REPUBLIC OF TÜRKİYE

ALTINBAŞ UNIVERSITY

Institute of Graduate Studies

Biomedical Sciences

ENCAPSULATION OF OILS IN MONTMORILLONITE CLAY: EXPLORING ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES FOR POTENTIAL WOUND HEALING

Dalya Adil Abdulmajeed ABDULMAJEED

Master's Thesis

Supervisor

Assoc. Prof. Dr. Hakan KAYGUSUZ

İstanbul, 2025

ENCAPSULATION OF OILS IN MONTMORILLONITE CLAY: EXPLORING ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES FOR POTENTIAL WOUND HEALING

Dalya Adil Abdulmajeed ABDULMAJEED

Biomedical Sciences

Master's Thesis

ALTINBAŞ UNIVERSITY

2025

The thesis titled ENCAPSULATION OF OILS IN MONTMORILLONITE CLAY: EXPLORING ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES FOR POTENTIAL WOUND HEALING prepared by DALYA ADIL ABDULMAJEED ABDULMAJEED and submitted on 02.07.2025 has been **accepted unanimously** for the degree of Master of Science in Biomedical Sciences.

Assoc. Prof. Hakan KAYGUSUZ
Supervisor

Thesis Defense Committee Members:

Assoc. Prof. Dr. Hakan KAYGUSUZ	Department of Basic Sciences, Altınbaş University	_____
Prof. Dr. Yasa Ekşioğlu ÖZOK	Department of Electrical and Electronics, Altınbaş University	_____
Assoc. Prof. Dr. Bircan DİNÇ	Department of Basic Sciences, Bahçeşehir University	_____

I hereby declare that this thesis meets all format and submission requirements for a master's thesis.

I hereby declare that all information/data presented in this graduation project has been obtained in full accordance with academic rules and ethical conduct. I also declare all unoriginal materials and conclusions have been cited in the text and all references mentioned in the Reference List have been cited in the text, and vice versa as required by the abovementioned rules and conduct.

Dalya Adil Abdulmajeed ABDULMAJEED

Signature

DEDICATION

I would like to sincerely express my deepest gratitude to my esteemed Associate Professor, Dr. Hakan Kaygusuz, whose advice, knowledge, and unwavering support have been invaluable in guiding me complete this thesis. Along with enriching my journey, his insightful feedback and encouragement have motivated me to strive for excellence. Dr. Hakan's mentorship has been precious, and I am profoundly thankful for the expertise and wisdom he has imparted.

I would also like to express my sincere gratitude for Prof. Dr. Emrah Torlak's valuable contribution in assisting with the antibacterial test. His support and efforts have been instrumental in achieving meaningful results, and I really appreciate the time and dedication he provided.

This thesis is further dedicated to my family. My parents and my sister, whose sacrifices, encouragement, and unconditional support have been the foundation of my educational pursuits. Their belief in me has been a persistent source of strength and motivation.

PREFACE

This thesis is submitted in partial discharge of the requirements for the degree of Master of Biomedical Sciences at Altınbaş University. The research presented herein has been conducted under the supervision of Assoc. Prof. Dr. Hakan Kaygusuz, whose leadership and skill have been instrumental in shaping this work and the work described in this thesis has been supported by Altınbaş University.

ABSTRACT

ENCAPSULATION OF OILS IN MONTMORILLONITE CLAY: EXPLORING ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES FOR POTENTIAL WOUND HEALING

ABDULMAJEED, Dalya Adil Abdulmajeed

M.Sc., Biomedical Sciences, Altınbaş University,

Supervisor: Assoc. Prof. Dr. Hakan KAYGUSUZ

Date: 07/2025

Pages: 65

Vegetable oils and essential oils have advantageous uses in different fields of science and technology, such as antibacterial activities, antioxidant and anti-inflammatory properties. Encapsulation of these oils often require different formulations such as emulsifiers and/or different types of synthetic additives. On the other hand, there is still room for development of natural based formulations for oil encapsulation. In this study, montmorillonite clay is used for a one-pot encapsulation of different types of oils, which are tea tree oil, tamanu oil and safflower oil. Clay-oil mixtures were characterized using Fourier transform infrared spectroscopy (FTIR), ferric reducing antioxidant assay (FRAP) and antibacterial tests. Results show that these formulations are effective against Gram-positive and Gram-negative bacteria and have potential uses in different applications.

Keywords: Montmorillonite Clay, Encapsulation, Tea Tree Oil, Safflower Oil, Tamanu Oil, Antibacterial Effect, Antioxidant Activity.

TABLE OF CONTENTS

Pages

LIST OF TABLES

Pages

LIST OF FIGURES

Pages

ABBREVIATIONS

MTT	:	Montmorillonite
TTO	:	Tea Tree Oil
TMU	:	Tamanu Oil
CS	:	Chitosan
NF	:	Norfloxacin
MIC	:	Minimum Inhibitory Concentration
FTIR	:	Fourier Transform Infrared Spectroscpy
XPS	:	X-ray photoelectron Spectroscopy
ECO	:	Eucalyptus Oil
LGO	:	Lemongrass Oil
REO	:	Rosemary Essential Oil
NaCl	:	Sodium Chloride
SSW	:	Safflower Seed Waste
DNA	:	Deoxyribonucleic Acid
GC-MS	:	Gas Chromatography-Mass Spectrometry
MRSA	:	Methicillin-Resistant Staphylococcus Aureus
NTR	:	Neutral Tamanu Resin
ATR	:	Acidic Tamanu Resin
EtTO	:	Ethanol-Tamanu Oil
MeTO	:	Methanol-Tamanu oil

ELISA	:	Enzyme-linked Immunosorbent Assay
CIO	:	Calophyllum Inophyllum Oil
EVOOs	:	Extra Virgin Olive Oils
TO	:	Tamanu Oil
PLA-MMT	:	Poly Lactic Acid-Montmorillonite
XRD	:	X-Ray Diffraction Analysis
RT-PCR	:	Real-Time Polymerase Chain Reaction
PVA	:	Polyvinyl Alcohol
Zn-MMT	:	Zinc (II) Montmorillonite
UV	:	Ultraviolet Radiation
TGA	:	Thermogravimetric Analysis
EO	:	Essential Oil
EOC	:	Essential Oil Components
AITC	:	Allyl Isothiocyanate
CTMA	:	Cetyltrimethylammonium
LDPE	:	Low Density Polyethylene
FRAP	:	Ferric Reducing Antioxidant Power

LIST OF SYMBOLS

μ	:	Micro
λ	:	Wavelength
M	:	Molar (mol/L)
mmol	:	Millimole
mm	:	Millimetre
mM	:	Millimolar (mmol/L)
g	:	Gram
DW	:	Dry Weight
KHz	:	Kilo Hertz
rpm	:	Revolutions Per Minute
min	:	Minute
h	:	Hour
cm−1	:	Reciprocal Centimetre- Unit of Wavenumber
cm	:	Centimetre
°C	:	Degree Celsius
L	:	Liter
mg	:	Milligram
%	:	Percentage
log	:	Logarithm

CFU : Colony-Forming Unit

1. INTRODUCTION

The prevalence of diseases, including wounds, caused by pathogens like bacteria, fungi, viruses, and protozoa is rapidly increasing, and the rising incidence of their resistant nature has prompted researchers to create strong, affordable, non-toxic, and effective disinfection inhibitory compounds. Based on their chemical makeup, these antimicrobial compounds can be generically categorized as inorganic and organic substances (Mokhtar, 2023). Organic antibacterial substances have been extensively used due to their potent inhibitory effects on bacterial growth. Thus, the use of natural and herbal therapies for wound healing has gained popularity in recent years. The demand for effective alternative therapies that don't possess the negative side effects of synthetic treatments is what's driving this interest. Healthcare professionals face major difficulty when dealing with infectious wounds, which can be caused by a variety of bacteria (Mokhtar, 2023).

Preliminary wound care has been employed since the prehistoric period, features some of the earliest medical records known to exist detailing procedures like dressing and infection control that are comparable to those used in contemporary wound care. In spite of centuries of experience, nonhealing wounds reduce the quality of life for those who are impacted and their caregivers, and they pose a substantial and underestimated clinical and financial burden to health care systems worldwide. A growing elderly population, an increasing prevalence of diabetes, obesity, and systemic disease, all these considerations which predispose individuals to delay wound healing that make non-healing, chronic wounds a global concern (Uberoi, 2024).

Basically, a wound is developed when the skin barrier is harmed or compromised, and it’s characterized as acute and chronic wound. Without the skin's protective layer, the wound bed and underlying tissue are susceptible to microbial contamination, which can lead to infections. Coagulation, hemostasis, inflammation, cell proliferation, cell migration, and tissue remodeling are all linked and overlap in optimal wound repair system, which results in wound closure in 3–14 days. When this sequence is disrupted, dysregulated or imbalanced, a persistent wound may form and recovery may be delayed (Uberoi, 2024).

In addition to requiring the cooperation and participation of several host cell types (such as keratinocytes, fibroblasts, and immune cells), the skin wound healing process is also

mediated by microbiological components of the wound ecosystem. Although microbes colonize almost every wound, not all colonization led to infections. Microorganisms' effects on wound healing vary greatly depending on the condition. In fact, by stimulating host immune responses that promote healing, like cytokine production and epithelialization, certain microbes may even aid in the processes involved in skin restoration. However, by developing virulence factors and antibiotic resistance and/or encountering a host that is especially vulnerable, seemingly innocuous or commensal microbes can cause havoc (Uberoi, 2024).

Therefore, the use of natural substances, including oils with established antibacterial qualities, may present a viable way to address this issue, emphasizing the therapeutic healing benefits offered by the ability of roots, leaves, and stems. Herbal medications have the benefit of being inexpensive and easily accessible in nature, both of which are essential for their distribution.

The utilization of natural oils has been of great significance in the treatment of human and animal wounds, undertaking these compounds effectiveness. Tea tree, safflower, tamanu, and olive oil are notable for their medicinal qualities and have been thoroughly researched for their antibacterial capabilities. They are abundant in vitamins, antioxidants, and vital fatty acids, all of which are necessary for fostering healing and lowering inflammation (de Oliveira Soares, 2024).

For instance, tea tree oil *(Melaleuca alternifolia)* has received interest due to its antibacterial, antifungal, antioxidant, antiviral, and anti-inflammatory effects. TTO with a distinct inhibitory profile against bacteria has been mentioned in a huge number of scholarly articles. Studies employing antibacterial agents for TTO were frequently conducted on *Staphylococcus aureus* (Chin, 2013), (Swamy, 2016), (Carson, 2006), (Carson, 2002) *Escherichia coli* (Gustafson, 1998), (Cox, 1998) and *Pseudomonas aeruginosa* (Cox, 2000) and it has been proven to be efficient against them. According to findings in the literature, the oil's antibacterial properties may result from membrane integrity being lost and respiration being inhibited following application. Monoterpenes make up the majority of the oil's almost 100 distinct constituents (Erosh Yadav, 2017). It has a minimum of 30% terpinen-4-ol and a maximum of 15% 1, 8-cineole (Pazyar, 2013). Terpinen-4-ol is the main key ingredients that tea tree oil contains which offers potent antibacterial qualities, followed by

1, 8-cineole. Tea tree oil is a valuable addition to wound healing treatments since studies have shown that it can suppress the growth of several bacteria (National center of complementary and integrative health NIH, Tea tree oil: Usefulness and safety, 2022).

Additionally, the linoleic acid found in safflower oil *(Carthamus tinctorius L.)* or false saffron, also known as "Kafesheh" in (Persian). It has been demonstrated to prevent the growth of specific bacteria since it is an essential fatty acid that not only has antibacterial effects but also aids in inflammation reduction and revitalization of the skin (Rakhimov, 2018). Research has demonstrated strong antibacterial action as it demonstrated efficacy against three bacterial species: *Enterobacter cloacae*, *Streptococcus agalactiae*, and *Escherichia coli* from a four bacterial species in all (Khémiri, 2020).

Moreover, tamanu oil *(Calophyllum inophyllum L.)* has been shown to have antimicrobial characteristics against a variety of bacterial types. Calophyllolide, which is abundant in this oil, has strong antibacterial and anti-inflammatory properties (Raharivelomanana, 2018). The finding aligns with research conducted by (Yimdjo, 2004), which reported that calophyllolide has antimicrobial activity against *S. aureus* (Nguyen, 2016) who reported that by day seven posttreatment, wound closure reached 80%, and by day 14, it reached 97%. However, there have been no reports on the fatty acid profile of tamanu oil and its activity in wound healing (Rakhmawati, 2024). Furthermore, Tamanu oil can combat a number of pathogens, including *P. aeruginosa*, *methicillin-resistant S. aureus* (MRSA), and *S. aureus* (Nguyen, 2016).

However, these oils have several limitations that restrict their use in this field, including low stability, limited absorption, short shelf life, toxicity, and low thermal stability. Therefore, these oils are incorporated into other natural substances to enhance their action, effectiveness and to solve related issues. Montmorillonite, which is recognized for its high cation exchange capacity, enables it to efficiently adsorb and release active chemicals. Due to this feature, it works as an ideal medium for the oils, assuring their continuous release and long-lasting antibacterial action.

By encapsulating these oils into clay, we might be able to protect them from degradation, enhance their stability, and improve their bioavailability. This implies that the skin can absorb the oils more readily, improving the therapeutic results. Since the clay itself has antibacterial qualities as well, its special structure enables it to create a barrier of protection

around the wound. Research has demonstrated that montmorillonite can prevent several bacterial growths including *Escherichia coli* and *Staphylococcus aureus* by its ability to adsorb bacterial toxins and interrupt bacterial cell membranes. The overall antibacterial action is increased by the synergistic effect of the clay and oil combination since the necessity for alternative treatments has been further underscored by the rising incidence of bacteria resistant to antibiotics. In order to promote quicker wound healing by utilizing the combined advantages of this mixture, this study intends to represent natural oils as effective alternatives by investigating their potential and improving their action when coupled with montmorillonite clay and the discovery of novel and natural remedies for infectious wounds can be aided by investigating the synergistic effects of antibacterial oils and montmorillonite clay. Therefore, this study aimed to encapsulate different oils into montmorillonite clay to assess the possibility of developing a combination with antioxidant and antibacterial characteristics that can promote the healing of infected wounds. The research applied a quantitative and qualitative experimental design to systematically investigate the encapsulation of oils into montmorillonite clay. The study focused on encapsulation efficiency and the potential antibacterial activity of the resulting mixtures. Tea tree oil, olive oil, safflower oil, and tamanu oil were the oils used in the present research. This procedure was intended to ensure that oils were incorporated into the clay matrix, followed by validation of Fourier-transform infrared spectroscopy (FTIR). Likewise, the ASTM E2149 test and the FRAP assay were used to evaluate the encapsulated samples' antibacterial and antioxidant properties, respectively.

2. LITERATURE REVIEW

This study draw attention to the importance of encapsulating oils into montmorillonite clay, highlighting their potential applications in wound healing focusing on antibacterial properties and the relevance of these qualities on wounds. Examining research that investigated the advantages of encapsulation, the procedures involved, the antibacterial and some antioxidant qualities of the materials used particularly oils and their impact on the infectious wound healing process will be the main focus in this literature review. The studies used were thoroughly selected utilizing comprehensive keyword searches in academic databases such as Web of Science and Google Scholar. Keywords relevant to my research, such as "montmorillonite clay," "oil encapsulation", "tea tree oil", "olive oil", "safflower oil", "tamanu oil", "antibacterial activity", "antioxidant activity", and "wound healing" were applied to identify mostly recent experimental studies.

2.1 MONTMORILLONITE CLAY: COMPOSITION, PROPERITIES, AND WOUND HEALING POTENTIALS

Montmorillonite clay is a natural clay mineral that has distinctive qualities that have been traditionally applied in industrial applications. It's obtained from volcanic ash and lately, its possible biological uses, especially in dermatology, have attracted a lot of attention lately. MTT clay with the formula of (Na,Ca)0.33 (Al,Mg)2 (Si4 O10) (OH)2 ·nH2O, belongs to the smectite group which is mostly made up of hydrated sodium calcium aluminum magnesium silicate hydroxide (Emmerich, 2009). High cation exchange capacity, water swelling, and a layered structure that improves adsorption are some of this clay's unique features (Jemai, 2024). Numerous studies have shown that montmorillonite has powerful antibacterial potential against various pathogens like *Staphylococcus aureus* and *Escherichia coli* remarkably, especially when combined with other substances (Ling, 2013) (Ohashi, 1992).

