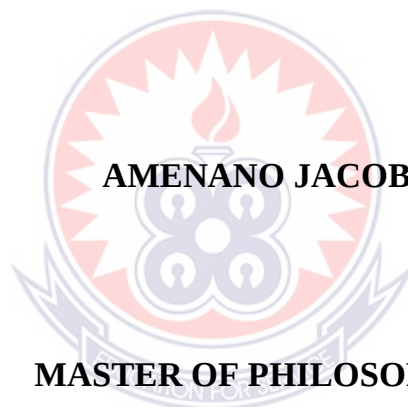


UNIVERSITY OF EDUCATION, WINNEBA

**GC-MS profiling and biological evaluation of ethyl acetate extract of the
bark of *morinda lucida* benth.**



AMENANO JACOB

MASTER OF PHILOSOPHY

UNIVERSITY OF EDUCATION WINNEBA

**GC-MS PROFILING AND BIOLOGICAL EVALUATION OF ETHYL
ACETATE EXTRACT OF THE BARK OF *MORINDA LUCIDA* BENTH.**



**AMENANO JACOB
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**A dissertation in the Department of Chemistry Education,
Faculty of Science Education, submitted to the School of
Graduate Studies in partial fulfillment
of the requirements for the award of the degree of
Master of Philosophy
(Chemistry Education)
in the University of Education, Winneba**

JUNE, 2024

DECLARATION

STUDENT'S DECLARATION

I Amenano Jacob, declare that this thesis, with the exception of quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my own original work, and it has not been submitted, either in part or whole, for another degree elsewhere.

Signature:

Date:

SUPERVISORS' DECLARATION

I hereby declare that the preparation and presentation of this work was supervised in accordance with the guidance for supervision of the thesis as laid down by the University of Education, Winneba.

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Date:

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I am overwhelmed with joy and gratitude as I finally complete this thesis. It has been a long and challenging journey, but the sense of accomplishment I feel today is truly exhilarating.

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To my lovely wife, Fortune Morkporkpor Adalevo, I thank you for all the encouragement

To my entire family, I extend my deepest appreciation for your love, support, and prayers.



DEDICATION

This work is first dedicated to my father Mr Francis K. Sawiri and Fortune Adalevo, my dearest wife. And also to my children, Jake Jr. Amenano-Sawiri and Lady Asieduwaah Amenano-Sawiri, for their prayers and support.



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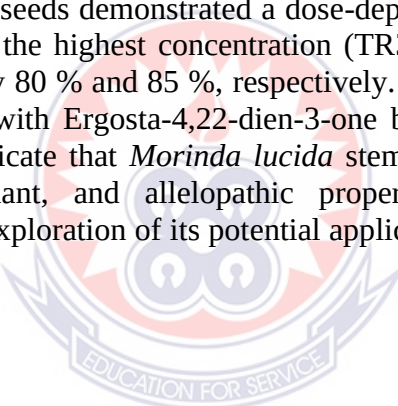


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ABSTRACT

This study investigates the allelopathic effects of *Morinda lucida* stem bark extract on seed germination, alongside its antimicrobial and antioxidant properties, as well as the Gas Chromatography-Mass Spectrometry (GC-MS) profiling of compounds found in the extract. The objectives included assessing antimicrobial activities against various microbial strains, evaluating antioxidant capacities using 1,1-diphenylpicrylhydrazyl (DPPH) and 2,2'-Azinobis-3-Ethylbenzothiazolin-6-sulfonic acid (ABTS) assays, conducting anti-germination bioassays, and profiling phytochemical compounds via Gas Chromatography-Mass Spectrometry (GC-MS). The antimicrobial evaluation, employing agar well diffusion and broth micro-dilution methods, revealed that the extract demonstrated bactericidal activity against *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli*, with minimum inhibitory concentrations (MICs) of 10 mg/mL and an MBC/MIC ratio of 2. *Phytophthora megakarya* had an Minimum Inhibitory Concentration (MIC) of 0.313 mg/mL and an MFC/MIC ratio of 2, indicating strong susceptibility and a fungicidal effect, meaning all the organisms were killed at this lower concentration. Antioxidant assays demonstrated a concentration-dependent raise in free radical scavenging, confirming potent antioxidant activity comparable to Vitamin C that was used as control. Phytotoxicity tests on *Lactuca sativa* seeds demonstrated a dose-dependent suppression of root and shoot development. At the highest concentration (TR3, 78.4 µg/µL), shoot and root lengths was decrease by 80 % and 85 %, respectively. GC-MS profiling identified 24 bioactive compounds, with Ergosta-4,22-dien-3-one being the most abundant (14.2 %). These findings indicate that *Morinda lucida* stem bark extract possesses strong antimicrobial, antioxidant, and allelopathic properties. The results provide a foundation for further exploration of its potential applications as a natural herbicide or in therapeutic contexts.



CHAPTER ONE

1.0 Overview

The knowledge of plants chemical constituents is key to the discovery of novel therapeutic values; hence the need for studies on plant phytochemical constituents cannot be over emphasized. All over Ghana and the world beyond, medicinal plant use in folk medicine keeps increasing by the day even as infectious diseases contribute significantly to high mortality rates judging from a global scale. Because of their availability, affordability, and indigenous knowledge, about 80 % of people in developing nations use folk medicines to treat a variety of illnesses. Although many studies have been done on the medicinal applications of African plants, some of these plants' therapeutic potential has not been fully realized (Okaiyeto et al., 2021). The therapeutic properties of plants have been used by mankind for centuries with zero or negligible signs of toxicity. These plants have been essential parts of indigenous traditional herbal medical practice and their role in primary health care systems can never be underrated in developing countries.

Also globally, weeds hinder crop production by competing for vital resources and by serving as reservoirs for pests and diseases, ultimately lowering yields (Oerke, 2006). With the global population projected to exceed 9 billion by 2050, minimizing crop yield losses from weed competition is essential (Chauhan, 2020). An estimated 854 million people worldwide suffer from chronic or acute malnutrition (Prendergast et al., 2014). Beyond poverty, factors such as limited knowledge in food production and preservation, along with pesticide use before and after harvest, contribute to food losses.

To prevent losses and maintain the superiority of foodstuff, synthetic pesticides are widely used in farming to control weeds, insect pests, plant diseases, and pathogens, thereby minimizing losses and preserving the quality of foodstuff (Damalas et al., 2011). Synthetic pesticides use provides certain environmental challenges. The fact that these chemicals damage species or organisms that are not intended targets is one area of serious concern. Because synthetic pesticides are frequently used throughout the whole agricultural field, >70% of synthetic insecticides and 95% of herbicides find themselves in locations other than the areas or species they are intended to control (Loewy et al., 2011). To manage weeds, agriculture in wealthy nations primarily uses synthetic herbicides. However, since the 1980s, herbicides with an outdated mode of action (MOA) have been developed. The persistent use of herbicides with similar MOA has led to 267 distinct species developing resistance towards their application (Hulme, 2023).

With more than 160 species, the United States (U.S.) is having most instances of herbicide resistance (Hulme, 2022). In addition, there are a number of other drawbacks associated with the frequent herbicide application that can present challenges, including high costs, risks of groundwater pollution from leaching and runoff, and complications in recycling irrigation water (Poudyal et al., 2019). A sustainable, eco-friendly instrument to control weeds must be created due to the evolution of herbicide-resistant weeds and other environmental problems associated with them. The use of plant-based natural molecules called allelochemicals to manage weeds is a wonderful area for finding such an alternative. These allelopathic substances have phytotoxic properties and may be used as natural pesticides to control some weeds.

Chemicals that are used to both attract and eliminate bugs are known as pesticides. Depending on the usage they are intended for; they come in a variety of shapes and sizes. According to Poudyal et al. (2019), pesticides include a variety of agents such as acaricides for controlling mites and ticks, algacides for managing algae, antiseptics for preventing microbial damage to materials, bactericides for eliminating bacteria, fungicides for controlling fungi, herbicides for managing weeds, insecticides for eradicating insects, and zoocides for controlling rodent populations. The word *pesticides* is so widely used that it is often treated as interchangeable with *crop protection products* (Melnikov, 2012).

An analysis on the use of pesticides revealed that 20,747 tons of pesticides were imported into Ghana in 2011 (9216 tons of insecticides, 8986 tons of herbicides, and 2545 tons of fungicides), according to (Guy Bertrand 2019). Yadav et al. (2015) noted that the persistent use of synthetic chemicals remains likely, with minimal attempts to reduce their impacts. Many of these substances degrade slowly, and in some cases, their breakdown products are even more toxic than the original compounds.

Pests, which include insects, mites, and other arthropods, nematodes, weeds, and vertebrates, are main limitations to crop productivity by directly hurting the crop and causing plant stresses that reduce production. Pre- and post-harvest losses worldwide are significantly worsened by pests, diseases, and weeds, leading to over 30% yield reduction. In Africa, about 54% of potential crop yield is lost annually, with diseases, insect/animal pests, and weeds contributing 16%, 20%, and 18% respectively (Junaid et al., 2024). Similar losses have been observed for Central and South America (48%) and Asia (Shiferaw et al., 2011). There is, however, the need to develop novel pesticides that will be benign to the environment and human health (Bouri et al.,

2023). Allelopathy refers to the production of secondary metabolites by plants, microorganisms, and algae that can affect the growth and functioning of other organisms within agricultural and natural systems (Zeng, 2014). Secondary metabolites are compounds that are not required for the growth or reproduction of an organism but are produced to confer a selective advantage to the organism.

Allelopathy describes the direct or indirect impact of plants on their immediate surroundings or on the microflora or microfauna that they are associated with through the generation of allelochemicals that impede plant growth (IAS, 2018). Allelochemicals produced by plants act as a protective strategy, helping them resist microbial infections, deter herbivores, and compete with neighbouring plants (Kong et al., 2019). Following release, the allelochemicals may inhibit the germination, growth, and establishment of nearby plants or alter the rhizosphere's soil characteristics by modifying the microbial community (Zhou et al., 2013). Due to the fact that many plants produce chemicals that affect the growth and development of other plants, allelochemicals are used selectively for protecting other plants from the weeds they suppress. The impact on the surrounding plants' growth can be either positive or negative. The Greek terms *allelon*, which means mutual or together, and *pathos*, which means to suffer or feel, are used to create the phrase allelopathy. Although the term “allelopathy” was introduced by Austrian scientist Hans Molisch in 1937, the concept of chemical interactions among plants has been recognized for thousands of years (Tomes, 2013). However, a recent advancement in research is creating synthetic herbicide based on this theory, in which those chemicals are classified as derived from natural products. The discovery of plant materials with abundant sources, affordable prices, and significant allelopathic effects will undoubtedly have significant ecological effects as a biological herbicide.

Traditionally, allelopathy research focused on evaluating the phytotoxic effects of plant residues or crude extracts. Recent advancements in separation and structural analysis now enable the identification, isolation, and characterization of active compounds (Scavo et al., 2020). Secondary metabolites are made in microbes during decomposition or by plants as secondary metabolites. Based on their similarities in chemical composition, allelochemicals are divided into 14 types. (Rice, 2012). Water soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones are among the 14 categories, as are simple unsaturated lactones, long-chain fatty acids, polyacetylenes, benzoquinone (**1.0**), anthraquinone (**1.1**), and complex quinones (**1.2**). Other categories include simple phenols (**1.3**), benzoic acid (**1.4**) and its derivatives, cinnamic acid and its derivatives, tannins (**1.5**), terpenoids (**1.6**), and steroids (**1.7**). (Cheng & Cheng, 2015). Figure 1.0 shows these structures.

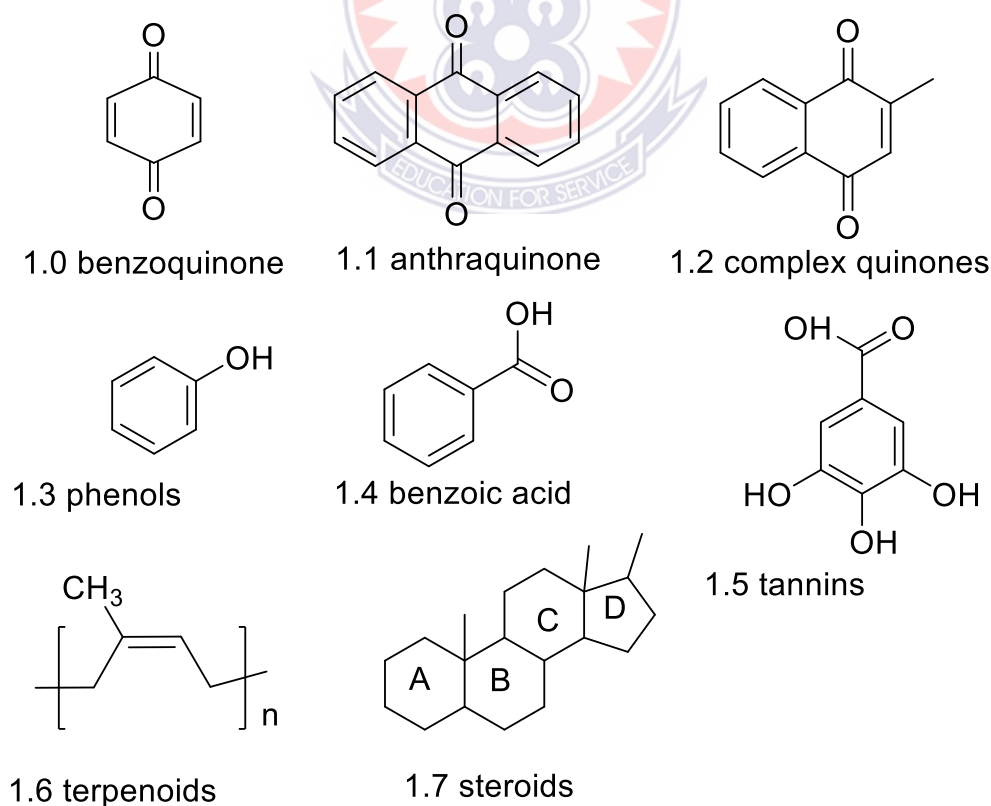


Figure 1.0 shows the general structures of compounds mentioned in text.

Although the efficacy and specificity of many secondary metabolites remain uncertain or limited, they present a viable alternative to synthetic herbicides.

1.10 Background to the Research

The control of weeds has been a challenge since man turned to farming instead of hunting. Since prehistoric times, peasant farming has been an integral part of human culture (Ioris, 2019). Through his own judgment, humans have chosen specific plant species from the thousands available in nature to serve as food crops and rely on them for sustenance (Meyer et al., 2012). Monoculture practice is essentially making competition for water, nutrients, and light very intense. Weeds which form part of nature make these competitions very intense (Arnold, 2020). The situation merits the development of farm inputs to enable larger production and ease the labouring of man. In the 19th century, farming systems mainly relied on crop rotation and manual hoeing to control weeds. Weed removal was essential not only to reduce competition with crops but also to prevent slower and less efficient harvesting, avoid poisoning from toxic weed seeds, reduce contamination of crops like oats that could lower their quality and market value, and eliminate potential reservoirs for plant pathogens (Alhassan, 2017). Urbanization and the expansion of farming reduced the effectiveness of traditional manual weed control, leading to the development of chemical solutions. Herbicides, or herbicide, are chemicals used to eliminate or suppress weeds and are classified as either selective—targeting specific species without harming desired crops—or non-selective, which destroy all vegetation ("Pest Box," 2021). While herbicides are commonly associated with agriculture, they are also widely used in non-agricultural settings such as parks, gardens, lawns, and railway areas to remove weeds (Mesnage et al., 2021).

The use of natural products in weed management offers a promising alternative to address the environmental and health risks linked with synthetic chemicals. This study focuses on exploring the potential of the plant *Morinda lucida* in this regard. The plant belongs to the family Rubiaceae. Several studies have been conducted on this plant with several researchers reporting several biological activities of the plant. Its stem bark extracts in Nigeria were experimented for antifungal, antibacterial and antimalarial properties (Oladeji et al., 2022). The plant is readily available throughout the year in south-western Africa. It contains secondary metabolites which perform as antibiotic, antiviral, anti- plasmodial and anti- parasitic (Adeleye et al., 2018).

The toxicity profile of *Morinda lucida* remains largely unestablished; however, one study indicates that high doses of its extract can be safely administered. Additionally, it possesses insecticidal, antifungal, and antimicrobial properties (Bezerra et al., 2020), and however, no research has reported the use of this plant as source of allelopathic agent which can serve as boost to the development of weed control agent.

1.20 Problem Statement of the Research

Weed control has been a persistent challenge as at the dawn of agriculture, with unmanaged weeds causing larger yield losses than any other pest. While synthetic herbicides is the primary solution, their environmental impact has prompted the search for alternatives. Plant-based weed control, using natural compounds—often secondary metabolites with unique mechanisms of action—is gaining attention. These natural products are more likely to exhibit biological activity than randomly developed synthetic chemicals and often degrade quickly in the environment, making them more eco-friendly (Mesnage et al., 2021; Li et al., 2015; Lengai et al., 2020).

1.30 Justification for Study

High-input farming methods—relying on synthetic fertilizers, irrigation, pesticides, herbicides, and improved crop varieties—have boosted global food production (Tudi et al., 2021). However, these advancements have also driven large-scale land conversion, biodiversity decline, ecosystem damage, and health concerns from chemical use. Currently, agriculture occupies about 40% of the world's arable land, a figure expected to grow with rising population and food demand (Tanentzap et al., 2015). Residues of these synthetic pesticides sometimes are found in our water bodies due to leaching. The persistent uses of these chemicals have resulted in some weeds developing resistance towards them. Research indicates that only about 0.3% of applied pesticides reach the intended pest, with the vast majority dispersing into the environment (Freidenreich, 2016). The indecent use of synthetic pesticides creates a circumstance where the bulk of chemical substance gets in touch with non-target organisms of which humans are part. There is therefore the need to find and develop alternative weed management tools to curtail these problems we are faced with hence the current study.

1.40 Aim and Objectives

1.5 Aim

The primary purpose of this study is to investigate the antimicrobial, antioxidant, and phytotoxic properties of the ethyl acetate crude extract of the stem bark of *Morinda lucida*, and to identify the bioactive compounds responsible for these effects using Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

1.60 Objectives

The objectives of the study include:

1. To determine the antimicrobial activities of *Morinda lucida* stem bark extract against a range of bacterial and fungal microbes.
2. To determine the antioxidant activities of *Morinda lucida* using DPPH and ABST
3. To examine the phytotoxic effects of *Morinda lucida* extract on seed germination and seedling growth.
4. To identify and characterize the bioactive compounds in the extract using GC-MS.

1.7 Significance of the study

The use of natural products in weed management offers a promising alternative to address the ecological and health concerns linked with synthetic chemicals. This study focuses on exploring the potential of the plant *Morinda lucida* in this regard. The significance of the study lies in its potential to contribute to the development of environmentally safe, plant-based herbicides. By identifying bioactive compounds with phytotoxic properties, this research may provide a foundation for sustainable agricultural practices, reduce dependency on harmful agrochemicals, and open new avenues in natural product research for both agricultural and medicinal applications.

1.8 Organization of the Thesis

This thesis comprises five chapters. Chapter 1 provides an overview and background to the study, including its aims and objectives, and significance of the study. Chapter

2 reviews relevant literature, while Chapter 3 details the materials and methods employed. Chapter 4 presents the results and discussions, and Chapter 5 concludes the study.



