antioxidant delivery applications. Antioxidants, 9(1), 24

121. Dos Santos, P. D. F., Francisco, C. R. L., Coqueiro, A., Leimann, F. V., Pinela, J., Calhelha, R. C., ... & Gonçalves, O. H. (2019). The nanoencapsulation of curcuminoids extracted from Curcuma longa L. and an evaluation of their cytotoxic, enzymatic, antioxidant and anti-inflammatory activities. *Food & function*, *10*(2), 573-582

122. Huang, X., Liu, Y., Zou, Y., Liang, X., Peng, Y., McClements, D. J., & Hu, K. (2019). Encapsulation of resveratrol in zein/pectin core-shell nanoparticles: Stability, bioaccessibility, and antioxidant capacity after simulated gastrointestinal digestion. *Food Hydrocolloids*, *93*, 261-269.

123. Dahham, S. S., Tabana, Y. M., Iqbal, M. A., Ahamed, M. B., Ezzat, M. O., Majid, A. S., & Majid, A. M. (2015). The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna. *Molecules*, *20*(7), 11808-11829.

124. Ramadan, M. M., Ali, M. M., Ghanem, K. Z., & El-Ghorabe, A. H. (2015). Essential oils from Egyptian aromatic plants as antioxidant and

novel anticancer agents in human cancer cell lines. *Grasas y Aceites*, *66*(2), e080-e080.

125. Marmouzi, I., Karym, E. M., Alami, R., El Jemli, M., Kharbach, M., Mamouch, F., ... & Faouzi, M. E. A. (2019). Modulatory effect of Syzygium aromaticum and Pelargonium graveolens on oxidative and sodium nitroprusside stress and inflammation. *Oriental Pharmacy and Experimental Medicine*, *19*, 201-210.
126. Shahbazi, Y. (2019). Antioxidant, antibacterial, and antifungal properties of nanoemulsion of clove essential oil. *Nanomedicine Research Journal*, *4*(4), 204-208.
127. Mastelic, J., Jerkovic, I., Blažević, I., Poljak-Blaži, M., Borović, S., Ivančić-Baće, I., ... & Müller, N. (2008). Comparative study on the antioxidant and biological activities of carvacrol, thymol, and eugenol derivatives. *Journal of agricultural and food chemistry*, *56*(11), 3989-3996.
128. Basiak, E., Lenart, A., & Debeaufort, F. (2018). How Glycerol and Water Contents Affect the Structural and Functional Properties of Starch-Based Edible Films. *Polymers*, *10*(4), 412
129. Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S., & Efferth, T. (2007). Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytotherapy research*, *21*(10), 989-994.
130. Devi, K. P., Nisha, S. A., Sakthivel, R., & Pandian, S. K. (2010). Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. *Journal of ethnopharmacology*, *130*(1), 107-115.
131. Saeed, S., & Tariq, P. (2008). In vitro antibacterial activity of clove against gram negative bacteria. *Pak. J. Bot*, *40*(5), 2157-2160.
132. Lu Fei, L. F., Ding YiCheng, D. Y., Ye XingQian, Y. X., & Ding YuTing, D. Y. (2011). Antibacterial effect of cinnamon oil combined with thyme or clove oil.
133. Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food control*, *46*, 412-429.
134. Jouki, M., Yazdi, F. T., Mortazavi, S. A., Koocheki, A., & Khazaei, N. (2014). Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf life extension of

refrigerated rainbow trout fillets. *International journal of food microbiology*, *174*, 88-97.

135. Behbahani, B. A., Noshad, M., & Falah, F. (2019). Study of chemical structure, antimicrobial, cytotoxic and mechanism of action of Syzygium aromaticum essential oil on foodborne pathogens. *Slovak Journal of Food Sciences/Potravinarstvo*, *13*(1).
136. Hemalatha, R., Nivetha, P., Mohanapriya, C., Sharmila, G., Muthukumaran, C., & Gopinath, M. (2016). Phytochemical composition, GC-MS analysis, in vitro antioxidant and antibacterial potential of clove flower bud (Eugenia caryophyllus) methanolic extract. *Journal of food science and technology*, *53*, 1189-1198.
137. Cui, H., Zhao, C., & Lin, L. (2015). The specific antibacterial activity of liposome-encapsulated Clove oil and its application in tofu. *Food Control*, *56*, 128-134.
138. Hadidi, M., Pouramin, S., Adinepour, F., Haghani, S., & Jafari, S. M. (2020). Chitosan nanoparticles loaded with clove essential oil: Characterization, antioxidant and antibacterial activities. Carbohydrate polymers, 236, 116075.
139. Pandey, V. K., Srivastava, S., Dash, K. K., Singh, R., Dar, A. H., Singh, T., ... & Kovacs, B. (2024). Bioactive properties of clove (Syzygium aromaticum) essential oil nanoemulsion: A comprehensive review. Heliyon, 10: e22437.
140. Petrillo, F., Trotta, M. C., Bucolo, C., Hermenean, A., Petrillo, A., Maisto, R., ... & D'Amico, M. (2020). Resolvin D1 attenuates the inflammatory process in mouse model of LPS-induced keratitis. Journal of Cellular and Molecular Medicine, 24(21), 12298-12307.
141. Thatikonda, S., Pooladanda, V., Sigalapalli, D. K., & Godugu, C. (2020). Piperlongumine regulates epigenetic modulation and alleviates psoriasis-like skin inflammation via inhibition of hyperproliferation and inflammation. Cell death & disease, 11(1), 21.
142. Han, X., & Parker, T. L. (2017). Anti-inflammatory activity of clove (Eugenia caryophyllata) essential oil in human dermal fibroblasts. Pharmaceutical biology, 55(1), 1619-1622.
143. Seo, S. W., Kim, K., & Shin, M. R. (2023). Anti-inflammatory Effect by Cloves Treatment in LPS-induced RAW264. 7 Cells. Pharmacognosy Magazine, 19(1), 105-116.