In terms of wound healing, montmorillonite clay decreases inflammation, encouraging fibroblast regeneration, and improving circulation, and therefore it's a possible alternative for traditional wound dressings (Zafar, 2024), (Ovincy, 2024).

In García-Villén et al., analysis, montmorillonite-norfloxacin (NF) nanocomposite was created to promote wound healing by treating infected wounds. The encapsulation method involved charge–charge interactions between negatively charged montmorillonite edges and protonated NF. Norfloxacin was adsorbed onto montmorillonite using an intercalation solution approach as part of the encapsulation process. Adsorption isotherms, solid-state characterization, and evaluations of drug loading capacity were used to characterize the nanocomposite. The antimicrobial activity was tested against *Pseudomonas aeruginosa, Escherichia coli,* A*spergillus niger, Bacillus subtilis,* and *Staphylococcus aureus*. Due to its controlled release characteristics and nanostructure, the nanocomposite demonstrated higher antibacterial efficacy than the free drug. The nanocomposite encouraged the growth of fibroblasts in vitro and was biocompatible. Given improved antibacterial activity and biocompatibility of montmorillonite-norfloxacin nanocomposite, García-Villén's assumptions that this nanocomposite is a prospective treatment for infected skin lesions, such as chronic ulcers and burns, appeared to be realistic (García-Villén, 2019).

Additional research by Magaña et al., examined the antibacterial properties of silver ion-modified montmorillonite nano clay. The encapsulation process entailed ion-exchange treatment of montmorillonite with silver, followed by either grinding for 300 seconds or calcination at 550°C for three hours. In the encapsulation process, silver was added to montmorillonite via ion exchange, and the mixture was then either ground for 300 seconds or calcined for three hours at 550°C. Significant antibacterial activity against *Escherichia coli* was demonstrated by the modified montmorillonite. Although the calcined sample displayed a bigger inhibition zone in disk susceptibility testing, the ground montmorillonite required a lower minimum inhibitory concentration (MIC). By using X-ray photoelectron spectroscopy (XPS) to verify that the Ag+ ions were in contact with the bacteria, the antibacterial activity was attributed to their presence. Since the method of modification and treatment greatly affects the antibacterial efficacy, the study found that silver-modified montmorillonite nanoclay is effective in inhibiting bacterial growth, especially against *Escherichia coli*. This made these nanocomposites promising for a variety of antimicrobial applications. Therefore, these studies were undertaken to support the growing interest in MTT clay as a natural material to be used for treating skin conditions due to its potent antibacterial characteristics (Magaña, 2008).

2.2 ESEENTIAL AND VEGETABLE OILS ANTIBACTERIAL AND WOUND HEALING PROPERITIES

2.2.1 Tea Tree Oil

Tea tree or Narrow-leaved paperbark *(Melaleuca alternifolia)* is a sort of tree or tall shrub in the myrtle family, *Myrtaceae* (Parviz, 2022). Tea tree oil is known for its strong antibacterial, antiviral, antioxidant, and antifungal activity against various types of bacteria as stated by multiple research studies.

For instance, Mumu et al., examined the antimicrobial activity of tea tree oil (TTO) against ten pathogenic bacteria, including *Streptococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus vulgaris, Aeromonas hydrophila, Escherichia coli, Streptococcus pneumoniae, Bacillus subtilis, Klebsiella pneumonia, and Streptococcus agalactiae*. The study also compared TTO efficacy with conventional antibiotics, eucalyptus oil (ECO), and lemongrass oil (LGO). Agar well diffusion and broth dilution were among the techniques employed to evaluate the inhibition of bacterial growth. In contrast to ECO and LGO, which demonstrated lesser inhibition percentages, the results demonstrated that TTO exhibited strong antibacterial activity, with 96.94% to 100% inhibition against the studied microorganisms after 24 hours of incubation. Tea tree oil proved to be the most effective antibacterial in the study. Indeed, TTO's 36.3 mm inhibition zone against *A. hydrophila* was the greatest in the entire study and appeared to be 1.1 times more potent than that of the control antibiotic, Cefepime. On top of that, TTO had strong action against all the bacteria that was examined. In comparison to the other oils and antibiotics, TTO also showed a greater activity index. According to the study's findings, TTO exhibits exceptional qualities and may be a better option for treating bacterial infections than traditional antibiotics as indicated below in Table 2.1 (Mumu, 2018).

Table 2.1: Average Zone of Inhibition of Traditional Antibiotics Vs. Tea Tree Oil (Mumu, 2018).

Antibiotic Name **Inhibition Zone (mm)**										
Bacteria	**Rifampicin**	**Cefepime**	**Cephalexin**	**Erythromycin**	**Amoxycillin**	**Sulfamethoxazole**	**Doxycycline**	**Cefuroxime Sodium**	**Clindamycin**	**Tea Tree Oil**
S. aureus	32.6	25.3	32.6	28.6	35.3	24.3	30.6	34.3	29.3	18.6
S. pyogenes	0	34.2	0	0	0	0	0	14.6	0	17.3
P. aeruginosa	0	31.6	0	0	0	0	0	16.3	0	18.6
P. vulgaris	17.6	35.3	19.3	14.3	0	21.6	27.6	28.3	0	21.3
A. hydrophila	8.6	31.3	19.6	0	0	24.3	21.3	23.6	0	30.6
E. coli	37.6	30.3	37.6	0	38.6	22.3	34.3	35.3	24.6	15.6
S. pneumoniae	25.6	17.6	28.6	26.3	40.3	27.3	10.3	35.3	23.3	17.6
B. subtilis	0	24.3	11.3	0	0	9.6	11.6	14.6	0	14.3
K. pneumoniae	26.6	10.3	35.6	30.3	36.6	38.3	35.3	16.6	28.3	16.6
S. agalactiae	20.3	18.3	24.3	24.3	32.3	29.6	16.6	31.3	24.3	16.3

Additionally, Carson's study used electron microscopy, time-kill, lysis, leakage, and salt tolerance experiments to investigate the mechanism of action of *Melaleuca alternifolia* (Tea tree oil TTO) on *Staphylococcus aureus*. TTO and its constituents (1,8-cineole, terpinen-4-ol, and α-terpineol) were administered to S. aureus at their least inhibitory concentrations (MICs) and twice their MICs. According to the results, TTO and its constituents considerably decreased S. aureus vitality, resulted in the loss of 260-nm-absorbing material, and decreased the bacteria's resistance to NaCl, indicating a damaged cytoplasmic membrane. Therefore, this investigation showed that TTO and its constituents killed *S. aureus* cells throughout their stationary phase of growth. Mesosome development and cytoplasmic content reduction were observed in treated cells using electron microscopy. The study revealed that TTO inhibits S. aureus's cytoplasmic membrane, indicating that it has the potential to be a strong antimicrobial agent (Carson, 2002).

Moreover, the study by Cox and Mann, (2000) examined the degree to which (*Melaleuca alternifolia)* tea tree oil (TTO) inhibited the growth of *Candida albicans, Staphylococcus aureus*, and *Escherichia coli*. These organisms were exposed to bactericidal/fungicidal and minimum inhibitory concentrations of TTO, and the effects on potassium ion leakage, respiration, and membrane permeability were measured. The findings demonstrated that TTO produced potassium ion leakage in *E. coli* and *S. aureus*, reduced respiration, and enhanced membrane permeability as measured by propidium iodide uptake. *E. coli* was most vulnerable to the effects of tea tree oil at minimum inhibitory and minimum lethal concentrations, followed by *C. albicans and S. aureus.* The study also reported that TTO exhibits broad-spectrum antibacterial activity by breaking down the permeability barrier of cell membranes, which results in chemiosmotic control loss and cell death (Cox, 2000).

Likewise, Gustafson's tested the impacts of tea tree oil (TTO) once more on *Escherichia coli*. The techniques included employing electron microscopy to observe changes and inducing autolysis in *E. coli* cells in both the exponential and stationary phases. The findings revealed that TTO resulted in extracellular bleb development, cytoplasmic coagulation, and loss of electron-dense material. Compared to exponentially growing cells, stationary phase cells showed a higher resistance to TTO-induced cell death and less TTO-stimulated autolysis. Increased resistance to the bactericidal effects of TTO was seen by a subset of stationary phase cells. According to the study's outcomes, TTO influences cell integrity and promotes autolysis, with stationary phase cells exhibiting more resistance to its effects (Gustafson, 1998).

2.2.2 Safflower Oil

The flowers of safflower *(Carthamus tinctorius)* are thistle-like annual plants with many branches that belong to the Asteraceae family. They are used extensively in medicine, particularly medical disorders including dysmenorrhea, amenorrhea, postpartum abdominal pain and mass, trauma, and joint pain. The floral extract was found to enhance antibacterial and antioxidant activities (Delshad, 2018).

In a study in 2022, Choudhary discovered that a bio surfactant derived from safflower seed waste (Ssw) has antibacterial action against bacterial strains that cause skin diseases and *Malassezia furfur*, which causes dandruff. The techniques used to assess the efficacy of the

bio surfactant included minimum inhibitory concentration (MIC) and kill-time experiments. The main ingredient, saponin, interacted with and damaged the pathogens' cell walls and membranes, causing cell death, according to the results. Saponin additionally broke through the cytoplasmic membrane to prevent the creation of proteins and deoxyribonucleic acid (DNA), which are essential for bacterial development. According to the study's findings, safflower seed waste bio surfactant is a strong antibacterial agent that works at the molecular level to combat bacterial and fungal infections (Choudhary, 2022).

Similarly, Karimkhani's research examined the antibacterial and antioxidant properties of methanolic extracts from four different safflower cultivars: Mahali, Padide, Isfahan-28, and Ionic liquid IL111. Total phenolic and flavonoid content, antibacterial capacity was evaluated using Minimum inhibitory concentration (MIC) tests, also antioxidant activity was measured employing two different techniques. Depending on the results, the IL111 cultivar had the highest levels of flavonoids and phenols, and its antioxidant activity was on par with that of synthetic antioxidants. The Isfahan-28 cultivar displayed the greatest antibacterial action, with MIC values of 30 mg/ml against *Staphylococcus aureus* and 60 mg/ml against *Salmonella enterica serovar Typhi*. On the basis of the conclusion, safflower extracts, especially those from the IL111 and Isfahan-28 cultivars have strong antibacterial and antioxidant qualities, which makes them attractive options for natural medicinal agents (Karimkhani, 2016).

Moreover, Ozkan assessed the extracts' antibacterial, antioxidant, and bio accessibility in vitro from three safflower genotypes: Arizona SC III, Dincer 5-18-1, and Remzibey-05. Among the techniques were extract preparation, in vitro gastrointestinal digestion, and an antimicrobial test. The extract from Dincer 5-18-1 exhibited the strongest antibacterial activity against *Listeria monocytogenes*, corresponding to the results. Consequently, Ozkan et al., concluded that safflower extracts have strong antibacterial and antioxidant qualities, and their bioactive components are crucial for bio accessibility (Ozkan, 2021).

Finally, Abuova's study examined the antibacterial activity and component makeup of an ophthalmic emulsion made from safflower (*Carthamus tinctorius L.*) flowers. The techniques comprised antimicrobial testing against two strains of Gram-positive bacteria, one strain of Gram-negative bacteria, and one fungal culture which are (*S. aureus*, *E. coli*, *P. aeruginosa*, *and C. albicans*), and gas chromatography-mass spectrometry (GC-MS) to

identify the chemical components of the flowers. In line with the results, there were notable concentrations of tridecane, tricosane, hexacosane, dodecanoic acid, pentacosane, and linoleic acid in the emulsion. With a growth suppression zone of 9.0 ± 0.0 mm, the emulsion showed bactericidal action, especially against *Staphylococcus aureus*. In conclusion, the ophthalmic emulsion derived from safflower exhibits encouraging antibacterial qualities, which could make it a viable option for treating eye infections (Abuova, 2022).

2.2.3 Tamanu Oil

Tamanu or *Calophyllum inophyllum L.* belonging to the *Calophyllaceae* family. It is a pantropical evergreen tree. For millennia, people have used it for medical and cosmetic purposes. This tree, known locally as "tamanu" in French Polynesia, was once thought to be sacred. Traditionally, the oil extracted from the nuts, known as "tamanu oil," has been applied topically to skin and mucous membrane lesions for a variety of purposes. This oil is particularly suggested for the treatment of different skin conditions said (Raharivelomanana, 2018).

Crucially, Nguyen et al., examined the chemical makeup of tamanu seed oil extracted using supercritical CO2 (sCO2) technology and assessed its antibacterial and anti-inflammatory properties. The techniques included diffusion methods to assess antibacterial activity against pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and gas chromatography-mass spectrometry (GC-MS) to identify fatty acids and coumarins. A mouse ear model was used to evaluate the anti-inflammatory efficacy. As stated in the results, fatty acids (72.62%) and coumarins were more abundant in tamanu oil extracted using sCO2 than in the Soxhlet method. The mice's thickness and weight has been decreased because of the oil's strong anti-inflammatory action while potent antibacterial activity was seen especially against *methicillin-resistant Staphylococcus aureus* (MRSA). Thus, tamanu oil derived using CO2 has strong antibacterial and anti-inflammatory properties, which makes it useful for both medical and cosmetic purposes (Nguyen, 2016).

Whereas Cassien's created resinous ethanol-soluble tamanu oil extracts EtTO to enhance the antioxidant qualities of (*Calophyllum inophyllum*) seed oil. Diffusion techniques were employed to measure antibacterial activity, and the results demonstrated that the extracts exhibited strong antimicrobial activity against a range of bacterial species. The study

examined the antibacterial properties of tamanu oil (TO) separated fractions and extracts made from ethanol. The extracts of tamanu oil TO and methanol tamanu oil MeTO, which were inactive, demonstrated noticeably stronger antibacterial activity at 20µg per disc. Neutral tamanu resin NTR and acidic tamanu resin ATR were the most active portions. As a consequence of the oil phenolic derivatives and OH-bearing pyranocoumarins, NTR had greater antibacterial efficacy than the other fractions, which were especially efficient against *Staphylococcus aureus* and *Mycobacterium tuberculosis*. Significant antibacterial action was found in EtTO, ATR, NTR, and pyranocoumarins when the study also assessed the minimum inhibitory concentration (MIC) against *M. tuberculosis* strain. The capacity of coumarin and phenolic chemicals in tamanu oil to penetrate bacterial walls and disrupt membrane potential has been linked to their antibacterial qualities. Cassien and his team assumed that TO resin extracts' biological characteristics make them promising as novel sources of natural anti-infectious agents (Cassien, 2021).

Furthermore, several emulsions consisting of natural oils were examined by Makiej to determine how effective they were at fighting bacteria. Significant antibacterial action was shown by those that contained 2.5% Tamanu oil stabilized with casein. The analysis showed a relationship between stability as determined by the samples' creaming index and oil concentration. The sample with a 2.5% concentration of tamanu and inca inchi oil showed the maximum stability in lecithin-stabilized emulsions. Gram-positive bacterial strains such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Bacillus cereus* were inhibited in their growth by tamanu oil, whereas *Escherichia coli* and *Candida albicans* were inhibited in their growth in the zone below 5 mm and thus the findings illustrated the way tamanu oil can be used to create antibacterial systems (Makiej, 2023).

Further work focused on isolating and describing the chemical components of *Calophyllum inophyllum's* nut and root bark. The study used a qualitative antimicrobial assay, which is the traditional agar disc dilution method using Mueller Hinton agar, to assess these compounds' in vitro cytotoxicity against the KB cell line and antibacterial activity against a variety of microorganisms, including Gram- (+) *Staphylococcus aureus, Vibrio angillarium,* Gram- (-) *Escherichia coli,* and *yeast Candida tropicalis*. Significant inhibitory action against S. aureus was found in the results, but not against other microbes. Yimdjo's study

findings suggested that these substances may be used as cytotoxic and antibacterial agents (Yimdjo, 2004).

