FORMULATION OF NATURAL REMEDY WITH AQUEOUS EXTRACT OF POLYHERBAL MIXTURE FOR THE THERAPEUTIC EFFECT ON SORE THROAT

BY

Etinosa OSAGIE

LSC1907387

BIOTECHNOLOGY TECHNIQUE (BTT)

A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

FEBRUARY 2025.

FORMULATION OF NATURAL REMEDY WITH AQUEOUS EXTRACT OF POLYHERBAL MIXTURE FOR THE THERAPEUTIC EFFECT ON SORE THROAT

BY

Etinosa OSAGIE

LSC1907387

A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

FEBRUARY 2025.

CERTIFICATION

This is to certify that this project work was carried out and submitted by **Etinosa OSAGIE**, with the matriculation number **LSC1907387** in the epartment of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Prof. E.O. Oshomoh	**Date**
(Project Supervisor)	
Dr. P.O. Alonge	**Date**
(Project Coordinator)	
Prof. E.O. Oshomoh	**Date**
(Head of Department)	
(External Examiner)	**Date**

DEDICATION

This project work is dedicated to God Almighty, my parents, my caring family and my friends.

ACKNOWLEDGEMENT

I am grateful to God Almighty for his guidance, protection and preservation throughout my research work.

My profound gratitude goes to my superb supervisor **PROF. E.O. OSHOMOH** for his unwavering love, support, care and patience.

My immerse gratitude goes to my dad **Mr. OSAGIE ERHAUYI**, my mum **Mrs. OMOLARA OGUNTIMEHIN, CAPT. STEVE MOBOLAJI OGUNTIMEHIN** for their unending and unwavering support during the course of study.

I am grateful to the entire staff of Science Laboratory Technology Department and to the Project Coordinator – **Dr. P.O. Alonge**, I am indeed grateful.

TABLE OF CONTENT

LIST OF PLATES

LIST OF TABLES

ABSTRACT

This study explores the antimicrobial and phytochemical properties of four medicinal plants—*Vernonia amygdalina* (bitter leaf), *Zingiber officinale* (ginger), *Citrus aurantiifolia* (lime), and *Curcuma longa* (turmeric)—to assess their potential in treating throat infections. The research aims to determine their antimicrobial effectiveness, identify bioactive compounds, establish optimal dosages, and scientifically validate traditional medicinal use. Aqueous and ethanol extracts of the plants were tested against bacterial strains, including *Staphylococcus aureus, Streptococcus mutans, Lactobacillus acidophilus, Klebsiella pneumoniae, Escherichia coli,* and *Pseudomonas aeruginosa.* The Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) were evaluated, showing that the extracts demonstrated significant antimicrobial effects, especially against Gram-positive bacteria. The inhibition zones increased with higher concentrations, though the extracts were less potent than ciprofloxacin, the control antibiotic. Phytochemical screening confirmed the presence of phenolics, flavonoids, saponins, tannins, and alkaloids, with phenolics being the most abundant. Elemental analysis identified essential macro- and microelements such as potassium, sodium, calcium, and iron, which may contribute to the therapeutic potential of these plants. The study concludes that these plant extracts exhibit notable antimicrobial properties, likely due to their rich phytochemical composition, and could serve as complementary or alternative treatments for throat infections. However, further studies are necessary to refine formulations, elucidate mechanisms of action, and assess clinical applications. This research highlights the relevance of medicinal plants in combating antibiotic resistance and underscores their potential role in natural healthcare.

CHAPTER ONE

1.1. INTRODUCTION

Many plants are used in phytomedicine to treat various illnesses, and the practice of using medicinal plants for infectious diseases dates back to ancient times. In Africa, traditional and herbal medicine has been in use for over 4,000 years. Before the advent of modern or conventional medicine, phytomedicine was the primary form of healthcare on the continent. Even today, herbal and traditional medicine remains widely utilized in Africa and other regions. However, factors such as the influence of Western lifestyles, the decline in traditional healers, and the younger generation's waning interest in preserving this knowledge have led to a rapid decline in the understanding of medicinal plants (Dyubeni *et al*., 2012).

For the past century, plant-based phytochemicals have played a crucial role in medical treatments. The biological activity of plant-derived compounds has drawn significant scientific attention due to their importance in agriculture and medicine. Despite extensive research, only a limited number of plant species have been thoroughly studied, and their full potential in nature remains largely unexplored. Therefore, to advance phytomedicine, comprehensive research into the biological activities of medicinal plants and their key phytochemicals is essential (Moghadamtousi *et al*., 2015).

Traditional medicine has a rich history, shaped by theories, beliefs, and cultural experiences accumulated over time. It is often based on unexplained principles and is used for maintaining health, preventing diseases, diagnosing conditions, and improving ailments. While herbal medicine differs in some ways from conventional pharmacological treatments, it has gained

widespread acceptance as a healthcare option. However, it must be scientifically tested for efficacy through conventional trial methodologies, as certain herbal extracts have proven effective for specific conditions. A common misconception among the public is that all natural remedies are completely safe, but herbal treatments do pose risks, necessitating further research in this field. One key issue that remains unresolved is understanding the absorption, metabolism, and effectiveness of herbs and their extracts—factors essential for evaluating their claimed health benefits (Firenzuoli *et al*., 2007).

Ethnobotany serves as an effective initial approach to studying plant use, highlighting the close relationship between science and tradition. Scientific research often stems from traditional knowledge, as many plant-based remedies passed down through generations have been validated for their effectiveness across various industries. Over the past few decades, the study of medicinal plants and their traditional applications has gained momentum. However, ethnobotanical research remains vital for preserving and utilizing biological resources, as it documents indigenous knowledge. The development of traditional medicines and the application of medicinal plants for treating various illnesses offer significant economic benefits. Due to poverty, lack of modern healthcare facilities, limited access to medical services, and inadequate communication, many individuals, particularly those in rural areas, continue to rely on traditional medicine for everyday ailments. Unfortunately, these individuals often represent the most vulnerable link in the medicinal plant trade.

Regions where plant-based treatments remain widely used hold valuable knowledge about their applications for different health conditions. Plant-derived compounds form the basis of approximately 25% of pharmaceuticals in developed countries. In 1976, a group of experts from

the World Health Organization (WHO) convened in Congo Brazzaville to define traditional African medicine, recognizing it as the culmination of various practices, materials, and techniques historically used by Africans to prevent illnesses, alleviate suffering, and promote healing. Indigenous knowledge of medicinal plants is invaluable not only for current and future drug development but also for preserving cultural traditions and biodiversity (Idu *et al*., 2009).

Natural products, particularly those sourced from plants, have played a fundamental role in human health throughout medical history. The identification of phytochemicals in plants has significantly contributed to medical advancements over the past century (Patel and Patel, 2016). Herbal medicine, also known as phytotherapy, continues to be widely used for treating numerous illnesses. Compared to synthetic compounds, dietary phytochemicals offer several advantages, including better oral bioavailability, affordability, and safety (Llango *et al*., 2022).

This discussion highlights several significant natural remedies, including bitter leaf (Vernonia amygdalina), ginger (Zingiber officinale), lime (Citrus aurantiifolia), and turmeric (Curcuma longa).

Ginger is widely used to alleviate nausea but also serves as an anti-inflammatory, pain reliever, warming agent, and cholesterol-lowering herb. It is extensively utilized to treat fevers, stomachaches, malaria, and indigestion due to its stimulating, carminative, and pungent properties. Traditionally, it is used to address ailments caused by Kapha and Vata imbalances. A combination of ginger, lime juice, and rock salt is known to stimulate gastric juice secretion and enhance appetite. It has been used to manage a wide range of conditions, including abdominal pain, anorexia, arthritis, indigestion, respiratory issues, hyperacidity, nausea, and rheumatism,

among others. Additionally, ginger extracts possess strong antioxidant properties comparable to synthetic antioxidant preservatives (Moghaddasi *et al*., 2012).

Lime is extensively used in West Africa, particularly in Nigeria, where it plays a crucial role in herbal medicine for treating various ailments. It is an essential ingredient in many herbal preparations. In Nigeria, lime juice is mixed with sugar, honey, and palm oil to treat coughs. In Malayan medicine, it is used as an antidote for poisoning and as a libido tonic. Lime juice is also employed to manage dysfunctional uterine hemorrhage, enhance endurance, rejuvenate the skin, treat epistaxis, and address conditions such as atherosclerosis, diabetes, and joint pain. When diluted, it serves as an effective mouthwash for soothing sore throats and oral irritations. Additionally, lime juice has been found useful in treating mosquito bite-related swelling, diarrhea, and pain. In some cases, it is mixed with oil and used as a vermifuge or included in diets for weight loss (Enejoh *et al*., 2015).

Bitter leaf (Vernonia amygdalina) is known by various names, including olubu (Igbo), ewuro (Yoruba), shikawa (Hausa), suwaka (Dagaare), and oriwo (Edo). In phytomedicine, its roots and leaves are traditionally used to treat fever, hiccups, kidney disease, and stomach pain. The plant is recognized for its strong antimalarial and antihelminthic properties, as well as its antitumorigenic effects and notable antiparasitic efficacy in zoo pharmacognosy. Various parts of the plant, including the stem, bark, roots, and leaves, are commonly used as purgatives, antimalarial agents, and treatments for eczema in both alcoholic and aqueous extracts (Adetunde *et al*., 2017).

Turmeric is a stimulant, carminative, aromatic, and mild digestive aid. It is considered one of nature's most powerful medicinal herbs. While turmeric has long been valued for its anti-inflammatory properties, recent studies have revealed its potential in treating a wide range of conditions, including cancer and Alzheimer's disease (Debjit *et al*., 2009). Over time, its therapeutic benefits have become increasingly recognized, solidifying its role as a vital natural remedy.

1.2. AIM OF STUDY

This research aims to examine the effects of bitter leaf, ginger, turmeric, and lime fruit on throat infections.

1.3. OBJECTIVE OF THE STUDY

The objectives of this research or study are to:

i. To evaluate antimicrobial properties.
ii. To identify active compounds.
iii. To determine optimal dosage and formulation.
iv. To document traditional knowledge and validate scientifically.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. THROAT INFECTION

The *Streptococcus* genus consists of Gram-positive, catalase-negative, and coagulase-negative cocci that typically form pairs or chains. These bacteria can colonize various body sites, including the pharynx, anus, and vaginal mucosa. Transmission occurs through multiple pathways, such as consuming contaminated food, direct skin contact with infected sores, exposure to nasal secretions via hand contact or contaminated surfaces, and airborne droplets. As significant human pathogens, *Streptococcus* species—particularly *Streptococcus pyogenes*—are β-hemolytic and responsible for several acute infections, including acute pharyngitis (strep throat) (Kanwal and Vaitla, 2022). Recurrent infections or prolonged exposure to *S. pyogenes* can lead to severe and potentially life-threatening complications (Castro and Dorfmueller, 2021). This bacterium is estimated to cause over 500,000 deaths globally and 1.78 million new infections annually (Wijesundara *et al*., 2021).

Streptococcus pyogenes is a major contributor to infections worldwide, accounting for approximately 37% of sore throat cases in children and 5–10% in adults (Wijesundara *et al*., 2021). While *Streptococcus* species are generally susceptible to β-lactam antibiotics, such as Cephalosporins and Penicillin, standard treatment involves antibiotic therapy (Cattoir, 2016). However, reports of treatment failures have been documented since the 1940s (Cattoir, 2016). Moreover, antibiotic misuse has led to a rise in resistance, posing a significant public health concern and increasing healthcare costs (Dadgostar, 2019; Ventola, 2015). The emergence of

antibiotic resistance in *Streptococcus* strains is attributed to mechanisms such as efflux pump activity and modifications of antimicrobial targets (Alves-Barroco *et al*., 2020).

2.2.0. Bitter Leaf (*Vernonia amygdalina*)

2.2.1. Introduction

With over 1,000 shrub species, *Vernonia amygdalina*, commonly known as bitter leaf, is the most widely cultivated species within its genus. It belongs to the *Asteraceae* family and is predominantly found in tropical Africa, where it grows naturally or is cultivated extensively across sub-Saharan regions. It is particularly common in Nigeria, Cameroon, Gabon, and the Democratic Republic of the Congo, where it is propagated vegetatively by cutting stems at a 45-degree angle. Named after the English botanist William Vernon, it is also referred to as ironweed. Unlike plants with specific habitat requirements, *V. amygdalina* thrives in diverse environments, including home gardens, cultivated fields, and savannah regions. Although primarily consumed as food, bitter leaf has long been recognized for its medicinal properties. True to its name, it has a distinctively bitter taste, yet it is frequently used in various traditional dishes. It is known by different names across Africa, including Onugbo (Igbo), Ewuro (Yoruba), Etidot (Ibibio), Ityuna (Tiv), Oriwo (Edo), and Chusa-doki Shiwaka (Hausa) (Agbogidi and Akpomorine, 2013).

