PHYTOCHEMICAL CONTENT, ANTIMICROBIAL AND ANTIOXIDANT COMPARISON OF THE AQUEOUS AND ETHANOLIC EXTRACTS OF *Allium sativum.*

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A DERSATATION WRITTEN IN THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE AWARD OF THE DEGREE OF POSTGRADUATE DEPLOMA OF SCIENCE (PGD) IN MICROBIOLOGY TECHNIQUE, UNIVERSITY OF BENIN, BENIN CITY.

19th DECEMBER, 2025.

CERTIFICATION

This is to certify that this research was carried out by Esther Agbonmere AKHIGBE (Miss) in the Department of Science Laboratory Technology, Faculty of Life Science, University of Benin, Benin City.

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CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of Esther Agbonmere AKHIGBE (Miss) titled; Phytochemical content, antimicrobial and antioxidant comparison of the aqueous and ethanolic extracts of *Allium sativum*. Has successfully passed the anti-plagiarism test and does not violate any copyright regulations.

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DEDICATION

This work is dedicated to God Almighty for His grace, mercy and provision granted unto me to complete this research. May His name be highly exalted.

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ABSTRACT

This study scientifically validated the traditional therapeutic use of *Allium sativum* by comparing the extraction yield, phytochemical composition, antimicrobial activity, and antioxidant potential of aqueous and ethanol extracts. Although the aqueousss extract produced a higher yield (22.87%) than the ethanol extract (14%), biological activity was greater in the ethanol extract, highlighting the importance of solvent polarity in selectively extracting bioactive compounds. Both extracts showed concentration-dependent antimicrobial activity against selected bacterial and fungal isolates, with greater susceptibility observed in Gram-positive bacteria and Candida albicans; however, the ethanol extract demonstrated superior efficacy, supported by lower Minimun Inhibitory Concentrattion(MIC), Minimum Bactericidal Concentration(MBC), and Minimum Fungicidal Concentration(MFC) values. Antioxidant assays 2,2 – diphenyl-l-picrylhydrazyl(DPPH) and Ferric Reducing Antioxidant Power(FRAP) revealed significant activity in both extracts, with the ethanol extract exhibiting higher ssradical scavenging ability and reducing power than the aqueous extract. Qualitative and quantitative phytochemical analyses confirmed the presence of key secondary metabolites, particularly phenolics and flavonoids, which were more abundant in the ethanol extract and strongly associated with enhanced antibacterial and antioxidant effects. Overall, despite its lower extraction yield, the ethanol extract of A. sativum showed greater biological potency, providing strong scientific support for the medicinal use of garlic and its potential application in pharmaceutical and nutraceutical development**.**

CHAPTER ONE

INTRODUCTION

Nature is always a golden sign to show the prominent phenomena of coexistence. Natural products from plants, animals, and minerals are the basis for treating human diseases (Atanasov *et al.,* 2021). Human beings have depended on nature for their simple requirements as sources of medicines, shelters, foodstuffs, fragrances, clothing, flavors, fertilizers, and means of transportation throughout the ages. (Fabricant and Fransworth, 2001). For a large proportion of the world's population, medicinal plants continue to play a dominant role in the healthcare system, and this is especially true in developing countries where herbal medicine has a long history of continuous use. The development and recognition of the medicinal and economic value of these plants are increasingly rising in both industrialized and developing nations (Ekor *et al*., 2021).

Traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health-care systems of resource-poor communities. Local populations have a long history of using plants for medicinal purposes. The medicinal use of plants is ancient, with historical accounts showing that therapeutic plant use dates back thousands of years. Early Chinese medical practices relied heavily on natural herbal preparations, while in India some of the earliest references to medicinal plants appear in the Rig-Veda. Over time, the properties and therapeutic applications of medicinal plants were studied systematically by ancient physicians, laying the foundation of traditional medical systems in Asia (Khan *et al*., 2018).

Medicinal plants remain important components of indigenous medical systems worldwide. Ethnobotanical knowledge provides a rich resource for natural drug discovery, serving as a bridge between traditional practices and modern pharmacological research (Wang *et al*.,

2020). Traditional use of herbal medicines signifies extensive historical reliance, and in many developing countries a large segment of the population still depends on traditional healers and their repertoire of medicinal plants to meet health-care needs. Even where modern medicine is available, the popularity of herbal remedies often persists due to cultural familiarity and perceived efficacy (Ekor *et al*., 2021).

Natural products play a key role globally in the treatment and prevention of human diseases. These products originate from diverse biological sources including terrestrial plants, microorganisms, marine organisms, and various animal species (Newman and Crag, 2020). Their importance in contemporary medicine has been extensively described in numerous scientific reports and reviews (Atanasov *et al*., 2021). The value of natural products can be observed through:

1. the continuous introduction of structurally diverse bioactive compounds serving as leads for synthetic and semi-synthetic drugs;
2. the variety of diseases they help treat or prevent; and
3. their widespread use in pharmacotherapy.

In recent years, scientific interest in traditional medicinal knowledge and plant-based research has increased significantly (Gakuya *et al*., 2020). Heightened attention to natural product chemistry is driven by unmet therapeutic needs, the exceptional diversity of natural metabolites, their utility as biochemical probes, advancements in analytical and isolation techniques, and improved methods for identifying and producing complex natural compounds (Harvey *et al*., 2023).

The World Health Organization (WHO) has acknowledged the essential role of traditional medicine and continues to promote global strategies aimed at strengthening standards, safety,

and efficacy in herbal medicine production. WHO emphasizes the need for appropriate agro-industrial technologies to support the cultivation, processing, and pharmaceutical development of medicinal plants (WHO, 2019).