CHAPTER TWO

LITERATURE REVIEW

2.0 Overview

This chapter presents a comprehensive review of literature relevant to the current research, with the aim of establishing a solid theoretical foundation and highlighting the study's significance. It summarizes existing knowledge on *Morinda lucida*, its antimicrobial and antioxidant activities, the herbicidal potential of plant-based compounds, common extraction techniques in natural product chemistry, and the instrumentation used for the characterization of natural compounds

2.1 Herbicide

Herbicides are farm input used to destroy weeds. They can be classified as **selective**, which target specific plant species without harming desired crops, or **non-selective**, which eliminate all vegetation (Britannica, 2019).

Weeds have long disrupted human activities, especially agriculture, and are a leading cause of crop losses worldwide, surpassing damage from animals and pathogens (Trejo-Téllez et al., 2024). The advent of the "chemical era" in herbicide development began during World War II. Today, herbicides are the most widely produced pesticides, making up 47.6% of global sales, followed by insecticides (29.4%), fungicides (17.5%), and others (5.5%) (Quandahor et al., 2024). Managing weeds have been a challenge ever since agriculture first emerged. More than any other agricultural pest, uncontrolled weeds may significantly lower crop output (Alhassan, 2017). The historical evolution of herbicides is critical to understanding their current applications. Herbicides, agrochemicals designed to hinder plant growth, have

evolved to become indispensable tools for various sectors including agriculture, landscaping, and government initiatives. Notably, they replace labour-intensive weeding methods and enhance efficiency in managing unwanted vegetation.

2.1.1 History of Herbicides

Herbicides have a long history that dates back to the beginning of farming. Historically, humans used a significant amount of work clearing weeds from agricultural land in order to provide the ideal environment for the best possible development of desired crops. The farmers of the time began to think about weed management or control at the same time. The use of herbicidal substances has roots in ancient agricultural practices. Early civilizations, such as the Greeks and Romans, employed various techniques to control weeds, including manual removal and burning (Woyessa, 2022). Traditional herbicidal remedies often relied on naturally occurring substances, showcasing an early awareness of the need for weed control.

Initially, weeds were removed by hand, but around 6000 B.C., primitive tools replaced bare-hand weeding. By 1000 B.C., animals like oxen and horses were used to pull harrows, and in 164 B.C., the Romans began applying salt to eliminate weeds on farmland (Vats, 2016). English farmers in sixteenth century used salt also to selectively kill thistle in wheat fields and remove weeds from garden paths (Vats, 2016).

Chemicals have long been used as herbicides. To get rid of weeds from railway tracks, vehicle highways, and lumber yards, people have used crude chemicals such as rock salts (Vats, 2016). These herbicides are non-selective since they are used to kill all plants. Due to their negative effects on agricultural plants, these chemicals could not be employed in agricultural fields. This led to the development of selective

herbicides that only specifically destroy weeds. Development of selective herbicides was based on growth regulator (Gupta et al., 2023).

Early botanists focused heavily on studying plant growth. Notably, Darwin in England and Boysen-Jensen in Denmark conducted key experiments on oat coleoptiles to investigate phototropism (Holland et al., 2009).

In 1993, France used DNOC, a dinitro compound contact herbicide, for selective control of annual weeds, effectively managing them without significantly harming cereal crops (Vats, 2014). The effectiveness of these herbicides convinced scientists and businesses to invest time, money, and resources in ongoing herbicide-related research. Glyphosate, one of the most widely known herbicides of the new millennium, is perhaps the best option. It was synthesised first several decades prior to the discovery of its herbicide action (Werner et al., 2021).

2.1.2 Classification of Herbicides

The agrochemical used to stop or obstruct typical plant growth and development. The following group of workers also rely on the use of herbicides, railroad companies, landscapers, green keepers, governments, and gardeners (Mesnage et al., 2021). The application of herbicides, target the physiological processes of weeds which lead to their death or improper growth (Parven et al., 2024).

Herbicides can be categorized or classified in a number of ways, including activity, chemical family, application technique, location of action, and time. Herbicides are also classified as pre-emergent or post-emergent depending on when they are applied. Pre-emergent chemicals stop the germination and early growth of weed seeds and are typically applied to the soil. Since they would also prevent crop germination, they

must be used before planting. **(2.0)** Dithopyr and **(2.1)** Pendimethalin are Pre-emergence herbicides (Vats, 2014). The other category based on herbicide application is the selective and nonselective nature of some of them. The selective herbicides are applied to selectively eliminate weeds while the desired crop grows. The nonselective herbicides on the other hand, kill weeds including desired crops.

Post-emergent herbicides are applied after crops have emerged and are designed to selectively target weeds, typically focusing on either monocot species like Bermuda grass and quack grass or dicot species such as ragweed and thistles (Mesnage et al., 2021). The features of herbicide's absorbance are also used to classify it. Contact herbicides only destroy the plant tissue that they come into contact with (the leaves) after absorbing them not after becoming translocated from there. They therefore only have a temporary impact on weed control and are less effective against perennial weeds that might reappear from their roots, tubers, or rhizomes for example, glufosinate-ammonium **(2.2)** and paraquat **(2.3)** (Mesnage et al., 2021).

Contrary to contact herbicides, systemic herbicides have to be absorbed by the weed and transported by mechanisms which may occur by rising with the xylem to the leaves, shoots, and flowers (acropetal) and/or downward with the phloem to the roots (basipetal). Systemic herbicides are more effective against perennials because their phytotoxic effects are typically (but not always) slower than those of contact herbicides (Barbaś et al., 2023). Examples are glyphosate **(2.4)**, atrazine **(2.5)**, dicamba **(2.6)** and 2,4-D **(2.7)**.

Herbicides are also conveniently categorized based on their mode of action (MOA), and their application ("Herbicides, Physiology of Action, and Safety," 2015).

Herbicides are frequently branded depending on their site of action, since herbicides in the same site of action class typically create similar effects in plants that are vulnerable to them (Vats, 2014).

Classifying herbicides by their site of action is considered more effective for managing herbicide resistance. Each site-of-action class is assigned a group number, while the International Herbicide Resistance Action Committee (HRAC) also uses letter-based groupings (Forouzesh et al., 2015).

Examples of some of the classes of herbicide are, **(2.8)** Imidazolinone, **(2.9)** Sulfonylurea, **(2.10)** Triazoloprimidine, **(2.11)** Pyrimidinylthio. The chemical structures of the compounds mentioned above are.



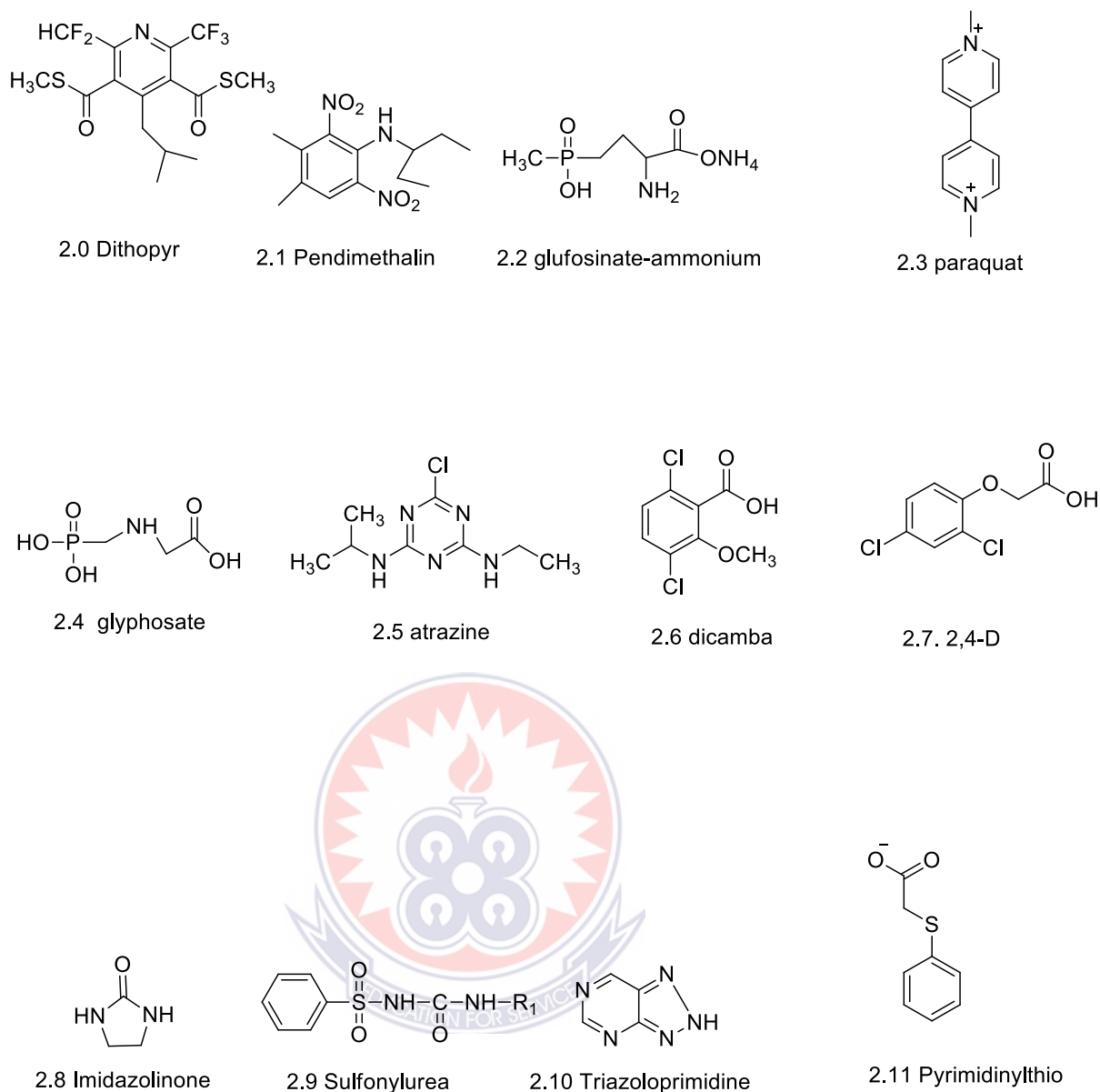


Figure 2.0 shows the general structures of compounds mentioned in text.

2.2 Review on the Significance of Natural Products Derived Herbicides.

The rising global food demand led to the creation and extensive use of synthetic pesticides for rapid pest and disease control. However, overreliance on these chemicals poses risks to the environment, human health, and contributes to the development of resistant pests and pathogens (Nicolopoulou-Stamati et al., 2016). Nature provides the largest chemical laboratory which houses all the needs of man.

Natural products which are from nature provide us with diverse opportunity to develop hit and also find leads toward new herbicides discovery (Chaachouay et al., 2024). There are considerable advantages associated with the use of natural products as herbicides.

Secondary metabolites from living organisms, shaped by biotic interactions, often exhibit biological activity even at low concentrations, making them more potent than many synthetically derived compounds (Moh et al., 2023).

Biological activities of most natural product compounds are premised on to developing new principles (Atanasov et al., 2021). Most drugs and active principles were discovered based on this principle. Researchers have explored the possibilities of engaging nature with this approach owing to the fact that many natural products are yet to be discovered. The most in the field of agriculture has been phytotoxicity. Examining a huge number of undiscovered secondary metabolites that are likely to have biological activity is striking (Mathur et al., 2023). Natural products are known to exert diverse functionalities also easy availability makes them affordable, allowing for seamless integration into agricultural production systems (Atanasov et al., 2015). This gives them a comparative advantage over synthetic chemicals since it's difficult for plants/organism to develop resistance towards them (Cheng & Cheng, 2015). The few natural product derived commercial herbicides (2.12) triketones, (2.13) cinmethylin, (2.14) bialaphos and (2.15) glufosinate introduced molecular target sites that had not been previously utilized. Literature reviews indicate that phytotoxic natural products affect many herbicide target sites that remain largely unexploited (Dayan et al., 2014)

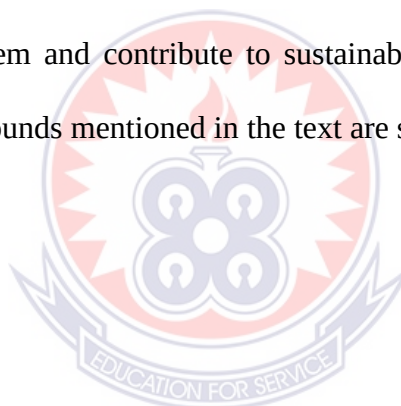
The need to develop or find new molecules that would target new sites calls for researchers to explore nature which houses diverse compounds in living organisms as alternative. The diversity of molecular structures from living sources offers new structures that are impossible to be produced synthetically (Schenone et al., 2013).

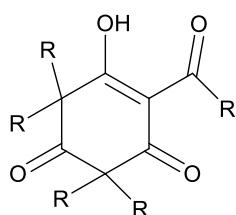
Because natural products are derived from nature, it is thought that because they are ecologically benign, using them will not harm the environment or people. Researchers believe that these compounds possess shorter half-life and are part of nature. In fact, certain natural compounds rank among the most powerful toxins affecting mammals (Sörqvist et al., 2019). Examples of natural product-based herbicides and their corresponding trade names include, (2.16) Artemisinin, (2.17) Dehydrozalanin C, (2.18) Leptospermone, (2.19) Cinmethylin, (2.20) 1,4-cineole, (2.21) AAL Toxin, (2.22) Hydrantocidin etc.

Herbicidal derivative of 1,4-cineole, was used in Europe and Asia. Shell Chemicals made the discovery and developed this chemical into herbicide (Campe et al., 2018). The cineole ring appears to become much less volatile after being substituted with benzyl ether, making it more suitable for use as herbicide and for the management of monocotyledonous weeds, cinmethylin is a growth inhibitor with a modest level of effectiveness (Sołtys et al., 2013). Although these herbicides have been studied for many years in both commercial and experimental settings, its precise mode of action is still unknown and laboratory work has indicated that 1,4- cineole has being a strong inhibitor of an enzyme involved in amino acid biosynthesis (He et al., 2022). A number of the more phytotoxic substances originating from plants include diterpene lactones, such chaparrinone and artemisinin (Sülsen, 2021).

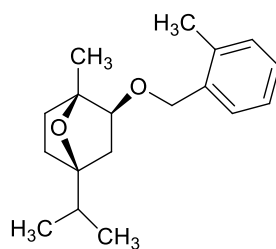
Due to the detection of harmful pesticide residues in food and increased public concern over safety, some chemical pesticides have been banned, leading to a growing adoption of plant-based pesticides in organic farming. Also, prolonged reliance on synthetic pesticides has led to issues like pollution, health risks, and biodiversity loss, whereas using botanical pesticides promotes environmental health and supports sustainable farming (Lengai et al., 2020).

Natural product pesticides are effective against various crop pests, cost-efficient, readily biodegradable, widely available, and generally safe for non-target organisms. Their diverse modes of action stem from the unique phytochemical compositions of different plants (Saravanan, 2022). Therefore, they can be incorporated into integrated pest management system and contribute to sustainable agricultural production. The structures of the compounds mentioned in the text are shown in figure 2.1.

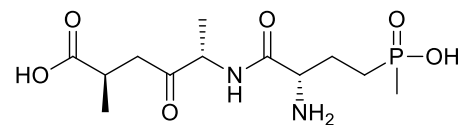




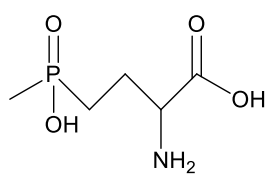
2.12 triketones



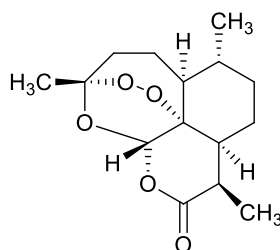
2.13 cinmethylin



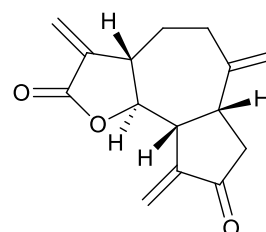
2.14 bialaphos



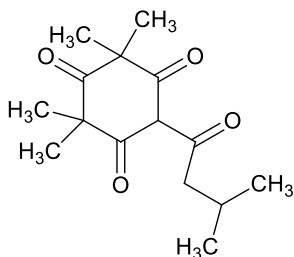
2.15 glufosinate



2.16 Artemisinin



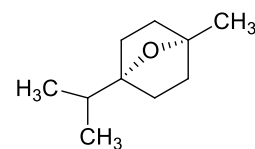
2.17 Dehydrozalanin C



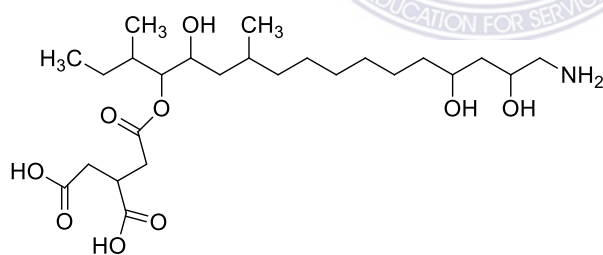
2.18 Leptospermone



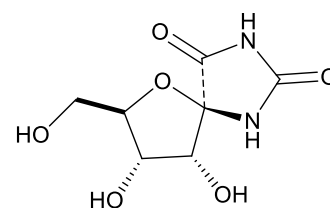
2.19 Cinmethylin



2.20 1,4-cineole



2.21 AAL Toxin



2.22 Hydrantocidin

Figure 2.1 shows the general structures of compounds mentioned in text

2.3 *Morinda lucida*

Morinda lucida Benth is a tree with a height of about 3 m – 23 m and native to Western Africa, it is widely dispersed throughout the tropical and subtropical regions like Ghana, Nigeria, Senegal and Congo (Adewole et al., 2021). *Morinda lucida* is an ethno pharmacologically vital plant that has traditionally been used to treat malaria in Ghana and other African countries (Oladeji et al., 2022).

Morinda lucida is an evergreen tree with bent branches and dark, glossy leaves on its upper surface. The reason for the name "brimstone tree" is the golden color of the wood (Adewole et al., 2021). It is a widely used medicinal plant in African traditional medicine, commonly found in tropical areas of Central and West Africa (Letsyo et al., 2023).

Despite its extreme bitterness, the entire plant, including its leaves, stem, stem bark, and roots, are believed to provide therapeutic benefits (Abubakar et al., 2022). The numerous health benefits of *Morinda lucida* are attributed to its rich content of vitamins A, K, and E, along with alkaloids and potent antioxidant phytochemicals such as flavonoids, which possess free radical scavenging, anti-allergic, anti-inflammatory, antiviral, anti-proliferative, and anti-carcinogenic properties (Joshua et al., 2018). Its phenolic compounds also contribute to anti-aging, cardiovascular protection, anti-atherosclerosis, improved endothelial function, and the inhibition of angiogenesis and cell proliferation (Abu et al., 2016). Alkaloids found in the plant are among the most important types of phytochemicals, commonly used as anti-parasitic agents. The plant also contains steroids, which exhibit antibacterial and anti-plasmodial properties (Kuete, 2023). Numerous classes of bioactive compounds have been identified from *Morinda* plants, and the study shows that various parts of the

plants have been widely used for folkloric therapeutic purposes. There are also numerous nutritional or mineral benefits and secondary metabolites (Oladeji et al., 2022). *M. lucida* extracts were more effective against all tested bacteria than the common antibiotics, chloramphenicol and ciprofloxacin, even at the same concentrations of 5, 10, and 20 mg/mL in an in vitro experiment using the agar well diffusion and disc diffusion methods with Gram-negative enterobacteria (Fakoya et al., 2014). In other studies, *Amaranthus spinosus* and *Amaranthus hybridus* growth was negatively impacted by *M. Lucida* leaf extract, with the effect being substantially greater at higher extract concentrations (Okeke et al., 2015).