This perennial, soft-woody shrub grows between 1 to 6 meters in height and is highly adaptable to a range of climatic conditions. Its bitterness is attributed to the presence of anti-nutritional compounds. However, its leaves are nutritionally valuable, containing essential vitamins, fiber, carbohydrates, and minerals. Phytochemical analysis has revealed the presence of alkaloids,

tannins, saponins, flavonoids, polyphenols, anthraquinones, edotides, xanthones, coumarins, glycosides, and sesquiterpenes (Ugbogu *et al*., 2021).

In ethnomedicine, the roots and leaves of *V. amygdalina* are traditionally used to treat conditions such as fever, hiccups, kidney disorders, and stomach pain. Aqueous and alcoholic extracts of the stems, roots, and leaves are commonly employed as purgatives, antimalarials, and remedies for eczema. Pharmacological studies suggest that the plant's leaf extract exhibits hypoglycemic and hypolipidemic properties in experimental animals, indicating potential benefits for managing diabetes mellitus. The bioactive compounds in *V. amygdalina* contribute to a range of pharmacological activities, including antimicrobial, antimalarial, antithrombotic, antioxidant, antidiabetic, laxative, hypoglycemic, antihelminthic, anti-inflammatory, cathartic, anticancer, antifertility, antifungal, and antibacterial effects (Ali *et al*., 2019).

Plate 2.1*: Vernonia amygdalina* leaves

Source: (Yeap *et al*., 2010)

2.2.2. Taxonomic classification of *Vernonia amygdalina*

Kingdom Plantae

Class Angiospermae

Order Asterales

Family Asteraceae

Genus Vernonia

Species Vernonia amygdalina (Agbogidi *et al*., 2013)

2.2.3. PHYTOCHEMISTRY

Alabi and Adeyemi (2021) reported that ethanol-based extracts of *Vernonia amygdalina* contain various flavonoids, including luteolin 7-O-β-glucuronide and luteolin 7-O-β-glucoside. Among these flavones, luteolin (3′,4′,5,7 tetrahydroxyflavone) exhibits particularly strong antioxidant properties. Additionally, the extracts contain other phytochemicals such as alkaloids, anthraquinones, steroids, phenols, phytates, oxalates, cyanogenic glycosides, tannins, and saponins. Using LC-MS/MS analysis, Hasibuan *et al*. (2020) identified flavonoids such as apigetrin, apigenin, luteolin, diosmetin, baicalein, rhoifolin, and scutellarin in *V. amygdalina*. Similarly, Toyang and Werpoorte (2013) isolated various bioactive compounds and flavonoids from *V. amygdalina* extracts, including vernonioside A3, vernodalol, vernolepin, vernodalin, 11,13-dihydrovernodalin, and hydroxyvernolide.

Adaramoye *et al*. (2008) suggested that a reduction in lipid peroxidation (LPO) levels in irradiated animals pretreated with *V. amygdalina* extracts might be linked to an increase in flavonoids, particularly luteolin 7-O-glucoside, which plays a protective role against liver

damage in mice. Using LC-MS analysis, Erukainure *et al*. (2018) identified phytochemicals such as nicotinic acid, cumidine, and 3-methyl-isoquinoline in *V. amygdalina*. Omojokun *et al*. (2019) characterized alkaloids in *V. amygdalina*, analyzing their composition using GC-MS. The identified alkaloids included 1,3-cyclooctadiene, 1-fluorononane, 1-hexanamine, dimethylamine, and hexadecanamide. Additionally, Iwalokun (2008) found that the plant extract contains phytoconstituents with antiplasmodial properties, including quinoline alkaloids such as cephantharin, cryptolepine, isocryptolepine, and neocryptolepine, along with terpenoids and coumarins.

Ifedibalu Chukwu *et al*. (2020) successfully isolated various compounds from *V. amygdalina* extracts, including vernodalin, vernomygdin, vernoniosides A1, A2, A3, B1, A4, B2, B3, D, and E, as well as vernodalol, epivernodalol, phytol, 4-methyl-vinyl butyrate, and (Z,Z,Z)-methyl ester-9,12,15-octadecatrienoic acid. Additionally, chromatographic techniques facilitated the separation of compounds such as glucuronolactone (CMP3), 10-geranilanyl-O-β-D-xyloside (CMP2), 11α-hydroxyurs-5,12-dien-28-oic acid-3α, 25-olide (CMP1), 1-heneicosenol O-β-D-glucopyranoside (CMP4), and 6β,10β,14α-trimethylheptadecan-15α-olyl-15-O-β-D-glucopyranosyl-1,5β-olide (CMP5), also known as vernoniaolide glucoside, from methanolic stem-bark extracts.

Hasibuan *et al*. (2020) further identified phytochemicals in *V. amygdalina* using LC-MS/MS, revealing the presence of coumarins (7-hydroxycoumarin, 4-methylumbelliferone, and 4-methylumbelliferyl glucuronide), phenolics (chlorogenic acid and 4-methoxycinnamic acid), and diterpenes (ingenol-3-angelate). Alara *et al*. extracted bioactive compounds from ethanolic *V. amygdalina* extracts using the Soxhlet method and microwave-assisted extraction (MAE). The

compounds were further analyzed and confirmed using gas chromatography-mass spectrometry (GC-MS) and Fourier-transform infrared spectroscopy (FTIR). Some of the identified and isolated bioactive compounds include two-pentanol, pentanoic acid, 2-methyl-3-hexanol, and ethyl ester linoleic acid.

2.2.4. Pharmacological activities of *Vernonia amygdalina L.*

2.2.4.1. Antidiarrhoeal activity

Degu *et al*. (2020) investigated the antidiarrheal effects of *Vernonia amygdalina* extracts on castor oil-induced diarrhea in mice. The plant extracts were obtained through cold maceration using 80% methanol. The study found that *V. amygdalina* only reduced the onset of diarrhea, stool frequency, and stool weight at the highest tested dose (400 mg/kg body weight). These inhibitory effects suggest that *V. amygdalina* has potential as an antidiarrheal agent.

Similarly, Shittu *et al*. (2016) evaluated the antidiarrheal properties of *V. amygdalina* extracts in mice infected with *Vibrio cholerae*. Experimental rats received a single dose of 100 µL of *V. cholerae*, while mice treated with 250 mg/kg of *V. amygdalina* extract exhibited notable anti-inflammatory and anti-secretory effects. The study's results further emphasize the inhibitory and antidiarrheal potential of *V. amygdalina*.

2.2.4.2. Antimicrobial Activity

Studies on the antibacterial properties of *Vernonia amygdalina* (Ngatu *et al*., 2012; Dumas *et al*., 2020) have shown that its extracts exhibit inhibitory effects against various bacterial strains, including *Salmonella enterica*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Dégbé *et al*.

(2018) further documented its activity against *Toxoplasma gondii*, the protozoan responsible for toxoplasmosis. Notably, chloroform extracts of *V. amygdalina* exhibited strong efficacy against *S. aureus*, producing a 21 mm inhibitory zone. According to Habtamu and Melaku (2018), all tested bacterial pathogens were susceptible to isorhamnetin and acetone extracts.

The antifungal properties of *V. amygdalina* leaf extracts were evaluated by Yusoff *et al*. (2020) against *Botrytis cinerea*. Crude extracts prepared using hexane, dichloromethane, methanol, and water at concentrations between 100 and 500 mg/mL were tested, with *V. amygdalina* extracts proving most effective against the fungus. Dichloromethane extracts at 400–500 mg/mL exhibited moderate antifungal activity. Similarly, Chukwuemeka *et al*. (2018) demonstrated that *V. amygdalina* extracts inhibited the growth of *Salmonella typhi*, *Bacillus subtilis*, *S. aureus*, and *Pseudomonas aeruginosa* in mice.

The plant's antiparasitic effects were examined by Ademola and Eloff (2011) and Abay *et al*. (2015), who found that acetone extracts of *V. amygdalina* inhibited *Haemonchus contortus* egg hatching and larval development. Additionally, Omoregie and Pal (2016) assessed its antiplasmodial activity against *Plasmodium berghei*-infected male Swiss rats, with in vivo results showing significant inhibition. Oral administration of *V. amygdalina* extract at 100 mg/kg and 1000 mg/kg reduced *P. berghei* activity by 23.7% and 82.3%, respectively, by day four.

2.2.4.3. Antioxidant Activity

Several studies have highlighted the antioxidant potential of *V. amygdalina* (Ifedibalu Chukwu *et al*., 2020). Iwalokun *et al*. (2006) investigated its antioxidative effects in mice exposed to acetaminophen-induced toxicity, where pretreatment with 50–100 mg/kg of *V. amygdalina*

extract mitigated oxidative stress caused by 300 mg/kg of paracetamol administered over seven days.

Ifedibalu Chukwu *et al*. (2020) further examined the antioxidative properties of isolated compounds from *V. amygdalina* methanolic stem-bark extracts using DPPH, nitric oxide, and hydrogen peroxide radical scavenging assays in mice, confirming mild antioxidative activity. Erukainure *et al*. (2018) also found that incubation of brain tissues with *V. amygdalina* reduced oxidative stress markers, including 2-keto-glutaramic acid and cysteinyl-tyrosine metabolites.

The chemoprotective effects of methanolic *V. amygdalina* extracts (250 mg/kg and 500 mg/kg) were studied by Adesanoye *et al*. (2015) in rats exposed to 2-acetylaminofluorene-induced hepatotoxicity, revealing an upregulation of antioxidant enzymes. Similarly, Ugbaja *et al*. (2021) reported antioxidative activity in flavonoid fractions of *V. amygdalina* in arsenic-induced oxidative stress in rats.

Erasto *et al*. (2006) compared the antioxidant efficacy of *V. amygdalina* extracts prepared with acetone, methanol, and water. Antioxidant activity was assessed by monitoring the reduction of DPPH and ABTS radicals at 519 nm and 734 nm, respectively. The methanol extracts exhibited the strongest antioxidative effects, scavenging 75.9%–99.3% of DPPH radicals at concentrations of 0.01–0.1 mg/mL. Acetone extracts scavenged between 63.3% and 91.7% of radicals, further supporting *V. amygdalina's* antioxidative potential.

Lolodi and Eriyamremu (2013) also examined the antioxidative properties of methanolic *V. amygdalina* extracts in rats induced with oxidative stress using a diet containing 5% *Cycas revoluta* (cycads). Administration of 200 mg/kg of *V. amygdalina* extract resulted in increased

malondialdehyde (MDA) levels and decreased superoxide dismutase (SOD) activity compared to control groups. Omojokun *et al*. (2019) found that *V. amygdalina* extracts (0–30.51 g/mL) inhibited arginase activity, with its alkaloid fractions reducing Fe^{2+}-induced lipid peroxidation.

2.2.4.4. Immunological Effect

These studies highlight *Vernonia amygdalina's* potential role in immune modulation.

Momoh *et al*. (2012) examined its effects on the CD4+ cell count of HIV-positive patients undergoing antiretroviral therapy (ART) for one year. Patients received varying doses of *V. amygdalina* in combination with the immune-boosting supplement Immunace. The study found a significant increase in CD4+ cell count, suggesting *V. amygdalina* may enhance immune function in immunocompromised individuals.

Similarly, Im *et al*. (2016) investigated the immune-modulatory effects of *V. amygdalina* by assessing its influence on lipid and hematological parameters in *Rattus norvegicus*. Rats received twice-daily doses of 50, 100, 200, 400, and 800 mg/kg of *V. amygdalina* extract for three weeks. The results showed a dose-dependent increase in CD4+ cell count, but at the highest dose (800 mg/kg), a decline was observed. Additionally, *V. amygdalina* supplementation led to an increase in white blood cells and lymphocytes, further demonstrating its potential immune-enhancing properties.

