INVESTIGATION OF PODOCARPUS FALCATUS SEED OIL AND ALOE VERA EXTRACT FOR DEVELOPMENT OF ANTIMICROBIAL AND ANTIOXIDANT OINTMENT

Workneh Ensermu Bayisa

A Thesis Submitted to the Department of Chemical Engineering

School of Mechanical, Chemical and Material Engineering

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Chemical Engineering (Process Engineering)

Office of Graduate Studies

Adama Science and Technology University

February, 2024

Adama, Ethiopia

INVESTIGATION OF *PODOCARPUS FALCATUS* SEED OIL AND *ALOE VERA* EXTRACT FOR DEVELOPMENT OF ANTIMICROBIAL AND ANTIOXIDANT OINTMENT

Workneh Ensermu Bayisa

Advisor Alemu Gonfa (PhD)

Co-Advisor Mulugeta Yilma (PhD)

A Thesis Submitted to the Department of Chemical Engineering

School of Mechanical, Chemical and Material Engineering

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Chemical Engineering (Process Engineering)

Office of Graduate Studies

Adama Science and Technology University

February, 2024

Adama, Ethiopia

DECLARATION

I hereby declare that this thesis proposal entitled “Investigation of *Podocarpus falcatus* seed oil and *Aloe vera* extract for development of antimicrobial and antioxidant ointment” is my own work and has not been submitted to any academic degree, diploma or certificate in any other university. The references used in this proposal are duly recognized by proper citations.

Workneh Ensermu Bayisa		
Name of student	Signature	Date

RECOMMENDATION

I the advisors of this research thesis, hereby certify that I have read the revised version of the thesis entitled "Investigation of *Podocarpus falcatus* seed oil and *Aloe vera* extract for development of antimicrobial and antioxidant ointment" prepared under my guidance by Workneh Ensermu submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Chemical Engineering (Process Engineering). Therefore, I recommend the submission of revised version of the thesis to the department following the applicable procedures.

Major Advisor	Signature	Date
Co-Advisor	Signature	Date

APPROVAL

We, the advisors of the thesis entitled "Investigation of *Podocarpus falcatus* seed oil and *Aloe vera* extract for development of antimicrobial and antioxidant ointment" and developed by Workneh Ensermu Bayisa, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Workneh Ensermu have read and evaluated the thesis entitled Investigation of *Podocarpus falcatus* seed oil and *Aloe vera* extract for development of antimicrobial and antioxidant ointment and examined the candidate during the open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Chemical Engineering (Process Engineering).

Chairperson	Signature	Date
Internal Examiner	Signature	Date
External Examiner	Signature	Date

Finally, approval and acceptance of the thesis is contingent upon the submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

Department Head	Signature	Date
School Dean	Signature	Date
Office of Postgraduate Studies, Dean	Signature	Date

ACKNOWLEDGEMENT

Primarily, I am deeply thankful to God, whose boundless wisdom, strength and grace have illuminated my path in the moments of uncertainty and challenge. I offer my deepest gratitude to my advisors for their unwavering support, precious guidance, and insightful feedback throughout the entire research process. I am immensely grateful to my family, whose encouragement, love and sacrifices have been the bedrock of my academic pursuits. I am also grateful to my friends and colleagues who provided encouragement, shared ideas, and offered assistance whenever I faced challenges.

LIST OF FIGURES

LIST OF TABLES

TABLE CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

DMSO	Dimethyl Sulfoxide
MOs	Microorganisms
TPC	Total Phenol Content
TFC	Total Flavonoid Content
AA	Ascorbic Acid
IC_{50}	Half Maximum Inhibition Concentration
FTIR	Fourier Transform Infrared Spectroscopy
GC– MS	Gas Chromatography Mass Spectroscopy
UV-Vis	Ultraviolet- Visible Spectroscopy
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
GAE	Gallic Acid Equivalent
MIC	Minimum Inhibitory Concentration
QE	Quercetin Equivalent
PEG	Polyethylene Glycol
RSM	Response Surface Methodology
ANOVA	Analysis of Variance
SXE	Soxhlet Extraction
MAU	Microwave Assisted Extraction
UAE	Ultrasound Assisted Extraction
AAU	Adama Science and Technology University

ABSTRACT

Natural herbal ointments consist of many beneficiary properties that are promising for antimicrobials and antioxidants as alternative agents, thereby exerting fewer side effects and toxicity compared to synthetic ointments. The present study involved extracting the oil, quantifying the phytochemical, antioxidant, and antimicrobial activities of P. falcatus seed oil and A. vera extract, and incorporating its extract into an ointment excipient. P. falcatus oil was extracted using ethanol solvent by the Soxhlet extraction method at the optimum extraction temperature of (70°C), time (4 hr), solvent-to-solid ratio (17.5 v/w) and yields 29.49% by RSM. Whereas, A.vera extract was obtained by employing a couple of oven driers and vacuum rotary evaporation after a clear a vacuum pump filtered liquid juice. From the results of phytochemical and antioxidant investigations, P. falcatus seed oil, A. vera gel, and its extract contain the TPC (10.25, 1.2, and 22.8) mg GAE/g of extract; TFC (1.414, 0.208, and 5.29) mg QE/g of extract; and IC_{50} values of 4.23, 22.48, and 2.68% mg/mL, respectively. The potential diameter inhibition activities of the extracts against MOs were determined via an agar diffusion assay. Aloe vera extract has exhibited a significant inhibition zone against all the tested MOs, especially E. coli and P. aeruginosa, as compared to its gel. However, P.falcatus seed oil did not exhibit the minimum inhibition diameter of the tested MOs. The crude extracts of P. falcatus oil and A. vera extract were instrumentally characterized by FT-IR and GC-MS to identify the functional group and biologically active compound present in the extracts. From the FTIR spectral study, the existence of different functional groups that might be assigned to alcohols, phenols, fatty acids, and amines was observed. Whereas the GC-MS analysis revealed the presence of fifteen and nine biologically active components in A.vera and P. falcatus seed oil, respectively. Those instruments justified the presence of various phytoconstituents that promised therapeutic activities and applicability in different medicinal values. The phytochemical, antimicrobial, and antioxidant activities of the formulated ointment were evaluated. From its physiochemical analysis, it has a pH of 6.67, spreadability of 56.78 g∗cm/s, and viscosity of 4122.15 cp, whereas its mean inhibition diameter from the least resistance to highest sensitive against MOs in ranges are 16.66 mm to 21 mm. The half-maximum inhibition concentration against free radicals is 1.12 mg/mL.

Keywords *P.falcatus* seed oil, *A.vera* extract, antimicrobial, antioxidant, ointment

CHAPTER ONE

1 INTRODUCTION

1.1 Background of the Study

Topical drugs are formulations that comprise dermatologic substances to achieve the desired drug concentrations at the proper site of action to treat skin ailments. Topical formulation is an attractive approach to treating skin disease rather than oral drug delivery. Skin drug delivery systems are extensively used to provide surface effects and dermal effects(Kaci et al., 2018). A topical therapeutic drug might be prepared in the form of gels, creams, or ointments, depending on the proposed application, the preferences of the patient, stability, and compatibility.

The ointment is the kind of topical medication that shows the characteristic of being viscous to semi-solid and offers a sustained release of the active ingredient for longer periods to the point of action. It is essential to choose a suitable excipient that enhances the permeation of active substances across the skin layers and exhibits compatibility with bioactive agents(Hussain et al., 2018). PEG bases are kinds of vehicle systems that have the properties of high absorption capacity, promote skin tissue regeneration, and are nontoxic, biocompatible, and biodegradable. It is usually to contain and deliver a drug used to treat skin infections, such as skin irritations, bacteria or fungal infections, surgical wounds, minor cuts, scrapes, burn wounds, inflammation, and other skin injuries (Ghosh et al., 2019). Antimicrobial ointments are kinds of topical medications that contain ingredients with antibiotic, antifungal, or antiviral agent properties. These substances inhibit the growth of specific traits of microorganisms, preventing the risk of infection and promoting the healing of the skin.

Microbial infections are one of the biggest threats to healthy skin in the current century. The frequencies of relapsing infectious disease and antibiotic resistance have greatly increased recently. The most widely spread skin infectious bacterial species are *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli,* and *Pseudomonas aeruginosa* (Saddiqe et al., 2020). Those pathogenic wounds are always observed around the area of the mucous membrane, incision, laceration, puncture, abrasion, burn, and closed wounds (Ghosh et al., 2019).

Furthermore, bacterial infections typically result in the generation of reactive oxygen compounds (ROS) from oxygen metabolism, which harm cells and tissues (Saddiqe et al., 2020). On the other hand, the development of ROS promotes oxidative stress that contributes to the aging process, cancer, and wrinkles (Ghazi, 2022).

Moreover, extensive skin tissue damage occurs during burns, which impairs collagen reorganization and granulation tissue formation and induces free radical-mediated damage, which results in delayed tissue repair at the area of the wound, incision, and burn. Recovery from any burn wound needs a long hospital stay, expensive medications, and a long rehabilitation period (Heidari et al., 2019). One of the alternatives to treating ROS is the use of a topically applied antioxidant agent. Antioxidants contain several health-related benefits for the skin by reducing the appearance of wrinkles, aging, inflammation, and microbial growth and boosting the endogenous antioxidant capacity of the skin.

Several antibiotics have been developed over time; however, the majority of these drugs have proven ineffective due to the emergence of multidrug-resistant pathogens. Moreover, synthetic drugs have often been associated with toxicity, severe side effects, and resistance (Palaniyappan et al., 2023). The occurrence of these unreal antimicrobial agents in ointments can disrupt the natural balance of the microbiome on the skin and increase the risk of skin corruption. Some of the synthetic antioxidants used in topical applications contain adverse effects that cause redness, itching, allergic reactions, poor chemical stability, the development of contact dermatitis, and skin irritations (Otang & Afolayan, 2016). The foundation of new antimicrobial and antioxidant formulations from natural sources has been innovative for the recent breakout of the resisted antibiotics of microorganisms and to avoid the drawbacks of synthetic antioxidants.

According to the World Health Organization, around 70% to 95% of the world's population uses medicinal plants mainly for their health care (Salhi et al., 2019). At the beginning of the 20th century, synthetic drugs were replaced by plant-derived medicinal extracts because of their harmlessness and fewer side effects (Khogta et al., 2020). Natural medicines are becoming increasingly important in medical and healthcare products. They provide a potential source of different types of active constituents in the creation of an alternative, cheap and

effective novel therapeutic medicines (Chellathurai et al., 2023). Biologically active ingredients establish new functions in cosmetics since they have antioxidant, antimicrobial, and anti-aging properties (Agung et al., 2019; Ferreira et al., 2022). The complex mixture of these compounds exerts synergistic activity against multiple ranges of the targeted microorganisms as well as skin ROS. Recently, about 2% to 15% w/w herbal extracts were employed in the ointment formulation to reduce the side effects (Farhan et al., 2021).

Plant antioxidants, such as polyphenols and flavonoids, reduce collagen degradation by inhibiting the concentration of free radicals in the tissues (Zouboulis et al., 2019). Phenolic compound components have several roles due to the enrichment of antioxidant, antibacterial, and antifungal properties(Ansari et al., 2019). In this circumstance, the selection of antimicrobial as well as antioxidant potential agents is required to inhibit both microbial growth and reactive species molecules.

Moreover, polyherbal employment in the formulation would reduce the duration of therapy for skin infections (Dev et al., 2019). Combination uses of plant material have shown stronger antibacterial and antioxidant activities than their individual effects. Synergistic is the combination of two or more biologically active components of an extract to induce more chemically complex substances and aggressive actions by its diverse active principles.

Traditionally, aloe vera extract and *P.falcatus* plant parts have been used to treat various ailments for several years. *P.falcatus* plant parts were employed in the treatment of stomachache, gonorrhea, deworming, cough, and vomiting (El-Sayed et al., 2018). In folk medicine, the shoot apex of its extract has shown significant anticancer activity, TPC and TFC (Meharie & Tunta, 2020). *Aloe vera* gel or extract can also provide unlimited opportunities for a new drug formulation with different modes of action as they contain several phytoconstituents. Some of the therapeutic properties of this leaf are antibacterial, antioxidant, anti-inflammatory, anticancer, and UV protection (Jales et al., 2022). However, *P.falcatus* seed oil has not been extensively explored for its biological activity to make a more valuable product. Until now, there has been no evidence about the formulation of these combined extracts for their topical skin care purposes. Formulation of ointment from these two extracts for the topical for topical antimicrobial drug could bring a new remedy to the resistance of clinical antibiotics in the trend year.

1.2 Statement of Problem

Human skin is a complex process that is influenced by a combination of intrinsic and extrinsic factors. Among those factors, microorganisms and reactive oxygen compound have been caused a significant damage to the human skin. Synthetic antimicrobials and antioxidants were commonly known to treat those skin infections for several years. However, synthetic ingredients have derived various problems to the normal human skin and adapted with MOs.

Currently, several synthetic antibiotics and antioxidant are becoming less effective due to the development of antibiotic resistance and pose several side effects.

Many investigations have been reported regarding to synthetic antimicrobial that resisted by the species of *Escherichia coli, Pseudomonas aeruginosa,* and *S. aureus.* In addition, synthetic antimicrobial were developing a number of related skin infections (Añibarro-Ortega et al., 2019; Palaniyappan et al., 2023).

Moreover, antioxidants are more widely incorporated in cosmetics due to their ability to prevent oxidative stress on the skin and reduce cellular damage(Herbal et al., 2022). Synthetic antioxidants, such as propyl gallate, butylated hydroxytoluene, and butylhydroxyanisole, are frequently used in cosmetic care products. However, it causes several skin infections, such as irritation, carcinogenesis, and pulmonary toxicity (Saddiqe et al., 2020).

Therefore, finding a new and alternative drug from a natural plant component that is suspected to have antimicrobial and antioxidant potential is the solution to this problem. By focusing on natural, it can be minimized the potential side effects, multidrug resistance and long-term consequences associated with synthetic antioxidants and antimicrobial agents.

1.3 Objectives

1.3.1 General Objective

The overall objective of this study was to extract and characterization of A.vera extract, and *P. falcatus* seed oil and development of antioxidant and antimicrobial ointment.

1.3.2 Specific Objectives

- Preparing and extracting *P. falcatus* seed oil and *A.vera* extract
- Studying the phytochemical, antioxidant and antimicrobial activities of *P. falcatus* seed oil and *A. vera* extract
- Characterizing and evaluating the physiochemical, antimicrobial and antioxidant activities of ointment

1.4 Significance of the Sudy

Natural herbal ointment has offered many promising properties, such as anti-microbial, antioxidant, anti-aging, and fewer side effects compared to synthetic therapy. Currently, herbal antimicrobial and antioxidant topical formulations are getting attention due to several reasons for their application in drug formulation. Therefore, incorporating these active agents into the drug delivery system could improve therapeutic activities and reduce the side effects that have been raised by patient compliance.

Therefore, *A.vera* and *P.falcatus* seed oils are non-toxic, non-edible, and distributed in various parts of Ethiopia. These two plants were commonly known for their medicinal value in numerous ailment treatments by traditional way. The oil obtained from these plant extracts has desirable biological active substances that are promising in developing cosmetic care products that serve as antioxidants and antimicrobials. Developing synergistic activities from these two plant extracts into a novel industrial antimicrobial and antioxidant ointment manufacturing is intended to enhance the potential utilization of the plant extracts to bring economic benefit, minimize the impact of synthetic antibiotics, and provide an alternative for antibacterial, antifungal, and antioxidant agents.

1.5 Research Question

- ✓ Is it possible to produce antibacterial and antioxidant ointment by incorporating *P.falcatus* seed oil and *A.vera* extract?
- ✓ At what concentration of *P. falcatus* seed oil and *A.vera* gel extract combination to be effective and desire in the selected ointment base.

1.6 Scope of Study

This study was to demonstrate the possibility of producing herbal ointment based formulation from *P.falcatus* seed oil and *A.vera* extract into the ointment excipient. A number of experimental were performed in this study based on the required number of equipment, raw materials, chemical and reagents. *P.falcatus* seed and *A.vera* leaf were collected and prepared for the extraction of extract into the laboratory. The moisture content of both the *P.falcatus* seed powder and *A.vera* gel were carried out by employing an oven dryer method. The *P.falcatus* seed oil was extracted at the optimum extraction condition by using Soxhlet extractor. While, the filtered liquid fraction of mucilage *A.vera* was concentrated by a rotary vacuum evaporator at 50℃ followed oven dried by 40℃ to get an extract of it. The phytochemical (TPC and TFC) and antioxidant content of *P. falcatus* seed oil and *A.vera* extract were investigated to determine the biological activity by using ultraviolet-visible spectroscopy (UV-Vis). Gas chromatography-mass spectrophotometers (GC-MS) and Fourier transform infrared spectroscopy (FT-IR) analyzers have been employed for the identification of essential compounds and functional groups of the extracted oil and extract, respectively. The antimicrobial activities of the extracted oil and extract, as well as the formulated ointment, were investigated to find out the potential inhibition of the extract against microorganisms. The physiochemical properties of the formulated ointment were evaluated (viscosity, spreadability, pH, and stability).

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Overview of P. Falcatus

2.1.1 Morphology

Podocarpus falcatus (P. falcatus), also known as Afrocarpus falcatus, is one of the two endogenous conifers of Ethiopia. It is a species of coniferous tree that belongs to the family Podocarpaceae and is commercially known as East African Yellow Wood (Assefa et al., 2015). It is an evergreen tree up to 46 m in length that grows in the altitude range of 1500 m to 2500 m with an average rainfall area of 1200 to 1800 mm per year (Bruno et al., 2022). This inedible plant is one of the indigenous plant species found in Ethiopia and is intensively used for its timber purposes. The wood is also suitable for the manufacture of furniture, bakery boards, and confectionery trays for which non-tainting is required.

Medicinal uses

Traditionally, *P.falcatus* has important medicinal value for the treatment of different ailments. The stem and bark of *P.falcatus* were used to treat stomachache, cattle disease, gonorrhea, deworming and cancer(El-Sayed et al., 2018). The gum and shoot parts of the plant have been employed for the treatment of diabetes, cough, lung problems, and vomiting (Meharie & Tunta, 2020). *P.falcatus* also has several pharmacologically proven activities. The leaves contain significant antioxidants, antimicrobials, polyphenols, anticancer drug taxol, diterpenoids, totarol, flavonoids, and quercetin-3-O-glycoside and flavonol glycosides. Quantitatively, the TPC and TFC content of *P.falcatus* shoot apexes were 142.23 mg QE/g and 196.84 mg GAE/g, respectively(Meharie & Tunta, 2020). Recently, some studies have confirmed that it contains antitumor, antimicrobial, anti-inflammatory, and antioxidant activities. The crude extract of stem barks showed strong activity against *S.aureus*, Endophytic fungi of *P.falcatus* display remarkable activity against various phytopathogens(El-Sayed et al., 2020).

2.2 Overview of Aloe Vera

2.2.1 Property of Aloe vera Leaves

Aloe barbadensis Miller, more commonly known as *Aloe vera* (A. vera). *A. vera* plants can survive continuously for more than seven years without any water, which has the capacity to absorb moisture from dew environments. *A.vera* leaves can be also categorized into three basic components; latex (aloin) is a bitter yellow liquid beneath the epidermis of the leaf, which has an active compound and a mixture of glucosides that is responsible for the skin healing properties (Jana et al. 2019). The second is the fleshy inner part of the leaf, which includes the cell walls and organelles called pulp (parenchyma tissue), and the last component, known as gel or mucilage, is the viscous clear liquid found in the parenchyma cells. The viscosity of *A. vera* gel is due to the content of the sugar glucomannan (Reham et al., 2022). The pulp contains approximately greater than 98.5% of water, while the gel consists of about 99% water (Forno-bell et al., 2021). The left portion contains several compounds, including polysaccharides, phenolic compounds, vitamins, enzymes, and organic.

2.2.2 Application of *A.Vera* Extract in Topical Formulations

Aloe vera extract is a popular ingredient in ointment formulations due to its various beneficial properties, particularly for skin health. *A.vera* is the richest source of nutrients and bioactive compounds, which are responsible for their medicinal properties. Recently, about 95% of the manufacturing ointment industry has incorporated aloe vera extract or gel into the formulation (Javed & Atta-Ur, 2014). In cosmetic products, aloe vera has been used as an antioxidant booster, antimicrobial, penetration enhancer, wound healing, moisturizer, anti-inflammatory, anti-acne, sunburn relief, antiphotoaging, and cooling skin irritation effects (Reham et al., 2022). The gel has the capability of altering the solubility properties of the stratum corneum and disrupting the nature of the skin lipids, thereby influencing drug diffusion across the skin. Provide effective curing activity and reduce the drug dose that causes side effects in the treatment of plaque, psoriasis, seborrheic dermatitis, acne vulgaris, and antifungal ointments.

2.2.3 Methods of Extracting the Concentrated *A.Vera* Gel Extract

The types of drying and extraction techniques can be affects the active components of the extract (Hossen et al., 2022; Saifullah et al., 2019). Dried aloe vera gel powder is prepared by the technique of either sun drying, air-drying or shed drying. sunshade and air-drying is more

favorable than solar drying due to maintaining the availability of bioactive component in the leave(Donkor et al., 2020). Freeze drying and oven drying are the two most commonly employed techniques to obtain a concentrated extract of either liquid form of gel (Solaberrieta et al., 2022). These bioactive substance are exposed to oxidation and degradation due to unsuitable extraction techniques (Solaberrieta et al., 2022).

Table 2.1 Extraction methods of aloe vera gel extract

Methods of extracting gel	Methods of maceration in the selected solvents	Methods of Concentrating	Reference
Gel extract	Cold maceration with occasional stirring for 24 hours in deionized water and ethanol and filtrated	Concentrated by a water bath set at 60 ^{o} C	(Yavagal & SV, 2019)
Gel dried in oven at 80$^{o}_{C}$ for 48 hr	About 20 g of powder dissolved in 200 ml of ethanol	Evaporated by Rotary evaporator	(Bhaskar et al., 2018)
Pulp filets were cut into small pieces	Ethanol is added to gel at the ratio of 3:1 v/v and filtered through a nylon-cloth	Placed in Petri dishes and dried at 60 ◦ C in oven for 24 h	(Otálora et al., 2021)
Gel were cut into pieces	400 ml of slime of the gel was prepared	gel concentrated using a rotary evaporator at 50 ◦C to get 12 g	(Activities et al., 2022)
inner gel homogenized by mechanical mixer	parenchyma was centrifuged at 10,000 rpm for 30 min.	The resulting mucilage was lyophilized with a freeze dryer	(Alvandi et al., 2023)

A.vera gel extract were effectively concentrated from 0.9 brix to 3.8 brix in rotator vacuum evaporation at the optimum extraction parameter of temperature (55^{o}C) and time (1hr) without affecting the ascorbic acid that is added to the fresh gel to observe the effect of process variable on the most heat labile components (Mahapatra et al., 2017).

2.3 Chemical Properties of Seed Oil

Peroxides are intermediate products of oil oxidation that reduce the shelf life and affect consumer acceptance of the product. A small amount of peroxide value indicates that the oil

has strong antioxidant content. The iodine value measures the number of unsaturated contents of fatty acids and the purity of the oil. During extraction and storage, the oil can be altered by the release of free fatty acids because of the hydrolysis of triglycerides, which increases the free acidity. The acid value is often a good measure of the breakdown of the triacylglycerol into free fatty acids and an indication of adverse effects on the quality of the oils (Kellens et al., 2007). A high acid value implies that the oil has a high susceptibility to decomposition, whereas pharmaceutical oil must not contain any acidity at all.

2.4 Advantages of Plant Seed Oil in Ointment Formulation

Plant seed oil has been suggested as a good option in ointment formulations due to its ability to offer several advantages, such as skin barrier, skin nourishment, acne alleviation, moisturizing, and hydration as a result of fatty acids (Lin et al., 2018; Talianu et al., 2020). Short chains of free fatty acids such as palmitic acid, cis-9-oleic acid, and cis-9, 12-linoleic acid are good candidates for having moisturizing and antioxidant properties (A. Singh et al., 2023; Zouboulis et al., 2019). The combination of different plants containing antioxidants exhibited a synergistic effect to protect the human skin against oxidative stress. The presence of monounsaturated free fatty acids like oleic acid enhances the permeability of other compounds present in plant oil into the skin by disrupting the barrier. One of the main problems with topical application of active ingredients is the impermeability of the stratum corneum barrier. In order to have the desired effect, the active ingredient not only has to cross this barrier, but a sufficient amount must also reach the deeper layers of the skin (Costa & Santos, 2017). Oleic acid is a ω-9 unsaturated fatty acid that contains the properties of solubilization, percutaneous absorption enhancer, formulation stabilization, moisturizing, and softening of the skin (Dario et al., 2018). Olive oil, in particular, contains a huge amount of oleic acid rather than linoleic acid, which aids as a penetration enhancer in ointment formulation (Ghodke et al., 2019). Oleic acid is an enriched source of antioxidants that strengthen cell membrane integrity and help replace cells and tissues (Choudhury et al., 2017). Linoleic acid, an unsaturated ω-6 fatty acid, provides several important benefits to the skin by improving the skin barrier function by reducing trans-epidermal water loss, providing better moisturizing, significant antimicrobial activity, and promoting cutaneous regeneration. Moringa oliefera oil and sunflower oil are enriched with linoleic acid concentrations, which

are frequently incorporated into a topical formulation for the aforementioned reasons(C Shaikh et al., 2018).

2.5 Phenolic Compound

Natural plant antioxidants, especially polyphenolic compounds, are the most important that are used for topical applications. Natural occurring polyphenols had remarkably exhibited the capacity to scavenge free radicals without causing any side effects to the skin (Farhan et al., 2021). Phenolic acid and flavonoids are the most abundant bioactive constituents that exert multiple pharmacological activities (Chellathurai et al., 2023). Polyphenolic compounds has a great role in healing of wound (Heidari et al., 2019). Recently, flavonoid and phenolic compounds have received great attention due to their higher antibacterial and antioxidant activity (Royani et al., 2023). Have diverse antimicrobial activity and exhibit strong antimicrobial activity against a wide spectrum of pathogens(Herman, 2019; Kaseke et al., 2021). Flavonoids also contain a strong antimicrobial property(Chellathurai et al., 2023; Palaniyappan et al., 2023).

2.6 Methods of Plant Oil Extraction

An extraction technique is a method used to obtain an intended biologically active compound from a plant cell with high yield, quality, and minimal changes to the functional attributes of the constituents(Dhanani et al., 2017). There are many different processes and techniques, such as chemical, biochemical, and mechanical methods of extraction, employed to separate the oil from the seed. Therefore, it is crucial to select the appropriate extraction technique based on biomass properties. To date, the most important classification is between conventional and non-conventional extraction.

2.6.1 Convectional Extraction Method

Oil extraction by mechanical presses requires physical agents to release the oil from the cells of plants. This method cannot completely extract the oil from the cells of the seeds, yields oil turbidity, and leaves some residual oil in the cake (Topare et al., 2011). In addition, the total yield of polyphenols and flavonoids in the oil extracted was less than that obtained by Soxhlet extraction. Mechanical extraction could provide a lower yield of TPC (121.20 mg GAE/g) compared to the extraction yield by UAE and SXE, which gave the TPC recovery of 301.81 mgGAE/g and 85.59 mgGAE/g (Thilakarathna et al., 2023).

An infusion is commonly used for the extraction of flavor compounds by steeping the fresh or dry herbal material in hot water for a period of less than thirteen minutes (<30 min). Flowers, leaves, and other plant parts are used for this preparation. This method is favorable for the extraction of glycosides and essential oils from delicate herbs and soft parts of plants (Bitwell et al., 2023; Malca-Garcia et al., 2019). A decoction is another method of extraction used to extract the tougher, harder fibrous roots and non-aromatic plant material that does not decompose at a higher extraction temperature in hot water for a longer period to soften the harder material and release its active constituents. The main limitation of decoction is yielded a high level of impurities within the crude extract and heat-sensitive components could not be operated under this extraction method(A. Kumar et al., 2023). This technique extracts more total glycosides as compared with other extraction methods, such as maceration, percolation, and Soxhlet extraction (Malca-Garcia et al., 2019).

Infusion and decoction are also the footprints of maceration and employing heat with a water solvent to extract. However, maceration employs a cold infusion to extract volatile and thermolabile bioactive compounds. The biomass was steeped in any type of liquid for a period of a few weeks to months, depending on the desired maceration. The disadvantage of this technique is that it requires a longer duration of extraction and low efficiency (Awad et al., 2021). The yield of extract obtained by these methods was lowest compared to MAE and UAE (Bitwell et al., 2023). A tincture is a type of convection extraction that is well known for the extraction of active chemical compounds by alcoholic solvents at the plant-to-solvent ratio (1:4) for 2–6 weeks.

Steam and water distillation are the two main categories of hydrodistillation techniques. Steam distillation is a technique that employs a satellite boiler as a steam inducer and is maintained with a reflux controller to reduce the loss of sensitive volatile components prone to hydrolysis and polymerization. However, some constituents of oil require a high-boiling range and a longer period of times for extraction due to low pressure (Talati, 2017). Whereas in water distillation, the biomass is steeped in hot water by providing heat through a closed or open steam coil within the stirrer to prevent settling and thermal degradation at the bottom. The laboratory apparatus recommended for trial distillations is the Clevenger system. Incomplete extraction, susceptibility to hydrolysis (esters), and proneness to polymerization of sensitive

components such as aldehydes and monoterpene hydrocarbons are some of the shortcomings of this method (Dhanani et al., 2017). In summary, the antioxidant activity obtained by hydro-distillation extraction was lower (3.77%) compared to Soxhlet extraction with ethanol solvent (36.33%) from eucalyptus oil. This could be due to the affinity of hydro-distillation to extract the volatile compounds rather than the high molecular compounds (Zhao & Zhang, 2014).

