PREDICTION OF THE PHYSICOCHEMICAL POTENTIAL OF ETHANOLIC EXTRACT OF STINGING NEETLE LEAVE

An *In-silico* Approach

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CERTIFICATION

This is to certify that this project work was carried out by NAME OF STUDENT with matriculation number ND/23/SLT/PT/ and has been read and approved as meeting the requirements for the award of National Diploma (ND) in Science Laboratory Technology (SLT), Institute of Applied Sciences (IAS), Kwara State Polytechnic, Ilorin, Kwara State.

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DEDICATION

This project is dedicated to Almighty God for his merciful and blessing throughout the completion of this programme.

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I sincerely appreciate the Almighty God for His blessing, strength, sustenance and above all his faithfulness and love from the beginning of my academic life up to this level. His benevolence has made me excel and successful in my academic pursuits.

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ABSTRACTS

The importance of natural products in drug discovery remains undeniable, with over 50% of all FDA-approved drugs being derived from natural sources. In particular, plant-derived antimicrobials have gained significant attention due to their broad-spectrum activity against both gram-positive and gram-negative bacteria, as well as fungi and viruses. Among the vast array of medicinal plants, stinging nettle has attracted attention for its diverse pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Despite its widespread use in traditional medicine, limited scientific research has been conducted to fully explore its antimicrobial potential and the mechanisms underlying its bioactivity. While some studies have suggested its efficacy against bacterial and fungal infections, there remains a gap in understanding the full scope of its antimicrobial activity, particularly against multi-drug-resistant strains. The GC-MS analysis of the ethanolic extract derived from the leaves of Urtica dioica, commonly known as stinging nettle, disclosed an extensive phytochemical variety, consisting of 26 unique compounds characterized by different retention intervals and peak measurements. Some certain compounds display violations in Lipinski's rules or exhibit low solubility; the overall assessment indicates their potential applicability in the realm of drug discovery and the development of phytopharmaceuticals. It is recommended that further studies involving varying concentration, isolation of key active ingredients and collaborative trials with traditional antibiotics could assist in unlocking its complete medicinal benefits.

CHAPTER ONE

1.0 Introduction

1.1 Background of the Study

Natural products have long served as an essential source of medicinal compounds, contributing significantly to the development of therapeutic agents in modern medicine. Plants, in particular, are known for their remarkable ability to synthesize bioactive secondary metabolites that exhibit diverse pharmacological activities. The historical use of plant-based remedies spans across ancient civilizations, from Egypt to China, and continues to form the basis of many contemporary pharmaceutical preparations.

The importance of natural products in drug discovery remains undeniable, with over 50% of all FDA-approved drugs being derived from natural sources (Newman & Cragg, 2016). In particular, plant-derived antimicrobials have gained significant attention due to their broad-spectrum activity against both gram-positive and gram-negative bacteria, as well as fungi and viruses (Ncube et al., 2012). The increasing prevalence of antimicrobial resistance (AMR), which compromises the effectiveness of synthetic antibiotics, underscores the need for alternative therapies derived from natural sources. Plants represent a promising avenue for the discovery of novel antimicrobial agents capable of overcoming resistance and mechanisms.

Plants produce a diverse range of secondary metabolites that help them protect against various environmental stressors, including herbivores, pathogens, and UV radiation. These metabolites include alkaloids, flavonoids, terpenoids, glycosides, and phenolic acids, all of which have demonstrated varying degrees of antimicrobial activity. The search for new antimicrobial agents from plants is crucial in light of the global health threat posed by resistant bacterial strains, such as Methicillin-resistant Staphylococcus aureus (MRSA) and Vancomycin-resistant Enterococci (VRE) (Bauer et al., 2019).

1.2 Stinging Nettle

Stinging nettle, is a perennial herb native to Europe, Asia, and North Africa, and is now widely distributed in temperate regions globally. Belonging to the Urticaceae family, Urtica dioica is easily identified by its opposite, serrated leaves, and stinging hairs (trichomes) on the stems and leaves, which release histamine, acetylcholine, and formic acid upon contact with the skin, resulting in a burning and irritating sensation (Maiz-Tome, 2009; Brodal, 2004).

Despite its unpleasant sting, Urtica dioica has a long history of medicinal use in various cultures, particularly in the treatment of inflammatory disorders, arthritic pain, urinary tract infections, and skin ailments. More recently, scientific research has highlighted its antioxidant, anti-inflammatory, and antimicrobial properties, which can be attributed to the presence of a wide array of bioactive compounds.

The leaves of stinging nettle are rich in flavonoids, phenolic compounds, alkaloids, terpenoids, and essential minerals such as iron, calcium, and magnesium (Taheri et al., 2022). These compounds not only contribute to the plant's antioxidant capacity but also its antimicrobial activity. Studies have demonstrated that Urtica dioica extracts exhibit significant antimicrobial activity against a broad spectrum of bacteria, fungi, and viruses (Semalty et al., 2017). The antimicrobial activity has been particularly effective against Staphylococcus aureus, Escherichia coli, and Candida species, highlighting the potential of Urtica dioica as a natural antimicrobial agent.

The ethanolic extraction of Urtica dioica leaves has been widely used in research due to the solvent's ability to extract both polar and non-polar compounds, ensuring a comprehensive profile of the plant’s bioactive components.

Gas Chromatography-Mass Spectrometry (GC-MS) is commonly employed to analyze these extracts, enabling the identification of individual phytochemicals and their quantification (Kumari et al., 2015). Given the rising concerns over antimicrobial resistance, Urtica dioica presents a promising alternative for the development of novel therapeutic agents. The bioactive compounds identified in its leaves could offer a complementary approach to conventional antibiotics, contributing to the search for new antimicrobial drugs. Maiz-Tome, L. (2009).