2.3 GINGER (*Zingiber officinale*)

2.3.1. INTRODUCTION

For over two centuries, traditional Chinese medicine has incorporated ginger (*Zingiber officinale*) as both a spice and a medicinal ingredient. This plant holds significant value in Asian and Chinese traditional medicine due to its numerous nutritional and therapeutic properties. Ginger has long been utilized as a herbal remedy for various ailments, including nausea, pain, and cold symptoms. Its bioactive compounds—such as iron (Fe), magnesium (Mg), calcium (Ca), vitamin C, flavonoids, and phenolic compounds (gingerdiol, gingerol, gingerdione, and shogaols), along with sesquiterpenes and paradols—exhibit a range of pharmacological activities. These include anti-inflammatory, anti-apoptotic, anti-tumor, antipyretic, anti-platelet, anti-tumorigenic, anti-hyperglycemic, antioxidant, anti-diabetic, anti-clotting, analgesic, cardiotonic, and cytotoxic properties. Ginger has been traditionally used to manage several health conditions, including rheumatism, arthritis, muscle cramps, sprains, sore throats, indigestion, vomiting, constipation, fever, dementia, and infectious diseases. Additionally, its leaves are commonly employed in Asian traditional medicine, particularly in China, and as a culinary spice. Ginger oil serves as a flavor enhancer in soft drinks, baked goods, pickles, sauces, and confectionery, as well as a food preservative. There are three primary forms of ginger: dried ginger, preserved ginger, and fresh root ginger. Its key phytocompounds, such as 6-gingerol, 6-shogaol, and zingerone, are primarily responsible for its pharmacological effects, particularly its antioxidant and anti-inflammatory properties. Ginger is an integral component of more than half of herbal prescriptions in both traditional and modern Chinese medicine. Traditional medicinal plants, including ginger, are often consumed raw or as simple herbal formulations, offering an affordable and accessible means of treatment. Research suggests that ginger extract has potential applications in both

medicine and culinary practices. The primary health benefits of ginger include relief from nausea, colds, and fever; expelling excess gas; promoting healthy digestion; reducing arthritis pain; protecting the liver; alleviating asthma; preventing obesity; relieving muscle soreness and menstrual cramps; enhancing cognitive function; and offering protection against cancer. Additionally, ginger helps regulate diabetes, supports heart health, boosts immunity, detoxifies the body, promotes skin health, treats diarrhea, improves sexual function, and maintains blood sugar balance (Shahrajabian *et al*., 2019).

For thousands of years, nature has been a rich source of medicinal compounds, with many essential modern drugs being derived from natural substances. The continuous search for novel treatments has been deeply rooted in traditional knowledge of medicinal plants. These natural remedies, whether used raw or as simple medical formulations, are often cost-effective, regionally available, and easy to consume. Due to their bioactive components, these formulations frequently yield positive health outcomes. Medicinal plants are often regarded as "chemical goldmines" because their natural compounds are compatible with both human and animal systems—many of which cannot be artificially synthesized in laboratories. Ginger, scientifically classified as *Zingiber officinale Roscoe* from the Zingiberaceae family, is one of the most valuable plants due to its diverse medicinal, nutritional, and ethnomedical applications. It is widely used across the globe as a spice, flavoring agent, and herbal remedy. Traditionally, *Z. officinale* has been a key component in medicinal systems such as Ayurveda, Siddha, Chinese, Arabian, African, and Caribbean medicine. It has been employed to treat ailments like nausea, vomiting, asthma, cough, heart palpitations, inflammation, dyspepsia, loss of appetite, constipation, indigestion, and pain (Dhanik *et al*., 2017).

Plate 2.2: Fresh Rhizome of *Zingiber officinale* (Ginger)

Source: Britannica, (2020)

2.3.2. Taxonomic classification of *Zingiber officinale*

Kingdom: Plantae

Subkingdom: Viridiplantae

Infrakingdom: Streptophyta

Superdivision: Embryophyta

Division: Tracheophyta

Subdivision: Spermatophytina

Class: Magnoliopsida

Order: Zingiberales

Family: Zingiberaceae

Genus: Zingiber

Species: *Zingiber officinale* (Teimoory *et al*., 2013)

2.3.3. Phytochemistry

Phytochemical studies have confirmed that the ginger rhizome contains several bioactive compounds with medicinal properties. The primary phytochemical constituents of *Zingiber officinale* include essential oils, phenolic compounds, flavonoids, proteins, carbohydrates, alkaloids, glycosides, saponins, steroids, terpenoids, and tannins (Mukjerjee and Karati, 2022). The ginger rhizome contains two main categories of compounds: essential oils, which contribute to its aroma, and pungent components known as gingerols. Ginger also comprises mucilage,

starch, 5–8% resinous substances, and 1–2% volatile oil. Ginger oil consists of over 24 components, including sesquiterpenes such as zingiberene and bisabolene, as well as monoterpenes like borneol, citral, camphene, phellandrene, and cineole. The secondary metabolites present in the ginger rhizome are of particular interest due to their significant biological activities. These compounds can generally be classified into volatile and non-volatile phenolic compounds, with the latter responsible for ginger's characteristic pungency. The pharmacological effects of the ginger rhizome are primarily attributed to its non-volatile pungent phenolic compounds. The term "oleoresin" refers to the combination of volatile oils, potent chemical constituents, and other bioactive substances extracted using solvents like acetone or ethanol (Ashraf *et al*., 2017).

2.3.4. Pharmacological Activity

2.3.4.1 Antimicrobial Activity

Ginger possesses strong antibacterial properties and, to a lesser extent, antifungal effects. Research has shown that methanol extracts from *Zingiber officinale* rhizomes exhibit significant antibacterial activity against *Salmonella enteritidis*, *Staphylococcus aureus*, and *Escherichia coli*. Recent findings suggest that zingerone provides protection against *E. coli*-induced diarrhea, a major cause of mortality in developing nations. Additionally, gingerone has demonstrated protective effects against hypermotility-induced diarrhea by inhibiting gastrointestinal motility. A recent study also found that juvenile Pacific white prawns (*Litopenaeus vannamei*) fed with zingerone showed enhanced immunity and resistance against *Vibrio alginolyticus*. The antibacterial potential of ginger essential oil was assessed using the paper agar diffusion method

against *Aspergillus niger*, *Saccharomyces cerevisiae*, *Mycoderma* species, *Lactobacillus acidophilus*, and *Bacillus cereus* (Mukjerjee and Karati, 2022).

2.3.4.2 Anticancer Activity

Ginger has garnered significant research attention due to its potential in combating various cancers, including colorectal, prostate, cervical, and breast cancer. Its anticancer mechanisms primarily involve promoting apoptosis and inhibiting cancer cell proliferation. Studies have indicated that ginger and its bioactive compounds play a crucial role in the suppression of colorectal cancer. In an in vitro study, a polyphenol-rich fraction of dried ginger powder was found to hinder the growth of colorectal and stomach adenocarcinoma cells. The primary anticancer mechanisms of ginger involve inducing apoptosis and restricting cancer cell proliferation. Experimental evidence suggests that ginger may aid in the prevention and treatment of multiple malignancies, such as colon, prostate, breast, cervical, liver, and pancreatic cancers (Mukjerjee and Karati, 2022).

2.3.4.3 Anti-inflammatory and Immunomodulatory Activities

Chronic inflammation is a significant contributor to the development of numerous diseases, including rheumatoid arthritis, diabetes, cancer, atherosclerosis, and age-related disorders. Extensive research has demonstrated that ginger and its active compounds exhibit strong anti-inflammatory properties. Initially, it was believed that ginger's anti-inflammatory effects were primarily due to its ability to suppress the production of prostaglandins and leukotrienes. However, further studies have shown that extracts from dried ginger, which are rich in shogaols,

and fresh ginger, predominantly containing gingerols, can inhibit the production of prostaglandin E2 (PGE2) induced by lipopolysaccharide (LPS) (Mukjerjee and Karati, 2022).

2.3.4.4 Antioxidant Activity

Antioxidants play a crucial role in neutralizing free radicals that contribute to cellular oxidative stress. Natural sources, including plants, are rich in antioxidant compounds. The antioxidant activity of plants is attributed to their diverse bioactive compounds, including flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, and isocatechins. Numerous studies have demonstrated the ability of *Z. officinale* to counteract various free radicals. Active components such as gingerols, shogaols, and zingerone exhibit strong antioxidant properties. The primary mechanism involves inhibiting the enzyme xanthine oxidase, which is responsible for generating reactive oxygen species (ROS). Gingerone has been shown to protect in vitro DNA from oxidative damage caused by ROS generated by stannous chloride. Furthermore, ginger provides an adaptogenic effect by reducing oxidative stress on smooth muscle tissue in the gut. Research has confirmed that *Z. officinale* possesses potent antioxidant properties by scavenging DPPH radicals. The total phenolic content in the alcoholic extract of the dried rhizome was found to be 870.1 mg/g of dry extract. With an IC50 value of 0.64 g/mL, the extract exhibited a 90.1% DPPH radical scavenging activity (Mukjerjee and Karati, 2022).

2.4. Lime (*Citrus aurantifolia*)

2.4.1 Introduction

Citrus aurantifolia, belonging to the Rutaceae family, is primarily utilized for juice production, daily consumption, and in various culinary traditions worldwide. It is well known for its medicinal properties, including antibacterial, anticancer, antidiabetic, antifungal, antihypertensive, anti-inflammatory, anti-lipidemic, and antioxidant effects. Additionally, it plays a role in preventing urinary disorders and supporting the health of the heart, liver, and bones. The plant's beneficial effects are largely due to its secondary metabolites, which include alkaloids, carotenoids, coumarins, flavonoids, phenolic acids, essential oils, and triterpenoids. Important bioactive compounds such as limonoids, apigenin, hesperetin, kaempferol, quercetin, naringenin, nobiletin, and rutin contribute to its therapeutic potential.

Citrus fruits are cultivated extensively in tropical and subtropical regions and are often considered "miracle fruits" due to their distinct aroma and delightful flavor. According to the USDA, in 2015, the world's largest producers of lemons and limes included Mexico (2,270 metric tons), Argentina (1,450 metric tons), the United States (784 metric tons), Turkey (668 metric tons), South Africa (330 metric tons), and Israel (60 metric tons). Citrus fruits contain a diverse range of naturally occurring compounds with potential health benefits. Their by-products, such as fruit peel pectin and essential oil, have significant applications in the pharmaceutical and cosmetic industries.

C. aurantifolia is a perennial evergreen tree that typically grows between 3 and 5 meters in height. Its stem is irregularly shaped, slender, and branched, often featuring short, stiff spines or thorns that measure about 1 cm or less. The leaves are elliptical to oval in shape, measuring 4.5

to 6.5 cm in length and 2.5 to 4.5 cm in width, with petioles that are 1 to 2 cm long and narrowly winged. The tree produces short, axillary racemes bearing a few fragrant, white flowers with five oblong petals, each approximately 10 to 12 mm long. The fruits are small, round, and green, measuring 3 to 5 cm in diameter, and turn yellow as they ripen. Like all citrus fruits, *C. aurantifolia* possesses a characteristic anatomical structure, including the flavedo—the outermost layer of the fruit— which is rich in flavonoids (Naranga and Jiraungkoorskul, 2016).

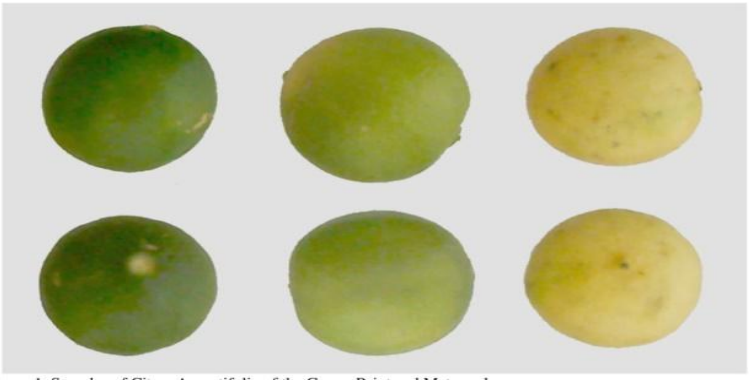

Plate 2.4: Different degree of ripeness of *Citrus aurantifolia* Fruits

Source: Tuesta-Monteza *et al.,* (2020)

2.4.2 Taxonomic classification of *Citrus aurantifolia*

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae
Genus	Citrus
Species	*Citrus aurantifolia* (Al Namani *et al*., 2018)

2.4.3 Phytochemistry

Historically, the health benefits of citrus fruits were primarily associated with their high vitamin C content. However, over the past decade, research has shifted towards identifying their bioactive compounds. *Citrus aurantifolia* contains several significant chemical groups, including flavonoids, limonoids, coumarins, and phytosterols. Preliminary phytochemical screening has detected the presence of alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycosides, carbohydrates, phenols, and reducing sugars in various parts of the plant, including its fruit.

Advanced analytical techniques such as gas chromatography and high-performance liquid chromatography (HPLC) have been used for an in-depth examination of *C. aurantifolia*. Mass spectrometry analysis has identified a wide range of phytochemicals, including 9,10-dimethyl-1,2-benzanthracene, 2,4,6-trichloroanisole, 5-geranoxy-7-methoxycoumarin, 6,7-dimethoxycoumarin, 8-geranoxypsoralen, α-bergamotene, α-phellandrene, α-pinene, α-terpineol, β-bisabolene, β-caryophyllene, β-pinene, d-limonene, camphene, γ-terpinene, p-cymene, apigenin, bergapten, bergamottin, citral, citronellol, fenchol, germacrene B, imperatorin, isoimperatorin, isopimpinellin, isovitexin, kaempferol, kaempferol derivatives, limonene, nobiletin, o-cymene, oxypeucedanin hydrate, phellopterin, quercetin, rutin, sabinene, and terpinolene. Additionally, citrus fruits contain other bioactive compounds such as pectin, furocoumarins, coumarins, lycopene (especially in grapefruit), pyranocoumarins, sitosterol, monoterpenes, and sesquiterpenes, which contribute to their medicinal properties (Enejoh *et al*., 2015).