Soxhlet extraction (SXE) is the latest among the other convectional extraction methods and is better known for the extraction of vegetal-rich oil. The sample placed in a thimble holder has been extracted by freshly recycled evaporated solvent from the distillation flask. This method is advantageous over the others extraction due to its lower processing cost, effectiveness at the lab scale, and good total recovery of extracts as compared to other conventional methods (Alara et al., 2018). This method yields more phytochemical compounds than the maceration technique(Dhanani et al., 2017). However, a lower antioxidant and TPC content were observed (13.67% and 49.11 mg GA/g) compared to UAE, which extracts a substantially higher activity of antioxidants (22.8% and 63.13 mg GA/g)(Mohammadpour et al., 2019).

The performance of the UAE is almost equivalent to that of SXE (Thilakarathna et al., 2023).

2.6.2 Non-Convectional Extraction Method

Microwave-assisted extraction (MAE) is the technique used to extract essential compounds by providing energy to the molecules based on ionic conduction and dipole rotation to destroy the plant cells with the aid of small traces of moisture contents. The heated moisture was evaporated and generated pressure on the cell wall, which helped in leaching the active constituents from the ruptured cells into the medium solvent. One of the main difficulties associated with microwave treatment is the separation of finer particles from the solvent(Talati, 2017). MAE is less fast, unfavorable to acidic conditions, and easily exposed to contamination as compared with the UAE. However, as compared to other extraction methods, it is more economical, environmentally friendly, and less time-consuming in terms of solvent amount(Kaseke et al., 2021). Maximum antioxidant activity of extract was observed by MAE extraction rather than Soxhlet extraction(Karami et al., 2015). Nevertheless, it has some limitations in the extraction of polyphenol compounds and heat-sensitive polyphenols such as anthocyanins and tannins (Kumar et al., 2023).

Ultra-sound-assisted extraction (UAE) is another non-convection extraction method that is available as an ultrasonic bath or ultrasonic probe based on the choice of extraction. It uses a high-pressure difference wave, which induces an alternative compressing and stretching of molecular structure near the liquid-solid interfaces, to break down the cell. Operating at suitable extraction temperatures for thermolabile components and lower extraction periods with high extraction efficiency (Belwal et al., 2018). However, ultrasound-assisted leaching, a lack of solvent recovery system, extended the duration of rinsing equipment after extraction, and increased the risk of losses or contamination of the extract(Kaseke et al., 2021). Moreover, the UAE also generates ROS, which results in lowering the concentration and degradation of some of the suspected compounds (Belwal et al., 2018).

2.7 The Effect of Extraction Parameters against Phytochemical activities

The quantity and quality of oil from the plant depend on the selection of parameters, such as temperature, time, particle size, and types of ideal solvents. The ratio of liquid to solid had no statistically significant effects on the yield of phytochemicals, but sufficient solvent volume is required to enable proper hydration and swelling of solid particles (Che Sulaiman et al., 2017; Solaberrieta et al., 2022). Smaller particle size and drying have been chosen for oil extraction due to the greater interfacial area, softening the tissues and lowering the viscosity of the oil (Daniel Alemu et al., 2022).

Criteria for an ideal solvent for a certain pharmacologically active constituent should be highly selective for the compound, not react, have a low price, and be harmless to man and the environment. The polarity degrees of the solvents significantly affected a single bioactive constituent that was present in the plant extracts. Solvent types determine the type and quantity of active constituents in plant materials (Hossen et al., 2022). Polar solvents are used to extract phenolic compounds, glycosides, and saponins, whereas non-polar solvents are used to used to extract a fatty acids and steroids. Most polar solvents are used in the extraction of a wide range of polar compounds as they interact with polar molecules. Ethanol is the best appropriate extraction solvent for polyphenol extraction and is safe for human consumption, whereas methanol solvent is more suitable for the extraction of lower molecular weight polyphenols (Do et al., 2014).

Temperature increases the diffusivity of oil from the cells, reducing the surface tension and oil viscosity. In that way, it improves the extraction rate and the yield of oil recovery. Much of the total polyphenol compounds are extracted at higher extraction temperatures. This is due to the breakdown of lignin-phenolic acid bonds, which gives rise to more phenolic acid. Mostly, the highest total polyphenol and oil yield are observed in the temperature range of 60°C to 80°C by the Soxhlet extraction method(A. Kumar et al., 2023). However, some phenolic compounds are reduced to greater than 70°C due to the thermal degradation of some phenolic compounds. According to some previous studies conducted on P. falcatus seed oil extracted by aqueous methods, a significant oil yield was shown at an extraction temperature of 70°C to 80°C(Daniel Alemu et al., 2022).

Time is a key point for the minimization of costs and the development of a better process. Extraction times that are too long or too short can lead to either lower yields or lower-quality extracts. Exposing the biomass to the solvent for a sufficient time would allow the desired compounds to migrate into the solvent(Che Sulaiman et al., 2017). The maximum yield of phenolic compounds was observed at the extraction time of six hours (6 hr) by the Soxhlet extraction method from licorice root(Karami et al., 2015). Too short an extraction time might lead to incomplete extraction of oil (under-extraction), which can result in a lower yield and a poorer quality extract. Whereas too long an extraction time (over-extraction) causes the extract to degrade and remove thermolabile compounds (Mahmud et al., 2019).

2.8 Optimization Methods of Oil Extraction

There are two most commonly used optimization studies. These are one factor at a time and response-surface methodology (RSM). In a single-parameter study, a one-factor-at-a-time approach was used to find the best operating conditions and achieve the desired limits for each factor(Mohammadpour et al., 2019). It is the technique of changing a single factor at a time while the other factors are set to be constant. The objectives of this are to determine the ultimate working ranges of level for one factor that provides the desired response. The optimum level of a factor is determined based on certain levels of other factors. However, it does not estimate the interaction between the levels of each factor. To determine the interaction effect of the process variable, an RSM is employed. It uses several mathematical and statistical techniques to model, analyze, and optimize the response by evaluating the

relative significance of variables that influence the interest response(Zhao & Zhang, 2014). Reduce the number of experimental runs and generate reliable results. A regression model can correlate the correlation between the input parameters and response. To confirm the results, sets of experiments were carried out in triplicate under the selected optimized conditions(Ngomade et al., 2023).

2.9 Agar Well Diffusion Assay

The agar-well diffusion method is used to determine the antimicrobial activity of plant extracts. Disc diffusion offers advantages over the others due to its simplicity, low cost, and ability to test an enormous number of microorganisms. About 6 mm of paper discs were placed in a beaker covered with aluminum foil and sterilized in an autoclave at 121°C for 15 minutes. The paper disc was placed into four variable extract concentrations for a given time in order to allow the solvent to diffuse. The paper discs were transferred by sterile loop to media seeded with a spore suspension of MOs. The Petri dishes were incubated at 27°C for 3 days for fungus and at 37°C for 24 h for bacterial tests (Guluma et al., 2020). The wells are separated by not less than 20 mm from each other and 10 mm from the edge of the plate. The potential strengths of antimicrobial agents were categorized into four and explained in the range of less than 8 mm as being less sensitive, 8 mm–14 mm as active, 14–19 mm as very active, and those greater than 19 mm as extremely sensitive (Bendjedid et al., 2021).

2.10 Skin Infection

Skin guards the internal organs of the human body from numerous harmful stresses induced by environmental factors. The main skin ailments that affect humans' skin are psoriasis, microbial infection, aging, wrinkles, allergies, seborrheic, and discoid eczema. Among them, skin aging, fungus, and bacterial infection are the most frequently observed on human skin.

Nowadays, about 70% of the bacteria that can cause an infection are resistant to at least one of the drugs mostly used for treatment. Prolonged or excessive use of antimicrobial ointments can lead to the development of antimicrobial resistance. This occurs when microorganisms adapt and become resistant to the effects of the active ingredients in the ointments. Many investigations have been reported regarding multidrug-resistant bacteria; among them, *Escherichia coli, Pseudomonas aeruginosa,* and *S. aureus* species are currently developing a broad spectrum of high resistance to antibiotics and causing a number of skin infections

(Añibarro-Ortega et al., 2019). These bacteria commonly causing various cutaneous infections such as cellulites, impetigo, bacteremia, folliculitis, furuncle, endocarditis, carbuncle, and abscess(Otang & Afolayan, 2016; Saddiqe et al., 2020). Candidiasis is considered to be the second most prevalent fungal skin infection in humans caused by Candida species, which are observed in the dermatomycoses of nails, hands, and feet. Fungal infections general characterized by eczema, ringworm, dermatitis, skin rashes, and inflammation. Fungal skin infections may be classified as superficial or subcutaneous mycoses. A superficial fungal infection involves the outer layer of skin, i.e., the stratum corneum, mucous membrane, nails, and cuticle of the hair shaft (Ansari et al., 2019).

2.11 Topical Drug Delivery

A topical drug is a formulation that contains a dermatologic agent to achieve the desired drug concentrations at the proper site of action to treat cutaneous disorders of the skin with a reduced systemic drug concentration. It prevents the reaction between the encapsulated ingredient and other molecules in the product matrix(Costa & Santos, 2017). Skin drug delivery systems have been widely used and provide for several purposes, such as surface effects, dermal effects, and systemic effects, as well as deeper tissue anti-inflammatory drugs (Kaci et al., 2018). The selection of the route of treatment depends on the severity of the disease and the comorbidities of the patient. The topical route is largely preferred due to the dermal nature of the disease, ease of application for the patient, minimization of systemic side effects, and lifelong use of medication to manage the disease.

Topical and transdermal application is an attractive strategy to treat disease rather than oral drug delivery. Topical products have a localized effect rather than systemic effects. In the transdermal delivery system, the drug is transported through the epidermis layer, followed by diffusion into the viable dermis, and ultimately reaches the systemic circulation to achieve therapeutic levels(Manna & Rudra, 2020). However, the delivery of drugs across the skin is a laborious task and requires the aid of external agents or carrier systems for efficient permeation. Burst and sustained release are two major features that should be associated with cosmetic delivery systems. Burst release is important to improve the penetration of active molecules, while sustained release is required to supply the skin for long periods. Therapeutic

drugs might be prepared in the form of gel, lotion, cream, or ointments to satisfy the preferences of the patient and physicians.

Herbal cream and ointment formulations were used in burn wound healing(Heidari et al., 2019), simple ointment in the incision and excision wound healing(Ghosh et al., 2019; Mahajan & Itankar, 2020). However, in the drug release study, herbal antifungal hydrophobic base showed minimal release or diffusion of the flavonoids compared to the PEG-based hydrophilic formulation(Ansari et al., 2019).

2.11.1 Emulsion

Emulsions are colloidal mixtures of two immiscible phases: a dispersed and an external phase. Stable emulsion comes with the aid of high-energy approaches such as ultrasonication, high-pressure homogenous emulsion, microemulsion, emulsification solvent evaporation, emulsification solvent diffusion and solvent injection, and phase inversion(Kumari et al., 2022). However, emulsion has some drawbacks, such as poor storage stability, size distribution, poor drug payload, and high manufacturing costs(Garg et al., 2020). To reach kinetically stable emulsification, increasing the viscosity and adding electrolytes are used to attain the immobilized dispersed phase droplets.

Cream

Creams are semisolid emulsions that are thermodynamically unstable. It might be prepared as hydrophilic, which contains large amounts of water in its external phase and offers advantages such as moisturizing, hydrating, and emollient. While hydrophobic creams contain a water droplet in the internal phase of oil that can provide intensive moisturization and a barrier effect for dry skin, Cream requires the inclusion of an emulsifier agent that is responsible for keeping the two immiscible phases together in a stable form. A cream has less thickness, less greasy consistency, and better spreadability over the absorption site than ointments. However, sometimes it causes severe allergic reactions and clogs the pores, resulting in blackheads (Ojha et al., 2022). There are different types of creams, like cleansing, cold, vanishing, massage, hand, and body creams.

2.11.2 Gel

The gelling vehicles used in the gelation formulation are mostly at concentrations below 15% w/v to increase the surface tension and prevent the flow of the solvent. The movement of the

dispersed phase is restricted in the three-dimensional networks of gels. Based on the physical state of the gelling agent, the gels are classified as hydrogels, which are prepared from water-soluble materials, have the potential to take up and hold significant quantities of liquids and fluids, and can encapsulate and distribute therapeutic agents of hydrophilic drugs. The two types of natural gelling materials for hydrogel formulation are polysaccharides like dextran, hyaluronic acid, and chitosan, and proteins like gelatin and collagen. Hydrogel is an attractive delivery system as it can be easily manufactured and suitable for drug delivery; however, hydrophobic drug delivery is limited and less able to cross the stratum corneum(Ojha et al., 2022). Organogels are capable of dissolving hydrophobic drugs and increasing their permeability through the stratum corneum. The main drawback of organogels is their oily nature, which hinders their removal from the skin after application because of their stickiness and oily residues, leading to lower patient compliance. The manufacture of bigels includes the separate preparation of the hydrogel and organogels, thereby subsequently mixing. Organic solvents that have been employed in the formulation of bigels for drug delivery are jojoba oil, palm oil, liquid paraffin, isopropyl palmitate, caprylic/capric triglyceride and PEG-400 (Martín-Illana et al., 2022). Bigels have also been developed containing organogels based on Carbopol and polyethylene glycol (PEG-400) (Martín-Illana et al., 2022).

2.11.3 Ointment

Ointments exhibit excellent thermodynamic stability and less likely phase separation. Ointments are classified into emollient, medicated, and non-medicated types based on their uses. Ointments are semisolid formulations that could be desired to treat a certain topical ailment. Ointments are more commonly used for the delivery of medication than skin care products(Palefsky, 2010). A compound ointment formulation allows you to blend a wider range of active and non-active ingredients than a simple ointment. The synthetic ointment contains less than 20% water and greater than 50% hydrocarbons, waxes, or polyethylene glycols as the vehicle for external application.

2.11.3.1 Properties of Ointment

The selection of the proper vehicle, pH, and viscosity is important to achieve the desired drug agent without affecting the skin condition or the formulation. Temperature and relative humidity are the main factors that affect the stability of an ointment. The pH of ointments is

another quality factor that can affect the stability of products. Extremely acidic or basic conditions of the ointment hurt the normal body of the skin. Therefore, a pH that is compatible with the human skin usually ranges from six to seven (Odeniyi et al., 2020). The viscosity of an ointment plays a major role as it maintains ingredients in the applied area of the skin and affects its spreadability, thereby facilitating drug diffusion through the ointment. extending the residence time of the formulation on the skin by increasing viscosity (Ugur Kaplan et al., 2019). Less sticky nature of ointment easily distributed to the damaged skin area by small force of action. Evenly distribution of mixing the ingredients renders to reduce the granules and control releases of biological active component.

2.11.3.2 Types of Ointments Bases

The ointment can be prepared into three or four types depending on the type of ingredient used. Therefore, the amount and type of ingredient employed in the formulation determine the types of ointments produced. Hydrocarbon bases provide emollient and protective properties and remain for prolonged periods on the site of action. It is difficult to incorporate aqueous phases into hydrocarbon bases and is limited in use due to its greasy and sticky nature(Ojha et al., 2022). Whereas, absorption bases has relatively less emollient properties, and contain a small amount of water. Both hydrocarbons and absorption bases are difficult to remove from the skin due to their hydrophobic nature. A water-removable base is a type of oil-in-water emulsion that has a large proportion of aqueous with the aid of suitable emulsifying agents. A water-soluble base is a type of hydrophilic ointment that is easily removed from the skin due to its good water solubility. Ultimately, the choice of ointment excipients will depend on the specific formulation and application.

2.11.3.3 Selection of Appropriate Ointment Base

The choice of appropriate excipients or vehicles for an ointment formulation depends on the specific requirements of the medication, compatibility with active components, stability, contact duration on skin and its intended use (Farhan et al., 2021). The main advantage of bases in the ointment formulation is to provide thickening to control consistency, maintaining stability, emulsion integrity and be sure of compatibility with the other ingredients. Some of viscosity increasing bases are; some fatty esters, paraffin types, vegetable gums, polyethylene

glycol derivatives, and mineral thickeners. The following table describes physicochemical properties, stability and percent of base used in ointment formulation.

Table 2.2 Characteristic and percentage of base used in ointments formulation

Synonyms	Physiochemical properties	Reference
Propylene glycol	Emollient (5 – 15)%	(Anjum et al., 2023; Uronnachi et al., 2022)
Hard paraffin	Used in hydrophobic or water in oil creams insoluble in water Melting from 47 – 65 ° C 5% is used in ointment	(Ajala et al., 2016)
soft paraffin	Insoluble in water avoids water evaporation and emollient Up to 50% are used in ointment Melting in range of 38 – 60 ° C	(Elzayat et al., 2018; Talekar et al., 2017)
Polyethylene glycol (PEG)	Preparation of hydrophilic ointments and creams PEG < 1000 melted over 37 – 40 ° C; PEG > 8000 melted from 60 ° C– 63 ° C PEG 400 (7.5 - 69.9) % in cream formulations PEG 4000 (84%) used in ointments	(D'souza & Shegokar, 2016)
Emulsifying Wax	Insoluble in water 30% used in ointment and 10% creams Emulsifying agent, stabilizer and gives thickness Melting from (61 – 65)° C	(Talekar et al., 2017; Krishanu, 2021)
Cetostearyl alcohol	Insoluble in water and melts over 55 – 60 ° C Stiffening, viscosity enhancing, and emollient Weak emulsifying properties (5 – 8)% is used	(Joshi et al., 2019)

Hydrocarbon bases are anhydrous substance particularly has some important characteristic, such as inert, protective barrier, improve skin hydration, emollient and non-reactive nature. Paraffin is a set of saturated alkane of hydrocarbons that may be observed in the form of hard, soft, and liquid paraffin. Generally, it is not desirable to incorporate a water phase into an anhydrous ointment base due to not contain the needed emulsifiers resulting in attempts instability, promotes microbial growth and susceptible to an individual who having a skin sensitivities.

Polyethylene glycol (PEG) base has an emulsifying property that helps to solubilize both water-soluble and oil-soluble ingredients to make stable emulsion (D'souza & Shegokar, 2016). It has a high absorption capacity, promote the regeneration of skin tissue, nontoxic, biocompatible, better stability in the swelling environment and during storage, (Vasowala et al., 2024). Depend on its molecular weight and concentration, PEG bases can provide a range of consistencies with diverse characteristics, which allowing for the creation of ointments, creams and lotions. The ointment formulated at the ratio a mixture of 70 % PEG-400 and 30 % PEG 4000 is to prepare a semi solid base. PEG ointment base has an excellent carrier and exhibits a synergistic action when loaded along with a therapeutic agent. Incorporating vegetables oils into a PEG base offer benefits such as enhances moisturization, emollience and occlusiveness. PEG has been much more explored with other co-polymers in the form of topical medication to treat diseases namely wound healing, antimicrobial activity and psoriasis. It can be applicable in the treatment of wound pathogenic microorganisms (A. M. Donkor et al., 2020), skin infection bacterial and fungus (Donkor et al., 2016; Punareewattana et al., 2023) and psoriasis (Vasowala et al., 2024) in the form of ointment. Its efficacy of wound healing potential is better effective than Carbopol 940 gel formulations (Shukla & Kashaw, 2019).

2.11.3.4 Basic Ointment Formulation

Ointment formulations made from herbal medicines can be prepared by extracting the desired extract from the plant parts and incorporating them into an ointment base. A suitable base for herbal ointment formulation is selected based the compatibility of extract (Fahimi et al., 2016). The three most important techniques of ointment preparation are levigation, trituration and fusion are frequently used to incorporate the drugs, aqueous and oil phase components into the

ointment base. Selections of those methods are depending on the type of bases, solubility and stability characteristics of active pharmaceutical ingredients. Levigating is the method usually used for the incorporation of small quantities of dried insoluble coarse substance into molten bases with a continuous rubbing. In other hand, fushion is the method applied to when drugs and other solid medicaments are melted together in their descending order of melting point. At the end, trituration is the techniques employing for insoluble kinds of finely medicated agent are grounded in small amount within a bases followed by geometric dilution. Oleaginous ointments are prepared by both levigation and fusion process. When the ointment base is a type of hydrophilic preparation, a levigating agents, such as alcohol, glycerin, liquid PEG or propylene glycol are used to incorporate or disperses the hydrophobic components into the internal aqueous phase otherwise it should be use a hydrophobic levigating agents(Choudhury et al., 2017). A hydrophobic emulsifying agent is sometimes employed in the case of large amount of water incorporation into hydrocarbon bases under levigation. The aqueous phase and oil phase were separately heated in the temperature range of 70℃ to 80℃ and finally mixed with continues stirring in less than five minutes in a water bath.

CHAPTER THREE

3 MATERIAL AND METHODS

3.1 Equipment and Materials

Round bottom flask, electric weighting balance, digital pH meter, beaker, test tubes, aluminum foil, pipette, Petri-dish, spatula, what- man filter paper, measuring cylinder, sieve, Erlenmeyer flask, thermometer, oven drier, conical flask and heating mantle

3.1.1 List of Reagents and Chemicals

Distilled water, 96 % Ethanol, 99% Methanol, hexane, chloroform, 2,2-diphenyl-1-picrylhydrazyl, phenolphthalein, glacial acetic acid, Folic Ciocalteu, Gallic acid, sodium carbonate, starch, potassium iodide, sodium thiosulphate, Aluminum chloride, potassium hydroxide and Mueller Hinton agar.

3.1.2 Apparatus / Instruments

Soxhlet extractor, Rotary evaporator, Viscometer, Ultraviolet-visible spectrophotometer, Gas Chromatography - Mass Spectrometry, and Fourier Transform Infra-Red Spectrophotometer.

3.1.3 Microorganisms

The activities of extracts and ointment were tested on gram-positive strains (*Staphylococcus aureus*, *Enterococcus faecalis*) and gram-negative strain (*Escherichia coli*, *Pseudomonas aeruginosa*) and a single fungus (*Candida albican*). These microorganisms were obtained from Microbiology Laboratory of Biology department of ASTU.

3.2 Methods

3.2.1 Raw Material Collection of P. Falcatus Seeds

The matured seeds of *P. falcatus seed* were collected from the ground surface around the *P. falcatus* tree that found in ASTU. The seed dried using sun drier with frequent spreading to control fungal growth for two weeks. The hard-outer shells of epicarp and mesocarp manually decorticated. The seed was pulverized to a powder using an electrical grinder into four ranges of particle size.

Figure 3.1 The process of matured pod seed to the kernel seed of *P.falcatus* seed

3.2.2 Size Reduction

Particle size analysis was conducted to attain the desired size and achieving the maximum oil yield. The powdered seed was sieved with different sieves sizes starting from 0.75 mm to 2 mm in range.

Electrical grinder **Grounded seed** **Sieve shaker**

Figure 3.2 The grounded seed of *P.falcatus* that sieved at different particle size

3.3 Overview of Experimental Frame Work

In this study, various experimental work was conducted by extracting P. *falcatus* seed oil and *A.vera* extract under different extraction methodologies. The extracted plants extracts were investigated for its phytochemical, antioxidant and antimicrobial contents. The extracts were also characterized instrumentally by GC-MS and FT-IR spectra for the identification of essential compound and functional group of a molecule's. The desired amounts of plant extract were incorporated into PEG ointment for the development of herbal-based antioxidant and antimicrobial ointments. The following process block diagram (figure 3.3) describes the experimental design of the whole process carried out at the laboratory stages.

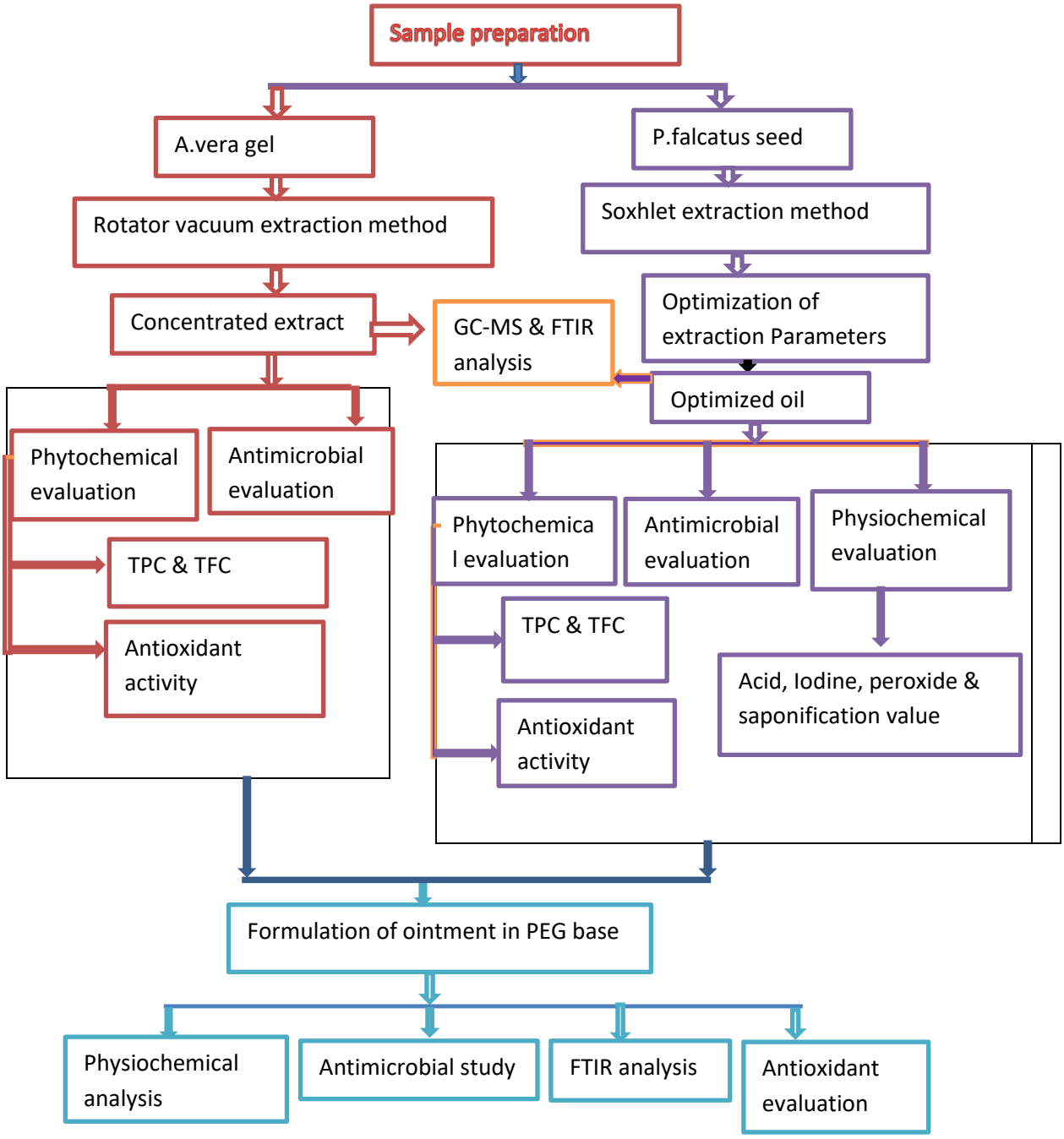


Figure 3.3 Process block diagram of the experimental work

3.4 Optimization of the Extraction Parameters of *P.Falcatus* Seed Oil

3.4.1 Study of One Factor at a Time

Before studying the interaction factor, a one-factor-at-a-time was conducted to reduce the number of experiments required to optimize the oil yield. The five mostly affecting the extraction parameters within their ranges are; time (2 - 8) hr, temperature (60 – 80)℃ , solvent to solid ratio (5– 25)v/w, particle size in ranges (0.75 - 2) mm, and solvents type (methanol, ethanol, chloroform & hexane) were selected for the extraction of optimum TPC and oil yield. The three most important parameters that were exerting a greater impact against TPC yield were further studied for the extraction of maximum oil yield at their suitable levels by RSM.

3.4.2 Response Surface Design

Box-bhenken design (BBD) is used to investigate the effects of the three independent factors to get the maximum oil yield. The three major factors affecting the yield were selected based on their preliminary extraction study. Ethanol solvent selected for the extraction of *P.falcatus* seed oil.

Table 3.1 Optimization of the extraction parameters for *P.falcatus* seed oil

Factor	Name	Units	Low level	High level
A	Time	Hr.	2	4
B	Temperature	℃	65	75
C	Ratio	V/W	12.5	17.5

Generally, 20 g of *P.falcatus* seed powder (grounded) was employed at each trial of extraction with a variable ratio of ethanol solvent at 250 mL, 300 mL, and 350 mL in a 500 mL flask according to the Soxhlet extraction method. After the extraction, the solvent was removed from the desired extract by rotary vacuum evaporation at a temperature of 40 °C to obtain the final free ethanol oil extract.

3.5 Preparation of *A. Vera* Extract

3.5.1 Extraction of *A.Vera* Extract

The preparation of crude *A.vera* extract was carried out according to (Manye et al., 2023) with slight modifications. *A.vera* leaves were transported to the laboratory in the plastic bag and any soil residues was washed with distilled water. The roots, rind, and shoot of the leaves were

removed, and the inner gel-like tissue was isolated with a sterilized knife as shown in Figures 3 .4.

