AVOCADO HEXANE LEAF EXTRACT: EXPLORING THE EFFECTS OF BALM FORMULATION ON ANTIMICROBIAL ACTIVITY AGAINST SELECTED PATHOGENIC MICROORGANISMS

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FACULTY OF PHARMACY,

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NOVEMBER, 2025

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY , FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQIREMENTS FOR THE AWARD OF THE DOCTOR OF PHARMACY (PHARM.D) DEGREE

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

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UNIVERSITY OF BENIN,

BENIN CITY.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work was carried out by Edemakhiota Gift Michelle with matriculation number PHA1908478 in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin-City, in partial fulfillment of the requirements for the award of Doctor of Pharmacy (Pharm.D) degree.

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(HEAD OF DEPARTMENT)

DEDICATION

This project is dedicated to my beloved mother, for being the most relentless, hardworking, and positive person I have ever known. You are the center of everything and the reason I chose this path. This work is also dedicated to every young girl with a dream. This serves as a reminder that with determination and perseverance, nothing is impossible.

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ABSTRACT

Persea americana (avocado) leaves are reported to exhibit antimicrobial properties. As herbal products become increasingly popular, it is crucial to investigate whether formulation excipients affect the antimicrobial efficacy of plant extracts such as avocado leaves**.** This study investigates the antimicrobial efficacy of *Persea americana* leaf extract formulated into balms, comparing its effectiveness to that of the crude n-hexane extract.

Fresh leaves of *Persea americana* were harvested, dried, pulverized and macerated in n-hexane. The extract obtained was subjected to phytochemical screening, then formulated into balms (0, 100 and 200 mg/mL) and tested against clinical strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis* using the ditch method. Ciprofloxacin was used as a positive control. Statistical significance was determined using a One Way ANOVA, with a significance level set at $p \leq 0.05$.

The hexane extract contained alkaloids, triterpenoids, steroids, phenolics, and proteins. At 100 mg/mL, the balm exhibited antimicrobial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus,* while the hexane extract showed no activity. At 200 mg/mL, the extract showed broader antimicrobial activity against the five test organisms compared to the balm. The balm containing no extract showed no activity against any of the test organisms. The crude n-hexane extract exhibited dose-dependent antibacterial activity, producing inhibition zones of 17–20 mm at 200 mg/mL and 22–24 mm at 400 mg/mL, corresponding to approximately 50–77% of ciprofloxacin (4 mg/mL) activity. When formulated into a balm, antimicrobial potency of the extract decreased slightly, indicating possible interactions between the plant extract and the components of the balm.

Formulating the n-hexane extract of *Persea americana* leaves into balm reduced the extract's antimicrobial activity. This suggests that excipient-extract interactions are essential factors to consider in the development of herbal formulations.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AMR – Antimicrobial Resistance

ATCC – American Type Culture Collection

CFU – Colony Forming Unit

MIC – Minimum Inhibitory Concentration

MHA – Mueller–Hinton Agar

NCCLS – National Committee for Clinical Laboratory Standards

SD – Standard Deviation

WHO – World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Persea americana

Persea americana, commonly known as avocado, is a perennial tree belonging to the family Lauraceae. It is an economically and medicinally important plant native to Central America and now widely cultivated in tropical and subtropical regions. In Nigeria, it is locally known as Ewe pia (Yoruba), Akwukwo Ube Oyibo (Igbo), and Ganyen Piya (Hausa). The leaves, seeds, and fruits of *Persea americana* have long been employed in traditional medicine for the management of hypertension, diabetes, gastrointestinal disorders, and microbial infections (Ejimofor *et al.*, 2022). The plant's therapeutic properties are largely attributed to its diverse phytochemical constituents, which include alkaloids, flavonoids, phenolics, steroids, and triterpenoids (Ngbolua *et al.*, 2019).

Scientific classification:

Kingdom – Plantae

Division – Magnoliophyta

Class – Magnoliopsida

Order – Laurales

Family – Lauraceae

Genus – Persea

Species – *Persea americana* Mill.

Common names – Avocado, Alligator pear, Palta pear

FIGURE 1.1: Avocado growing in its natural habitat

1.1.1 Phytochemistry, Ethno-Medicinal Uses, and Pharmacological Profile of *Persea americana* Leaves

The leaves of *Persea americana* contain numerous bioactive compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, and sterols, which contribute to its pharmacological activities (Duarte *et al.*, 2016). Phytochemical investigations have revealed the presence of compounds like β-sitosterol, stigmasterol, lupeol, squalene, gallic acid, quercetin, rutin, and apigenin. These compounds are responsible for antioxidant, anti-inflammatory, antimicrobial, and lipid-lowering effects (Ngbolua *et al*., 2019). Traditionally, *Persea americana* leaves are used in the treatment of ailments such as hypertension, toothache, diarrhea, and parasitic infections. Decoctions of the leaves are commonly consumed to alleviate fever, promote wound healing, and manage diabetes (Tcheghebe *et al.*, 2016). Ethnomedicinal reports further highlight its application as a

topical agent for skin conditions and as a natural flavoring in traditional diets (Marrero-Faz *et a*l., 2014).

1.1.2 Pharmacological Actions of *Persea americana* Leaves

The leaves of *Persea americana* (avocado) have been extensively studied for their wide spectrum of pharmacological activities, attributed to their diverse phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, saponins, triterpenoids, and sterols. These bioactive metabolites act synergistically to produce antimicrobial, antihypertensive, anti-inflammatory, analgesic, anticonvulsant, antidiabetic, wound-healing, antiviral, and antioxidant effects (Bello *et al.*, 2025).

1.1.2.1 Antimicrobial Activity

Several studies have demonstrated the antimicrobial potential of *Persea americana* leaves against both Gram-positive and Gram-negative bacteria, as well as fungi of medical importance. Ethanolic and methanolic extracts have shown significant inhibition against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis,* and *Pseudomonas aeruginosa* (Nasri *et al.*, 2024). The antibacterial mechanism is believed to involve disruption of microbial cell membranes, protein denaturation, and inhibition of nucleic acid synthesis, attributed to the presence of phenolic acids, flavonoids, and alkaloids (Adeyemi *et al.*, 2020). Similarly, antifungal activity has been observed against *Candida albicans* and *Aspergillus niger,* suggesting potential use in the management of fungal skin infections. The lipophilic n-hexane extracts demonstrate moderate antimicrobial efficacy, which is enhanced when combined with more polar solvents

(Okwu & Emenike, 2021). These findings validate the traditional use of avocado leaves in the treatment of infectious diseases.

1.1.2.2 Antihypertensive Activity

The antihypertensive effect of *Persea americana* leaves is well documented and is primarily associated with their vasorelaxant and diuretic properties. The aqueous leaf extract has been shown to cause dose-dependent relaxation of isolated rat aorta, mediated through endothelium-derived relaxing factors such as nitric oxide (NO) and prostacyclin (Owolabi *et al.*, 2020). Furthermore, phytosterols and flavonoids in the extract modulate calcium influx across smooth muscle cell membranes, thereby reducing vascular resistance. Clinical and experimental studies have also revealed significant reductions in serum total cholesterol, triglycerides, and low-density lipoproteins (LDL), alongside increased high-density lipoproteins (HDL), suggesting cardioprotective benefits (Olaniyan, 2014). This dual antihypertensive and lipid-lowering action positions Persea americana as a valuable natural agent for managing hypertension and related cardiovascular disorders.