2.4.4 Pharmacological Activity

2.4.4.1 Antibacterial Activity

Research has shown that root extracts of C. aurantifolia effectively inhibit the growth of Neisseria gonorrhoeae, Beta-haemolytic streptococci, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, and Klebsiella pneumoniae. Additionally, the fruit extract has demonstrated inhibitory effects on facultative anaerobic bacteria. Notably, C. aurantifolia exhibits strong antimycobacterial properties, particularly against isoniazid-resistant Mycobacteria strains. The antimicrobial activity of C. aurantifolia has been attributed to the

presence of bioactive compounds such as palmitic acid, linoleic acid, oleic acid, 5-geranyloxypsoralen, 5-dimethoxypsoralen, 4-hexan-3-one, and citral (Enejoh *et al*., 2015).

2.4.4.2 Antifungal and Antiaflatoxigenic Activity

Studies on the essential oil of C. aurantifolia indicate its inhibitory effects against fungal species such as Candida albicans, Aspergillus niger, Phaeoramularia angolensis, and Aspergillus parasiticus, along with its aflatoxins. The plant is currently employed as a natural fungicide for citrus fruit crops, and its antifungal properties have been linked to the presence of monoterpenes. Furthermore, C. aurantifolia has been proposed as a promising candidate for preventing aflatoxin contamination and fungal toxicity in food and animal feed (Enejoh *et al*., 2015).

2.4.4.3 Anticancer/Cytotoxic Activity

C. aurantifolia has demonstrated anticancer potential by inhibiting the growth of prostate, pancreatic, breast, colon, and neuroblastoma cancer cells. The anticancer properties of the plant are attributed to key phytochemicals such as limonoids, flavonoids, D-limonene, and D-dihydrocarvone. Research findings indicate that the essential oil of C. aurantifolia inhibits human colon cancer cells by 78%, induces DNA fragmentation, and promotes apoptosis, suggesting its potential as a preventive agent, particularly against colon cancer (Enejoh *et al*., 2015).

2.4.4.4 Antioxidant Activity

Studies on the juice, fruit peels, and leaves of C. aurantifolia have shown that it inhibits low-density lipoprotein (LDL) oxidation in a concentration-dependent manner. Its antioxidant properties have been attributed to its ability to donate hydrogen, which is linked to the presence

of flavonoids, carotenoids, and vitamin C. The flavonoids in C. aurantifolia fruit juice and peels act by blocking enzymes involved in the production of superoxide anion, such as xanthine oxidase and protein kinase C. Additionally, these flavonoids inhibit the generation of reactive oxygen species by suppressing enzymes like cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase (GST), mitochondrial succinoxidase, and NADH oxidase. Vitamin C, a key constituent of C. aurantifolia, exhibits antioxidant activity both in vitro and in vivo, protecting LDL and plasma lipids from oxidative damage caused by different forms of cancer-related oxidation (Enejoh *et al*., 2015).

2.4.4.5 Immunomodulatory Activity

Research on the effects of C. aurantifolia juice on mitogen-activated mononuclear cell cultures has demonstrated its immunomodulatory potential. At concentrations of 250 and 500 micrograms per liter, the juice extract significantly inhibited the proliferation of phytohaemagglutinin-activated mononuclear cells. Additionally, studies on the impact of sweet orange (Citrus sinensis) peel extract on the humoral immune response in broiler chickens revealed that citrus peel supplementation enhanced immune response and disease resistance. Vitamin C, a major component of citrus juice and peels, plays a crucial role in immune system function by neutralizing free radicals that cause muscle damage. Immune cells, particularly phagocytes and T-cells, accumulate vitamin C and rely on it for proper function. A deficiency in vitamin C reduces resistance to infections, while adequate levels enhance various immune system markers (Enejoh *et al*., 2015).

2.5. Turmeric (Curcuma longa)

2.5.1. Introduction

Turmeric, an evergreen herbaceous plant belonging to the Zingiberaceae (ginger) family, is extensively cultivated across Asia, particularly in India and China. It is believed to have originated in India, where its use dates back at least 2,500 years. Turmeric plants thrive in tropical and subtropical climates and are cultivated throughout India. While its precise origin remains uncertain, it is widely thought to have originated in Southeast Asia, most likely India. Apart from India, turmeric is also grown in southern China, Taiwan, Japan, Burma, Indonesia, and some regions of Africa. In Brazil, the increasing demand for turmeric is driven by its ability to enhance food aroma and impart vibrant color. Curcuma longa is a sterile plant that does not produce seeds. It grows up to 3–5 feet in height and features dull yellow flowers. The rhizome, a thick, fleshy underground stem surrounded by remnants of old leaves, is the most significant part of the plant due to its medicinal properties.

The rhizomes are boiled, dried, and ground to produce turmeric powder, a bright yellow spice with a warm, slightly bitter, and peppery flavor, along with a fragrance reminiscent of orange and ginger. Turmeric powder is best known as a key ingredient in curry spice and is also responsible for the bright yellow color of ballpark mustard (Verma *et al*., 2018).

Throughout history, plants and herbs have played a crucial role in treating illnesses. The Indian subcontinent is home to a vast array of medicinal and aromatic plants, which have been extensively utilized in traditional medicine. The Rigveda, composed between 4500 and 1600 BC, contains some of the earliest references to medicinal plant usage in India. Turmeric, known as Haridra in Sanskrit, is one such plant that is well-documented in ancient Indian medical texts,

particularly Dravyaguna Sastra. In Hindu traditions, turmeric holds great cultural significance. Women apply it as an auspicious mark on their foreheads daily, and it plays a central role in wedding rituals, where brides are adorned with turmeric paste. Ayurvedic literature extensively discusses the medicinal properties of turmeric, highlighting its applications in Dashemani Lekhaniya (weight management), Kusthagna (treatment of skin disorders), and Visaghna (detoxification and antidote for poisons) (Krup *et al*., 2013).

Plate 2.5: A turmeric rhizome cut in half

Source: Britannica, (2020)

2.5.2. Taxonomic classification of Turmeric (*Curcuma longa*)

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Subclass: Zingiberidae

Order: Zingiberales

Family: Zingiberaceae

Genus: Curcuma

Species: longa

Scientific name: Curcuma longa (Chanda and Ramachandra, 2019)

2.5.3. Phytochemistry

Turmeric contains a wide range of phytochemicals, including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols. Among these, curcumin, comprising 2–5% of turmeric, is the most significant bioactive compound, responsible for its yellow color and many medicinal properties. Being hydrophobic, curcumin dissolves easily in oils, ethanol, dimethylsulfoxide, acetone, and chloroform, but it is insoluble in water. The oxidation of curcumin produces vanillin, and its maximum absorption wavelength is approximately 420 nm (Chanda and Ramachandra, 2019).

Qualitative chemical tests used in chemical evaluations have identified multiple phytoconstituents in powdered crude turmeric. Researchers have performed preliminary phytochemical screening on aqueous, acetone, ethanolic, chloroform, and methanolic extracts of *Curcuma longa* rhizomes using common precipitation and color reactions. These analyses revealed the presence of proteins, carbohydrates, alkaloids, glycosides, terpenes, steroids, flavonoids, tannins, and saponins (Adinew, 2012).

2.5.4. Pharmacological Activity

2.5.4.1. Antimicrobial Activity

Extracts and essential oils from turmeric (*Curcuma longa*) demonstrate inhibitory effects against numerous bacteria, parasites, and fungi. Studies on chicks infected with *Eimeria maxima* revealed that turmeric-enriched diets facilitated weight gain and reduced intestinal lesions. Additionally, turmeric oil, when applied topically to guinea pigs infected with dermatophytes or pathogenic fungi, suppressed fungal growth, leading to the disappearance of lesions within a week. Curcumin has also exhibited moderate efficacy against *Leishmania* species and *Plasmodium falciparum* (Chanda and Ramachandra, 2019).

When tested for microbial susceptibility, ethanolic extracts of turmeric showed antibacterial activity against various bacteria, with *Staphylococcus epidermidis* exhibiting the lowest inhibition zone and *Shigella flexneri* the highest. The antibacterial properties of turmeric are attributed to its phytochemicals, including tannins, alkaloids, phenols, steroids, flavonoids, phlorotannin, cardiac glycosides, terpenoids, triterpenes, and saponins. Sulfur-functionalized carbon dots (S-CDs) derived from turmeric displayed potent antioxidant and antibacterial

activity, with sulfur-functionalized CDs generating more reactive oxygen than non-functionalized ones. Gram-positive bacteria (*L. monocytogenes*, *E. coli*) were more susceptible than Gram-negative bacteria. Turmeric extracts (N-hexane, water, chloroform, and ethanol) have been evaluated against *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Water-based extracts significantly inhibited *Salmonella typhi* and *Escherichia coli*, while methanolic extracts showed a broader antimicrobial spectrum. Aqueous preparations were particularly effective against *Staphylococcus aureus*. Turmeric extracts have also exhibited antifungal properties against *Mucor* species, suggesting potential applications as preservatives for antibiotics. Additionally, turmeric-derived curcumin-loaded hydrogels combined with UV-A light serve as antimicrobial coatings that enhance food safety and shelf life. Curcuminoids—curcumin, bisdemethoxycurcumin (BDC), and demethoxycurcumin (DMC)—have demonstrated antifungal and antibacterial activities against *Aspergillus niger*, *Candida albicans*, *Bacillus subtilis*, and *Staphylococcus aureus* (Jyotirmayee and Mahalik, 2022).

2.5.4.2. Anticancer Activity

Numerous studies have explored the effects of turmeric on carcinogenesis using in vitro human cell lines and animal models. Research indicates that curcumin can regulate all three stages of carcinogenesis: tumor initiation, promotion, and angiogenesis. Studies on prostate and colon cancer have shown that curcumin inhibits tumor formation and cell proliferation. Additionally, turmeric and curcumin counteract the effects of several known carcinogens and mutagens.

The anticancer properties of turmeric are attributed to its antioxidant and free-radical scavenging activities, as well as its ability to increase glutathione levels, which aid in detoxifying carcinogens. Curcumin has also been found to reduce UV-induced mutations. Experiments on

Swiss mice indicate that dietary turmeric may serve as a chemopreventive agent against stomach tumors induced by benzo-(alpha)-pyrene. Topical applications of turmeric ethanolic extracts and curcumin-based ointments have shown significant symptom reduction in patients with external malignant tumors. Turmeric's antioxidant properties neutralize cancer-causing free radicals. Additionally, studies have shown that turmeric inhibits the expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) and induces apoptosis in human myeloid leukemia cells (HL-60) (Chanda and Ramachandra, 2019).

2.5.4.3. Antioxidant and Anti-Inflammatory Activity

Turmeric's antioxidant properties stem from its ability to scavenge free radicals and activate nuclear factor erythroid 2-related factor 2 (Nrf2), promoting antioxidant responses. These effects make turmeric beneficial in conditions such as diabetic microangiopathy, endothelial dysfunction, and inflammation. The bioactive component tumerone in turmeric essential oil also exhibits anticancer activity. Turmeric oleoresin, extracted during curcumin production, contains oil, resin, and non-extractable curcumin, all of which have demonstrated strong antioxidant and antimutagenic effects. Studies suggest that curcumin protects against neurological damage caused by pesticide exposure, reducing oxidative stress in the hippocampus of rats exposed to organophosphates. Additionally, turmeric extracts have exhibited strong antioxidant activity in ABTS and DPPH assays. Curcumin has also been shown to modulate the neuroendocrine system, reducing chronic stress-related disorders. By affecting nitric oxide production and brain-derived neurotrophic factor (BDNF) expression, curcumin alleviates anxiety responses. Its anti-inflammatory effects are linked to pathways involving Wnt/β-catenin, NF-κB, mitogen-activated protein kinases, and redox regulation. Curcumin suppresses NACHT, LRR, and PYD domain-

containing protein 3 inflammasome activation, contributing to its anti-inflammatory benefits. Additionally, it influences signal transducers and activators of transcription-3 and peroxisome proliferator-activated receptor-γ (PPARγ) (Ahmad *et al*., 2020).