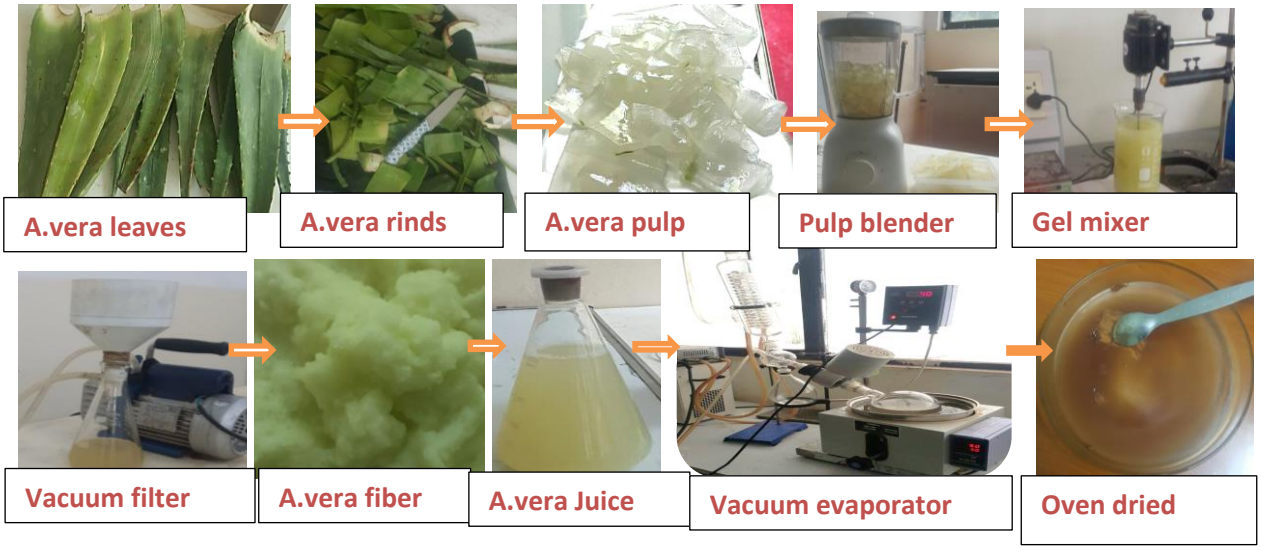


Figure 3.4 The process pictures of extracting aloe vera gel extract

A.vera gel were homogenized using a blender and dissolved in distilled water at the ratio of 1:1 for 48 hr. Thereafter, the filtered aloe vera juice was concentrated in a rotary evaporator at the temperature of 50°C. The extract were further dried in oven at the temperature of 45℃. The dried crude extract was then placed in a refrigerator for further use.

3.6 Moisture Content Determination

The dried petridish in the oven at 105°C for 20 minutes was weighed after it cooled. The samples were dried in an oven at 105°C by frequently measured until a constant weight was maintained. Whereas, A.vera gel was dried at 50°C for 24 hours, and finally, the percentage weight of A. vera gel was determined in both water and dry matter contents.(Palaniyappan et al., 2023).

$$\text{Moisture contents (\%)} = \frac{Wo - W1}{Wo} * 100 \quad \text{..... (1)}$$

W_o = Initial mass of the sample + crucible (g)

W_1 = Mass of crucible + sample after drying (g)

3.7 Determination of the Extract and Oil Yield

The *P.falcatus* seed oil yield was expressed as the weight of the extracted oil per grounded seed weight (Bruno et al., 2022). Whereas, aloe vera extract was determined as the percentage weight of the extract to the gel weight and expressed as the following formula:

$$\text{Oil yield (\%)} = \frac{\text{Mass of extracted oil}}{\text{Initial mass of sample}} \times 100 \quad \text{..... (2)}$$

3.8 Physiochemical Determination of P. Falcatus Seed Oil

3.8.1 Specific Gravity Measurement

The density of oil samples was determined using a pycnometer with a 50 mL capacity. The dry empty pycnometer, with oil and with water was weighed. The specific gravity was determined according to the following equation (Nduka et al., 2021).

$$Specific\ gravity = \frac{W1 - W0}{W2 - W0} \quad \text{..... (3)}$$

W_0 = dry empty glass weight,

W_1 = glass within oil

W_2 = glass within a distilled water

3.8.2 Acid Value Determination

A 0.5 g of an oil sample was added to 20 mL of absolute ethanol. Around 3 drops of phenolphthalein was added and the solution titrated using 0.1 M potassium hydroxide until pink colour observed. The acid value was calculated by the following equation.

$$\text{Acid value} = \frac{56.1 \times \text{Titre value} \times \text{N of KOH}}{\text{Sample weight}} \quad \text{..... (4)}$$

Titre value = Milliliters of KOH used

N = Normality of KOH

3.8.3 Determination of Iodine Value

The visual method was used with some modification based on the procedure of (Tubino & Aricetti, 2013). A 0.15 g of oil was dissolved in 15 mL of 96% ethanol under vigorous magnetic stirring for five minutes. A, 20 mL of 0.1 M of ethanol iodine, 200 mL of distilled water and starch solution were added. Titration was carried out with 0.1 M of sodium thiosulfate solution until the solution shows the disappearance of the blue to a milky color solution.

$$\text{Iodine Value} = \frac{12.69 \times C \times (V_1 - V_2)}{M} \quad \text{..... (5)}$$

C = concentration of $Na_2S_2O_3$

V_1 = volume of $Na_2S_2O_3$ for blank

V_2 = volume of $Na_2S_2O_3$ for sample

M = mass of the sample

NB : 12.69 is molecular weight of iodine used to convert $Na_2S_2O_3$ to I.

3.8.4 Peroxide Value Determination

One g (1 g) of oil was weighted into a conical flask and 20 mL of solvent mixture of glacial acetic acid and chloroform (2:1) was added after addition of 1 g of potassium iodide. The mixture was boiled for 1 minute and hot solution quickly transferred into a flask containing 20 mL of 10 % potassium iodide. One percent starch solution was dropped and titrated with 0.025 N of $Na_2S_2O_3$ until the color is changed from blue to colourless(Nduka et al., 2021).

$$\text{Peroxide value} = \frac{S \times M \times 1000}{\text{Sample weight}} \quad \text{..... (6)}$$

S = M of $Na_2S_2O_3$

M = Molarity of $Na_2S_2O_3$ solution

NB: 1000 is the amount of peroxide value in a kilogram of P. falcatus seed oil.

3.8.5 Determination of Saponification Value

Two gram (2 g) of oil with 25 mL of alcoholic KOH was mixed in flask and refluxed condenser for a 1 hr. After a while, a few drops of phenolphthalein indictor was added after

the flask was removed and titrated with 0.5 N hydrochloric acid until the pink color of the indictor just disappears (Nduka et al., 2021).

$$\text{Saponification Value} = \frac{A-B*56.1}{W} \quad \text{..... (7)}$$

A = HCl used for blank (mL), B = HCl used for sample (mL)

W = weight of sample (g), N = normality of HCl

3.9 Quantitative Determination of Phytochemical Contents

3.9.1 Evaluation of Antioxidant Capacity

A 2 mg of DPPH was prepared in a 20 mL of methanol as reagent solution. About 180 µL was transferred into a test tube containing a sample to get the final volume of 200 µl. The control was prepared in the same manner by replacing the sample with methanol. The reaction mixture were incubated for 1hr and absorbance of was taken at 517 nm (Odeniyi et al., 2020).

$$\text{DPPH inhibition}(\%) = \frac{[\text{control}]-[\text{sample}]}{[\text{control}]} \times 100 \quad \text{..... (8)}$$

Control = Absorbance of DPPH + methanol

Sample = Absorbance of DPPH radical + sample

3.9.2 Quantification of Total Phenolic Contents

The total phenolic contents of extracts was done according to the method done by (Mehmood et al., 2022). Briefly, 20 µL of crude plant extract (10 mg/mL), 100 µL of Folin-Ciocalteaus reagent and 80 ml of 7.5% anhydrous sodium carbonate was mixed for about 5 min in vortex. After 30 min incubation period, absorbance was taken at 750 nm and the total phenolic content was expressed as milligram of Gallic acid equivalent per gram of extract.

$$\text{TPC} = \frac{C \times V}{M} \quad \text{..... (9)}$$

V = Volume of the extract (mL)

M = Weight of plant extracts (g)

C = Gallic acid concentration from calibration curve (mg/mL)

3.9.3 Quantification of Total Flavonoid Contents

The flavonoid content of extract was determined using the method adopted from (Saifullah et al., 2019), slightly amended. Series of reference quercetin solutions was prepared. Crude extracts of 1 mg was dissolved in 200 mL methanol. About 20 mL 10 % aluminum chloride was added to the solution in respectively. Measured at 510 nm of absorbance and result was expressed in milligram of quercetin equivalent per gram of extract.

$$TFC = \frac{C \times V}{M} \quad \text{..... (10)}$$

C = Quercetin concentration from the calibration curve (mg/mL)

V = Volume of the extract (mL)

M = Weight of extract (g)

3.10 Characterization of Chemical Composition

3.10.1 Fourier Transform Infrared Spectroscopy (FT-IR)

The functional groups of extracts and ointment were analyzed by FT-IR (JASCO FT/IR-6600 type A) at the scanning speed of 2 mm/sec and resolution of 4 cm^{-1}. The frequency ranges of wave number were used from 500 cm^{-1} - 4000 cm^{-1}. A 2 mg of sample was mixed with KBr powder and pressed into pellet (Minjares-Fuentes et al., 2016).

3.10.2 Gas Chromatography Mass Spectroscopic (GC-MS) Analyzer

A Gas chromatography mass spectroscopic(GC14-A) contains capillary column of having 30 m length, 0.25 mm internal diameter and 0.25 μm film thickness. An initial temperature was set at 45 ℃ for 2 min then linearly increased to 300 ℃ at five ℃/min for 5 min. Samples was diluted in a n-hexane solvent at the concentration of 1% v/v. The injector temperature, detector temperature, sample injection amount, split ratio and helium flow rate were maintained at 250℃, 280 ℃, 1 μL, 1:15 and 1.41 ml/min respectively (Gad et al., 2021).

3.11 Determination of Inhibition Zone of the Extract

Antibacterial activity of the two extracted was tested by agar well diffusion method. Four concentrations of *A.vera* extract (12.5 mg/mL – 100 mg/mL) (Nsofor et al., 2023) were reconstituted in distillate water and 1 mL/mL, 2 mL/mL, 3 mL/mL and 1 mL of *P.falcatus*

seed oil were dissolved in DMSO (El-gied, 2015). The culture plates were incubated at 27 ºc for 48 hours in case of fungi and 37 ℃ in 24 hr for bacteria.

3.12 Formulation and Optimization of Ointment

Development of an effective ointment was prepared by optimizing the ointment bases and active ingredients at various concentrations to attain a proper consistency and good efficacy.

Primarily, it is important to choose a compatible ointment base for the formulation of ointment containing aloe vera extract and P.falcatus seed oil.

3.13 Optimization of PEG Ointments Bases

The excipients used in the preparation were chosen based on the previous studies. Formulations of bases were done through trial and error before the proper formulation was achieved at different formulation ratio of PEG-400 to PEG-4000 at a six level of optimization starting from 85:15 to 60:40 within a five interval. In this method, each of proposed bases was added to the labelled beakers in water bath at 60 °C with continuous stirring by rod glass.

3.14 Optimization of Antimicrobial Ointments

Once a proper combination of excipients determined depend on the desired viscosity, spreadability, texture and smoothness; a one factor at a time was applied to both aloe vera extract and *P.falcatus* seed oil at various concentration in order to achieve the most effective in both antibacterial and antioxidant activities. Initially, the antimicrobial activities of the ointment were evaluated according to the methodology done by Donkor et al., (2021). The synergetic activities of *A.vera* extract and *P.falcatus* seed oil were studied in a PEG ointment base at a different percentage of 2.5%, 5%, 10, and 15% w/w in respectively.

Table 3.2 Antimicrobial optimization of ointment

Composition of ointments	$F_{1,1}$	$F_{2,1}$	$F_{3,1}$	$F_{2,2}$	$F_{2,3}$	$F_{2,4}$
P.falcatus seed oil (mg)	50	100	150	100	100	100
A.vera (mg)	50	50	50	25	100	150
PEG-ointment base (g)	1					

Where, 25 mg (2.5%) (, 50 mg (5%), 100 mg (10%) and 150 mg (15%)

Formulation of ointment containing different concentration of active components were incorporated at the required quantity of extract in the optimized ointment base under continuous stirring until a smooth consistency and an elegant product was formed at the temperature of 70 °C for about 5 min by trituration method.

3.15 Physiochemical Evaluation of Formulated ointment

3.15.1 PH of Ointment

The pH meter was calibrated at pH of 4, 7 and 10 standard buffers before use. A 10% w/v of ointment was prepared in distilled water. The pH measurement was measured within a second round of test having an interval gap of one month in triplicate (Herbal et al., 2022).

3.15.2 Viscosity

The viscosity of the different ointment formulations was determined without dilution using a Brookfield viscometer with spindle no. 64 at 100 rpm at temperature of 25 ℃.

3.15.3 Spreadability

A 2 g of ointment was sandwiched between a glass slide provided with the hook. A 1 kg sample weight was placed on the upper glass slid for a 5 min to expel air and provide a uniform film of ointment. The top plate subjected to pull about 80 g with the help of string attached to the hook. The spreadability value was determined according to the (Fatima et al., 2017) and expressed in g.cm/s.

$$\text{Spreadability} = \frac{L*M}{T} \quad \text{..... (11)}$$

Where, M = Weight (80 g), L = length (10 cm) and T = Time taken to separate the slide

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Physiochemical Property of Oil

The physicochemical properties of *P.falcatus* seed oil, such as iodine value, Saponification value and Peroxide value are in line with almost of the values recorded in the literature. The results of the Physical and chemical properties of the extracted seed oil were shown in Table 4.1.

Table 4.1 Physiochemical results of *P.falcatus* seed oil

Physical property		Chemical property	
Specific gravity @ 40 ℃	0.95	Acid value	1.9 mgKOH/ g
Density	0.97 g/mL	Iodine value	135.36 g I_2/100 g oil
Moisture content	3.80 ± 17 %	Saponification value	180.9 mgKOH/ g
Viscosity	72.84 ± 0.6× 10^{-6} m^2/s	Peroxide value	7.03 meq O_2/kg
		Free fatty acid	0.95 mg KOH/g

The result values were expressed as the means of three independent experiments ± SD.

The physicochemical properties of *P. falcatus* seed oil (specific density, iodine, saponification, and acid value) were in good agreement with the results reported by Bruno et al., (2022). Some of the reported chemical properties, such as iodine values and specific gravity (Daniel Alemu et al., 2022), density, Peroxide and viscosity value (Gupta et al., 2022), were in close agreement with the present work. However, in contrast to this experiment, the disagreement paper reported on the contents of moisture and specific density(Feleke et al., 2012).

4.2 Functional Study of the Oil and Extract Using FT- IR

The presence of some major essential functional groups, such as the phenolic, fatty acid, and amine groups, was detected and confirmed the existence of biologically active functional groups. The functional groups of *P. falcatus* seed oil and *A. vera* extract were analyzed by FT-IR, as shown in figure 4.1.

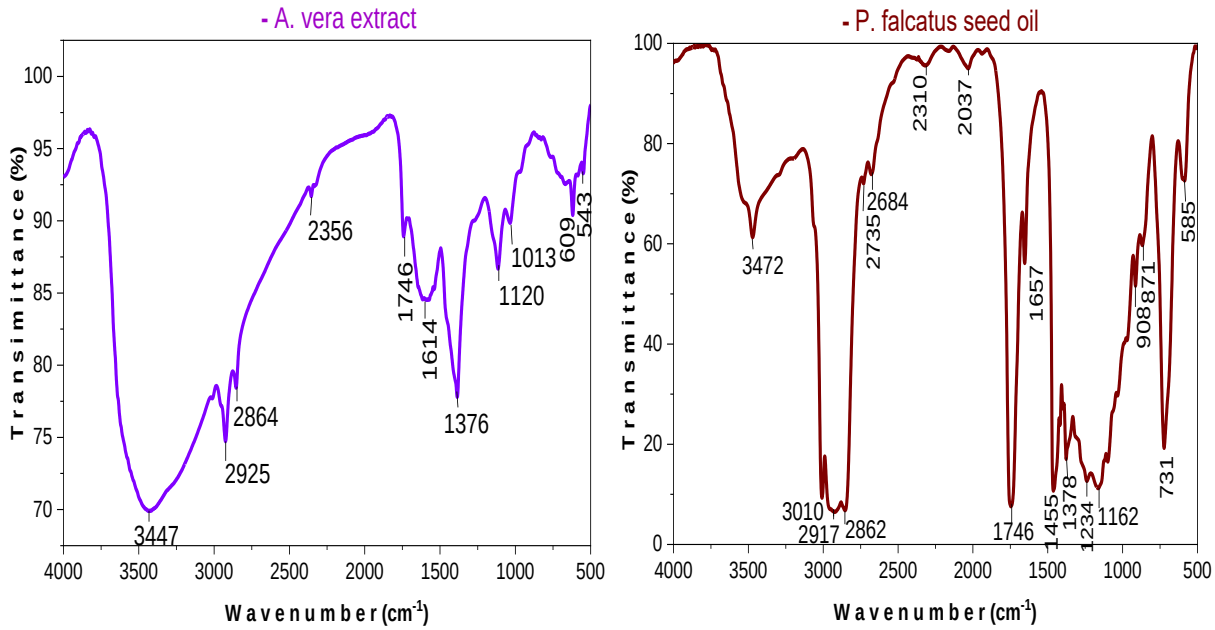


Figure 4.1 FT- IR spectrum of *A. vera* extract and *P. falcatus* seed oil

The IR spectra of the oil and extract were found in the region of 4000 to 500 cm^{-1}. The results of the FT-IR study revealed the existence of distinct patterns of functional groups that should be assigned as carboxyl, hydroxyl, fatty acid, alcohols, amides, and phenolic compounds.

Table 4.2 Description of functional group with its assigned chemical compounds

IR region (cm^{-1})	Peak (cm−1)	Functional group(bond)	Type of compounds assigned
4000–2500 (X–H) region	3447 cm^{-1} and 3472	O–H, and N–H stretching	alcohol or phenol and amine group
	3010	C–H adjacent to a double bond	unsaturation fatty acids of triglycerides(alkenes)
	2925, 2917,	C–H	symmetric and asymmetric

	2864 and 2862 cm^{-1}		stretching of fatty acids and their methyl esters(alkanes)
2500–2000 (≡) region	2356, 2310 and 2037	C≡C	alkynes
2000–1500 (=) region	1742	C=O	carboxylic acid, ketones, aldehydes
1500–600 (fingerprint) region	1614 and 1667	C=C cis stretching	
	1455, 1378, 1376 and 1234 cm^{-1}	bending vibrations of CH_2 and CH_3	presence of fatty acids
	1120 and 1162	C - O and C-N stretching vibration	aliphatic amines

Those peaks found in the region between 3200 cm^{-1} - 3500 cm^{-1} indicates the presence of the alcohol or phenol (O - H stretch) and amine group. The well-known parabola-shaped peak at 3447 cm^{-1} and 3472 cm^{-1} with stretching vibrations suggests the existence of different phenolic compounds like as flavonoids, phenolic acid, anthraquinones and chromones (Bhattacharyya et al., 2019; Jales et al., 2022; Solaberrieta et al., 2022). This functional group can also be appeared in the protein and fatty acid structures. The peak in the region of 3000 - 2700 cm^{-1} shows the presence of the carboxylic acid. The spectra typical absorption peaks at 3010 cm^{-1} of the stretched vibration shows the presence of unsaturation fatty acids of triglycerides. The unique peaks at 2925, 2917, 2864 and 2862 cm^{-1} is assigned to symmetric and asymmetric stretching of fatty acids and their methyl esters within the carboxyl groups (Solaberrieta et al., 2022). The stretching vibrations in the fingerprint region at 1614, 1667, 1455, 1378, 1376 and 1234 cm^{-1} are also implies the presence of fatty acids. The band at 1742 cm^{-1} is associated to C=O from ester bonds linkage (Jales et al., 20220), while the small band at 1614 cm^{-1} and 1667 cm^{-1} is due to the absorption of C=C cis stretching(Donkor et al., 2021). The CH absorption of bending vibrations CH_2 and CH_3 bands can be noticeably seen in the region of 1455, 1378 and 1376 cm^{-1} respectively (Palaniyappan et al., 2023). The band at 1120 cm^{-1} and 1162 cm^{-1} is due to the absorption of C- O ester bonds, whereas the band at 731 cm^{-1} is

corresponding to CH_2 rocking vibration(Donkor et al., 2021). In addition, according to the authors who characterized the enriched extracts of polyphenols, the peaks at 1614 and 1667, 1234 and 908 could be attributed to the ring of C=C stretching vibration, ester C- O stretching and C-N stretching vibration of aliphatic amines respectively (Solaberrieta et al., 2022). The peak absorption at 1013 cm^{-1} revealed the presence of S = O stretch sulfoxide(Palaniyappan et al., 2023)

4.3 GS–MS analysis of *P. Falcatus* Seed Oil

Gas chromatography-mass spectrometry analysis of ethanol extract of *P.falcatus seed oil* were revealed the presence of eight major bioactive compounds. Some of the major essential compound of *P.falcatus* seed oils that exerting the therapeutic activities are stated below in the table 4.3.

Table 4.3 Therapeutic activities of some fatty acid of *P.falcatus* seed oil

Compound name	**Pharmacological properties**	**References**
Hexadecanoic acid, methyl ester	Antifungal, Antioxidant &Antibacterial	(Tyagi & Agarwal, 2017; Ukwubile et al., 2019)
9-Octadecenoic acid, methyl ester, (E)-	Antibacterial, fungi antioxidant and anticancer	(Hagr et al., 2019; Ukwubile et al., 2019)
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Antibacterial, antifungal & Nematicida	(Marrez et al., 2022)
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Antifungal, anti-inflammatory, anti-acne & antieczemic	(F. H. Ferdosi et al., 2021; (Yamuna et al., 2017)
Methyl stearate	Antibacterial	(Ayyandurai et al., 2022)

P.falcatus seed oil contains less saturated fatty acids than unsaturated fatty acids. From the analyzed results, the relative concentrations of 9-octadecenoic acid and 12-octadecadienoic acid are the most abundant in the extracted oil. These biologically active compounds have been used in the cosmetic and pharmaceutical industries due to their powerful antimicrobial,

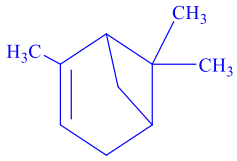
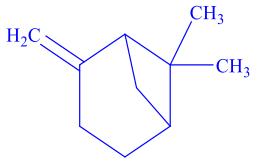
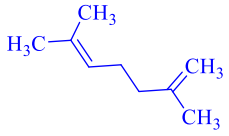
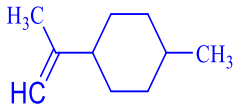
antioxidant, and antiviral properties in several ointment formulations, especially 9,12-octadecadienoic acid

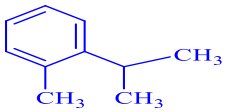
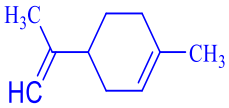
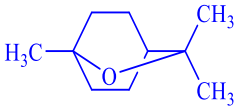
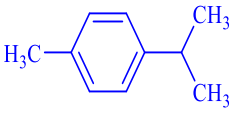
(Zheng et al., 2019). Fatty acid derivatives, especially linoleic and oleic acids, have excellent antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus*(Donkor et al., 2021). A portion of 9,12-octadecadienoic acid is employed in personal care products to nourish the skin(Zheng et al., 2019). A higher percentage of unsaturated fatty acids usually reveal the presence of antioxidant and anti-inflammatory potentials. Eicosanoid acid has the potential to balance the skin's natural sebum and repair excessively dry skin. This oil contains almost all components of glyceryl monolete, which can sometimes be employed in the ointment formulation(Mahalingam et al., 2008).

4.4 GC-MS Analyzed of Aloe Vera Extract

Some of the promising therapeutic activities among the fifteen essential compounds identified by GC – MS has been described in the given table 4.4.

Table 4.4 Therapeutic activities of some assured of chemical compound of *A.vera* extract

Compound name	Compound structures	Therapeutic effect	Reference
α-Pinene		Antifungal and antibacterial	(Guluma et al., 2020)
β-Pinene		Anti-cancerous, antimicrobial, antioxidant and anti-inflammatory	(Patil et al., 2021)
β-Myrcene		Flavors,vitamins, Antioxidant, anti-ageing and antibacterial	(Połeć et al., 2020)
Alpha -Phellandrene		Antioxidant, antibacterial and anti-cancer	(Thangaleela et al., 2022; Wojtunik-Kulesza et al.,

			2019)
O-Cymene		Antifungal	(Feng et al., 2021; Zouirech et al., 2022)
D-Limonene		Antibacterial, antifungal, anti-inflammatory and antioxidant	(Thangaleela et al., 2022)
Eucalyptol		Antifungal activity	(Panikar et al., 2021)
Gamma -Terpinene		Inflammatory, Antibacterial and antioxidant	(Patil et al., 2021)

The higher content of terpenes, α-phellandrene, and β-myrcene indicates that the extract contains higher antioxidant activity (Wojtunik-Kulesza et al., 2019). B-myrcene provides a flavor when combined with other volatile compounds such as limonene and 3-carene, which have an important role in the aroma and flavor production of products(Surendran et al., 2021). Aromatic compounds such as alpha-phellandrene, myrcene, γ-terpinene, α-terpinene, and α-pinene showed strong inhibitory activity against antimicrobial features among the major bioactive compounds presented(Ogidi et al., 2021).

4.5 Preliminary Screening of Phytochemical

Preliminary phytochemical evaluation is an indication for the detection of secondary metabolites. Secondary metabolites are active constituents that are found naturally in plants and are accountable for their combating and preventing disease properties(Guluma et al., 2020). The preliminary phytochemical screening of the ethanolic extract of *P.falcatus* seed oil and *A.vera* extract was carried out to determine the existence of various polyphenol components, such as alkaloids, flavonoids, terpenoids, saponins, and tannins, by using the standard procedures as described below.

Table 4.5 Phytochemical tested of *P.falcatus* seed oil and *A.vera* extract

Phytochemical	Test used	Observed colour	*A.vera extract*	*P. falcatus seed oil*
Phenol	Ferric-chloride	bluish black	+	+
Flavonoid	Zinc-chloride	Yellow precipitate	+	+
Tannin	Ferric-chloride	brownish green	-	+
Alkaloid	Mayer's test	brown precipitate	+	-
Terpenoids	Salkowski's	reddish brown	+	+
Saponins	Foam test	Froth test	+	+
Sign of representation; + = Present, − = absent				

Crude ethanol extracts of *P.falcatus* seed oil and *A.vera* extract have shown the presence of saponins, tannins, terpenoids, flavonoids, and phenols. However, alkaloids and tannins were not detected in aloe vera extract, except alkaloids in the crude ethanol extracts of *P. falcatus* seed oil (S. Kumar et al., 2017).

4.6 The Effect of Extraction Parameters against the Yield and TPC of *P. Falcatus* Seed Oil

4.6.1 The Effect of Particle Size and Feed to Solvent Ratio on Extraction Oil Yield

The effects of particle size and solid-to-solvent ratio on the extraction of oil yield were optimized by one factor at a time at a constant extraction of temperature and time. The range of particle size (0.75 - 2) mm and solvent-to-solid ratio (5- 25) v/w were selected at the maximum extraction temperature and time of 70 for 6 hr based on the previous result reported(Ngomade et al., 2023). Ethanol solvent is used from the primarily detection among the various solvent extraction that more affect simultaneously on the yield of TPC and oil recovery.