Once more, researchers have assessed five ethnomedical *Calophyllum inophyllum* oils (CIO) from Indonesia, Tahiti, Fiji, and New Caledonia for its ability to heal wounds and eliminate bacteria that cause skin infections. Numerous Gram-positive and Gram-negative microorganisms, including multidrug-resistant *Staphylococcus aureus* (MRSA), were used to test the antibacterial activity. β-defensin 2 release was measured for antibacterial activity using enzyme-linked immunosorbent assay (ELISA), and wound healing capabilities were assessed using the scratch test assay. Tested strains of aerobic Gram + and Gram - bacteria, including *Staphylococcus aureus* (a multidrug resistant bacteria linked to nosocomial and skin infections), *Bacillus cereus* (linked to wound infections in postoperative patients and cutaneous infections following trauma), *Staphylococcus epidermidis* and *Staphylococcus hemolyticus* (linked to catheter-associated infections), and *Corynebacterium minutissimum* (linked to erythrasma), demonstrated very intriguing antibacterial activities. The findings indicated that, depending on the oil's source, CIO could be applied to keratinocyte cells at concentrations ranging from 2.7% to 11.2% without causing harm. In vitro, all CIO tests accelerated wound closure, with healing factors 1.3–2.1 times greater than the control. CIO demonstrated two different antibacterial effects: first, it directly inhibited the mitotic growth of Gram-positive bacteria and second, it stimulated the release of β-defensin 2 against Gram-negative bacteria. Also, the MIC values of all the CIOs evaluated against Gram+ bacterial species are comparable to or less than those of ofloxacin, which was used as positive control alongside bacterial strains involved in acne (Propionibacterium species) such as *Propionibacterium acnes* and *Propionibacterium granulosum*, hence suggesting the potential of CIO for acne treatment (Léguillier, 2015).

2.2.4 Olive Oil

Olive oil or Olea Europaea fruit oil is the fixed oil extracted from the ripe fruit of the olive, Olea europaea, which belongs to the Oleaceae family. Numerous studies have shown that it includes chemicals that may suppress or kill dangerous germs.

In 2020, Guo et al., examined the antibacterial properties of olive oil polyphenol extract against *Staphylococcus aureus* and *Salmonella Typhimurium*. Using solvent extraction procedures, polyphenols were extracted from olive oil, and their antibacterial qualities were assessed using agar diffusion methods to assess the inhibition zones and microdilution assays to ascertain the minimum inhibitory concentration (MIC). Significant antibacterial activity was demonstrated by the olive oil polyphenol extract against *Staphylococcus aureus* and *Salmonella Typhimurium*. The agar diffusion tests revealed distinct inhibition zones, and the MIC values suggested robust inhibition. It was believed that the rupture of bacterial cell membranes and interference with metabolic activities were the causes of the antibacterial activity. Hence, they concluded that olive oil polyphenol extract has strong antibacterial properties against *Staphylococcus aureus* and *Salmonella Typhimurium*, and it may find use in food preservation and safety (Guo, 2020).

Likewise, another study examined the antimicrobial properties of polyphenolic extracts from three types of extra virgin olive oils (EVOOs) from the Southern Italian area of Campania: Ogliarola, Ravece, and Ruvea antica. Using microdilution assays to determine the minimum inhibitory concentration (MIC) and agar diffusion methods to measure inhibition zones, the polyphenols in these EVOOs were extracted, and their antimicrobial properties were evaluated against both Gram-positive (*Bacillus cereus DSM 4313, Bacillus cereus DSM 4384, Staphylococcus aureus DMS 25923, Enterococcus faecalis DSM 2352, and Listeria innocua DSM 20649*) and Gram-negative bacteria (*Escherichia coli DSM 8579, and Pseudomonas aeruginosa ATCC 50071*). Among the three EVOO types, the polyphenolic extracts showed notable antibacterial activity, albeit to differing degrees. Escherichia coli was especially susceptible to the extracts' effects. A link between the antimicrobial activity and the total polyphenol content was found by statistical analysis, suggesting that stronger antibacterial effects were typically associated with higher polyphenol concentrations. The research ultimately reached the conclusion that EVOOs' high polyphenol content gave them

strong antibacterial qualities. These EVOOs may find application as natural antibacterial agents in food safety and preservation (Nazzaro, 2019).

On the other hand, a 2021 report assessed the antibacterial qualities of phenolic compounds present in olive oil as well as their capacity to regenerate fibroblast cells. Cultured human fibroblasts (CCD-1064Sk) were treated for 24 hours with a variety of olive oil phenolic compounds, such as luteolin, apigenin, ferulic acid, coumaric acid, and caffeic acid, at a concentration of 10^-6 M. Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) spectrophotometric assay and real-time polymerase chain reaction (RT-PCR) for genes like VEGF, TGF-β1, PDGF, and COL-I 1, the effects on cell proliferation, migration, and gene expression associated with wound healing were evaluated. Research conducted that every chemical, except for ferulic acid, markedly increased fibroblast migration and proliferation while also upregulating the expression of genes related to wound healing. Phenolic compounds have antibacterial activity against *Candida albicans*, *Escherichia coli*, *Proteus* species, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Phenolic compounds found in olive oil have demonstrated strong antibacterial and bio stimulatory actions on fibroblasts. These results showed that these substances would be very useful in encouraging the healing of infected wounds. (Melguizo-Rodríguez, 2021).

2.3 ENCAPSULATION OF OILS: TECHNIQUES, ADVANTAGES AGAINST DIFFERENT BACTERIAS, AND WOUND HEALING CHARACTERISTICIS

The encapsulation of different types of oils (Essential oils & Vegetable oils) in clay matrices or polymers is a method that shows promise for improving the functional qualities of oils and expanding their uses in a variety of industries.

Recent evidence investigated examined the impact of incorporating tea tree oil (TTO) into poly (lactic acid)-montmorillonite (PLA-MMT) clay bio nanocomposites and its antibacterial properties on the process of infectious wound healing. The process of integrating tea tree oil into MMT clay involved sonication accompanied by melt mixing. Tween 80 or polysorbate 80 “a nonionic surfactant and an emulsifier” was added in a 0.25/1 g proportion of essential oil to a mixture of 10 g of MMT, 30 g of glycerol, and various concentrations of tea tree essential oil to create the samples. Using an Ultra-Turrax T25 in a closed bottle with an ice bath, each mixture was dissolved in 50 mL of deionized water and

agitated for one minute at 19,000 rpm. After that, the suspension was subjected to 30 minutes of 20 KHz sonication in an ice bath using a 400 W Hielscher UP400S tip ultrasonicator. The samples were then dried for 24 hours at 110°C. Some tea tree oil probably evaporated during production due to the high essential oil concentrations. Five samples were created using ultrasonication. Disk diffusion was also used to achieve antibacterial activity against chosen bacterial strains. The results demonstrated that TTO had strong antibacterial action, especially against *Escherichia coli* and *Staphylococcus aureus*. The bacteria under investigation also demonstrated comparable susceptibility to tea tree oil, which was verified by repeating the experiments. TTO's thermal stability and sustained release were enhanced by its encapsulation in PLA-MMT clay, which further resulted in long-lasting antibacterial activity. X-Ray diffraction (XRD) examination of the ultrasonicated samples revealed the combination of oil and glycerol and the sonication method used offered improved clay intercalation outcomes. The presence of clay in the bio nanocomposites limited the evaporation of the antibacterial tea tree oil. Based on the observations, TTO encapsulated in PLA-MMT bio nanocomposites was a promising strategy that requires further research to create antibacterial efficient wound healing materials (Proença, 2020).

In another study, Labib et al., explored chitosan-based topical preparations wound healing capability that is loaded with essential oils *Rosmarinus officinalis* (rosemary) and *Melaleuca alternifolia* (tea tree) in vivo. High concentrations of oxygenated monoterpenes were found in the essential oils after GC-MS analysis. Six groups of rats (Group III: plain (chitosan), Group IV: tea tree oil in chitosan, Group V: rosemary oil in chitosan, and Group VI: tea tree and rosemary essential oils blend (1:1) in chitosan) were used to examine the preparations in vivo using an excision wound model. Delivering chitosan-based topical treatments to the wounds using tea tree oil, rosemary oil, or a combination of the two was one of the techniques. In order to create the essential oil-loaded chitosan (CS) film, a 2% (w/w) viscous CS solution was made in 1% (v/v) acetic acid while being magnetically stirred overnight. A 2% (w/v) Polyvinyl Alcohol (PVA) solution was made then slightly heated and filtered. To create a uniform film-forming solution, the CS and PVA solutions were mixed with glycerol in a 3:1:1 ratio and stirred for an hour. After adding essential oils drop by drop until the concentration reached 10% (v/v), the mixture was stirred for one more hour. After 10 minutes of ultrasonography to eliminate air bubbles, the solution was poured onto Plexiglas plates and allowed to dry for 48 hours at 25 ± 2°C and 50±2% relative humidity. On days 7 and 14

of the experiment, each of the groups' wound areas and the wound healing processes (inflammation, proliferation, and remodeling) were noted and documented. Assessment was done on day 0 as well as days 7, and 14 immediately following the wound. The outcome of wound measurements was expressed as the percentage of wound contraction, which was determined using Wilson's formula, as follows:

$$\%Wound\ Contraction = \frac{¼\ Day\ 0\ wound\ area - Wound\ area\ on\ particular\ day}{Day\ 0\ wound\ area} \times 100\% \quad (2.1)$$

Histopathological analysis revealed full re-epithelialization and activated hair follicles in the combination group, and the loading process of rosemary oil with tea tree into chitosan added significant antioxidants and antiseptic wound healing properties, effectively promoting various stages of wound healing. Ultimately, the films were peeled off, and the results demonstrated that the high percentage of oxygenated monoterpenes in both essential oils played a crucial role and significantly increased wound contraction compared to individual oils and the untreated group (Labib, 2019).

Along those lines, a very recent probe discovered the antibacterial characteristics of a synergistic sustained-release system that uses zinc (II) montmorillonite (Zn-MMT) and chitosan (CS) laden with essential oils particularly tea tree oil. In order to guarantee homogeneity and eliminate air bubbles, the procedure comprised making a 2% CS solution in acetic acid, mixing it with Zn-MMT, and adding tea tree oil using magnetic stirring and ultrasonic treatment. For the creation of antibacterial material, the resultant film-forming solution was cast and allowed to dry. Considering the prolonged release profile that increased its efficacy over time, the results confirmed that the CS/Zn-MMT system loaded with essential oil had strong antibacterial activity against *Escherichia coli*. Pure TTO had an inhibitory zone diameter of 11.8 ± 0.1 mm. Additionally, the antibacterial activity of TTO volatilization led to a considerable reduction in bacterial density. Pure MTT and CS, however, showed little bactericidal activity. Conversely, the inhibition zones of CS-Zn-OM, Zn-OMt, and Na-OMt had diameters of 17.7 ± 0.1 mm, 19.3 ± 0.1 mm, and 20.4 ± 0.1 mm, respectively. The diameter of the inhibition zones clearly increases after the use of montmorillonite-based materials loaded with TTO. The loading process of essential oils into the CS/Zn-MMT matrix added notable antibacterial properties and improved the material's overall performance via inhibiting bacterial growth of *E. coli*. Therefore, the study confirmed

that chitosan-modified montmorillonite composite could be used as a promising drug carrier to provide an effective future reference for the sustained-release delivery of drugs. With regard to these outcomes, this synergistic system of CS-Zn-OMt was successfully created using affordable and eco-friendly materials, and it has potential uses in antibacterial coatings and wound healing said (Zhan, 2023).

In the same way, researchers aimed to develop and analyze bio nanocomposites using chitosan reinforced with montmorillonite (MMT) and essential oil from Rosmarinus officinalis (rosemary essential oil REO). Prior to adding rosemary essential oil to the chitosan/MMT composites in different concentrations (0%, 0.5%, 1%, and 2%), the encapsulation method required preparing chitosan/MMT composites reinforced with various commercial nano clays (Cloisite®Na+, Cloisite®Ca++, and Cloisite®20). The composite's physical properties are then assessed through characterization techniques utilizing XRD, mechanical testing, and other analytical methods. Researchers demonstrated that adding MMT enhanced the composites' mechanical qualities, with natural MMT offering more reinforcement. Additionally, the addition of REO rendered the films opaquer and more color-saturated while decreasing their tensile strength. Along with increased surface hydrophilicity, water solubility, and lower swelling index, the films also exhibited enhanced Ultraviolet (UV) light blocking properties. The study found that adding varying concentrations of REO to chitosan/MMT bio nanocomposites improved their physical characteristics when compared to pure chitosan, which made them appropriate for use as food industry packaging materials (Souza, 2018).

Just as important, Essifi and his team identified a green way to add essential oils (EO) including carvacrol, thyme oil, and thymol to sodium-exchanged montmorillonite (Na+-Mt). Through hydrogen interactions between the EO's OH groups and the clay surface, these EO molecules were adsorbed onto the Na+-Mt surface during the encapsulation process. Provided evidence showed that the release of EO molecules took place above 180°C and that the adsorption had no effect on the Na+-Mt interlayer space. They determined that Na+-Mt delivers a regulated and selective release of EO, preserving their chemical stability and shielding them from external conditions and that this encapsulation approach might have interesting applications in a number of industrial domains (Essifi, 2022).

Additionally, Hammoudi conversely focused on creating and developing films of alginate-montmorillonite nanocomposite that contains lemon essential oil (LEO) for antifungal and antibacterial purposes. Montmorillonite (MMT) nano clay was added to alginate-based films that had been activated with different concentrations of LEO (0.5%, 1%, and 1.5% v/v) as part of the encapsulating process. The films' microstructure, surface morphology, and the strong interactions between alginate and MMT in the presence of LEO were examined using optical microscopy, Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). In contrast, LEO-containing films showed greater breakdown temperatures than pure alginate films, according to thermogravimetric analysis (TGA). Strong antibacterial and antifungal properties were observed in testing against (*Aspergillus niger and Candida albicans*) and (*Escherichia coli, Staphylococcus aureus, Bacillus cereus,* and *Salmonella enteritidis*). Notably, *E. Coli* and *S. enteritidis*, two Gram-negative bacteria, exposed greater resistance to active films among the studied bacterial strains. This can be explained due to their extra protective outer membrane that made them more susceptible to antimicrobial chemicals. Incidentally, the disc diffusion test on Gram-positive bacteria (i.e., *B. cereus, S. aureus*) revealed that the essential oil had the best antibacterial action, with the best activity occurring at a concentration of 1.5% LEO. Also, Aspergillus *niger* was more successfully suppressed by the films than by any other fungus tested. For these reasons, it was concluded that alginate-MMT/LEO nanocomposite films possessed enhanced antimicrobial and antifungal properties, making them promising candidates for active packaging materials in the food industry (Hammoudi, 2020).

Again, another work checked how essential oil components (EOCs) put into montmorillonite nano clay inhibited the growth of *Staphylococcus aureus* and *Aspergillus niger* effectively. By mixing 500 mg of MMT with 100 mg of the chosen substance, the encapsulating procedure was carried out. Upon that, the vials were placed in a vortex and shaken for a full day at 40 °C in an oven. The final powdered nano clay materials, MMT-AITC, MMT-Ca, MMT-Ci, MMT-DD, MMT-Eu, and MMT-Thy, were prepared using the above technique. They were loaded with eugenol, thymol, carvacrol, cinnamaldehyde, diallyl disulphide, and allyl isothiocyanate, correspondingly. Agar-based lab test that simulate surface treatment and microdilution assays to ascertain the minimum inhibitory concentration (MIC) were employed to evaluate the antibacterial properties of these nanomaterials. When compared to their free forms, the encapsulated EOCs exhibited much higher antifungal efficacy against

Aspergillus niger. The MICs of encapsulated EOCs were much lower than those of the free forms, indicating decreased MICs against *Staphylococcus aureus*.

According to the findings, MMT can be utilized for sorption or encapsulation, nonetheless given its hydrophilic character, it is typically employed for polar compounds. While nonpolar compounds, organo modified clays can be used. Hence, they suggested that low-molecular-weight nonpolar volatile chemicals can be encapsulated using non-modified MMT, which is in line with earlier reports of *Ocimum gratissimum* essential oil being encapsulated in polymer-MMT combinations each natural and modified. Putting it all together, EOCs encapsulated in montmorillonite nanoclay exhibited superior antimicrobial properties compared to their pure forms, highlighting their potential as effective antimicrobial agents in various applications (Bernardos, 2019).