2.5.4.4. Antidiabetic Activity

Studies have shown that turmeric plays a significant role in diabetes management. Extracts containing ar-turmerone and curcuminoids have been found to promote adipocyte differentiation in a dose-dependent manner. The hypoglycemic effects of turmeric extracts, particularly ethanol-based ones, suggest that both curcuminoids and sesquiterpenoids contribute to its blood sugar-lowering properties. Turmeric also impacts insulin levels and postprandial plasma glucose. Consumption of *Curcuma longa* does not significantly affect glycemic response, but it increases insulin levels 30–60 minutes after oral glucose tolerance tests. Additionally, turmeric and curcumin have demonstrated protective effects against diabetes-related complications, as evidenced by studies on albino rats with alloxan-induced diabetes, showing reduced blood sugar levels and polyol pathway modulation (Chanda and Ramachandra, 2019).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

The following materials were utilized in this study:

3.1.1 Equipment/Apparatus

The equipment used includes a hot air oven (Size 2, Gallenkamp, U.K.), an incubator (Size 2), and a bench autoclave (Gallenkamp, U.K.). Additionally, an H80 weighing balance (Mettler, Switzerland), an MSE High Speed 18 centrifuge, and a spectrophotometer (SP8-400 UV/visible, PYE UNICAM, England) were employed. Other laboratory instruments used include a water bath (Gallenkamp, U.K.), cheesecloth, a blender, a knife, a cutting board, a juicer, and a fine strainer. Various glassware such as beakers, pipettes, Pyrex burettes, microscopic slides, glass petri dishes, flasks, measuring cylinders, separating funnels, bijou bottles, universal bottles, and Macartney bottles were also used.

3.1.2 Microbiological Media

The microbiological media utilized in this study include Nutrient Broth (BIOTECH, TM 341, India), Mueller Hinton Agar (BIOTECH, TM 339, India), Nutrient Agar (BIOTECH, TM 350, India), Sabouraud Dextrose Broth (BIOTECH, TM 361, India), and Potato Dextrose Agar (BIOTECH, TM 387, India).

3.1.3 Chemicals/Reagents

All chemicals used were of analytical grade, including distilled water, Analar Grade Methanol (50%), Folin-Denis reagent, tannic acid, vanillin, Folin-Ciocalteu reagent, sodium carbonate (obtained from Local Chemie), Tween-80 (10%), sulfuric acid (70%), ammonia (1%), sodium acetate trihydrate, aluminum chloride, ciprofloxacin (Sigma-Aldrich Biochemika, USA), nitric acid, and perchloric acid.

3.1.4 Antimicrobial Agents

Ciprofloxacin (Sigma-Aldrich Biochemika, USA) was used alongside distilled water extracts of bitter leaf (*Vernonia amygdalina*), lime (*Citrus aurantiifolia*), ginger (*Zingiber officinale*), and turmeric (*Curcuma longa*).

3.1.5 Source of Test Microorganisms

The microbial isolates used were sourced from clinical stock cultures at the University of Benin Teaching Hospital, Edo State, Nigeria. These cultures were preserved in Sabouraud Dextrose Agar (PDA) and nutrient agar slants. The selected bacterial isolates include *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans, Lactobacillus acidophilus, Escherichia coli,* and *Klebsiella pneumoniae*.

3.1.6 Sterilization of Materials

The autoclave and hot air oven were used to sterilize various materials. Glassware such as test tubes, glass rods, pipettes, measuring cylinders, beakers, and conical flasks were thoroughly

washed with detergent, rinsed multiple times with distilled water, and dried after being wrapped in aluminum foil. The drying process was conducted at 160–170°C for 45–60 minutes.

3.2 METHODS

3.2.1 Study Design

This study aimed to assess the efficacy of four medicinal plants bitter leaf (*Vernonia amygdalina*), lime (*Citrus aurantiifolia*), ginger (*Zingiber officinale*), and turmeric (*Curcuma longa*) in treating throat infections. The medicinal properties of these plants, both individually and in combination, were analyzed using an in vitro experimental approach. Chemical analysis was employed to identify the bioactive compounds in the plant extracts, while biological assays were conducted to evaluate their antibacterial activity. Additionally, the study investigated potential synergistic effects that could enhance the therapeutic efficacy of these plants.

3.2.2 Plant Collection and Identification

The plant materials collected for this study include ginger and turmeric rhizomes, bitter leaf, and lime fruits. The lime fruits and bitter leaf were sourced from Ekosodin village, Edo State, while the ginger and turmeric rhizomes were obtained from Benin City markets. Identification and authentication of the plant specimens were performed by Prof. Akinnibosun Henry Adewale at the Herbarium Unit of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. The voucher numbers assigned were: Bitter leaf (UBH-V342), Lime (UBH-C516), Ginger (UBH-Z384), and Turmeric (UBH-Z397).

3.2.3 Other Materials Used

Solvents used include distilled water for extraction and 70% ethanol (analytical grade). Reagents included aluminum chloride for flavonoid determination, hydrogen peroxide (H_2O_2), and Folin-Ciocalteu reagent for phenolic content analysis. Laboratory equipment used included a High-Performance Liquid Chromatography (HPLC) system for phytochemical profiling, a flame atomic absorption spectrophotometer (FAAS) for elemental analysis, a rotary evaporator, a blender, a Soxhlet apparatus, a pH meter, and a UV-visible spectrophotometer.

3.2.4 Preparation of Plant Extracts

Fresh bitter leaf was thoroughly washed to remove contaminants and air-dried in a shaded area for two weeks. The dried leaves were ground into a fine powder, and 20 g of this powder was soaked in 200 mL of 70% ethanol for 48 hours. The mixture was then filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. The final extract was stored in amber-colored bottles at 4°C to maintain its bioactive properties.

Lime fruits were washed, sliced, and manually pressed to extract the juice. The juice was filtered through a muslin cloth to remove seeds and pulp, then mixed with an equal volume of ethanol to form the ethanolic extract. This solution was stored in amber bottles at 4°C to prevent oxidation and preserve bioactive compounds.

Ginger rhizomes were washed, sliced, and air-dried for over 30 days. The dried slices were further dried at 60°C for 30 minutes in an oven and ground into a fine powder. A 10 g portion of the powder was soaked in 200 mL of 70% ethanol in a conical flask, which was sealed with

aluminum foil and shaken at 200 rpm for eight hours. The extract was then filtered, concentrated at 40°C using a rotary evaporator, and stored in amber vials at 4°C.

Turmeric rhizomes were washed, sliced, and air-dried for 14 days. The dried pieces were further dried at 60°C for 30 minutes and ground into powder. A 25 g portion of the turmeric powder was mixed with 150 mL of ethanol and stirred at 450–500 rpm for three hours at room temperature. The mixture was filtered, concentrated with a rotary evaporator at 40°C, and stored in amber-colored bottles at 4°C to preserve bioactive properties.

3.2.5 Antimicrobial Assay of the Extracts

The antibacterial activity of *D. concentrica* ethanol and aqueous extracts was assessed using a modified agar well diffusion technique, following CLSI (2010) and Cheesbrough (2006). Mueller-Hinton Agar and Potato Dextrose Agar plates were prepared, and microbial isolates were cultured in broth before inoculation. The inoculum was standardized to 10^6 CFU/ml using a spectrophotometer and cytometer.

Using a sterile cork borer, 6 mm diameter wells were made in the agar plates, and 100 µL of the stock solution at concentrations of 100, 60, and 20 mg/mL was introduced into the wells. Ciprofloxacin (1µg/ml) served as the positive control, while distilled water (1µL/ml) was used as the negative control. After incubation at 38°C for 24 hours, microbial inhibition zones were measured using a millimeter-calibrated ruler. The mean inhibition zone diameters (IZDs) were then recorded.

3.2.6 Determination of Minimum Inhibitory Concentration (MICs) of the selected Antimicrobial Agent

According to Firas *et al*. (2008), a modified broth dilution approach was used to measure the extracts' minimum inhibitory concentrations (MICs) against the test isolates. In sterile sealed tubes, 10 mL of Mueller-Hinton broth was used to create various concentrations of the antimicrobial agent, ranging from 0.02 to 10µg/mL, from the stock solution. Using a micropipette, a 100 µL aliquot of the test organisms' overnight broth culture was added. This aliquot was diluted 1:100 to meet a 0.5 McFarland turbidity standard, or around 1×10^8 CFU/mL. In each experimental round, a control tube containing only the broth and inoculum and no antimicrobial agent was used. The tubes were then incubated under the proper conditions. Following incubation, bacterial growth was evaluated using turbidity, and the MIC was defined as the lowest concentration at which no visible growth was observed.

3.2.7 Determination of Minimum Bactericidal Concentration (MBC) of the Extracts.

Following MIC determination, broth tubes with no apparent growth were streaked onto fresh Nutrient Agar plates with a sterilised inoculating loop. Three experimental tubes having concentrations ranging from the MIC to progressively higher were chosen. The inoculation plates were then incubated under suitable conditions, with bacterial cultures kept at 37°C for 24 hours. Following incubation, the plates were checked for bacterial growth. The minimum bactericidal concentration (MBC) was defined as the lowest extract concentration that resulted in total microbial cell killing, as evidenced by no apparent growth on the agar plates. In other words, the MBC was defined as the lowest dose at which no bacterial growth was detected following subculturing and incubation (Lalitha, 2004; CLSI, 2010; Dowe *et al*., 2016).

3.3 Phytochemical Analysis

The extracts were quantitatively analysed to evaluate the presence of important bioactive components such as curcuminoids, gingerols, flavonoids, polyphenols, and alkaloids. Standard qualitative techniques, such as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), were used to identify compounds.

3.3.1 Determination of Total Phenolic Content

The amount of total phenolics in the extract was quantified using the Folin-Ciocalteu reagent, following the method of Singleton and Rossi (1965), with a modest modification that used tannic acid as a standard.

Briefly, 1.0ml of extract solution (250Ug/ml) was put to a test tube. Then, 1.0ml of Folin-Ciocalteu reagent was added, and the flask contents were thoroughly mixed. After 5 minutes, 15.0 mL of Na_2CO_3 (20%) was added and allowed to stand for 2 hours. The absorbance was measured at 760nm with a UV-Vis spectrophotometer. The total phenolic content was calculated as Ug tannic acid equivalent (TAE) using an equation derived from the standard tannic acid calibration graph.

3.3.2 Determination of Alkaloids Content

The total alkaloid content was determined using the method outlined by Harborne (1973). 5g of the extract was weighed into a 250ml beaker, and 100ml of 20% acetic acid in ethanol was added before being covered and let to stand for two hours. The extract was filtered and concentrated to one-quarter of its original volume in a water bath. Concentrated ammonium hydroxide was applied drop by drop to the extract until it precipitated completely. The entire solution was

allowed to settle before the precipitate was collected via filtering, rinsed with 1% ammonia solution, dried, and weighed. All samples were analysed in duplicate.

$$\textbf{Alkaloid (\%)} = \frac{\textbf{Weight of residue}}{\textbf{Weight of sample}} \times 100$$

$$\textbf{Alkaloids (\%)} = \frac{(W2-W1)}{\textbf{Weight of sample}} \times 100$$

Where:

$(W_2 - W_1)$ = Weight of residue

3.3.3 Determination Flavonoid Content

The flavonoid content of the homogeneous cabbage extract (1.5 g) was measured using triplicate aliquots (Ilahy *et al*., 2011). The flavonoid content of the methanolic extract was determined using 30-microliter aliquots. The samples were diluted with 90 µl methanol, 6 µl of 10% aluminium chloride (AlCl3), 6 µl of 1mol/l sodium acetate (CH3CO2Na), and 170 µL of methanol. The absorbance was measured at 415 nm after 30 minutes. Quercetin served as a reference for estimating flavonoid content (Ug Qe/g).

3.3.4 Estimation of Total Saponin Content

The total saponin content was estimated using the method reported by Makkar *et al*., which is based on a vanillin-sulphuric acid colorimetric reaction with certain modifications. 50 µL of plant extract was mixed with 250 µL of distilled water. To this, add approximately 250 µL of vanillin reagent (800mg of vanillin in 10mL of 99.5% ethanol). Then 2.5mL of 72% sulphuric acid was added and stirred thoroughly. This solution was maintained in a water bath at 60°C for

ten minutes. After 10 minutes, it was chilled in ice water and the absorbance was measured at 570 nm. Standard saponin solutions ranging from 0 to 25 ppm were produced from the saponin stock solution. Standard solutions were treated similarly to test samples. The values were represented as PPM.

3.3.5 Estimation of Tannins Content

Quantitative Determination of Tannin

To conduct the experiment, 0.20 mL of sample was mixed with 20 mL of 50% methanol and agitated in a water bath at 77 - 80 ˚C for 1 hour. The extract was quantitatively filtered through a double-layered Whatman No.1 filter paper, then 20 mL of distilled water, 2.5 mL of Folin-Denis reagent, and 10 mL of 17% Na2CO3 were added and combined. The mixture was left to stand for 20 minutes. A series of standard tannic acid solutions were produced in methanol, and their absorbance, as well as samples, were measured using a UV/Visible spectrophotometer at 760 nm following colour development. Total tannin content was estimated using the calibration curve.