2.3.1 Classification of *Morinda lucida*

Morinda lucida, commonly called the Brimstone tree, is a medium-sized evergreen tree which belongs to kingdom plantae, phylum tracheophytes, class Magnoliopsida, order Gentianales, family *Rubiaceae*, and is of the *Morinda lucida* genus which is the most diverse genus classified in classification of plants (Tindall, 2019).

Morinda lucida is commonly known by various names across West Africa: as Oruwo in Nigeria, Atak ake or Ewe amake in Togo, Ewi or Konkroma in Ghana, and Sagogo or Bondoukou alongua in Ivory Coast (Oladeji et al., 2022). Compounds such as anthraquinones, glycosides, alkaloids, terpenoids, iridoids, fatty acids, essential or volatile oils, tannins and flavonoids have been reported in *M Lucida* (Oladeji et al., 2022). Sub-Saharan Africa hosts about 1,900 *Rubiaceae* species across 164 genera, with more than half being native to the region (Adewole et al., 2021).

2.3.2 Ethno-Medicinal Uses of *Morinda lucida*

The study of traditional facts, knowledge and healing methods of treatments for jaundice, prevention and cure of diseases among ethnic tribal and rural populations is known as ethnomedicine (Raghuvanshi, 2021). The ethnic or tribal society has relied on the indigenous flora for medicinal and other uses from the beginning of human existence. Based on their experiences keeping themselves well, rural and tribal groups' indigenous beliefs and practices are represented in the traditional medicine system (Burnside et al., 2023). Drug-resistant pathogens are becoming more common, so it is critical to find and separate new bioactive compounds from medicinal plants using standardized, contemporary analytical techniques. Compounds derived from medicinal plants may offer new, simple strategies to combat harmful bacteria (Vaou et al., 2021).

Ethnomedicinal plants have been crucial in drug discovery and production, with research indicating that nature provides 60% of current cancer drugs and 75% of anti-infective agents (Khan et al., 2022; Mandhata et al., 2023). The WHO estimates that about 65% of the global population—and 80% in developing nations—rely on plants for primary healthcare, while they also indirectly contribute to healthcare in developed countries (Potet et al., 2021). *Morinda lucida* Benth, a member of the Rubiaceae family, is among the most important and widely utilized ethnomedicinal plants in sub-Saharan Africa. *Morinda lucida* exhibits multiple modes of action, including repellence, protein inhibition, and denaturation, which vary with the type of botanical compound and target pest. Plants containing such bioactive compounds have been successfully used to control various crop pests and treat human infections (Dah-Nouvlessounon et al., 2023).

Morinda lucida has been effectively used as a source of secure pesticides for controlling insect and pests (Rahmatullah et al., 2013). Management of pests using plant-based products was practised over time until technology took over and synthetic pesticides were developed (Brunner, 2014).

The secondary metabolites present in crude extracts, fractions or isolates of Brimstone tree includes, anthraquinones found in wood and stem bark, the red colorants 1-methylether-alizarin, rubiadin, ucin, soranjidiol, damnacanthal, nordamnacanthal, morindin, munjistin and purpuroxanthin, two anthraquinols, oruwal and oruwalol, have also been found; these give a yellow colour and in addition to anthraquinones, tannins and flavonoids have also been isolated (Dah-Nouvlessounon et al., 2023). The bioactive compounds in *Morinda lucida* may account for its diverse properties, including anti-diabetic, antimicrobial, anti-trypanosomal, anti-inflammatory, antioxidant, antipyretic, and anti-plasmodial effects across different plant parts (Sarkar et al., 2021). One of the most prevalent classes of phytochemicals, phenols are thought to be natural antioxidants that function as radical scavengers to reduce the likelihood of oxidative stress-induced damage to big biological molecules like proteins, DNA, and lipids (Ghasemzadeh et al., 2018). Multiple studies have investigated the antiplasmodial properties of the leaves, roots, and stem bark of the Brimstone tree (*Morinda lucida*) (Oladeji et al., 2022). *Morinda lucida* is widely used in Nigeria to treat fever and other ailments such as malaria, diabetes, hypertension, ulcers, and infections (Agyare et al., 2013).

Phenolic compounds, naturally found in higher plants, are well known for their antioxidant properties and have been used to inhibit pathogenic bacterial growth and biofilm formation. They also serve as a source for discovering new therapeutic agents

(Canli et al., 2023). Research on Asian and Pacific Angiosperms has revealed several phenolic compounds with in vivo antibacterial activity (Sulaiman et al., 2024). For instance, oral administration of ethyl gallate at 50 mg/kg/day increased the survival rate of *S. typhimurium*-infected mice by over 70% (Choi et al., 2014). Notably, phenolic compounds often exhibit multiple pharmacological effects, including antibacterial, anti-inflammatory, and immunomodulatory properties. The root bark of *Morinda lucida* is traditionally used to produce a scarlet red dye. Among the Ashanti people of Ghana, the root and stem bark are used to dye cotton cloths, known as "kobene," which are worn as mourning attire by officials and the bereaved family during periods of national mourning or the death of a chief (Forster, 2013).

2.3.3 Phyto chemicals in *Morinda lucida*

Phytochemicals are natural plant compounds that aid physiological functions and promote health (Kumar et al., 2023). Phytochemicals substances are vital for shielding the plant from infections and environmental stresses, but they are not necessary for the plant's growth or development (Rabizadeh et al., 2022). Phytochemicals offer health benefits when consumed and have been utilized in medicinal plants for treating microbial diseases, a practice documented since ancient times (Matotoka et al., 2023).

Morinda lucida is rich in phytochemicals and nutrients, comprising ten anthraquinones as well as alkaloids, tannins, flavonoids, saponins, glucosides, and triterpenoids, tannins, phenolics and organic acids have been isolated (Adeleye et al., 2018). These classes represent only a fraction of the diverse array of phytochemicals found in plants.

Each class encompasses a variety of compounds with unique structures and functions. The health benefits associated with phytochemicals include anti-oxidant activity, anti-inflammatory effects, anti-microbial performance and potential protection against chronic diseases, such as cancer and cardiovascular diseases (Kumar et al., 2023).

Research on *Morinda lucida* revealed notable differences in phytochemical levels across plant parts, with tannin content measuring 1.49 mg/100 g in the leaves (Kumar et al., 2023) and 1.37 mg/100 g in the stem bark (Abu et al., 2016).

Flavonoids are a broad class of plant-derived phytonutrients present in nearly all fruits and vegetables. Together with carotenoids, they contribute to the vibrant colours of these foods and represent the largest group of plant phytonutrients, with over 6,000 identified types (Panche et al., 2016). Glucosides, which contain an aglycone derived from anthraquinone, exhibit laxative properties. Saponins are naturally occurring plant glycosides—phytochemicals with soap-like characteristics—that can produce foam when mixed with water (Ashour et al., 2019).

Terpenoids, when consumed regularly, may help manage metabolic disorders such as diabetes. Phenolic compounds, abundant in plants, often possess antioxidant properties, enabling them to neutralize harmful free radicals before these reactive molecules can damage vital biomolecules (Siddiqui et al., 2024). Research on the wood and stem bark of *Morinda lucida* has identified 18 anthraquinones, including 1-methyl etheralizarin, rubiadin and its derivatives, lucidin, soraanjidiol, damnacanthal, nordamnacanthal, morindin, munjistin, and purpuroxanthin (Siddiqui et al., 2024). Quinazolinones and their derivatives constitute an important class of heterocyclic compounds occupying an important position in medicinal chemistry. Natural Quinazolinone has been shown to exhibit various pharmacological activities, which

primarily include anti-cancer, anti-inflammatory, anti-fungal, anti-hyperlipidemic, anti-convulsant, anti-malarial and anti-microbial activities. The naturally occurring quinazolinones are medicinally and pharmaceutically important (He et al., 2017).

Studies have shown that crude leaf extracts of *Morinda lucida* adversely affect the germination and growth of *Amaranthus spinosus* and *Amaranthus hybridus*, with stronger effects observed at higher extract concentrations. These extracts significantly reduced leaf number, leaf area, stem height, and both fresh and dry weights of the plants compared to the control (Okeke et al., 2015; Huang et al., 2019). Much is not known about the allelopathy effect of the stem bark of the tree; hence this research seeks to bridge that gap.

2.40 Extraction Methods in Natural Products Chemistry

In natural products chemistry, extraction methods are crucial processes employed to isolate and obtain bioactive compounds from various natural sources such as plants, fungi, bacteria, and marine organisms (Zhang et al., 2018). These compounds often have therapeutic, medicinal, or industrial significance. The extraction methods play a pivotal role in the isolation of these compounds by separating them from the complex matrix of the natural source. Extraction is the process of selectively removing a substance from a mixture using a suitable solvent or method. In natural products chemistry, extraction methods involve the separation of bioactive compounds from the raw natural material, which may contain a complex mixture of chemicals (Nam et al., 2015). The goal is to obtain a concentrated extract enriched with the desired compounds.

Since the discovery of fire, natural product extraction has most likely been exploited (Asiamah et al., 2023). Innovative extraction methods developed by ancient

civilizations—including the Egyptians, Phoenicians, Jews, Arabs, Indians, Chinese, Greeks, Romans, Mayas, and Aztecs—were applied for producing food, medicines, and perfumes (Shankar et al., 2022).

In the perfume, cosmetic, pharmaceutical, agricultural, biofuel, and fine chemicals sectors today, there is not a manufacturing process that does not involve extraction methods such as maceration, microwave extraction method, Soxhlet extraction, decoction, infusion etc (Uahomo et al., 2023). Recent extraction methods aim to reduce solvent use while ensuring efficient, cost-effective production of high-quality extracts (Rapinel et al., 2017). Technological breakthroughs that split from the past are needed to meet the difficulties posed by the competitiveness of the worldwide market and environmental fortification agencies (Ezeigweneme et al., 2024). Extraction processes should be developed with a focus on minimizing energy use, utilizing alternative and reusable solvents, relying on renewable natural resources, and ensuring that the resulting extract or product is both safe and of high quality (Rapinel et al., 2017).

Significance of extraction methods in Natural products chemistry includes, Isolation of bioactive compounds such as alkaloids, flavonoids, terpenoids, and polyphenols, which have pharmacological activities. Extraction methods help in isolating these compounds in a pure and concentrated form (Touahria et al., 2022). The methods facilitate the analysis of Phytochemical compounds, helping researchers identify and characterize the chemical constituents of plants (Devanesan & AlSalhi, 2023). Extracts obtained through extraction methods are used in biological studies to understand the mechanisms of action of bioactive chemicals. This is necessary for evaluating the potential therapeutic applications of these compounds (Sasidharan, 2011).

Natural product extracts can have various industrial applications, such as in the food and cosmetic industries. Extraction methods enable the isolation of compounds that contribute to the flavors, fragrance, and other properties of these products (Bolouri et al., 2022). To sum up, in the field of natural product chemistry, extraction techniques are essential procedures that facilitate the separation, refinement, and examination of bioactive substances derived from various natural origins. These techniques greatly advance our knowledge of the chemical makeup of natural items and the range of possible uses for them.

2.4.1 Microwave Extraction Method

Microwave-assisted extraction (MAE) is a current extraction technique that utilizes microwave energy to improve the extraction of bioactive compounds from various samples (Llompart et al., 2019). This method has gained popularity in recent years due to its efficiency, speed, and potential for increased yields compared to traditional extraction methods.

The principle behind Microwave-Assisted Extraction involves the use of microwave radiation to heat the solvent or water within a sample. Unlike traditional methods that rely on conduction or convection heating, microwaves provide rapid and volumetric heating, penetrating the sample and causing molecules to vibrate, generating heat (Hu et al., 2021). This rapid and selective heating accelerates the extraction process, leading to improved efficiency and reduced extraction times, the microwave radiation is delivered to the sample during microwave-assisted extraction, which causes the solvent or water in the sample to heat up quickly (Llompart et al., 2019). The greater temperature and pressure enable compounds to be extracted from the matrix into the solvent more easily. The extraction process is accelerated and made more efficient by

the rapid and selective heating generated by microwaves, compared to traditional methods (Mao et al., 2021).

Microwave is an electromagnetic wave, which consists of electric field and magnetic field which oscillate perpendicularly to each other in frequency ranging from 0.3 to 300 GHz (Sun et al., 2016)

Microwave energy can penetrate specific materials and interact with their polar components to produce heat. In a closed Microwave-Assisted Extraction (MAE) system, heating occurs directly at the molecular level within sealed vessels, using various modes of microwave radiation (Destandau et al., 2013). Typically, extraction is done with high operating pressure, temperature, and homogeneous microwave heating; this enables the system to remove fat quickly and effectively (Hewavitharana et al., 2020). In microwave-assisted extraction, the vessel's internal pressure is carefully regulated to remain within its operational limits, while the temperature can be adjusted above the solvent's normal boiling point. Advances in closed-system technology have introduced high-pressure microwave-assisted extraction (HPMAE). The combination of elevated temperature and pressure enhances the efficiency of the process by improving the solvent's capacity to absorb microwave energy (Chan, 2014).

When compared to conventional techniques, microwave-assisted extraction drastically shortens extraction times, thus decrease in extraction time from several hours to 3 min was also observed when applying MAE (Karami et al., 2014). It may finish extractions in a matter of minutes or hours that would need many more hours if traditional methods were used. It also improves effectiveness, as the selective energy transfer and rapid heating enhance extraction efficiency, leading to higher yields of

bioactive compounds (Nour et al., 2021). It is obvious that the extraction yield of MAE is higher and required shorter extraction time when compared with other extraction techniques.

A study found that microwave radiation damages the extraction sample matrix, enabling rapid extraction as active compounds are released and dissolved in the solvent once the cell structure is broken (Liu et al., 2022). Also non-polar solvents cannot be used in MAE because of their poor absorbents for microwave heating. Another drawback of MAE is that additional time for clean up steps is required and the extracted active compounds may be lost during the procedures (Chan, 2014). Compared with ultrasonic-assisted extraction, apparatuses and equipment of microwave assisted extraction are more expensive and their operation is more difficult in many cases (Ince et al., 2012).

2.4.2 Soxhlet Extraction Method

In organic chemistry, Soxhlet extraction is a popular technique for removing compounds from solid materials. Lipids, essential oils, and other organic substances are extracted from plants, seeds, and other natural materials using this approach (Zhang et al., 2018). Since Franz von Soxhlet initially presented the Soxhlet extraction device in 1879, it has become a common procedure in many labs. Soxhlet extraction of solid samples is commonly known as “solid–liquid extraction”, it is one of the oldest techniques of solid sample preparation.

Soxhlet Extractor, has a flask attached to the bottom of a vertical column, at the top, it has a thimble holder or siphon attached. A condenser is attached to the top of the extractor, and its function is to condense the solvent vapors from the stem bark into

liquid form, allowing them to drip back into the sample. The solvent is kept in this flask, which is positioned beneath the extractor. During the extraction process, the solvent is heated, evaporated, and then condensed stem bark into liquid form.

Soxhlet Extraction Procedure:

- A solid sample containing the desired compound is placed in a porous thimble made of materials like cellulose or glass wool. The thimble is then loaded into the Soxhlet extractor.
- The boiling flask is filled with a suitable solvent. Common solvents include hexane, ethanol, or methanol, depending on the nature of the compounds being extracted.
- The solvent is heated, vaporizing and rising through the column. Once the solvent reaches the top of the column, it condenses in the condenser and drips back into the sample, creating a continuous extraction cycle.
- This cycle continues until the extracted compounds are dissolved in the solvent, forming a concentrated solution in the boiling flask.
- The concentrated extract is then recovered from the boiling flask by evaporating the solvent, leaving behind the extracted compounds.

Apart from extracting and separating the compounds of interest from insoluble high-molecular-weight fractions, it also assists to eliminate and segregate additional compounds that can impede the analytical process in its later stages (Lama-Muñoz & Del Mar Contreras, 2022). Conventional Soxhlet extraction offers several benefits. The method repeatedly exposes the sample to fresh solvent, enhancing the displacement of the transfer equilibrium (De Castro et al., 2010). Heat from the distillation flask maintains a relatively high temperature in the extraction chamber,

improving efficiency. Additionally, the low cost of equipment allows multiple extractions to be conducted simultaneously, increasing sample throughput and eliminating the need for filtration after leaching (Redfern et al., 2014).

Moreover, Soxhlet extraction is a straightforward technique that requires minimal expertise and can yield a larger sample mass compared to many modern alternatives. There is a wide variety of official methods involving a sample preparation step based on the type of Soxhlet extraction.

Comparing Soxhlet extraction to alternative methods for preparing solid samples, the two biggest disadvantages are the lengthy extraction process and the significant amount of extractant waste, which not only increases disposal costs but also contributes to environmental issues (Yu et al., 2023). Target species that are thermolabile may undergo thermal decomposition as a result of the prolonged extraction of samples at the solvent boiling point (Gil-Martín et al., 2022). In addition, the large amounts of extractant used call for an evaporation–concentration step after extraction. Finally, the Soxhlet technique is limited by extractant and difficult to automate (Zygler et al., 2012). Conventional Soxhlet extraction, despite its strengths and limitations, has served as the foundation for numerous modifications designed to minimize its drawbacks while maintaining or enhancing its benefits (Phong et al., 2022). In recent decades, most improvements have focused on making Soxhlet more comparable to modern solid sample preparation techniques by reducing leaching times through auxiliary energy sources and incorporating automation into the extraction setup (Yu et al., 2023). Figure 2.2 below shows the diagram of Soxhlet.

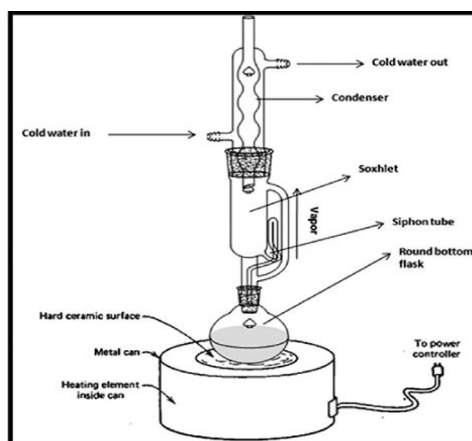


Figure 2.2. The diagram of Soxhlet extraction set-up.

2.4.3 Maceration Method of Extraction

Maceration is a process used in various fields, including culinary, pharmacy, and herbal medicine, to extract Flavors, colours, or active compounds from plant materials (Stéphane et al., 2022). It is considered as a widely and low-cost way to get natural products from plant material. The principles of maceration involve the soaking of the plant material in a liquid, allowing the solvent to absorb the desirable components (Abubakar et al., 2020).

In this process, the powdered solid materials are placed in a closed vessel, and the solvent is added. It is allowed to stand for hours or days, with occasional shaking and sufficient time is allowed for the solvent to diffuse through the cell wall to solubilize the constituent present in plant (Bitwell et al., 2023). Once the predetermined duration has elapsed, the solvent is strained out, and the solid residue is compressed to extract the maximum amount of solvent. When the solvent is water and the maceration process takes a long time, a little quantity of alcohol may be added to halt the growth of microorganisms (Spigno et al., 2007).

Maceration involves three principal steps. Firstly, plant materials are converted to powder form by grinding. This allows good contact between solvent and materials. After grinding, a chosen solvent is added in a closed vessel. Then, the liquid is strained off but the solid residue of this extraction process is pressed to recover large amount of solvent (Plášková & Mlček, 2023). Maceration has a lot of advantages over the other extraction methods and this includes, Skilled operator not required, Maceration is a simple method using non-complicated utensil and equipment, it is an energy saving process and a suitable method for less potent and cheap drugs (Ponphaiboon et al., 2023).