1.3 Statement of the Problem

The increasing prevalence of antimicrobial resistance (AMR) has become one of the most pressing public health challenges of the 21st century. With the overuse and misuse of conventional antibiotics, many pathogenic microorganisms have developed resistance to commonly prescribed drugs, rendering them ineffective in treating infections. This problem is further compounded by the limited discovery and development of new antibiotics over the past few decades, leading to a growing "antibiotic crisis." The World Health Organization (WHO) has warned that, without urgent action, AMR could cause an additional 10 million deaths annually by 2050, surpassing cancer as the leading cause of death (WHO, 2019).

The urgent need for new antimicrobial agents is more critical than ever. While synthetic antibiotics have been the cornerstone of infectious disease management for decades, there is a growing realization of the limitations and risks associated with over-relying on these agents. In this regard, natural products offer a promising solution. Historically, plant-derived compounds have provided a significant source of antimicrobial agents, with several modern drugs being based on natural plant extracts.

Among the vast array of medicinal plants, stinging nettle has attracted attention for its diverse pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Despite its widespread use in traditional medicine, limited scientific research has been conducted to fully explore its antimicrobial potential and the mechanisms underlying its bioactivity. While some studies have suggested its efficacy against bacterial and fungal infections, there remains a gap in understanding the full scope of its antimicrobial activity, particularly against multi-drug-resistant strains.

Furthermore, the chemical composition of stinging nettle leaves, including the identity and concentration of bioactive compounds responsible for its antimicrobial effects, has not been comprehensively studied using modern analytical techniques. Gas Chromatography-Mass Spectrometry (GC-MS) offers a robust tool for profiling the chemical constituents of plant extracts, yet few studies have applied this technology to Urtica dioica extracts for a detailed chemical analysis.

Additionally, physicochemical properties, such as solubility, stability, and toxicity, need to be evaluated before they can be considered as viable candidates for pharmaceutical use. A computational approach, incorporating molecular docking and in silico analysis, is necessary to predict the biological activity and potential drug-like properties of these compounds.

This research aims to address these gaps by investigating GC-MS analysis to identify the active components, and evaluating the physicochemical properties of the extract. Furthermore, a computational approach will be employed to predict the interactions of the identified compounds with key microbial targets, contributing to the discovery of novel antimicrobial agents and advancing the development of natural products as alternatives to synthetic antibiotics.

1.4 Justification of the Study

The ethanol extract of stinging nettle leaves offers an excellent opportunity to study the plant's bioactive compounds in relation to antimicrobial efficacy. Ethanol is a commonly used solvent in

phytochemical extractions, providing a broad spectrum of both polar and non-polar compounds that may contribute to the plant's therapeutic effects (Kumari et al., 2015). Utilizing Gas Chromatography-Mass Spectrometry (GC-MS) for the analysis of the extract will enable the identification and quantification of specific phytochemicals responsible for the plant's antimicrobial activity. GC-MS has proven to be an invaluable tool in plant research, providing detailed and accurate data on the chemical composition of plant extracts, which can then be correlated with their biological activity.

By addressing the critical gaps in the current literature, this study will contribute valuable insights into the use of natural products as an alternative to synthetic antibiotics. The findings could pave the way for the development of new plant-based therapies, offering a safer, more sustainable solution to the problem of antimicrobial resistance.

1.5 Aims and Objectives

The primary aim of this study is to evaluate the physicochemical properties of ethanolic extracts of stinging nettle leaves using a computational approach. This study seeks to evaluate the bioactive compounds present in the plant and their antimicrobial activities, which could contribute to the development of novel natural antimicrobial agents.

Objectives of the Study:

To achieve the aim of this study, the following specific objectives will be pursued:

1. To carry out ethanolic extraction of stinging nettle leaves, isolating the bioactive components from the plant material for further analysis.
2. To analyze the bioactive compounds, present in Urtica dioica ethanolic extract using Gas Chromatography-Mass Spectrometry (GC-MS), identifying and quantifying the chemical constituents responsible for the antimicrobial activity.
3. 5. To predict the physicochemical properties of the bioactive compounds in Urtica dioica extract using the SwissADME server (Swiss Analysis of Molecular Drug-like Properties), assessing key parameters such as solubility, lipophilicity, and toxicity, to evaluate their suitability as potential drug candidates.

1.6 Scope of the Study

This study is focused on evaluating the GC-MS analysis, and physicochemical properties of the ethanolic extract of stinging nettle leaves. The research will be conducted within the following parameters:

This study will be based on the extraction of stinging nettle leaves sourced from [insert location if necessary, or state "locally sourced" or "commercially available"]. The plant material will be authenticated to ensure the correct species is used in the research. Only the leaves of Urtica dioica will be used in this study, as they are traditionally known for their medicinal properties. The extraction will be carried out using ethanol as a solvent, which is effective in isolating both polar and non-polar bioactive compounds.

The GC-MS analysis will be used to identify and quantify the bioactive compounds present in the ethanolic extract of Urtica dioica. Only volatile compounds detected through this method will be considered. The analysis will focus on determining the chemical composition and identifying potential active constituents responsible for antimicrobial properties.

The physicochemical properties of the bioactive compounds in the ethanolic extract will be assessed using the SwissADME server. Parameters such as solubility, lipophilicity, toxicity, and drug-likeness will be predicted, and only compounds with high potential for drug development will be considered. The study will not delve into the in vivo toxicity or long-term stability of the compounds. The study will include molecular docking studies to predict the interaction of identified compounds with microbial targets. The research will be limited to computational predictions, and experimental validation of molecular interactions will not be conducted.

1.7 Significance of the Study

This study holds significant potential in contributing to the growing body of knowledge surrounding natural products and their application in combating antimicrobial resistance (AMR). The antimicrobial properties of Urtica dioica, a plant traditionally used in folk medicine, could offer a novel approach to addressing the urgent need for new antimicrobial agents. With increasing resistance to conventional antibiotics, there is a critical demand for alternative therapies, particularly those derived from natural sources.