3.4 Elemental Analysis

The elements sodium and potassium were measured with a flame photometer, whereas calcium, magnesium, iron, copper, and zinc were measured with an Atomic Absorption Spectrophometer.

CHAPTER FOUR

4.0. RESULTS

Table 4.1 contains several plants have been traditionally used to treat throat infections (pharyngitis) due to their antimicrobial, anti-inflammatory, and soothing properties. Here are some notable plants:

Botanical Name	Common Name	Most Effective Plant Part	Method of Use	Common Locality
Glycyrrhiza glabra	Licorice	Root	Decoction, tea, or gargle	Asia, Europe
Ocimum gratissimum	African Basil	Leaves	Infusion, steam inhalation	Africa, Asia
Thymus vulgaris	Thyme	Leaves, stems	Tea, essential oil gargle	Europe, Mediterranean
Mentha piperita	Peppermint	Leaves	Tea, steam inhalation	Europe, North America
Eucalyptus globulus	Eucalyptus	Leaves	Steam inhalation, essential oil	Australia, Africa
Salvia officinalis	Sage	Leaves	Tea, gargle, essential oil	Europe, North America
Althaea officinalis	Marshmallow	Root	Tea, lozenge	Europe, North America
Allium sativum	Garlic	Bulb	Crushed in honey, raw consumption	Global
Matricaria chamomilla	Chamomile	Flowers	Tea, gargle	Europe, Asia
Camellia sinensis	Green Tea	Leaves	Tea, gargle	China, India
Andrographis paniculata	King of Bitters	Leaves	Tea, extract	India, China
Sambucus nigra	Elderberry	Flowers, berries	Tea, syrup	Europe, North America
Psidium guajava	Guava	Leaves	Infusion, tea	Tropical regions

Adhatoda vasica	Malabar Nut	Leaves	Decoction, syrup	India, Southeast Asia
Piper nigrum	Black Pepper	Fruit	Powder in warm water, decoction	India, Southeast Asia
Justicia adhatoda	Vasaka	Leaves	Tea, syrup	India, Sri Lanka
Bidens pilosa	Black-jack	Leaves, stems	Decoction, tea	Africa, South America
Plantago major	Plantain	Leaves	Tea, gargle	Global
Cinnamomum verum	Cinnamon	Bark	Tea, extract	Sri Lanka, India
Phyllanthus amarus	Stonebreaker	Whole plant	Decoction, tea	Asia, Africa
Azadirachta indica	Neem	Leaves, bark	Decoction, gargle	India, Africa
Hibiscus sabdariffa	Roselle	Calyx	Tea, infusion	Africa, Asia
Carica papaya	Papaya	Leaves	Decoction, syrup	Tropical regions
Annona muricata	Soursop	Leaves	Tea, decoction	Tropical regions
Xylopia aethiopica	African Pepper	Fruit	Decoction, powder	Africa
Lippia javanica	Fever Tea	Leaves	Tea, steam inhalation	Africa
Vernonia cinerea	Purple Fleabane	Whole plant	Decoction, infusion	Asia, Africa
Terminalia chebula	Haritaki	Fruit	Decoction, gargle	India, Southeast Asia
Scoparia dulcis	Sweet Broomweed	Whole plant	Tea, decoction	Africa, South America
Perilla frutescens	Perilla	Leaves, seeds	Tea, inhalation	Asia, Europe

Table 4.2: Minimum inhibitory concentrations (MICs) of the aqueous extract of polyherbal mixture (*Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L)* against the Test Organisms.

Organisms	Concentrations (mg/mL)															
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
S. aureus	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
L. acidophilus	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. mutans	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
K.pneumoniae	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
E. coli	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
P. aeruginosa	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

Key: Growth(+), No growth (-)

Table 4.3. Minimum Bactericidal Concentration (MBCs) of the aqueous extracts of polyherbal mixture (*Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L.)* against the Test organisms.

Organisms	Extracts (mg/mL)
L. Acidophilus	8
S. Mutans	10
S. aureus	10
K. pneumoniae	14
E. coli	15
P. aeruginosa	17

Table 4.3 shows the Minimum Bactericidal Concentrations (MBCs) of the aqueous extract of polyherbal mixture (*Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L.*) against the test Organisms. The result recorded the lowest MBCs of 8mg/mL of *Streptococcus mutans*, *Lactobacillus acidophilus*(10mg/mL), *Streptococcus mutans*(8mg/mL), *Staphylococcus aureus* (10mg/mL), *Klebsiella pneumonae*(14mg/mL) and *Escherichia coli* (15mg/mL) while *Pseudomonas aeruginosa* recorded the highest MBCs of 17mg/mL for the extract.

Table 4.4: Zones of Inhibition of the aqueous extract of polyherbal mixture (*Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L)* at different concentrations.

Organisms					
	Concentrations (mg/mL)			**Control**	
	20	**60**	**100**	**CIP 5µg/Ml**	**D.H_20 10µg/mL**
L.acidophilus	10±0	13.5±0.5	14.5±1.5	33.5±5.5	0.0±0.0
S.mutans	7.5±0.5	9.5±0.5	11.5±0.5	30.5±0.5	0.0±0.0
S. aureus	7.5±0.5	8.5±0.5	8.5±0.5	34.0±7.0	0.0±0.0
K.pneumoniae	4.5±0.5	6.0±1.0	10.5±1.5	31.5±1.5	0.0±0.0
E. coli	4.0±1.0	7.0±1.0	9.5±0.5	31.5±0.5	0.0±0.0
P. aeruginosa	4.0±1.0	5.5±0.5	9.5±0.5	33.5±2.5	0.0±0.0

Key: Mean of 3 replicates ± Standard Error Mean , 0.0 = No activity, CIP = ciprofloxacin

Table 4.4 presents the inhibition zones produced by the aqueous extract of the polyherbal mixture at different concentrations. The findings demonstrate a dose-dependent antimicrobial response, with the highest inhibition observed at 100 mg/mL.

.

Plate 4.4: Petri dishes showing Zone of Inhibition (IZD)

Photocredit: Osagie Etinosa

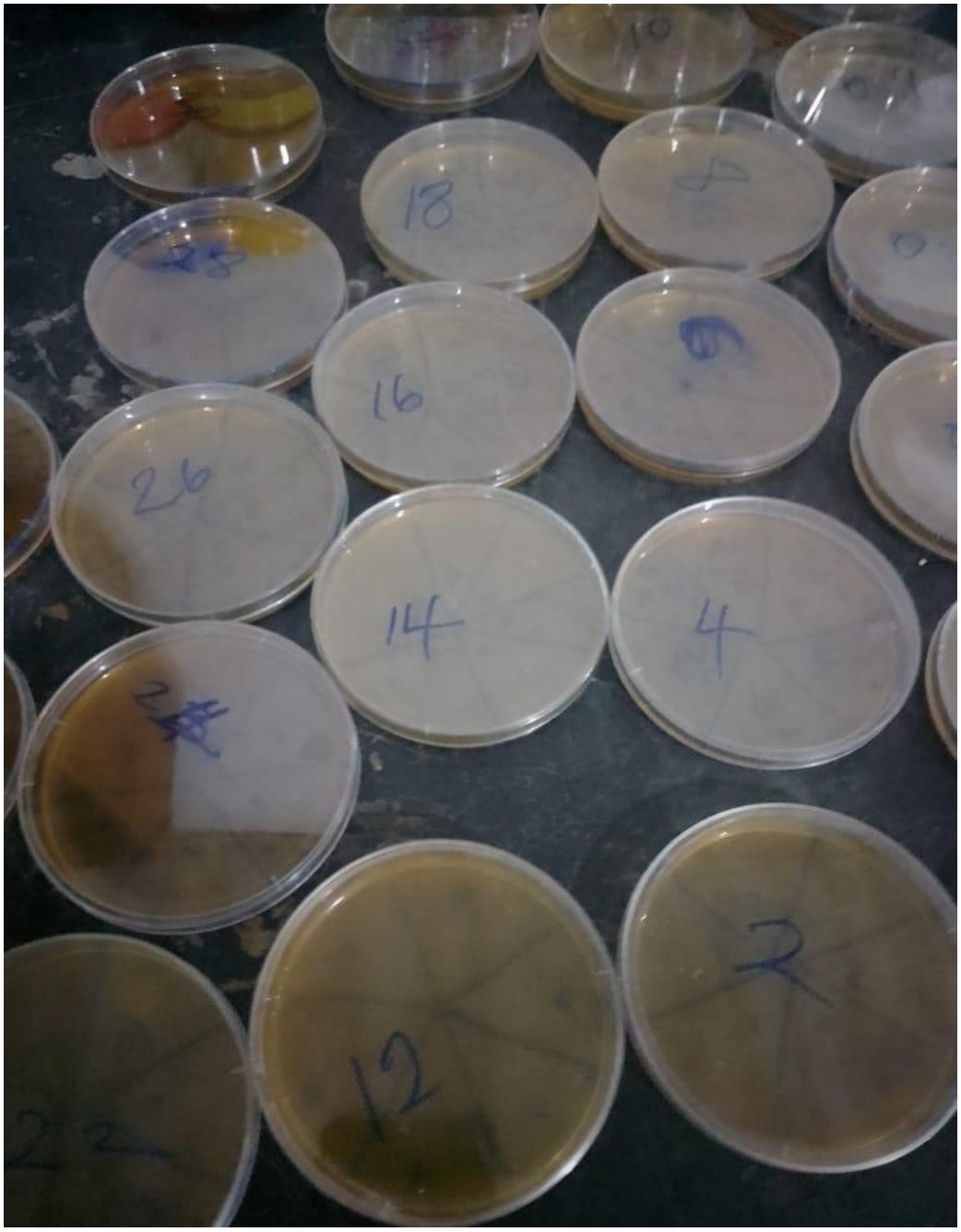

Plate 4.5: Petri dishes for Minimum inhibitory concentrations (MICs) and Minimum Bactericidal Concentration (MBCs) analysis

Photocredit: Osagie Etinosa

Table 4.5: Phytochemical Screeening of the aqueous extracts of Polyherbal mixture (*Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L)*

Plant constituents	**Aqueous**
Phenolics	+
Flavonoids	+
Saponin	+
Tannins	+
Alkaloids	+

Key: **+ positive (present), - = negative (absent)**

Table 4.5 shows the qualitative phytochemical screening present in the aqueous extract of *Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L*. The result shows the presence of phenolics, flavonoids, saponins, Tannins, alkaloids in aqueous extracts.

Table 4.6: Quantitative analysis of secondary metabolites in the polyherbal extracts (*Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L.)* of the ethanol extract [mean ± SEM (%)]

Plant constituents	Aqueous (%)
Phenolic	230.1375±7.013
Flavonoids	8.001±0.249
Saponins	6.732±0.072
Alkaloids	1.005±0.005
Tannins	28.683±3.975

Key: Mean±SEM (Standard Error Mean)

Table 4.6 shows the quantitative phytochemical compounds present in the ethanol extract Polyherbal mixture **(*Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L.*)**The result shows the aqueous extract to contain concentration of secondary metabolites which include phenolics (230.1375±7.013), flavonoids(8.001±0.249%), saponins (6.732± 0.072%), alkaloids (1.005 ±0.005%), tannins (28.683±3.975%),

Table 4.7. Elemental Analysis of Polyherbal mixture (*Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L.*)

ELEMENTS	CONCENTRATION (Mg/Kg)	WHO STANDARD	
		MALE	FEMALE
Sodium, Na	511.695	2300mg	2300mg
Potassium, K	2650.584	4700mg	4700mg
Magnesium, Mg	21.491	400mg	420mg
Manganese, Mn	33.480	2.3mg	1.8mg
Copper, Cu	2.924	900mcg	900mcg
Calcium, Ca	346.491	1000mg	1000mg
Zinc, Zn	28.947	11mg	8mg
Iron, Fe	390.351	8mg	18mg

Table 4.7 shows the concentraton of each element(mg/kg) present in aqueous extract of polyherbal mixture *Vernonia amygdalina*, *Zingiber officinale R, Citrus aurantiifolia, Curcuma longa L* with microelements Sodium (511.695mg/kg), Potassium (2650.584mg/kg), Magnesium (21.491mg/kg) and microelement Manganese (33.480mg/kg), Copper (2.924mg/kg), Calcium (346.491mg/kg), Zinc (28.947mg/kg), Iron (390.351mg/kg).

CHAPTER FIVE

5.0. DISCUSSION

The study aimed to evaluate the antimicrobial and phytochemical properties of aqueous and ethanol extracts from four medicinal plants: *Vernonia amygdalina* (bitter leaf), *Zingiber officinale* (ginger), *Citrus aurantiifolia* (lime), and *Curcuma longa* (turmeric). The results demonstrated significant antimicrobial activity against various bacterial strains, including *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The findings also revealed the presence of bioactive compounds such as phenolics, flavonoids, saponins, tannins, and alkaloids, which are known for their antimicrobial, antioxidant, and anti-inflammatory properties. The discussion below integrates the results with relevant citations from the literature review to provide a comprehensive analysis.