2.50 Allelopathy Phenomenon of Secondary Metabolites

Allelopathy is an ecological phenomenon, in which the chemicals produced by plants and microorganisms affect the growth, development, and fitness of other organisms (Aci et al., 2022). Allelopathy is also defined as a key plant-interference mechanism in which secondary metabolites, known as allelochemicals, are released into the soil rhizosphere by plants (Cheng & Cheng, 2015). In recent times, the International Allelopathy Society (IAS) has further expanded the definition as follows: “any process involving secondary metabolites produced by plants, microorganisms, viruses, and fungi that influence the growth and development of agricultural and biological systems” (Aci et al., 2022).

Initially, allelopathy was regarded as a subfield of ecological science, with research primarily emphasizing qualitative descriptions of dominant or invasive plant species (Hickman et al., 2023). Today, allelopathic research spans multiple disciplines, including natural product chemistry, plant physiology (such as mode-of-action investigations), applied agriculture, forestry, soil science, and more recently,

proteomics and genomics (Singh et al., 2021). The allelopathic impact of certain plants is caused by secondary metabolites, which are found in many tissues and plants and are often produced by processes including volatilization, decomposition, and root exudation (Shan et al., 2023). Allelochemicals exhibit diverse chemical compounds and modes of action; many are regarded as potential lead compounds for the development of next-generation weedicides (Alhassan, 2017).

Secondary metabolites are separated into 14 groups based on their biosynthetic source (Thirumurugan et al., 2018). These groups comprised of coumarins, terpenoids, sesquiterpenes, flavonoids, quinones, and simple phenolic. Phenolic compounds, which are the predominant allelochemicals in plants, have been widely studied to understand their allelopathic mechanisms in model species like *Arabidopsis thaliana* and *Lactuca sativa* (Bachheti et al., 2020). Simple phenolic compounds have been shown to affect morphophysiological processes in various crops, such as altering root morphology in maize (Hussain et al., 2022). In addition, more complex metabolites—such as mono-, di-, and triterpenoids, as well as sterols—have been investigated for their roles in allelopathic interactions, particularly in seed germination, oxidative damage, and plant defence (Wang et al., 2018).

2.5.1 Coumarins

Coumarins, which is part of lactones derived from *o*-hydroxycinnamic acid, often feature a variety of side chains, with isoprenoids being the most common (Ninkuu et al., 2021). Several plant-derived coumarins have demonstrated phytotoxic effects in laboratory bioassays, frequently suppressing seed germination and seedling growth (Govêa et al., 2020). Specifically, studies have shown that umbelliferone and psoralen can disrupt mitosis in *Allium cepa*, while scopoletin inhibits both germination and root development in oats (Bachheti et al., 2020).

2.5.2 Sesquiterpenes

Despite the large number of compounds classified as phytotoxic or allelopathic sesquiterpenes, mainly sesquiterpene lactones (2.26), little is known about their molecular targets and mechanisms of action (Cárdenas et al., 2022). Artemisinin is a typical sesquiterpene lactone bearing an endoperoxide moiety and without the common double bond in the lactone ring, and has a powerful inhibitory effect on plant growth (Ivănescu et al., 2015).

2.5.3. Quinones

Quinones represent a small class of allelochemicals, with juglone (2.27) being among the earliest identified in this group (Macías et al., 2019). Research indicates that juglone can suppress oxygen uptake and interact with the SH groups of cysteine, glutathione, and bovine serum albumin, leading to a reduction of SH group content in extracts from barley and bean seedlings (Macías et al., 2019).

2.5.4 Flavonoids

These compounds contribute to functions such as coloration and pollination (e.g., flavones, flavonols, chalcones, and catechins) as well as disease resistance (phytoalexins). Only a few have been reported to exhibit allelopathic activity. One example is kaempferol (2.28), which has been identified in soils beneath *Quercus mongolica* Fisch (Kumar et al., 2013).

2.5.5 Terpenoids

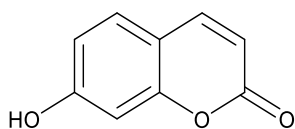
They are secondary metabolites present in many organisms, and they have a wide range of structural diversity. Due to their allelopathic action, they are now being explored as potential leads in the creation of novel agrochemicals based on natural

products (Sudatti et al., 2020). Inhibition of germination has been reported for several monoterpenes (e.g., camphor, pinenes, cineoles) in pure form (Chenyin et al., 2023). Furthermore, it has been confirmed that cinmethylin acts as a proherbicide that suffers metabolic breakdown to give rise to 2-hydroxy-1,4- cineole, the true herbicide (Ogawa et al., 2023).

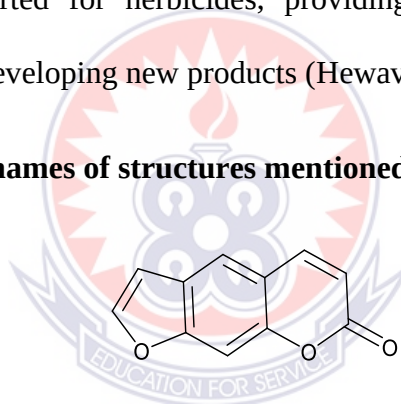
The identification of novel mechanisms of action for herbicide design is one of the primary benefits that allelochemicals may provide, as stated and emphasized several times over the past ten years (Hickman et al., 2023).

It is important to note that the newly identified targets and modes of action differ from those previously reported for herbicides, providing fresh insights and potential molecular targets for developing new products (Hewavitharana et al., 2020).

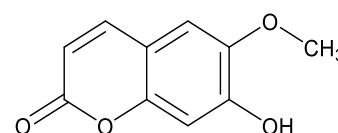
Figure 2.3 shows the names of structures mentioned in the text.



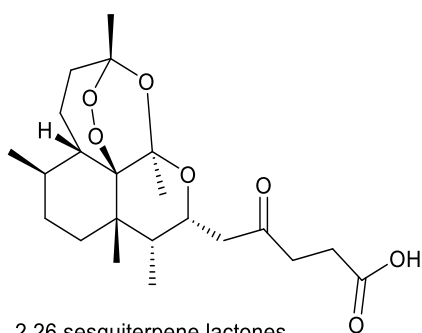
2.23 Umbelliferone



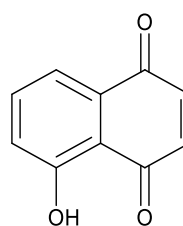
2.24 psoralen



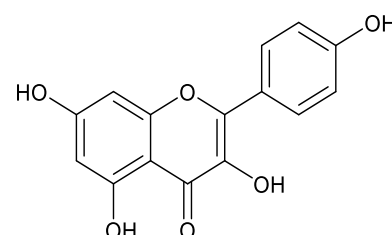
2.25 scopoletin



2.26 sesquiterpene lactones



2.27 juglone



2.28 kaempferol

Figure 2.3. Shows the general structures of compounds mentioned in text

2.60 Review on Instrumentation and on Characterization.

Natural products are synthesized by all types of organisms, including plants, animals, and microorganisms. Traditionally, they are classified into two main groups: (i) those occurring in mammals, such as metabolites, and (ii) those obtained from nonmammalian sources like bacteria, fungi, plants, marine organisms, and other animal species (Přichystal et al., 2016). In microorganisms, secondary metabolites are of great biological significance (Alam et al., 2022). These compounds, which can range from a few dozen to over 2,000 in number and vary in molecular weight, display diverse locations, functions, and biological activities (Farombi & Owoeye, 2011).

The process of tracking and categorizing already-known chemicals is called as "dereplication," and the majority of the data necessary to do so are produced utilizing mass spectrometry (MS), Nuclear Magnetic Resonance spectroscopy (NMR), High-performance liquid chromatography (HPLC) (Itô et al., 2014). For natural product databases steps incorporating traditional methods used for the discovery of novel natural compounds include

(1) Phenotypic screening,

(2) Compound isolation and characterization,

Compounds from plants are extracted from thoroughly dried portions of taxonomically classified species. Common extraction methods used to separate natural product mixtures from the plant matter include cold extraction, hot percolation, Soxhlet extraction, pressured liquid extraction, and supercritical fluid extraction (Kapadia et al., 2022). In some cases, fractions may be collected by thin

layer chromatography, high-performance liquid chromatography (HPLC), or size exclusion chromatography (Přichystal et al., 2016).

For a practical chemist, natural compounds have long been among their most fascinating items. The separation, purification, and structural elucidation of natural products really play a major part in many definitions of chemistry (Dias et al., 2012).

2.6.1 Nuclear Magnetic Resonance spectroscopy (NMR)

NMR was jointly discovered in 1946 by Bloch, Hansen, and Packard of Stanford University and Purcell, Pound, and Torrey of Harvard University (Giunta et al., 2020). The first observation that magnetic nuclei, such protons and phosphorus 31 (^1H and ^{31}P respectively), could absorb radio frequency energy in a nucleus-specific magnetic field of a certain intensity led to the discovery of NMR (Libretexts, 2022), and it operates on the principle that various atoms inside a molecule resonated at various frequencies upon absorption, causing the nuclei to start resonating. This finding made it possible to examine a molecule's structure in great detail. Since then, kinetic and structural investigations of solids, liquids, and gases have all been studied using NMR (Emwas et al., 2020).

Nuclear Magnetic Resonance (NMR) is a powerful analytical technique used in chemistry, physics, and medicine and is also widely used for determining the structure of organic compounds, studying the properties of materials, and even in medical imaging (commonly known as magnetic resonance imaging or MRI).

The application of NMR in natural products research is explored, emphasizing its ability to provide detailed information about molecular structure, stereochemistry, and dynamic behaviour (Emwas et al., 2020). Mass spectrometry can be quite selective

even though it is considerably more sensitive, for this reason that the full ^1H and ^{13}C NMR data assignment is required for all pertinent publications reporting novel natural product structures (Dayrit et al, 2017). The last 10 years have witnessed a huge increase in the technical and application possibilities of NMR spectroscopy; in the field of natural products, these applications divide into two broad categories: NMR-based metabolomics and structural identification of individual entities (Halabalaki et al., 2014). ^1H -NMR is capable of measuring intact samples and, in principle, ideal, is limited by a bias towards analytes with medium to high concentrations (García-García et al., 2017).

2.6.1.1 NMR Metabolomics

NMR metabolomics encompasses metabolite fingerprinting, profiling—identifying metabolite sets by comparing spectra with reference libraries—and targeted analysis of specific metabolites linked to particular metabolic reactions. Higher operating frequencies improve NMR spectrometer sensitivity; however, designing high-field instruments poses challenges due to the broader spectral range requiring uniform irradiation (Blümich, 2016). Advances in nanoscale NMR have enabled spectrometry and imaging for nuclei such as ^1H , ^{19}F , and ^{31}P , using chemical contrast agents, offering promising applications for future studies on natural compounds (DeVience et al., 2015). NMR has been critically important in both medicine and chemistry, with new applications continuing to emerge every day (Libretexts, 2020).

2.6.2 High-Performance Liquid Chromatography (HPLC)

For analyses and separations, high-pressure liquid-solid chromatography (HPLC) is quickly taking the lead in many domains. High-Performance Liquid Chromatography (HPLC) is a widely employed chromatographic technique for separating and

analyzing natural product components. This section introduces the principles of HPLC and its applications in natural product characterization.

HPLC column is able to separate almost everything that can dissolve. But along with this adaptability comes the need to consider the desired separation and the most effective means of achieving it (Rusli et al., 2022). High-Performance Liquid Chromatography (HPLC) is a versatile yet fundamentally straightforward separation technique that serves both analytical and preparative purposes. It enhances research efficiency by enabling faster analyses compared to slower separation methods. In principle, a sample dissolved in a suitable solvent is introduced into a stationary phase (a column packed with porous material), after which a mobile phase (liquid) is pumped through the column under high pressure. Separation occurs due to differences in the migration rates of the sample components, which result from their varying degrees of partitioning between the stationary and mobile phases. As a result, components elute at different times according to their specific partition behaviours (Skoog et al., 2018; Dong, 2013). A typical HPLC system is made up of the following primary parts:

Solvent Reservoirs: Storage of sufficient amount of HPLC solvents for continuous operation of the system.

Pump: This provides the constant and continuous flow of the mobile phase through the system.

Injector: This allows an overview of the analyte's mixture into the stream of the mobile phase before it enters the column.

Column: This is the heart of HPLC system; it actually produces a separation of the analytes in the mixture. A column is the place where the mobile phase is

in contact with the stationary phase, forming an interface with enormous surface.

Detector: This is a device for continuous registration of specific physical (sometimes chemical) properties of the column effluent. The most common detector used in pharmaceutical analysis is UV (ultraviolet).

A Computer-based system controls all parameters of HPLC instrument composition

And acquires data from the detector and monitors system performance.

To sum up, high-performance liquid chromatography (HPLC) is a highly adaptable and potent analytical method that is essential to many sectors, research, and quality assurance. It is a vital instrument because of its capacity to precisely and sensitively isolate, identify, and measure molecules. It does have several disadvantages, though, such as expense, operator expertise, and environmental issues.

2.6.3 Gas Chromatography-Mass Spectrometry (GC-MS)

The current gold standard in the determination and monitoring of Volatile Organic Compound VOCs is gas chromatography-mass spectrometry (GC-MS) (Savito et al., 2023). Gas chromatography coupled to mass spectrometry (GC-MS) has been regarded as the gold standard for analysing of many compounds (lipids, drug metabolites, and environmental contaminants), as well as for forensic science (Fiehn, 2016). GC-MS is generally considered a versatile analytical platform, and this is because of its dependability, superior capacity for separation, selectivity, sensitivity, and repeatability (Pilařová et al., 2019; Piechocka et al., 2020). It is widely regarded as the preferred technique for qualitative analysis of complex gaseous mixtures.

Advances such as multi-dimensional gas chromatography (MDGC) have further enhanced its resolution and reduced detection limits (Majchrzak et al., 2018).

In GC-MS analysis, the process begins with the gas chromatograph, where the sample is vaporized and separated into individual components using a capillary column coated with a stationary phase (liquid or solid) (Medeiros, 2018). Separation occurs as an inert carrier gas, such as hydrogen or nitrogen, propels the compounds through the column, with each eluting at a different time based on its boiling point and polarity. This elution time is known as the compound's retention time. Gas chromatography can resolve highly complex mixtures or extracts containing hundreds of compounds. Once the separated compounds exit the GC column, the mass spectrometer ionizes and fragments them using chemical or electron ionization. The resulting ions and fragments are then directed through the mass analyser—commonly an ion trap or quadrupole—for detection and analysis.

The process concludes in the identification and analysis of ions, where fragmented ions manifest according to their mass-to-charge ratios. On the other hand, peak regions correlate with the amount of the molecule. A complicated sample separated by GC-MS will yield a number of distinct peaks in the gas chromatogram, and each peak produces a distinct mass spectrum that may be used to identify the molecule (Rockwood et al., 2018; Rubakhin & Sweedler, 2010).

Gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), and nuclear magnetic resonance (NMR) spectroscopy are the primary analytical techniques used for metabolic profiling in scientific research, largely because of their significant analytical advantages (Gowda et al., 2014)

There are two main forms of ionization used in GC–MS are electron ionization (EI) and chemical ionization (CI). For many analysts, the preferred approach for GC-MS is EI-based due to the availability of several mass spectrum databases and libraries (Feeney et al., 2022).

The advantages of GC–MS include its user-friendliness in terms of analysis time and operating costs, as well as its strong ability to identify compounds, which can be accomplished even with a basic GC system paired with a single quadrupole MS detector (Li et al., 2015). Additionally, GC–MS can mitigate certain issues often encountered in LC–MS, such as matrix effects and ion suppression from co-eluting compounds, thereby offering improved chromatographic resolution (Tarakhovskaya et al., 2023).

The GC-MS technique is applicable to both qualitative and quantitative analysis. It offers quantitative data that is precise and repeatable, which is essential in many sectors including forensic research, medicines, and environmental studies. Before analysis, chemical derivatization must be used to identify polar, thermolabile, non-volatile metabolites (Tarakhovskaya et al., 2023). There are three types of GC-MS, thus

Single Quadrupole GC-MS; this contains a single quadrupole and is commonly called GC-MS, making it ideal for routine sample analysis.

The triple quadrupole MS provides a higher level of selectivity and is best suited to analyses where the highest sensitivity is required.

HRAM GC-MS/MS; this is for comprehensive characterization of samples in a single analysis with high-confidence compound discovery, identification and quantitation.

To sum up, GC-MS is a flexible and strong analytical instrument that has great sensitivity, selectivity, and the capacity to reveal structural details about molecules. These benefits make it a useful tool for a variety of applications, including drug development and environmental monitoring.



CHAPTER THREE

MATERIALS AND METHODS

3.1 Overview

This chapter outlines the materials and methods used in the study. It details the selection and preparation of *Morinda lucida* plant material, the extraction process using ethyl acetate, and the various assays conducted to assess antimicrobial, antioxidant, and phytotoxic activities. The methodology also includes the use of analytical-grade reagents, standard laboratory equipment, and consumables sourced from reputable suppliers in Accra, Ghana.

Additionally, the chapter highlights the use of Agilent GC-MSD apparatus equipped with fused silica capillary column for compound profiling and describes the data recording and analysis processes, which were carried out using Microsoft Excel and Word 2021 software. This methodology provides a comprehensive approach to evaluating the bioactivity and chemical composition of the plant extract.

3.2 Selection of *Morinda lucida* Stem Bark

Fresh stem bark of *Morinda lucida*, naturally growing in the forests around Birim Central municipality, was collected for study. The plant was chosen due to its traditional medicinal uses and abundance in the area. Although previous research has explored its medicinal properties, its potential as a source of new herbicides has received limited attention.

Morinda lucida is widely used in traditional medicine for ailments such as fever, jaundice, skin infections, and chronic diseases like diabetes and hypertension, with

specific applications varying across Nigeria, Côte d'Ivoire, and DR Congo (Adeleye et al., 2018).

3.3 Materials and Equipment

Materials

Solvents: Ethyl acetate in its pure state without purification was purchased from Sigma Chemicals (Huge Ltd, Accra, Ghana), and used for extracting compounds from the plant material. All reagents used were of analytical grade. Laboratory Glassware: includes Flasks, beakers, pipettes, and Petri dish. Filtration Equipment: Whatman™ No.1 filter paper of diameter 9 cm was purchased from Sigma Chemicals (Huge Ltd, Accra, Ghana). Laboratory Consumables: Personal protective equipment (PPE), such as gloves and safety glasses, Vials, syringes, plastic bags, and other consumables for sample storage and analysis, were purchased from Merck store in Accra. Petri dishes and Whatman filter papers were procured, while the seeds were sourced from a seed store in Medina, Accra, Ghana. Data Recording and Analysis: Was done using Excel and Word 2021 software.

GC-MS

GC-MS data were recorded on an Agilent GC-MS apparatus equipped with a DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused silica capillary column.

Plant Material

In June 2023, *Morinda lucida* bark was harvested from Akim Oda in Ghana's Eastern Region. The plant was verified by the curator of the UCC Botanical Garden, after which the bark was air-dried and milled into powder using an electric blender.

Test microorganisms

The microorganisms tested in this study—*Staphylococcus aureus* (NCTC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCTC 13440), *Pseudomonas aeruginosa* (ATCC 4853), *Salmonella typhi* (ATCC 14028), *Candida albicans* (ATCC 90028), *Aspergillus niger* (clinical), and *Phytophthora megakarya* (clinical)—were sourced from the Microbiology Laboratory, Department of Basic Sciences, School of Basic and Biomedical Sciences, UHAS.