The significance of this research lies in the following areas:

1. ***Contribution to Public Health:*** The findings of this study could provide essential data for the development of new antimicrobial agents derived from plant-based sources. As

antimicrobial resistance continues to rise, discovering natural alternatives to synthetic antibiotics is crucial for maintaining effective treatments for infections caused by resistant pathogens. If successful, Urtica dioica extracts could potentially be developed into new therapeutic options, offering a safer and more sustainable solution to combat bacterial and fungal infections.

2. ***Pharmaceutical Development:*** By evaluating the physicochemical properties of the identified bioactive compounds using SwissADME, this study will offer valuable information on their drug-likeness and potential suitability for further development as pharmaceutical agents. This data will be essential in determining whether the compounds can be formulated into effective antimicrobial drugs, thereby contributing to the ongoing efforts in drug discovery and development.
3. ***Informatics and Computational Approach:*** The integration of computational methods, such as molecular docking studies, enhances the predictive capability of the study, offering insights into the potential mechanisms of action of the identified compounds. The computational predictions of molecular interactions with microbial targets will serve as a preliminary guide for future experimental studies, thus optimizing the drug development process and reducing time and costs.
4. ***Sustainability and Environmental Impact:*** Natural products, such as those derived from Urtica dioica, represent an environmentally friendly alternative to synthetic chemicals in the development of medicinal compounds. The use of ethanol as an extraction solvent is relatively safe and biodegradable, further emphasizing the sustainability of plant-based solutions. This study will contribute to the understanding of how natural resources can be utilized responsibly for the development of effective, eco-friendly therapies.
5. ***Future Research Directions:*** The results of this study could pave the way for further research into the antimicrobial potential of other medicinal plants, contributing to the broader field of natural product drug discovery. Additionally, this research may serve as a foundation for future studies that explore the synergistic effects of plant extracts and synthetic antibiotics, enhancing the effectiveness of available treatments.

CHAPTER TWO

2.0 Literature Review

The literature review serves as the foundation upon which this study is built, providing a comprehensive understanding of previous research related to natural products, medicinal plants, and their applications in drug discovery. It contextualizes the present study within the broader scientific conversation surrounding the use of computational tools in natural product research.

In recent decades, there has been a resurgence of interest in plant-derived compounds due to the rising challenge of antimicrobial resistance and the limitations associated with synthetic drugs. Medicinal plants, such as Urtica dioica (commonly known as stinging nettle), have long been valued for their therapeutic properties and are now being re-examined through modern scientific lenses, including phytochemical analysis and computational predictions.

This chapter explores relevant scholarly work concerning the effectiveness of ethanolic extraction methods, the use of Gas Chromatography-Mass Spectrometry (GC-MS) in identifying bioactive compounds, and the role of in silico tools like SwissADME in drug-likeness prediction. The goal is to identify key insights, highlight research gaps, and establish the relevance of this study in addressing current needs in antimicrobial research and natural drug development.

2.1 Overview of Natural Products in Medicine

Natural products have served as a cornerstone in the development of medicinal agents for centuries, with traditional knowledge forming the basis of modern pharmacology. Derived from plants, animals, and microorganisms, natural products are composed of a wide variety of bioactive compounds, many of which exhibit significant pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Newman & Cragg, 2020).

Historically, natural remedies formed the bulk of early healthcare systems, especially in indigenous and traditional medicine. Over time, scientific research has confirmed the medicinal efficacy of many of these substances, leading to the isolation and synthesis of active compounds. Notable examples include morphine from Papaver somniferum (opium poppy), quinine from Cinchona bark, and artemisinin from Artemisia annua — all of which have shaped modern drug development (Atanasov et al., 2021).

Natural products continue to play a crucial role in contemporary drug discovery, particularly in the search for novel antimicrobial agents. As antimicrobial resistance (AMR) becomes a critical global health threat, attention has shifted towards bioactive compounds from natural sources, which are

perceived to have fewer side effects and are often structurally distinct from synthetic compounds, making them effective against resistant strains (Lahlou, 2013).

Plant-based natural products, especially those extracted from leaves, roots, seeds, and barks, are rich in secondary metabolites such as alkaloids, flavonoids, terpenoids, phenols, and saponins. These compounds contribute to the plants' defense mechanisms and often translate to therapeutic benefits in humans. Current pharmacological research increasingly incorporates modern analytical techniques, such as Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC), to identify these bioactive constituents.

In recent years, the integration of in silico approaches and computational biology into natural product research has further enhanced the efficiency of screening for potential drug candidates. Computational tools now enable researchers to predict drug-likeness, toxicity, bioavailability, and molecular interactions of natural compounds, making natural product research more cost-effective and time-efficient (Jiménez-Luna et al., 2021).

In summary, natural products remain indispensable in the field of drug development, especially as a response to global challenges such as antimicrobial resistance. Their unique chemical structures, coupled with centuries of traditional use and advancing analytical technologies, make them vital candidates for novel therapeutic agents.

2.2 Botanical and Pharmacological Overview of Stinging nettle

Stinging nettle, commonly known as stinging nettle, is a perennial herbaceous plant belonging to the Urticaceae family. Native to Europe, Asia, and North Africa, it has since spread globally, thriving in temperate regions worldwide.

Morphological Characteristics:

- Size: Urtica dioica typically grows between 1 to 3 meters in height during the summer months, with the aerial parts dying back in winter.
- Leaves: The plant bears opposite, ovate to heart-shaped leaves measuring 3 to 15 centimeters in length. These leaves have serrated margins and are sparsely covered with stinging hairs.
- Stems: The erect, four-sided stems are armed with stinging hairs that cause a burning sensation upon contact.
- Flowers: Urtica dioica produces small, greenish or brownish flowers arranged in dense axillary inflorescences.

Habitat and Distribution: Urtica dioica prefers moist, nitrogen-rich soils and is commonly found along streams, meadows, ditches, woodland clearings, and disturbed areas. Its widespread presence across various continents underscores its adaptability to diverse environmental conditions.

Pharmacological Properties: Urtica dioica has been utilized in traditional medicine for centuries, and modern research has validated many of its purported health benefits. The plant contains a variety of bioactive compounds, including flavonoids, tannins, sterols, fatty acids, and polysaccharides, contributing to its therapeutic effects.