On the whole, montmorillonite and other clays are common, more accessible, and inexpensive organic materials. Clays are materials that can be used in various manners. Its microstructure can be altered to meet the needs of chemists based on the intended application, just as it is able to be transformed into any shape. Montmorillonite alone has been used as a suitable live microbe adsorbent. Therefore, a number of quick, easy, and affordable methods can be used to directly modify montmorillonite by antimicrobial compounds. Essential oil encapsulation in MTT clay, modified MTT, and polymer/MTT nanocomposites presents a viable approach to create potent antibacterial agents for use in wound healing as well as food packaging or coating films, insecticide, drug delivery, and broader uses that go beyond wound healing due to their stability, biocompatibility, and controlled release properties (Tunc, 2011), (Pramanik, 2015), (Tornuk, 2015), (Fernández, 2020). The reviewed studies offered compelling proof of these formulation's improved antibacterial capabilities, opening the door for more investigation and potential medical uses.

Table 2.2 illustrates multiple encapsulation processes and their applications.

Table 2.2: Encapsulation Techniques of Mt/EO and Their Uses (de Oliveira, 2022).

Substance	Oils/Oils Components	Usage	Targeted Species	Source
Mt	Curcuma aromatica and Zanthoxylum limonella EO	Antibacterial and insecticide	insect: Aedes albopictus Bacteria: Pseudomonas aeruginosa, and Bacillus subtilis	(Pramanik, 2015)
Na-Mt- Functionalized with lecithin	EO components: Citronellol, citral, and linalool	Antifungal Coatings	Fungi: Chaetomium globosum, Alternaria alternata and Aspergillus fumigatus	(Fernández, 2020)
Bent	EO components: Allyl isothiocyanate, carvacrol, trans-cinnamaldehyde, diallyl disulfide, eugenol, and thymol	Antimicrobial	Fungi: Aspergillus niger and bacteria: Staphylococcus aureus	(Bernardos, 2019)
Nanocomposite Alginate/Mt	Lemon oil		Bacteria: Staphylococcus aureus; Salmonella enteritidis, Escherichia coli and Bacillus cereus. Fungus: Aspergillus baraziliensens, and Candida albicans	(Hammoudi, 2020)
Nanocomposite films based on cellulose acetate butyrate/ modified Mt (Cloisite®30B)	EO components: Carvacrol and cinnamaldehyde		Bacteria: Listeria innocua and Escherichia coli. Fungus: Saccharomyces cerevisiae	(Quintero, 2014)
Nanocomposite films based on LDPE/commercial Mt Dellite®72 T	EO component: Carvacrol		Bacteria: Escherichia coli e Listeria innocua; Fungi: Alternaria alternata	(Shemesh & Goldman, 2015)
Nanocomposite films based on cellulose acetate /AgNP-Ge-Mt	EO component: Thymol		Bacteria: Escherichia coli, Pseudomonas aeruginosa, Salmonella ent´erica, Staphylococcus aureus and Fungi: Aspergillus Niger and Aspergillus Flavus	(Dairi, 2019)
Nanocomposite films based on methylcellulose/Mt	EO component: Carvacrol	Antibacterial	Escherichia coli, and Staphylococcus aureus	(Tunç, 2011)
Nanocomposite LDPE/Mt (Na-Cloisita, Cloisite 10 A Cloisite 15 A, Cloisite 20 A, Nanomer I30P, Nanomer I44PA, and Dellite 72 T.			Escherichia coli	(Shemesh & Krepker, 2015)
Nanocomposite films based on starch /Na-Bent	Cinnamon EO		Escherichia coli, Salmonella typhimurium, and Staphylococcus aureus	(Iamareerat, 2018)

Table 2.2: Encapsulation Techniques of Mt/EO and Their Uses (de Oliveira, 2022) (Table Continued).

Nanocomposite Alginate/Mt	Origanum majorana EO		Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, and Bacillus cereus	(Alboofetileh, 2018)
Nanocomposite chitosan/Mt	Ginger and Rosemary EO		Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa	(Souza, 2019)
Nanocomposite films kappa-carrageenan/Mt (Na-Cloisite)	Zataria multiflora boiss EO		Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhimurium	(Shojaee-Aliabadi, 2014)
Nanocomposite PLA/ commercial Mt Cloisite®30B	EO components: Thymol and Cinnamaldehyde		Escherichia coli and Staphylococcus aureus	(Villegas, 2019)
Alginate/Na-Mt nanocomposite film	Clove, cumin, caraway, marjoram, cinnamon, and coriander EO		Escherichia coli, Staphylococcus aureus, and Listeria monocytogenes	(Alboofetileh, 2014)
Mt	Clausena anisata EO	Insecticide	Acanthoscelides obtectus (Say)	(Ndomo, 2008)
Na-Mt and CTMA-Mt	Ocimum gratissimum EO		Sitophilus zeamais	(Nguemtchouin, 2013)
Nanocomposite Bent/PVP	Syzygium aromaticum EO		Aedes aegypti	(Santos, 2020)
Nanocomposite films LPDE/ commercial Mt Nanomer ® I 0.44 P	EO components: Carvacrol and Thymol	Active films for food packaging (Strawberry)	Fungus: Botrytis cinerea	(Campos-Requena, 2015)
Nanocomposite films starch/ Na-Mt	EO components: Carvacrol and Thymol	Active films for food packaging (Strawberry)	Fungus: Botrytis cinerea	(Campos-Requena, 2017)
Nanocomposite chitosan/Na-Mt	Ginger EO	Active films for food packaging (chicken meat)	Total mesophilic and coliform aerobic bacteria	(Souza &Vieira, 2018)
Nanocomposite Chitosan/Montmorillonits (Mt, Na-Cloisite and Ca-Cloisite)	Rosemary and ginger EO			(Pires, 2018)
Nanocomposite films chitosan/Na-Mt and Org-Mt	Thyme EO		Escherichia coli	(Giannakas, 2020)
Nanocomposite LDPE/Mt and LDPE/Halloysite	EO components: Thymol, Eugenol, and Carvacrol	Active films for food packaging (meat)	Escherichia coli, aerobic mesophilic bacteria, lactic acid bacteria, yeast, molds, and Enterobacteriaceae	(Tornuk, 2015)
Nanocomposite chitosan/Mt	Rosemary EO	fish coating	psychrotrophic bacteria	(Abdollahi, 2014)

3. METHOD

3.1 DATA COLLECTION AND MATERIALS

Montmorillonite clay (purchased from Sigma-Aldrich), Olive oil, safflower oil, tamanu oil, tea tree oil (purchased from Talya herbal products website), Weighing balance (Radwag scales company & Ohaus Scales company), Magnetic stirrer (purchased from ISO Lab), Centrifuge (Nüve – NF 200) & (Hettich, Tuttlingen, Germany), Distilled water, Ultra-pure water (from Rephile Bioscience Ltd water system) FTIR spectrometer (purchased from Shimadzu- IRAffinity-1S) Hydrochloric acid (HCL) (purchased from Edu-Chem), Acetic acid (purchased from Edu-Chem) TPTZ (2,4,6-tri (2-pyridyl)-s-triazine) (purchased from Sigma-Aldrich), Sodium acetate (purchased from Edu-Chem), Iron (III) chloride (purchased from Edu-Chem), ferrous (II) sulphate hepta hydrate (FeSo4-7H2O) (purchased from Edu-Chem), UV spectrometer (purchased from Shimadzu– UV-1280), and Hot water bath (purchased from Nüve– ST 30) Tryptic soy broth (Liofilchem, Roseto degli Abruzzi, Italy), Orbital shaker (Biosan, Riga, Latvia), Plate count agar (Liofilchem), E. coli, ATCC 25922 (Gram-negative bacterium), S. aureus, ATCC 25923 (Gram-positive bacterium).

3.2 PROCEDURE

3.2.1 Encapsulation of Olive Oil

The method used was adapted from the technique described by (Essifi, 2022), and (Bernardos, 2019). 0.5 g of montmorillonite clay weighed using a weighing balance in order to encapsulate olive oil (*Olea europaea*). Concurrently, A graduated cylinder was utilized to measure 20 milliliters of olive oil, which was subsequently transferred to a beaker. The beaker was placed on a magnetic stirrer set to 650 rpm without heating. To ensure thorough mixing, the clay was added to the oil gradually and stirred for 2 hours. The mixture was later centrifuged at 2000 rpm for 5 minutes to separate the clay from the oil. With a spatula, the separated clay was collected and transferred on filter paper placed in a funnel on Erlenmeyer flask. After washing the clay with 10 milliliters of distilled water to get rid of any contaminants and extra oil, it was allowed to air for a day on a clean filter paper as shown in

Figure 3.1. To verify encapsulation by examining functional groups, the dried sample which resembled a paste or chunk of mud was transferred to a falcon tube and assigned for FTIR

analysis. The sole purpose of this experimental step was to find out whether encapsulation could be achieved using these straightforward techniques alone, without the addition of extra chemicals.

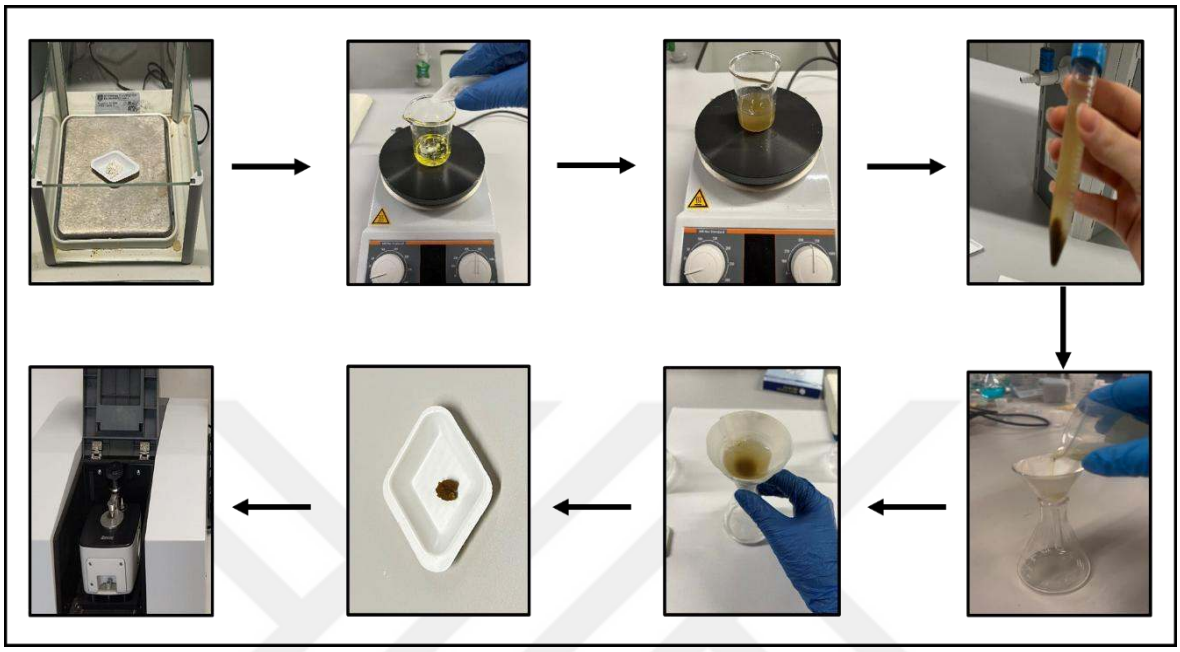

Figure 3.1: Encapsulation Process of Olive Oil *(Olea Europaea)*.

3.2.2 Encapsulation of Tea Tree Oil

For tea tree oil, 2 ml of essential oil was diluted with 24 ml of olive oil (as a carrier oil). Later, the diluted mixture was then combined with 1 g of montmorillonite clay. The encapsulation process followed the same steps as for the other olive oil: stirred on a magnetic stirrer at 650 rpm for 2 hours, centrifuged at 2000 rpm for 5 minutes, washed the separated clay with distilled water, and air dried.

3.2.3 Encapsulation of Safflower Oil

The encapsulation process for safflower oil followed the same steps as for previous ones. 20 milliliters of safflower oil were combined with one gram of montmorillonite clay. Since safflower is a vegetable oil it didn't need to be diluted with a carrier oil. After weighing the clay, the oil was measured and put into a beaker. After stirring the mixture with a magnetic stirrer for 2 hours at 650 rpm and centrifuge it for 5 minutes at 2000 rpm Figure 3.2, the

separate clay was cleaned with distilled water and left for air dry. FTIR was then used to evaluate the dried sample.

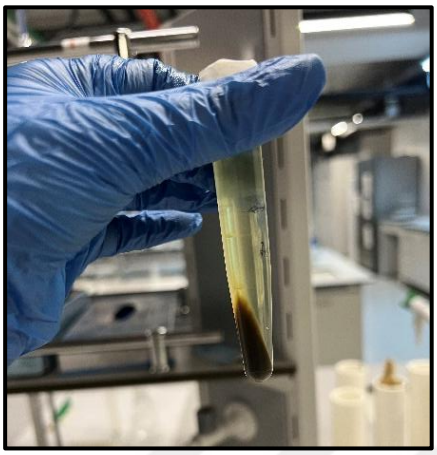

Figure 3.2: Centrifugation of Safflower Oil From MTT Clay at 2000 rpm for 5 Minutes.

3.2.4 Encapsulation of Tamanu Oil

Similarly, to encapsulate tamanu oil, 20 ml was measured using graduated cylinder and added to a beaker. After that, 1 g of montmorillonite clay was weighed and added to the beaker progressively. The combination was stirred on a magnetic stirrer at 650 rpm for 2 hours without heat, centrifuged at 2000 rpm for 5 minutes, and the separated clay was then washed with distilled water and left to dry in room temperature Figure 3.3.

Figure 3.3: Collection of Encapsulated Tamanu Oil/ MTT Clay Sample Left for Air Dry on a Filter Paper.

3.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

An infrared spectrum of a solid, liquid, or gas's absorption or emission can be obtained using the Fourier transform infrared spectroscopy (FTIR) technique. High-resolution spectral data is concurrently collected over a broad spectral range by an FTIR spectrometer. The Fourier Transform Infrared (FTIR) spectroscopy study was performed without apodization and with the measurement mode set to % transmittance. A spectral range of 400 cm^{-1} to 4000 cm^{-1} was covered by 65 scans to guarantee a sufficient signal-to-noise ratio with a resolution of 2 cm^{-1} and all samples were located into the FTIR spectrometer one by one. Subsequently, the spectral data that was visible on the screen was gathered and exported to Excel for examination. The peaks that corresponded to the different functional groups in the samples were found and analyzed to ensure incorporation.

3.4 FERRIC REDUCING ANTIOXIDANT POWER (FRAP ASSAY)

The antioxidant properties of the encapsulated samples were tested using the FRAP assay based on the technique described by (Benzie & Strain, 1996). The FRAP assay measures the ferric reducing ability which is indicative of the antioxidant potential of the samples. Initially, the FRAP solution reagent was prepared by mixing 10ml of pure water and 0.0312 g TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) reagent in solution with 40 mM hydrochloric acid), one volume of 0.054 g Iron (III) chloride hexahydrate, and ten volumes of 0.31 g sodium acetate buffer (pH 3.6) with 1.6g Acetic acid, all prepared in ratio of 1:1:10 and it was incubated for 30 minutes at 37°C, Figure 3.4. Eventually, distilled water was used to dilute solutions of ferrous (II) sulphate hepta hydrate (FeSo4-7H2O) in the range of (25, 50, 100, 200, 400 µM/liter) to get various final concentrations that were used to create the calibration curve to calculate the concentration value of the desired samples Figure 3.6, and the concentration were kept in the dark before measuring their absorbance. Afterward, the samples were mixed with 2ml of FRAP reagent and incubated at 37 °C for 30 minutes. At 593 nm, the absorbance of the standard concentrations and the (Tea tree oil + Olive oil/MMT and Tamanu oil/MMT) samples mixed with FRAP reagent were measured, Figure 3.5, The data obtained were determined and expressed as the FRAP value in µM Fe2+.