5.1. Antimicrobial Activity

The study found that the aqueous and ethanol extracts of the polyherbal mixture exhibited significant antimicrobial activity, particularly against Gram-positive bacteria such as *S. aureus* and *S. mutans*. The Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) indicated that the extracts were effective in inhibiting and killing bacterial strains at moderate concentrations. For instance, *L. acidophilus* showed the lowest concentration (8 mg/mL), while *P. aeruginosa* required the highest concentration (17 mg/mL) for inhibition. These results align with previous studies that have highlighted the antimicrobial properties of the individual plants. For example, *Vernonia amygdalina* has been reported to exhibit antibacterial activity against *Salmonella enterica*, *Klebsiella pneumoniae*,

and *Staphylococcus aureus* (Ngatu *et al*., 2012; Dumas *et al*., 2020). Similarly, *Zingiber officinale* (ginger) has been shown to possess strong antibacterial properties against *E. coli* and *S. aureus* (Mukjerjee and Karati, 2022). The antimicrobial activity of *Citrus aurantiifolia* (lime) has also been documented, particularly against *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa* (Enejoh *et al*., 2015). These findings suggest that the polyherbal mixture could serve as a complementary or alternative treatment for bacterial infections, especially in the context of rising antibiotic resistance.

The zones of inhibition observed in the study further validated the antibacterial efficacy of the extracts. The results showed a dose-dependent response, with larger inhibition zones at higher concentrations (20, 60, and 100 mg/mL). However, the extracts were less potent than the control antibiotic, ciprofloxacin, which exhibited significantly larger inhibition zones. This is consistent with previous research, which has shown that while plant extracts can be effective against bacterial pathogens, they often require higher concentrations to achieve similar effects as conventional antibiotics (Cattoir, 2016; Ventola, 2015). This highlights the need for further optimization of the polyherbal formulation to enhance its potency.

5.2.Phytochemical Composition

The phytochemical analysis revealed that the aqueous and ethanol extracts contained significant amounts of bioactive compounds, including phenolics, flavonoids, saponins, tannins, and alkaloids. Phenolics were the most abundant (230.1375 ± 7.013 mg/g), followed by tannins (28.683 ± 3.975 mg/g), flavonoids (8.001 ± 0.249 mg/g), saponins (6.732 ± 0.072 mg/g), and alkaloids (1.005 ± 0.005 mg/g). These compounds are known for their antimicrobial, antioxidant,

and anti-inflammatory properties, which likely contribute to the therapeutic effects of the extracts.

Phenolic compounds, in particular, have been widely studied for their antimicrobial activity. They are known to disrupt bacterial cell membranes, inhibit enzyme function, and interfere with microbial DNA synthesis (Patel and Patel, 2016). The high phenolic content in the extracts may explain their strong antibacterial activity, particularly against Gram-positive bacteria. Flavonoids, another major group of phytochemicals, have also been shown to exhibit antimicrobial properties by inhibiting bacterial growth and biofilm formation (Enejoh *et al*., 2015). The presence of these compounds in the polyherbal mixture supports its potential as a natural remedy for bacterial infections.

5.3. Elemental Composition

The elemental analysis of the polyherbal extract revealed the presence of essential macro- and microelements, including potassium (2650.584 mg/kg), sodium (511.695 mg/kg), calcium (346.491 mg/kg), iron (390.351 mg/kg), manganese (33.480 mg/kg), and zinc (28.947 mg/kg). These elements play crucial roles in human health, including immune function, enzymatic activity, and cellular processes. For example, potassium and calcium are essential for maintaining cellular homeostasis and immune function, while zinc and iron are critical for enzymatic activities and antimicrobial defense mechanisms (Adetunde *et al*., 2017). The presence of these elements in the polyherbal mixture suggests that it not only exhibits antimicrobial activity but also provides essential nutrients that support overall health and immune function.

5.4. Comparison with Conventional Antibiotics

While the polyherbal extracts demonstrated significant antimicrobial activity, they were less potent than the control antibiotic, ciprofloxacin. This is consistent with previous studies that have shown that plant-based antimicrobials often require higher concentrations to achieve similar effects as conventional antibiotics (Cattoir, 2016; Ventola, 2015). However, the extracts' ability to inhibit a broad spectrum of bacterial pathogens, including both Gram-positive and Gram-negative strains, suggests that they could serve as complementary or alternative treatments, particularly in the context of antibiotic resistance. The rising prevalence of antibiotic-resistant bacterial strains, such as *Streptococcus pyogenes* and *Pseudomonas aeruginosa*, underscores the need for alternative therapeutic options (Wijesundara *et al*., 2021; Alves-Barroco *et al*., 2020). The polyherbal mixture, with its broad-spectrum antimicrobial activity and rich phytochemical composition, could be a valuable addition to the arsenal of natural remedies for combating bacterial infections.

5.5. Potential Synergistic Effects

One of the key findings of the study is the potential for synergistic effects among the bioactive compounds in the polyherbal mixture. The combination of *Vernonia amygdalina*, *Zingiber officinale*, *Citrus aurantiifolia*, and *Curcuma longa* may enhance the overall antimicrobial efficacy of the extract. For example, the phenolic compounds in *Vernonia amygdalina* and *Curcuma longa* may work synergistically with the flavonoids in *Citrus aurantiifolia* and the alkaloids in *Zingiber officinale* to disrupt bacterial cell membranes and inhibit microbial growth. This is supported by previous research that has shown that combining different plant extracts can enhance their antimicrobial activity (Adetunde *et al*., 2017; Enejoh *et*

al., 2015). Further studies are needed to explore the specific mechanisms of action and potential synergistic interactions among the bioactive compounds in the polyherbal mixture.

5.6. CONCLUSION

The results show that the aqueous and ethanol extracts of *Vernonia amygdalina, Zingiber officinale R, Citrus aurantiifolia,* and *Curcuma longa L* have strong antibacterial action, most likely due to their high phytochemical content. While the extracts are less powerful than traditional antibiotics, they hold promise as additional or alternative medicines, particularly in light of rising antibiotic resistance. More study is needed to completely understand their mechanisms of action, optimise formulations, and assess potential therapeutic uses.

The results of this study highlight the potential *of Vernonia amygdalina, Zingiber officinale R, Citrus aurantiifolia, and Curcuma longa L* extracts as natural antibacterial agents. While more research is needed to improve their potency and investigate clinical applications, the findings underscore the importance of these medicinal plants in combating the growing challenge of antibiotic resistance, as well as their potential role in complementary medicine.

REFERENCES

Abay, SM, Lucantoni, L, Dahiya, N, Dori, G, Dembo, EG, Esposito, F, Lupidi, G, Ogboi, S, Ouédraogo, RK, Sinisi, A, Taglialatela-Scafati, O, Yerbanga, RS, Bramucci, M, Quassinti, L, Ouédraogo, JB, Christophides, G, Habluetzel, A., 2015. Plasmodium transmission blocking activities of Vernonia amygdalina extracts and isolated compounds. Malar J **14**:288. doi:10.1186/s12936-015-0812-2.

Adaramoye, O, Ogungbenro, B, Anyaegbu, O, Fafunso, M., 2008b. Protective effects of extracts of Vernonia amygdalina, Hibiscus sabdariffa and vitamin C against radiationinduced liver damage in rats. J Radiat Res **49**(2):123–131. doi:10.1269/jrr.07062.

Ademola, IO, Eloff, JN., 2011. Anthelminthic activity of acetone extract and fractions of Vernonia amygdalina against Haemonchus contortus eggs and larvae. Trop Anim Health Prod **43**(2):521–527. doi:10.1007/s11250-010-9727-7.

Adesanoye, OA, Farombi, EO., 2010. Hepatoprotective effects of Vernonia amygdalina (astereaceae) in rats treated with carbon tetrachloride. Exp Toxicol Pathol, **62**(2):197–206. doi:10.1016/j.etp.2009.05.008

Adetunde, L. A., Ninkuu, V., and Sacky, I. (2017). Evaluating the antimicrobial potency of crude extracts of Psidium guajava bark, leaves of Vernonia amygdalina, Carica papaya and whole plant of Phyllanthus niruri against specific pathogenic bacteria. *African Journal of Bacteriology Research*, ***9***(3):15-20.

Adinew, B. (2012). Phytochemistry of turmeric: An overview. *Chemistry*, **21**(6).

Agbogidi, O. M., and Akpomorine, M. O. (2013). Health and nutritional benefits of bitter leaf (Vernonia amygdalina Del.). *Int JA PS. BMS Hetero Group J*, ***2*****,** 164-70.

Ahmad, R. S., Hussain, M. B., Sultan, M. T., Arshad, M. S., Waheed, M., Shariati, M. A., ... and Hashempur, M. H. (2020). Biochemistry, safety, pharmacological activities, and clinical applications of turmeric: a mechanistic review. *Evidence-Based Complementary and Alternative Medicine*, ***2020***(1), 7656919.

Al Namani, J., Baqir, E., Al Abri, A., Al Hubaishi, T., Husain, A., and Khan, S. A. (2018). Phytochemical Screening, Phenolic Content and Antioxidant Activity of Citrus aurantifolia L. Leaves Grown in Two Regions of Oman: Antioxidant activity and quantification of phenolics in Omani lime. *Iranian Journal of Pharmaceutical Sciences*, ***14***(1), 27-34.

Alabi, QK, Adeyemi, WJ., 2021. Vernonia amygdalina (Del) as an antioxidant, aspirin toxicity, and oxidative stress. Toxicology Pp 491–504. doi:10.1016/B978-0-12-819092-0.00048-0.

Al-Ameri, A., Almoneafy, A. A., Oshaish, A., Almaweri, A. A., Al-Sanabani, A., Mashrh, A., ... and Al-Dhaheri, M. (2024). The Influence of Ginger Aqueous Extract on Tonsillitis-Causing Streptococcus pyogenes Bacteria. *Albaydha University Journal*, ***6***(3).

Ali, M., Diso, S. U., Waiya, S. A., and Abdallah, M. S. (2019). Phytochemical screening and antibacterial activity of bitter leaf (Vernonia amygdalina). *Annals Microbiol. Infectious Dis*, ***2***(4), 01-07.

Alves-Barroco, C., Rivas-García, L., Fernandes, A. R., and Baptista, P. V. (2020). Tackling multidrug resistance in Streptococci–From novel biotherapeutic strategies to nanomedicines. *Frontiers in microbiology*, ***11***, 579916.

Ashraf, K. A. M. R. A. N., Sultan, S. A. D. I. A., and Shah, S. A. A. (2017). Phychemistry, phytochemical, pharmacological and molecular study of Zingiber officinale Roscoe: a review. *Int J Pharm Pharm Sci*, ***9***(11), 8-16.

Britannica, T. (2020). Editors of encyclopaedia. *Argon. Encyclopedia Britannica*.

Castro, S. A., and Dorfmueller, H. C. (2021). A brief review on Group A Streptococcus pathogenesis and vaccine development. *Royal Society open science*, ***8***(3), 201991.

Cattoir, V. (2016). Mechanisms of antibiotic resistance.

Chanda, S., and Ramachandra, T. V. (2019). Phytochemical and pharmacological importance of turmeric (Curcuma longa): A review. *Research and Reviews: A Journal of Pharmacology*, ***9***(1), 16-23.

Cheesbrough, M**.** (2006). District Laboratory practice in tropical Countries. 2nd Edition.Canbridge University press, Cambridge. Pp 434.

Chukwuemeka, NO, Oyebisi, SO, Adebisi Oluwadare, OJ, Oladapo, RS, Berka, NP., 2018. Antibacterial assay and reversion of carbon tetrachloride induced liver damage on wistar mice by Vernonia amygdalina. Delile. Pak J Pharm Sci **31**(4):1311–1321.

Clinical and Laboratory Standards Institute **(**2010) *Performance Standards for Antimicrobial Susceptibility Testing*, 20th informational supplement, M100–S20. Wayne, PA: Clinical and Laboratory Standards Institute Laboratory manual Pp. 1453.

Dadgostar, P. (2019). Antimicrobial resistance: implications and costs. *Infection and drug resistance*, Pp 3903-3910.

Debjit Bhowmik, C., Kumar, K. S., Chandira, M., and Jayakar, B. (2009). Turmeric: a herbal and traditional medicine. *Arch. Appl. Sci. Res*, ***1***(2):86-108.