Key Pharmacological Activities:

- Anti-Inflammatory: Extracts from Urtica dioica have demonstrated the ability to inhibit pro-inflammatory pathways, offering potential relief for conditions like arthritis.
- Antioxidant: The plant exhibits significant antioxidant properties, scavenging free radicals and reducing oxidative stress, which is beneficial in preventing chronic diseases.
- Antimicrobial: Studies have shown that Urtica dioica possesses antibacterial and antiviral activities, making it a candidate for developing natural antimicrobial agents.
- Analgesic: The plant has been reported to alleviate pain, providing a natural alternative to synthetic analgesics.
- Anticancer: Preliminary studies suggest that Urtica dioica extracts can inhibit the proliferation of cancer cells and induce apoptosis, highlighting its potential as an anticancer agent.
- Hepatoprotective: Research indicates that the plant offers protective effects against liver damage, supporting its use in liver-related ailments.
- Antidiabetic: Some studies have observed that Urtica dioica can help regulate blood glucose levels, suggesting a role in managing diabetes.
- Antiallergic: The plant has been traditionally used to alleviate allergy symptoms, and some evidence supports its role in modulating allergic reactions.
- Neurological Benefits: There is emerging evidence that Urtica dioica may have neuroprotective effects, potentially aiding in the management of conditions like Alzheimer's disease.

Safety and Toxicity: While Urtica dioica offers numerous health benefits, it can cause adverse effects such as dermatitis upon direct contact due to its stinging hairs. Additionally, consumption

should be approached with caution in individuals on certain medications or with specific health conditions. Consulting with a healthcare provider before incorporating Urtica dioica into one's health regimen is advisable.

2.3 Phytochemical Composition of Stinging nettle

Urtica dioica, commonly known as stinging nettle, possesses a rich array of phytochemicals that contribute to its diverse pharmacological activities. The plant's various parts—leaves, stems, roots, and seeds—contain distinct bioactive compounds, including alkaloids, flavonoids, tannins, sterols, fatty acids, and vitamins. Understanding these constituents is essential for elucidating the therapeutic potential of Urtica dioica.

Alkaloids: Urtica dioica contains alkaloids such as histamine and acetylcholine, which are responsible for the dermatitis caused upon contact with the plant.

Flavonoids: The plant is rich in flavonoids, including quercetin, kaempferol, and rutin. These compounds exhibit antioxidant, anti-inflammatory, and anticancer properties.

Tannins: Tannins present in Urtica dioica contribute to its astringent properties and have been associated with antimicrobial and anti-inflammatory effects.

Sterols and Triterpenoids: Phytochemical analyses have identified the presence of sterols and triterpenoids in Urtica dioica, compounds known for their anti-inflammatory and analgesic properties.

Fatty Acids: The plant's fatty acid profile includes palmitic, stearic, oleic, linoleic, and α-linolenic acids. Notably, mature leaves contain about 40% α-linolenic acid, a valuable omega-3 fatty acid.

Vitamins: Urtica dioica is a source of vitamins A (as carotenoids), C, K1, and several B vitamins, including riboflavin and pantothenic acid. These vitamins play vital roles in antioxidant defense, blood clotting, and energy metabolism.

Minerals and Trace Elements: The plant contains essential minerals such as calcium, potassium, magnesium, phosphorus, sulfur, and chlorine, along with trace elements like titanium, manganese, copper, and iron.

Carotenoids: Leaf carotenoids, including lutein and β-carotene, contribute to the plant's antioxidant capacity. Mature leaves have a higher concentration of these compounds compared to young leaves.

Essential Oils: GC/MS analysis of Urtica dioica essential oil has identified compounds such as α-pinene, β-pinene, and β-caryophyllene, which exhibit antioxidant and anti-inflammatory properties.

Lignans: Lignans present in the plant have been associated with antioxidant and anticancer activities.

The diverse phytochemical profile of Urtica dioica underpins its traditional and contemporary medicinal applications. Ongoing research continues to uncover the therapeutic potentials of these compounds, offering insights into novel drug development.

2.4 Ethanolic Extraction and Its Relevance

Extraction is a fundamental step in the isolation and identification of bioactive compounds from medicinal plants. It plays a critical role in phytochemical research by helping to isolate compounds of interest in a concentrated and bioavailable form. Among various extraction methods, ethanol is widely recognized as one of the most effective and commonly used solvents due to its ability to extract a broad range of phytochemicals.

Ethanol as a Solvent: Ethanol is a polar organic solvent that is particularly suitable for extracting both polar and some non-polar compounds, including flavonoids, tannins, phenolics, saponins, alkaloids, and terpenoids (Tiwari et al., 2011). Its relatively low toxicity, ease of availability, and compatibility with food and pharmaceutical applications make it a preferred choice in both laboratory and industrial settings.

Advantages of Ethanolic Extraction:

- Wide Solubility Spectrum: Ethanol can extract a diverse group of phytochemicals, ensuring a more comprehensive phytochemical profile than many other solvents.
- Safety: It is considered safe for human use and suitable for pharmaceutical formulations.
- Antimicrobial Stability: Ethanolic extracts often exhibit greater stability and enhanced antimicrobial activities compared to aqueous extracts due to better solubilization of active compounds (Azwanida, 2015).
- Compatibility with Analytical Tools: Ethanol-based extracts are ideal for subsequent analyses using techniques like Gas Chromatography-Mass Spectrometry (GC-MS), which requires solvent systems that do not interfere with compound separation or detection.

Relevance in *Urtica dioica* Research: Ethanolic extraction has been widely applied in the phytochemical screening and pharmacological assessment of Urtica dioica. Studies have shown

that ethanolic extracts of the leaves possess significant biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects (Gülçin et al., 2004). These extracts typically show higher efficacy compared to those obtained with non-polar solvents due to the broader range of soluble phytochemicals present.