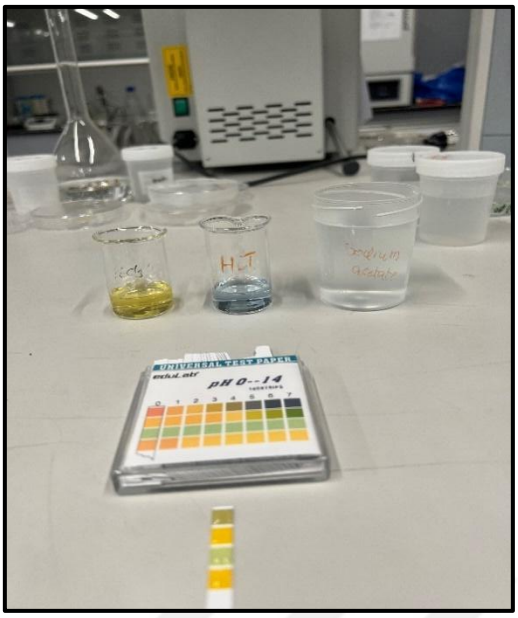

Figure 3.4: Ferric Reducing Antioxidant Power Reagent Preparation.

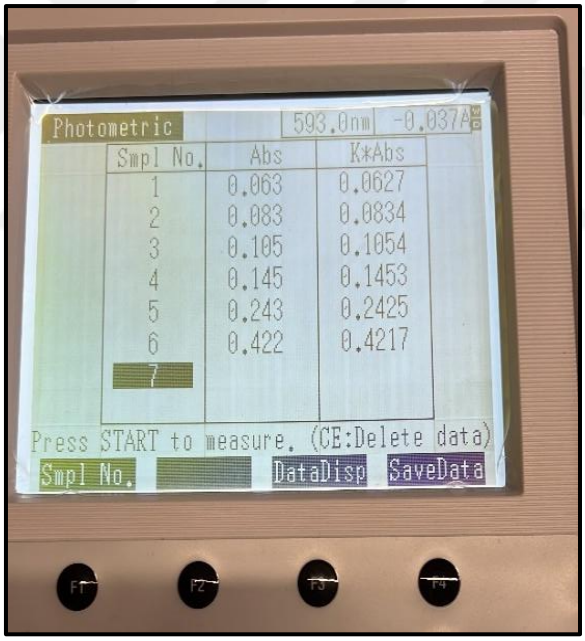

Figure 3.5: Concentrations Prepared Were Measured with UV Spectrometer at 593nm.

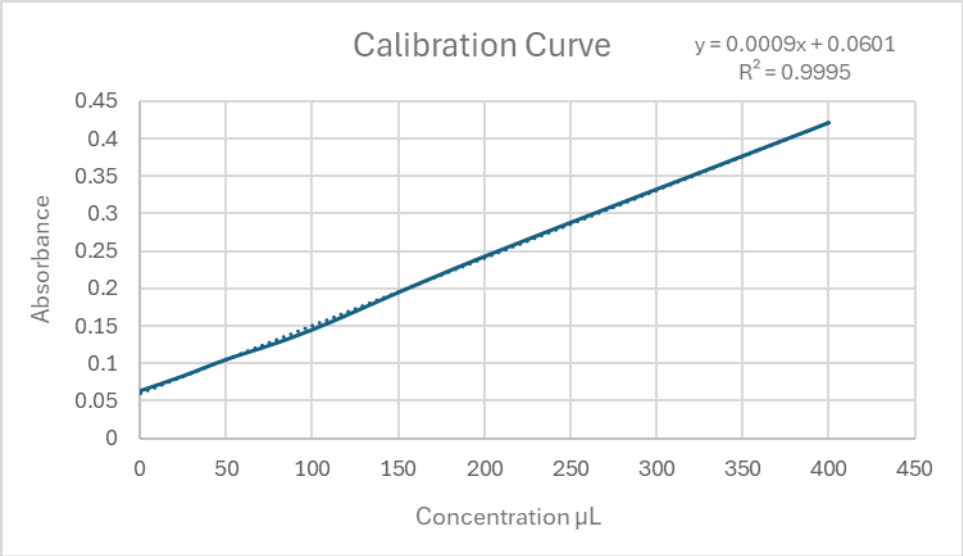

Figure 3.6: FRAP (Ferric Reducing Antioxidant Power) Assay Calibration Curve Expressed in µM Equivalent to FeSO4-7H2O.

Only two samples were assessed primarily for their antioxidant activity that were readily available at this stage: tea tree oil + olive oil/montmorillonite clay, and tamanu oil/montmorillonite clay. Each sample was measured three times to guarantee precision and repeatability. By measuring the samples' absorbance values and using them in the formula generated from the linear calibration curve, the concentrations of the samples were obtained. The antioxidant capacity of each sample was evaluated using its average concentration. Calculations of concentration and average concentration were made as follows:

$$\text{TTO1: } x = \frac{y-0.0601}{0.0009} = \frac{0.129-0.0601}{0.0009} = 76.56\ \mu M \tag{3.1}$$

$$\text{TTO3: } x = \frac{y-0.0601}{0.0009} = \frac{0.133-0.0601}{0.0009} = 81.00\ \mu M \tag{3.2}$$

$$\text{TTO3: } x = \frac{y-0.0601}{0.0009} = \frac{0.221-0.0601}{0.0009} = 178.78\ \mu M \tag{3.3}$$

$$\text{TTO Average } x = \frac{76.56+81.00+178.78}{3} = 112.11 \mu M \tag{3.4}$$

$$\text{TMU1: } x = \frac{y-0.0601}{0.0009} = \frac{0.148-0.0601}{0.0009} = 97.67 \mu M \tag{3.5}$$

$$\text{TMU2: x} = \frac{\text{y}-0.0601}{0.0009} = \frac{0.174-0.0601}{0.0009} = 126.56\ \mu\text{M} \quad (3.6)$$

$$\text{TMU3: x} = \frac{\text{y}-0.0601}{0.0009} = \frac{0.135-0.0601}{0.0009} = 83.22\ \mu\text{M} \quad (3.7)$$

$$\text{TMU Average x} = \frac{97.67+126.56+83.22}{3} = 102.48\ \mu\text{M} \quad (3.8)$$

Where:

TTO is tea tree + olive oil/MTT sample

TMU is tamanu oil/MTT sample.

3.5 ANTIBACTERIAL STANDARD TEST METHOD (ASTM E2149)

The antibacterial effects of the encapsulated samples were evaluated using the ASTM E2149 test or shake flask test. This test assesses the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions. The antibacterial activity of non-leaching, irregularly shaped, or hydrophobic surfaces is frequently measured using this sensitive technique. When antimicrobial surfaces are shaken around in a solution contaminated by microorganisms, it gauges their antibacterial activity. This approach is used on a regular basis since it's one of the few methods for uniformly testing an antimicrobial material with an irregular shape, like thread, powder, or 3D-molded plastic (Campos, 2016), (ASTM E2149, 2025, Microbe investigation Switzerland (mis)).

In this study, the antibacterial activities of the gel samples were evaluated against two microorganisms: *Escherichia coli* (*E. coli*, ATCC 25922, Gram-negative) and *Staphylococcus aureus* (*S. aureus*, ATCC 25923, Gram-positive), using the ASTM E2149 − 25 standard test method for determining the antimicrobial activity of antimicrobial agents under dynamic contact conditions (ASTM E2149, 2021).

Stock cultures of the microorganisms were transferred into Tryptic Soy Broth (Liofilchem, Roseto degli Abruzzi, Italy) and incubated at 35 °C overnight. The resulting cultures were centrifuged (Hettich, Tuttlingen, Germany) at 3600 × g for 10 minutes at 5 °C, washed twice with sterile phosphate buffer (0.3 mM KH_2PO_4, pH 7.2), and resuspended in the same buffer. The cell density of the suspensions was adjusted to the 0.5 McFarland turbidity standard, corresponding to approximately 1.5 × 10^8 colony-forming units (CFU)/mL. These

suspensions were then diluted in phosphate buffer to obtain a working concentration of approximately 1.5×10^5 CFU/mL.

Test portions (1 g) of the gel samples were placed into screw-cap flasks. One flask, containing no sample, served as the "inoculum only" control. Subsequently, 50 mL of the working bacterial suspension was added to each flask. The flasks were placed on an orbital shaker (Biosan, Riga, Latvia) and shaken at 220 RPM for up to 60 minutes.

At contact times of 30 and 60 min, aliquots from each flask were serially diluted and plated onto Plate Count Agar (Liofilchem). The plates were incubated at 35 °C for 24 hours. After incubation, the colonies were counted, and the results were expressed as log CFU/mL.

The antibacterial activity of the samples was expressed as the logarithmic reduction (R) value, calculated using the following equation:

$$R = logB - logA \tag{3.9}$$

where:

a. B is the CFU/mL count from the "inoculum only" control flask at the specified contact time

b. A is the CFU/mL count from the flask containing the test sample at the same contact time

3.6 DATA ANALYSIS TECHNIQUES

With the use of FTIR spectroscopy, the encapsulation efficiency was confirmed. In order to verify the oils' incorporation into the clay matrix, the FTIR spectra were examined to find distinctive peaks that corresponded to the oils' functional groups. The ASTM E2149 test was utilized to examine the antibacterial effects, and the FRAP assay was utilized to investigate the antioxidant capabilities.

3.7 JUSTIFICATION METHODS

Because of clay's high surface area and adsorption capacity, both of which are beneficial for encapsulating bioactive compounds, montmorillonite clay was selected as the encapsulating agent. Through the detection of particular functional groups, FTIR spectroscopy offered a

reliable technique for verifying the encapsulation of oils. The ASTM E2149 test and the FRAP assay were chosen because of their proven accuracy in assessing antibacterial and antioxidant qualities, correspondingly.

3.8 ETHICAL CONSIDERATIONS

This study did not involve human or animal subjects, and therefore, no ethical approval was required. However, all procedures were conducted following standard laboratory safety protocols.

4. RESULTS AND DISCUSSION

4.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The FTIR spectra of montmorillonite clay alone and montmorillonite clay encapsulated with oils were compared using measurements made with Shimadzu IRAffinity-1S. New peaks were seen, signifying successful encapsulation (Figure 4.1, Figure 4.2), Figure 4.2). Additionally, both spectra showed the distinctive montmorillonite clay peak at 1026 cm^{-1}, but no other peaks were observed in pure montmorillonite only. (Adikary. 2012), (Jha, 2019). The observed peak corresponds to the Si–O stretching vibration bonds, one of the most significant bonds between silicon (Si) and oxygen (O). The Si–O stretching vibration bond is a signature feature of silicate minerals. In the infrared spectrum, this vibration usually appears in montmorillonite between 1020 and 1040 cm^{-1}. It aids in recognizing the mineral and comprehending its composition and structure.

On the other hand, encapsulated oil/clay spectra showed the following peaks and shifts when observed with FTIR (Shimanouchi, 1967):

4.1.1 C=C Bonds

When a C=C bond is present in an aromatic ring, Fourier Transform Infrared (FTIR) spectroscopy usually shows a peak at about 1560 cm^{-1}. A peak at 1630 cm^{-1} as well usually indicates a C=C bond in alkene groups (IR Absorption Frequencies - NIU, n.d.).

-C=C double bonds can be found in tea tree oil. C=C double bonds are present in the structures of terpinen-4-ol, γ-terpinene, and α-terpinene, the main components of tea tree oil.

-Compounds with C=C double bonds can be found in tamanu oil as well because of which contains a variety of fatty acids and coumarins mostly extracted from the seeds of the *Calophyllum inophyllum* tree. This also holds true for olive and safflower oils.

4.1.2 C=O Bonds

In Fourier Transform Infrared (FTIR) spectroscopy, a peak at approximately 1743 cm^{-1} usually signifies the presence of a carbonyl group (C=O), which is frequently present in Esters (IR Absorption Frequencies - NIU, n.d.). Carbonyl groups are also found in Aldehydes, Ketones, Carboxylic acids, and Amides.

Tea tree oil may contain carbonyl groups, depending on the specific compounds it has such as “Camphor” which is a ketone or “Citral” an aldehyde.

For tamanu oil, olive and safflower they contain carbonyl groups in their structure due to compounds like triglyceride, linoleic, calophyllolide, xanthones, and fatty acids.

4.1.3 C-H Bonds

A peak around 2845 cm^{-1} and 2924 cm^{-1} in FTIR spectroscopy typically indicates the presence of C-H stretching vibrations in alkanes (IR Absorption Frequencies - NIU, n.d.).

Tea Tree Oil, Safflower Oil, Tamanu Oil, and Olive Oil contain C-H groups, so most probably the peaks around 2845 and 2924 cm^{-1} are indicated related to the oils.

4.1.4 O-H Bonds

A peak around 3623 cm^{-1} in FTIR spectroscopy generally demonstrates the existence of O-H stretching vibrations in alcohols. The stretching vibrations of the hydroxyl group in free (non-hydrogen-bonded) alcohols are linked to this peak (Textmap, 2021).

Tea tree oil encompasses O-H groups since the prime component terpinen-4-ol is alcohol and has a hydroxyl (O-H) group in its structure. As for tamanu oil, it has calophyllolide, a compound with anti-inflammatory properties that contributed to the presence of hydroxyl (O-H).

While safflower oil is abundant in various fatty acids, mainly linoleic acid and oleic acid, these fatty acids contributed to the occurrence of hydroxyl (O-H) groups. Moreover, olive oil primary components also include oleic acid (up to 83%), linoleic acid (up to 21%), and palmitic acid (up to 20%). All these fatty acids contributed to the existence of hydroxyl (O-H) groups in it (Uncu, 2019).

It’s also important to mention that the intensity of the clay's peaks was affected differently by each oil. The FTIR Figure 4.2 clearly indicated the difference in peak strength, which illustrates the interactions between various oils and the clay matrix. These variations in peak intensity offer important information on the nature of the interactions between the clay and the oils as well as the effectiveness of encapsulation (IR Absorption Frequencies - NIU, n.d.).

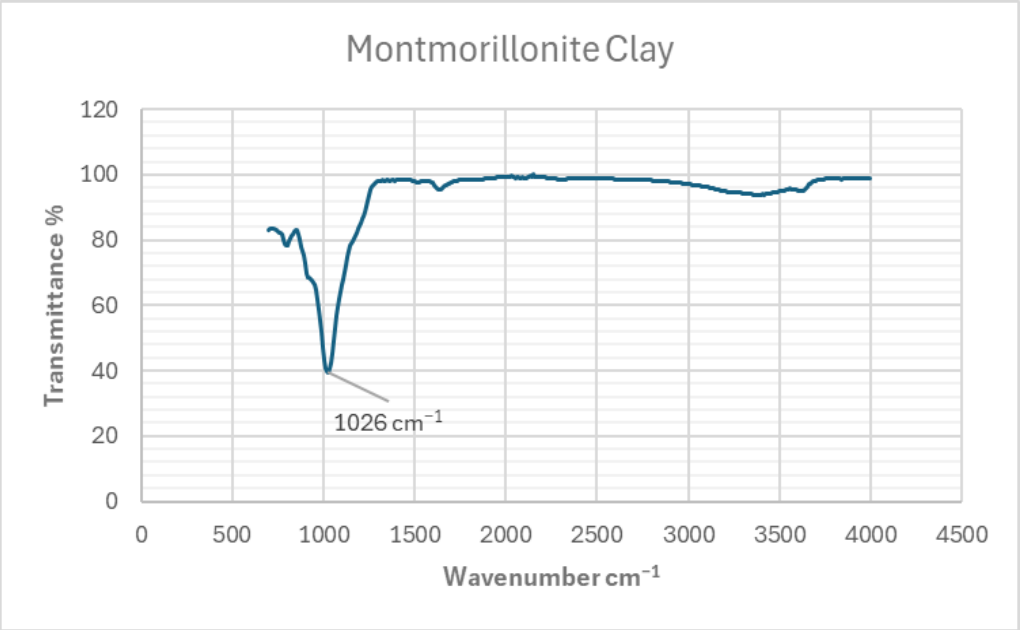

Figure 4.1: Pure Montmorillonite Clay Spectrum.