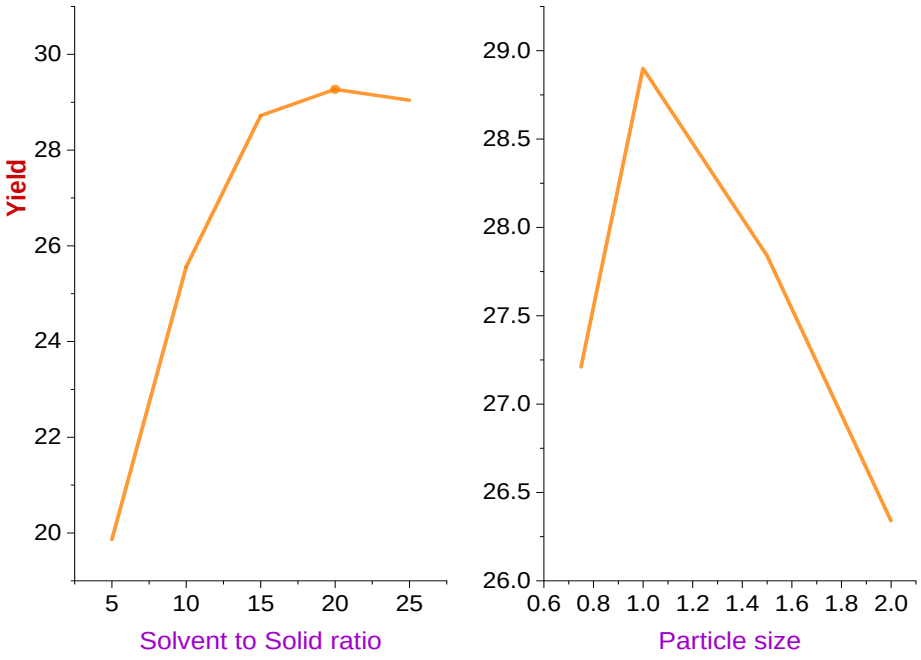


Figure 4.2 The effect of feed to solvent ratio and particle size on the yield oil extraction

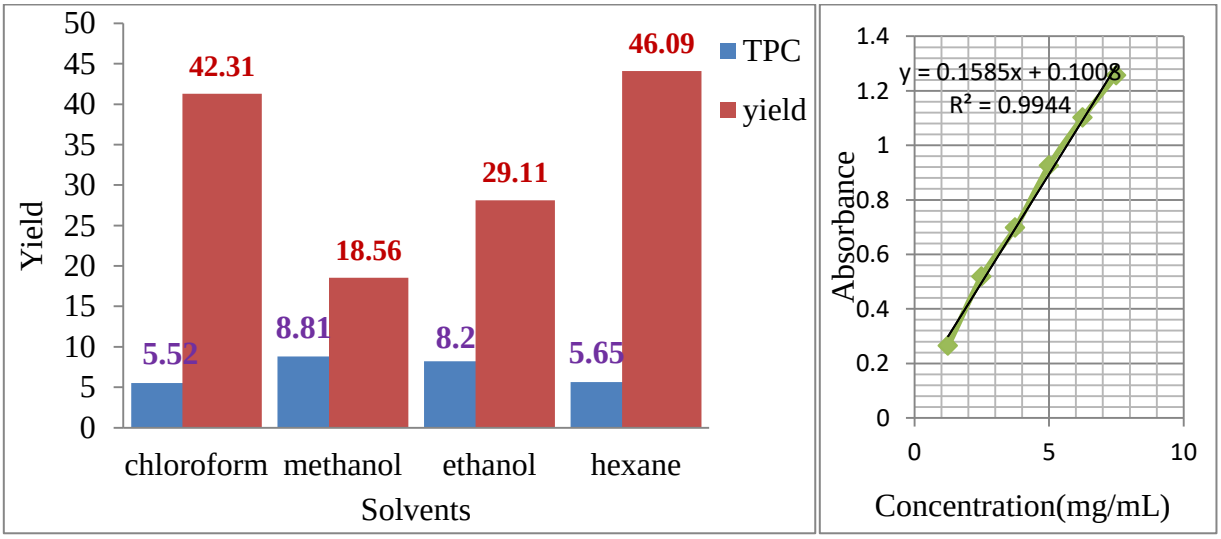
Based on the yield of maximum oil extraction, the particle sizes that are approximately to one millimeter (≅ 1 mm) was selected. Where those particle sizes are almost found between 1 mm and 1.5 mm (1 ≤ 1.5) mm, as can be observed from Figure 4.2. However, the formation of a lump-like powder seed was observed at smaller particle size (0.75 ≤ 1) mm due to the exposure of oil to the outer surface from its cell contents. A radical decrease was revealed at particle sizes that were greater than or equal to 1.5 mm (1.5 ≤ 2) mm. A larger particle size, especially those greater than 2 mm (≥ 2 mm), was not enough to yield much oil as a result of the very small surface area-to-volume ratio, and the yield of oil was continuously decreasing at this particle size range.

The smallest oil yield was found at the lowest extraction solvent because of the equilibrium concentration is achieved overnight at the lowest solvent volume (5 mL/g) and results some of the oil left in the cell that is not released into the medium. The rate of mass transfer decreases, and there is no longer mass transfer as the concentration gradients of the active components reach equilibrium with small extraction solvent. As the amount of extraction solvent is increasing up from this stage of extraction solvent volume, the yield of oil extraction extremely increasing until it reach the maximum extraction solvent (15 mL/g). Above this extraction solvent amount (15 mL/g), the extraction yields of oil gradually increased up to it

reach 20 mL/g of extraction solvent. However, after 20 mL/g of extraction solvent, the yield of oil were continuously decreased. Over use of a large solvent volume for extraction would lead to the degradation and evaporation of essential oils from the extract during solvent recovery. The optimum oil yield of extraction solvent was selected at 15 mL/g with some consideration of the flask volume and cost saving for the next extraction process.

4.6.2 Effect of Extraction Solvents against TPC and Oil Yield

The selection of appropriate solvents in the extraction of oil is the key step in the optimization of extraction parameters, as it has a substantial impact on the yield of extraction. From the comparative investigation of various organic solvents against oil yield and TPC, it was revealed that the highest concentration of polyphenolic compounds obtained had more dielectric properties. The favorable extraction temperature, time, and solvent-to-solid ratio were set at constant values of 70℃, 6 hr, and 20 v/w respectively.



Extraction solvents types against the yield of oil and TPC Calibration curve of standard GA

Figure 4.3 The effects of extraction solvents on the yield of oil and TPC

The above figure 4.3 illustrates the results of the TPC contents of *P. falcatus* seed oil that can be calculated based on the above calibration curve of the standard GA and expressed in mg of GA equivalent per g of extract. The calibration curve of a solution was prepared from 20 µL to 120 µL by two-fold dilution methods to plot the linear graph. Therefore, to plot the linear graph in terms of GA concentration, the amount of GA concentration (1.25–6.25)mg/mL in

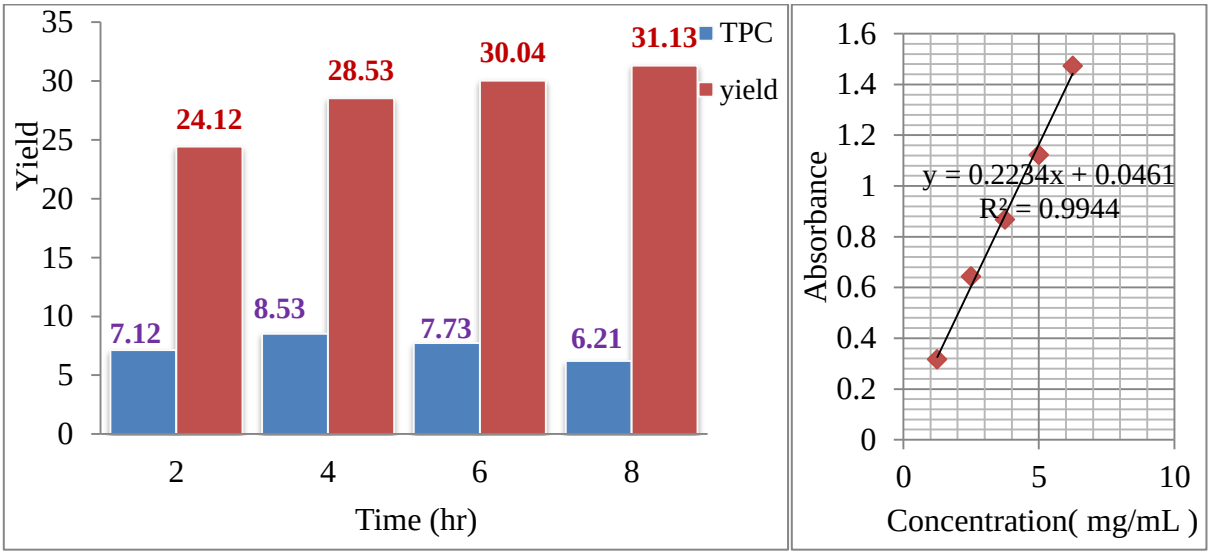
the solution was calculated from the initial amount of GA concentration (0.0625 mg/mL) that was used in the solution.

In this study, both polar (ethanol and methanol) and non-polar (chloroform and hexane) solvents were used for the extraction of *P. falcatus* seed oil. There is a huge difference in oil yields and TPC recovered depending on the dielectric properties of the extraction solvents used. The yield of *P.falcatus* seed oil decreased in the following order of extraction solvents: hexane > chloroform > ethanol > methanol. However, increasing the oil yield is not always means a high TPC value as it can be observed from the above figure 4.2. The oil yields of methanol extract is the least among the solvents employed for oil extraction. However, it contains a huge amount of TPC value than even those yields the highest oil recover solvents. which is positively agreement with the findings of Al-Ghanayem et al., (2022). However, methanol and ethanol were highly capable of extracting more biologically active components compared to non-polar solvents, as shown in the figure 4.2. The methanol extract of P.falcatus seed oil extracted more total phenol content, followed by ethanol among the solvents. This finding is in good agreement with the previously reported findings on different fixed seed and plant leaf solvent types investigated(Royani et al., 2023; Guluma et al., 2020; Shewale & Rathod, 2018). The ethanol solvent is selected for further optimization of the oil extraction by considering, from the point of view of its safety to the environment and low toxicity to humans, its capability of extracting both lipophilic and hydrophilic compounds (Gavan et al., 2022). Polar solvents are more favorable for the extraction of antimicrobial and antioxidant agents than non-polar solvents because of their affinity toward active constituents such as total polyphenols, steroid alkaloids, and flavonoids present in plant parts(Mehmood et al., 2022).

4.6.3 The Effect of Extraction Time against the TPC and Oil Yield

The effect of extraction time was studied by setting up the extraction temperature at a suitable constant level of 70°C. This extraction condition did not significantly affects the active chemical compound and the highest oil yield of *P.falcatus* seed oil that was obtained from the previous conducted finding (Daniel Alemu et al., 2022). The ethanol extraction of *P.falcatus* seed oil by Soxhlet extraction at a constant extraction temperature of 70℃ and solvent to solid ratio of 15 mL on TPC is shown in the figure 4.3. The selection of extraction time range is

based on the concept of previous study by RSM with Soxhlet extraction method (Ngomade et al., 2023).



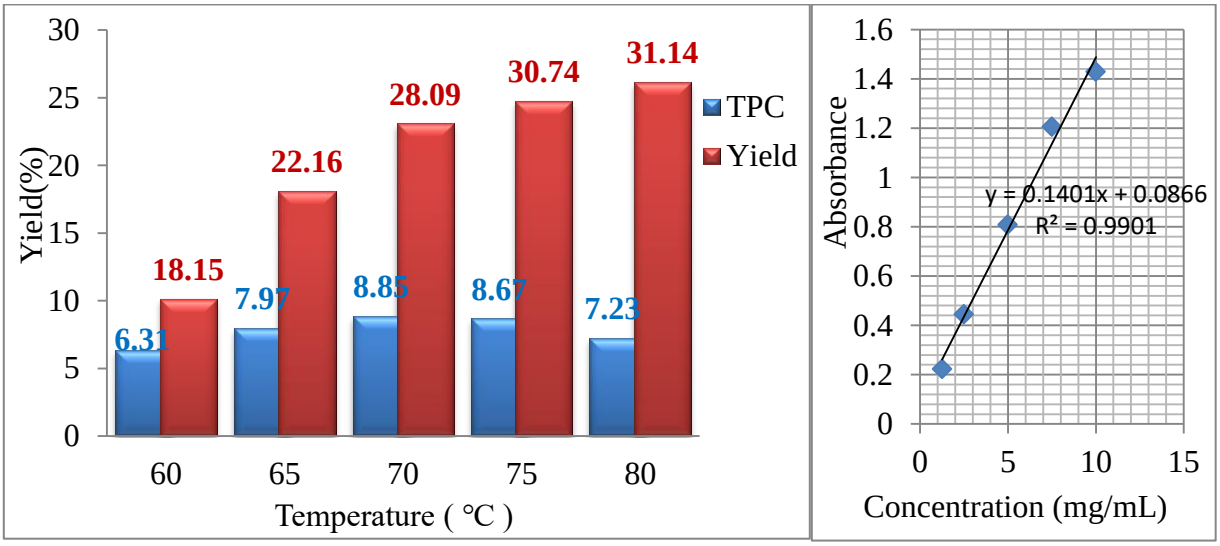
Extraction time variable on the yield of oil and TPC Calibration curve of Gallic acid

Figure 4.4 The effect of extraction time on the yield of oil and TPC

The variable of extraction time was highly affected by the TPC and oil yield, as can be seen in figure 4.4. Ethanolic extraction of oil for a two-hour extraction period provides a small amount of oil yield. The TPC and oil yield simultaneously increased with increasing extraction time; however, after four hours of extraction time, the TPC recovery decreased but the oil yield continuously increased. The increase in oil yield is due to the prolonged exposure of particle cells to the medium. This could provide a chance of sufficient time for the active chemical compound available in the cell to migrate to the extraction solvent as solvent penetration subsequently into the solute. Phenolic compounds were continuously decreasing after four hours (4 hr) of extraction time, resulting in degradation and oxidation formation due to prolonged extraction time(Mahmud et al., 2019). Therefore, by taking into account the cost of saving from the perspective of economics and quantification of TPC, the range of extraction time levels was selected, starting from 2 hours to 4 hours for the next optimization experiment conducted (RSM).

4.6.4 Effect of Extraction Temperature against the Oil Yield and TPC

Most phenolic compounds are heat-sensitive and easily oxidized. Therefore, the upper limiting temperature must be set to preserve the useful components in the extract. In the previous work, the yield of oil decreased after the extraction temperature reached 80°C(Daniel Alemu et al., 2022). The effects of temperature variables on the total phenolic content of oil were studied at different extraction temperatures. Five different extraction temperatures that ranged from 65°C to 80°C at a constant time of 4 hr and an ethanol-to-feed ratio of 15 mL were optimized against TPC, as shown in figure 4.5.



Extraction temperature variable on oil yield and TPC Calibration curve of Gallic acid

Figure 4.5 The effect of extraction temperature on the yield of oil and TPC

The calibration curve of a solution was prepared from 25 µL to 200 µL by two-fold dilution methods to plot the linear graph. Therefore, to plot the linear graph in terms of GA concentration, the amount of GA concentration (1.25–10) mg/mL in the solution was calculated from the initial amount of GA concentration (0.0625 mg/mL) that was used in the solution.

From in the figure 4.5, the extraction temperature revealed a significant effect on the extraction TPC and oil yield of *P. falcatus* seed. The oil yield increased in progress as the temperature increased; however, a maximum variation in oil yields was observed at the temperature of 75°C. On the contrary, the results of the previous study showed that the aqueous extract of *P falcatus* seed oil yielded the maximum oil at an extraction temperature of 70°C (Daniel Alemu et al., 2022). In this study, a higher TPC yield was obtained at a

temperature of 70, while the lowest TPC recovery was observed at an extraction temperature of 60. Similarly, this work is in agreement with the previous research conducted at the lower range of extraction temperature (60 - 65)°C, which had a little effect on the TPC yields (Che Sulaiman et al., 2017). The analyzed data results showed that an increase in temperature generally favors an increase in the TPC of the oil. This phenomenon is due to the breakage of the bond found between phenolic and lignin at elevated temperatures. Generally, a high extraction temperature has a positive effect on the extraction of phenolic compounds, but these increments are inconsistent. Moreover, an increase in temperature would likely cause a decrease in viscosity, an increase in intermolecular interactions, and an increase in diffusion rate in the cell, which causes the movement of compounds from the cell to the solvent more easily (Pods et al., 2022). Increasing the temperature up to 80°C can lead to a significant increase in the yield of oil; however, TPC yield is gradually reduced due to the possibility of degradation and decomposition of some heat-sensitive compounds (Che Sulaiman et al., 2017; Iryana et al., 2022). In addition, prolonged extraction time above 80°C decreases the TPC yield because the high extraction temperature results in the oxidation and degradation of some desired compounds(Mahmud et al., 2019).

4.7 Phytochemical and Antioxidant Contents of the Optimized *P. Falcatus* Seed Oil

The phytochemical composition (flavonoids, phenolic acids) and antioxidant potential of ethanolic extracts of *P.falcatus* seed oil are presented in the table 4.6.

Table 4.6 Shows the TPC, TFC and antioxidant content of *P.falcatus* seed oil.

Phytochemical compounds	Oil	Standard
Phenolic content	10.25 ± 021	Gallic acid (mg GAE /g)
Flavonoids	1.414 $\pm$ 033	Quercetin (mg QE/g)
Maximum radical scavenging (RSA_{max})	73. 3	(%)
IC_{50}	4.24	mg/mL

The total phenolic content of *P. falcatus* seed oil is a little higher than that of its stems and leaf extract, as well as that of the other Podocarpus species (Podocarpus elongates, Podocarpus henkelii, and Podocarpus latifolius), which were extracted with methanolic solvent and had a phenolic content ranging from 2.38 to 6.94 mg/g (Abdillahi et al., 2011). The leaf extract of *P.*

falcatus showed the highest TPC content compared to the current findings, as reported from the previous experiment (Meharie & Tunta, 2020). The present TPC result of *P.falcatus* seed oil is nearly the same as in the other different plant herbal extracts. Similarly, some of the plant seeds having the same range of total phenol content as *P. falcatus* seed oil are; Moringa oleifera seeds extracted by methanol solvent (Bhutada et al., 2016), Sesame seed with methanol extraction (Hussain et al., 2019). The TFC contents of *P.falcatus* seed oil is much lower to from the last reported paper done on its extracts of its leaf (Meharie & Tunta, 2020). The TFC value of the *P.falcatus* seed oil is also nearly the same value with the other plant variety of Giza extract (Abd Elhamid et al., 2022).

The antioxidant activities of *P.falcatus* seed oil are expressed in half-maximum inhibitory concentration (IC_{50}) and percent of radical scavenging activity (% RSA). The IC_{50} value is frequently used as it easily points out the comparative evaluation of antioxidant capacity among the samples. The highest value of the IC_{50} of the extract showed the lower contents of antioxidants in the sample. The IC_{50} value indicates that the concentration of the required sample for scavenging 50% of DPPH radical. The IC_{50} value of *P.falcatus* seed oil has scavenging potential at a concentration of 4.24 mg/mL. This means it requires this much concentration to reduce the total concentration of DPPH radical to half. The oil has the potential to inhibit up to 67.3% of the free radical at its maximum concentration.

4.8 Yield and Compositional Analysis of *A.Vera* Gel

The dry matter content of aloe vera gel is about 1.75% and the rest of volumes were occupied by water (98.24%). This work is go hand in hand with the result reported by (Stanley et al., 2014; Palaniyappan et al., 2023). The recovery of aloe vera extract yield is 2% obtained from 200 mL of aloe liquid fraction that are closer to the reported last year of paper (Alzaid et al., 2022). The pH values of gel and extract are 4.6 to 5.4 which is good in agreement with the range reported previously done(Flores-López et al., 2016).

4.9 Phytochemical and Antioxidant Contents of *A.Vera*

4.9.1 The potential Content of TPC, TFC and DPPH of *A.Vera* gel and Its Extracts.

The following figure 4.7 were describing the comparative evaluation of phytochemical and antioxidant activities of *A.vera* gel and its extract.

Table 4.7 Depict the TPC, TFC and antioxidant content of *A. vera* gel and its extracts

Phytochemical compounds	Extract	Gel	Standard
Phenolic content	22.34 ± 474	1.19 ± 282	mg GAE /g
Flavonoids	5.29 ± 023	0.208 ± 001	mg QE/g
Maximum radical scavenging (RSA_{max})	84.43	28	**%**
IC_{50} value	2.8	22	mg/mL

Aloe vera mucilage (gel) contains much lower TPC compared to its extract. However, the mucilage has higher TPC value than its rind as confirmed from previous paper (Añibarro-Ortega et al., 2019). This suggesting that, aloe vera gel contains high amount of water than its dry matter as confirmed from present work and literature.

The highest TPC contents of aloe vera extract is due to the enrichments of biological active constituents in the form of concentrated. The TPC contents of aloe vera extract has shown some similarity with the other previous conducted experiment (Manye et al., 2023), with those supplemented with various dose of nutritional (Chowdhury et al., 2021) and lower to thirteen investigated aloe vera species (A. Kumar et al., (2020).

Whereas, the TFC content of gel and its extract were showed a significant difference was observed on the TFC. The TFC content of extract is much far away from gel as the result of enrichment of active compound in the concentrated form. The TFC contents of extract has been a great significance compared to last work done (Manye et al., 2023) and slightly lower to the find of (Mehmood et al., 2022). Surprisingly, *A.vera* extract contain higher amount of TFC and TPC than its gel.

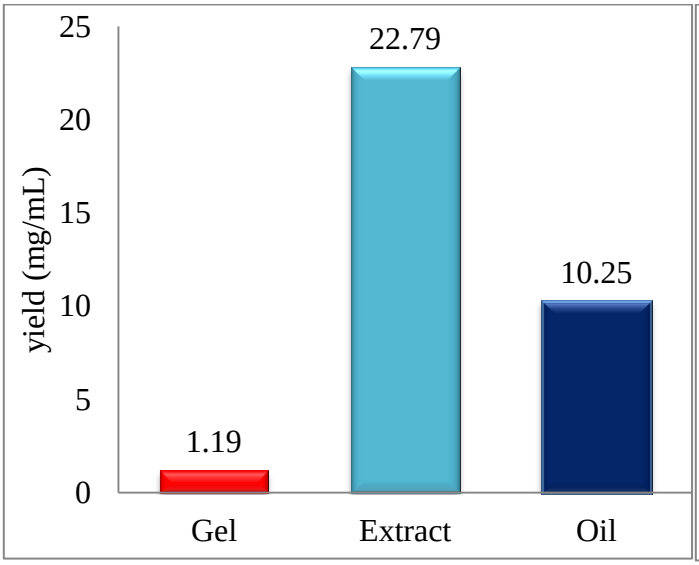
The total antioxidant activities *A.vera* gel and its extract were conducted to determine the antioxidant potential. The antioxidant potential is inversely proportional to IC_{50} value that was determined from the linear regression of the radical scavenging activity versus the extract concentration. The scavenging potential of *A.vera* gel and its extract in accord to IC_{50} value has 22 mg/mL and 4.24 mg/mL of the concentration. The half-maximum inhibition concentration of *A.vera* gel is much lower than its extract. It required more than six times of

concentration to achieve the IC_{50} value of *A.vera* extract. *A.vera* gel and its extract has the capacity to reduce the free radical up to 28% and 84.43% in the given solution. Here also great difference is made between those extracts as result of unmatched inhibition concentration contents. As tried to mention somewhere else, *A.vera* gel has low contents of active chemical compound due to its large portion of volume is occupied by water. The potential content of antioxidant of A.vera extract is slightly higher to the previous reported paper (Bagherian et al., 2021) and lower than macerated methanolic extract (Mehmood et al., 2022), ethanol extract by MAE (Solaberrieta et al., 2022). The present finding pronouncely confirmed to the previous studies that TCP and antioxidant activities ha significantly correlated (Mehmood et al., 2022). Difference of TPC, TFC and antioxidant is might be due to a number of intrinsic and extrinsic factories, such as plant variety, extracting solvents, extraction methods environmental, handling and development stage could be significantly influence on the phytochemical constituents (R. Kumar et al., 2018; Solaberrieta et al., 2022). Therefore, the *A.vera* extract would be a promising for the formulation of antimicrobial and antioxidant product in the drug delivery system (Nunes et al., 2021; Mehmood et al., 2022).

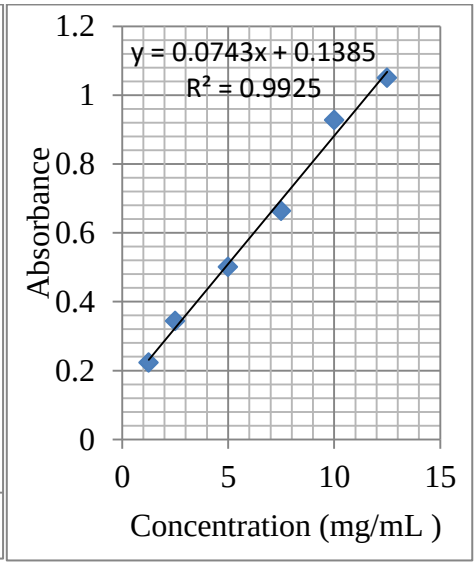
4.10 Comparative Studies of *P. Falcatus* Seed Oil and *A.Vera* Extracts

4.10.1 Total Polyphenol Contents of *P.Falcatus* Seed Oil, *A.Vera* Gel and Its Extract

The following figure 4.5 was explaining the comparative study of TPC yield of *P.falcatus* seed oil, *A.vera* gel and its extract.



Total Phenol contents of extracts



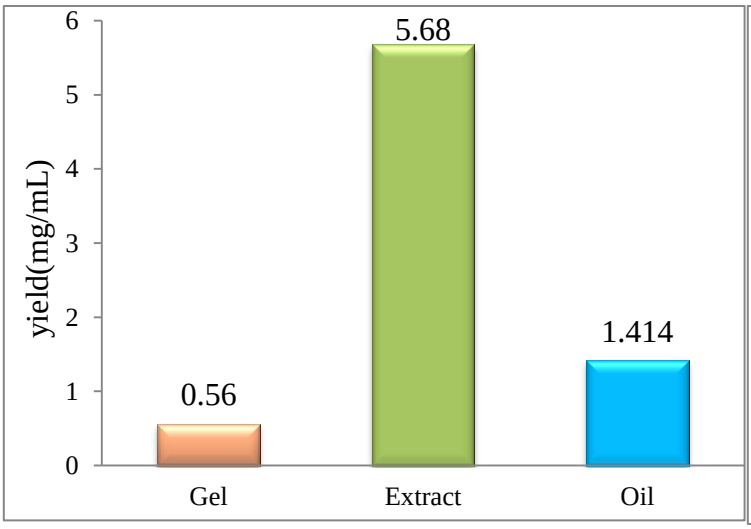
Calibration curve of Gallic acid

Figure 4.6 Illustrates the yields of TPC of *P.falcatus* seed oil, *A.vera* mucilage and extract

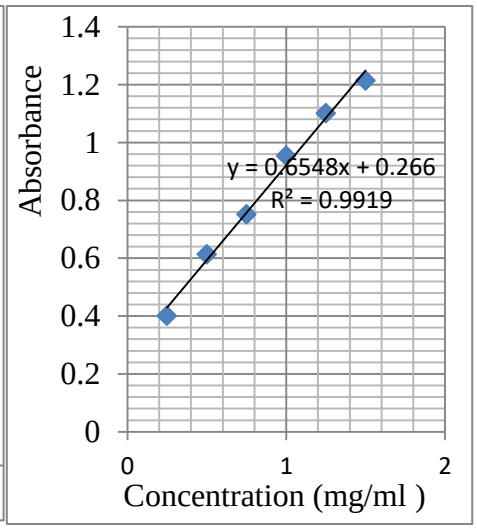
The total phenolic contents of *P.falcatus* seed oil, *A.vera* gel and its extract are depicted on the figure. *A.vera* extract has showed the highest amount of TPC than gel and oil. *A.vera* gel contain the lowest amount of TPC amongest the extracts. Confirmation from the previous work has been also stated that aloe vera extract were two fold greater than the gel (Flores-López et al., 2016). There is also a significance difference of TPC found in the extracts of *A.vera* extract and *P.falcatus* seed oil. Oil bearing plant seed are generaly contains a small amount of biological active componets because of its hydrophobic nature. Since most of the active constituents found in the plant extracts has hydrophilic characteristic. The total phenol compound is part of phytochemical which shows the attributes of hydrophilic nature. Phenolic contents of any plants are directly related to their antioxidant properties. In the present study, the presence of significantly amount of phenolics would be contribute to antioxidant.

4.10.2 Total Flavonoid Contents of *P.Falcatus* Seed Oil, *A.Vera* Mucilage and Extract

The following figure 4.5 was explaining the comparative study of TPC yield of *P.falcatus* seed oil, *A.vera* gel and its extract.



Total Flavonoid contents of extracts



Calibration curve of quercetin

Figure 4.7 Describes the TFC yields of P.falcatus Seed oil, A.vera mucilage and extract

The total flavonoid contents of *P.falcatus* seed oil, *A.vera* gel and its extract are shown on the figure above. *A.vera* extract again also contain the highest amount of TFC compared to its gel and oil. This could be as the result of high concentration and hydrophilic properties of different active phytoconstituents were present in the *A.vera* extract. *A.vera* gel has the least

amount of TFC as compared to aloe vera extract and *P.falcatus* seed oil. Flavonoid is associated with the colour and flavour of the plant natures. This colour and flovour is reduced with other heat sensitives components during the extraction process.

4.10.3 The IC_{50} Value of *P. Falcatus* Seed Oil, *A.Vera* Mucilage and Extract

The half-maximum inhibition concentration of *P.falcatus* seed oil, *A.vera* mucilage and extract were compared with each other's in the figure 4.8.