Extraction Procedure Overview: The process generally involves drying and pulverizing plant material, followed by maceration or Soxhlet extraction in ethanol for a specified duration. The extract is then filtered, concentrated, and stored for further analysis or biological testing.

In this study, the use of ethanolic extraction serves as a preparatory step to enable effective phytochemical analysis, antimicrobial screening, and GC-MS profiling of Urtica dioica leaves. The selection of ethanol ensures that the extract retains a rich composition of active compounds, providing a strong basis for understanding the plant's medicinal potential.

2.5 Application to Stinging nettle Leave

Urtica dioica has been widely studied for its antimicrobial effects. Research indicates that its ethanolic extracts possess notable inhibitory effects against both Gram-positive and Gram-negative bacteria, as well as certain fungal strains. The antimicrobial potential is largely attributed to the presence of flavonoids, phenolic compounds, tannins, and terpenoids in the leaves (Gülçin et al., 2004).

The relevance of studying the antimicrobial activity of Urtica dioica lies in its potential as a natural and accessible alternative to synthetic antibiotics, especially in the face of growing AMR.

2.6 Gas Chromatography-Mass Spectrometry (GC-MS) and Its Role in Phytochemical Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful and widely used analytical technique for identifying and quantifying volatile and semi-volatile compounds in complex mixtures, especially in plant-based research. It combines the features of gas chromatography (GC) and mass spectrometry (MS) to provide detailed information about the chemical composition of natural products, including medicinal plant extracts.

2.6.1 Principle of GC-MS

Gas Chromatography (GC): This component separates the different compounds in a mixture based on their volatility and interaction with the column's stationary phase. Compounds with lower boiling points or less interaction with the column elute faster.

Mass Spectrometry (MS): Once separated, the compounds are ionized and fragmented in the MS unit. These fragments are detected and presented as a mass spectrum, which acts as a unique "fingerprint" for each compound.

GC-MS is particularly effective in identifying volatile organic compounds such as terpenoids, fatty acids, esters, and phenolic compounds—many of which are bioactive constituents in medicinal plants (Miller, 2002).

Advantages of GC-MS in Natural Product Research

- *High Sensitivity and Accuracy:* GC-MS can detect even trace amounts of compounds with high specificity.
- *Qualitative and Quantitative Analysis:* It provides both the identification and relative concentration of compounds.
- *Extensive Libraries:* Software libraries (e.g., NIST) help match the spectral data with known compounds.
- *Time-Efficient:* Allows rapid screening of a wide array of phytochemicals from plant extracts.

2.7 Physicochemical Properties and Computational Analysis Using SwissADME

The evaluation of physicochemical properties is crucial in determining the drug-likeness and pharmacokinetic behavior of bioactive compounds. These properties influence the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles, which are key factors in drug discovery and development. In recent years, computational tools such as SwissADME have become indispensable for predicting these properties efficiently and reliably.

SwissADME is a free web-based computational platform developed by the Swiss Institute of Bioinformatics. It allows for the prediction of physicochemical descriptors, pharmacokinetic properties, drug-likeness, and medicinal chemistry friendliness of small molecules (Daina et al., 2017). This tool plays an essential role in virtual screening and helps streamline the selection of promising bioactive compounds from plant extracts for further drug development.

Parameters Predicted by SwissADME:

- *Lipophilicity (Log P):* Determines membrane permeability and solubility. Compounds with optimal lipophilicity are more likely to cross cell membranes effectively.
- *Water Solubility:* Essential for drug absorption and bioavailability.
- *Topological Polar Surface Area (TPSA):* Indicates the compound's ability to interact with biological membranes and transporters.
- *Molecular Weight:* Affects the compound's diffusion and transport.
- *Number of Hydrogen Bond Donors and Acceptors:* Influences solubility and interaction with biological targets.
- *Pharmacokinetics:* Includes predictions on gastrointestinal (GI) absorption, blood-brain barrier permeability, and interaction with cytochrome P450 enzymes.

CHAPTER THREE

3.0 Methodology

3.1 Materials

Stinging Leave; Ethanol; Mentholated spirit; Nutrient agar, measuring cylinder; Water bath; Distilled water; Beaker; Air tight bottle; weighing balance; Latex and rubber gloves; Funnel; Filter paper; Petri dish; Wire loop; Cork borer; Spatula; Conical flask; Foil paper; Cotton wool; stirring rod.

3.2 Methods

3.2.1 Collection of plant sample

Fresh *Urtica dioica* leaves was obtained from Kwara State Polytechnic, Ilorin. The leaves collected were cleaned, rinsed, and air-dried for 15 days. After drying, the leaves will be ground into fine powder using a mortar and pestle and preserved in a refrigerator.

3.2.2 Preparation and Extraction of plant sample

Using a weighing balance, 50g of dried *U. dioica* leave powder was weighed into a clean labelled beaker and 350ml of water was added and stirred thoroughly. The solution was left for 24hrs untouched. Another 50g of dried *U. dioica* leave powder was weighed into a clean labelled beaker and 250ml of ethanol was added and stirred thoroughly. The solution was left for 24hrs untouched. After 24 hours, those mixtures will be filtered with Whatman No.1 filter paper. Filtrates were then stored in an air tight bottle and were concentrated to dryness using water bath. The extraction method of Stinging nettle was carried out using the method described by Muhammed *et al.* (2018).