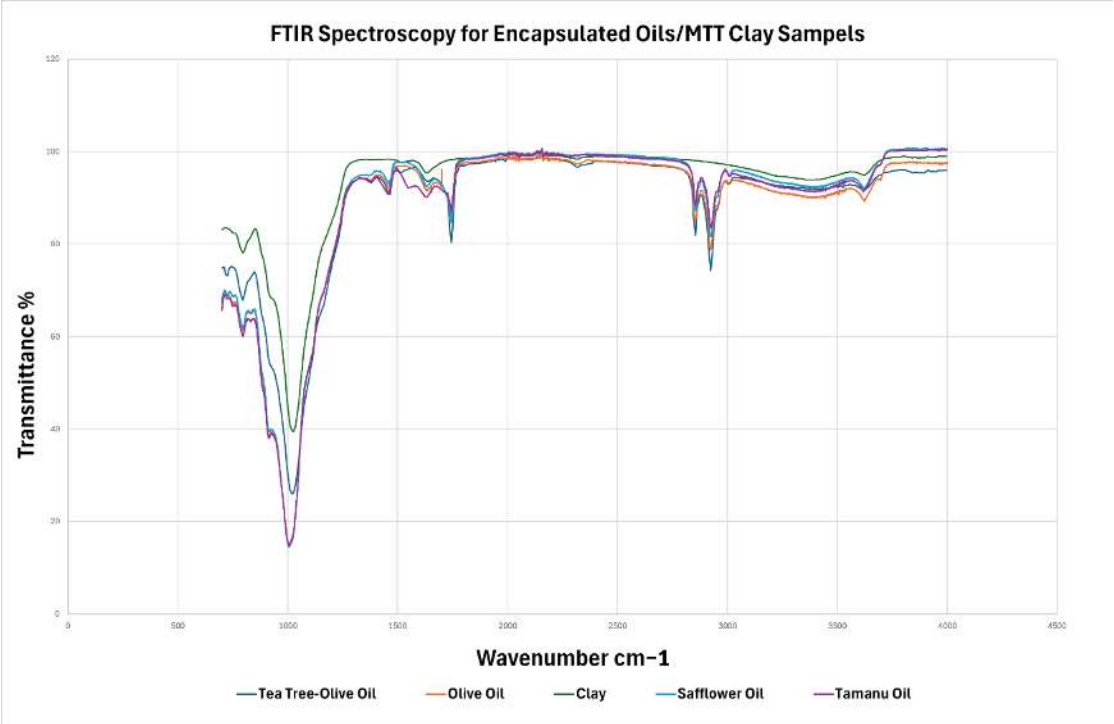

Figure 4.2: Oils Encapsulated Into Montmorillonite Clay Spectrum.

4.2 FERRIC REDUCING ANTIOXIDANT POWER (FRAP ASSAY)

The FRAP assay results were not as promising as predicted, which may be attributed to several factors such as, the FRAP test itself may not be totally appropriate for these types of semi-solid samples. Besides, the little time provided for the sample to mix with the FRAP reagent before measuring could have influenced the release and detection of antioxidant activity. Additionally, other environmental or experimental variations could have influenced the results. To ensure uniformity in the study, it is crucial to mention that the units of my sample's results were changed to correspond with those used in comparative research.

In contrast to tamanu oil's antioxidant activity with another study, the result obtained from the FRAP assay was 102.48 micromolar (μM), which was converted to 0.10248 mmol/fe2+/g for uniformity was significantly lower than the antioxidant activity stated by (Chambon, 2023) where the extracts of *C. inophyllum* leaves and nuts showed 2.89 mmol/fe2+/g and 0.89 mmol/fe2+/g, respectively. However, it's crucial to remember that the sample used in this investigation contained MTT clay, which is different from the liquid extracts utilized in earlier investigations. And that the absorbance was evaluated immediately which could have affected the antioxidant capacity that was recorded.

As for tea tree oil+ olive oil/MTT sample, the result was converted to 0.11211 mmol Fe2+/g and compared with the study figure of (Frangipane, 2023) that was around 0.45 mmol Fe2+/g. This comparison confirmed that the antioxidant capacity obtained is far lower than that of ordinary olive oil sample in the compared study.

Apart from this, the antioxidant activity of olive oil has also been observed by (Wang, 2021). In particular, their findings for the olive oil sample, represented as GL, showed 104.93 μM Fe2+/mg DW as the antioxidant capacity that is greater compared to the achieved outcome of 0.11211 μM Fe2+/mg.

The substantial variation in antioxidant effect draws attention to the variation in antioxidant content between samples and emphasizes the significance of experimental variations, sample preparation, and measurement settings. Figure 4.3, indicates TTO and TMU concentrations in (μM Fe2+).

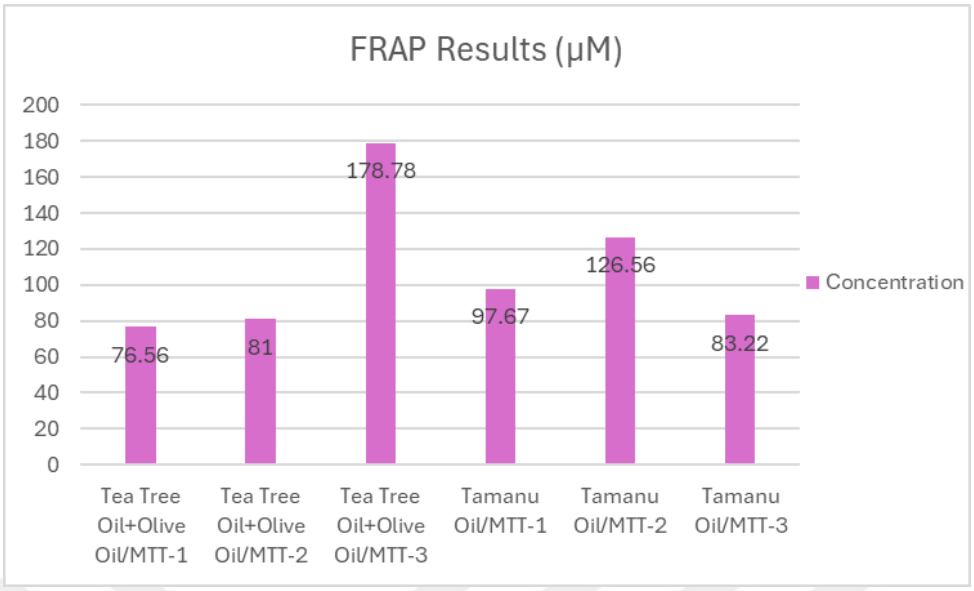

Figure 4.3: FRAP Concentrations Expressed in (µM Fe2+) for Tea Tree Oil & Olive Oil Mix/MTT, and Tamanu Oil/MTT Samples.

4.3 ANTIBACTERIAL TESTING (ASTM E2149 METHOD)

The antimicrobial activity of Oil/MTT Clay samples were evaluated against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 as stated in the method. Log Reduction and % reduction of each sample was calculated using the following formulas:

$$Reduction\ log\ = \ log\ B - log\ A \tag{4.1}$$

$$Reduction(\%)(CFU/mL) = \frac{Initial\ Count - Final\ Count}{Initial\ Count} \times 100 \tag{4.2}$$

4.3.1 *Escherichia Coli*

Tea tree+ olive oil/ Clay sample was possible to reach 0.77 log during 30 min and 2.14 log during 60 min against *E. coli* corresponding to % reduction values to 83.2% and 99.3% respectively which considers the highest comparing to tamanu oil/ Clay sample that was able to reach 0.54 log during 30 min and 1.67 log during 60 min corresponding to 71.4% and 97.9% reduction values respectively. As for safflower oil/ Clay, sample achieved the lowest % reduction values of 40.9% and 83.6% in which we considered very low as seen in (Table 4.1, Table 4.2, Table 4.3) and Figure 4.4

Table 4.1: Tamanu Oil/Clay Sample Against *E. Coli.*

Clay sample with tamanu oil				
30 min	***A***	63000	log ***A***	4.80
	B	220000	log ***B***	5.34
	log ***R***			**0.54**
60 min	***A***	4700	log ***A***	3.67
	B	220000	log ***B***	5.34
	log ***R***			**1.67**

Table 4.2: Safflower Oil/Clay Sample Against *E. Coli.*

Clay sample with safflower oil				
30 min	***A***	130000	log ***A***	5.11
	B	220000	log ***B***	5.34
	log ***R***			**0.23**
60 min	***A***	36000	log ***A***	4.56
	B	220000	log ***B***	5.34
	log ***R***			**0.79**

Table 4.3: Tea Tree+ Olive Oil/Clay Sample Against *E. Coli.*

Gel sample with tea tree oil				
30 min	***A***	37000	log ***A***	4.57
	B	220000	log ***B***	5.34
	log ***R***			**0.77**
60 min	***A***	1600	log ***A***	3.20
	B	220000	log ***B***	5.34
	log ***R***			**2.14**

4.3.2 *Staphylococcus Aureus*

Tea tree+ olive oil/ Clay sample reached 0.67 log during 30 min and 1.59 log during 60 min corresponding to 78.4% and 97.4% respectively which again considers the highest value. Whereas safflower oil/ Clay, sample achieved 0.47 log during 30 min and 1.29 log during 60 min corresponding to 66.3% and 94.9% reduction values which considered slightly higher than tamanu oil/ Clay sample that was capable to reach 0.42 log and 1.05 log corresponding to 62.1% and 91.1% reduction values that was assigned as the lowest against *S. aureus* bacteria strain as seen in (Table 4.4, Table 4.5, Table 4.6) and Figure 4.5 and which might refer to, some bacteria could be more resistant to some agents and more susceptible to other agents.

Table 4.4: Tamanu Oil/Clay Sample Against *S. Aureus.*

Gel sample with tamanu oil				
30 min	***A***	72000	log ***A***	4.86
	B	190000	log ***B***	5.28
	log ***R***			**0.42**
60 min	***A***	17000	log ***A***	4.23
	B	190000	log ***B***	5.28
	log ***R***			**1.05**

Table 4.5: Safflower Oil/Clay Sample Against *S. Aureus.*

Gel sample with safflower oil				
30 min	***A***	64000	log ***A***	4.81
	B	190000	log ***B***	5.28
	log ***R***			**0.47**
60 min	***A***	9700	log ***A***	3.99
	B	190000	log ***B***	5.28
	log ***R***			**1.29**

Table 4.6: Tea Tree+ Olive Oil/Clay Sample Against *S. Aureus.*

Gel sample with tea tree oil				
30 min	***A***	41000	log ***A***	4.61
	B	190000	log ***B***	5.28
	log ***R***			**0.67**
60 min	***A***	4900	log ***A***	3.69
	B	190000	log ***B***	5.28
	log ***R***			**1.59**

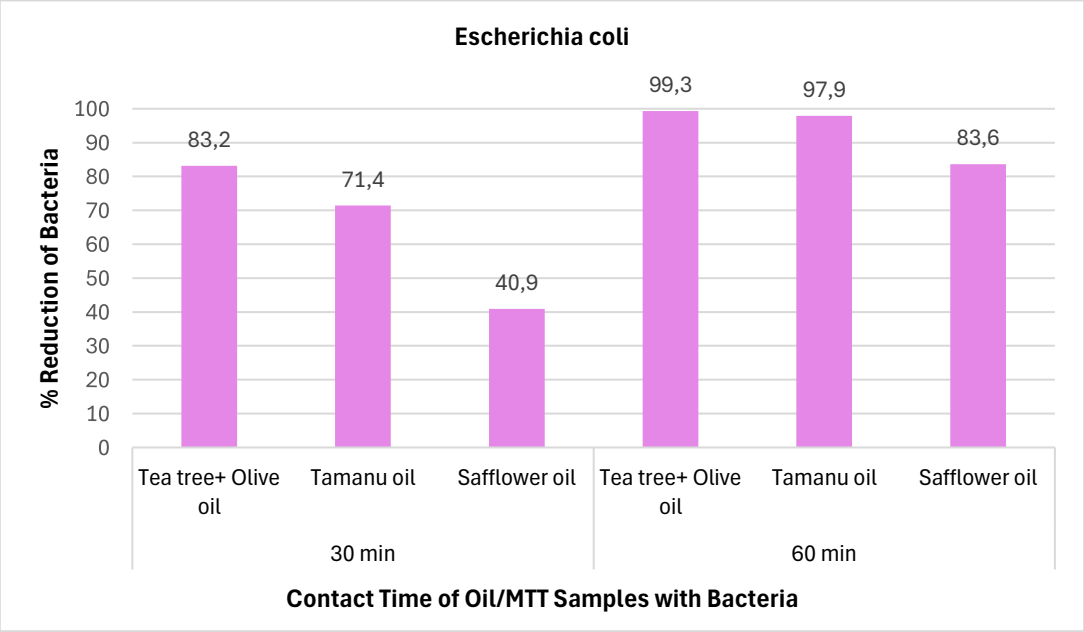

Figure 4.4: Samples %Reduction Values During 30 and 60 Minutes Against *E. Coli.*

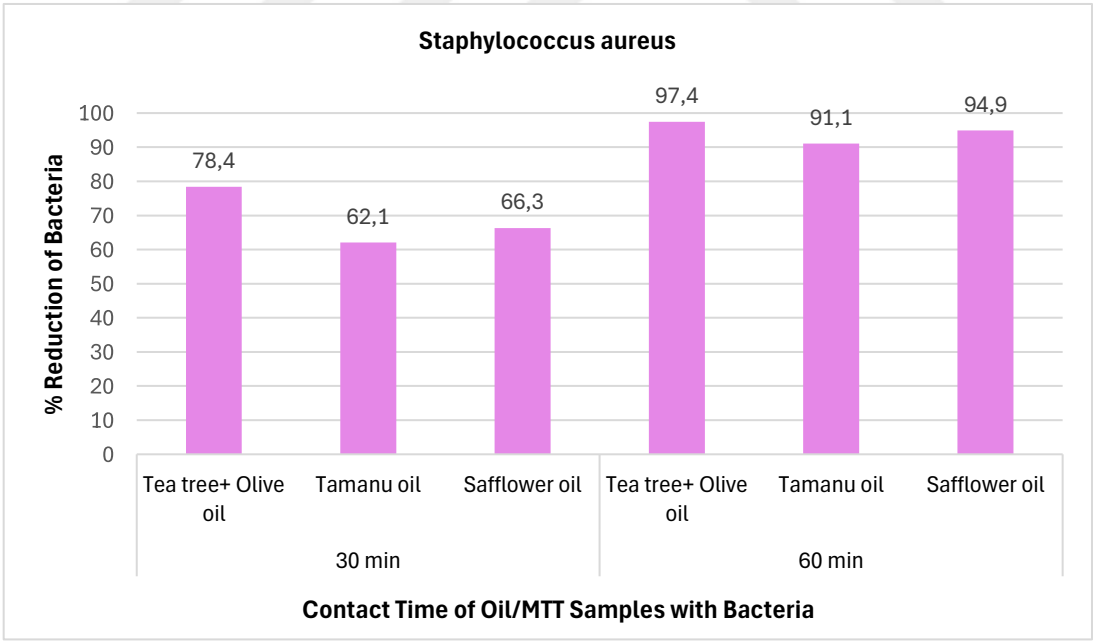

Figure 4.5: Samples %Reduction Values During 30 and 60 Minutes Against *S. Aureus.*

Generally, according to scientific studies, a log reduction of 3 or higher (reduction percentage of 99.9% or higher) is usually considered to indicate strong antibacterial activity as reported based on the various research studies (Bruna, 2015). However, the interpretation of antibacterial effectiveness can vary depending on the context of the study, the type of bacteria being tested, type of the sample used, the specific goals of the research, etc.

In this study, achieving these values with natural ingredients and simple steps is considered a positive outcome. In other research studies, some data have been interpreted as indicating significant antibacterial activity, while others have assessed them as poor (Pankey, 2004), (Savas, 2015), (Guzińska, 2018), (Harun, 2018), (Ristić, 2019). Specifically, we can consider a reduction percentage of 70-90%, which inhibits bacterial growth, as bacteriostatic. While the lowest drug concentration required to achieve a reduction value of (≥99.9%) for a predetermined period of time (typically 24 hours) under specific circumstances is classified as bactericidal, effectively killing the bacteria (Barry, 1999).

Thus, it is important to note that the contact time between the samples and the bacteria in this research was 30 and 60 minutes; extending this duration could potentially yield even better results. Additionally, the concentration/amount of the antimicrobial agent used, conditions/environment under which the test is conducted, and high baseline bacterial count might also influence the results. Therefore, in some cases, a 70% reduction might still represent a substantial decrease in bacterial load and hence might be seen as a strong effect.