1.1.2.3 Anti-inflammatory and Analgesic Activity

The anti-inflammatory and analgesic properties of *Persea americana* leaves have been attributed to flavonoids, steroids, and triterpenoids that modulate inflammatory mediators. In experimental models, aqueous and methanolic leaf extracts significantly reduced carrageenan-induced paw edema and acetic acid-induced writhing in mice (Ngbolua *et al.*, 2019). The mechanism involves inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways, leading to reduced synthesis of prostaglandins and leukotrienes.

Furthermore, the analgesic effect of *Persea americana* was shown to be comparable to standard analgesics such as aspirin and morphine in certain models, confirming its dual central and peripheral analgesic activities. This provides a scientific basis for its traditional use in the management of pain, rheumatism, and inflammatory disorders (Brai *et al*., 2020).

1.1.2.4 Anticonvulsant Activity

The anticonvulsant activity of *Persea americana* leaf extract has been demonstrated in several animal models, where aqueous extracts delayed seizure onset and reduced seizure duration in pentylene tetrazole (PTZ)-, picrotoxin (PCT)-, and bicuculline (BCL)-induced convulsions (Ngbolua *et al*., 2019). The mechanism is likely related to modulation of gamma-aminobutyric acid (GABA) neurotransmission, enhancing inhibitory synaptic activity in the central nervous system. This suggests that bioactive compounds such as alkaloids and triterpenes in the extract may act as natural GABAergic agents or calcium channel modulators. Consequently, Persea americana could serve as a potential therapeutic agent for epilepsy and related neurological disorders (Adeyemi *et al.*, 2020).

1.1.2.5 Antidiabetic Activity

The hypoglycemic and antidiabetic properties of Persea americana have been extensively reported. The aqueous and methanolic leaf extracts inhibit carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase, reducing postprandial glucose absorption (Jamshid *et al*., 2017). In addition, they exhibit protein tyrosine phosphatase 1B (PTP1B) inhibitory activity, which enhances insulin sensitivity and glucose uptake in peripheral tissues. In vivo studies have demonstrated significant reductions in fasting blood glucose,

glycosylated hemoglobin (HbA1c), and lipid peroxidation levels in diabetic rats treated with avocado leaf extract (Marrero-Faz *et al.*, 2014). These effects are likely mediated by flavonoids, saponins, and phenolics, which act synergistically to regulate carbohydrate metabolism and protect pancreatic β-cells from oxidative damage.

1.1.2.6 Wound-Healing Activity

The wound-healing potential of *Persea americana* leaf extract has been demonstrated in several preclinical studies. Topical application of ethanolic extract significantly increased fibroblast proliferation, collagen synthesis, and epithelialization rate in excision wound models (Khoswanto *et al.*, 2018). The acceleration of healing is attributed to the presence of saponins, tannins, and flavonoids, which promote tissue regeneration and angiogenesis. Histological studies further revealed reduced inflammatory cell infiltration and enhanced granulation tissue formation in treated wounds, indicating both anti-inflammatory and antioxidant contributions to the healing process. This validates the inclusion of avocado leaf extract in topical herbal balms for treating skin injuries and infections (Abdelnour *et al*., 2021).

1.1.2.7 Antiviral Activity

The antiviral activity of *Persea americana* leaves is attributed mainly to phenolic compounds such as catechins, flavonoids, and triterpenoids. These compounds exhibit inhibitory effects against viral enzymes involved in replication (Miranda *et al.*, 1997) reported that ethanolic extracts of avocado leaves demonstrated activity against Herpes simplex virus type 1 (HSV-1), Adenovirus, and HIV-1. The proposed mechanisms include inhibition of viral entry and replication, as well as disruption of viral envelope

integrity. Moreover, avocado leaf extracts have been shown to boost the host immune response by upregulating interferon production, thus enhancing viral clearance (Singh *et al*., 2022).

1.1.2.8 Antioxidant Activity

Persea americana leaves possess strong antioxidant properties, which are essential in mitigating oxidative stress-related diseases. The methanolic and aqueous extracts have demonstrated significant free radical scavenging activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radicals (Zhang *et al.,* 2023). These antioxidant effects are attributed to phenolic acids, flavonoids, and tocopherols, which neutralize reactive oxygen species (ROS) and inhibit lipid peroxidation. Consequently, the antioxidant action of avocado leaves underpins several of its pharmacological activities, including anti-inflammatory, cardioprotective, and hepatoprotective effects.

1.1.2.9 Safety Profile

Toxicological studies have shown that *Persea americana* leaf extracts are generally safe at moderate doses. However, high concentrations may induce hepatotoxic effects due to the presence of persin and certain alkaloids (Adeyemi *et al.,* 2020). Acute and sub-chronic toxicity tests in rodents demonstrated no mortality or significant biochemical alterations at doses below 1000 mg/kg, supporting its traditional use (Olaniyan *et al.*, 2014). Nevertheless, caution is advised in prolonged use, and further toxicokinetic studies are recommended to ensure human safety.

1.2 *Apis mellifera* (Beeswax)

Beeswax is a natural product secreted by worker bees (*Apis mellifera*) and used in constructing honeycombs. It is composed mainly of esters of fatty acids and long-chain alcohols. Apart from its primary function in the hive, beeswax has gained recognition in pharmaceutical, cosmetic, and food industries due to its biocompatibility, non-toxicity, and stability. Beeswax has been used for centuries in traditional medicine for its wound-healing, antimicrobial, anti-inflammatory, and emollient properties. In topical formulations, it serves both as an active ingredient and as an excipient, functioning as a thickening agent, stabilizer, and moisturizer (Fratini *et al*., 2016).

Scientific Classification

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hymenoptera

Family: Apidae

Genus: Apis

Species: *Apis mellifera*

Common Name: Honey bee

FIGURE 1.2: Picture of *Apis mellifera*

1.2.1 Phytochemistry, Ethno-Medicinal Uses, and Pharmacological Profile of *Apis mellifera* (Beeswax)

Beeswax is a complex mixture of hydrocarbons (approximately 14%), monoesters (35%), diesters (14%), free fatty acids (12%), and long-chain alcohols (1%). The predominant components include myricyl palmitate, cerotic acid, and hydrocarbons such as

hentriacontane (Muresan *et al*., 2021). Trace compounds such as propolis residues, flavonoids, and aromatic substances also contribute to its biological activity. From an ethnomedicinal perspective, beeswax has been incorporated into balms, salves, and ointments to treat burns, wounds, eczema, and skin irritation. In many African and Asian traditional practices, beeswax is used in combination with herbal extracts to enhance skin penetration and prolong the stability of active compounds. Pharmacologically, beeswax functions as an occlusive agent, creating a protective barrier on the skin that prevents moisture loss and allows for a sustained release of therapeutic compounds (Fratini *et al.*, 2016; Abdelnour *et al.,* 2021). The fatty acids and esters in beeswax also exhibit mild antibacterial and anti-inflammatory effects, making it a valuable component of topical herbal formulations.