Harvesting and preparation of plant material

- Tree stem bark was harvested from surrounding forest in Akim Oda, in the Birim Central municipality, Eastern region of Ghana. Plant samples were collected during the minor raining season in June; this is to ensure optimal phytochemical content. Figure 3.1
- The tree stem bark was cleaned under running water to remove impurities and dirt.
- Plant sample was shade-dried at 25 °C to avoid excessive heat and preserves the integrity of the compounds in the tree stem bark. The tree stem bark was well dried for three months, figure 3.2. Figure 3.2
- The dried stem bark of *M. lucida* was first pulverized and sealed in plastic bag and refrigerated at -4 °C until the extraction.



Figure 3.1 Harvested *Morinda lucida* stem bark



Fig 3.2 Air-dried *Morinda lucida* stem bark.

3.3 Extraction of Plant

Ethyl acetate was selected for the extraction process. This was because it has a moderate polarity, allowing it to dissolve a wide range of both polar and nonpolar organic compounds. Ethyl acetate is effective for extracting diverse substances from a mixture; additionally, it has a relatively low boiling point, enabling easy evaporation to recover the extracted compound, and is considered less toxic compared to other common solvents (Supang et al., 2022).

Extraction Method

The preserved powdered sample, sealed in a plastic bag, was taken and 200 grams were weighed and transferred into a flat-bottom flask. 450 mL of pure ethyl acetate was added to the flask, which was then preserved to prevent solvent loss. The mixture was allowed to stand for a day before being filtered through Whatman™ No. 41 filter paper. This filtration process was repeated two times, and the combined filtrates were

then concentrated using a rotary evaporator (see Figure 3.3). The final crude extract weighed 56.33 g, yielding 28 %.



Fig.3.3. Rotary evaporator used to concentrate the ethyl acetate extract.

3.4 Screening Methodology

Bioassays are tests that assess the effect of a secondary metabolite on a biological system (Alhassan, 2017). This is carried out on compounds to establish the activity of the compounds. In this study, phytotoxicity, anti-microbial and anti-oxidants bioassays were conducted on the ethyl acetate extract as described below.

3.4.1 Antimicrobial Analysis

The anti-oxidant and anti-microbial properties of various test samples were investigated using standardized methodologies. DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-Azinobis-3-Ethylbenzthiazolin-6-sulfonic acid) reagents of analytical grade sourced from Korea were utilized for the antioxidant

assays. Dimethyl sulfoxide (DMSO) from Sigma-Aldrich Inc., St. Louis, MO, USA, along with methanol procured from British Drug Houses (BDH) laboratory supplies, Merck Ltd, Lutterworth, UK, and distilled water, were employed in the antioxidant study. For the assessment of antimicrobial activity, Mueller-Hinton broth and agar, as well as tetracycline and ketoconazole antibiotics, were acquired from Thermo Fisher (Oxoid Limited, Hampshire, UK). Additionally, tetrazolium chloride (TTC) at a concentration of 0.1 % (w/v) from Sigma Aldrich, and Phosphate Buffered Saline (PBS) of analytical grade from Sigma Aldrich, were utilized. McFarland standard, consisting of barium chloride and sulphuric acid, was employed to standardize microbial inoculum density for the antimicrobial tests.

Determination of the antimicrobial activity of the extracts

The antimicrobial efficacy of the *Morinda lucida* extracts was evaluated through two established methods: the Kirby-Bauer agar well diffusion method, as detailed in the works of Eloff (1998) and Neglo et al., (2020), and the broth micro-dilution method, as per the methodologies outlined by (Eloff, 1998) and (Bonifácio et al., 2014).

Agar Well Diffusion

In this study, sterile Muller-Hinton agar (20 mL) was poured and allowed to solidify before being inoculated with a drop of the test microorganisms at a concentration of 1×10^6 colony-forming units (CFU)/mL. Before inoculation, each strain was cultured overnight at 37 °C in Muller-Hinton broth, followed by dilution to 0.5 McFarland standards with saline. Subsequently, the diluted cultures were inoculated onto Muller-Hinton agar plates. Using a cork borer (3.5 mm), five wells were created in each plate. Among these, three wells were filled with 70 µL of *Morinda lucida* extract solution at

a concentration of 100 mg/mL. As negative controls, 20 % DMSO was employed, while chloramphenicol (30 µg/disc) and Nystatin (100 µg/disc) served as positive controls for bacteria and fungi inoculum, respectively. Following the addition of extracts, a 15-minute diffusion period at room temperature ensued, after which the plates were incubated at 37 °C for 24/48 hours, and subsequently assessed for zones of inhibition. The experimental process was conducted in triplicate for each sample.

Minimum Inhibitory Concentration

The determination of Minimum Inhibitory Concentrations (MIC) for *Morinda lucida* extracts was conducted utilizing the micro broth dilution method, adhering to a protocol previously documented with minor adaptations Clinical and Laboratory Standards Institute, Bonifácio et al., 2019; 2011; Eloff, 1998;; Witt et al., 2021).

A stock solution of *Morinda lucida* extract at a concentration of 100 mg/mL, prepared in DMSO/Tween20, served as the initial reagent. Subsequently, a two-fold serial dilution of this stock solution was performed until ten distinct concentrations were attained. Each well of the 96-well microtiter plates (obtained from Citotest Labware Manufacturing Co. Ltd, Jiangsu, China) was filled with 100 µL of double-strength Mueller Hinton broth (Oxoid Limited, United Kingdom), mixed with 100 µL of the *Morinda lucida* extract, resulting in good concentrations ranging from 50.0 to 0.1 mg/mL. Within each column of the microtiter plate, wells 11 and 12 functioned as positive control (broth + organism only) and negative control (broth with no organism), respectively, for each microbial strain. Following this, 100 µL of the 0.5 McFarland-standardized test organisms were introduced into the respective wells and incubated at 37 °C for 24 hours for bacteria and 48 hours for fungi. Minimum inhibitory concentrations (MICs) were identified visually using 0.1% (w/v)

tetrazolium chloride (TTC) as an indicator, with the MIC recorded as the lowest concentration showing no colour change, signifying growth inhibition.

Determination of Minimum Bactericidal (MBC) and Fungicidal Concentration (MFC).

To ascertain the potential bactericidal or fungicidal effects of the isolated extract, Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFCs) were determined by transferring samples from each well of the susceptibility assay onto Nutrient Agar or Sabouraud Dextrose Agar (SDA) plates, followed by incubation at 37 °C for 24–48 hours. The presence or absence of microbial growth was then assessed, following the procedure described by De S. Santos et al. (2020)

ABTS radical scavenging activity

The (2,2'-Azinobis-3-Ethylbenzothiazolin-6-sulfonic acid) ABTS free radical scavenging potential of *Morinda lucida* extract was investigated following a methodology previously outlined (Ozgen et al., 2006); (Tai et al., 2016) with minor adaptations. To initiate the ABTS scavenging activity assay, a mixture of 10 mL ABTS and 2.4 mM potassium persulfate was prepared to produce the ABTS free radical. This stock solution was subsequently diluted in 50 mL methanol to obtain a working solution. Following this, 150 µL of the prepared solution was combined with 50 µL of various compound concentrations (ranging from 0.0625 to 2.0 mg/mL), vortexed, and then incubated at 30°C for 30 minutes. The absorbance of each concentration was measured at 734 nm using a UV spectrophotometer (Jenway, Bibby Scientific Ltd, Stafford, UK) to obtain readings for both control ABTS (Ao) and test samples (A). The experimental protocol was replicated for ascorbic acid within a concentration range of (0.0625-2.0 mg/mL). All experimental procedures were

conducted in triplicate. The free radical scavenging activity against ABTS was subsequently determined by analyzing the data using the following equation:

The IC_{50} (half maximal inhibitory concentration) of the *Morinda lucida* extract was calculated using Microsoft Excel through nonlinear regression analysis (curve fitting) with the Log (Inhibitor) vs. response (three parameters) method.

DPPH radical scavenging activity

The DPPH (1,1-diphenylpicrylhydrazyl) free radical scavenging ability of the extract was assessed following a methodology previously outlined (Baliyan et al., 2022) with minor adjustments. A 0.1 mM solution of DPPH was prepared by dissolving 394 μ g in 10 mL of ethanol to create a working solution. Subsequently, 0.5 mL of the freshly prepared solution was mixed with 1 mL of the extract at concentrations ranging from 0.0625 to 2.0 mg/mL. The mixtures were vortexed and then incubated at 30 °C for 30 minutes. Absorbance readings were obtained at 517 nm using a UV spectrophotometer (Jenway, Bibby Scientific Ltd, Stafford, UK). The same procedure was repeated for the reference standard (ascorbic acid) with concentrations ranging from 0.0625 to 2.0 mg/mL. All experimental steps were conducted in triplicate. The free radical scavenging activity of the compound against DPPH was determined by analyzing the recorded absorbance data using the following equation:

The absorbance of the *Morinda lucida* extract is represented by A, while A_0 denotes the absorbance of the DPPH solution. The IC_{50} (half maximal inhibitory concentration) of the *Morinda lucida* extract was calculated using Microsoft Excel through nonlinear regression analysis (curve fitting) with the Log (Inhibitor) vs. response (three parameters) method.

3.5 Phytotoxicity Bioassay

Phytotoxicity tests should be quick, relevant, and economical for evaluating both natural and synthetic compounds. These assessments can be carried out in the laboratory using either entire plants or specific plant parts (Bharali et al., 2019). There is a mutual relationship between germination and seedling development and anti-germination activity on seeds by an extract or single compound which can be used as a bioassay for phytotoxic activity (Rys et al., 2022). Parameters such as shoot and root lengths are accepted as a direct measure of phytotoxic effects.

Phytotoxic bioassay procedure for inhibition of germination

The viability of the seeds was checked before the set-up as seen in figure 3.4

In the set-up, 1 gram of the *M. lucida* extract was taken and dissolved in 1 milliliter (mL) of neat ethyl acetate solution making a stock concentration of 1000 mg/mL. To prepare treatments treatment 1 (TR1), treatment 2 (TR2) and treatment 3 (TR3), the following was done;

TR1: A 100 μ L portion of the stock solution was mixed with 5 mL of ethyl acetate, resulting in a final concentration of 19.61 μ g/ μ L.

TR2: A 200 μ L portion of the stock solution was combined with 5 mL of ethyl acetate to obtain a final concentration of 39.22 μ g/ μ L.

TR3: A 400 μ L portion of the stock solution was diluted with 5 mL of ethyl acetate, yielding a final concentration of 78.44 μ g/ μ L.

Three Petri dishes were set up, each containing a double layer of Whatman™ No. 41 filter paper. The filter papers were treated with plant extracts and placed in a fume hood for 24 hours to allow the ethyl acetate to fully evaporate. Subsequently, 5 mL of distilled water was added to each dish, and 20–30 lettuce seeds (*Lactuca sativa*) were randomly distributed on the filter papers. A control was prepared in parallel by

applying only ethyl acetate to the filter papers, allowing it to evaporate to dryness, then adding 5 mL of distilled water before placing 20–30 seeds. All dishes were maintained at room temperature for seven days. Germination was recorded when the radicle extended beyond 3 mm. The experiment was conducted in triplicate, and root and shoot lengths were measured, with the average values used as indicators of phytotoxic activity.

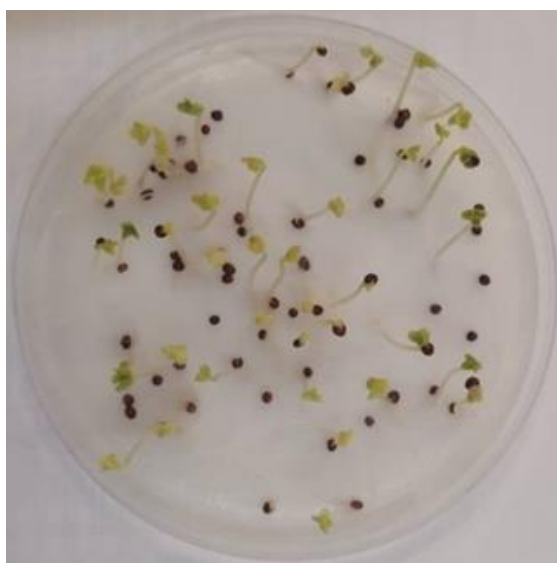


Fig 3.4. Show the results of viability test before anti germination test was conducted.

3.6 GC-MS Profiling

In the GC-MS profiling, the extract sample was prepared following the procedure below;

A 1 mg portion of the extract was dissolved in 1 mL of GC-grade ethyl acetate to obtain a concentration of 1 $\mu\text{g}/\mu\text{L}$ (0.001 mg/mL). GC-MS analysis was performed using an Agilent GC-MSD system fitted with a DB-5SIL MS fused silica capillary

column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness). Helium served as the carrier gas at a flow rate of 2 mL/min, with ethyl acetate as the solvent. The injector temperature was maintained at 250 $^{\circ}$ C, while the transfer line was set to 280 $^{\circ}$ C. The column temperature program began at 50 $^{\circ}$ C (held for 2 min), increased to 280 $^{\circ}$ C at 20 $^{\circ}$ C/min, and was held at this final temperature for 15 min. The mass spectrometer operated in electron ionization (EI) mode at 70 eV.



CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Overview

This chapter discusses the results of the study. It comprises results from the antimicrobial activity, antioxidant activity and phytotoxicity set-ups of *Morinda lucida* extract. It also presents the compounds identified through GC-MS analysis of the extract.

4.2 Results of Anti-Microbial Screening

Table: 4.1. Summary of the test results of the antimicrobial screening.

Organism	EXP1	EXP2	EXP3	MEAN MIC	MBC	MBC/MIC
	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	
<i>E.coli</i>	10	10	10	10	20	2 ^{bc}
<i>K. pneumonia</i>	1.25	1.25	1.25	1.25	2.5	2 ^{bc}
<i>S. aureus</i>	10	10	10	10	20	2 ^{bc}
<i>S.typhii</i>	10	10	10	10	20	2 ^{bc}
<i>P.aeruginosa</i>	1.25	1.25	1.25	1.25	5	4 ^{bc}
<i>C. albicans</i>	10	10	10	10	20	2 ^{bc}
<i>A.niger</i>	0.156	0.156	0.156	0.156	2.5	16 ^{bs}
<i>P.megakarya</i>	0.313	0.313	0.313	0.313	0.56	2 ^{bc}

The antibacterial activity of the extract showed the lowest MIC values of 1.25 mg/mL against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The MICs of 10

mg/mL against *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli* showed considerable activity. As shown in Table 4.1 below. An MBC/MIC ratio of ≤ 4 indicates a bactericidal or fungicidal effect, while a ratio greater than 4 suggests a bacteriostatic or fungistatic effect (Abdullayev et al., 2024). An antibacterial agent is classified as bactericidal if it kills bacteria, and bacteriostatic if it only inhibits their growth (Ishak et al., 2025). The extract showed bactericidal activity against most bacterial strains, with MBC/MIC ratios of 2 or 4.4. All bacterial strains showed an MBC/MIC ratio of 2, except *P. aeruginosa*, which had a ratio of 4, indicating that it is more resistant to the extract.

The antifungal activity of the extract against *Candida albicans* indicated a MIC of 10 mg/mL, similar to the bacterial strains, indicating moderate susceptibility. The most antifungal activity was against *Aspergillus niger* with a MIC of 0.156 mg/mL, but its MFC/MIC ratio was 16, suggesting a fungistatic effect rather than fungicidal. *P. megakarya* had a MIC of 0.313 mg/mL and an MFC/MIC ratio of 2, indicating strong susceptibility and a fungicidal effect meaning all the organisms were killed at this lower concentration. The fungicidal activity of the extract against *p. megakarya* at such a lower concentration is noteworthy and much more potent than the extract of *Thevetia peruviana* seeds recorded in previous literature (Ambang et al., 2010). This result suggests that crude extract *Morinda lucida* bark is an efficient biocide substance with antifungal activity (Fakoya et al., 2014).

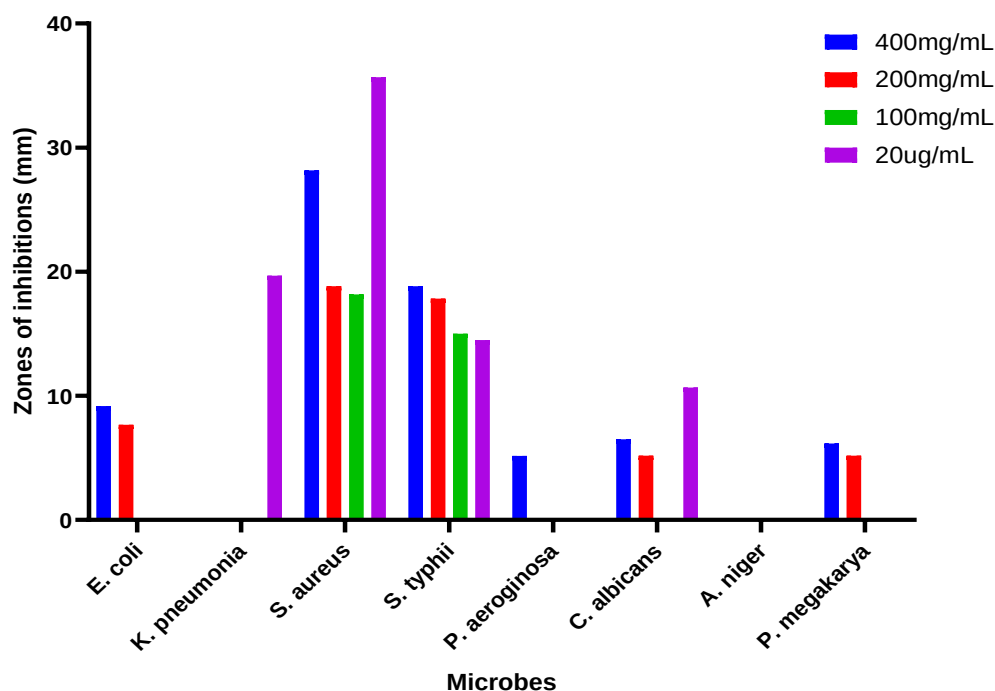


Fig. 4.1 Zones of inhibitions of *Morinda lucida* bark extracts against selected microbes

In Fig 4.1 the antimicrobial activity of the extract is concentration-dependent, with higher concentrations generally producing larger zones of inhibition. *Staphylococcus aureus* and *Salmonella typhi* exhibited the highest susceptibility to the extract, especially at 20 µg/mL and 400 mg/mL. *Klebsiella pneumoniae* showed no inhibition at any concentration, indicating resistance or lack of susceptibility to the extract. Fungal strains like *Candida albicans*, *Aspergillus niger*, and *Penicillium notatum* showed moderate to low susceptibility.

Morinda lucida extract exhibits strong antibacterial effects, particularly against *S. aureus* and *S. typhi*. The activity decreases with lower concentrations for most microbes, confirming a dose-response relationship. Fungal strains show some susceptibility, suggesting that the extract has both antibacterial and antifungal

potential. The absence of activity against *K. pneumoniae* suggests selectivity in the extract's antimicrobial spectrum.

