PREDICTION OF THE PHYSICOCHEMICAL POTENTIAL OF ETHANOLIC EXTR ACT OF STINGING NEETLE LEAVE

An *In-silico* Approach

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SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY
(CHEMISTRY UNIT)
INSTITUTE OF APPLIED SCIENCE (IAS), KWARA STATE POLYTECHNIC, ILORIN, K WARA STATE, NIGERIA.

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF
NATIONAL DIPLOMA (ND)
IN SCIENCE LABORATORY TECHNOLOGY

JULY, 2025.

CERTIFICATION

This is to certify that this project work was carried out by NAME OF STUDENT with matri culation number ND/23/SLT/PT/ and has been read and approved as meeting the requir ements 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 co
mpletion of this programme.

AKNOWLEDGEMENT

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.

I heartly thank my supervisor (Mr Ahmed A.F) for his support, patient, mentorship role an d understanding towards making this project an achievable work. May Almighty God ble ss and reward you abundantly.

To my amiable HOD, Coordinator and all Lecturers, thank you for impacting knowledge o n us. God will reward you all abundantly.

My Appreciation goes to my parents and all my siblings and relatives for all their respecti ve supports making this program a successful one. I pray that God Almighty will bless a nd reward you all abundantly.

I would not forget to acknowledge all those who have contributed to the success of this program, May God never leave you all (Amen).

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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 aga inst 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 p harmacological activities, including antioxidant, anti-inflammatory, and antimicrobial pro perties. Despite its widespread use in traditional medicine, limited scientific research has been conducted to fully explore its antimicrobial potential and the mechanisms underlyi ng its bioactivity. While some studies have suggested its efficacy against bacterial and f ungal 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 etha nolic extract derived from the leaves of Urtica dioica, commonly known as stinging nettl e, disclosed an extensive phytochemical variety, consisting of 26 unique compounds cha racterized by different retention intervals and peak measurements. Some certain compo unds 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 conc entration, isolation of key active ingredients and collaborative trials with traditional antibi otics 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, cont ributing significantly to the development of therapeutic agents in modern medicine. Plan ts, in particular, are known for their remarkable ability to synthesize bioactive secondary metabolites that exhibit diverse pharmacological activities. The historical use of plant-ba sed remedies spans across ancient civilizations, from Egypt to China, and continues to f orm 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 br oad-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 resista nce (AMR), which compromises the effectiveness of synthetic antibiotics, underscores th e need for alternative therapies derived from natural sources. Plants represent a promisin g avenue for the discovery of novel antimicrobial agents capable of overcoming resistan ce and mechanisms.

Plants produce a diverse range of secondary metabolites that help them protect against various environmental stressors, including herbivores, pathogens, and UV radiation. Thes e metabolites include alkaloids, flavonoids, terpenoids, glycosides, and phenolic acids, al l of which have demonstrated varying degrees of antimicrobial activity. The search for n ew antimicrobial agents from plants is crucial in light of the global health threat posed b y 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 wi dely 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 c ontact with the skin, resulting in a burning and irritating sensation (Maiz-Tome, 2009; Bro dal, 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 tra ct infections, and skin ailments. More recently, scientific research has highlighted its anti oxidant, anti-inflammatory, and antimicrobial properties, which can be attributed to the p resence of a wide array of bioactive compounds.

The leaves of stinging nettle are rich in flavonoids, phenolic compounds, alkaloids, terpe noids, 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 anti microbial activity. Studies have demonstrated that Urtica dioica extracts exhibit significa nt antimicrobial activity against a broad spectrum of bacteria, fungi, and viruses (Semalt y et al., 2017). The antimicrobial activity has been particularly effective against Staphylo coccus 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 t he solvent's ability to extract both polar and non-polar compounds, ensuring a comprehe nsive profile of the plant's bioactive components.

Gas Chromatography-Mass Spectrometry (GC-MS) is commonly employed to analyze th ese extracts, enabling the identification of individual phytochemicals and their quantific ation (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 mo

st pressing public health challenges of the 21st century. With the overuse and misuse of conventional antibiotics, many pathogenic microorganisms have developed resistance t o commonly prescribed drugs, rendering them ineffective in treating infections. This prob lem 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 Orga nization (WHO) has warned that, without urgent action, AMR could cause an additional 1 0 million deaths annually by 2050, surpassing cancer as the leading cause of death (WH O, 2019).

The urgent need for new antimicrobial agents is more critical than ever. While synthetic a ntibiotics have been the cornerstone of infectious disease management for decades, ther e is a growing realization of the limitations and risks associated with over-relying on thes e agents. In this regard, natural products offer a promising solution. Historically, plant-der ived 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 di verse pharmacological activities, including antioxidant, anti-inflammatory, and antimicro bial properties. Despite its widespread use in traditional medicine, limited scientific resea rch has been conducted to fully explore its antimicrobial potential and the mechanisms underlying its bioactivity. While some studies have suggested its efficacy against bacteri al and fungal infections, there remains a gap in understanding the full scope of its antim icrobial activity, particularly against multi-drug-resistant strains.

Furthermore, the chemical composition of stinging nettle leaves, including the identity an d concentration of bioactive compounds responsible for its antimicrobial effects, has no t been comprehensively studied using modern analytical techniques. Gas Chromatograp hy-Mass Spectrometry (GC-MS) offers a robust tool for profiling the chemical constituen ts 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 us

e. A computational approach, incorporating molecular docking and in silico analysis, is n ecessary to predict the biological activity and potential drug-like properties of these com pounds.