Therefore, the results indicated that tea tree + olive oil and safflower oil samples showed promising antibacterial properties against Gram-positive and -negative bacteria. For the case of tamanu oil findings, they showed good antibacterial activity against Gram-negative bacteria (*E. coli*) than in Gram-positive (*S. aureus*).

4.4 LIMITATIONS

Potential limitations of this study include the variability in the encapsulation efficiency due to differences in the physicochemical properties of the oils. Additionally, the study did not evaluate the long-term stability of the encapsulated mixtures, which would require further investigation.

4.5 DISCUSSION

The combination of oil and clay is believed to create a synergistic effect that holds promise for medical applications as a promising treatment. According to the Fourier-transform infrared spectroscopy (FTIR) results, this research has confirmed that encapsulating oils in a clay matrix can be accomplished through a straightforward technique devoid of the inclusion of chemical additions. Although the detected antioxidant activity is relatively low, it nonetheless exhibits a measurable degree of efficacy. This activity could possibly be augmented through the improvement of procedural precision, the expansion of sample amounts, or the prolongation of mixing durations with FRAP reagent prior to assessment. On top of that, the individual measurement of each constituent (oils, clay, and their combination) may yield stronger outcomes and enable comparative analysis. Alternatively, the application of different antioxidant assays could more successfully expose the antioxidant properties. It's unclear how antibacterial activity was observed in all samples, even though the reduction percentages were not uniformly high. Remarkably, the tea tree + olive oil/clay sample displayed significant antibacterial effects, with reduction percentages of 99.3% for *E. coli* and 97.4% for *S. aureus* over 60 minutes, respectively. These results indicate a strong bactericidal effect, particularly in the tea tree + olive oil/clay sample. It is necessary to consider that prolonging the interaction time between the samples and the microorganism could potentially improve the antibacterial activity further, leading to even more noticeable declines in bacterial counts.

Bactericidal/or bacteriostatic properties of the clay-oil samples can be of use in different fields of application. Most commercial topical applications are intended to be broad-spectrum, that are able to target both Gram-positive and Gram-negative bacteria. However, they have a tendency to be more effective against Gram-positive bacteria. Interestingly, tamanu oil and tea tree+ olive samples showed better activity against Gram-negative bacteria than safflower oil, which can likely be related to the active compound present inside and hence can be of use in further specific applications. Although tamanu oil is generally more effective against Gram-positive bacteria, the calophyllolide and inophyllums it contains, might contribute to this activity, while terpinen-4-ol and polyphenols from tea tree and olive oil might be the key ingredients that eradicated Gram-negative bacteria. These compounds can disrupt bacterial cell walls or interfere with essential bacterial processes. Examples of

Gram-negative bacteria are the ones in *Vibr*io species. Although rare, *Vibrio vulnificus* are commonly known as flesh-eating bacteria and have significant mortality rates when the wound is infected. Although studies of tea tree oil and tamanu oil against this type of bacteria are very limited or almost not presented, a study in 2020 showed the effect of a developed agent derived from tea tree oil on different kinds of pathogens responsible for skin and soft tissue infections, including *Vibrio vulnificus* in which further research may be necessary to approve this effect (Johansen, 2020).

Despite natural antibacterial characteristics of oils/clay models, it is necessary to conduct additional trials on wound or artificial models, coupled with more comprehensive investigations, to determine the viability of this combination in the treatment of infectious wounds.

5. CONCLUSIONS

This study demonstrates the encapsulation of tea tree and olive oil, tamanu oil and safflower oil in montmorillonite clay powder. Resulting mixture has a paste-like appearance, hence can be used on surfaces or skin. Efficiency against Gram-positive and Gram-negative bacteria are important for different fields of pharmaceutical and biomedical applications. As a conclusion, this method provides efficient encapsulation of different oils on clay powder with good antibacterial properties. Due to its strong potential, this encapsulated material can be used in further applications such as wound dressing and controlled release agents. For instance, if a drug delivery device has an antibacterial property it can be efficient in topical applications.

REFERENCES

Abdollahi, M., Rezaei, M., & Farzi, G. (2014). Influence of chitosan/clay functional bionanocomposite activated with rosemary essential oil on the shelf life of fresh silver carp. International journal of food science & technology, 49(3), 811-818. https://doi.org/10.1111/ijfs.12369

Abuova, Z., Turgumbayeva, A., Jumagaziyeva, A., Rakhimov, K., & Jussupkaliyeva, A. (2022). Study of component composition and antimicrobial activity of the ophthalmic emulsion based on the safflower flowers (Carthamus tinctorius L.). International Journal of Microbiology, 2022(1), 3181270. https://doi.org/10.1155/2022/3181270

Adikary, S. U., & Wanasinghe, D. D. (2012). Characterization of locally available Montmorillonite clay using FTIR technique. Annual Transactions of Institution of Engineers Sri Lanka, 1(part B), 140-145.

Alboofetileh, M., Rezaei, M., Hosseini, H., & Abdollahi, M. (2014). Antimicrobial activity of alginate/clay nanocomposite films enriched with essential oils against three common foodborne pathogens. Food control, 36(1), 1-7. https://doi.org/10.1016/j.foodcont.2013.07.037

Alboofetileh, M., Rezaei, M., Hosseini, H., & Abdollahi, M. (2018). Morphological, physico-mechanical, and antimicrobial properties of sodium alginate-montmorillonite nanocomposite films incorporated with marjoram essential oil. Journal of food processing and preservation, 42(5), e13596. https://doi.org/10.1111/jfpp.13596

ASTM E2149. (2025). Swiss Anti-Bacterial & Anti-Viral Testing Laboratory, ISO Certified.

ASTM E2149-20. (2021). Standard test method for determining the antimicrobial activity of antimicrobial agents under dynamic contact conditions.

Barry, A. L., Craig, W. A., Nadler, H., Reller, L. B., Sanders, C. C., & Swenson, J. M. (1999). Methods for determining bactericidal activity of antimicrobial agents. Approved guidelines,19,1-3. Clinical and Laboratory Standards Institute.

Benkhaira, N., Koraichi, S. I., & Fikri-Benbrahim, K. (2022). In vitro methods to study antioxidant and some biological activities of essential oils: a review. Biointerface Res. Appl. Chem, 12(3), 3332. https://doi.org/10.33263/BRIAC123.33323347

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry, 239(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Bernardos, A., Bozik, M., Alvarez, S., Saskova, M., Perez-Esteve, E., Kloucek, P., ... & Martinez-Manez, R. (2019). The efficacy of essential oil components loaded into montmorillonite against Aspergillus niger and Staphylococcus aureus. Flavour and Fragrance Journal, 34(3), 151-162. https://doi.org/10.1002/ffj.3488

Bruna, J. E., Quilodrán, H., Guarda, A., Rodríguez, F., Galotto, M. J., & Figueroa, P. (2015). Development of antibacterial MtCu/PLA nanocomposites by casting method for potential use in food packaging. Journal of the Chilean Chemical Society, 60(3), 3009-3014. http://dx.doi.org/10.4067/S0717-97072015000300007

Campos, M. D., Zucchi, P. C., Phung, A., Leonard, S. N., & Hirsch, E. B. (2016). The activity of antimicrobial surfaces varies by testing protocol utilized. PLoS One, 11(8), e0160728. https://doi.org/10.1371/journal.pone.0160728

Campos-Requena, V. H., Rivas, B. L., Pérez, M. A., Figueroa, C. R., & Sanfuentes, E. A. (2015). The synergistic antimicrobial effect of carvacrol and thymol in clay/polymer nanocomposite films over strawberry gray mold. LWT-Food Science and Technology, 64(1), 390-396. https://doi.org/10.1016/j.lwt.2015.06.006

Campos-Requena, V. H., Rivas, B. L., Pérez, M. A., Figueroa, C. R., Figueroa, N. E., & Sanfuentes, E. A. (2017). Thermoplastic starch/clay nanocomposites loaded with essential oil constituents as packaging for strawberries− In vivo antimicrobial synergy over Botrytis cinerea. Postharvest Biology and Technology, 129, 29-36. https://doi.org/10.1016/j.postharvbio.2017.03.005

Carson, C. F., Hammer, K. A., & Riley, T. V. (2006). Melaleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties. Clinical microbiology reviews, 19(1), 50-62. https://doi.org/10.1128/cmr.19.1.50-62.2006

Carson, C. F., Mee, B. J., & Riley, T. V. (2002). Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrobial agents and chemotherapy, 46(6), 1914-1920. https://doi.org/10.1128/aac.46.6.1914-1920.2002

Cassien, M., Mercier, A., Thétiot-Laurent, S., Culcasi, M., Ricquebourg, E., Asteian, A., ... & Pietri, S. (2021). Improving the antioxidant properties of Calophyllum inophyllum seed oil from French Polynesia: development and biological applications of resinous ethanol-soluble extracts. Antioxidants, 10(2), 199. https://doi.org/10.3390/antiox10020199

Chambon, M., Ho, R., Baghdikian, B., Herbette, G., Bun-Llopet, S. S., Garayev, E., & Raharivelomanana, P. (2023). Identification of antioxidant metabolites from five plants (Calophyllum inophyllum, Gardenia taitensis, Curcuma longa, Cordia subcordata, Ficus prolixa) of the polynesian pharmacopoeia and cosmetopoeia for skin care. Antioxidants, 12(10), 1870. https://doi.org/10.3390/antiox12101870

Chin, K. B., & Cordell, B. (2013). The effect of tea tree oil (Melaleuca alternifolia) on wound healing using a dressing model. The Journal of Alternative and Complementary Medicine, 19(12), 942-945. https://doi.org/10.1089/acm.2012.0787

Choudhary, M., Verma, V., Saran, R., Bhagyawant, S. S., & Srivastava, N. (2022). Natural biosurfactant as antimicrobial agent: strategy to action against fungal and bacterial activities. Cell Biochemistry and Biophysics, 80(1), 245-259. https://doi.org/10.1007/s12013-021-01045-1

Cox, Gustafson, Mann, Markham, Liew, Hartland, ... & Wyllie. (1998). Tea tree oil causes K+ leakage and inhibits respiration in Escherichia coli. Letters in applied microbiology, 26(5), 355-358. https://doi.org/10.1046/j.1472-765X.1998.00348.x

Cox, S. D., Mann, C. M., Markham, J. L., Bell, H. C., Gustafson, J. E., Warmington, J. R., & Wyllie, S. G. (2000). The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). Journal of applied microbiology, 88(1), 170-175. https://doi.org/10.1046/j.1365-2672.2000.00943.x

Dairi, N., Ferfera-Harrar, H., Ramos, M., & Garrigós, M. C. (2019). Cellulose acetate/AgNPs-organoclay and/or thymol nano-biocomposite films with combined antimicrobial/antioxidant properties for active food packaging use. International Journal of Biological Macromolecules, 121, 508-523. https://doi.org/10.1016/j.ijbiomac.2018.10.042

de Oliveira Soares, F., Bizinoto, L. B., de Quadros, E. A., Rosado, I. R., Martin, I., de Rezende, R. S., ... & Alves, E. G. L. (2024). Use of vegetable oils in wound treatment. https://doi.org/10.53660/CLM-3032-24E13

de Oliveira, L. H., Trigueiro, P., Souza, J. S. N., de Carvalho, M. S., Osajima, J. A., da Silva-Filho, E. C., & Fonseca, M. G. (2022). Montmorillonite with essential oils as antimicrobial agents, packaging, repellents, and insecticides: An overview. Colloids and Surfaces B: Biointerfaces, 209, 112186. https://doi.org/10.1016/j.colsurfb.2021.112186

Delshad, E., Yousefi, M., Sasannezhad, P., Rakhshandeh, H., & Ayati, Z. (2018). Medical uses of Carthamus tinctorius L. (Safflower): a comprehensive review from traditional medicine to modern medicine. Electronic physician, 10(4), 6672. https://doi.org/10.19082/6672

Emmerich, K., Wolters, F., Kahr, G., & Lagaly, G. (2009). Clay profiling: the classification of montmorillonites. Clays and clay minerals, 57(1), 104-114. https://doi.org/10.1346/CCMN.2009.0570110

Erosh Yadav, Sunil Kumar, Sheefali Mahant, Sarita Khatkar & Rekha Rao, (2017) Tea tree oil: a promising essential oil, Journal of Essential Oil. https://doi.org/10.1080/10412905.2016.1232665

Essifi, K., Hammani, A., Berraaouan, D., El Bachiri, A., Fauconnier, M. L., & Tahani, A. (2022). Montmorillonite nanoclay based formulation for controlled and selective release of volatile essential oil compounds. Materials chemistry and physics, 277, 125569. https://doi.org/10.1016/j.matchemphys.2021.125569

Fernández, M. A., Roque, L. B., Espinosa, E. G., Deyá, C., & Bellotti, N. (2020). Organo-montmorillonite with biogenic compounds to be applied in antifungal coatings. Applied Clay Science, 184, 105369. https://doi.org/10.1016/j.clay.2019.105369

Frangipane, M. T., Costantini, L., Merendino, N., & Massantini, R. (2023). Antioxidant profile and sensory analysis in olive oils of different quality grades. Agriculture, 13(5), 993. https://doi.org/10.3390/agriculture13050993

García-Villén, F., Faccendini, A., Aguzzi, C., Cerezo, P., Bonferoni, M. C., Rossi, S., ... & Viseras, C. (2019). Montmorillonite-norfloxacin nanocomposite intended for healing of infected wounds. International journal of nanomedicine, 5051-5060. https://doi.org/10.2147/IJN.S208713

Giannakas, A., Stathopoulou, P., Tsiamis, G., & Salmas, C. (2020). The effect of different preparation methods on the development of chitosan/thyme oil/montmorillonite nanocomposite active packaging films. Journal of Food Processing and Preservation, 44(2), e14327. https://doi.org/10.1111/jfpp.14327

Guo, L., Gong, S., Wang, Y., Sun, Q., Duo, K., & Fei, P. (2020). Antibacterial activity of olive oil polyphenol extract against Salmonella Typhimurium and Staphylococcus aureus: Possible Mechanisms. Foodborne pathogens and disease, 17(6), 396-403. https://doi.org/10.1089/fpd.2019.2713

Gustafson, Liew, Chew, Markham, Wyllie, & Warmington. (1998). Effects of tea tree oil on Escherichia coli. Letters in applied microbiology, 26(3), 194-198. https://doi.org/10.1046/j.1472-765X.1998.00317.x

Guzińska, K., Kaźmierczak, D., Dymel, M., Pabjańczyk-Wlazło, E., & Boguń, M. (2018). Anti-bacterial materials based on hyaluronic acid: Selection of research methodology and analysis of their anti-bacterial properties. Materials Science and Engineering: C, 93, 800-808. https://doi.org/10.1016/j.msec.2018.08.043

Hammoudi, N., Ziani Cherif, H., Borsali, F., Benmansour, K., & Meghezzi, A. (2020). Preparation of active antimicrobial and antifungal alginate-montmorillonite/lemon essential oil nanocomposite films. Materials Technology, 35(7), 383-394. https://doi.org/10.1080/10667857.2019.1685292

Harun, N. H., Mydin, R. B. S., Sreekantan, S., Saharudin, K. A., Basiron, N., Radhi, F., & Seeni, A. (2018). Shape-Dependent Antibacterial Activity against Staphylococcus aureus of Zinc Oxide Nanoparticles. Malaysian Journal of Medicine & Health Sciences, 14.