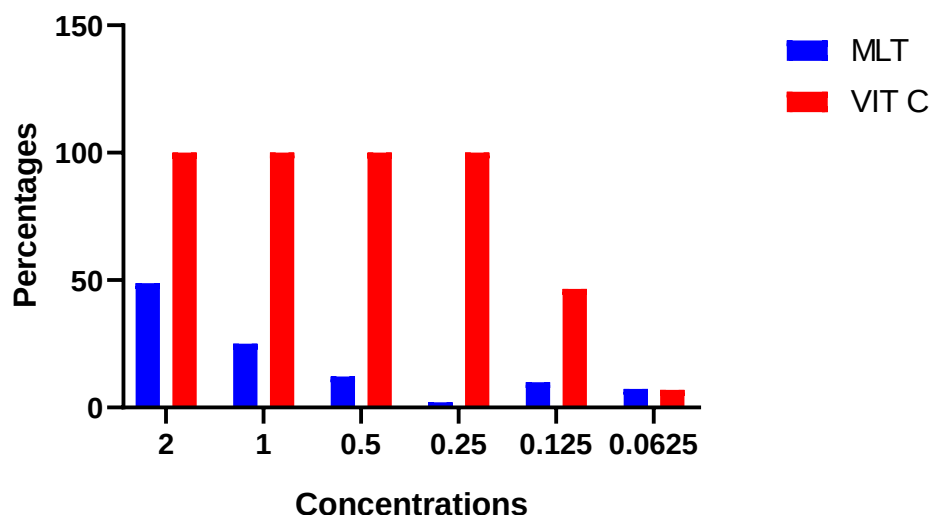


Fig. 4.2: 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity of *Morinda lucida* extracts

Vitamin C (Red Bars): Shows consistently high antioxidant activity (nearly 100%) at all concentrations from 2 to 0.25 mg/mL. Activity begins to drop below 100% only at 0.125 mg/mL and sharply declines at 0.0625 mg/mL. This indicates strong and effective antioxidant potential even at low concentrations.

Morinda lucida extract (MLT - Blue Bars): Demonstrates moderate antioxidant activity, highest at 2 mg/mL (~55%). The antioxidant effect decreases steadily with decreasing concentration. At concentrations below 0.5 mg/mL, MLT shows minimal to low antioxidant activity.

This indicating the potent antioxidant properties of *Morinda lucida* bark extracts compared to Vitamin C, graph shown in figure 4.2. Antioxidant activity has been directly linked to the presence of phenolic moieties present in the molecular structure of natural antioxidants. Many phytochemicals having phenolic moieties have been shown to exhibit antioxidant activity (Ghasemzadeh et al., 2018).

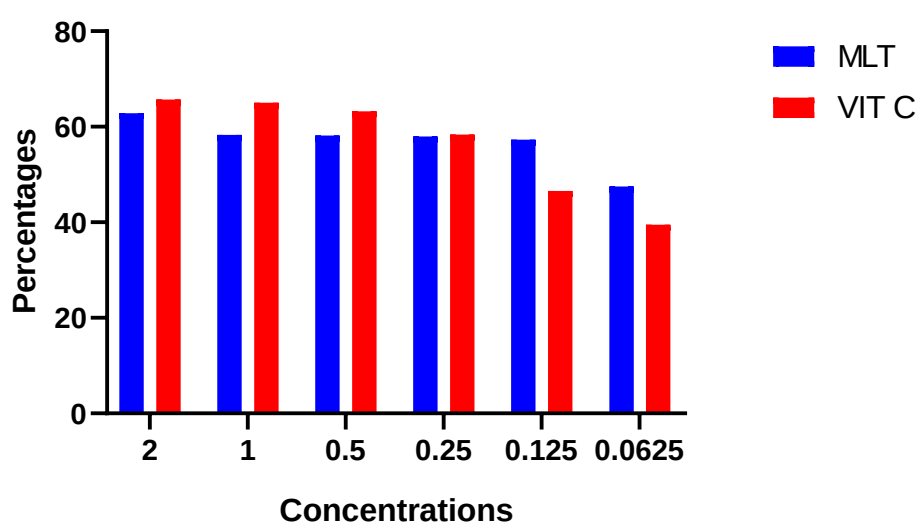


Fig. 4.3: Suggest *Morinda lucida* extract antioxidant activity on 2,2'-Azinobis-3-Ethylbenzthiazolin-6-sulfonic acid (ABTS).

ABTS assay results for *Morinda lucida* extracts, revealing a similar trend of concentration-dependent antioxidant activity, figure 4.3. The percentage of ABTS scavenging increases with higher concentrations of the extract, further highlighting the strong antioxidant potential of *Morinda lucida* extracts compared to Vitamin C.

4.3 comparing the antioxidant activity results of both DPPH and ABTS assays

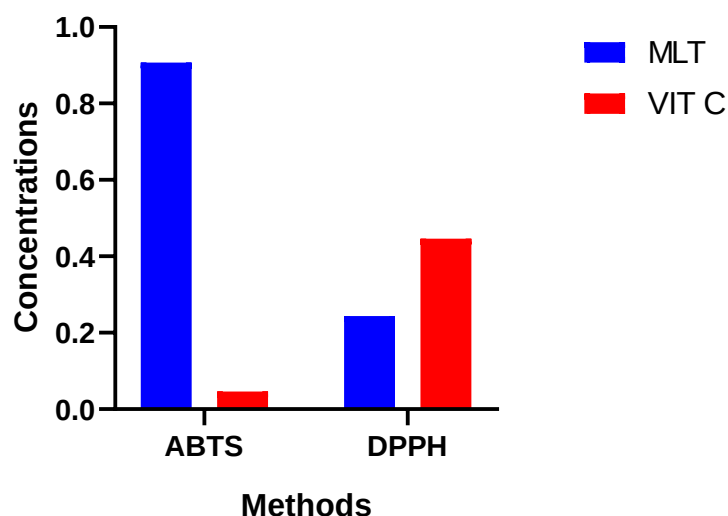


Fig. 4.4: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azinobis-3-Ethylbenzthiazolin-6-sulfonic acid (ABTS) antioxidant activities

Figure 4.4 combines the results of both DPPH and ABTS assays, showcasing the antioxidant activities of *Morinda lucida* extracts in a comprehensive manner.

ABTS Assay:

Morinda lucida (MLT) extract exhibits a very high antioxidant activity with a concentration close to 0.9. Vitamin C, in comparison, shows minimal activity with concentration close to 0.05. In the ABTS assay, *Morinda lucida* extract outperformed Vitamin C, indicating it is highly effective in scavenging ABTS radicals.

DPPH Assay:

Vitamin C demonstrated higher antioxidant activity (concentration ≈ 0.45) compared to MLT (concentration ≈ 0.3). This suggests that Vitamin C was more efficient in neutralizing DPPH radicals than MLT.

The graph illustrates the antioxidant effects of different concentrations of *Morinda lucida* extracts compared to Vitamin C, providing a holistic view of the plant extract's antioxidant capabilities.

4.4 Discussion

Plants are said to hold solutions to the antimicrobial resistance menace of various pathogens (Vaou et al., 2021). Currently, antimicrobial resistance is said to be a global pandemic and exploring novel therapeutic agents from floral sources is a necessity as this current study explored the potentials of *Morinda lucida* extracts from Birim Central Municipality against a range of microbes. The results of this study highlight the trends in inhibition levels of *Morinda lucida* extracts against selected human pathogens. *S. aureus* emerged as the most susceptible microbe to the extracts, while *A. niger* exhibited the lowest level of inhibition. These trends provide valuable insights into the differential effects of *Morinda lucida* extracts on various pathogens and underscore the importance of considering microbial susceptibility when evaluating the antimicrobial potential of natural products.

The antioxidant activities of *Morinda lucida* extracts were investigated in this study using DPPH and ABTS assays. The results demonstrated a concentration-dependent increase in antioxidant activity, with higher concentrations of the extract showing greater scavenging percentages in both assays. These findings suggest that *Morinda lucida* extracts possess potent antioxidant properties that can effectively neutralize free radicals and oxidative stress. The combination of DPPH and ABTS assays provided a comprehensive overview of the antioxidant activities of *Morinda lucida* extracts compared to Vitamin C, highlighting their efficacy in scavenging free radicals and protecting against oxidative damage.

Comparing these results with findings from other studies in the field, it is evident that natural plant extracts, including *Morinda lucida*, contain bioactive compounds with significant antioxidant potentials. Studies by (Oladeji et al., 2022) and (Chaves et al., 2020) have also demonstrated the antioxidant activities of plant extracts using DPPH and ABTS assays, showcasing the diverse sources of natural antioxidants and their health-promoting properties. These collective findings underscore the importance of exploring natural antioxidants for potential applications in various industries, including pharmaceuticals, nutraceuticals, and cosmetics.

Further research into the specific compounds present in *Morinda lucida* extracts and their mechanisms of action could provide valuable insights into the antioxidant effects observed in this study. Understanding the underlying pathways through which these compounds exert their antioxidant activities may lead to the development of novel antioxidant therapies and products. In all, the study of *Morinda lucida* extract antioxidant activities contributes to the growing body of evidence supporting the use of natural antioxidants for promoting health and combating oxidative stress-related diseases.

4.5 Results of Phytotoxic Bioassay Screening Experiments

Table 4.2 shows the root and shoot length of germinated seedling which was treated with M. Lucida bark extract at various concentrations. The table indicates that comparatively, the seedlings in the control reported the highest root and shoot length from the samples treated with 19.61 $\mu\text{g}/\mu\text{L}$, 39.22 $\mu\text{g}/\mu\text{L}$ and 78.44 $\mu\text{g}/\mu\text{L}$ of the extract.

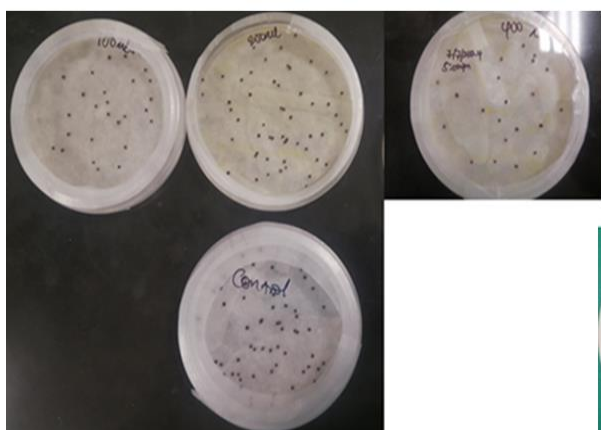


Fig. 4.5 The figure above shows the set-up of the antigermination experiment of the *Morinda lucida* at temp of 25 °C (day one).

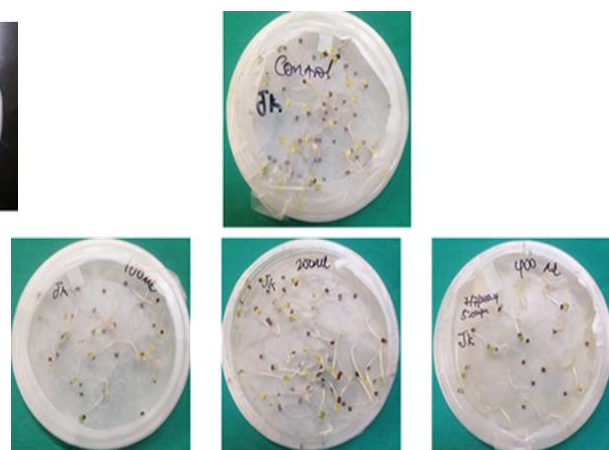


Fig. 4.5 Germinated seeds after 7 days at laboratory temperature at 25 °C. The radicle of each seed was above 3 mm which was considered germinated

Table 4.2 Impact of crude extracts on the shoot and root lengths of germinating *Lactuca sativa* (lettuce) seeds.

Treatments	Extract concentrations	Average Shoot length (mm)	Average root length (mm)
Control	No extract/ Only H ₂ O	22	39
TR1	19.61 µg/µL	19	33
TR2	39.22 µg/µL	12	23
TR3	78.44 µg/µL	4	7

The data in Table 4.2 show that the ethyl acetate extract of *Morinda lucida* suppressed seed germination and hindered both root and shoot development when compared with the control. Treatment one (TR1) displayed only slight growth suppression, which may be attributed to its lower concentration of phytotoxic constituents. This treatment resulted in approximately a 10% decrease in shoot length and an 18% decrease in root length.

Treatment two (TR2) resulted in a more pronounced reduction in plant growth, as it contained twice the concentration used in TR1. This greater suppression could be attributed to the corresponding increase in phytotoxic compounds within the extract. Specifically, shoot length decreased by 45 % and root length by 43 % compared to the control. In treatment three (TR3), which had double the concentration of TR2, shoot and root lengths were reduced even further—by 80 % and 85 %, respectively, relative to the control. The heightened inhibition in TR3 is likely a consequence of the elevated phytotoxic compound levels associated with the increased concentration.

Figure 4.6 presents a bar chart comparing the control with the three treatments.

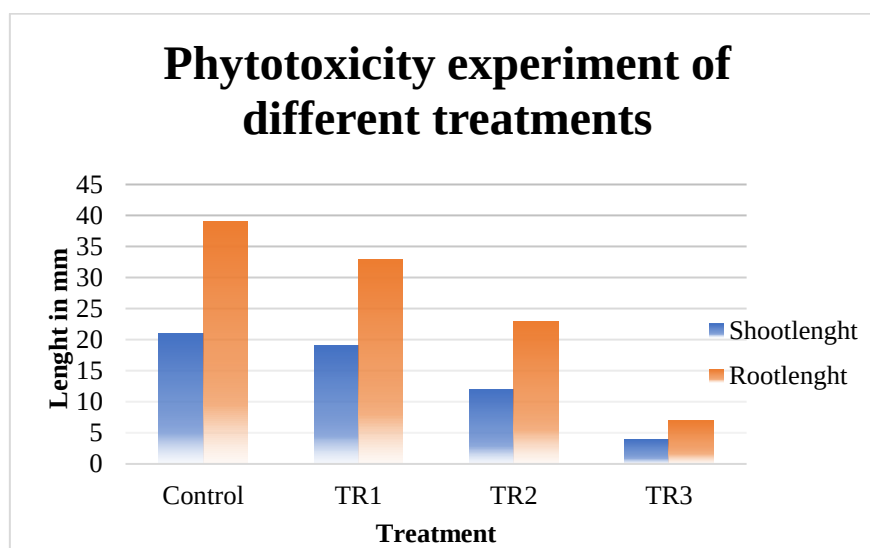


Fig 4.6. A bar chart showing the lengths of the shoot and roots of lettuce seed (*Lactuca sativa*), the control and the three Treatments

The percentage reduction of the root and shoot lengths was calculated using the formula below.

Where, N_c = Length of the shoot/root in control experiment

N_e = Length of the shoot/root in experimental set-up

The roots of the seedlings were noticeably more affected across all three treatments compared to the control. This is likely because the roots serve as the initial point of contact for the phytotoxic compounds. These compounds may then be transported through the stem, a process that could take several days to complete. The presence of the phytotoxic agents in the extract may interfere with water and nutrient absorption. The variation in the degree of reduction between root length and shoot length may also be attributed to structural differences between the two plant parts.

4.6 Conclusion

The antibacterial activity of the extract showed the lowest MIC values of 1.25 mg/mL against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The MICs of 10 mg/mL against *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli* showed considerable activity. [MBC/MIC ratio ≤ 4 indicates a bactericidal/fungicidal effect, whereas a ratio > 4 suggests a bacteriostatic/fungistatic effect (Abdullayev et al., 2024)]. An antibacterial agent is considered bactericidal if it kills bacteria or bacteriostatic if it inhibits their growth (Ishak et al., 2025). The extract demonstrated bactericidal activity against most bacterial strains with MBC/MIC = 2 or 4. All bacterial strains showed an MBC/MIC ratio of 2, except *P. aeruginosa*, which had a ratio of 4, indicating that it is more resistant to the extract.

The antifungal activity of the extract against *Candida albicans* indicated a MIC of 10 mg/mL, similar to the bacterial strains, indicating moderate susceptibility. The most antifungal activity was against *Aspergillus niger* with a MIC of 0.156 mg/mL, but its MFC/MIC ratio was 16, suggesting a fungistatic effect rather than fungicidal. *P. megakarya* had a MIC of 0.313 mg/mL and an MFC/MIC ratio of 2, indicating strong susceptibility and a fungicidal effect meaning all the organisms were killed at

this lower concentration. The fungicidal activity of the extract against *p. megakarya* at such a lower concentration is noteworthy and much more potent than the extract of *Thevetia peruviana* seeds recorded in previous literature (Ambang et al., 2010). This result suggests that crude extract of *Morinda lucida* is an efficient biocide substance with antifungal activity.

4.7 GC-MS profiling

The ethyl acetate extract of *Morinda lucida* contains potent allelochemicals that inhibit seed germination and growth, with up to 85 % root length reduction at the highest concentration tested. GC–MS profiling identified 24 compounds, including several known phytotoxins, with Ergosta-4,22-dien-3-one being the most abundant. The findings suggest its potential as a source for developing new herbicides, warranting further bio-guided isolation to identify the key active compound.

4.7 GC-MS results

Tables 4.2 show the compounds present in the ethyl acetate extract obtained from *M. lucida* stem bark. the three major compounds by abundance were Ergosta-4,22-dien-3-one (11.51%), Cholest-4-en-3-one (10.35%) and Stigmasta-5,22-diene,-3-methoxy, (3.beta.,22E)- (8.30%) among other compounds.

Table 4.3. List of identified compounds from the ethyl acetate extract of *Morinda lucida* bark with their retention time and molecular mass of

S/N	Retention time (tr)	Name of Compound	M/z
1	38.1	Pentadecanoic acid	242.4
2	39.4	4(1H)-Quinazolinone	146.1
3	41.4	9-Octadecenoic acid	282.4
4	41.5	cis-13-Docosenoic acid	338.5
5	41.8	n-Octadecanoic acid	284.5
6	43.4	Benzeneacetic acid	136.2
7	44.2	Eicosanoic acid	312.5
8	44.5	9,10-Anthracenedione, 1,8-dimethoxy-	268.3
9	44.7	5-(p-hydroxyphenyl)-5-phenyl-	268.3
10	45.1	9,10-Anthracenedione, 2-methyl-1-nitro-	267.2
11	46.1	Docosanoic acid	340.6
12	46.3	Diethyl 4,4'-biphenyldicarboxylate	298.3
13	46.4	Spiro[2,5-cyclohexadiene-1,7'(1'H)-cyclopent[ij]isoquinolin]-4-one, 2',3',8',8'a-tetrahydro-5'-hydroxy-6'-methoxy-, (R)-	283.3
14	48.1	Flavone, 4',5,6,7-tetramethoxy-	342.4
15	48.7	Acetamide, N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)-	399.4
16	49.0	Diethyl-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate	327.4
17	50.5	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	430.7
18	51.5	Cholest-5-ene, 3-methoxy-, (3.beta.)-	400.7
19	51.8	Stigmasta-5,22-diene, -3-methoxy, (3.beta.,22E)-	426.7
20	53.3	Ergosta-4,22-dien-3-one	396.7
21	54.2	Cholest-4-en-3-one	384.7
22	54.4	1,5-Diphenyl-1,5-pentanedione	252.3
23	54.6	Androstan-3-one, 17-hydroxy-2-(hydroxymethylene)-17-methyl-, (5.alpha.,17.beta.)-	332.5
24	55.2	Androstan-3-one, 17-hydroxy-2-methyl-, (2.alpha.,5.alpha.,17.beta.)-	304.5

4.8.0 Profiling of compounds found in the analysis

The saturated fatty acid composition of *M. lucida* stem bark were Pentadecanoic, Docosanoic acid *n*-Octadecanoic acid, Benzeneacetic acid, and Eicosanoic acid with retention times 38.1, 46.1, 41.8, 43.4, and 44.2 min respectively (Table 4.3) while there are two unsaturated fatty acids 9-Octadecenoic acid, and Cis-13-Docosenoic acid with retention times 41.4 and 41.5 min, respectively

4.8.1 Pentadecanoic acid

Pentadecanoic acid, a saturated fatty acid found in various natural sources, such as dairy fat and some plants, has drawn interest due to its potential bioactive properties. Higher dietary intake and circulating levels of Pentadecanoic acid have been associated with lower risks of adiposity, chronic inflammation, cardiovascular disease, metabolic syndrome, type 2 diabetes, nonalcoholic steatohepatitis (NASH), chronic obstructive pulmonary disease, pancreatic cancer and others (Venn-Watson et al., 2020). Pentadecanoic acid exhibits antimicrobial effects, particularly against certain bacterial strains. Its ability to disrupt bacterial cell membranes contributes to its activity and makes it of interest in studies on antimicrobial agents derived from natural sources (Ricciardelli et al., 2020).