Dégbé, M, Debierre-Grockiego, F, Tété-Bénissan, A, Débare, H, Aklikokou, K, DimierPoisson, I, Gbeassor, M., 2018. Extracts of Tectona grandis and Vernonia amygdalina have anti-

Toxoplasma and pro-inflammatory properties in vitro. Parasite **25**:11. doi:10.1051/parasite/2018014.

Degu, A, Kefale, B, Alemayehu, D, Tegegne, GT., 2020. Evaluation of the Antidiarrheal Activity of Hydromethanol Crude Extracts of Ruta chalepensis and Vernonia amygdalina in Mice. Evid Based Complement Alternat Med doi:10.1155/2020/8318713.

Dhanik, J., Arya, N., and Nand, V. (2017). A review on Zingiber officinale. *Journal of Pharmacognosy and phytochemistry*, ***6***(3), 174-184.

Dowe, E**.,** Ahonkhai, I., Ayinde, B.A. and Uwumarongie, H.O. (2016). Phytochemical and antimicrobial evaluation of the methanol stem extract and fraction of *Massularia acuminata* G. Don (Rubiaceae) against isolated odontopathogens. *Ewemen Journal of Microbial Research*. **2**(1):13-21.

Dumas, NGE, Anderson, NTY, Godswill, NN, Thiruvengadam, M, Ana-Maria, G, Ramona, P, Crisan, GC, Laurian, V, Shariati, MA, Tokhtarov, Z, Emmanuel, Y., 2020. Secondary metabolite contents and antimicrobial activity of leaf extracts reveal genetic variability of Vernonia amygdalina and Vernonia calvoana morphotypes. Biotechnol Appl Biochem doi:10.1002/bab.2017

Dyubeni, L., and Buwa, L. V. (2012). An ethnobotanical study of plants used for the treatment of ear, nose and throat (ENT) infections in Nkonkobe Municipality, South Africa. *Journal of Medicinal Plants Research*, ***6***(14), 2721-2726.

Enejoh, O. S., Ogunyemi, I. O., Bala, M. S., Oruene, I. S., Suleiman, M. M., and Ambali, S. F. (2015). Ethnomedical importance of Citrus aurantifolia (christm) swingle. *The Pharma Innovation*, ***4***(8, Part A), 1.

Erasto, P, Grierson, DS, Afolayan, AJ., 2006. Bioactive sesquiterpene lactones from the leaves of Vernonia amygdalina. J Ethnopharmacol **106**(1):117–120. doi:10.1016/j.jep.2005.12.016.

Erukainure, OL, Chukwuma, CI, Sanni, O, Matsabisa, MG, Islam, MS, 2018. Histochemistry, phenolic content, antioxidant, and anti-diabetic activities of Vernonia amygdalina leaf extract. J Food Biochem doi:10.1111/jfbc.12737.

Firas, A., Bayati, L., Sulaiman, R. and Khudir, D.S. (2008). *In vitro* antimicrobial activity of *Salvadora persica* extracts against some isolated oral pathogens in Iraq. *Turkish Journal of Biol*ogy **32**(409): 57-62.

Firenzuoli, F., and Gori, L. (2007). Herbal medicine today: clinical and research issues. *Evidence-Based Complementary and Alternative Medicine*, ***4***:37-40.

Habtamu, A, Melaku, Y., 2018. Antibacterial and Antioxidant Compounds from the Flower Extracts of Vernonia amygdalina. Adv Pharmacol Sci doi:10.1155/2018/4083736.

Harborne, J. B. (1973). *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall.

Hasibuan, P, Harahap, U, Sitorus, P, Satria, D., 2020. The anticancer activities of Vernonia amygdalina Delile. leaves on 4T1 breast cancer cells through phosphoinositide 3-kinase (PI3K) pathway. Heliyon **6** (7). doi:10.1016/j.heliyon.2020.e04449

Idu, M., Obaruyi, G. O., and Erhabor, J. O. (2008). Ethnobotanical uses of plants among the binis in the treatment of ophthalmic and ENT (ear, nose and throat) ailments. *Ethnobotanical leaflets*, ***2009***(4),9.

IfedibaluChukwu, EI, Aparoop, D, Kamaruz, Z., 2020. Antidiabetic, anthelmintic and antioxidation properties of novel and new phytocompounds isolated from the methanolic stem-bark of Vernonia amygdalina Delile (Asteraceae). Scientific African 10. doi:10.1016/j.sciaf.2020.e00578

Ilahy, R., Hdider, C., Lenucci, M. S., Tlili, I., and Dalessandro, G. (2011). Phytochemical composition and antioxidant activity of high-lycopene tomato (Solanum lycopersicum L.) cultivars grown in Southern Italy. *Scientia Horticulturae*, ***127***(3):255-261.

Im, E, Ae, A, Bn, U, Po, U., 2016. Immuno-modulatory properties of prebiotics extracted from Vernonia amygdalina. Afr J Tradit Complement Altern Med **13**(6), 11–17. doi:10.21010/ajtcam.v13i6.3.

Iwalokun, BA., 2008. Enhanced antimalarial effects of chloroquine by aqueous Vernonia amygdalina leaf extract in mice infected with chloroquine resistant and sensitive Plasmodium berghei strains. Afr Health Sci **8** (1), 25–35

Jyotirmayee, B., and Mahalik, G. (2022). A review on selected pharmacological activities of Curcuma longa L. *International Journal of Food Properties*, ***25***(1), 1377-1398.

Kanwal, S., and Vaitla, P. (2020). Streptococcus pyogenes.

Krup, V., Prakash, L. H., and Harini, A. (2013). Pharmacological activities of turmeric (Curcuma longa Linn): a review. *J Homeop Ayurv Med*, ***2***(133): 2167-1206.

Ilango, S., Sahoo, D. K., Paital, B., Kathirvel, K., Gabriel, J. I., Subramaniam, K., ... and Nirmaladevi, R. (2022). A review on annona muricata and its anticancer activity. *Cancers*, ***14***(18), 4539.

Lalitha, M. K. (2004). Manual on antimicrobial susceptibility testing. *Performance standards for antimicrobial testing: Twelfth Informational Supplement*, ***56238***: 454-456.

Lolodi, O, Eriyamremu, GE., 2013. Effect of methanolic extract of Vernonia amygdalina (common bitter leaf) on lipid peroxidation and antioxidant enzymes in rats exposed to cycasin. Pak J Biol Sci **16**(13): 642–646. doi:10.3923/pjbs.2013.642.646.

Makkar, H. A., and Becker, K. (1996). Nutrional value and antinutritional components of whole and ethanol extracted Moringa oleifera leaves. *Animal feed science and technology*, ***63***(1-4): 211-228.

Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M., and Kadir, H. A. (2015). Annona muricata (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. *International journal of molecular sciences*, ***16***(7), 15625-15658.

Moghaddasi, M. S., and Kashani, H. H. (2012). Ginger (Zingiber officinale): A review. *Journal of Medicinal Plants Research*, ***6***(26), 4255-4258.

Momoh, MA, Muhamed, U, Agboke, AA, Akpabio, EI, Osonwa, UE., 2012. Immunological effect of aqueous extract of Vernonia amygdalina and a known immune booster called immunace(®) and their admixtures on HIV/AIDS clients: a comparative study. Asian Pac J Trop Biomed **2**(3), 181–184. doi:10.1016/S2221-1691(12)60038-0.

Mukjerjee, S., and Karati, D. (2022). A mechanistic view on phytochemistry, pharmacognostic properties, and pharmacological activities of phytocompounds present in Zingiber officinale: A comprehensive review. *Pharmacological Research-Modern Chinese Medicine*, ***5***:100173.

Narang, N., and Jiraungkoorskul, W. (2016). Anticancer activity of key lime, Citrus aurantifolia. *Pharmacognosy reviews*, ***10***(20): 118.

Ngatu, NR, Okajima, MK, Yokogawa, M, Hirota, R, Takaishi, M, Eitoku, M, Muzembo, BA, Sabah, AB, Saruta, T, Miyamura, M, Kaneko, T, Sano, S, Suganuma, N., 2012. Anti-allergic effects of

Vernonia amygdalina leaf extracts in hapten-induced atopic dermatitis-like disease in mice. Allergol Int **61**(4): 597–607. doi:10.2332/allergolint.11-OA-0393

Omojokun, OS, Famurewa, AJ, Jaiyeoba, OA, Oboh, G, Agbebi, OJ., 2019. Alkaloid extracts from Bitter leaf (Vernonia amygdalina) and Black nightshade (Solanum nigrum) inhibit phosphodiesterase-5, arginase activities and oxidative stress in rats penile tissue. J Food Biochem. doi:10.1111/jfbc.12889.

Omoregie, ES, Pal, A., 2016. Antiplasmodial, antioxidant and immunomodulatory activities of ethanol extract of Vernonia amygdalina del. Leaf in Swiss mice. Avicenna J Phytomed **6**(2), 236–247.

Patel, V., and Patel, R. (2016). The active constituents of herbs and their plant chemistry, extraction and identification methods. *Journal of chemical and pharmaceutical research*, ***8***(4): 1423-1443.

Singleton, V. L., and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, ***16***(3): 144-158.

Shahrajabian, M. H., Sun, W., and Cheng, Q. (2019). Clinical aspects and health benefits of ginger (Zingiber officinale) in both traditional Chinese medicine and modern industry. *Acta agriculturae scandinavica, section b—Soil and Plant Science*, ***69***(6): 546-556.

Shittu, OB, Ajayi, OL, Bankole, SO, Popoola, TO., 2016. Intestinal ameliorative effects of traditional Ogi-tutu, Vernonia amygdalina and Psidium guajava in mice infected with Vibrio cholera. Afr Health Sci **16**(2): 620–628 10.4314/ahs.v16i2.33.

Teimoory, H., Azizi, M., Najafi, M. F., Behzadi, A., and Rezaei, M. (2013). Antibacterial activity of Myrtus communis L. and Zingiber officinale rose extracts against some Gram positive pathogens.

Toyang, NJ, Verpoorte, R., 2013. A review of the medicinal potentials of plants of the genus Vernonia (Asteraceae). J Ethnopharmacol **146**(3): 681–723. doi:10.1016/j.jep.2013.01.040

Tuesta-Monteza, V., Alcarazo, F., Mejiá-Cabrera, H. I., and Forero, M. G. (2020, August). Automatic classification of citrus aurantifolia based on digital image processing and pattern recognition. In *Applications of Digital Image Processing XLIII* **11510**: 60-72. SPIE.

Ugbaja, RN, Akinhanmi, TF, James, AS, Ugwor, EI, Babalola, AA, Ezenandu, EO, Ugbaja, VC, Emmanuel, EA, 2021. Flavonoid-rich fractions from Clerodendrum volubile and Vernonia amygdalina extenuates arsenic-invoked hepatorenal toxicity via augmentation of the antioxidant system in rats. Clinical Nutrition Open Science **35**, 12–25. doi:10.1016/j.nutos.2020.12.003.

Ugbogu, E. A., Emmanuel, O., Dike, E. D., Agi, G. O., Ugbogu, O. C., Ibe, C., and Iweala, E. J. (2021). The phytochemistry, ethnobotanical, and pharmacological potentials of the medicinal plant-Vernonia amygdalina L.(bitter Leaf). *Clinical Complementary Medicine and Pharmacology*, ***1***(1): 100006.

Ugbogu, E. A., Emmanuel, O., Dike, E. D., Agi, G. O., Ugbogu, O. C., Ibe, C., and Iweala, E. J. (2021). The phytochemistry, ethnobotanical, and pharmacological potentials of the medicinal plant-Vernonia amygdalina L.(bitter Leaf). *Clinical Complementary Medicine and Pharmacology*, ***1***(1): 100006.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and therapeutics*, ***40***(4): 277.

Verma, R. K., Kumari, P., Maurya, R. K., Kumar, V., Verma, R. B., and Singh, R. K. (2018). Medicinal properties of turmeric (Curcuma longa L.): A review. *Int. J. Chem. Stud*, ***6***(4): 1354-1357.

Wijesundara, N. M., Lee, S. F., Cheng, Z., Davidson, R., and Rupasinghe, H. V. (2021). Carvacrol exhibits rapid bactericidal activity against Streptococcus pyogenes through cell membrane damage. *Scientific reports*, ***11***(1): 1487.

Yeap, S. K., Ho, W. Y., Beh, B. K., Liang, W. S., Ky, H., Yousr, A. H. N., and Alitheen, N. B. (2010). Vernonia amygdalina, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. *Journal of medicinal plants research*, ***4***(25): 2787-2812.

Yusoff, SF, Haron, FF, Mohamed, TMM, Asib, N, Sakimin, SZ, Kassim, Abu, F, Ismail SI., 2020. Antifungal Activity and Phytochemical Screening of Vernonia amygdalina Extract against Botrytis cinerea Causing Gray Mold Disease on Tomato Fruits. Biology **9**(9): 286. doi:10.3390/biology9090286.