1.2.2 Pharmacological Actions of Apis mellifera (Beeswax)

1.2.2.1 Antimicrobial Activity

Beeswax has demonstrated moderate antimicrobial activity against various Gram-positive and Gram-negative bacteria, as well as some fungal species. Studies have shown inhibitory effects against *Staphylococcus aureus, Bacillus subtilis,* and *Candida albicans*, largely due to its minor constituents such as free fatty acids, phenolic residues, and traces of propolis (El-Sohaimy *et al*., 2020). When incorporated into herbal formulations, beeswax enhances the physical stability and antimicrobial retention of the active plant extracts. Its hydrophobic nature facilitates the entrapment of lipophilic phytochemicals, promoting their gradual release on the skin surface. This sustained-release property amplifies the antimicrobial efficacy of topical formulations containing herbal extracts (Fratini *et al*., 2016).

1.2.2.2 Anti-inflammatory and Wound-Healing Activity

Beeswax is well recognized for its potent wound-healing and anti-inflammatory properties. Its mechanism of action is primarily attributed to its ability to maintain a moist wound environment, which promotes re-epithelialization and fibroblast proliferation. Studies have shown that beeswax-based formulations significantly accelerate wound contraction and collagen deposition compared to conventional bases (Abdelnour *et al.,* 2021). In an animal study, beeswax-containing ointments reduced inflammatory cell infiltration and edema, with results comparable to standard anti-inflammatory drugs (Sharma *et al*., 2022). This effect is enhanced when beeswax is combined with plant extracts rich in flavonoids and phenolics, as these compounds act synergistically to suppress pro-inflammatory mediators such as TNF-α, IL-1β, and COX-2 (Muresan *et al*., 2021).

1.2.2.3 Antioxidant and Emollient Properties

Beeswax exhibits mild antioxidant activity due to the presence of unsaturated fatty acids and trace amounts of phenolic compounds derived from propolis residues. These compounds scavenge reactive oxygen species (ROS), thereby protecting skin lipids and proteins from oxidative damage (Al-Waili *et al.*, 2020). Its emollient action, derived from the presence of natural esters and waxes, helps to soften and hydrate the skin, enhancing comfort and reducing irritation. This property explains its inclusion in dermatological and cosmetic formulations aimed at treating eczema, psoriasis, and dermatitis.

1.2.2.4 Role in Herbal Formulations

In addition to its intrinsic pharmacological activities, beeswax serves a vital pharmaceutical function in herbal formulations. It acts as a base and consistency enhancer in ointments, creams, and balms. Its lipophilic character makes it particularly suitable for encapsulating hydrophobic plant extracts such as *Persea americana* n-hexane leaf extract, thereby improving formulation stability and patient compliance. However, while its hydrophobic matrix helps prolong drug release, it can also limit the immediate bioavailability of the active compounds, a phenomenon observed in avocado leaf balm formulations where antimicrobial activity was reduced compared to the crude extract. This effect results from the partial encapsulation of lipophilic phytochemicals within the wax matrix, reducing their diffusion to the target site (Etukudo and Akinmoladun, 2022).

1.3 Background of the Study

For centuries, medicinal plants have been a cornerstone of healthcare across cultures, providing remedies for both common and complex ailments (Bussmann *et al*; 2018). They remain a significant source of novel bioactive compounds in modern drug discovery (Bolger, C.A *et al*; 2017). The global burden of infectious diseases, coupled with the alarming rise in antimicrobial resistance, has necessitated a renewed focus on natural products as alternatives or complements to synthetic drugs (Newman, D.J. *et al*; 2020).

Avocado leaf (*Persea americana*), a member of the *Lauraceae* family, is a widely cultivated plant known for its nutritional and therapeutic importance (Giddings, L.A *et al*; 2017). While the fruit is valued as a rich source of vitamins, minerals, and unsaturated fatty acids, its leaves have been traditionally used in ethnomedicine for the treatment of hypertension, diabetes, gastrointestinal disturbances, and microbial infections (Cragg,

G.M. *et al*; 2018). Phytochemical investigations of avocado leaves have revealed the presence of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins, and saponins, which are known to exert pharmacological activities (DeGoey, D.A *et* al; 2018). Infectious diseases caused by bacteria and fungi remain a leading cause of morbidity and mortality worldwide. Organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* are implicated in conditions ranging from superficial skin infections to life-threatening systemic illnesses. The challenge is further compounded by the rise of multidrug-resistant strains, rendering many conventional antibiotics less effective. Given this context, there is an urgent need to explore plant-based antimicrobials that may provide safe, effective, and affordable alternatives. Avocado leaves, which are widely available and traditionally used for treating infections, present an attractive candidate for such studies.

1.4 Statement of the Problem

The problem of antimicrobial resistance has been described as one of the greatest threats to public health in the 21st century (Tarassoli, Z *et al*; 2021). Resistance mechanisms such as efflux pumps, enzymatic degradation of drugs, and biofilm formation have rendered many first-line antibiotics ineffective. As a result, infections that were once easily treatable have become major clinical challenges (Solis-Salis *et al*; 2021). At the same time, the discovery pipeline for new antibiotics has slowed dramatically, with few novel classes introduced in recent decades. This has created an urgent need for alternative approaches, including the search for antimicrobial agents from natural products (Agunloye M.O *et* al; 2025). Furthermore, while crude extracts may demonstrate activity

in vitro, there is a need to investigate whether such activity is retained when incorporated into pharmaceutical formulations designed for practical application, such as balms.

1.5 Rationale of the Study

The rationale for this study is grounded in three main considerations. First, avocado leaves are an underexplored resource with potential to provide bioactive compounds that can address the growing problem of antimicrobial resistance. Second, n-hexane extraction preferentially isolates lipophilic compounds such as steroids and certain alkaloids, which may exhibit antimicrobial effects different from those of polar extracts. Third, translating crude extract activity into a usable formulation is essential for practical application, as patients are more likely to benefit from standardized topical products than from unprocessed plant materials. By comparing the antimicrobial activity of the crude extract with that of a formulated balm, this study provides insight into the impact of formulation on the bioactivity of herbal extracts. This dual approach not only validates traditional claims but also advances the scientific basis for developing avocado-based antimicrobial products.

1.6 Aim of the Study

The aim of this study is to evaluate the phytochemical constituents and antimicrobial activity of the n-hexane extract of avocado (*Persea americana*) leaves, and to investigate the effect of incorporating the extract into a balm formulation.

1.7 Objectives of the Study

The specific objectives of the study are to:

1. Extract the leaves of *Persea americana* using n-hexane by maceration method.

2. Qualitative phytochemical screening of the n -hexane extract.

3. Evaluation of the antimicrobial activity of the hexane extract against selected clinical isolates.

4. Incorporation of different concentrations of the n hexane extract into a balm .

5. Determination of the antimicrobial activity of the formulated balms

6. To compare the antimicrobial activity of the hexane extract and formulated balms

1.8 Significance of the Study

This research holds significance for both science and society. Scientifically, it contributes to the growing body of knowledge on the phytochemistry and antimicrobial potential of avocado leaves. It also provides preliminary evidence for the effect of formulation on the antimicrobial activity of herbal extracts, highlighting the importance of dosage form design in phytomedicine research (Bongo, G.N *et al;* 2024). Socially, the study has implications for the development of affordable, accessible, and natural remedies for common infections, particularly in resource-limited settings where access to conventional antibiotics may be restricted. The ability to incorporate avocado extract into a balm formulation adds practical value, as balms are easy to apply, well-accepted by patients, and particularly suited for treating skin infections (Sharma, A *et al;* 2024).