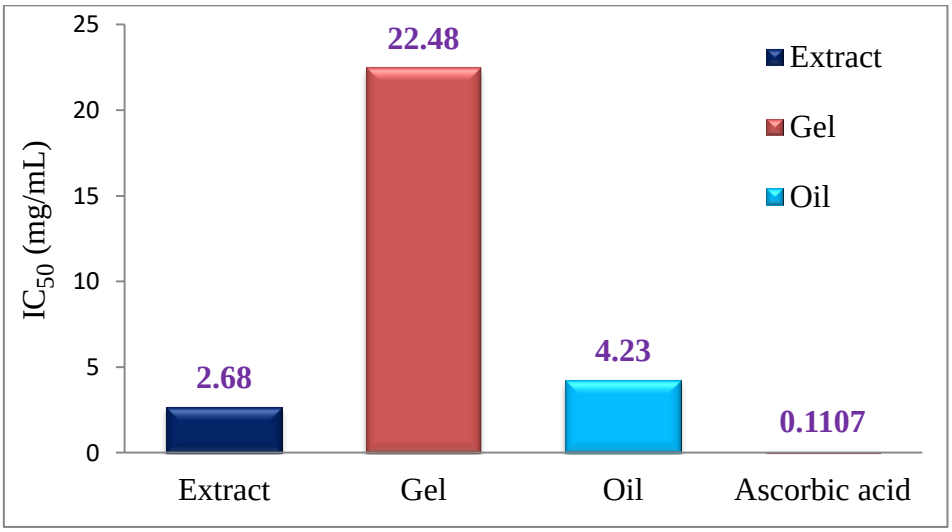


Figure 4.8 The half Inhibition concentration of plant extracts and ascorbic acid

The half-maximum inhibition concentration (IC_{50}) of *P.falcatus* seed oil, *A.vera* gel and its extract were illustrated on the above figure 4.8. High amount of IC_{50} value is not enough to reduce the half free radical at smallest dosage of extract. Based on this concept, the IC_{50} value of aloe vera extract and *P.falcatus* seed oil has revealed a lower value of IC_{50} as comparative to the aloe vera gel and higher to the standard ascorbic acid. Therefore, *A.vera* extract and *P.falcatus* seed oil has capacity to reduce the free radical by half percent at the concentration value of 2.68 mg/mL and 4.24 mg/mL in a given solution. However, the IC_{50} value of aloe vera gel is not enough to scavenge the free radical below the concentration of 22.48 mg/mL. Therefore, *A.vera* extract had the strongest antioxidant activity among the plants examined with the lowest IC_{50} value and somewhat closest to standard ascorbic acid (0.11 mg/mL). Therefore, a combination use of *P.falcatus* seed oil and aloe vera extract is possible to reach the standard ascorbic acid inhibition concentration at the lowest dosage.

4.10.4 The Potential DPPH Radical Inhibition Percentage of *P. Falcatus* and *A.Vera*

The capacity of free radical inhibition percentages of *P.falcatus* and *A.vera* were presented in the figure 4.9.

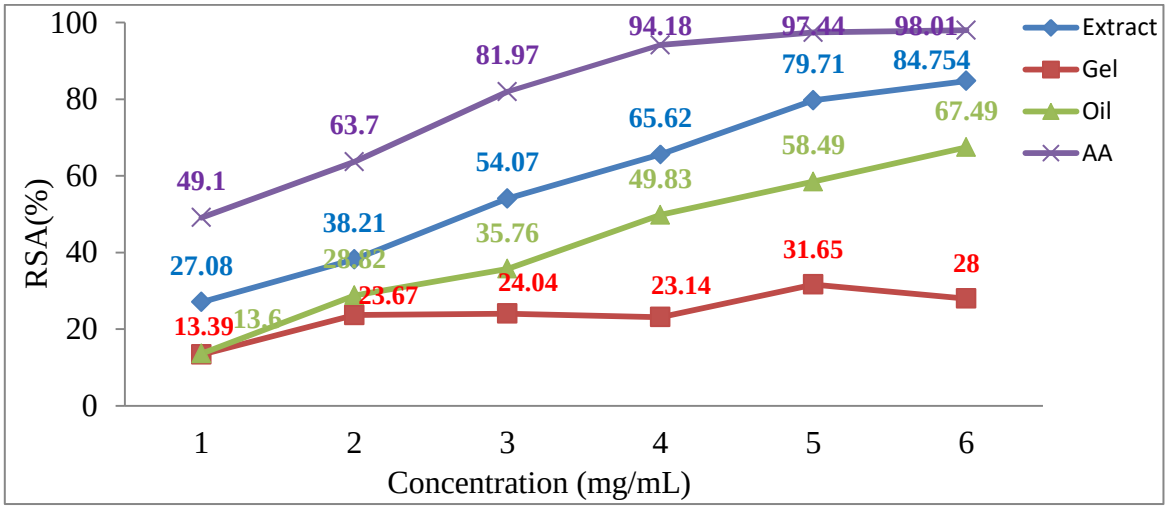


Figure 4.9 Percentage of DPPH scavenging activities of plant extracts and Ascorbic acid

The total antioxidant potential of *P.falcatus* seed oil, *A.vera* gel and its extract were shown on the figure 4.9 In this case, the potential scavenging of free radical were expressed in percentage compared to the standard ascorbic acid. The maximum radical scavenging capacity of *P.falcatus* seed oil, *A.vera* gel and its extract at its maximum concentration are 67.49%, 28% and 84.54% in respectively. Aloe vera extract contain the highest antioxidant compared to the other extracts. The highest reduction of free radical is observed from the extract of aloe vera followed by the oil of *P.falcatus* seed but higher than gel. Whereas, the standard asorbic acid has the potencial to inhibit 81.97% of the radical even at its lower concentration of 3 mg/mL. The potential inhibtion of each extracts is depend on their concentration dosage employed in the solution as it observed from the figure 4.9 (Bendjedid et al., 2021). The potential inhabition of DPPH radical is because of hydrogen or electron donation from active chemical compound present in the extract. In general, both *P.falcatus* seed and *A.vera* extract contain enough amount of antioxidant and it can be applicable in the area of product.

4.11 Model Fitting with Response Surface Methodology

Figure 4.9 demonstrates the relation between predicted and actual value of the experiment carried out on the oil yield of *P.falcatus* seed oil.

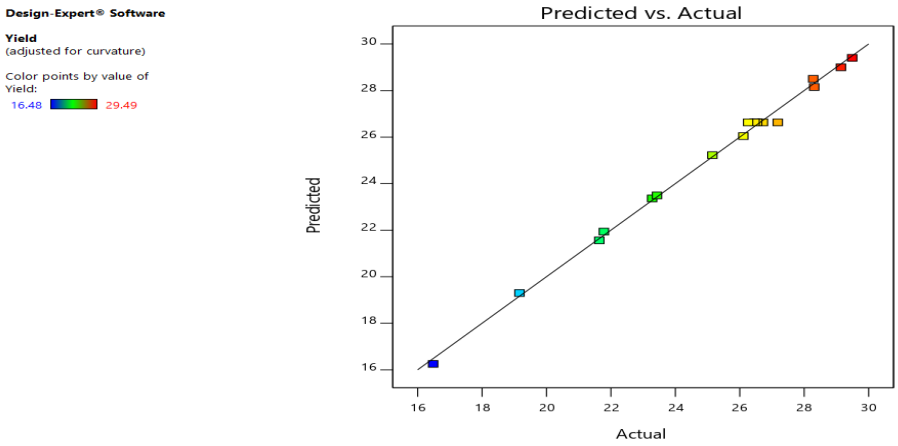


Figure 4.10 Presented the normal plot of experimental versus predicted on the oil yield.

4.11.1 Development of Regression Model Equation

Final Equation in Terms of Coded Factors

Yield = +26.63 + 3.98*A + 2.15*B + 0.8725 C - 0.5075*AB - 0.4475*AC + 0.8075*BC - 2.36 -1.38*B^2 +0.3278*C^2

Final Equation in Terms of Actual Factors

Yield = -548.74275 +14.86560 Temperature +12.70100 Time + 0.312800 Ratio - 0.101500 Temperature* Time - 0.035800 Temperature* Ratio + 0.323000 Time * Ratio - 0.094490 Temperature ^{2} -1.38225 Time^2 + 0.052440 Ratio^2

4.12 ANOVA for Quadratic Model

Analysis of variance was performed to determine the significant differences between the independent variables against the oil yields.

Table 4.8 Regression analysis and ANOVA employed in the model fitting for oil yields

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	207.13	9	23.01	226.81	< 0.0001
A-Temperature	126.56	1	126.56	1247.32	< 0.0001
B-Time	36.89	1	36.89	363.60	< 0.0001
C-Ratio	6.09	1	6.09	60.02	0.0001
AB	1.03	1	1.03	10.15	0.0154
AC	0.8010	1	0.8010	7.89	0.0262
BC	2.61	1	2.61	25.70	0.0014
A^2	23.50	1	23.50	231.56	< 0.0001
B^2	8.04	1	8.04	79.28	< 0.0001
C^2	0.4523	1	0.4523	4.46	0.0727
Residual	0.7103	7	0.1015		
Lack of Fit	0.258	3	0.0753	0.6214	0.6374
Pure Error	0.4845	4	0.1211		
Cor Total	207.84	16			

Where; A = Time, B = Temperature & C = Ratio

4.12.1 Model Adequacy Check and Statistical Analysis

According to the experimental data illustrated in the Table 4.9, the value for determination of coefficient, Predicted and adjusted value for the response are 0.99, 0.97 and 0.96 in respectively. The difference of the Predicted and adjusted value was less than 0.2, whereas the adequate precision value was 53.83, which is greater than four are. All the aforementioned statistics confirming that the obtained experimental results and predicted values were in good correlation without any significant differences. This result confirms that the predictability of

the models in determining the optimum extraction conditions required to maximize the extraction yields of *P.falcatus* seed oil is well fitted.

Almost all the independent variables had exerted a significant effect against the extraction oil yield. All the extraction temperatures, solvent to solid and time variables were highly affect the recovery of oil at less than 0.05 probabilities. The linear and quadratic extraction factors has been showed a significant oil recovery at less than 0.0001 ($p < 0.01$), whereas the interaction process variable exhibits a greater effect at less than 0.005 ($p < 0.5$). The extracted oil yield is highly affected by the Linear and its quadratic variables than its interaction. This indicates that the extraction parameters employed in this study were significantly important for the optimization process.

4.13 Response Surface Analysis of Oil Yield

Response surface methodology was used to determine the optimum processing conditions that give maximum extraction yield. The relationship between independent and dependent variables is illustrated below in three-dimensional representations of the response surface plot.

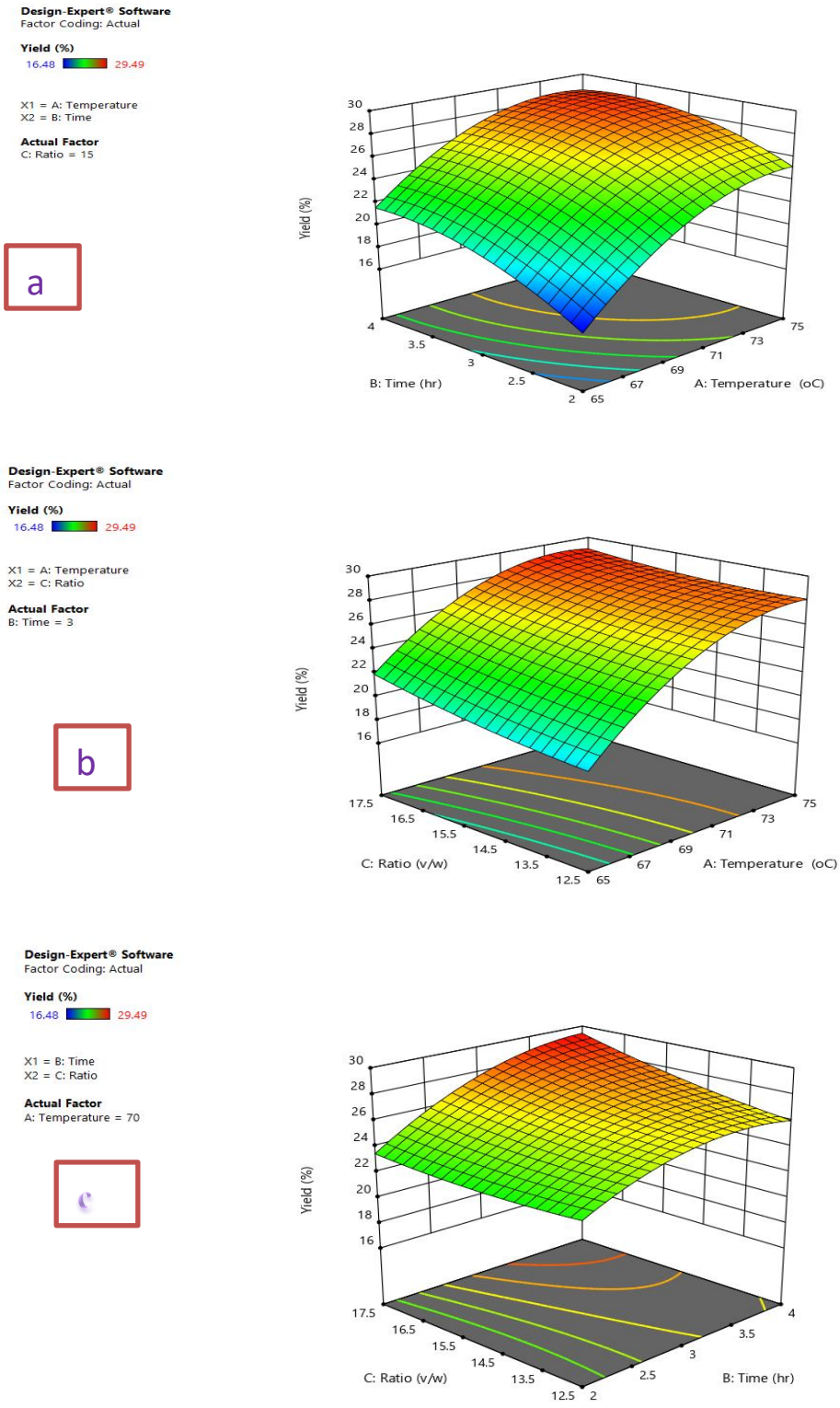


Figure 4.11 The plot of response surface in 3D for oil yields

Based on the 3-dimensional plot illustrated on the above figure 4.12, the extraction yields of the oil are ranging from 16.48% to 29.49% from the total runs of 17 experimental conducted. The maximum oil yield was obtained at an extraction temperature of 70°C, extraction time of 4 hr and solvent to solute ratio of 17.5 v/w. The current work were yield higher than the previously reported paper conducted on *P.falcatus* seed, which is extracting with water solvent at an extraction temperature of 70°C yields 22% (Daniel Alemu et al., 2022).

4.14 The Effect of Extraction Variables against Oil Yield

Increasing of extraction yield is generally due to the phenomenon of mass transfer that occurs in the extraction process.

Figure 4.2a, represents the effect of extraction temperatures and time on the yields o oil at a constant extraction solvent to solid ratio of 15 mL. Both of the process variables are a significant effect on the extraction oil yield. At the lowest levels of the interaction between temperature and time were yields the minimum oil recovery; whereas, at their highest levels provide the maximum oil. Since at higher temperature, the cell membrane is destroyed and phytoconstituents are exposed into the medium(Pods et al., 2022). Moreover, having a longer extraction period can also provide a chance to reduce its surface tension, viscosity and increases diffusion rate to the medium from the left portion of components in the cell of the particle. These two factories were simultaneous provided the highest oil yield at its highest extraction level.

Figure 4.10b, represents the effect of extraction temperatures and solid to solvent ratios on the extraction yields at a constant extraction time of 3 hr. Both of the extraction process variable has effectively affecting the oil yield. The maximum oil recovery was obtained at the highest extraction level from both sides of the process. The oil yield increases as the temperature and solid solvent ratio is increases. As it can be observed from the figure, the temperature variable is exerted greater impact on the oil yield than solvent to solid ratio. This might be due to the reason that under a high temperature, the plant tissues are becomes more softened and weak interactions of the cell membranes is becomes (Pods et al., 2022). In addition, the increased of extraction solvent amount would resulting of increases the concentration difference between

the solvent and solid to facilitate easily the mass exchange. The yields of oil were increased with simultaneous increasing of extraction solvent volume and temperature up to it reaches the maximum level of extraction. This confirms that a large volume of solvent and high amount of extraction temperature induces a high concentration difference between the solvent and biomass cell.

Figure 4.10c, illustrates the effect of solvent ratios and extraction times on the yields of oil at a constant extraction temperature of 70°C. The combination extraction of both variables at their lowest and highest extraction level has been found a better oil recovery. At the minimum extraction solvent ratio interaction with the longer extraction period were provide the minimum oil recovery. However, the maximum oil yields were obtained at longer extraction time and highest solvent to biomass amount. This confirms that an extended period of extraction provides a chance for various active constituents available in the cell of biomass to transfer gradual to the solvent by concentration difference (Che Sulaiman et al., 2017). Enough amount of solvent would bring a concentration difference in the cell of particle to diffuse into the media in shorter period.

4.15 Comparative Studies of Antimicrobial Contents of Herbal Extracts

The sensitivity of microbial against extracts was determined based on inhibition diameter using agar diffusion method. In this study, the antimicrobial activity of *A.vera* extract and *P. falcatus* oil were investigated against four bacteria species and one-fungus strains. Their effectiveness were determined by the presence or absence of inhibition zones.

The minimum inhibition concentrations (MIC) of the extracts were determined at a different concentration to identify the minimum active concentration that should be incorporated in to the ointment formulation.

Table 4.9 The inhibition zones of *A.vera* extract against the MOs

MOS	*A.vera* extract(mg/mL)				*A.vera* gel concentration (mg/mL)				
	100	50	25	12.5	100	50	25	12.5	+ve(50 mg)
S. aureus	14	12	10	7.66	10.33	7.33	6	4.66	26
E. faecalis	19.66	18.66	16.33	15	13.66	11	10.3	9.33	28
E. coli	25	23	19.66	16	14.33	12.33	11	9	30
P.aeruginosa	20.33	18.33	16	14.66	15	13	11	9	31
C. albican	24	19.33	15.66	14	13.66	12	9	8	29

In this work, the drug reference as positive control for anti-bacterial and antifungal test were Ciprofloxacin and gentamicin employed. As expected, DMSO and distilled water were employed as negative controls and no inhibition zone observed against to all of the bacteria tested.

A.vera gel and its extract were showed a maximum inhibition diameter against four of the species except of *S. aureus*. *S. aureus* is revealed a characteristic of little resistance to the tested extracts at all its concentration. Decreasing of the inhibition diameters related to the fall in the concentration of *A.vera* extract indicates that the antimicrobial potential is depend on the dose. The maximum diameter inhibitions to the minimum inhibition of the A.vera gel and its extract against bacteria strains are; *S. aureus* (10.33- 4.66 & 14 -7.66) mm, *E. faecalis* (13.66 - 9.33 &19.66-15) mm, *E. coli* (14.33 - 9 & 25 -16) mm, *P.aeruginosa* (15- 9 & 20.33-14.66) mm and fungus species of *C. albican* (13.66-8 &19.33-14) mm respectively. Therefore, the minimum inhibition concentration of *A.vera* extract and its gel are 12.5 mg/mL to all the tested MOs except resisted *S. aureus*. Is the concentration at which *A.vera* has attained the minimum inhibition concentration (susceptibility) to the selected microorganism. The MIC of *A.vera* extract were showed an inhibition diameter of 15 mm, 16 mm, 14.66 mm and 14 mm against *E. faecalis, E. coli, P.aeruginosa* and *C. albican* respectively. While the MIC of *A. vera* gel were observed an inhibition diameter of 9.33 mm, 9, 9 mm and 8 mm to *E. faecalis, E. coli, P.aeruginosa* and *C. albican*. However, the MIC of S. aureus for *A.vera* extract and gel are 25 mg /mL and 100 mg/mL that can inhibit 10 mm and 10.33 mm in diameter.

As it can be seen from the figure 4.10, *A.vera* extract has been showed a significant inhibition diameter to the selected bacteria and fungus species as compared to gel. The result of the present finding are in strongly agreed with the previous work (Cataldi et al., 2015). Its extract exhibits a stronger inhibition against *Pseudomonas aeruginosa, Escherichia coli, C. albican* and *E. faecalis* except *Staphylococcus aureus* with a little resistance. Similarly, several others results were confirmed that the antibacterial activity of *A.vera* extract against *E. coli* showed the greatest zones of inhibition even at the lowest concentration of extract (Haque et al., 2020; Arsene et al., 2022). The inhibition zone of *P. Aeruginosa* and *E. Coli* of the present study is showed the highest as compared to the other author's papers (Nalin Pagi & Payal Patel, 2017; Nsofor et al., 2023). Previous study demonstrated that the effectiveness of inhibition toward *Escherichia coli* might be due to presence of excess amount of alkaloids in the extract (S. Singh et al., 2021). In contrast, Nejatzadeh-Barandozi, (2013) reported that the maximum suppression growth has been observed on the *P. Aeruginosa* and lowest inhibition diameter was observed on to *E. coli*.

This research finding are confirmed that the extracted aloe vera extract has a potential substitution in place of ingredient that can be serve as antimicrobial in the product production. Hence, these results suggest that antibacterial ointment formulation that containing the extracts of *Aloe vera* has good promising in the treatment of dermal bacterial infection. The antibacterial activity of *P.falcatus* seed oil was not revealed any inhibition against to the four tested bacteria. This might be due to the presence of inadequate quantities of active chemical compounds in *P.falcatus* seed oil required to inhibit the selected microbial. However, *A.vera* extract exerted a strong inhibition zone to the three of bacteria strains except one with a little resistance and one fungal strains as shown above in the table format. The reason for the highest antibacterial activity of *A.vera* might be due to the presence of certain bioactive compounds as it contains a diversity of biological active compound.

4.16 Formulated Ointment

In this case, PEG 400 was served as a levigating agent (cosolvent), which has the tendency to mix insoluble substance into the formulation. Whereas, the solid PEG (PEG-4000) was used to increases the viscosity of the formulation up to the desired concentration as it can be combined with the liquid form of PEG (PEG-400). Six formulation of ointment bases were formulated at

different concentration ratio of PEG depend on the previous reported result as the starting point for this optimization (Piranaghl et al., 2023). Among the six formulation the ratio of 9: 3 of PEG (PEG-400:PEG-4000) was selected based on its evaluation of its smoothness, consistence (viscosity), crack formation and spreadability. As the amount of PEG-4000 increased, the spreadability of ointment consequently decreased and more ointment that is viscous were formed. The optimized PEG ointment base was showed a better smoothness, no phase separation, viscosity (4786 cp) and spreadability (18.12g.cm/s). Some considerations have been taken into account during the optimization of the excipient. Hence, high viscosity content would render the ointment to be have the characteristic of lower potency. This phenomenon will be affects the spreadability, diffusion rate and availability of active chemical compounds. The selected vehicle was for further optimization with plant extracts in order to evaluate the activities extract against antimicrobial and antioxidant potenct.

4.16.1 Antimicrobial Activities

4.16.1.1 Effect of *P. Falcatus* Seed Oil against MOs

The antimicrobial activities of different formulation were evaluated against four clinical bacterial and one fungal skin strains. The activities of ointment were studied under various concentration of *P. falcatus* seed oil (50, 100,150) mg/g of PEG ointment base with constant amount of *A. vera* extract (50 mg) based on the idea of Donkor et al., (2023).

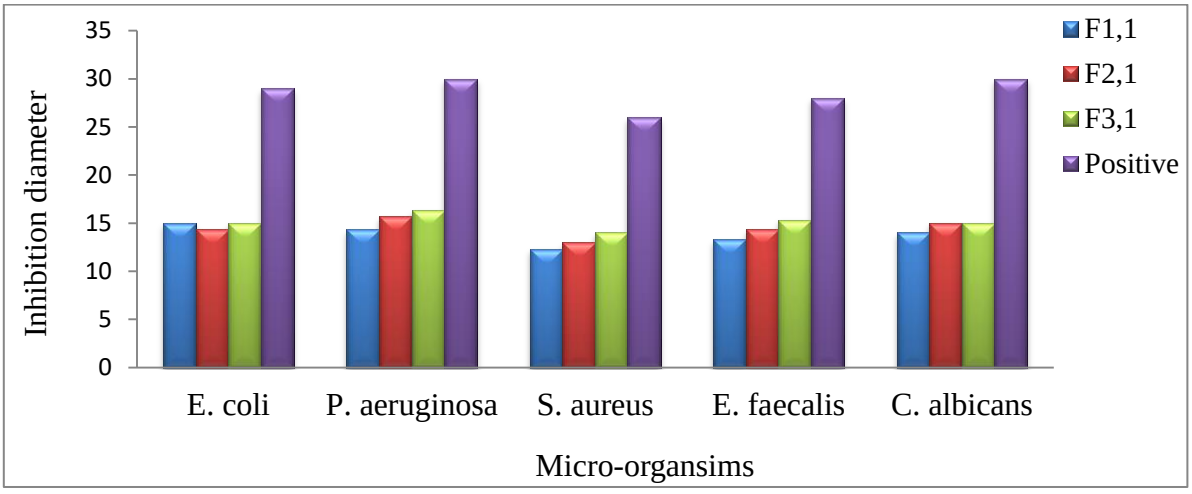


Figure 4.12 The inhibition zone of ointments at different amount of oil against MOs

The antibacterial and antifungal activities of the formulation were compared with commercial antibacterial and antifungal ointment respectively. The positive control of streptomycin and

fluconazole were used for bacterial and fungal strains. The PEG ointment base was used as a negative control did not exhibits any activity of inhibition in all case of tests.

All the formulated ointment at different amount of oil were found between in the range of active diameter inhibition (10 - 15) mm. There is no significant difference of inhibition diameter is observed among of the formulated ointment. A little differences of inhibition diameter revealed between each of the formulated ointment is the result of an increment of *P.falcatus* seed oil concentration across the formulation. Therefore, the increased in small diameter is due to in a dose-dependent manner against all the test organisms. However, clear zones of inhibition were induced to all of the suspected pathogens even to the resisted S. aureus bacteria. This indicates that the synergic activities of the two extracts would bring these kinds of advantage over a single extract employment in the formulation. When compared it with the commercial antibacterial and fungal that a huge difference of inhibition diameter is revealed.

4.16.1.2 The Effect of *A.Vera* Extract against MOs

Aloe vera extract were evaluated at three levels of optimization by a one factor method and the optimum amount of *P. falcatus* seed oil at 10% was selected based on biocompatibility by considering the property of PEG ointment base will not compromise with hydrophobic agents at maximum amount of incorporation(D'souza & Shegokar, 2016).

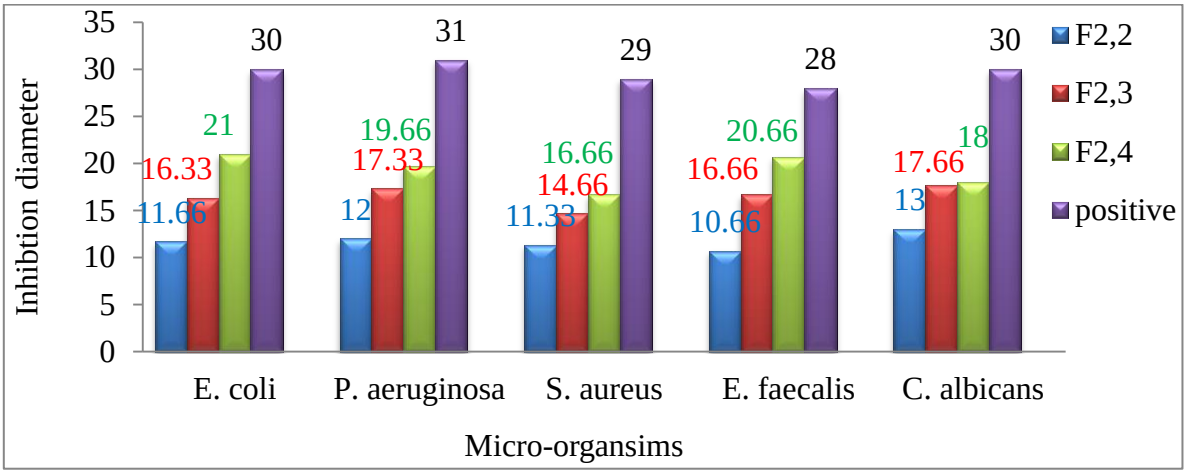


Figure 4.13 The inhibition zone of ointments against MOs at variable extract of A.vera

The activities of the formulated ointment were evaluated at a constant concentration of *P. falcatus* seed oil (100 mg) with a different concentration of *A.vera* extract (25, 100 & 150)

mg/g incorporated in PEG ointment base. The changes of *A.vera* concentration had showed a better antimicrobial potential compared to the formulated *P.falcatus* seed oil. The highest inhibition zone were obtained at the highest incorporation of extract into the excipient.

Formulated $F_{2,4}$ revealed a better efficacy that is closely similar to that of commercial antimicrobial ointment and categorized under the range of extremely sensitive to the tested pathogen except to the resisted *S. aureus*. Whereas, formulated $F_{2,\ 2}$ and $F_{\ 2,\ 3}$ have showed from sensitive to very sensitive against to the suspected clinical isolated pathogens. However, in all formulation, the dose dependent inhibition diameters exhibited as concentration was increased from 25 mg/g to 150 mg/g. All of the tested fungus and bacteria strains especial *E. coli* and *P. aeruginosa* were sensitive to the formulated ointments. In addition, formulating ointment that has exhibit antimicrobial activity with similar to that of the reference drug is possible to achieve by incorporating higher than the maximum concentration used in the formulation.

4.16.2 Antioxidant Activity of Ointments

This study were formulate different candidate of ointment containing antioxidant potential of extracts at a various concentration. Evaluation for its antioxidant activities is based on the consideration of antimicrobial activities of the ointment. From its antimicrobial activities, formulation $F_{2,4}$ and $F_{3,\ 4}$ were studied to see the effect of *P.falcatus* oil. The formulated ointments that contain 10% w/w & 15% w/w of *P.falcatus* seed oil with and without incorporation of *A.vera* extract at its maximum concentration (15%) were evaluated to find out which formulation had the highest antioxidant activity. The percentage inhibition of DPPH free radicals by the developed ointment were shown below in figure 4.13.