3.2.3 GC-MS Analysis of Extracted Root and Leaves of *U. dioica*

The GC-MS of *U. dioica* was carried out using the method described by Ibrahim *et al.* (2022). The analysis involved the use of an Agilent 6890 gas chromatograph equipped with a mass spectrometric detector (MSD) model Agilent 5973. A fused silica capillary column (HP-5MS), 5% phenyl polysiloxane as non-polar stationary phase (30 m60.25 mm6i.d) and 0.25 mm film thickness was used. Operating conditions were as follows: injector port temperature, 2508C. Helium was used as a carrier gas at a flow rate of 1.0 ml/min pulsed split less mode programmed at 88C/min to 2608C, and held for 18 min. The total analysis time was 41 min. A 1 ml volume was injected into the MSD. The mass MSD was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from m/z 50–500. The ion source temperature was 2308C and the quadruple temperature was 1508C. The electron multiplier voltage (EM voltage) was

maintained at 1100 V above auto tune, and a solvent delay of 3 min was employed. The instrument was manually tuned using perfluorotributylamine (PFTBA). Identification was based on comparison with the MS computer library (NIST Software Package, Finnigan) and on the respective retention indices. The separated components were identified by matching data with those of the data published by Wiley (Wiley7n.1) library of mass spectra and literature comparison. This was carried out in Chemical Engineering Laboratory in University of Ilorin.

3.3.4 Evaluation of the *in silico* Physicochemical Properties of Ethanolic Extracts of *Urtica dioica* Leaves.

SwissADME (https://www.swissadme.ch/) was used for the prediction of the physicochemical properties of compounds characterized from aqueous and methanolic extracts of *C. odorata* roots and leaves. The address was launched on google chrome and the canonical smiles of the compounds downloaded from PubChem were pasted on the SwissADME dialogue box to run the prediction.

CHAPTER FOUR

4.0 Results

4.1 Ethanolic Extracts Yield

The concentration of ethanolic extracts of stinging nettle leaves yielded dark green sample. The yield obtained from ethanolic extracts of stinging nettle leaves were 5.2ml.

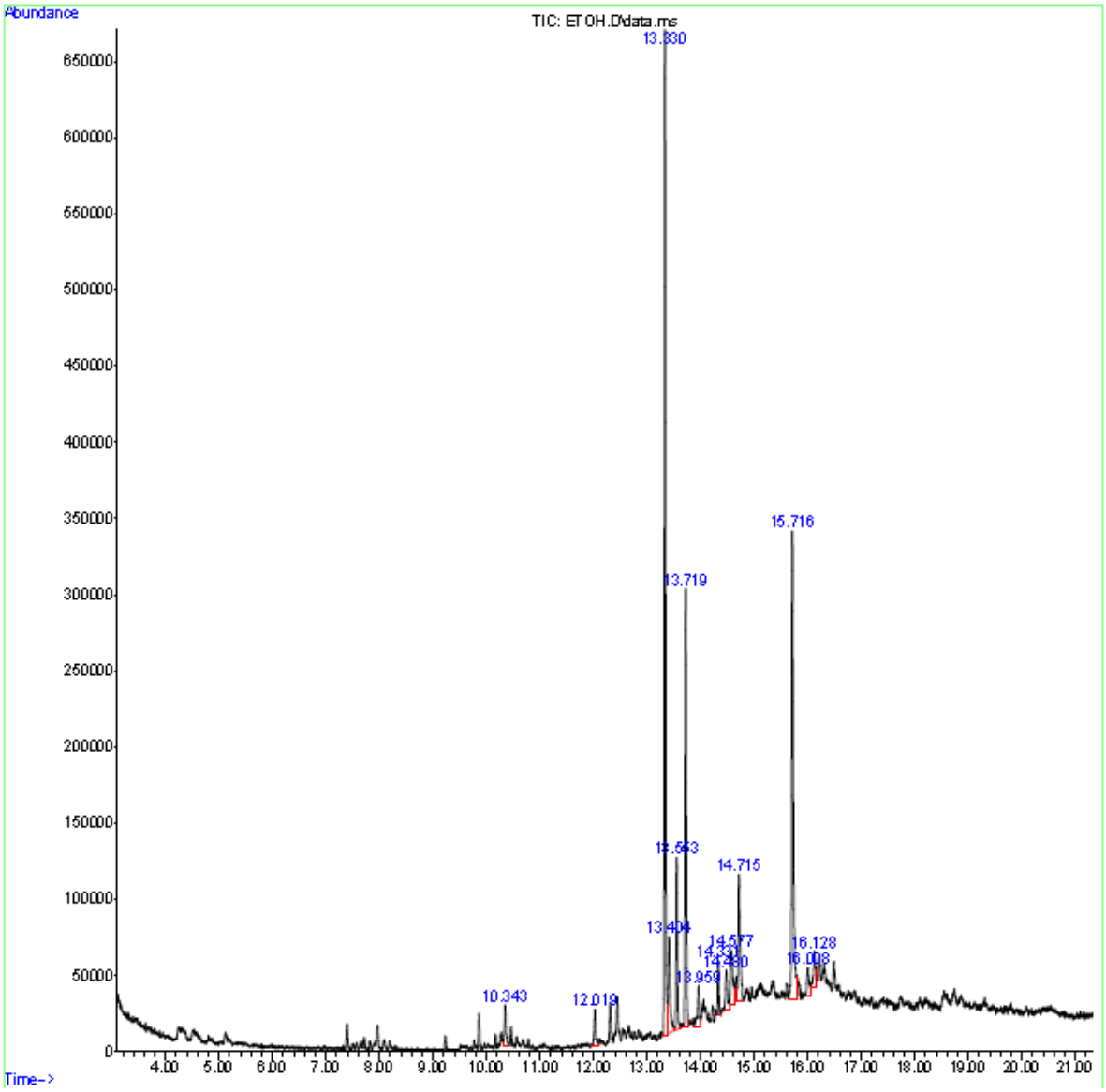
4.2 GC-MS Analysis for the Bioactive Compounds present in the Ethanolic Extract of Stinging Nettle Leaves.