1.9 Scope of the Study

This study focused on the phytochemical screening and antimicrobial evaluation of the n-hexane extract of avocado leaves. The antimicrobial assay was limited to selected bacterial and fungal clinical isolates of medical relevance. The study also included the formulation of a herbal balm containing the extract and the evaluation of its antimicrobial

activity. The research did not include fractionation of the extract, in vivo studies, or toxicity evaluation, which are recommended for future work.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1. MATERIALS

Leaves of *Persea americana*, Sabourand dextrose agar (Titan Biotech), Mueller hinton agar (Titan Biotech), Mylar agar (Titan Biotech), Yellow Beeswax, Cocoa Butter, Shea Butter, Olive oil, Vitamin E, Pepper mint oil.

2.2. EQUIPMENTS

Water bath (Stuart®), Rotary evaporator (Stuart®), Glass rod, Foil paper, Maceration jar, 250ml beaker, Porcelain dish, Measuring cylinder, Spatula, Masking tape, Sample tubes, Oven (Gallenkamp, England), Electronic weighing balance (M-METLAR M4 11R), Autoclave (Gallenkamp, England), Dispensing container, White handkerchief, Whatmann No 1 filter paper, Tissue paper, Micropipette, Test tubes, Loop, Petri dishes, EDTA bottle, Cork borer, Incubator (Gallenkamp, England), Metre rule.

2.3. REAGENTS AND SOLVENTS

Ethanol (GH-TECH), Tween-80 diluent (Titan Biotech), Distilled water.

2.4. Study Area and Duration

This study was conducted in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The laboratory work spanned a period of four months, from May to September 2025. All experiments, including

extraction, phytochemical screening, antimicrobial assays, and balm formulation, were carried out under standard laboratory conditions.

2.5 METHODOLOGY

2.5.1 Plant Collection

Fresh leaves of avocado (*Persea americana*) were harvested from a farm located in Obagie community, Ikpoba Okha local government area of Edo State.

2.5.2 Preparation of Plant Material

The leaves were thoroughly washed with clean water to remove dirt and debris. They were air-dried at room temperature and were ground into coarse powder using a mechanical grinder. The powdered sample was weighed and stored in an airtight container until further analysis.

2.5.3 Extraction Procedure

The powdered avocado leaves were subjected to maceration using n-hexane. Exactly 500 g of the powdered leaves were soaked in 2.5 L of n-hexane for seven days with intermittent shaking. After seven days, the mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated *in* a rotary evaporator at 40 oC to remove the solvent, yielding a dark green sticky residue.

2.5.4 Phytochemical Screenings

The n-hexane extract of *Persea americana* (avocado) leaves was subjected to a series of qualitative phytochemical tests to identify the major classes of secondary metabolites present in the extract. The screening was carried out according to the standard methods described by Harborne (1998), Sofowora (2008), and Evans (2009). The tests were

performed to detect the presence or absence of alkaloids, carbohydrates, reducing sugars, saponins, steroids/triterpenes, phenolics, tannins, flavonoids, proteins, and deoxy sugars. The phytochemical screening serves as a preliminary step in determining the bioactive constituents that may be responsible for the observed pharmacological effects of the extract. Each test was based on characteristic color changes or precipitate formation resulting from the interaction of the extract with specific reagents.

2.5.5 Test for Alkaloids

To detect alkaloids, 2 mL of the n-hexane extract was transferred into three separate test tubes, and a few drops of Wagner's, Mayer's, and Dragendorff's reagents were added respectively. Each tube was gently shaken and allowed to stand for 5 minutes. The formation of reddish-brown precipitate with Wagner's reagent, cream precipitate with Mayer's reagent, or orange-brown precipitate with Dragendorff's reagent indicated the presence of alkaloids. The appearance of such reactions was noted, confirming that alkaloidal bases were extractable even in the lipophilic n-hexane medium, possibly due to their non-polar free base forms.

2.5.6 Test for Carbohydrates (Molisch's Test)

To test for the presence of carbohydrates, 2 mL of the extract was mixed with two drops of Molisch's reagent, followed by careful addition of concentrated sulfuric acid down the side of the test tube to form a separate layer. The mixture was observed for a violet or purple ring at the interface of the two layers, which would indicate the presence of carbohydrates. No coloration was observed, signifying that carbohydrates were absent in the n-hexane extract.

2.5.7 Test for Reducing Sugars (Fehling's Test)

For reducing sugars, 2 mL each of Fehling's solution A and Fehling's solution B were mixed with 2 mL of the extract in a test tube and heated in a boiling water bath for 5 minutes. The appearance of a brick-red precipitate would indicate the presence of reducing sugars. However, the reaction yielded no such precipitate, confirming the absence of reducing sugars in the extract.

2.5.8 Test for Saponins (Frothing Test)

The presence of saponins was determined using the froth test. Two milliliters of the extract were diluted with 10 mL of distilled water in a test tube and shaken vigorously for 30 seconds. The mixture was allowed to stand for 10 minutes and observed for the formation of a stable froth layer. The absence of a persistent honeycomb froth indicated that saponins were not present in the n-hexane extract.

2.5.9 Test for Steroids and Triterpenes (Liebermann–Burchard Test)

To detect steroids and triterpenes, 2 mL of the extract was mixed with 2 mL of acetic anhydride, followed by careful addition of 1 mL of concentrated sulfuric acid down the side of the test tube. The appearance of a blue-green color indicated the presence of steroids, while a red or pink coloration indicated triterpenes. The reaction produced a greenish coloration, confirming the presence of steroidal compounds in the extract, consistent with the non-polar extraction medium used.

2.5.10 Test for Phenolic Compounds (Ferric Chloride Test)

Phenolic compounds were detected by adding 2 mL of 10% ferric chloride solution to 2 mL of the extract. The formation of a deep blue or green coloration indicated the presence of phenolic compounds. This reaction was positive, confirming that phenolic

compounds were extractable even in n-hexane, possibly due to the partial solubility of certain phenolic derivatives in semi-polar environments.

2.5.11 Test for Tannins

To test for tannins, 2 mL of the extract was mixed with 2 mL of distilled water, boiled for 5 minutes, and filtered. Two drops of 10% ferric chloride were added to the filtrate and observed for color change. The absence of blue-black or greenish coloration indicated that tannins were not present in the extract.

2.5.12 Test for Flavonoids (Alkaline Reagent Test)

Flavonoids were tested by mixing 2 mL of the extract with 2 mL of 10% sodium hydroxide solution. The appearance of an intense yellow color that disappears upon acidification with dilute hydrochloric acid would confirm the presence of flavonoids. The test yielded no color change, showing that flavonoids were absent in the n-hexane extract.

2.5.13 Test for Proteins (Biuret Test)

The Biuret test was employed to detect proteins. Two milliliters of the extract were mixed with 1 mL of 10% sodium hydroxide solution and 2 drops of 1% copper sulfate solution. The mixture was shaken and observed for a violet or purple coloration, indicating the presence of peptide linkages. A light violet coloration was observed, suggesting the presence of trace proteins or proteinaceous materials.

2.5.14 Test for Deoxy Sugars (Keller–Killiani Test)

The Keller–Killiani test was used to identify the presence of deoxy sugars associated with cardiac glycosides. Two milliliters of the extract were mixed with 2 mL of glacial acetic acid containing a trace amount of ferric chloride, followed by careful addition of

concentrated sulfuric acid down the side of the test tube. The appearance of a brown ring at the interface would confirm the presence of deoxy sugars. No brown ring was observed, indicating the absence of deoxy sugars in the extract.