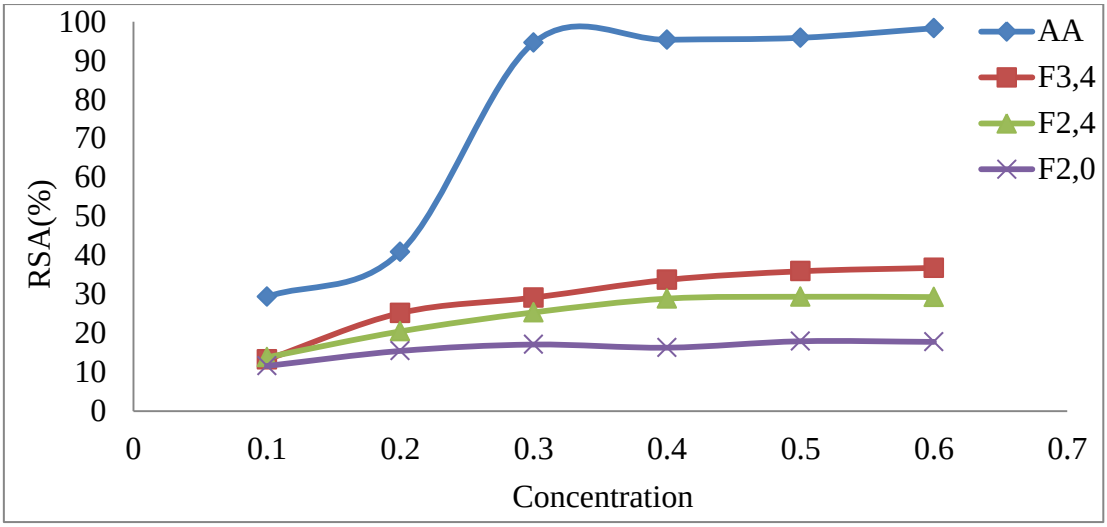


Figure 4.14 DPPH radical inhibition of formulated ointments

The antioxidant activity of the developed ointment was determined using DPPH scavenging method. The results of developed formulation showed some scavenging activities against DPPH radicals in a dose dependent manner. The antioxidant activities of ointment enhanced at a two different level of *P.falcatus* oil incorporation. The percentage of DPPH radical inhibition were reduced gradually as the incorporation amount of *P. falcatus* seed oil raised from 100 mg to 150 mg at a constant set of *A. vera* extract amount (150 mg). The percentage reduction is 29% and 36% at its minimum and maximum amount incorporation. Whereas, the lowest inhibition percentage has been found in the formulation $F_{2,0}$ in which *A.vera* extract did not encapsulated within the *P.falcatus* oil. So, the absence and as well as the dose variability of the plant extract in the formulation has been rendering the ointment to be have a lower or higher content of antioxidant potential.

The radical inhibition is not enough to describe the antioxidant potential; therefore, an IC_{50} value was applied to achieve to the point of inhibition. A comparative study of each formulation was done based on IC_{50} value.

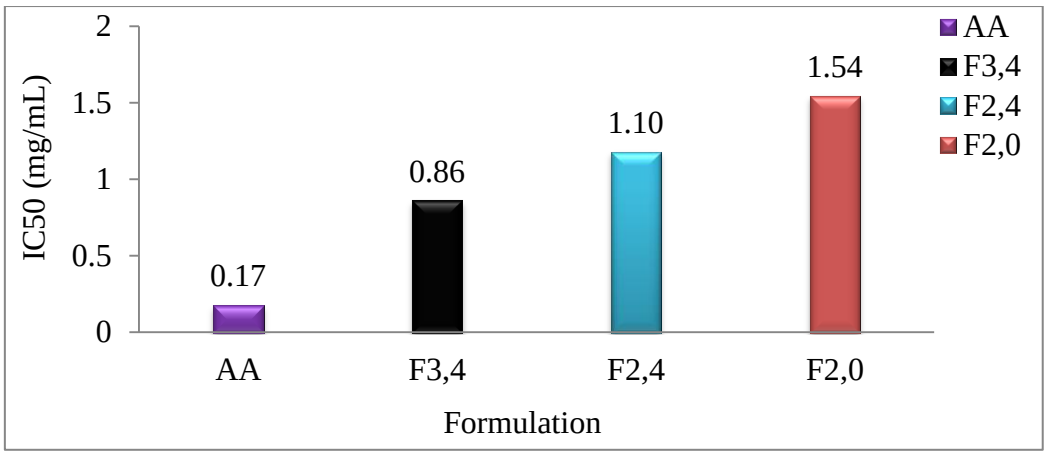


Figure 4.15 shows the half maximum inhibition concentration of ointments

The IC_{50} value has inversely relationship with radical scavenging percentage. From the above figure 4.14, the IC_{50} values of formulated ointments were compared to each other as well as within the standard ascorbic acid. The combination effect of extracts in the formulation was shown a significant antioxidant activity according to the ranges given for standard antioxidant. Therefore, it can be concluded that the formulated ointment has a medium of antioxidant activity as it had an IC_{50} value in the range of 1.54 mg/mL to 0.86 mg/mL at different concentration plant extract in the PEG ointment base. Surprisingly, the formulated ointment was revealed a moderate antioxidant activity resulting synergies from the two plant extracts.

4.16.3 Physiochemical Properties of Formulated Ointments

The organoleptic properties of ointments, such as color, physical appearance, texture, homogeneity, phase separation, and immediate skin feel were analyzed. The prepared ointments were checked by visual eye to identify the color of each formulation and its homogeneity were observed by rubbing a small quantity of the ointment between the thumb and index finger. The presence of undissolved coarse materials indicated that whether the formulated ointment is a homogenous and smooth or not.

Table 4.10 The results of physiochemical of ointments

Formulation coded	$F_{1,1}$	$F_{2,1}$	$F_{3,1}$	$F_{2,2}$	$F_{2,3}$	$F_{2,4}$
pH	5.955	6.11	6.01	6.17	6.33	6.67
Spreadability(g.cm/s)	20.64	27.12	37.36	34.47	45.15	56.78
Viscosity (cp)	4450.34	4245.25	3861.81	3920.46	4014.62	4122.15

The results of all of the test samples revealed that no greatness, no sign of phase separation or cracking, homogenous, smooth texture, opaque in colour and shows a slight kind of aromatic sensing odor. The opaque appearance resulting mainly due to the presence of phytoconstituents added in the form of extracts. The spreadability results of the ointment pointing out that the viscosity ointment revealed sufficient and evenly applied to the skin by a small amount of shear or force. All of the formulated ointments were in line with the previous reported result ranges (Ajala et al., 2016) and higher than the last recorded result (Avish & Swaroop, 2019).The ointments were dissolved in all the selected solvents from both lipophilic and hydrophilic solutions. Water and ethanol solution were highly dissolved the ointment without shows any precipitate at the bottom of the beaker. Hexane solvent was revealed a significant colour changes among the dissolving solvents. Therefore, all the formulated ointment fulfilled the main criteria required to have to after the formulation to be effectively employed for the commercial.

4.16.4 Drug-Excipients Compatibility

In cosmeceutical product, the active components were found to be in a small concentration in the product. However, it is important to determine the excipient drug interaction(incompatibility) to predict whether the delivery system is effectively carrying the drug agent to the proposed area without reacting with active components. FTIR analysis is an effective way of identifying whether or not an interaction is formed between the encapsulated extract in the excipient matrix. The FT-IR spectra of PEG base and ointment containing the extract were illustrated on Figure. 4.15.

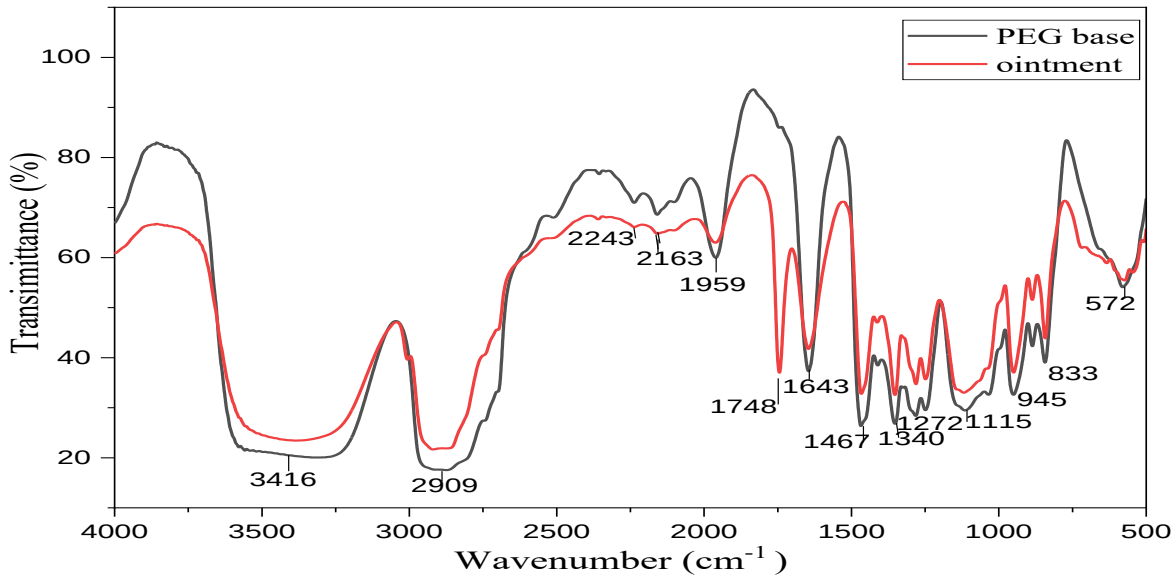


Figure 4.16 FT-IR spectra of PEG ointment base and ointment

In the FTIR spectrum of ointment, the major characteristic bands corresponding to *A.vera* extract and *P.falcatus* seed oil were disappeared or buried in the peaks of PEG base. Some spectra peaks of the extract were also revealed in the formulation. A few minor changes observed in the spectra were attributed to overlying of the peaks of active components and corresponding base. There is no significant variation of chemical group formation between the extracts and excipient. Nevertheless, a new peak was observed at the wave number of 1748 cm^{-1}, which indicates the interaction of extracts and PEG base. Sometimes, PEG bases were form an interaction with active pharmaceutical ingredients, such as ibuproxam ketoprofen and calcium phosphomycin. This causes to degrade the drug in a short period than the expected shelf life for the formulated ointment. The rate of decomposition was considerably higher when a fatty acid components are percent in ointment (Bharate et al., 2010). This confirms that the PEG excipient is suspected with incompatibility for effective delivering of active components that present in the extract even if a little change of displacement and intensity.

CHAPTER FIVE

5 Conclusion and Recommendation

5.1 Conclusion

This study were comparatively evaluated the phytochemical, antioxidant and antimicrobial activities of *P. falcatus* seed oil and *A. vera* gel extract to develop herbal based ointment. *P. falcatus* seed oil was optimized under various extraction parameters to get the optimum TPC and oil yield. The optimum oil yield and TPC (29.48% and 10.25 mg GAE/g) were obtained at the extraction time of 4 hr, temperature 70^{o}C , ethanol to biomass 17.5 v/w, and particle size of 1 mm. *A. vera* extract was extracted with a couple of vacuum rotary evaporator followed by oven drying at the temperature of 50℃ and 4℃. Quantitatively the phytochemical, antioxidant and antibacterial activities of *P.falcatus* seed oil, aloe vera mucilage and its extract have been compared. *A.vera* extract had shown the highest contents of phytochemical, antibacterial and antioxidant activities flowed by *P.falcatus* oil except in antimicrobial activity. However, *A.vera* gel revealed a stronger antimicrobial inhibition than *P.falcatus* oil, which did not exhibit a clear inhibition zone. The two plant extracts were further instrumental analyzed by FTIR and GS-MS to identify the active functional group of the components and essential organic compound that are promising for natural antimicrobial and antioxidant. As confirmation obtained from the instrumental analysis, aloe vera extract contain more essential compound than *P.falcatus* seed oil that responsible for the antimicrobial activity and antioxidant.

By considering the problems and limitation associated with the current market, an herbal based drug formulation for skin infection were suggested. Herbal based drug ointment were formulated by encapsulating the desired amount of both plant at different level of optimization against antimicrobial and antioxidant in PEG ointment base. The formulated ointments were found to be a good antimicrobial and antioxidant activity to the selected MOs and DPPH radical reduction. By considering the biocompatibility, spreadability, viscosity and maximum inhibition diameter, a 10% of *P.falcatus* seed oil and 15% of *A.vera* extract was selected from the optimized ointments. In general, from the present experiment it can be summarized that the oil and extract had shown a synergistic activities in the formulated ointment.

5.2 Recommendation

Further research work is required on the following points:

- Optimization of extraction parameters such as solvents, temperature are much needed for *A.vera* extract.
- It should be best to select a freeze drier for the extraction of *A.vera* extract
- The fiber sometimes named as bagasse left from *A.vera* juice by filtration has the capacity to reabsorb a high amount and will promises in the production of diaper babies and spongy
- Formulation in the form of nano-emulsion is more advisable for this kind of ingredients
- Hence, *A.vera* extract combined with *P.falcatus* seed oil can be employed as a source of natural antimicrobial and antioxidant that can serve as a medicinal ingredients from the present work. The extracted *P.falcatus* seed oil did not shows enough microbial inhibition to all of the test bacteria.

6 **Reference**

Abdillahi, H. S., Finnie, J. F., & Van Staden, J. (2011). Anti-inflammatory, antioxidant, anti-tyrosinase and phenolic contents of four Podocarpus species used in traditional medicine in South Africa. *Journal of Ethnopharmacology*, *136*(3), 496–503.

Agung, S. R. I., Kusuma, F., Yusuf, D., & Baitariza, A. (2019). *The effect of different storage temperatures on antiseptic gel stability containing green tea extract formulated with aloe vera gel*. *11*(4), 4–9.

Ajala, T. O., Femi-Oyewo, M. N., Odeku, O. A., Aina, O. O., Saba, A. B., & Oridupa, O. O. (2016). The physicochemical, safety and antimicrobial properties of Phyllanthus amarus herbal cream and ointment. *Journal of Pharmaceutical Investigation*, *46*(2), 169–178.

Al-Ghanayem, A. A., Alhussaini, M. S., Asad, M., & Joseph, B. (2022). Effect of Moringa oleifera Leaf Extract on Excision Wound Infections in Rats: Antioxidant, Antimicrobial, and Gene Expression Analysis. *Molecules*, *27*(14).

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2018). Soxhlet extraction of phenolic compounds from Vernonia cinerea leaves and its antioxidant activity. *Journal of Applied Research on Medicinal and Aromatic Plants*, *11*(June), 12–17.

Alvandi, H., Rajati, H., Naseriyeh, T., Rahmatabadi, S. S., Hosseinzadeh, L., & Arkan, E. (2023). Incorporation of Aloe vera and green synthesized ZnO nanoparticles into the chitosan/PVA nanocomposite hydrogel for wound dressing application. *Polymer Bulletin*,

Alzaid ,Activities, A., R., Al-rajhi, A. M. H., S. Z., Abboud, M. A. Al, Almuhayawi, M. S., Jaouni, S. K. Al, Selim, S., Ismail, K. S., & Abdelghany, T. M. (2022). *Molecular Docking and Efficacy of Aloe vera Gel Based on Chitosan Nanoparticles against Helicobacter pylori and Its.*

Añibarro-Ortega, M., Pinela, J., Barros, L., Ćirić, A., Silva, S. P., Coelho, E., Mocan, A., Calhelha, R. C., Soković, M., Coimbra, M. A., & Ferreira, I. C. F. R. (2019). Compositional features and bioactive properties of aloe vera leaf (Fillet, mucilage, and rind) and flower. *Antioxidants*, *8*(10), 1–21

Anjum, H., Sofi, G., Shahwan, M., Khan, M. S., Shamsi, A., & Shamsi, S. (2023). In vitro and In vivo study targeting the development of Unani Antidermatophytic Cream: Implication of Herbal Formulations in Treatment of Dermatophytosis. *Heliyon*, *9*(5), e16154.

Ansari, M. T., ibrahim, N. binti daud, Sami, F., Majeed, S., Hasnain, M. S., & Badgujar, V. B. (2019). Design and evaluation of topical herbal antifungal stick containing extracts of Rhinacanthus nasutus. *Journal of Herbal Medicine*, *17–18*(3), 100290.

Arsene, M. M. J., Viktorovna, P. I., Sergei, G. V., Hajjar, F., Vyacheslavovna, Y. N., Vladimirovna, Z. A., Aleksandrovna, V. E., Nikolayevich, S. A., & Sachivkina, N. (2022). Phytochemical Analysis, Antibacterial and Antibiofilm Activities of Aloe vera Aqueous Extract against Selected Resistant Gram-Negative Bacteria Involved in Urinary Tract Infections. *Fermentation*, *8*(11), 626.

Assefa, A., Abate, D., & Stenlid, J. (2015). Corynelia uberata as a threat to regeneration of Podocarpus falcatus in Ethiopian forests: Spatial pattern and temporal progress of the disease and germination studies. *Plant Pathology*, *64*(3), 617–626.

Avish d maru, & swaroop r lahoti. (2019). Formulation and evaluation of ointment containing sunflower wax. *Asian Journal of Pharmaceutical and Clinical Research*.

Awad, A. M., Kumar, P., Ismail-fitry, M. R., Jusoh, S., Faris, M., Aziz, A., & Sazili, A. Q. (2021). *Green Extraction of Bioactive Compounds from Plant Biomass and Their Application in Meat as Natural Antioxidant*. 1–39.

Azubuike, C. P., Alalor, C. A., & Igwilo, C. I. (n.d.). *Evaluation of the Antibacterial activity of Herbal ointments formulated with Methanolic extract of Cassia alata*.

Bagherian, T., Tackallou, S. H., & Mohammadgholi, A. (2021). Quantitative measurement of Bax and Bcl2 genes and protein expression in MCF7 cell-line when treated by Aloe Vera extract. *Gene Reports*, *23*(March), 101123.

Belwal, T., Ezzat, S. M., Rastrelli, L., Bhatt, I. D., Daglia, M., Baldi, A., Devkota, H. P., Orhan, I. E., Patra, J. K., Das, G., Anandharamakrishnan, C., Gomez-Gomez, L., Nabavi, S. F., Nabavi, S. M., & Atanasov, A. G. (2018). A critical analysis of extraction techniques used for botanicals: Trends, priorities, industrial uses and optimization strategies. *TrAC - Trends in Analytical Chemistry*, *100*, 82–102.

Bendjedid, S., Lekmine, S., Tadjine, A., Djelloul, R., & Bensouici, C. (2021). Analysis of phytochemical constituents, antibacterial, antioxidant, photoprotective activities and cytotoxic effect of leaves extracts and fractions of Aloe vera. *Biocatalysis and Agricultural Biotechnology*, *33*(December 2020).

Bharate, S. S., Bharate, S. B., & Bajaj, A. N. (2010). Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: A comprehensive review. *Journal of Excipients and Food Chemicals*, *1*(3), 3–26.

Bhaskar, R., Ola, M., Patil, P. H., Nawandar, K. S., Sampson, I. E., Senchi, Elinge, Wilberforce, O., Ilochi, J. O. E., Olivia, N., Bista, R., Ghimire, A., Subedi, S., Nejatzadeh-Barandozi, F., Gebrehiwot, T. Y., Krishna Chaithanya, K., Hagos, Z., Hiruy, M., Poornima, K., … Wang, D. (2018). Physico-Chemical Properties of Neem Seed Kernel Extract. *Journal of Physics: Conference Series*, *13*(1), 297–306.

Bhattacharyya, R., Medhi, K. K., & Borkataki, S. (2019). Phytochemical analysis of Drymaria cordata (l.) willd. ex schult. (whole plant) used by tea tribes of erstwhile Nagaon district of Assam, India. *International Journal of Pharmaceutical Sciences and Research*, *10*(9), 4264–4269.

Bitwell, C., Indra, S. Sen, Luke, C., & Kakoma, M. K. (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African*, *19*, e01585.

Bruno, S., Ngomade, L., Donald, R., Tchuifon, T., Fregue, R., Tagne, T., Laloi, M., Ngueteu, T., Patai, H. M., Nche, G. N., & Anagho, S. G. (2022). *Optimization by Response Surface Methodology of Biodiesel Production from Podocarpus falcatus Oil as a Cameroonian Novel Nonedible Feedstock*. *2022*.

C Shaikh, S., Sanap, D., Bhusari, D. V, Jain, S., Kochar, P. P., & Memon, F. S. (2018). Fabrication and evaluation of herbal ointment formulations of moringa olifera for topical delivery. *Universal Journal of Pharmaceutical Research*.

Cataldi, V., Di Bartolomeo, S., Di Campli, E., Nostro, A., Cellini, L., & Di Giulio, M. (2015). In vitro activity of Aloe vera inner gel against microorganisms grown in planktonic and sessile phases. *International Journal of Immunopathology and Pharmacology*, *28*(4), 595–602.

Che Sulaiman, I. S., Basri, M., Fard Masoumi, H. R., Chee, W. J., Ashari, S. E., & Ismail, M. (2017). Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of Clinacanthus nutans Lindau leaves by response surface methodology. *Chemistry Central Journal*, *11*(1), 1–11.

Chellathurai, B. J., Anburose, R., Alyami, M. H., Sellappan, M., Bayan, M. F., Chandrasekaran, B., & Chidambaram, K. (2023). *Development of a Polyherbal Topical Gel for the Treatment of Acne*. 1–14.

Choudhury, H., Gorain, B., Pandey, M., Chatterjee, L. A., Sengupta, P., Das, A., Molugulu, N., & Kesharwani, P. (2017). Recent Update on Nanoemulgel as Topical Drug Delivery System. *Journal of Pharmaceutical Sciences*, *106*(7), 1736–1751.

Chowdhury, T., Chowdhury, M. A. H., Qingyue, W., Enyoh, C. E., Wang, W., & Khan, M. S. I. (2021). Nutrient uptake and pharmaceutical compounds of Aloe vera as influenced by integration of inorganic fertilizer and poultry manure in soil. *Heliyon*, *7*(7).

Costa, R., & Santos, L. (2017). Delivery systems for cosmetics - From manufacturing to the skin of natural antioxidants. *Powder Technology*, *322*, 402–416.

D'souza, A. A., & Shegokar, R. (2016). Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications. *Expert Opinion on Drug Delivery*, *13*(9), 1257–1275.

Daniel Alemu, G., Solomon, A., Anbesse Girma, S., & Sisay, F. (2022). Effects of Extraction Temperature and Particle Size on Quality of Edible Oil from Podocarpus Falcatus Seed by Aqueous Method, Ethiopia. *Journal of Food Science and Nutrition Therapy*, *8*(1), 006–010.

Dario, M. F., Oliveira, F. F., Marins, D. S. S., Baby, A. R., Velasco, M. V. R., Löbenberg, R., & Bou-Chacra, N. A. (2018). Synergistic photoprotective activity of nanocarrier containing oil of Acrocomia aculeata (Jacq.) Lodd. Ex. Martius—Arecaceae. *Industrial Crops and Products*, *112*(September 2017), 305–312.

Dev, S. K., Choudhury, P. K., Srivastava, R., & Sharma, M. (2019). Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation. *Biomedicine and Pharmacotherapy*, *111*(December 2018), 555–567.

Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield , phytochemical constituents and antioxidant activity of Withania somnifera. *Arabian Journal of Chemistry*, *10*, S1193–S1199.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and*

Drug Analysis, *22*(3), 296–302.

Donkor, A. M., Ahenkorah, B., Wallah, T. A., & Yakubu, A. (2023). Evaluation of extracts from Sida acuta, Phyllanthus amarus, Parkia biglobosa and their herbal ointment for therapeutic and biological activities. *Heliyon*, *9*(9), e19316.

Donkor, A. M., Donkor, M. N., & Kuubabongnaa, N. (2020). Evaluation of anti-infective potencies of formulated aloin a ointment and aloin a isolated from Aloe barbadensis miller. *BMC Chemistry*, *14*(1).

Donkor, A. M., Oduro-Mensah, D., & Fiazorli, M. (2016). Extracts of Euphobia hirta linn and physalis angulata L. and their amalgamation demonstrate potency against Staphylococcus aureus and pseudomonas aeruginosa. *International Journal of Pharmacy and Pharmaceutical Sciences*, *8*(4), 322–326.

Donkor, M. N., Kuubabongnaa, N., & Donkor, A.-M. (2021). Effectiveness of isolated compound from Aloe barbadensis Miller and its formulated ointment against bacteria and fungi. *Journal of Applied Biotechnology & Bioengineering*, *8*(2), 47–53.

El-gied, A. A. A. (2015). *Investigation of cream and ointment on antimicrobial activity of Mangifera indica extract*. *6*(2), 53–57.

El-Sayed, A. S. A., El Sayed, M. T., Rady, A., Zein, N., Enan, G., Shindia, A., El-Hefnawy, S., Sitohy, M., & Sitohy, B. (2020). Exploiting the biosynthetic potency of taxol from fungal endophytes of conifers plants; genome mining and metabolic manipulation. *Molecules*, *25*(13), 1–21.

El-Sayed, A. S. A., Safan, S., Mohamed, N. Z., Shaban, L., Ali, G. S., & Sitohy, M. Z. (2018). Induction of Taxol biosynthesis by Aspergillus terreus, endophyte of Podocarpus gracilior Pilger, upon intimate interaction with the plant endogenous microbes. *Process Biochemistry*, *71*.

Elzayat, E. M., Auda, S. H., Alanazi, F. K., & Al-agamy, M. H. (2018). Evaluation of wound healing activity of henna , pomegranate and myrrh herbal ointment blend. *Saudi Pharmaceutical Journal*, *26*(5), 733–738.

Fahimi, S., Alireza, S., Abdollahi, M., & Hajimehdipoor, H. (2016). *Formulation of a Traditionally Used Polyherbal Product for Burn Healing and HPTLC Fingerprinting of Its Phenolic Contents*. *15*(September 2014), 95–105.

Farhan, A., Alsuwayt, B., Alanazi, F., Yaseen, A., & Ashour, M. A. (2021). Evaluation and HPLC characterisation of a new herbal ointment for the treatment of full-thickness burns in rats. *Journal of Taibah University Medical Sciences*, *16*(2).

Fatima, S., Zaman, R., Haider, N., Shamsi, S., & Alam, A. (2017). Design and development of Unani anti-inflammatory cream. *Journal of Ayurveda and Integrative Medicine*, *8*(3), 140–144.

Feleke, S., Haile, F., Alemu, A., & Abebe, S. (2012). Characteristics of seed kernel oil from podocarpus falcatus. In *Source: Journal of Tropical Forest Science* (Vol. 24, Issue 4).

Feng, Y. X., Zhang, X., Wang, Y., Chen, Z. Y., Lu, X. X., Du, Y. S., & Du, S. S. (2021). The potential contribution of cymene isomers to insecticidal and repellent activities of the essential oil from Alpinia zerumbet. *International Biodeterioration and Biodegradation*, *157*, 105138.

Ferreira, S. M., Falé, Z., & Santos, L. (2022). Sustainability in Skin Care: Incorporation of Avocado Peel Extracts in Topical Formulations. *Molecules*, *27*(6), 1–16.

Flores-López, M. L., Romaní, A., Cerqueira, M. A., Rodríguez-García, R., Jasso de Rodríguez, D., & Vicente, A. A. (2016). Compositional features and bioactive properties of whole fraction from Aloe vera processing. *Industrial Crops and Products*, *91*, 179–185.

Forno-bell, N., Munoz, M. A., & Cornejo, J. (2021). *Efficacy Prediction of Four Pharmaceutical Formulations for Intramammary Administration Containing Aloe vera (L .) Burm . f . Combined With Ceftiofur or Cloxacillin in Lactating Cows as an Alternative Therapy to Treat Mastitis Caused by Staphylococcus au*. *8*(March), 1–12.

Gad, H., Al-Sayed, E., & Ayoub, I. (2021). Phytochemical discrimination of Pinus species based on GC–MS and ATR-IR analyses and their impact on Helicobacter pylori. *Phytochemical Analysis*, *32*(5), 820–835.

Garg, A., Sharma, G. S., Goyal, A. K., Ghosh, G., Si, S. C., & Rath, G. (2020). Recent advances in topical carriers of anti-fungal agents. *Heliyon*, *6*(8), e04663.

Gavan, A., Colobatiu, L., Hanganu, D., Bogdan, C., Olah, N. K., Achim, M., & Mirel, S. (2022). Development and Evaluation of Hydrogel Wound Dressings Loaded with Herbal Extracts. *Processes*, *10*(2).

Ghazi, S. (2022). Do the polyphenolic compounds from natural products can protect the skin from ultraviolet rays? *Results in Chemistry*, *4*(May), 100428.

Ghodke, A., Amol, G. D., Shirish, J. P., Dipak, B. V, Umesh, B. J., Chetana Bhalke Meena V, K. R., & Pooja, K. P. (2019). Formulation and evaluation of antiinflamatory & analgesic herbal ointment of punicagranatum peel. *Www.Wjpps.Com*, *8*(8).

Ghosh, D., Mondal, S., & Ramakrishna, K. (2019). A topical ointment formulation containing leaves extract of Aegialitis rotundifolia Roxb ., accelerates excision , incision and burn wound healing in rats. *Wound Medicine*, *26*(1), 100168.

Guluma, T., G, N. B., Teju, E., & Dekebo, A. (2020). Testing for antifungal activity Paper discs of about 6 mm in diameter were cut from Whatman-No.1 filter paper and placed in a beaker covered with aluminums foil and sterilized in an oven at 180 °C for 1 h. Then 10 and 20 μL of a solution of samples were p. *Chemical Data Collections*, *28*.