4.8.2 4(1H)-Quinazolinone

4(1H)-Quinazolinone and its derivatives have demonstrated phytotoxic activity in certain studies. Quinazolinone compounds are known for their diverse bioactivities, and some derivatives have shown the ability to inhibit the growth of various plants and algae, acting as allelopathic agents (Karan et al., 2021). They have been studied for potential applications in agriculture as natural herbicides due to their selective inhibition effects.

Research has highlighted that specific quinazolinone derivatives interfere with plant growth by inhibiting cell division or disrupting normal physiological processes in plants. However, the exact level of phytotoxicity can depend on the compound's structure and functional groups.

4.8.3 9-Octadecenoic acid

9-Octadecenoic acid, often referred to in its methyl ester form as oleic acid, has both bioactive and phytotoxic properties. It is recognized for its antifungal, antimicrobial, and anticancer activities, particularly as found in extracts of various plants (Muzahid et al., 2022). Studies highlight its role in inhibiting fungal pathogens and other microorganisms, thus showcasing its bioactivity. Additionally, the phytotoxicity of 9-Octadecenoic acid is noted in some contexts, where it can act as an allelopathic compound, meaning it may inhibit the growth of certain plant species by interfering with their physiological processes (Đorđević et al., 2022). This dual nature makes it valuable in specific agricultural and medicinal applications for its protective qualities against pathogens and pests

4.8.4 Cis-13-Docosenoic acid

Cis-13-Docosenoic acid, also known as erucic acid, is a monounsaturated fatty acid primarily found in plants such as rapeseed. This fatty acid has some bioactive properties, especially in industrial and food applications, and has raised concerns in high concentrations due to potential health risks. Erucic acid does not have well-documented phytotoxic effects, but its bioactivity in various biological systems, such as antimicrobial or antifungal activity, is a subject of ongoing research. It is mainly used industrially for lubricants and in polyamide synthesis rather than for agricultural phytotoxic applications (Knutsen et al., 2016).

4.8.5 *N*-Octadecanoic acid

N-Octadecanoic acid (stearic acid) exhibits notable bioactivity with applications in antimicrobial, anti-inflammatory, and antioxidant treatments. It has been found in various medicinal plants and is used for its therapeutic potential due to these properties (Canli et al., 2023). Research demonstrates that this compound can effectively scavenge free radicals, thereby potentially reducing oxidative stress and protecting cells against damage. While primarily non-toxic, it does exhibit mild phytotoxicity under specific conditions, affecting plant growth at high concentrations. These findings highlight *n-Octadecanoic acid* as a promising candidate for medicinal applications, particularly within formulations designed to manage oxidative stress and microbial infections.

4.8.6 Benzeneacetic acid

Benzeneacetic acid, also known as phenylacetic acid, has been studied for its bioactive properties and various applications in both medical and agricultural fields. It is an aromatic carboxylic acid with a benzene ring linked to an acetic acid group, and it plays a significant role in certain biosynthetic pathways and industrial applications.

Phenylacetic acid exhibits antibacterial and antifungal properties and has been investigated for its potential in treating infections. In certain studies, derivatives of benzeneacetic acid have been synthesized and tested for enhanced antimicrobial activity, particularly against gram-positive and gram-negative bacteria (Panda & Duarte-Sierra et al., 2022). In plants, benzeneacetic acid functions as a precursor to phenylacetaldehyde and other compounds involved in growth regulation. It can impact plant metabolic pathways and has been linked to auxin-like activity, which

influences root formation and cell elongation in various plant species (Casanova-Sáez et al., 2021).

Studies have shown that it can have a mild inhibitory effect on some plant species when applied in high concentrations, mainly due to its interference with cell wall structure and metabolic processes in plants and at certain concentrations, it can inhibit seed germination and root growth in plants, which indicates a phytotoxic effect.

4.8.7 Eicosanoic acid

Eicosanoic acid belongs to the group of *saturated fatty acids*. It is a straight-chain fatty acid with 20 carbon atoms, also known as arachidic acid.

Saturated fatty acids, such as eicosanoic acid, have no double bonds in their carbon chain, which contributes to their stability and relatively high melting points. Eicosanoic acid has limited documented bioactive properties compared to shorter-chain fatty acids or unsaturated long-chain fatty acids. However, it does have some functional roles in biological contexts, primarily associated with cell membrane structure and lipid metabolism. In terms of bioactivity, eicosanoic acid is not known for potent antibacterial, antifungal, or anti-inflammatory effects, unlike other fatty acids with shorter or unsaturated chains.

4.8.8 9,10-Anthracenedione, 1,8-dimethoxy-

9,10-Anthracenedione, 1,8-dimethoxy-, also known as 1,8-dimethoxyanthraquinone, is a compound found in several plants and is recognized for its potential biological and pharmacological properties. Compounds in the anthraquinone class, including 1,8-dimethoxy-anthraquinone, are known for their antimicrobial effects, showing activity against certain bacteria and fungi (Maiyo et al., 2024).

Research indicates that 1,8-dimethoxyanthraquinone has moderate antimicrobial effects. 1, 8-dimethoxyanthraquinone inhibits the growth of bacterial strains like *Staphylococcus aureus* and *Escherichia coli*. Additionally, it has shown antifungal properties, which may be useful in combating specific fungal pathogens that affect human health (Sulaiman et al., 2024).

Anthraquinones can exhibit antioxidant properties, helping to neutralize free radicals, and may also have anti-inflammatory effects (Zhao et al, 2023). 1,8-Dimethoxyanthraquinone is phytotoxic and can affect plant growth, especially in certain concentrations. As with other anthraquinones, this compound can inhibit seed germination, root growth, and cellular functions in sensitive plants, leading to growth suppression or even plant death (Hook et al., 2020).

4.8.9 5-(p-hydroxyphenyl)-5-phenyl-

Studies have shown that 5-(p-hydroxyphenyl)-5-phenyl- have been reported to show some weedicial property via distracting growth of plant in such a way that it interact with the plant hormones.

4.8.10 9,10-Anthracenedione, 2-methyl-1-nitro-

9,10-Anthracenedione, 2-methyl-1-nitro-, also known as 2-methyl-1-nitroanthraquinone, is a derivative in the anthraquinone family, and research on this compound has explored its bioactivity and potential phytotoxic effects. Like many nitro-anthraquinone compounds, 2-methyl-1-nitroanthraquinone has shown activity against bacterial and fungal pathogens (Amorim et al., 2023). The nitro group on the anthraquinone ring is often associated with an increase in antimicrobial efficacy, allowing it to disrupt microbial cell walls or inhibit enzymatic activities (Amorim et

al., 2023). Research have highlighted its potential against certain bacterial strains, particularly in the context of drug-resistant microbes.

The presence of a nitro group in this compound could enhance its phytotoxicity by disrupting plant cellular structures or metabolic processes. Studies have shown that nitro-substituted anthraquinones can affect seed germination and root elongation in test plants.

4.8.11 Docosanoic acid

Docosanoic acid, also known as behenic acid, is a long-chain saturated fatty acid commonly found in plant oils. Behenic acid has some antimicrobial properties, though its activity is generally mild compared to other fatty acids. This compound has been used in formulations for skincare products due to its protective and emollient effects on skin (Purnamawati et al., 2017). Behenic acid is not widely recognized as phytotoxic. Unlike some shorter-chain fatty acids or compounds with more reactive groups, behenic acid has a stable structure that does not readily interfere with plant cellular processes at typical concentrations.

4.8.12 Diethyl 4,4'-biphenyldicarboxylate

Diethyl 4, 4'-biphenyldicarboxylate is a biphenyl derivative and an ester that has been explored for its bioactive properties, although direct studies on its phytotoxicity are limited. Biphenyl compounds and their derivatives, such as diethyl 4,4'-biphenyldicarboxylate, have shown promise in antimicrobial studies (Hemeg et al., 2020). Biphenyl esters are known for their probable antimicrobial properties against a variety of bacterial strains (Angelini, 2024). In particular, they have been explored for activity against gram-positive and gram-negative bacteria, making them candidates

for use in antibacterial agents or surface coatings. For diethyl 4,4'-biphenyldicarboxylate specifically, studies suggest moderate efficacy in inhibiting bacterial growth, particularly when combined with other bioactive agents in a formulation (Abdallah et al., 2023). Although specific research on the phytotoxicity of diethyl 4,4'-biphenyldicarboxylate is limited, related biphenyl derivatives have been shown to impact seed germination and plant growth.

4.8.13 Spiro[2,5-cyclohexadiene-1,7'(1'H)-cyclopent[ij]isoquinolin]-4-one, 2',3',8',8'a-tetrahydro-5'-hydroxy-6'-methoxy-, (R)-

This is an intricate molecule with a spiro-fused structure often found in certain types of natural products and synthetic analogs, particularly in alkaloids. Compounds with similar isoquinoline or spiro structures have shown bioactivity and, in some cases, phytotoxic properties. However, direct evidence of this compound's bioactivity and phytotoxicity in the literature is not abundant, likely due to its specificity and structural complexity.

Isoquinoline derivatives and spiro-fused compounds are known for their diverse biological activities, including antimicrobial, anticancer, and anti-inflammatory effects (Mahadeviah et al., 2024). Spiro compounds are generally studied for their pharmacological applications, as their rigid structures often make them effective enzyme inhibitors or bioactive molecules (Acosta-Quiroga et al., 2021).

4.7.14 Flavone, 4',5,6,7-tetramethoxy

Flavone, 4',5,6,7-tetramethoxy- (also known as 4',5,6,7-tetramethoxyflavone) is a naturally occurring methoxylated flavone. It belongs to a group known as Polymethoxyflavones (PMFs). PMFs have received increasing attention due to their

multiple bioactivities, such as antioxidant, anti-inflammatory, anti-cancer, metabolic regulatory, immunoregulatory, neuroprotective, and skin protective effects (Gan et al., 2023) .

Several hydroxylated PMFs exhibit strong anti-inflammatory properties. For instance, 5-demethylnobiletin has been shown to alleviate drug-induced ear and acute paw edema in mice, and to suppress delayed-type hypersensitivity by limiting cell infiltration and reducing inflammatory mediators, including IL-2, IL-4, TNF- α , and NO (Galea et al., 2017). Further studies by Mushtaq et al. (2023) show that Polymethoxyflavones (PMFs) are dietary bioactive phytochemicals, naturally present in all forms of citrus or plants. Li et al (2022), stated in their work that methoxylated flavones can possess antimicrobial properties, potentially inhibiting bacterial and viral growth, which makes them candidates for use as natural preservatives or anti-infective agents but more research needs to be done on this topic. Although less studied for direct phytotoxicity, flavonoids and methoxylated flavones can act as allelopathic agents, potentially inhibiting the growth of competing plants. They may affect cellular processes such as seed germination, root elongation, and enzyme activity in susceptible plant species (Shukla et al., 2023).

4.7.15 Acetamide, N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)

Acetamide, N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)- is a synthetic compound with potential pharmacological and agricultural applications due to its structural features, which include multiple methoxy groups and a benzo[a]heptalene scaffold.

Research on compounds with similar structures has shown activities that span several bioactive properties, though specific data on this particular acetamide derivative may still be developing (Zaoui et al., 2021).

Due to its aromatic and methoxylated structure, this compound may exhibit allelopathic properties, which are common in many secondary plant metabolites (Yang et al., 2022).

4.7.16 Diethyl-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate

Diethyl-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate is a synthetic compound within the pyridine dicarboxylate family, and related compounds have demonstrated various bioactivities, particularly in pharmacological applications (Ali et al, 2022). The ester and phenyl functional groups in the compound have a say in the potential antioxidant and anti-inflammatory effects. Similar compounds are known to scavenge free radicals and reduce oxidative stress, possibly making this compound relevant in oxidative stress-related disorders (Kumar et al., 2023).

Compounds in the pyridine dicarboxylate group may exhibit allelopathic effects, which impact neighbouring plants by inhibiting root elongation and seed germination. This could position Diethyl-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate as a potential natural herbicide or growth inhibitor in agricultural applications (Singh et al, 2021)

4.7.17 2H-1-Benzopyran-6-ol,3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R(4R,8R*)]]-**

2H-1-Benzopyran-6-ol,3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R(4R,8R*)]]-, also commonly known as α -Tocopherol (a form of Vitamin E),

is well-studied for its bioactive properties, particularly its antioxidant activity. Alpha-tocopherol is a lipid-soluble antioxidant that protects cell membranes and nervous tissues. It works by scavenging hydroperoxyl radicals and inhibiting the production of new free radicals. Alpha-tocopherol is most effective when combined with vitamin C, which neutralizes lipid hydroperoxyl radicals (Dasgupta et al, 2014). Also, α -Tocopherol is a potent antioxidant that protects cell membranes from oxidative damage by scavenging free radicals, and is widely used in both medical and cosmetic applications due to these properties (Upadhyay et al., 2023).

With regard to the anti-inflammatory properties of vitamin E, several studies have demonstrated that vitamin E could inhibit the secretion of inflammation-mediating molecules such as eicosanoids and cyclooxygenase-2 enzyme (COX-2) (Kanchi et al., 2017).

α -Tocopherol has demonstrated anti-inflammatory and neuroprotective effects, reducing inflammation markers and supporting nerve cell health. It has been studied for its potential in managing chronic conditions, including neurodegenerative diseases (Zaffarin et al., 2020).

Recent studies have noted that α -Tocopherol and other tocopherols can influence plant growth, showing mild allelopathic effects when introduced in high concentrations. This suggests it could have a phytotoxic effect under certain conditions, impacting seed germination and seedling growth (Yilmaz et al, 2021). Due to its ability to inhibit oxidative stress, α -Tocopherol is often used in skin care for its protective effects on skin integrity. It also supports immune health by protecting immune cells from oxidative damage.

4.7.18 Cholest-5-ene, 3-methoxy-, (3 β -)

Cholest-5-ene, 3-methoxy-, (3 β -), also known as 3 β -Methoxycholest-5-ene, is a sterol derivative that has garnered attention for its potential bioactivity, primarily in relation to its structural similarities with cholesterol and other bioactive sterols. Similar to other sterol compounds, 3 β -Methoxycholest-5-ene has demonstrated antifungal and antimicrobial properties. Research suggests that it disrupts cell membrane integrity in fungal and bacterial cells, making it effective in inhibiting their growth (Upadhyay et al., 2023). Preliminary studies indicate that sterol derivatives like 3 β -Methoxycholest-5-ene may have an anti-inflammatory effect, as they can reduce inflammatory marker expression in certain cell types, although more research is needed to confirm these effects (Rahman et al., 2022).

While studies specifically on 3 β -Methoxycholest-5-ene's phytotoxicity are limited, sterols and sterol derivatives have been known to exhibit allelopathic effects, which could potentially apply to these compound similar sterols have shown inhibitory effects on seed germination and early seedling growth in some plant species, suggesting that 3 β -Methoxycholest-5-ene may have potential phytotoxic effects if introduced in sufficient concentrations (Singh et al., 2021).

4.7.19 Stigmasta-5,22-diene-3-methoxy, (3 β ,22E)-

Stigmasta-5,22-diene-3-methoxy, (3 β ,22E)-, commonly known as a methoxylated derivative of stigmasterol, has been explored in recent research for its bioactive properties, including anti-inflammatory and anticancer activities. Methoxylated sterol compounds are noted for their anti-inflammatory effects, which arise from inhibiting key inflammatory mediators (Bakrim et al., 2022). This compound, through similar structural mechanisms as stigmasterol, may exhibit similar activity by downregulating

pro-inflammatory cytokines. Preliminary evidence suggests that Stigmasta-5,22-diene-3-methoxy exhibits antimicrobial effects, particularly against gram-positive bacteria, which further supports its potential for therapeutic use in infection control (Carcione et al., 2023).

As a sterol derivative, this compound is likely to exhibit allelopathic effects. Research on similar sterol compounds shows that they can slow down seed germination and seedling growth in plants, suggesting possible phytotoxic activity for Stigmasta-5,22-diene-3-methoxy (Kim et al., 2021).

4.7.20 Ergosta-4,22-dien-3-one

Ergosta-4,22-dien-3-one, a sterol derivative commonly found in fungi and some plants, has been investigated for its bioactive properties, particularly in the areas of anticancer, anti-inflammatory, and antimicrobial effects. Research indicates that Ergosta-4,22-dien-3-one has notable antimicrobial effects, particularly antifungal properties. This compound disrupts fungal cell membrane integrity, making it effective against fungal pathogens, including those that affect crops and are resistant to other treatments (Wang et al, 2022). There is evidence that compounds structurally related to Ergosta-4,22-dien-3-one can interfere with plant growth, particularly in terms of root development and cellular differentiation, supporting its potential as a natural herbicidal or phytotoxic agent. These references indicate that Ergosta-4,22-dien-3-one possesses significant bioactivity with antifungal properties and also holds potential phytotoxic activity, making it valuable for both therapeutic and agricultural research contexts.

4.7.21 Cholest-4-en-3-one

Cholest-4-en-3-one, a cholesterol-derived steroidal ketone, has been studied for its bioactivity, particularly due to its structural similarity to other cholesterol derivatives that exhibit pharmacological properties. In a study to investigate the antibacterial activity of cholest-4-en 3-one (cholestenone), a cholesterol analog, against *H. pylori* both in vitro and in vivo. The presence of cholesterol did not change the inhibitory effects of cholestenone, and when the *H. pylori* standard strain ATCC 43504 was cultured in the presence of cholestenone, microbial growth was considerably reduced dose-dependently in contrast to microorganisms cultured with cholesterol (Kobayashi et al., 2021). This compound has shown moderate antibacterial and antifungal activity, possibly due to its ability to disrupt microbial cell membranes, similarly to other cholesterol derivatives (Cheng et al, 2022).

Cholest-4-en-3-one has been explored for its anti-inflammatory potential by modulating inflammatory pathways and reducing the release of pro-inflammatory cytokines. This bioactivity could make it useful in treating inflammation-related conditions (Nagao et al., 2021).

Cholest-4-en-3-one has been associated with allelopathic effects, such as inhibition of seed germination and suppression of root and shoot growth in certain plant species (Kumar et al., 2021). This may be attributed to its interference with plant hormonal pathways, particularly affecting auxin transport and cell differentiation. Due to its growth-inhibiting effects, Cholest-4-en-3-one is studied for its potential as a natural herbicidal agent in agriculture. It affects early stages of plant development, suggesting its utility in controlling weed growth.

This goes to confirm that Cholest-4-en-3-one is both bioactive, with cytotoxic and antimicrobial properties, and phytotoxic, affecting seed germination and plant growth, making it valuable in medical and agricultural research contexts.