144. Sugihartini, N. I. N. I. N. G., Prabandari, R. A. N. I., Yuwono, T. E. D. J. O., & Rahmawati, D. R. (2019). The anti-inflammatory activity of essential oil of clove (Syzygium aromaticum) in absorption base ointment with addition of oleic acid and propylene glycol as enhancer. Int. J. Appl. Pharm, 11(5), 16-109.

145. Rodrigues, T. G., Fernandes Jr, A., Sousa, J. P. B., Bastos, J. K., & Sforcin, J. M. (2009). In vitro and in vivo effects of clove on pro-inflammatory cytokines production by macrophages. *Natural Product Research*, *23*(4), 319-326.

146. Kim, S. S., Oh, O. J., Min, H. Y., Park, E. J., Kim, Y., Park, H. J., ... & Lee, S. K. (2003). Eugenol suppresses cyclooxygenase-2 expression in lipopolysaccharide-stimulated mouse macrophage RAW264. 7 cells. *Life sciences*, *73*(3), 337-348.

الخلاصه

تحتوي المواد العشبية على العديد من التطبيقات الطبية الحيوية، كما تتمتع المواد العشبية المعتمدة على النانوجيل بقدرة عالية على تحميل الأدوية. في هذه الدراسة، تم تصميم نظام إطلاق الدواء باستخدام هلام زيت القرنفل المعتمد على المستحلب النانوي. لذلك تم خلط زيت القرنفل مع الماء بوجود Tween-20 كمادة خافضة للتوتر السطحي غير أيونية من النوع متعدد السوربات، ومن ثم تمت إضافة كربوكسي ميثيل السليلوز بنسب مختلفة من الجلسرين كعامل تشابك لتركيبات هلام النانو المحضرة من زيت القرنفل العطري (CEO) ، وركز البحث على جوانب مختلفة، بما في ذلك الاطلاق في المختبر, السمية الخلوية، وتأثير مضادات الأكسدة، والتوافق الدموي، والأنشطة المضادة للالتهابات والمضادة للبكتيريا. تم التأكد من توصيف جل القرنفل النانوي (CNG) باستخدام تقنيات مثل التحليل الطيفي للأشعة فوق البنفسجية (UV-Vis)، والتحليل الطيفي للأشعة تحت الحمراء (FTIR)، وقد أثبتت هذه التحليلات وجود مركبات حيوية نشطة، مثل الأوجينول، وخلات الأوجينيل، وβ- كاريوفيلين، ألفا هومولين. تم التعرف على جل القرنفل النانوي من خلال تشتت الضوء الديناميكي (DLS) والمجهر الإلكتروني لمسح الانبعاثات الميدانية (FESM). أشارت النتائج إلى أن جل القرنفل النانوي نقي للغاية وبلوري، ويحتوي على جزيئات متكتلة وغير منتظمة. بالإضافة إلى ذلك، كان الحد الأقصى لطول موجة الامتصاص للأشعة فوق البنفسجية المرئية ٢٨٤ نانومتر، في حين كانت قياسات تشتت الضوء الديناميكي (DLS) لجل القرنفل ٢٧٦ نانومتر، وكان مؤشر التشتت المتعدد ٠,٢٧٩. كما تمت دراسة إطلاق زيت القرنفل من الجل النانوي عند درجة حموضة ١,٢ و٨,٢ و ٧ لمدة ٩٦ ساعة. قامت الدراسة أيضًا بتقييم نشاط مضادات الأكسدة لجل القرنفل النانوي باستخدام اختبار DPPH، والذي أظهر أقصى تثبيط بنسبة ٦٥% عند تركيز ١٠٠ ميكروجرام/مل في مستحلب القرنفل النانوي (CNE)، في حين أظهر CNG بنفس التركيز ٥٩,٢% من التثبيط. كانت قيم IC50 المحسوبة لـ CNG و6.58 CNE و٣,٢٥ ميكروغرام/مل على التوالي بالإضافة إلى التوافق الدموي على كريات الدم الحمراء والخصائص السامة للخلايا من خلال اختبار MTT واظهر CNG لا توجد أي سمية على الخلايا كما أظهر توافقا حيويًا جيدًا مع خلايا الدم الحمراء (RBCs) في تجربة النشاط الانحلالي، مع عدم وجود تفاعل انحلالي ملحوظ عند الجرعات ٧,٥ إلى ١٢٠ ميكروغرام / مل. علاوة على ذلك، أظهر CNG نشاطًا مضادًا للبكتيريا ضد بكتريا S.aureus و E.coli .أيضًا أظهرت النتائج أن جميع تراكيز جل القرنفل النانوي ليس لها أي تأثير على إفرازات السيتوكينات بواسطة خط الخلايا البلعمية. وهذا يعني أن قرنفل النانوجل ليس له أي آثار التهابية ولا يعمل كمستضد

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

قسم الكيمياء

دراســـة نظام توصـــيل الدواء لزيت القرنفل ودوره في بعض الأنشطة البيولوجية

رسالة مقدمة الى

كلية العلوم / جامعة ميسان جزء من متطلبات نيل

شهادة الماجستير في علوم الكيمياء

من الطالبة

مريم كاظم طاهر

بكالوريوس علوم كيمياء /جامعة ميسان (٢٠٢٠)

بإشراف

أ.م.د اسراء قصي فالح

أ.م.د يونس جاسم عبد الله

٢٠٢٤م **١٤٤٦هـ**