Hagr, T. E., Adam, I. A., Almain, A. A., & Mohammed, M. M. (2019). Phytochemical Screening , GC - MS Analysis , Antibacterial and Antioxidant Activity of Seeds Oil of Annona Squmosa L . Sudanese Medicinal Plant. *Open Science Journal of Pharmacy and Pharmacology*, *7*(1), 1–6.

Haque, S. D., Al Amin, A. M. M., Islam, B., Nazneen, N., Karim, S. N., & Rahman, M. A. (2020). Antibacterial effect of Dimethyl sulfoxide extract of Aloe vera (Aloe barbadensis) leaf gel against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumoniae. *Mediscope*, *7*(2), 67–74.

Hayouni, E. A., Miled, K., Boubaker, S., Bellasfar, Z., Abedrabba, M., Iwaski, H., Oku, H., Matsui, T., Limam, F., & Hamdi, M. (2011). Hydroalcoholic extract based-ointment from Punica granatum L. peels with enhanced in vivo healing potential on dermal wounds. *Phytomedicine*, *18*(11), 976–984.

Heidari, M., Bahramsoltani, R., Abdolghaffari, A. H., Rahimi, R., Esfandyari, M., Baeeri, M., Hassanzadeh, G., Abdollahi, M., & Farzaei, M. H. (2019). Efficacy of topical application of standardized extract of Tragopogon graminifolius in the healing process of experimental burn wounds. *Journal of Traditional and Complementary Medicine*, *9*(1), 54–59.

Herbal, C., Cream, F., Standardized, C., Tan, P. L., Rajagopal, M., Chinnappan, S., Tan, L. F.,

& Yap, V. L. (2022). *Formulation and Physicochemical Evaluation of Green Cosmeceutical Herbal Face Cream Containing Standardized Mangosteen Peel Extract*.

Herman, A. (2019). Antimicrobial Ingredients as Preservative Booster and Components of Self-Preserving Cosmetic Products. *Current Microbiology*, *76*(6), 744–754.

Hossen, M., Lokman, M., Mitra, K., & Hossain, B. (2022). c. *Journal of Agriculture and Food Research*, *10*(September), 100460.

Hussain, S. A., Hameed, A., Ajmal, I., Nosheen, S., Suleria, H. A. R., & Song, Y. (2018). Effects of sesame seed extract as a natural antioxidant on the oxidative stability of sunflower oil. *Journal of Food Science and Technology*, *55*(10), 4099–4110.

Iryana, S., Gunawan, S., Ibrahim, R., & Wirawasista, H. (2022). Extract with high 1 , 1-diphenyl-2-picrylhydrazyl (DPPH) inhibitory capability from pericarp and seed of mangosteen (Garcinia mangostana L .) using microwave-assisted extraction (MAE) two-phase solvent technique. *Arabian Journal of Chemistry*, *15*(12), 104310.

Jales, S. T. L., Barbosa, R. D. M., Albuquerque, A. C. De, Duarte, L. H. V, Silva, G. R., Meirelles, L. M. A., Silva, T. M. S., Alves, A. F., Viseras, C., Raffin, F. N., & Moura, T. F. A. D. L. (2022). *Development and Characterization of Aloe vera Mucilaginous-Based Hydrogels for Psoriasis Treatment*.

Javed, S., & Atta-Ur, R. (2014). Aloe vera gel in food, health products, and cosmetics industry. In *Studies in Natural Products Chemistry* (1st ed., Vol. 41). Elsevier B.V.

Joshi, V., Yashaswini, G., Acharya, A., Bheemachari, Annegowda, H. V., & Niraula, B. (2019). Formulation and evaluation of semisolid dosage forms of an anti-inflammatory drug. *3 Biotech*, *9*(7), 1–12.

Kaci, M., Belhaffef, A., Meziane, S., Dostert, G., Menu, P., Velot, Desobry, S., & Arab-Tehrany, E. (2018). Nanoemulsions and topical creams for the safe and effective delivery of lipophilic antioxidant coenzyme Q10. *Colloids and Surfaces B: Biointerfaces*, *167*, 165–175.

Karami, Z., Emam-Djomeh, Z., Mirzaee, H. A., Khomeiri, M., Mahoonak, A. S., & Aydani, E. (2015). Optimization of microwave assisted extraction (MAE) and soxhlet extraction of phenolic compound from licorice root. *Journal of Food Science and Technology*, *52*(6), 3242–3253.

Kaseke, T., Opara, U. L., & Fawole, O. A. (2021). Novel seeds pretreatment techniques: effect on oil quality and antioxidant properties: a review. *Journal of Food Science and Technology*, *58*(12), 4451–4464.

Khogta, S., Patel, J., Barve, K., & Londhe, V. (2020). Herbal nano-formulations for topical delivery. *Journal of Herbal Medicine*, *20*(December 2017), 100300.

Kumar, A., Mahajan, A., & Begum, Z. (2020). Phytochemical screening and in vitro study of free radical scavenging activity of flavonoids of aloe vera. *Research Journal of Pharmacy and Technology*, *13*(2), 593–598.

Kumar, A., Nirmal, P., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., Sneha, V., & Oz, F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. *Molecules*, *28*(2), 1–41.

Kumar, R., Sharma, S., & Devi, L. (2018). Investigation of Total Phenolic, Flavonoid Contents and Antioxidant Activity from Extracts of Azadirachta indica of Bundelkhand Region. *International Journal of Life-Sciences Scientific Research*, *4*(4), 1925–1933.

Kumar, S., Yadav, A., Yadav, M., & Yadav, J. P. (2017). Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm.f. *BMC Research Notes*, *10*(1), 1–12.

Kumari, S., Goyal, A., Gürer, E. S., Yapar, E. A., Garg, M., Sood, M., & Sindhu, R. K. (2022). Bioactive Loaded Novel Nano-Formulations for Targeted Drug Delivery and Their Therapeutic Potential. *Pharmaceutics*, *14*(5).

Lin, T. K., Zhong, L., & Santiago, J. L. (2018). Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. In *International Journal of Molecular Sciences* (Vol. 19, Issue 1). MDPI AG.

Mahajan, R., & Itankar, P. (2020). Antioxidant, Antimicrobial and Wound Healing Potential of Helicteres isora Linn. Leaf Extracts. *Digital Chinese Medicine*, *3*(3), 188–198.

Mahalingam, R., Li, X., & Jasti, B. R. (2008). *SEMISOLID DOSAGES : OINTMENTS , CREAMS , AND GELS*. 267–312.

Mahapatra, S., Srivastav, P. P., & Halder, K. (2017). Optimisation of process parameters for vacuum concentration and foaming behaviour of Aloe vera (Aloe barbadensis Miller) gel. *Agricultural Engineering International: CIGR Journal*, *19*(2), 149–158.

Mahmud, I. A., S Mirghani, M. E., Yusof, F., & Al-khatib, A. (2019). Effects of Time, Temperature, and Solvent Ratio on the Extraction of Non-Extractable Polyphenols with Anticancer Activity of Barhi Date Palm Kernels Extracts Using Response Surface Methodology. *Journal of Biotechnology Engineering*, *1*(1), 1–36.

Malca-Garcia, G. R., Zagal, D., Graham, J., Nikolić, D., Friesen, J. B., Lankin, D. C., Chen, S. N., & Pauli, G. F. (2019). Dynamics of the isoflavone metabolome of traditional preparations of Trifolium pratense L. *Journal of Ethnopharmacology*, *238*(March), 111865.

Manna, M., & Rudra, A. (2020). *GSC Biological and Pharmaceutical Sciences Development and formulation of Aloe vera emulgel*. *12*(02), 161–166.

Manye, S. J., Saleh, J. S., Ishaya, H. B., Chiroma, S. M., Attah, M. O. O., & Dibal, N. I. (2023). Phytochemical screening and in-vitro antioxidant activities of aqueous and methanol extracts of Aloe vera. *Pharmacological Research - Modern Chinese Medicine*, *8*(May), 100291.

Martín-Illana, A., Notario-Pérez, F., Cazorla-Luna, R., Ruiz-Caro, R., Bonferoni, M. C., Tamayo, A., & Veiga, M. D. (2022). Bigels as drug delivery systems: From their components to their applications. *Drug Discovery Today*, *27*(4), 1008–1026.

Meharie, B. G., & Tunta, T. A. (2020). Evaluation of diuretic activity and phytochemical contents of aqueous extract of the shoot apex of Podocarpus falcactus. *Journal of Experimental Pharmacology*, *12*, 629–641.

Mehmood, A., Javid, S., Khan, M. F., Ahmad, K. S., & Mustafa, A. (2022). In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. *BMC Chemistry*, *16*(1), 1–10.

Meskini, M., & Esmaeili, D. (2018). The study of formulated Zoush ointment against wound infection and gene expression of virulence factors Pseudomonas aeruginosa. *BMC Complementary and Alternative Medicine*, *18*(1), 1–10.

Minjares-Fuentes, R., Femenia, A., Comas-Serra, F., Rosselló, C., Rodríguez-González, V. M., González-Laredo, R. F., Gallegos-Infante, J. A., & Medina-Torres, L. (2016). Effect of different drying procedures on physicochemical properties and flow behavior of Aloe vera (Aloe barbadensis Miller) gel. *Lwt*, *74*, 378–386.

Mohammadpour, H., Sadrameli, S. M., Eslami, F., & Asoodeh, A. (2019). Optimization of ultrasound-assisted extraction of Moringa peregrina oil with response surface methodology and comparison with Soxhlet method. *Industrial Crops and Products*, *131*(September 2018), 106–116.

Nalin Pagi, D. D., & Payal Patel, H. J. (2017). Antimicrobial Activity and Phytochemical Screening of Aloe vera (Aloe barbadensis Miller). *International Journal of Current Microbiology and Applied Sciences*, *6*(3), 2152–2162.

Nduka, J. K. C., Omozuwa, P. O., & Imanah, O. E. (2021). Effect of heating time on the physicochemical properties of selected vegetable oils. *Arabian Journal of Chemistry*, *14*(4), 103063.

Nejatzadeh-Barandozi, F. (2013). Antibacterial activities and antioxidant capacity of Aloe vera. *Organic and Medicinal Chemistry Letters*, *3*(1), 5.

Ngomade, S. B. L., Rawat, N., Ngueteu, M. L. T., Anensong, C. S. D., Bopda, A., Nche, G. N. A., Anagho, S. G., & Atray, N. (2023). Optimization of the oil extraction process by response surface methodology from Podocarpus falcatus seed as a low-grade substitute feedstock for biodiesel applications. *Biomass Conversion and Biorefinery*, *0123456789*.

Nsofor, O. U., Ogochukwu, A. P., Gabriel, A. O., Emmanuel, E., & Linus, C. M. (2023). *Evaluation of antibacterial activity of aloe vera extract on some bacterial pathogens*. *3*(1), 26–29.

Nunes, A., Gonçalves, L., Marto, J., Martins, A. M., Silva, A. N., Pinto, P., Martins, M., Fraga, C., & Ribeiro, H. M. (2021). Investigations of olive oil industry by-products extracts with potential skin benefits in topical formulations. *Pharmaceutics*, *13*(4), 1–20.

Nwamaka Henrietta, I., Olusola, I. A., Uthman Bayonle, A., Aminat, O. B., & Kingsley, I. (2020). Antibacterial profiling of methanolic leaf extracts and herbal cosmetic cream formulations containing the leaf extracts of Urtica dioica, Amaranthus viridis and Aloe vera. *World Journal of Biology Pharmacy and Health Sciences*, *2020*(03), 2582–5542.

Odeniyi, M. A., Okumah, V. C., Adebayo-Tayo, B. C., & Odeniyi, O. A. (2020). Green synthesis and cream formulations of silver nanoparticles of Nauclea latifolia (African peach) fruit extracts and evaluation of antimicrobial and antioxidant activities. *Sustainable Chemistry and Pharmacy*, *15*(September 2019), 100197.

Ogidi, C. O., Ojo, A. E., Ajayi-Moses, O. B., Aladejana, O. M., Thonda, O. A., & Akinyele, B. J. (2021). Synergistic antifungal evaluation of over-the-counter antifungal creams with turmeric essential oil or Aloe vera gel against pathogenic fungi. *BMC Complementary Medicine and Therapies*, *21*(1).

Ojha, B., Jain, V. K., Gupta, S., Talegaonkar, S., & Jain, K. (2022). Nanoemulgel: a promising novel formulation for treatment of skin ailments. *Polymer Bulletin*, *79*(7), 4441–4465.

Otálora, M. C., Wilches-Torres, A., & Gómez Castaño, J. A. (2021). Extraction and physicochemical characterization of dried powder mucilage from opuntia ficus-indica cladodes and aloe vera leaves: A comparative study. *Polymers*, *13*(11).

Otang, W. M., & Afolayan, A. J. (2016). Antimicrobial and antioxidant efficacy of Citrus limon L. peel extracts used for skin diseases by Xhosa tribe of Amathole District, Eastern Cape, South Africa. *South African Journal of Botany*, *102*, 46–49.

Palaniyappan, S., Sridhar, A., Arumugam, M., & Ramasamy, T. (2023). Bioactive Analysis of Antibacterial Efficacy and Antioxidant Potential of Aloe barbadensis Miller Leaf Extracts and Exploration of Secondary Metabolites Using GC – MS. In *Applied Biochemistry and Biotechnology* (Issue 0123456789). Springer US.

Palefsky, I. (2010). Creams, Lotions, and Ointments. *Cosmetic Dermatology: Products and Procedures*, 71–74.

Panikar, S., Shoba, G., Arun, M., Sahayarayan, J. J., Usha Raja Nanthini, A., Chinnathambi, A., Alharbi, S. A., Nasif, O., & Kim, H. J. (2021). Essential oils as an effective alternative for the treatment of COVID-19: Molecular interaction analysis of protease (Mpro) with pharmacokinetics and toxicological properties. *Journal of Infection and Public Health*, *14*(5), 601–610.

Patil, S. M., Ramu, R., Shirahatti, P. S., Shivamallu, C., & Amachawadi, R. G. (2021). A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of Thymus vulgaris Linn. *Heliyon*, *7*(5), e07054.

Piranaghl, H., Golmohammadzadeh, S., Soheili, V., Noghabi, Z. S., Memar, B., Jalali, S. M., Taherzadeh, Z., & Fazly Bazzaz, B. S. (2023). The potential therapeutic impact of a topical bacteriophage preparation in treating Pseudomonas aeruginosa-infected burn wounds in mice. *Heliyon*, *9*(7), e18246.

Pods, C., Medjroubi, K., & Nieto, G. (2022). *Process Optimization of Phytoantioxidant and Photoprotective Ultrasonic Assisted Extraction Method*.

Połeć, K., Broniatowski, M., Wydro, P., & Hąc-Wydro, K. (2020). The impact of β-myrcene – the main component of the hop essential oil – on the lipid films. *Journal of Molecular Liquids*, *308*.

Punareewattana, K., Borlace, G. N., Seubsasana, S., Thongkham, E., & Aiemsaard, J. (2023). In vitro antimicrobial examination and efficacy of Eryngium foetidum L. extract for skin ointment in animal infectious dermatitis treatment. *ScienceAsia*, *49*(2), 248.

Reham Yahya 1, 2 , Aisha M. H. Al-Rajhi 3,*, S. Z. A. 4, 5, M. A. A. A., Mohammed S. Almuhayawi 6,*, S. K. A. J. 7, , Samy Selim 8,*, K. S. I. 5, & Abdelghany, and T. M. (2022). Molecular Docking and Efficacy of Aloe vera Gel Based on Chitosan Nanoparticles against Helicobacter pylori and Its Antioxidant and Anti-Inflammatory Activities. *Polymers*, *14*.

Royani, A., Hanafi, M., Julistiono, H., & Manaf, A. (2023). Materials Today : Proceedings The total phenolic and flavonoid contents of Aloe vera and Morinda citrifolia extracts as antibacterial material against Pseudomonas aeruginosa. *Materials Today: Proceedings*, *72*, 2796–2802.

Saddiqe, Z., Naeem, I., Hellio, C., Patel, A. V., & Abbas, G. (2020). Phytochemical profile, antioxidant and antibacterial activity of four Hypericum species from the UK. *South African Journal of Botany*, *133*, 45–53.

Saifullah, M., McCullum, R., McCluskey, A., & Vuong, Q. (2019). Effects of different drying methods on extractable phenolic compounds and antioxidant properties from lemon myrtle dried leaves. *Heliyon*, *5*(12), e03044.

Salhi, N., Bouyahya, A., Fettach, S., Zellou, A., & Cherrah, Y. (2019). Ethnopharmacological study of medicinal plants used in the treatment of skin burns in occidental Morocco (area of Rabat). *South African Journal of Botany*, *121*, 128–142.

Shewale, S., & Rathod, V. K. (2018). Extraction of total phenolic content from Azadirachta indica or (neem) leaves: Kinetics study. *Preparative Biochemistry and Biotechnology*, *48*(4), 312–320.

Shukla, R., & Kashaw, V. (2019). Development, characterization and evaluation of poly-

herbal ointment and Gel formulation containing Nerium Indicum Mill, Artocarpus Heterophyllus Lam, Murraya Koenigii Linn, Punica Granatum Linn. *Journal of Drug Delivery and Therapeutics*, *9*(2).

Singh, A., Ansari, V. A., Haider, M. F., Ahsan, F., Mahmood, T., & Wasim, R. (2023). Development and Evaluation of Topical Liposome Preparation of Flax Seed Oil and Tamarind Seed Oil against Skin-Protective Activity. *Pharmaceutical Chemistry Journal*, *57*(6), 918–931.

Singh, S., Dodiya, T. R., Singh, S., & Dodiya, R. (2021). Topical wound healing, antimicrobial and antioxidant potential of Mimosa pudica Linn root extracted using n-hexane followed by methanol, fortified in ointment base. *International Journal of Pharmaceutical Sciences and Nanotechnology*, *14*(3), 5472–5480.

Solaberrieta, I., Jiménez, A., & Garrigós, M. C. (2022). Valorisation of Aloe Vera Skin By-Products to Obtain Bioactive Compounds by Microwave-Assisted Extraction: Antioxidant Activity and Chemical Composition. *SSRN Electronic Journal*.

Surendran, S., Qassadi, F., Surendran, G., Lilley, D., & Heinrich, M. (2021). Myrcene—What Are the Potential Health Benefits of This Flavouring and Aroma Agent? *Frontiers in Nutrition*, *8*(July), 1–14.

Talati, A. (2017). *Extraction methods of natural essential oils*. *February*.

Talekar, Y. P., Apte, K. G., Paygude, S. V., Tondare, P. R., & Parab, P. B. (2017). Studies on wound healing potential of polyherbal formulation using in vitro and in vivo assays. *Journal of Ayurveda and Integrative Medicine*, *8*(2), 73–81.

Talianu, M. T., Dinu-Pîrvu, C. E., Ghica, M. V., Anuţa, V., Jinga, V., & Popa, L. (2020). Foray into concepts of design and evaluation of microemulsions as a modern approach for topical applications in acne pathology. *Nanomaterials*, *10*(11), 1–43.

Thangaleela, S., Sivamaruthi, B. S., Kesika, P., Tiyajamorn, T., Bharathi, M., & Chaiyasut, C. (2022). A Narrative Review on the Bioactivity and Health Benefits of Alpha-Phellandrene. *Scientia Pharmaceutica*, *90*(4), 57.

Thilakarathna, R. C. N., Tang, L. F. S. T.-K., Char, E.-S., & Lee, Y.-Y. (2023). Physicochemical and antioxidative properties of ultrasound-assisted extraction of mahua (Madhuca longifolia) seed oil in comparison with conventional Soxhlet and mechanical

extractions. *Journal Homepage: Www.Elsevier.Com/Locate/Ultson*, *92*(Ultrasonics Sonochemistry).

Tubino, M., & Aricetti, J. A. (2013). A green potentiometric method for the determination of the iodine number of biodiesel. *Fuel*, *103*, 1158–1163.

Tyagi, T., & Agarwal, M. (2017). Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of Pistia stratiotes L. and Eichhornia crassipes (Mart.) solms. *~ 195 ~ Journal of Pharmacognosy and Phytochemistry*, *6*(1), 195–206.

Ugur Kaplan, A. B., Cetin, M., Orgul, D., Taghizadehghalehjoughi, A., Hacımuftuoglu, A., & Hekimoglu, S. (2019). Formulation and in vitro evaluation of topical nanoemulsion and nanoemulsion-based gels containing daidzein. *Journal of Drug Delivery Science and Technology*, *52*(March), 189–203.

Ukwubile, C. A., Ahmed, A., Katsayal, U. A., Ya'u, J., & Mejida, S. (2019). GC–MS analysis of bioactive compounds from Melastomastrum capitatum (Vahl)Fern. leaf methanol extract: An anticancer plant. *Scientific African*, *3*, 10–17.

Uronnachi, E., Atuegwu, C., Umeyor, C., Nwakile, C., Obasi, J., Ikeotuonye, C., & Attama, A. (2022). Formulation and evaluation of hair growth enhancing effects of oleogels made from Rosemary and Cedar wood oils. *Scientific African*, *16*, e01223.

Vasowala, T., Gharat, S., Mhase, M., & Momin, M. (2024). Advances in hydrogels based cutaneous drug delivery system for management of psoriasis. *European Polymer Journal*, *202*(2), 112630.

Wojtunik-Kulesza, K. A., Kasprzak, K., Oniszczuk, T., & Oniszczuk, A. (2019). Natural Monoterpenes: Much More than Only a Scent. *Chemistry and Biodiversity*, *16*(12).

Yadav Abhishek, & Samanta Krishanu. (2021). Formulation and evaluation of herbal ointment using Emblica officinalis extract. *World Journal of Advanced Research and Reviews*, *9*(2), 032–037.

Yamuna, P., Abirami, P., Vijayashalini, P., & Sharmila, M. (2017). GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of Gomphrena decumbens Jacq. *Journal of Medicinal Plants Studies*, *5*(3), 31–37.

Yavagal, P. C., & SV, S. (2019). In vitro evaluation of antibacterial potential of Aloe vera against Enterococcus faecalis. *Journal of Ayurvedic and Herbal Medicine*, *5*(3), 96–99.

Zhao, S., & Zhang, D. (2014). Supercritical CO2 extraction of Eucalyptus leaves oil and comparison with Soxhlet extraction and hydro-distillation methods. *Separation and Purification Technology*, *133*, 443–451.

Zheng, Y. F., Liu, X. M., Zhang, Q., Lai, F., & Ma, L. (2019). Constituents of the Essential Oil and Fatty Acid from Rare and Endangered Plant Magnolia kwangsiensis Figlar & Noot. *Journal of Essential Oil-Bearing Plants*, *22*(1), 141–150.

Zouboulis, C. C., Ganceviciene, R., Liakou, A. I., Theodoridis, A., Elewa, R., & Makrantonaki, E. (2019). Aesthetic aspects of skin aging , prevention , and local treatment ☆. *Clinics in Dermatology*, *37*(4), 365–372.

Zouirech, O., Alyousef, A. A., El Barnossi, A., El Moussaoui, A., Bourhia, M., Salamatullah, A. M., Ouahmane, L., Giesy, J. P., Aboul-Soud, M. A. M., Lyoussi, B., & Derwich, E. (2022). Phytochemical Analysis and Antioxidant, Antibacterial, and Antifungal Effects of Essential Oil of Black Caraway (Nigella sativa L.) Seeds against Drug-Resistant Clinically Pathogenic Microorganisms. *BioMed Research International*, *2022*.

APPENDIX

Appendix A Experimental preliminary screening of phytochemical photo

Figure a. Preliminarily phytochemical identification

Appendix B Experimental Result Photo of Chemical Property of oil

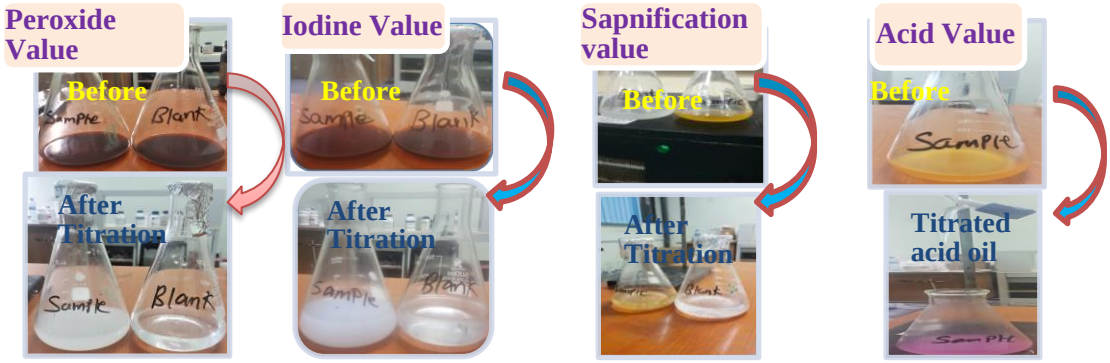


Figure b. Determination of chemical properties of P.falcatus seed oil

Appendix C Experimental photo of agar well diffusion assay

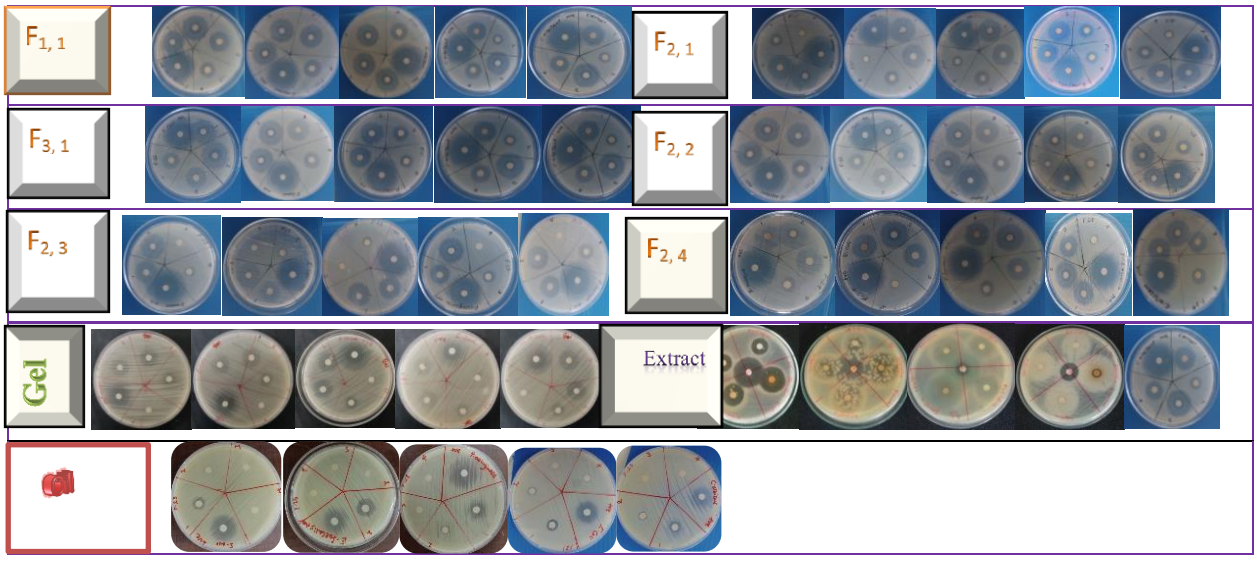


Table c. Inhibition activities of extract and ointments

Appendix D Physiochemical of evaluation of ointment

Figure d. Solubility, spreadability and viscosity measurement

Appendix E Extraction variable and yield oil by response surface

Table e. Optimization of oil yield at different variables level

Std	Run	A:Temperature (^{o}C)	B:Time (hr)	C:Ratio (V/W)	Yield (%)
9	1	70	2	12.5	23.28
12	2	70	4	17.5	29.49
7	3	65	3	17.5	21.78
14	4	70	3	15	26.54
17	5	70	3	15	26.26
5	6	65	3	12.5	19.16
4	7	75	4	15	28.28
15	8	70	3	15	27.18
3	9	65	4	15	21.64
2	10	75	2	15	25.15
6	11	75	3	12.5	28.31
16	12	70	3	15	26.46
10	13	70	4	12.5	26.11
8	14	75	3	17.5	29.14
11	15	70	2	17.5	23.43
1	16	65	2	15	16.48
13	17	70	3	15	26.72

Appendix F Calculation result of the TPC of *P.falcatus* oil

Table f.1. Calibration curve of standard Gallic acid for phenol detection of solvents

Solution used for detection(µL)	20	40	60	80	100	120

GA concentration solution (mg/mL)	1.25	2.5	3.75	5	6.25	7.5
Absorbance	0.2641	0.5187	0.6985	0.9251	1.1022	1.2964
Standard concentration of Gallic acid is 0.0625 g/solution						

Table f.2. Results of extraction solvents on TPC of *P.falcatus seed oil*

Solvents	Volume(mL)	Mass(g)	$\bar{X} = \frac{y-c}{m}$ at different Abs.	TPC(mg/mL)	± SD
Chloroform	8.72	8.46	5.35226078	5.516751	0.107821028
Methanol	4.2	3.71	7.74553102	8.768526	0.151797927
Ethanol	6	5.82	6.79621451	8.181623	0.169654706
Hexane	9.5	9.23	5.47570978	5.635888	3.541633771

Table f.3. Calibration curve of the standard Gallic acid for phenol detection of temperature

Solution used for detection(µL)	25	50	100	150	200
GA concentration solution (mg/mL)	1.25	2.5	5	7.5	10
Absorbance	0.2231	0.445	0.8089	1.2056	1.4291