2.6 Test Microorganisms

Clinical isolates of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Klebsiella pneumoniae* were employed in this study. These microorganisms were selected based on their clinical and epidemiological relevance as common etiological agents of human infections, including skin, respiratory, gastrointestinal, and urinary tract infections (Okorie *et al*; 2022). The isolates were obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, where they had been previously identified and preserved under standardized laboratory conditions. The bacterial isolates were maintained on sterile nutrient agar slants to ensure viability and stability of their morphological characteristics (Onwuka *et al*.; 2023). All cultures were subcultured every 72 hours to maintain their viability and prevent contamination. Prior to each antimicrobial assay, the bacterial isolates were inoculated into nutrient broth and incubated at 37 °C for 24 hours. Subculturing and inoculum preparation were conducted aseptically using sterile inoculating loops and pipettes under laminar flow conditions to minimize contamination risks. Each culture was examined microscopically and macroscopically for purity before use. Only viable and pure cultures were employed for the antimicrobial studies.

2.7 Antimicrobial Assay of Crude Extract

The antimicrobial activity of the crude n-hexane extract of *Persea americana* leaves was determined using the ditch plate method, which is widely recognized for evaluating antimicrobial efficacy of plant-derived substances (Adebayo et al., 2021; Cheesman et al., 2022). Mueller–Hinton agar (MHA) served as the growth medium for bacterial isolates. Microbial inocula were standardized to match 0.5 McFarland turbidity standard (approximately 1×10^{8} CFU/mL for bacteria). Sterile cotton swabs were used to evenly distribute the standardized suspensions across the surface of prepared agar plates to ensure uniform microbial lawn formation. A sterile glass spreader was used to ensure even distribution of the inoculum across the surface (Eze *et al.*, 2024). Using a sterile spatula, a narrow ditch (approximately 3-4 mm wide) was aseptically cut through the center of each inoculated plate. The ditch was then filled with 0.1 mL of the crude extract at two different concentrations (100 mg/mL and 200 mg/mL). The plates were left at room temperature for 30 mintes to allow diffusion of the extract into the agar medium before incubation.

Ciprofloxacin (4 mg/mL) was used as a standard antibacterial agent. Distilled water was used as the negative control. The inoculated plates were incubated at 37 °C for 24 hours for the bacterial isolates. After incubation, the antimicrobial activity was assessed by measuring the width of the zones of inhibition in millimeters using a transparent ruler. The experiment was carried out in triplicate, and the mean values were calculated to ensure accuracy and reproducibility of results. The results were expressed as mean ± standard deviation (SD) and compared with those of the positive controls to evaluate the

relative potency of the extract. Larger inhibition zones indicated stronger antimicrobial activity, while the absence of zones indicated resistance or inactivity

2.8 FORMULATION OF BALM AND THE ANTIMICROBIAL ASSAY OF FORMULATED BALM

2.8.1 Formulation of Herbal Balm

The crude extract was formulated into a balm using beeswax, etc. Evaluate its activity in a topical dosage form. The balm base was prepared using a combination of natural excipients with emollient and stabilizing properties. Ingredients used in the balm base: Beeswax, Cocoa butter, Olive oil, Vitamin E oil and peppermint oil. The excipients were melted together using a water bath and stirred until uniform. The avocado extract was incorporated into the molten base at concentrations corresponding to 100 mg/mL and 200 mg/ml. The mixtures were poured into containers and allowed to solidify at room temperature. A balm without the extract was also prepared which served as negative control.

2.8.2 Preparation of Extract Stock Solution

A stock solution of the n-hexane extract of *Persea americana* leaves was prepared by accurately weighing 2 g of the crude extract and dissolving it in 4 mL of Tween-80 to enhance solubility, resulting in a concentration of 500 mg/mL. This served as the mother solution from which subsequent dilutions were prepared. From this stock, 0.2 mL and 0.4 mL aliquots were taken and further diluted appropriately to obtain working concentrations of 100 mg/mL and 200 mg/mL, respectively.

2.8.3 Antimicrobial Assay of Formulated Balm

The antimicrobial activity of the formulated balm containing the n-hexane extract of Persea americana leaves was evaluated using the ditch plate method as described by standard microbiological protocols. Sterile Mueller Hinton Agar (MHA) plates were prepared and inoculated with standardized bacterial suspensions equivalent to the 0.5 McFarland standard. The bacterial isolates used included *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.* A sterile scalpel was used to aseptically create a straight ditch (approximately 1 cm wide) across the center of each agar plate. The prepared ditches were carefully filled with 0.1 mL of the formulated balm samples at concentrations of 100 mg/mL and 200 mg/mL. A blank balm (without extract) served as control, while Ciprofloxacin (2 mg/mL) was used as a standard antibacterial reference. The plates were allowed to stand at room temperature for 30 minutes to facilitate proper diffusion of the balm components into the agar medium. After pre-diffusion, the plates were incubated at 37 °C for 24 hours. Upon incubation, the zones of inhibition, the clear regions around the ditch indicating bacterial growth suppression, were measured in millimeters (mm) using a transparent ruler. All tests were conducted in triplicate, and results were recorded as mean ± standard deviation (SD). The antimicrobial performance of the formulated balm was compared with that of the blank balm, commercial balm, and standard antibiotic control to evaluate the influence of formulation on antimicrobial efficacy.

CHAPTER THREE

3.0 RESULTS

3.1 Phytochemical Screening of n-Hexane Extract of Avocado Leaves

The qualitative phytochemical screening showed the following results:

Table 3.1: Phytochemical Screening of n-hexane Extract of Avocado Leaves

TEST	RESULT
Alkaloids	+
Carbohydrates	-
Reducing sugars	-
Saponins	-
Steroids/Triterpenes	+
Phenolic compounds	+
Tannins	-
Proteins	+
Deoxy sugars	-
Flavonoids	-

Key: (+) means Present, (–) means absent

3.2 Antimicrobial Activity of Crude Extract of Avocado Leaves

Table 3.2: Antimicrobial activity of crude n-Hexane Extract and formulated balm

Organisms	Hexane 100mg/ml	Hexane 200mg/ml	Balm 100mg/ml	Balm 200mg/ml	Control balm (no extract)	Ciprofloxacin 4mg	Ketoconazole 60mg/ml
Pseudomonas aeruginosa	NZ	20 mm	12 mm	12 mm	NZ	32 mm	NZ
*Escherichia coli (*G -ve)	NZ	18 mm	NZ	NZ	NZ	30 mm	NZ
Klebsiella Pneumoniae (G -ve)	NZ	17 mm	NZ	NZ	NZ	34 mm	NZ
Bacillus subtilis (G +ve)	NZ	18 mm	12 mm	14 mm	NZ	30 mm	NZ
Staphylococcus aureus	NZ	18 mm	14 mm	15 mm	NZ	36 mm	NZ