Table 4.1: Characterized Compounds present in the Ethanolic Extract of Stinging Nettle Leaves

The ethanolic extracts of stinging nettle leaves contained 26 compounds having 14 peaks (table 4.1). The below table shows the result obtained:

S/N	Peak	Compound name	Retention time	% Area
1.	1	Nonyl-2-propyl ester	10.343	1.67
2.	1	Sulfurous acid	10.343	1.67
3.	1	10-methylnonadecane	10.343	1.67
4.	2	Hentriacontane	12.019	1.61
5.	2	3,8-dimethyl decane	12.019	1.61
6.	3	Neophytadiene	13.330	31.69
7.	3	2,6,6-trimethyl Bicyclo [3.1.1] heptane	13.330	31.69
8.	3	2-Dodecyne	13.330	31.69
9.	4	6,10-dimethyl-2-undecanone	13.404	4.49
10.	4	1-bromo-octadecane	13.404	4.49
11.	6	7-methyl-3,4-octadiene	13.719	15.08
12.	6	13-tetradece-11-yn-1-ol	13.719	15.08
13.	7	2-methyloctacosane	13.959	1.99
14.	8	Tetrapentacontane	14.331	1.83

15.	9	5-ethyl-1,3-dioxan-5-yl pentyl ester phthalic acid	14.480	2.01
16.	9	Hexyl tridec-2-yn-1-yl ester	14.480	2.01
17.	10	n-Hexadecanoic acid	14.577	3.30
18.	10	(E)-5-Octadecene	14.577	3.30
19.	11	Ethyl ester Hexadecanoic acid.	14.715	6.28
20.	12	3,7,11,15-tetramethyl-2-hexadecen-1-ol	15.716	20.76
21.	12	2-methyl butanoic acid	15.716	20.76
22.	12	3,7-dimethyl-6-octenyl ester	15.716	20.76
23.	13	Undecyl vinyl ester carbonic acid	16.008	1.64
24.	13	Nonahexacontanoic acid	16.008	1.64
25.	14	Eicosyl vinyl ester	16.128	1.83



Total Ion Chromatogram for the Characterized Bioactive Compounds of the Ethanolic Extract of Stinging Nettle Leaves

Table 4.2: The Physicochemical properties and Drug likeness of Hits Compounds in Ethanolic Extract of Stinging Nettle Leaves.

Compounds	**Water solubility (ESOL)**	**Solubility Class**	**Lipophilicity (iLOGP)**	**Medicinal Chemistry**	**Lipinski drug likeness**
Neophytadiene (M.W. 278.5g/mol)	- 6.77	Poorly soluble	5.05	P: 0 alert LL: No SA: 4.08	1 violation
2,6,6-trimethyl Bicyclo[3.1.1] hepatane (M.W. 138.25g/mol)	-3.15	Soluble	2.68	P: 0 alert LL: No SA: 4.03	1 violation
2-Dodecyne (M.W 166.30g/mol)	-3.96	Soluble	3.66	P: 0 alert LL: No SA: 4.10	1 violation
3,7,11,15-Tetramethyl-2-Hexadecen-1-ol (M.W. 296.53g/mol)	-5.98	Moderately soluble	4.66	P: 0 alert LL: No SA: 4.30	1 violation
7-methyl-3,4-octadiene (M.W 124.22g/mol)	-2.26	soluble	2.88	P: 0 alert LL: No SA: 3.07	0 violation
13-tetradece-11-yn-1-ol (M.W 208.34g/mol)	-3.69	soluble	3.71	P: 0 alert LL: No SA: 3.78	0 violation
n-hexadecanoic acid (M.W 256.42g/mol)	-5.02	Moderately soluble	3.85	P: 0 alert LL: No SA: 2.31	1 violation

ethyl ester hexadecanoic acid (M.W 394.53g/mol)	-5.78	Moderately soluble	3.43	P: 0 alert LL: No SA: 3.70	1 violation
2-methyl butanoic acid (M.W 104.12g/mol)	-1.10	Very soluble	1.31	P: 0 alert LL: No SA: 1.07	0 violation
3,7-dimethyl-6-octenyl ester (M.W 238.37g/mol)	-4.08	Moderately soluble	3.94	P: 0 alert LL: No SA: 3.31	0 violation

P: PAINS: Pan-assay Interference Compounds; LL: Lead Likeness; SA: Synthetic Accessibility.

CHAPTER FIVE

5.0 Discussion, Conclusion and Recommendations

5.1 Discussion

5.1.1 GC-MS Analysis for the Bioactive Compounds present in the Ethanolic Extract of Stinging Nettle Leaves.

The GC-MS analysis (refer to Table 4.1) of the ethanolic extract derived from the leaves of Urtica dioica, commonly known as stinging nettle, disclosed an extensive phytochemical variety, consisting of 26 unique compounds characterized by different retention intervals and peak measurements. This assortment includes various chemical types such as esters, hydrocarbons, acids, alkenes, and alcohols, highlighting the intricate chemical composition of the plant extract.

Particularly, peak 3 revealed three compounds that had the highest percentage area, approximately 31.69% which include Neophytadiene, 2,6,6-trimethyl Bicyclo[3.1.1] heptane, and 2-Dodecyne suggesting these may represent the most prevalent or functional elements of the extract.

Furthermore, noteworthy fatty acids and their derivatives were identified, among which n-Hexadecanoic acid (Palmitic acid) at 3.30%, Ethyl hexadecanoate at 6.28%, and 3,7,11,15-tetramethyl-2-hexadecen-1-ol at 20.76% were significantly present. These substances are recognized for their abilities to combat inflammation, provide antioxidants, fend off microbes, and inhibit cancer, indicating their potential therapeutic implications.

Additional important bioactive substances include Nonyl-2-propyl ester (observed in peak 1), 13-tetradecene-11-yn-1-ol, and 2-methyl octacosane. The diverse array of long-chain hydrocarbons and esters emphasizes the importance of stinging nettle as a source of compounds with pharmacological significance.

5.1.2 The Chromatographic Profile – Total Ion Chromatogram

The Total Ion Chromatogram (TIC) illustrated in the accompanying image depicts a visual summary of the volatile components found in the ethanolic extract from the stinging nettle leaves as analyzed by GC-MS. Each peak in the chromatogram signifies a unique compound that elutes at a designated retention time, while the elevation or intensity of these peaks indicates the quantity of each respective compound.