Iamareerat, B., Singh, M., Sadiq, M. B., & Anal, A. K. (2018). Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material. Journal of food science and technology, 55, 1953-1959. https://doi.org/10.1007/s13197-018-3100-7emm

IR Absorption Frequencies - NIU - Department of Chemistry and Biochemistry. (n.d.). Northern Illinois University. https://www.niu.edu/clas/chembio/research/analytical-lab/ftir/ir-frequencies-table.shtml

Jemai, R., Chalghaf, R., Boubakri, S., Djebbi, M. A., Naamen, S., Rhaiem, H. B., & Amara, A. B. H. (2024). Montmorillonite: Properties, Characteristics, and Its Harnessing in Environmental Applications. In Recent Advances in Montmorillonite. IntechOpen. https://doi.org/10.5772/intechopen.1004763

Jha, A. K., & Majumder, S. (2019). Characterization of Montmorillonite minerals. International Journal of Emerging Technologies and Innovative Research. 6(3), 585. https://doi.org/10.1729/Journal.22282

Johansen, B., Duval, R. E., & Sergere, J. C. (2020). Antimicrobial spectrum of Titroleane™: a new potent anti-infective agent. Antibiotics, 9(7), 391. https://doi.org/10.3390/antibiotics9070391

Karimkhani, M. M., Shaddel, R., Khodaparast, M. H. H., Vazirian, M., & Piri-Gheshlaghi, S. (2016). Antioxidant and antibacterial activity of safflower (Carthamus tinctorius L.) extract from four different cultivars. Quality assurance and safety of crops & foods, 8(4), 565-574. https://doi.org/10.3920/QAS2015.0676

Khémiri, I., Essghaier, B., Sadfi-Zouaoui, N., & Bitri, L. (2020). Antioxidant and antimicrobial potentials of seed oil from Carthamus tinctorius L. in the management of skin injuries. Oxidative Medicine and Cellular Longevity, 2020(1), 4103418. https://doi.org/10.1155/2020/4103418

Labib, R. M., Ayoub, I. M., Michel, H. E., Mehanny, M., Kamil, V., Hany, M., ... & Mohamed, A. (2019). Appraisal on the wound healing potential of Melaleuca alternifolia and Rosmarinus officinalis L. essential oil-loaded chitosan topical preparations. PloS one, 14(9), e0219561. https://doi.org/10.1371/journal.pone.0219561

Leguillier, T., Lecsö-Bornet, M., Lemus, C., Rousseau-Ralliard, D., Lebouvier, N., Hnawia, E., ... & Rat, P. (2015). The wound healing and antibacterial activity of five ethnomedical Calophyllum inophyllum oils: an alternative therapeutic strategy to treat infected wounds. PloS one, 10(9), e0138602. https://doi.org/10.1371/journal.pone.0138602

Ling, Y., Luo, Y., Luo, J., Wang, X., & Sun, R. (2013). Novel antibacterial paper based on quaternized carboxymethyl chitosan/organic montmorillonite/Ag NP nanocomposites. Industrial Crops and Products, 51, 470-479. https://doi.org/10.1016/j.indcrop.2013.09.040

Magana, S. M., Quintana, P., Aguilar, D. H., Toledo, J. A., Angeles-Chavez, C., Cortés, M. A., ... & Sánchez, R. T. (2008). Antibacterial activity of montmorillonites modified with silver. Journal of Molecular Catalysis A: Chemical, 281(1-2), 192-199. https://doi.org/10.1016/j.molcata.2007.10.024

Makiej, A., Hochór, Z., Smułek, W., & Kaczorek, E. (2023). The bioactivity and physicochemical properties of emulsions based on tamanu, Moringa, and inca inchi oils. Foods, 13(1), 62. https://doi.org/10.3390/foods13010062

Melguizo-Rodríguez, L., Illescas-Montes, R., Costela-Ruiz, V. J., Ramos-Torrecillas, J., de Luna-Bertos, E., García-Martínez, O., & Ruiz, C. (2021). Antimicrobial properties of olive oil phenolic compounds and their regenerative capacity towards fibroblast cells. Journal of Tissue Viability, 30(3), 372-378. https://doi.org/10.1016/j.jtv.2021.03.003

Mikulec, K. (2022, July 15). *How to use tea tree oil: 8 Uses, dilution, application, and risks*. Derm Collective.https://dermcollective.com/how-to-use-tea-tree-oil/

Mokhtar, A., Ahmed, A. B., Asli, B., Boukoussa, B., Hachemaoui, M., Sassi, M., & Abboud, M. (2023). Recent advances in antibacterial metallic species supported on montmorillonite clay mineral: a review. Minerals, 13(10), 1268. https://doi.org/10.3390/min13101268

Mumu, S. K., & Hossain, M. M. (2018). Antimicrobial activity of tea tree oil against pathogenic bacteria and comparison of its effectiveness with eucalyptus oil, lemongrass oil and conventional antibiotics. American Journal of Microbiological Research, 6(3), 73-78. https://doi.org/10.12691/ajmr-6-3-2

Nazzaro, F., Fratianni, F., Cozzolino, R., Martignetti, A., Malorni, L., De Feo, V., ... & d'Acierno, A. (2019). Antibacterial activity of three extra virgin olive oils of the Campania region, Southern Italy, related to their polyphenol content and composition. Microorganisms, 7(9), 321. https://doi.org/10.3390/microorganisms7090321

Ndomo, A. F., Ngamo, L. T., Tapondjou, L. A., Tchouanguep, F. M., & Hance, T. (2008). Insecticidal effects of the powdery formulation based on clay and essential oil from the leaves of Clausena anisata (Willd.) JD Hook ex. Benth. (Rutaceae) against Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae). Journal of pest science, 81, 227-234. https://doi.org/10.1007/s10340-008-0211-3

Nguemtchouin, M. G., Ngassoum, M. B., Chalier, P., Kamga, R., Ngamo, L. S., & Cretin, M. (2013). Ocimum gratissimum essential oil and modified montmorillonite clay, a means of controlling insect pests in stored products. Journal of Stored Products Research, 52, 57-62. https://doi.org/10.1016/j.jspr.2012.09.006

Nguyen, H. H., & Tran, T. T. M. (2016). Chemical composition analysis and antibacterial-antiinflammatory activity tests of tamanu seed oil extracted by supercritcial fluid technology. Science and Technology Development Journal, 19(3), 146-154. https://doi.org/10.32508/stdj.v19i3.573

Ohashi, F., & Oya, A. (1992). Antimicrobial and antifungal agents derived from clay minerals. Journal of Materials Science, 27, 5027-5030. https://doi.org/10.1007/BF01105269

Ovincy, C., Babel, S., Baral, S., Poudel, S., & Jain, S. (2024). Clay Therapy in Wound Healing: A Brief Review of the Literature. Journal of Wound Management and Research, 20(1), 1-8. https://doi.org/10.22467/jwmr.2023.02558

Ozkan, K., Bekiroglu, H., Bayram, Y., Sagdic, O., & Erbas, S. (2021). In vitro bioaccessibility, antioxidant and antibacterial activities of three different safflower (Carthamus tinctorius L.) genotypes. Food Science and Technology, 42, e08921. https://doi.org/10.1590/fst.08921

Pankey, G. A., & Sabath, L. D. (2004). Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. Clinical infectious diseases, 38(6), 864-870. https://doi.org/10.1086/381972

Parviz, G., Kosar, M., & Demirci, F. (2022). Tea Tree (Melaleuca alternifolia (Maiden & Betche) Cheel) Oil an important medicinal essential oil. EMU Journal of Pharmaceutical Sciences, 5(1), 57-74. https://doi.org/10.54994/emujpharmsci.1030526

Pazyar, N., Yaghoobi, R., Bagherani, N., & Kazerouni, A. (2013). A review of applications of tea tree oil in dermatology. International journal of dermatology, 52(7), 784-790. https://doi.org/10.1111/j.1365-4632.2012.05654.x

Pires, J. R. A., de Souza, V. G. L., & Fernando, A. L. (2018). Chitosan/montmorillonite bionanocomposites incorporated with rosemary and ginger essential oil as packaging for fresh poultry meat. Food Packaging and Shelf Life, 17, 142-149. https://doi.org/10.1016/j.fpsl.2018.06.011

Pramanik, S., Gopalakrishnan, R., Barua, N., Buragohain, A. K., & Karak, N. (2015). Montmorillonite immobilized Curcuma aromatica/Zanthoxylum limonella oil nanoconjugate as a green antibacterial and biocompatible material with mosquito repellent attributes. Applied Clay Science, 109, 33-38. https://doi.org/10.1016/j.clay.2015.03.008

Proença, L. B., Pena, C. A. P., da Silva, G. V., Camargo, I. L. B. D. C., & Branciforti, M. C. (2020, December). Study of the Antibacterial Property of Tea Tree Oil and Its Incorporation Into Poly (Lactic Acid) -montmorillonite Clay Bionanocomposites. In

Macromolecular Symposia (Vol. 394, No. 1, p. 2000073). https://doi.org/10.1002/masy.202000073

Quintero, R. I., Galotto, M. J., Rodriguez, F., & Guarda, A. (2014). Preparation and characterization of cellulose acetate butyrate/organoclay nanocomposites produced by extrusion. Packaging Technology and Science, 27(6), 495-507. https://doi.org/10.1002/pts.2043

Raharivelomanana, P., Ansel, J. L., Lupo, E., Mijouin, L., Guillot, S., Butaud, J. F, ... & Pichon, C. (2018). Tamanu oil and skin active properties: from traditional to modern cosmetic uses. OCL 25 (5): D504. https://doi.org/10.1051/ocl/2018048

Rakhimov, K. D., Turgumbayeva, A. A., Ustenova, G. O., Abuova, Z. B., & Ross, S. A. (2018). Study of safety and antimicrobial, anti-inflammatory and anti-inflammatory properties ointment from the safflower extract. Bulletin of national academy of science of the republic of Kazakhstan. 2 (372), 17 – 21. https://rmebrk.kz/journals/3923/11853.pdf#page=17

Rakhmawati, R., Wahyuni, D. S. C., Ainurofiq, A., Hadi, S., Choiri, S., Retnaningtyas, E., & Pratama, T. D. S. (2024) In vivo Wound Healing Activity of Tamanu Oil (Calophyllum inophyllum l.) Extracted by Cold-Pressed and Hot-Pressed Methods and Their Fatty Acids Composition. Majalah Obat Tradisional, 29(3), 357-365.Research, 29:3, 201-213. https://doi.org/10.22146/mot.97146

Ristić, T., Persin, Z., Kralj Kuncic, M., Kosalec, I., & Zemljic, L. F. (2019). The evaluation of the in vitro antimicrobial properties of fibers functionalized by chitosan nanoparticles. Textile research journal, 89(5), 748-761. https://doi.org/10.1177/0040517518755785

Santos, A. J., Pina, L. T., Galvão, J. G., Trindade, G. G., Nunes, R. K., Santos, J. S., ... & Nunes, R. S. (2020). Clay/PVP nanocomposites enriched with Syzygium aromaticum essential oil as a safe formulation against Aedes aegypti larvae. Applied Clay Science, 185, 105394. https://doi.org/10.1016/j.clay.2019.105394

Savas, L. A., & Hancer, M. (2015). Montmorillonite reinforced polymer nanocomposite antibacterial film. Applied Clay Science, 108, 40-44. https://doi.org/10.1016/j.clay.2015.02.021

Shemesh, R., Goldman, D., Krepker, M., Danin-Poleg, Y., Kashi, Y., Vaxman, A., & Segal, E. (2015). LDPE/clay/carvacrol nanocomposites with prolonged antimicrobial activity. Journal of Applied Polymer Science, 132(2). https://doi.org/10.1002/app.41261

Shemesh, R., Krepker, M., Goldman, D., Danin-Poleg, Y., Kashi, Y., Nitzan, N., ... & Segal, E. (2015). Antibacterial and antifungal LDPE films for active packaging. Polymers for Advanced Technologies, 26(1), 110-116. https://doi.org/10.1002/pat.3434

Shimanouchi, T. National Bureau of Standards. (1967). III. Tables of Molecular Vibrational Frequencies, Page 7- 54. In National Bureau of Standards (Vols. V6–1967). U.S. Department of commerce. https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds6.pdf

Shojaee-Aliabadi, S., Mohammadifar, M. A., Hosseini, H., Mohammadi, A., Ghasemlou, M., Hosseini, S. M., ... & Khaksar, R. (2014). Characterization of nanobiocomposite kappa-carrageenan film with Zataria multiflora essential oil and nanoclay. International journal of biological macromolecules, 69, 282-289. https://doi.org/10.1016/j.ijbiomac.2014.05.015

Souza, V. G. L., Pires, J. R., Rodrigues, P. F., Lopes, A. A., Fernandes, F. M., Duarte, M. P., ... & Fernando, A. L. (2018). Bionanocomposites of chitosan/montmorillonite incorporated with Rosmarinus officinalis essential oil: Development and physical characterization. Food packaging and shelf life, 16, 148-156. https://doi.org/10.1016/j.fpsl.2018.03.009

Souza, V. G. L., Rodrigues, C., Ferreira, L., Pires, J. R. A., Duarte, M. P., Coelhoso, I., & Fernando, A. L. (2019). In vitro bioactivity of novel chitosan bionanocomposites incorporated with different essential oils. Industrial crops and products, 140, 111563. https://doi.org/10.1016/j.indcrop.2019.111563

Souza, V. G., Pires, J. R., Vieira, É. T., Coelhoso, I. M., Duarte, M. P., & Fernando, A. L. (2018). Shelf-life assessment of fresh poultry meat packaged in novel bionanocomposite of chitosan/montmorillonite incorporated with ginger essential oil. Coatings, 8(5), 177. https://doi.org/10.3390/coatings8050177

Swamy, M. K., Akhtar, M. S., & Sinniah, U. R. (2016). Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evidence-Based Complementary and Alternative Medicine, 2016(1), 3012462. https://doi.org/10.1155/2016/3012462

Tea tree oil: Usefulness and safety. (n.d.). NCCIH. https://www.nccih.nih.gov/health/tea-tree-oil

Textmap, Wade. (2021). Map: Organic Chemistry 1. 11.5: Infrared Spectra of Some Common Functional Groups. Chemistry Libre text. https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Map%3A_Organic_Chemistry_(Wade)_Complete_and_Semesters_I_and_II/Map%3A_Organic_Chemistry_(Wade)/11%3A_Infrared_Spectroscopy_and_Mass_Spectrometry/11.05%3A_Infrared_Spectra_of_Some_Common_Functional_Groups

Tornuk, F., Hancer, M., Sagdic, O., & Yetim, H. (2015). LLDPE based food packaging incorporated with nanoclays grafted with bioactive compounds to extend shelf life of some meat products. LWT-Food Science and Technology, 64(2), 540-546. https://doi.org/10.1016/j.lwt.2015.06.030

Tunç, S., & Duman, O. (2011). Preparation of active antimicrobial methyl cellulose/carvacrol/montmorillonite nanocomposite films and investigation of carvacrol release. LWT-Food Science and Technology, 44(2), 465-472. https://doi.org/10.1016/j.lwt.2010.08.018

Uberoi, A., McCready-Vangi, A., & Grice, E. A. (2024). The wound microbiota: microbial mechanisms of impaired wound healing and infection. Nature Reviews Microbiology, 22(8), 507-521. https://doi.org/10.1038/s41579-024-01035-z

Uncu, O., Ozen, B., & Tokatli, F. (2019). Use of FTIR and UV–visible spectroscopy in determination of chemical characteristics of olive oils. Talanta, 201, 65-73. https://doi.org/10.1016/j.talanta.2019.03.116

Villegas, C., Arrieta, M. P., Rojas, A., Torres, A., Faba, S., Toledo, M. J., ... & Valenzuela, X. (2019). PLA/organoclay bionanocomposites impregnated with thymol and cinnamaldehyde by supercritical impregnation for active and sustainable food packaging. Composites Part B: Engineering, 176, 107336. https://doi.org/10.1016/j.compositesb.2019.107336

Wang, L., Ahmad, S., Wang, X., Li, H., & Luo, Y. (2021). Comparison of antioxidant and antibacterial activities of camellia oil from Hainan with camellia oil from Guangxi, olive oil, and peanut oil. Frontiers in Nutrition, 8, 667744. https://doi.org/10.3389/fnut.2021.667744

Yimdjo, M. C., Azebaze, A. G., Nkengfack, A. E., Meyer, A. M., Bodo, B., & Fomum, Z. T. (2004). Antimicrobial and cytotoxic agents from Calophyllum inophyllum. Phytochemistry, 65(20), 2789-2795. https://doi.org/10.1016/j.phytochem.2004.08.024

Zafar, K., Lee, A., Wang, J. Y., Bitterman, D., Kabakova, M., Patel, P., ... & Jagdeo, J. (2024). The potential cutaneous benefits of bentonites and montmorillonites. Archives of Dermatological Research, 316(10), 1-10. https://doi.org/10.1007/s00403-024-03456-5

Zhan, J., Chen, H., Zhou, H., Hao, L., Xu, H., & Zhou, X. (2023). Essential oil-loaded chitosan/zinc (II) montmorillonite synergistic sustained-release system as antibacterial material. Journal of Dispersion Science and Technology, 44(2), 288-298. https://doi.org/10.1080/01932691.2021.1947848