Key: (NZ) means No Zone of Inhibition, mm means millimeters

3.3 N-HEXANE EXTRACT BALM pH

pH	VALUES
Control	5.11
100mg/ml Balm	5.75
200mg/ml Balm	5.69
Extract	6.40

Table 3.3: pH of n-Hexane extract balm

3.4 Antimicrobial Activity of Formulated Balm Compared with Controls

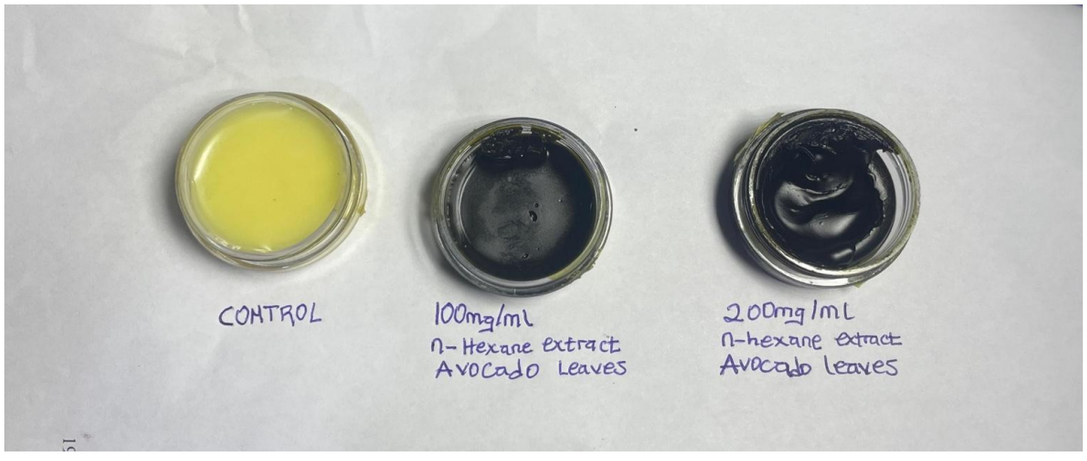


Figure 3.4: Formulated herbal balm

3.5 Some Compounds found in n-hexane extract of *Persea americana*

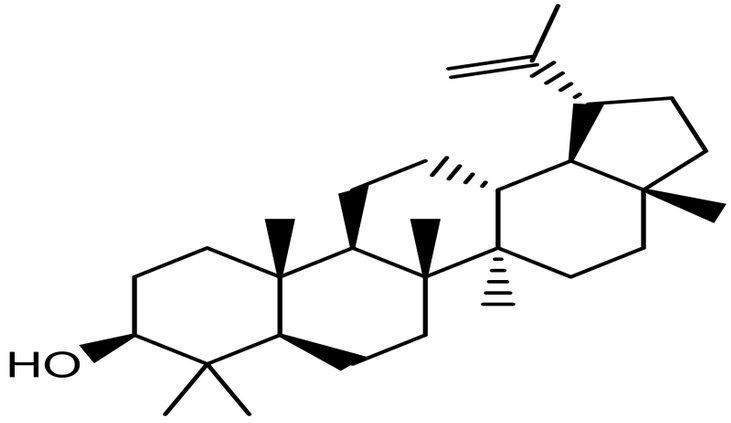


Figure 3.5.1: Chemical structure of Lupeol

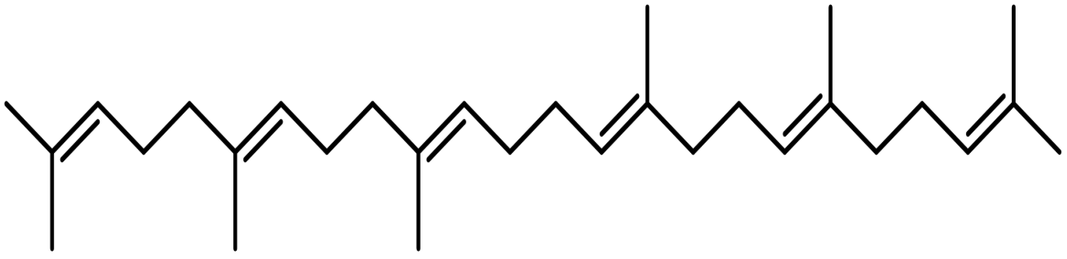


Figure 3.5.2: Chemical structure of Squalene

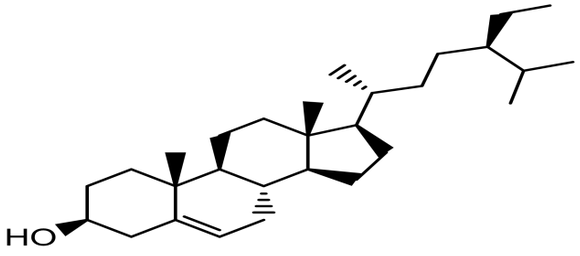


Fig 3.5.3 Chemical structure of Beta -Sitosterol

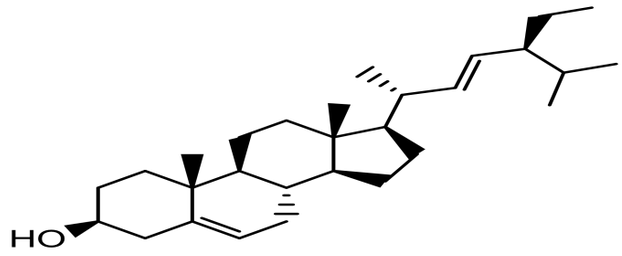


Fig 3.5.4 Chemical structure of Stigmasterol

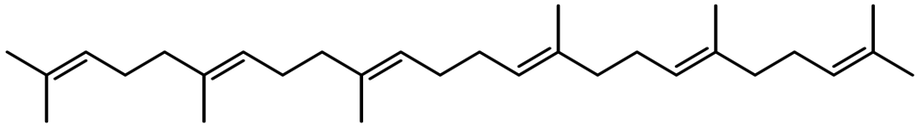


Fig 3.5.5 Chemical structure of Squalene

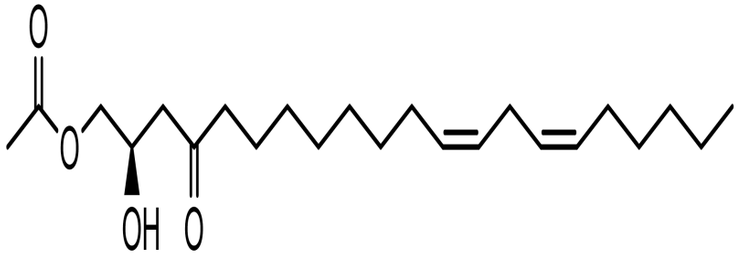
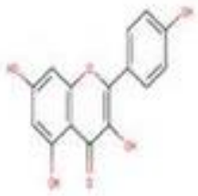
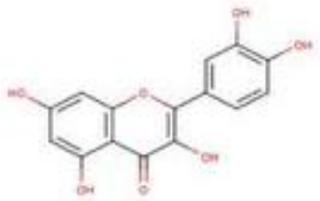