Table f .4. Effect of extraction temperature on the TPC of *P.falcatus seed oi*

Temperature (℃)	Volume(mL)	Mass(g)	$\bar{X} = \frac{y-c}{m}$ at different Abs.	TPC(mg/mL)	± SD
60	3.74	3.63	6.12824173	6.31	0.31468372
65	4.56	4.43	7.74732334	7.97	0.273915774
70	5.8	5.52	8.42398287	8.85	0.074019625
75	6.34	6.15	8.413276231	8.67	0.150910294

80	6.56	6.3	6.97811087	7.23	0.48333444

Table f.5. Calibration curve of the standard Gallic acid for phenol detection of time

Solution used for detection(µL)	20	40	60	80	100
GA concentration solution (mg/mL)	1.25	2.5	3.75	5	6.25
Absorbance	0.6423	0.8673	0.9873	1.221	1.484

Table f .6. The results of extraction time against TPC of *P.falcatus seed oil*

Time (hr)	Volume(mL)	Mass(g)	$\bar{X} = \frac{y-c}{m}$ at different Abs.	TPC(mg/mL)	± SD
2	4.96	4.82	6.89136646	7.091530631	0.258042953
4	5.88	5.71	8.24436853	8.483831516	0.021113952
6	6.2	6	7.481014493	7.730381643	0.0698757
8	6.4	6.23	6.039813665	6.204623989	0.174824585

Appendix G TPC, TFC and antioxidant activities of optimized oil and A.vera

Table g.1. Calibration curve of the standard Gallic acid for phenol detection

Solution used for detection(µL)	10	20	40	60	80	100
GA concentration solution (mg/mL)	1.25	2.5	5	7.5	10	
Absorbance	0.2236	0.3445	0.5008	0.6637	0.9274	1.0501

Table g.2. TPC content of *P.falcatus* seed oil, *A.vera* mucilage and its extracts

Extracts	volume	Mass(g)	$\bar{X} = \frac{y-c}{m}$ at different Abs.	TPC(mg/mL)	± SD
A.vera extract	-	12	4.443243243	88.56	0.476178328
A.vera gel	74	73.99	1.19021983	1.19021983	0.028281593
Optimized oil	6.25	6	10.145382866	10.58648649	0.49054057

Table g.3. Calibration curve of the standard quercetin acid for flavonoid detection

Solution used for detection(µL)	25	50	75	100	125	150
Quercetin concentration solution (mg/mL)	0.25	0.5	0.75	1	1.25	1.5
Absorbance	0.40	0.613	0.75	0.953	1.1013	1.2135

Table g. 4. TFC content of *P.falcatus* seed oil, *A.vera* mucilage and its extracts

Extracts	volume	Mass(g)	$\bar{X} = \frac{y-c}{m}$ at different Abs.	TFC(mg/mL)	± SD
A.vera extract	-	4	1.167837508	21.168	0.000543906
A.vera gel	74	73.99	0.208155162	1.19021983	0.028281593
Optimized oil	6.25	6	1.414477703	1.473414274	0.033284434

Appendix H Calculation results of DPPH for the extracts, oil and ointments

Table h.1. DPPH scavenging activities of P.falcatus oil, aloe vera gel and its extract

Volume of solution used (µL)	Concentration of samples (mg/mL)	RSA(%)			
		gel	extract	oil	AA
20	1	13.39	27.08	13.6	71.97
40	2	23.67	38.21	28.82	84.18
80	3	24.04	54.07	35.76	97.44
120	4	23.14	65.62	49.83	98.01
160	5	31.65	79.71	58.49	97
200	6	28	84.754	67.49	98
IC_{50} =		22.48	2.68	4.23	0.11

At the concentration samples of 1 mg /20 mL

Table h.2. DPPH scavenging of formulated ointments

Volume of solution used (µL)	Concentration of samples (mg/mL)	RSA(%)			
		F2,4	F3,4	F2,0	AA
100	0.1	13.82	13.23	11.59	29.37945733
200	0.2	20.47	25.198	15.46	40.86400525
300	0.3	25.36	29.158	17.099	94.64710222
400	0.4	28.89	33.707	16.29	95.39306501
500	0.5	29.37	35.92	17.94	95.90130339
600	0.6	29.29	36.789	17.78	98.35232396
IC_{50} =		1.1	0.85	1.54	0.17
At the concentration samples of 1 m g /10 mL					

Appendix I GS–MS results of *P.falcatus* seed oil and *A.vera* extract

Table I.1. The identified essential compound of *P.falcatus* seed oil by GS–MS

Compound Name	Formula	Rt.	Area	% Area	%
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	12.2507	130813	3.3273	10.119
9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	15.0224	155752	42.9891	12.048
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	15.112	153874	35.5586	11.902
Methyl stearate	$C_{20}H_{40}O$	15.2519	157883	3.3397	12.213
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	15.3933	152040	1.4067	11.761
Methyl 5,11,14-eicosatrienoate	$C_{21}H_{38}O_2$	18.5119	179091	5.2819	13.853
cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	18.687	182570	1.5545	14.122
cis-11,14-Eicosadienoic acid, methyl estera	$C_{21}H_{38}O_2$	18.8228	180766	6.5421	13.983

Table I.2. The major essential compound of *A. vera* extract identified by GS–MS

Compound name	Molecular Formula	Rt.	Area	%Area	%
Toluene	C_7H_8	3.21	570156	1.63	12.01
Bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylethyl)-	$C_{10}H_{16}$	5.44	261951	0.75	5.52
α-Pinene	$C_{10}H_{16}$	5.58	118089	33.77	2.49
β-Phellandrene	$C_{10}H_{16}$	6.20	222652	0.64	4.69
β-Pinene	$C_{10}H_{16}$	6.30	129769	3.71	2.74
β-Myrcene	$C_{10}H_{16}$	6.43	856010	2.45	18.04
Alpha -Phellandrene	$C_{10}H_{16}$	6.74	111923	0.32	2.36
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	6.92	206031	0.59	4.34
O-Cymene	$C_{10}H_{16}$	7.04	929289	2.66	19.58
D-Limonene	$C_{10}H_{16}$	7.12	188181	5.38	3.96
Eucalyptol	$C_{10}H_{18}o$	7.20	157164	44.95	3.31
3-Carene	$C_{10}H_{16}$	7.38	148382	0.42	3.12
Gamma -Terpinene	$C_{10}H_{16}$	7.61	655912	1.88	13.82
alpha-Terpinyl acetate	$C_{12}H_{20}O_2$	13.37	189700	0.54	3.99
Heptacosane	$C_{24}H_{50}S_2$	20.72	110133	0.31	2.32

Appendix J GC–MS analysis of P.falcatus seed oil and A.vera extract profile

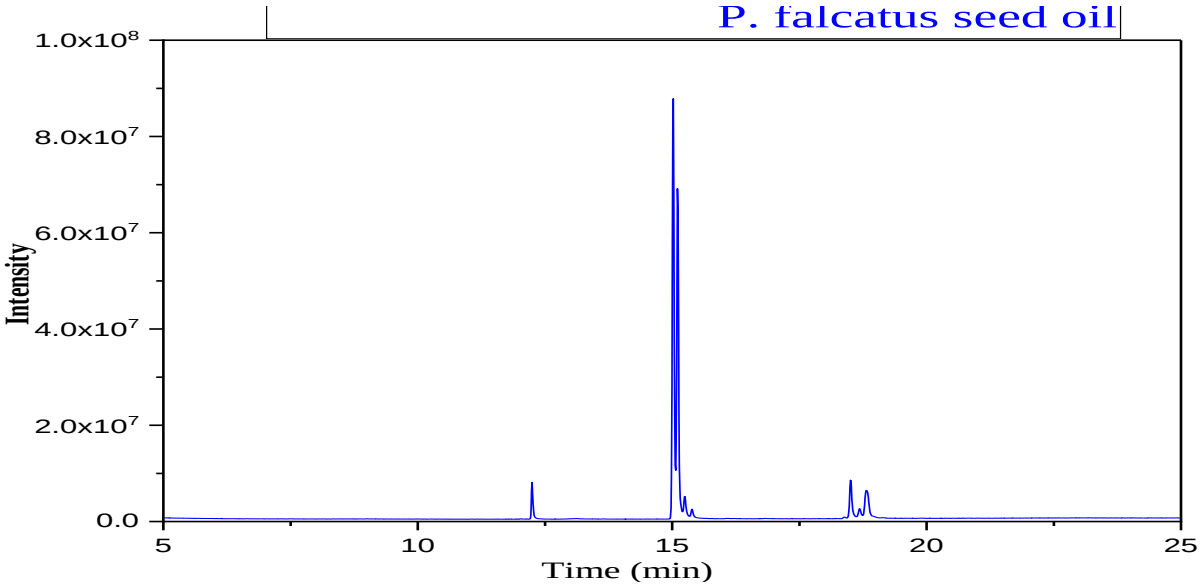


Figure g.1. GC–MS profile of ethanolic extract of P.falcatus seed oil

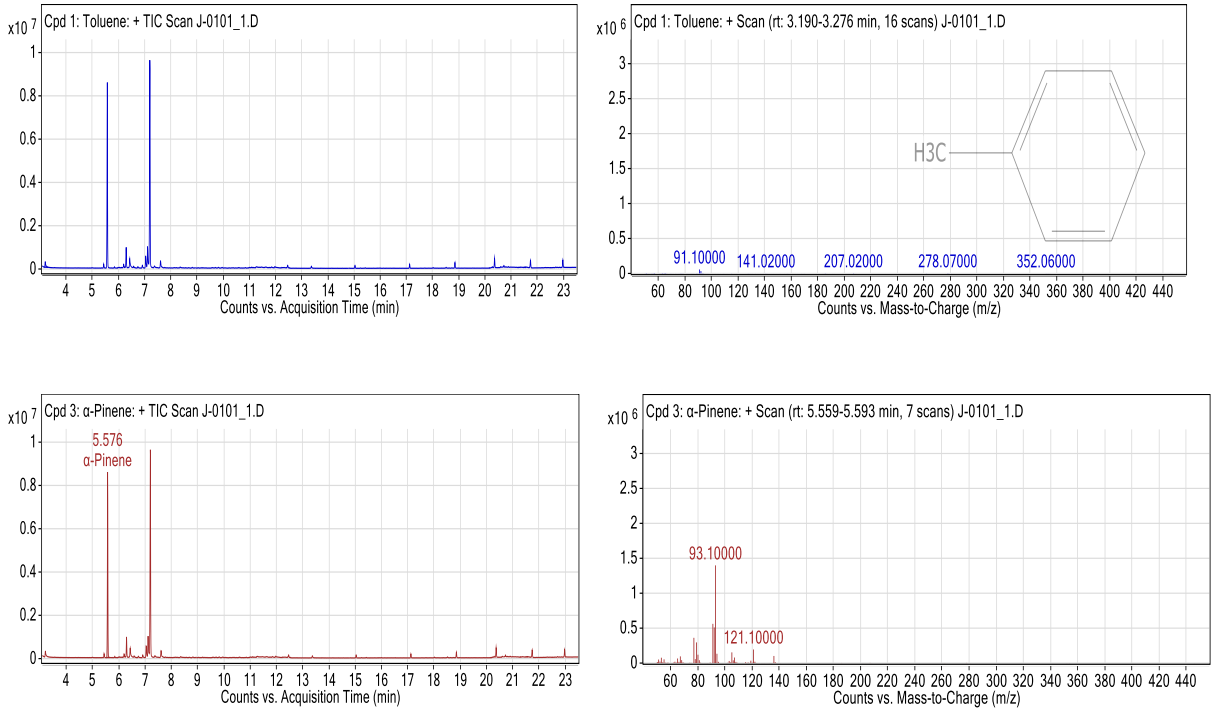


Figure g.4. GC–MS spectra of alph-pinene essential oil found in the extract of aloe vera extract

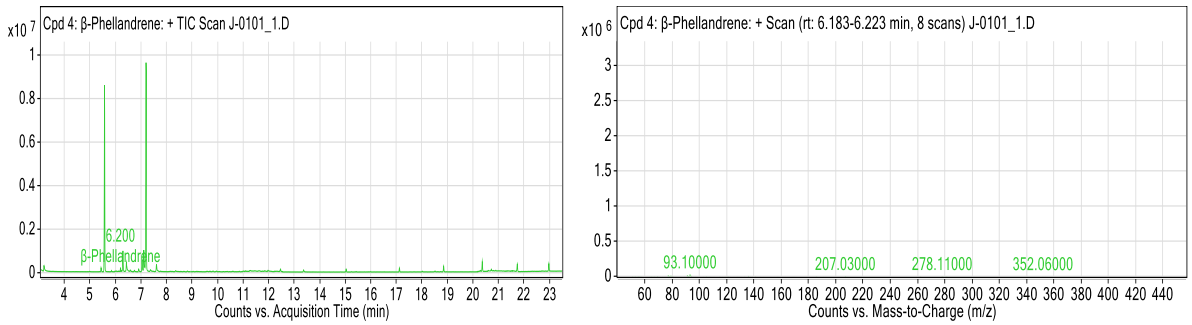


Figure g.5. GC–MS spectra of beta phellandrene essential oil found in the extract of aloe vera extract

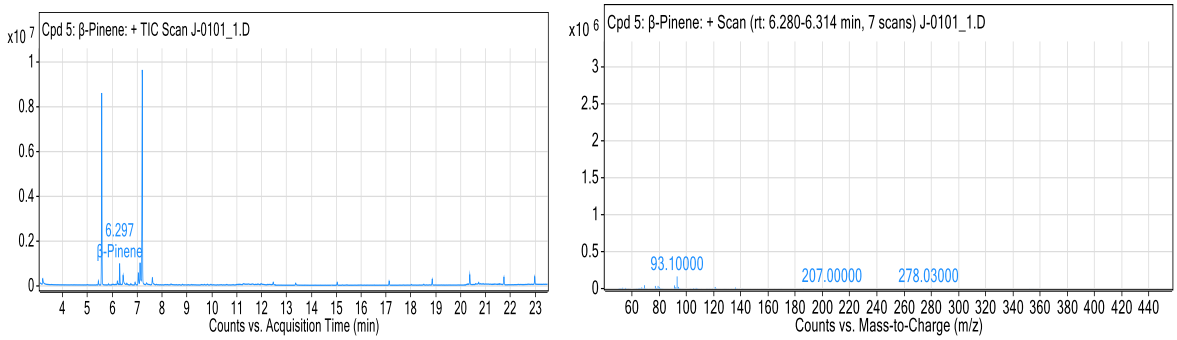


Figure g.6. GC–MS spectra of beta-pinene essential oil found in the extract of aloe vera extract

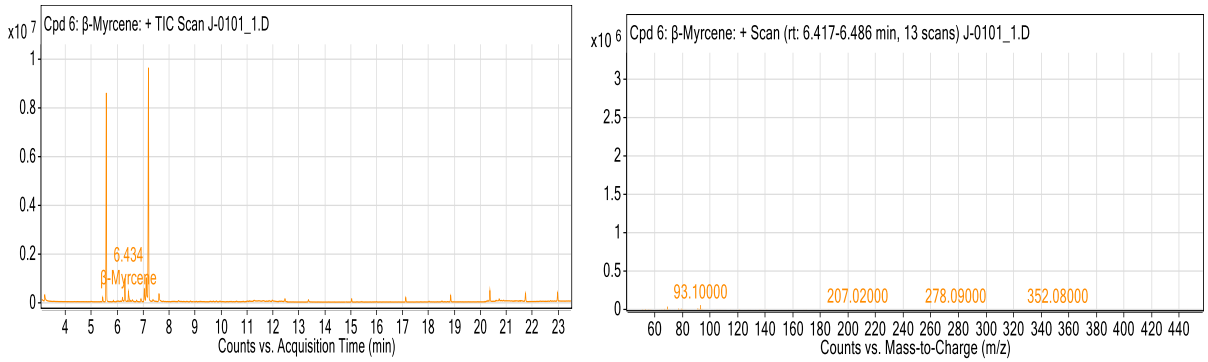


Figure g.7. GC–MS spectra of beta-myrcene essential oil found in the extract of aloe vera extract

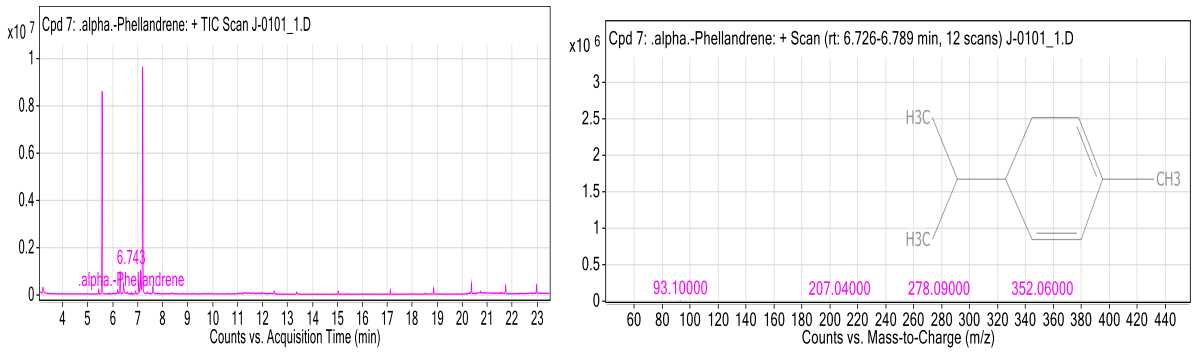


Figure g.8. GC–MS spectra of alph-pellandrene essential oil found in the extract of aloe vera extract

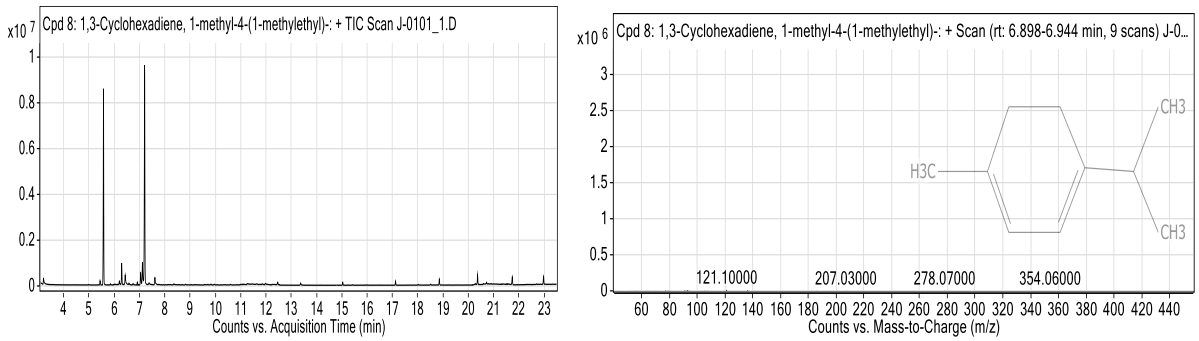


Figure g. 9 GC–MS spectra of toluene essential oil found in the extract of aloe vera extract

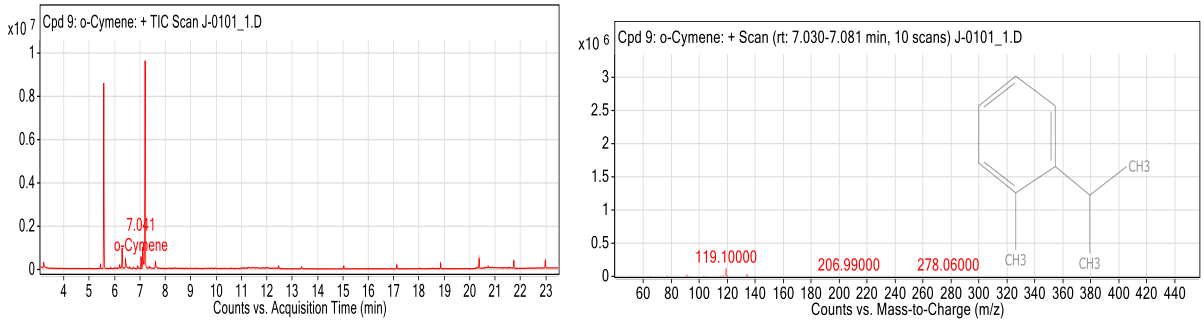


Figure g.10. GC–MS spectra of o-Cymene essential oil found in the extract of aloe vera extract

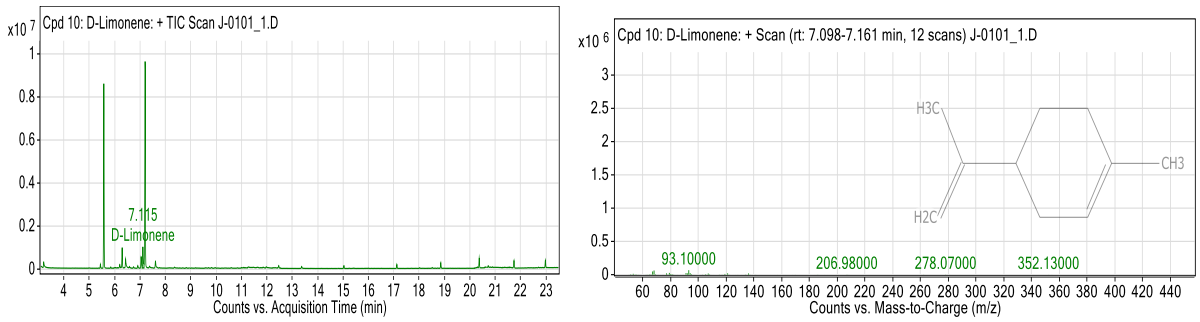


Figure g.11. GC–MS spectra of D-limonene essential oil found in the extract of aloe vera extract

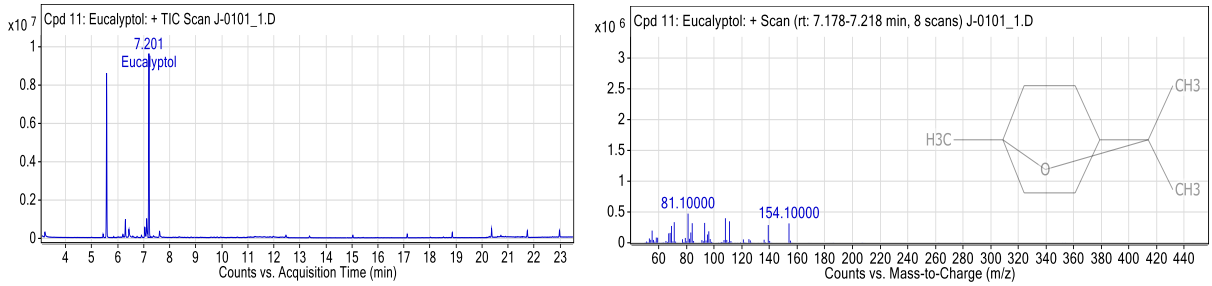


Figure g.12. GC–MS spectra of Eucalyptol essential oil found in the extract of aloe vera extract

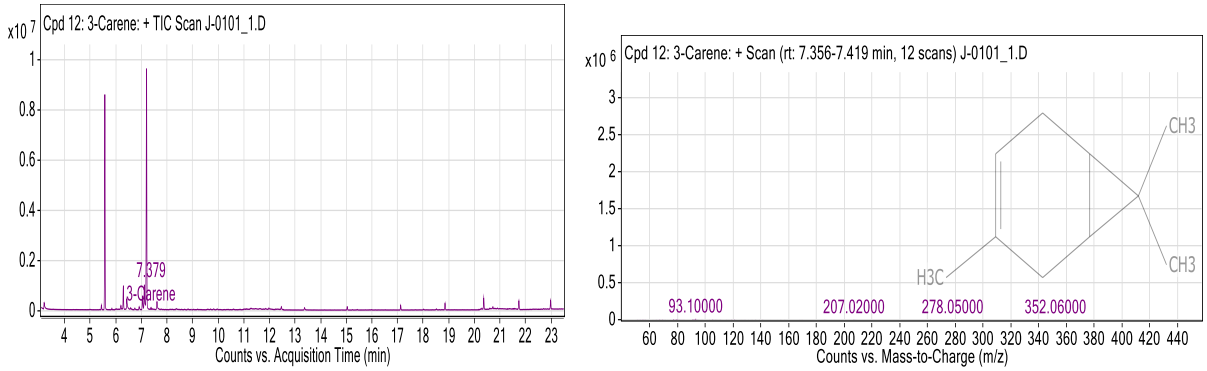


Figure g.13. GC–MS spectra of 3-carene essential oil found in the extract of aloe vera extract

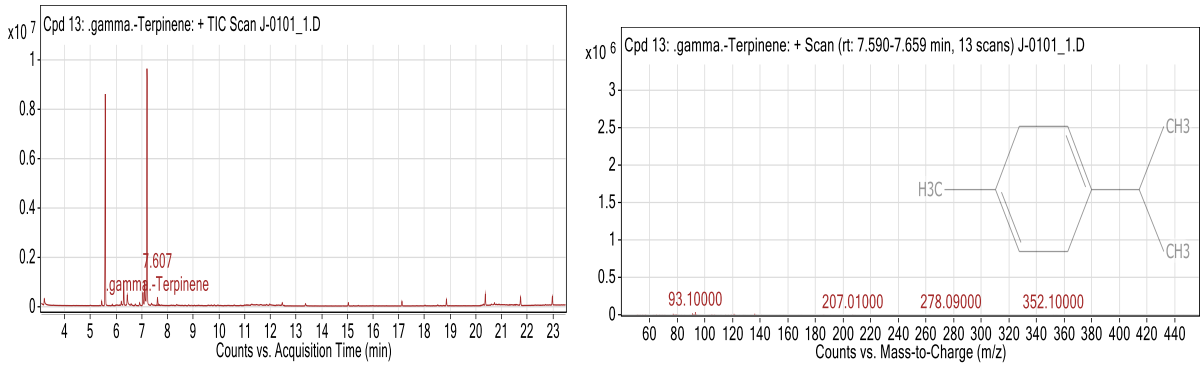


Figure g.14. GC–MS spectra of gamma-Terpinene essential oil found in the extract of aloe vera extract

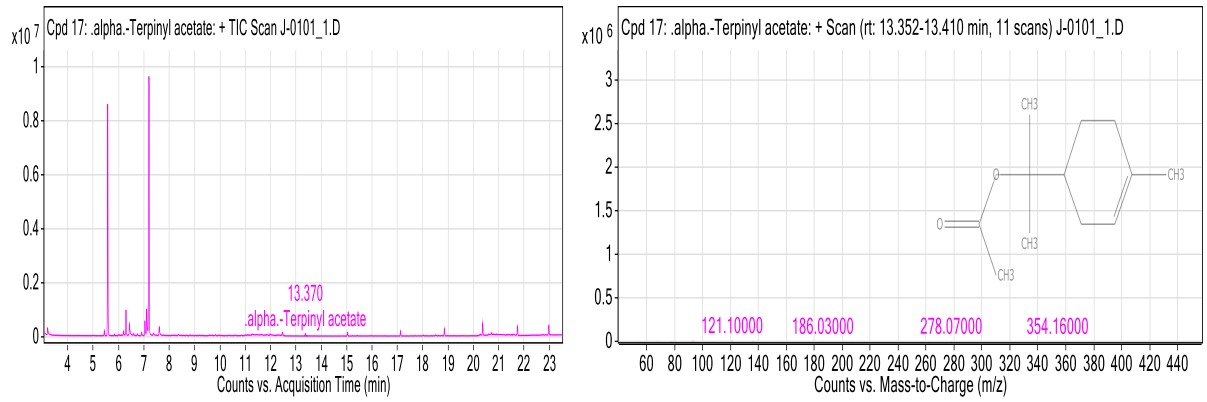


Figure g.15. GC–MS spectra of alpha-Terpinyl acetate essential oil found in the extract of aloe vera extract

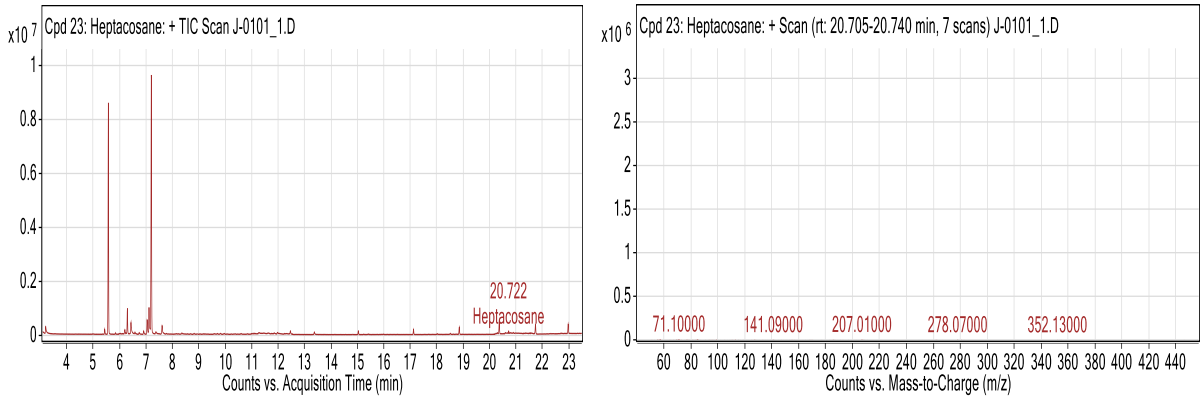


Figure g.16. GC–MS spectra of Heptacosane essential oil found in the extract of aloe vera extract