This research aims to address these gaps by investigating GC-MS analysis to identify th e active components, and evaluating the physicochemical properties of the extract. Furth ermore, a computational approach will be employed to predict the interactions of the ide ntified compounds with key microbial targets, contributing to the discovery of novel anti microbial agents and advancing the development of natural products as alternatives to s ynthetic 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 a l., 2015). Utilizing Gas Chromatography-Mass Spectrometry (GC-MS) for the analysis of t he extract will enable the identification and quantification of specific phytochemicals res ponsible 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 p lant 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 fin dings could pave the way for the development of new plant-based therapies, offering a s afer, 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 e valuate the bioactive compounds present in the plant and their antimicrobial activities, w hich 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 bioacti ve 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 quan tifying the chemical constituents responsible for the antimicrobial activity.
3. 5. To predict the physicochemical properties of the bioactive compounds in Ur tica 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 emph

asizing the sustainability of plant-based solutions. This study will contribute to th e understanding of how natural resources can be utilized responsibly for the d evelopment of effective, eco-friendly therapies.

5. ***Future Research Directions:*** The results of this study could pave the way for f urther research into the antimicrobial potential of other medicinal plants, contri buting to the broader field of natural product drug discovery. Additionally, this r esearch may serve as a foundation for future studies that explore the synergis tic effects of plant extracts and synthetic antibiotics, enhancing the effectiven ess 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 a

ctive 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 m onths, with the aerial parts dying back in winter.
- Leaves: The plant bears opposite, ovate to heart-shaped leaves measuring 3 to 15 centi meters in length. These leaves have serrated margins and are sparsely covered with stin ging hairs.
- Stems: The erect, four-sided stems are armed with stinging hairs that cause a burning s ensation upon contact.
- Flowers: Urtica dioica produces small, greenish or brownish flowers arranged in dense a xillary 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 wi despread presence across various continents underscores its adaptability to diverse envi ronmental conditions.

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

Key Pharmacological Activities:

- Anti-Inflammatory: Extracts from Urtica dioica have demonstrated the ability t o inhibit pro-inflammatory pathways, offering potential relief for conditions lik e arthritis.
- Antioxidant: The plant exhibits significant antioxidant properties, scavenging f ree radicals and reducing oxidative stress, which is beneficial in preventing chr onic diseases.
- Antimicrobial: Studies have shown that Urtica dioica possesses antibacterial a nd antiviral activities, making it a candidate for developing natural antimicrobi al agents.
- Analgesic: The plant has been reported to alleviate pain, providing a natural alt

ernative to synthetic analgesics.

- Anticancer: Preliminary studies suggest that Urtica dioica extracts can inhibit t he proliferation of cancer cells and induce apoptosis, highlighting its potential as an anticancer agent.
- Hepatoprotective: Research indicates that the plant offers protective effects ag ainst 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 symptom s, 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 lik e Alzheimer's disease.

Safety and Toxicity: While Urtica dioica offers numerous health benefits, it can cause ad verse effects such as dermatitis upon direct contact due to its stinging hairs. Additionall y, consumption should be approached with caution in individuals on certain medications or with specific health conditions. Consulting with a healthcare provider before incorpora ting 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 phytochemic als that contribute to its diverse pharmacological activities. The plant's various parts—le aves, stems, roots, and seeds—contain distinct bioactive compounds, including alkaloid s, flavonoids, tannins, sterols, fatty acids, and vitamins. Understanding these constituent s is essential for elucidating the therapeutic potential of Urtica dioica.

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

Flavonoids: The plant is rich in flavonoids, including quercetin, kaempferol, and rutin. Th ese 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 stero ls and triterpenoids in Urtica dioica, compounds known for their anti-inflammatory and a nalgesic properties.

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

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

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

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

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

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

The diverse phytochemical profile of Urtica dioica underpins its traditional and contemp orary medicinal applications. Ongoing research continues to uncover the therapeutic pot entials 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 compoun ds from medicinal plants. It plays a critical role in phytochemical research by helping to i solate compounds of interest in a concentrated and bioavailable form. Among various e xtraction methods, ethanol is widely recognized as one of the most effective and commo

nly 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 e xtracting both polar and some non-polar compounds, including flavonoids, tannins, phen olics, saponins, alkaloids, and terpenoids (Tiwari et al., 2011). Its relatively low toxicity, e ase 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 phytochemic als, ensuring a more comprehensive phytochemical profile than many other so lvents.
- Safety: It is considered safe for human use and suitable for pharmaceutical fo rmulations.
- Antimicrobial Stability: Ethanolic extracts often exhibit greater stability and en hanced antimicrobial activities compared to aqueous extracts due to better sol ubilization of active compounds (Azwanida, 2015).
- Compatibility with Analytical Tools: Ethanol-based extracts are ideal for subse quent analyses using techniques like Gas Chromatography-Mass Spectrometr y (GC-MS), which requires solvent systems that do not interfere with compoun d 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 hav e shown that ethanolic extracts of the leaves possess significant biological activities, inc luding antimicrobial, antioxidant, and anti-inflammatory effects (Gülçin et al., 2004). The se extracts typically show higher efficacy compared to those obtained with non-polar sol vents due to the broader range of soluble phytochemicals present.

Extraction Procedure Overview: The process generally involves drying and pulverizing pl ant material, followed by maceration or Soxhlet extraction in ethanol for a specified dura tion. The extract is then filtered, concentrated, and stored for further analysis or biologica l testing.

In this study, the use of ethanolic extraction serves as a preparatory step to enable effect ive 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 act ive compounds, providing a strong basis for understanding the plant's medicinal potenti al.

2.5 Application to Stinging nettle Leave

Urtica dioica has been widely studied for its antimicrobial effects. Research indicates tha t 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 l argely attributed to the presence of flavonoids, phenolic compounds, tannins, and terpen oids 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 gro wing AMR.

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

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