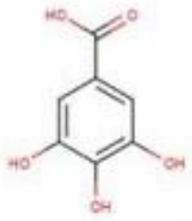
Fig 3.5.6 Chemical structure of Persin



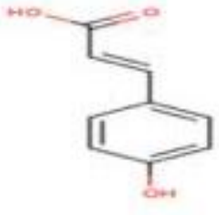
Quercetin



Kaempferol



Gallic acid



p-Coumaric acid

CHAPTER FOUR

4.0 DISCUSSION

4.1 Overview of Findings

This study evaluated the phytochemical composition and antimicrobial activity of the n-hexane extract of *Persea americana* (avocado) leaves and the formulated balm to determine the effect of incorporating the extract into a topical preparation. Phytochemical analysis revealed the presence of alkaloids, steroids/triterpenes, phenolic compounds, and proteins, while carbohydrates, reducing sugars, saponins, tannins, flavonoids, and deoxy sugars were absent. The antimicrobial assay using the ditch plate method showed that the n-hexane extract exhibited inhibitory activity against *Pseudomonas aeruginosa* (20 mm), *Escherichia coli* (18 mm), *Klebsiella pneumoniae* (17 mm), *Bacillus subtilis* (18 mm), and *Staphylococcus aureus* (18 mm) at 200 mg/mL, while no inhibition was observed at 100 mg/mL. The formulated balm containing the extract also demonstrated antibacterial activity, though at a reduced level. The 100 mg/mL balm inhibited *Pseudomonas aeruginosa* (12 mm), *Bacillus subtilis* (12 mm), and *Staphylococcus aureus* (14 mm), while the 200 mg/mL balm showed slightly higher zones of inhibition, *Pseudomonas aeruginosa* (12 mm), *Bacillus subtilis* (14 mm), and *Staphylococcus aureus* (15 mm). No inhibitory effect was observed against *Escherichia coli* and *Klebsiella pneumoniae* at either concentration. The control balm (without extract) produced no inhibition zones, confirming that the antimicrobial activity was due to the presence of *Persea americana* extract. The slightly reduced activity of the balm compared to the crude extract may be attributed to interactions between the lipophilic excipients and the active compounds, which could have affected their diffusion or bioavailability in the agar medium.

4.2 Phytochemical Constituents of the Extract

The phytochemical analysis indicated the presence of bioactive secondary metabolites such as alkaloids, steroids/triterpenes, phenolics, and proteins. Alkaloids are well-known for their broad spectrum of biological activities, including antibacterial, antifungal, and anticancer properties. Their presence in the extract could contribute significantly to the antimicrobial effects observed, particularly against *Staphylococcus aureus* and *Escherichia coli* because steroids and triterpenes are lipophilic in nature, they can integrate into microbial cell membranes, disrupting their structure and increasing permeability, which leads to leakage of vital cellular components. This mechanism likely contributed to the antimicrobial activity observed, particularly against Gram-positive bacteria such as Staphylococcus aureus. Steroids and triterpenes, on the other hand, have been associated with membrane-disrupting properties, enabling them to exert antimicrobial activity by increasing the permeability of microbial cell membranes (Ikpefan, E.O., *et al.,* 2022). Phenolic compounds are strong antioxidants and possess well-documented antimicrobial properties, often linked to their ability to denature proteins and disrupt cell walls (Baraka, B., 2022). The absence of carbohydrates, reducing sugars, saponins, tannins, and flavonoids is also noteworthy. Flavonoids, in particular, are often reported in polar solvent extracts such as methanol or ethanol fractions, but may be absent in non-polar solvents such as n-hexane, which preferentially extracts lipophilic components. The profile observed in this study therefore reflects the selectivity of n-hexane as an extraction solvent, favoring non-polar constituents such as steroids, triterpenes, and certain alkaloids.

4.3 Antimicrobial Activity of the Crude Extract and Formulated Balm

The antimicrobial evaluation of the n-hexane extract o*f Persea americana* leaves revealed that the crude extract demonstrated inhibitory activity against *Pseudomonas aeruginosa* (20 mm), *Escherichia coli* (18 mm), *Klebsiella pneumoniae* (17 mm), *Bacillus subtilis* (18 mm), *and Staphylococcus aureus* (18 mm) at a concentration of 200 mg/mL, while no inhibition was observed at 100 mg/mL. The activity of the extract was therefore concentration-dependent, indicating that the antimicrobial constituents exert greater efficacy at higher concentrations. These organisms are important clinical pathogens frequently associated with respiratory, urinary, gastrointestinal, and wound infections (Mahmoud *et al*., 2023). Among the tested bacteria, *Staphylococcus aureus* and *Bacillus subtilis* (both Gram-positive organisms) showed notable susceptibility, suggesting that the extract's bioactive compounds, particularly alkaloids, steroids/triterpenes, and phenolics may disrupt bacterial cell wall synthesis or membrane integrity. The inhibition of *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative bacteria) is particularly significant, as these organisms are often more resistant due to their outer lipopolysaccharide membrane, which limits drug permeability (Hashempur *et al*., 2025). The observed sensitivity therefore suggests that the lipophilic nature of the extract allowed better penetration through bacterial membranes, leading to growth inhibition. The formulated balm also exhibited antimicrobial activity, though at a reduced level compared to the crude extract. The 100 mg/mL balm inhibited *Pseudomonas aeruginosa* (12 mm), *Bacillus subtilis* (12 mm), and *Staphylococcus aureus* (14 mm), while the 200 mg/mL balm produced slightly higher inhibition zones , *Pseudomonas aeruginosa* (12 mm), *Bacillus subtilis* (14 mm), and *Staphylococcus aureus* (15 mm). No inhibition was

observed against *Escherichia coli* and *Klebsiella pneumoniae* for either concentration. The control balm showed no inhibitory activity, confirming that the antimicrobial effect originated from the avocado leaf extract. The reduction in activity of the balm compared to the crude extract may be attributed to the physicochemical interactions between the extract and the excipients used in formulation. Being lipophilic substances, these excipients could encapsulate or reduce the diffusion of the active compounds through the agar medium, thereby lowering the observable inhibition zones. However, since the active compounds themselves are also lipophilic, some level of activity was retained, suggesting partial compatibility between the extract and the balm matrix. This finding supports the potential of *Persea americana l*eaf extract as a valuable bioactive ingredient in topical antimicrobial formulations, though optimization of excipient composition is necessary to maximize its efficacy.

4.5 Comparison Between Crude Extract and Balm Formulation

A comparative evaluation of the antimicrobial activity of the n-hexane leaf extract of *Persea americana* and its formulated balm revealed notable differences in efficacy across the tested bacterial isolates. The crude extract exhibited significant antibacterial activity, particularly at 200 mg/mL, showing inhibition zones of 20 mm for *Pseudomonas aeruginosa,* 18 mm *for Escherichia coli,* 17 mm *for Klebsiella pneumoniae,* 18 mm *for Bacillus subtilis,* and 18 mm *for Staphylococcus aureus*. No inhibition was observed at 100 mg/mL. In contrast, the formulated balm demonstrated moderate antibacterial activity, with inhibition zones of 12 mm for *Pseudomonas aeruginosa* at both concentrations, 12–14 mm for *Bacillus subtilis*, and 14–15 mm for *Staphylococcus*

aureus, while no activity was observed against *Escherichia coli* and *Klebsiella pneumoniae.* These findings indicate that the crude extract displayed a higher potency compared to the balm formulation, corroborating the concentration-dependent relationship commonly observed in plant-derived antimicrobial studies (Okoro *et al*., 2023). The reduction in activity following formulation may be attributed to the lipophilic nature of the excipients, which can encapsulate or limit the diffusion of active compounds into the agar medium (Osei-Djarbeng *et al.,* 2021). Despite this, the retention of moderate antibacterial activity in the balm suggests that the active phytoconstituents such as alkaloids, phenolics, and triterpenoids remained stable and biologically active within the formulation matrix (Amin *et al*., 2022). From a pharmaceutical perspective, while the crude extract exhibited greater in vitro inhibition, the balm formulation represents a more clinically applicable dosage form for managing topical infections, especially those caused by *S. aureus, B. subtilis, and P. aeruginosa,* which are major etiological agents of wound and skin infections (Tantawy *et al*., 2023). The reduced but retained activity underscores the need for optimization of the formulation to enhance bioavailability and release kinetics of the active ingredients (Kumar et al., 2024). This aligns with global phytopharmaceutical development trends, emphasizing the transition from crude extract testing to standardized, patient-friendly herbal preparations with reproducible therapeutic profiles (Bello et al., 2025).