4.7.22 1,5-Diphenyl-1,5-pentanedione

1,5-Diphenyl-1,5-pentanedione, also known as dibenzoylmethane, is a diketone compound that has been explored for bioactive properties, including anticancer, antioxidant, and antimicrobial activities (Youssef et al., 2023). Dibenzoylmethane has demonstrated strong antioxidant properties, which may help protect cells from oxidative damage, thereby contributing to its potential as a therapeutic agent against diseases associated with oxidative stress (Lee et al., 2018).

Dibenzoylmethane has been observed to interfere with seed germination and early plant development, indicating phytotoxic potential. These effects are hypothesized to result from disruption of cellular redox balance or inhibition of key enzymes in plants (Choi et al, 2021). This compound has been studied for its effects on surrounding plants when present in soil, showing potential as an allelopathic agent that can suppress weed growth in agricultural settings (Choi et al., 2021). These references highlight that 1,5-Diphenyl-1,5-pentanedione exhibits notable bioactive properties, including anticancer, antioxidant, and anti-inflammatory activities, as well as phytotoxic effects, which could be useful for agricultural applications.

4.7. 23 Androstan-3-one,17-hydroxy-2-(hydroxymethylene)-17-methyl-, (5 α ,17 β)

Androstan-3-one,17-hydroxy-2-(hydroxymethylene)-17-methyl-, (5 α ,17 β)-, commonly referred to as a modified androstanolone or dihydrotestosterone (DHT)

derivative, is a steroidal compound that has been studied primarily for bioactivities related to hormone function, especially in animal and cell-based models. As a derivative of dihydrotestosterone (DHT), this compound exhibits androgenic effects, influencing androgen receptors and showing potential roles in the regulation of male sexual characteristics, muscle development, and secondary male features (Kinter et al., 2023).

4.7. 24 Androstan-3-one,17-hydroxy-2-methyl-, ($2\alpha,5\alpha,17\beta$)-

Androstan-3-one,17-hydroxy-2-methyl-, ($2\alpha,5\alpha,17\beta$)- is a steroid compound structurally related to androstanolone derivatives and modified dihydrotestosterone (DHT). This compound has been examined primarily for bioactivity related to its hormonal and androgenic effects. Androstan-based steroids can show anti-inflammatory effects by interacting with androgen receptors that mediate inflammatory pathways. This property can be beneficial in wound healing and tissue repair, as well as in reducing inflammation in some immune responses (Miller et al 2022).

Androstan derivatives have been examined for their role in inhibiting cancer cell growth, especially in hormone-sensitive cancers such as prostate and breast cancers in other studies.

Androstan-3-one,17-hydroxy-2-methyl-, ($2\alpha,5\alpha,17\beta$)- exhibits bioactive properties mainly in androgenic, anti-inflammatory, and potential anticancer applications. There is limited evidence regarding any direct phytotoxicity of this compound.

In conclusion, previous research has shown that extract of *Morinda lucida* contains several secondary metabolites, which are active compounds that have potential

therapeutic properties, with some isolated compounds exhibiting antimicrobial properties (Achukwu et al 2024). The identified compounds include terpenoids, flavonoids, alkaloids, and phenolic compounds. Some of these compounds have been reported to exhibit various biological activities. Further studies are needed to isolate and characterize the individual compounds identified in the GC-MS analysis and to evaluate their safety and efficacy *in vivo*.



CHAPTER FIVE

SUMMARY, CONCLUSION, RECOMMENDATION AND SUGGESTIONS

5.1 Overview

This chapter discusses the key findings of the study on antimicrobial activities of *Morinda lucida* stem bark extract against a range of bacterial and fungal microbes, antioxidant activities of *Morinda lucida* stem bark using DPPH and ABST, phytotoxic effects of *Morinda lucida* stem bark extracts on *Lactuca sativa* seeds and GC-MS profiling of compounds present in *Morinda lucida*. It interprets the results in the context of existing literature, acknowledges study limitations, and suggests practical applications and directions for future research.

5.2 Summary of Findings

Eight microbes were treated with *Morinda lucida* extract of different concentrations using both Agar well diffusion method and broth micro-dilution method, in this study to ascertain the medicinal efficacy of *Morinda lucida* stem bark extracts. The zone of inhibition of *Morinda lucida* stem bark extracts varied among the different microbes at different concentrations. *S. aureus* exhibited the highest level of inhibition, while *A. niger* showed the lowest susceptibility with the rest of the microbes in-between.

Free radical scavenging potential of the *Morinda lucida* stem bark extract was investigated in this study using ABTS and DPPH solution. ABTS assay results for *Morinda lucida* stem bark extracts, reveal a similar trend of concentration-dependent antioxidant activity. The percentage of ABTS scavenging increases with higher concentrations of the extract, further highlighting the strong antioxidant potential of

Morinda lucida extracts compared to Vitamin C. DPPH assay results for *Morinda lucida* stem bark extracts show a concentration-dependent increase in antioxidant activity. As the concentration of the extract increases from 0.0625 to 2 mg/mL, the percentage of DPPH scavenging also increases, indicating the potent antioxidant properties of *Morinda lucida* extracts compared to Vitamin C.

Plant secondary metabolites have been giving much attention by researchers as an alternative source of weedicide and have the possible to eradicate these damaging effects of synthetic herbicide (Divekar et al., 2022). In this study, the crude extract's phytotoxicity was evaluated using three treatments—TR1, TR2, and TR3—with increasing concentrations of 19.61 $\mu\text{g}/\mu\text{L}$, 39.22 $\mu\text{g}/\mu\text{L}$, and 78.44 $\mu\text{g}/\mu\text{L}$, respectively. TR3 showed the strongest effect, reducing shoot length by 80% and root length by 85% compared to the control.

This indicates the presence of phytotoxic compounds in the plant extract and suggests that *Morinda lucida* could potentially be developed as a natural herbicide.

GC-MS profiling analysis indicated the presence of terpenoids, flavonoids, alkaloids, organic acids and phenol compounds. Some of these compounds have been reported to exhibit various biological activities

5.3 Conclusions

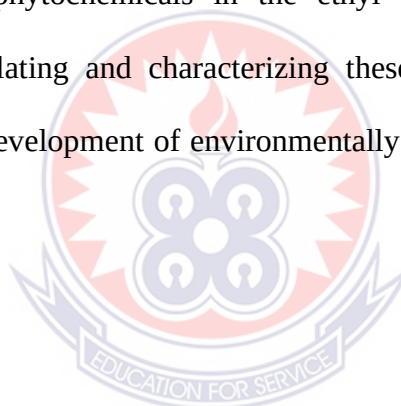
The study revealed that the ethyl acetate crude extract of the stem bark of *Morinda lucida* exhibited strong antimicrobial activities and has a potential to be developed as an antimicrobial principle. It also revealed strong antioxidants properties of the crude extract. The phytotoxicity study revealed that, the *Morinda lucida* extract has strong phytochemical which inhibits the germination and growth of *Lactuca sativa* seedlings.

A GC-MS profiling of the extract revealed the presence of 24 compounds with several biological activity reports on them in literature. This study provides a basis for further investigation into the potential of *Morinda lucida* extract as natural herbicides.

5.4 Recommendations

Further studies are needed to isolate and characterize the individual compounds identified in the GC-MS analysis and to evaluate their safety and efficacy in vivo. This would provide a guide in the development of an antimicrobial principle.

The strong phytotoxic activity exhibited by the extract suggests the presence of significant bioactive phytochemicals in the ethyl acetate crude extract. Further research aimed at isolating and characterizing these compounds could provide a valuable lead for the development of environmentally friendly, natural product-based herbicides.



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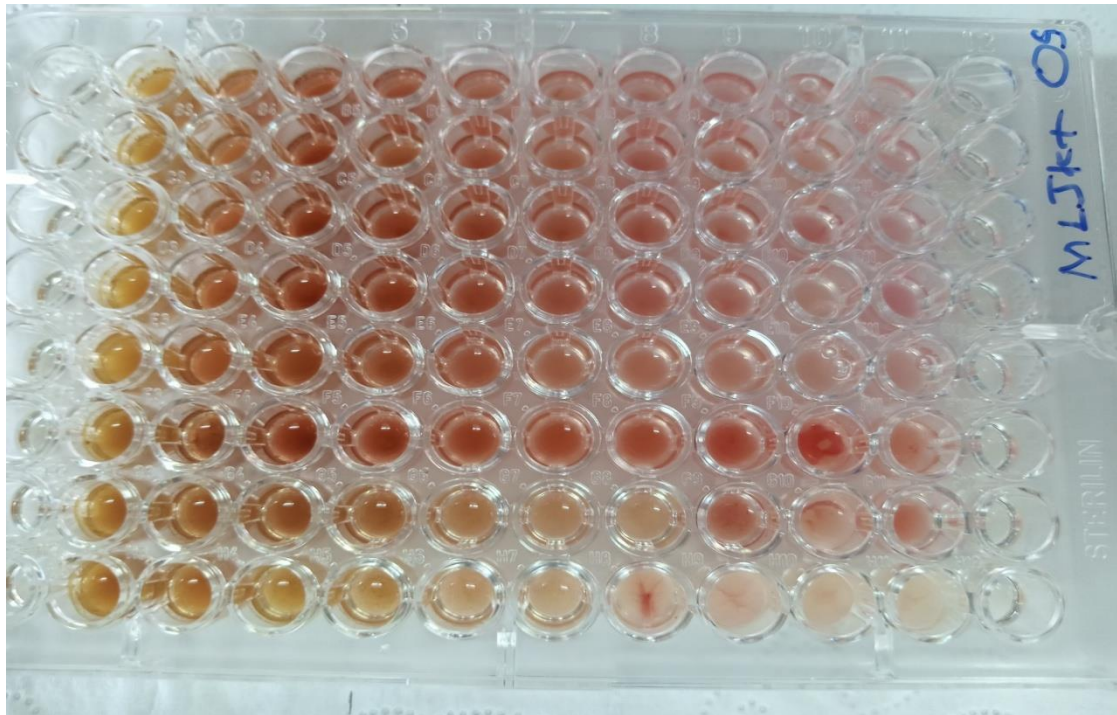


APPENDIX A

Antimicrobial Activity

Organism	EXP1	EXP2	EXP3	MEAN MIC	MBC	MBC/MIC
<i>E.coli</i>	10	10	10	10	20	2 ^{bc}
<i>K.</i>	1.25	1.25	1.25	1.25	2.5	2 ^{bc}
<i>pneumonia</i>						
<i>S. aureus</i>	10	10	10	10	20	2 ^{bc}
<i>S.typhii</i>	10	10	10	10	20	2 ^{bc}
<i>P.aeruginosa</i>	1.25	1.25	1.25	1.25	5	4 ^{bc}

<i>C. albicans</i>	10	10	10	10	20	2 ^{bc}
<i>A.niger</i>	0.156	0.156	0.156	0.156	2.5	16 ^{bs}
<i>P.megakarya</i>	0.313	0.313	0.313	0.313	0.56	2 ^{bc}



Antimicrobial activities

APPENDIX B

Antioxidant Activities

Antioxidant Activities of Test Samples

ABTS

Conc		MLT	
2		0.163	0.13
1		0.231	0.198
0.5		0.263	0.239
0.25		0.27	0.29
0.125		0.272	0.243
0.0625		0.275	0.255
Cotrol		0.317	0.255

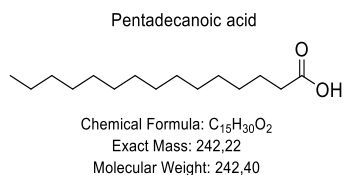
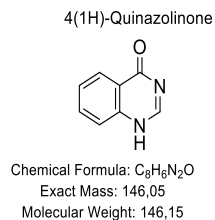
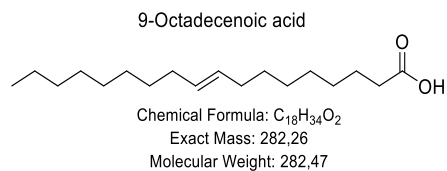
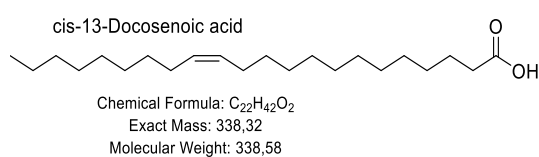
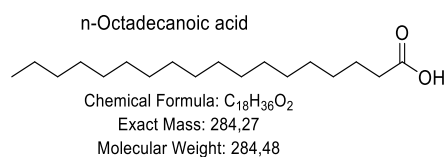
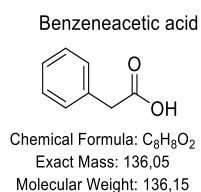
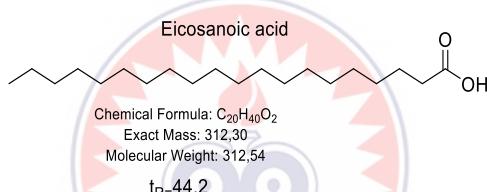
DPPH

Conc			
2	0.338	0.345	
1	0.387	0.379	
0.5	0.388	0.381	
0.25	0.389	0.383	
0.125	0.395	0.389	
0.0625	0.486	0.479	
Cotrol	0.858	0.979	

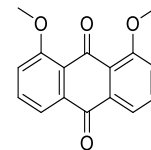
APPENDIX C

GC-MS Profiling

Structures of Compounds

 $t_R=38.1$  $t_R=39.4$  $t_R=41.4$  $t_R=41.5$  $t_R=41.8$  $t_R=43.2$  $t_R=44.2$

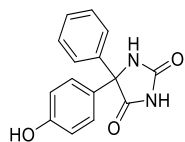
9,10-Anthracenedione, 1,8-dimethoxy-



Chemical Formula: $C_{16}H_{12}O_4$
Exact Mass: 268,07
Molecular Weight: 268,27

 $t_R=44.5$

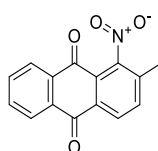
Hydantoin, 5-(p-hydroxyphenyl)-5-phenyl-



Chemical Formula: $C_{15}H_{12}N_2O_3$
Exact Mass: 268,08
Molecular Weight: 268,27

$t_R=44.7$

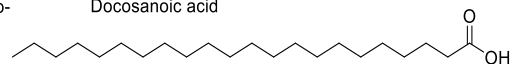
9,10-Anthracenedione, 2-methyl-1-nitro-



Chemical Formula: $C_{15}H_9NO_4$
Exact Mass: 267,05
Molecular Weight: 267,24

$t_R=45.1$

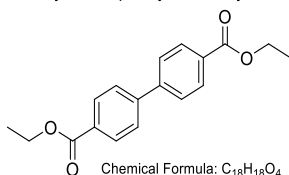
Docosanoic acid



Chemical Formula: $C_{22}H_{44}O_2$
Exact Mass: 340,33
Molecular Weight: 340,59

$t_R=46.1$

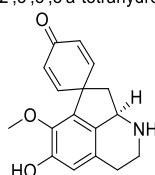
Diethyl 4,4'-biphenyldicarboxylate



Chemical Formula: $C_{18}H_{18}O_4$
Exact Mass: 298,12
Molecular Weight: 298,34

$t_R=46.3$

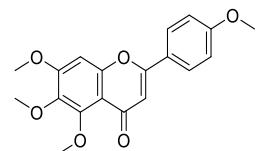
Spiro[2,5-cyclohexadiene-1,7'-(1'H)-cyclopent[ij]isoquinolin]-4-one, 2',3',8',8'a-tetrahydro-5'-hydroxy-6'-methoxy-, (R)-



Chemical Formula: $C_{17}H_{17}NO_3$
Exact Mass: 283,12
Molecular Weight: 283,33

$t_R=46.4$

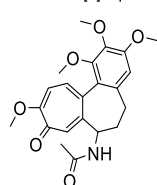
Flavone, 4',5,6,7-tetramethoxy-



Chemical Formula: $C_{19}H_{18}O_6$
Exact Mass: 342,11
Molecular Weight: 342,35

$t_R=48.1$

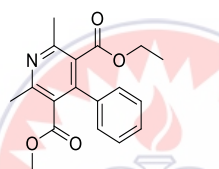
Acetamide, N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)-



Chemical Formula: $C_{22}H_{25}NO_6$
Exact Mass: 399,17
Molecular Weight: 399,44

$t_R=48.7$

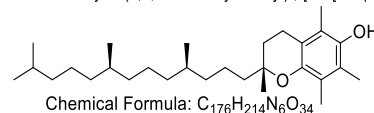
Diethyl 2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate



Chemical Formula: $C_{19}H_{21}NO_4$
Exact Mass: 327,15
Molecular Weight: 327,38

$t_R=49.0$

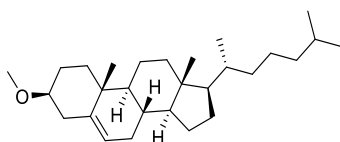
2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R'*(4R',8R*)]]-



Chemical Formula: $C_{176}H_{214}N_6O_{34}$
Exact Mass: 2955,52
Molecular Weight: 2957,66

$t_R=50.5$

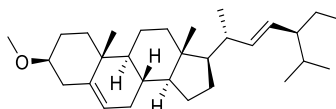
Cholest-5-ene, 3-methoxy-, (3.β.)-



Chemical Formula: C₂₈H₄₈O
Exact Mass: 400,37
Molecular Weight: 400,69

t_R=51.5

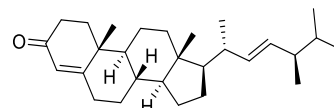
Stigmasta-5,22-diene, 3-methoxy-, (3.β.,22E)-



Chemical Formula: C₃₀H₅₀O
Exact Mass: 426,39
Molecular Weight: 426,73

t_R=51.8

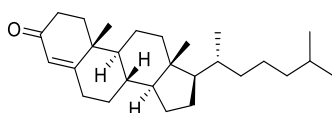
Ergosta-4,22-dien-3-one



Chemical Formula: C₂₈H₄₄O
Exact Mass: 396,34
Molecular Weight: 396,66

t_R=53.3

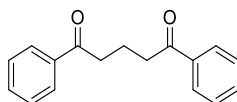
Cholest-4-en-3-one



Chemical Formula: C₂₇H₄₄O
Exact Mass: 384,34
Molecular Weight: 384,65

t_R=54.2

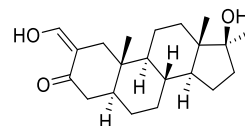
1,5-Diphenyl-1,5-pentanedione



Chemical Formula: C₁₇H₁₆O₂
Exact Mass: 252,12
Molecular Weight: 252,31

t_R=54.4

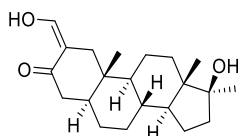
Androstan-3-one,17-hydroxy-2-(hydroxymethylene)-17-methyl-, (5.α.,17.β.)-



Chemical Formula: C₂₁H₃₂O₃
Exact Mass: 332,24
Molecular Weight: 332,48

t_R=54.6

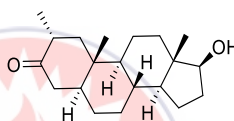
Androstan-3-one,17-hydroxy-2-(hydroxymethylene)-17-methyl-, (5.α.,17.β.)-



Chemical Formula: C₂₁H₃₂O₃
Exact Mass: 332,24
Molecular Weight: 332,48

t_R=54.6

Androstan-3-one, 17-hydroxy-2-methyl-, (2.α.,5.α.,17.β.)-



Chemical Formula: C₂₀H₃₂O₂
Exact Mass: 304,24
Molecular Weight: 304,47

t_R=55.2