The TIC derived from the ethanolic extract of stinging nettle leaves shows a complex pattern with numerous peaks, underscoring the existence of various bioactive constituents. Each peak

identifies a specific compound that elutes at a certain retention time, suggesting varying degrees of polarity and affinities toward the mobile and stationary phases utilized during the chromatographic process.

Significant peaks emerge notably between the retention times of 12.7 to 16.5 minutes, with compounds such as Neophytadiene and comparable terpenoid-like substances likely eluting in this range. The intensity or abundance of these peaks denotes the relative concentration of these compounds within the extract.

Prominent peaks are located around 13.33 minutes and 15.716 minutes, pointing to the occurrence of highly prevalent compounds like Neophytadiene, 2,6,6-trimethyl Bicyclo[3.1.1]heptane, and 2-Dodecyne, which together comprise approximately 31.69% area at 13.33 minutes, while 3,7,11,15-tetramethyl-2-hexadecen-1-ol accounts for 20.76% at 15.716 minutes.

The Early Eluting Peaks (10–12 minutes) likely indicate the presence of lighter, more volatile compounds such as Nonyl-2-propyl ester and Sulfurous acid. The Later Eluting Peaks (subsequent to 14 minutes) showcase more massive, less volatile compounds including n-Hexadecanoic acid, Ethyl hexadecanoate, and Undecyl vinyl ester carbonic acid. The baseline remains distinctively stable with minimal noise, signaling effective separation and top-notch sample preparation.

5.1.3 Physicochemical properties and Drug likeness of Hits Compounds in Ethanolic Extract of Stinging Nettle Leaves.

Table 4.2 presents a summary of the physicochemical attributes and drug-like characteristics of the recognized compounds, reinforcing the extract's bioactive capabilities. Significant findings include:

Lipophilicity (iLOGP):

Lipophilicity refers to the tendency of a substance to dissolve in lipid or fat molecules. It is one of the important physicochemical properties predicted in our study. It helps us to identify the compounds that have the ability to interact with biological membrane and pass-through cellular barriers to reach its target site. (Sarat; 2008). The majority of the compounds exhibit logP values between 1.31 and 5.05, reflecting varying degrees of hydrophobic characteristics. For instance, Neophytadiene (logP = 5.05) demonstrates a notable lipophilic quality, which can improve membrane permeability, although it might also decrease solubility.

Water Solubility (ESOL):

The solubility varies from highly soluble (2-methyl butanoic acid) to poorly soluble (Neophytadiene), affecting how well the compounds can be absorbed. Most substances are classified as either soluble or moderately soluble, which is advantageous for their biological use.

Lipinski's Rule of Five

Lipinski drug likeness is a set of guidelines used in drug discovery and medical chemistry to assess the likelihood of a compound being an orally active drug. The importance of Lipinski drug likeness lies in its utility as an initial filter to prioritize potential drug candidates which can progress further in preclinical testing and optimization. Certain compounds like Neophytadiene and 2-Dodecyne display a single violation, while others such as 2-methyl butanoic acid and 3,7-dimethyl-6-octenyl ester fully adhere to the rule, indicating a more promising potential as oral pharmaceutical options.

Medicinal Chemistry Alert (PAINS, SA, LL):

Pan-assay Interference Compounds

This refers to a type of compound that interfere with a wide range of targets and biological system. Every compound has a "P: 0 alert," indicating the lack of troublesome substructures.

Synthetic accessibility

This refers to the feasibility and ease of synthesizing a chemical compound in the laboratory. Application of synthetic accessibility lies in its ability to identify compounds that can be efficiently prepared in the laboratory and can accelerate the drug development process, reducing cost and allowing faster progress through preclinical and clinical test (Joh, 2004). Synthetic accessibility (SA) as indicated in table 4.2 scores range from 1.07 to 4.10, indicating that for most compounds, the synthesis is moderately straightforward.

Lead-Likeness

Lead-Likeness refers to a set of criteria used in drug discovery to identify chemical compounds that possess certain resemblance to the bioactive compounds. The relevance of lead-likeness lies in its ability to identify hit compounds efficiently and provide valuable insights into the potential target of the hit compounds, also increase the chances of identifying compounds that have a higher probability of exhibiting the desired pharmacological effects (Alex ;2008). Lead-likeness (LL) indicators are mostly marked as "No," highlighting some departure from ideal lead-like features but not ruling out bioactivity entirely.

5.1.4 Pharmacological and Drug-Likeness Implications

Potential Bioactive Roles

Structures related to **neophytadiene** and **terpenoids** frequently demonstrate capabilities for reducing inflammation, combating microbes, or providing antioxidant defense. **Esters of fatty acids** and certain **aliphatic hydrocarbons**, including n-hexadecanoic acid, might play a role in anti-inflammatory responses and cytotoxicity.

Substances like **2-methyl butanoic acid**, characterized by their high solubility and compliance with drug likeliness standards, could be strong candidates for drug development.

Formulation Considerations

Compounds that are not easily soluble yet possess significant bioactivity, such as neophytadiene may necessitate the implementation of strategies to enhance solubility in formulations. Substances exhibiting moderate deviations from optimal parameters can still be viable options for topical applications or targeted delivery methods.

5.2 Conclusion

The analysis conducted through GC-MS and the subsequent physicochemical assessment of the ethanolic extract derived from Stinging Nettle leaves indicate the existence of various promising bioactive entities. The chromatogram validates the existence of numerous bioactive phytochemicals in the stinging nettle extract, with several key compounds making substantial contributions to the overall profile. These significant peaks merit additional scrutiny due to their potential pharmacological relevance.

Although certain compounds display violations in Lipinski's rules or exhibit low solubility, the overall assessment indicates their potential applicability in the realm of drug discovery and the development of phytopharmaceuticals.

5.3 Recommendations and Suggestion for Further Study

It is recommended that further studies involving varying concentration, isolation of key active ingredients and collaborative trials with traditional antibiotics could assist in unlocking its complete medicinal benefits.

It is also advisable to pursue future in silico docking studies, biological testing, and formulation research to substantiate these candidates for therapeutic use.

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