4.6 Influence of Bioactive Compounds on Balm Activity

The presence of key lipophilic bioactive compounds such as lupeol, squalene, β-sitosterol, stigmasterol, and persin in the n-hexane extract of *Persea americana* likely played a

significant role in determining the antimicrobial efficacy of both the crude extract and the formulated balm. These compounds are predominantly triterpenoids and phytosterols, well documented for their antimicrobial, anti-inflammatory, and wound-healing effects (Akinpelu *et al.,* 2022; Bhuyan *et al.,* 2023). Lupeol, a pentacyclic triterpenoid, exhibits broad-spectrum antimicrobial and anti-inflammatory activity by disrupting bacterial cell walls and modulating oxidative stress pathways (Alam *et al.,* 2022). Its hydrophobic nature enhances its ability to integrate into microbial membranes, leading to leakage of intracellular contents and subsequent cell death (Torky *et al.,* 2024). Squalene, a naturally occurring triterpene and precursor of cholesterol, contributes to the skin-conditioning and antioxidant potential of the balm. However, due to its highly hydrophobic and bulky structure, squalene can reduce the diffusion rate of other bioactives within the semisolid matrix, resulting in steric hindrance that limits the availability of some compounds at the site of microbial action (Alves *et al.,* 2023). Similarly, β-sitosterol and stigmasterol, both plant sterols structurally similar to cholesterol, possess membrane-stabilizing and anti-inflammatory properties that support wound repair and tissue regeneration (Omoruyi *et al.,* 2021). Yet, these sterols may also form lipid-rich microdomains with the balm excipients (such as beeswax), reducing the overall diffusion and surface release of antimicrobial constituents, a phenomenon characteristic of sterically hindered, lipophilic systems (Singh *et al.,* 2023). Persin, a unique aliphatic compound specific to P. americana, is known for its antifungal, cytotoxic, and antibacterial properties, attributed to its ability to disrupt lipid membranes and inhibit protein synthesis in microbial cells (Mahmoud *et al.,* 2022). Within the balm formulation, persin's lipophilicity ensures strong partitioning into the waxy base, which may have reduced its free diffusion but

simultaneously enhanced its prolonged retention and localized effect on the skin surface. Thus, while the extract's potency decreased in the formulated balm due to steric and lipophilic constraints, these same factors may contribute to a sustained-release antimicrobial effect, beneficial for topical applications (Amin *et al.,* 2022). Collectively, the interaction of these lipophilic compounds within the balm matrix reflects a balance between efficacy and formulation stability. Their presence supports the observed antimicrobial and wound-healing properties of the formulation, while also explaining the reduced inhibition zones compared to the crude extract. Optimizing the concentration of such bioactives and modifying the balm's lipid composition could therefore improve diffusion, bioavailability, and therapeutic performance in future formulations (Bello *et al.,* 2025).

4.7. Limitations of the Study

Several limitations must be acknowledged. First, the study used only n-hexane as the extraction solvent, which restricted the range of phytochemicals extracted. Future studies should compare extracts obtained with solvents of varying polarity to provide a broader phytochemical and antimicrobial profile. Second, the antimicrobial assay was limited to in vitro testing. The in vivo efficacy and safety of the extract and balm remain to be established. A follow-up study could involve the application of the formulated balm on a suitable in vivo model (e.g., animal skin or human volunteers with ethical approval) to assess antimicrobial efficacy and local skin safety. Prior to application, physicochemical characterisation of the balm (pH, spreadability, viscosity, stability) should be performed, followed by skin permeation or tape-strip studies to quantify active agent delivery. Microbial load reduction at treated sites, irritation testing, and formulation stability under

ICH-recommended conditions should be monitored (Ekom, S.E., *et al.*, 2022). Comparisons should be made between the crude extract, formulated balm, blank balm, and a commercially available standard product to determine the effect of formulation on antimicrobial activity and bioavailability. Third, the interaction between excipients and the extract in the balm formulation requires further exploration, including studies on release kinetics and stability (Rosa, Y., *et al.*, 2025).

4.8. Implications for Future Research

The findings of this research open avenues for further studies. Comparative solvent extraction could reveal additional bioactive compounds. Bioassay-guided fractionation may identify the specific phytochemicals responsible for antimicrobial activity. Furthermore, optimization of balm formulation using different excipient ratios or nanotechnology-based delivery systems could enhance efficacy. Lastly, in vivo studies and clinical trials would be required to validate the therapeutic potential of avocado-based formulations.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study investigated the phytochemical constituents and antimicrobial activity of the n-hexane extract of avocado (*Persea americana*) leaves, as well as the effect of incorporating the extract into a balm formulation. The phytochemical screening revealed the presence of alkaloids, steroids/triterpenes, phenolic compounds, and proteins, which are known to possess antimicrobial properties. The crude extract exhibited dose-dependent inhibition against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, while showing no activity against *Klebsiella pneumoniae*, *Candida albicans*, and *Aspergillus niger*.

When formulated into a balm, the extract retained antimicrobial activity against the same organisms, albeit at a slightly reduced potency compared to the crude extract. This difference was attributed to possible interactions between the extract and excipients in the balm base. Nonetheless, the findings confirm that avocado leaves possess bioactive compounds with antimicrobial potential and that these can be incorporated into practical dosage forms such as balms for topical use.

The study therefore validates the traditional use of avocado leaves in managing infections, particularly skin-related conditions such as boils, cellulitis, and superficial wounds. It also highlights the potential role of avocado-based formulations in contributing to the fight against antimicrobial resistance.

5.2 Recommendations

Based on the findings of this study, the following recommendations are made:

1. Further Phytochemical Studies:

More extensive phytochemical analyses should be carried out using solvents of varying polarities (e.g., methanol, ethanol, aqueous) to capture a wider range of bioactive compounds.

2. Bioassay-Guided Fractionation:

Fractionation of the crude extract should be performed to isolate and identify the specific phytochemicals responsible for the antimicrobial activity.

3. Optimization of Balm Formulation:

The formulation of the herbal balm should be optimized by varying the ratios of excipients and considering advanced delivery systems (such as liposomes or nanoparticles) to enhance the release and bioavailability of active compounds.

4. In Vivo Studies:

Animal models should be employed to evaluate the safety, efficacy, and pharmacokinetic properties of the crude extract and formulated balm, providing stronger evidence for clinical application.

5. Clinical Trials:

Upon successful preclinical evaluation, clinical trials should be designed to confirm the therapeutic potential of avocado leaf formulations in managing bacterial skin infections.

6. Exploration of Synergy with Antibiotics:

The potential synergistic effects between avocado extract and conventional antibiotics should be investigated, as such combinations may reduce antibiotic resistance and improve treatment outcomes.

7. Standardization:

Standardization of the extract and balm formulation should be pursued to ensure safety, and efficacy, which are critical for eventual commercialization.

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