Medicinal plants continue to serve as sources of new drugs, with many modern medicines derived directly or indirectly from botanical sources. With over 250,000 flowering plant species estimated globally, studying medicinal plants enhances our understanding of plant toxicity and helps safeguard humans and animals from natural poisons (Ngarivhume *et al.,* 2021). This review therefore examines the historical and contemporary value of medicinal plants such as *Thymus vulgaris* in both traditional and modern medical practices, particularly their significance as sources of bioactive natural compounds.

Even today, plants are not only indispensable in health care but also remain one of the most promising sources for future safe medicines (Harvey *et al.,* 2023). Despite the availability of numerous modern drugs, there is still an urgent need to discover and develop new therapeutic agents. Current estimates indicate that effective therapies exist for only about one-third of known human diseases, which underscores the need for continuous research and innovation (Atanasov *et al.,* 2021). Therefore, the fight against diseases must be pursued with sustained effort. Traditional plant medicines continue to hold significant value in modern drug industries due to their relatively low side effects and the synergistic actions of their diverse natural compounds (Ekor *et al.,* 2021).

Most of the major drugs developed over the past several decades—many of which revolutionized modern medical practice—were isolated or chemically modified from natural sources, particularly plants. These bioactive constituents demonstrate the therapeutic potential inherent in botanical and zoological species (Newman and Cragg, 2020). The World Health Organization (WHO) supports and encourages the integration of herbal medicines into

national healthcare systems because these remedies are widely accessible, cost-effective, and historically validated for their safety and efficacy (WHO, 2019). Thus, research on pharmacologically active agents derived from natural sources, including plant extracts, has led to the discovery of many valuable drugs that play a crucial role in treating human diseases (Atanasov *et al.,* 2021). Recent phytochemical and pharmacological studies have also provided effective solutions for certain conditions where synthetic drug development has fallen short (Mohd *et al*., 2022).

In many developing countries, spices are widely incorporated into daily diets as natural food ingredients due to their flavour-enhancing properties and associated health benefits. *Allium cepa* (onion) and *Allium sativum* (garlic) remain among the most commonly consumed spices because of their broad availability and their diverse bioactive constituents. Recent studies have demonstrated that *Allium sativum* contains substantial quantities of carbohydrates, proteins, minerals, dietary fibre and essential vitamins, making it a valuable nutritional resource (Sharma *et al.,* 2020; Adeoye and Adebayo, 2019). However, the full utilization of these nutrients may be hindered by the presence of anti-nutritional factors naturally present in plant tissues.

Anti-nutrients are secondary metabolites that plants synthesize for protective and physiological functions. These compounds can limit nutrient bioavailability by interacting with proteins, vitamins, and minerals, thereby reducing the effective nutritional value of consumed food materials. Some anti-nutritional compounds have been shown to exert negative health effects when ingested in high concentrations; nevertheless, increasing evidence indicates that many of them also possess beneficial physiological roles when consumed in moderate amounts (Mwangi et al., 2021; Hassan and Musa, 2020).

Allium sativum L., commonly known as garlic, remains one of the oldest cultivated plants used for therapeutic and dietary purposes. For centuries, garlic has served as both a culinary spice and a medicinal herb, valued for its diverse health-promoting properties. As a member of the *Amaryllidaceae* family, garlic belongs to a group that includes more than 250 genera and thousands of species distributed across different ecological zones. Members of this family survive harsh environmental conditions, including cold and drought, owing to their morphological adaptations such as bulbs, rhizomes, and tubers. The genus *Allium*, comprising over 450 species, is widely distributed across the northern hemisphere, with more than 300 cultivated varieties of garlic currently grown globally (Rana *et al.,* 2021).

Several *Allium* species besides common garlic are widely utilized for culinary and nutritional purposes. These include leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), shallot (*Allium ascalonicum*), wild garlic (*Allium ursinum* L.), elephant garlic (*Allium ampeloprasum* var. *ampeloprasum*), chives (*Allium schoenoprasum* L.), and Chinese chives (*Allium tuberosum* L.) (Mokhtari *et al.,* 2020).

The biological, pharmacological, and therapeutic properties of garlic are attributed mainly to its abundant organosulfur compounds. Key sulfur-containing bioactives include alliin, allicin, ajoene, S-allylcysteine, diallyl disulfide, and various thiosulfinates. Additionally, garlic contains non-sulfur phytochemicals such as saponins, flavonoids, vitamins, minerals, and Maillard reaction products, all of which contribute to its health functions (Shang *et al.,* 2019).

Garlic has long been recognized for its antimicrobial activity. Recent studies confirm that its sulfur-containing derivatives—particularly allicin—are responsible for broad-spectrum antibacterial, antifungal, and antiviral effects (Abdel-Daim *et al.,* 2020). Similarly, garlic exhibits notable antioxidant potential due to its phenolic and flavonoid constituents, which play essential roles in neutralizing oxidative stress and protecting cellular components (Zeng

et al., 2022). As a result, garlic continues to serve as a functional food and traditional therapeutic agent widely used to enhance physical well-being and prevent various diseases (Hossain *et al.,* 2021).

Therefore, keeping in view the importance of garlic as an important medicinal food, the present study was conducted to determine the phytochemical content and antimicrobial/antioxidant comparison of the aqueous and ethanoic extracts of *Allium sativum.*

1.2 Aim and Objectives

The objectives of the study was to determine the phytochemical content antimicrobial and antioxidant comparison of the aqueous and ethanoic extracts of *Allium sativum.*

Aim

The aim of the study is to;

determine the yield of the ethanol and aqueous extracts of *A. sativum*

i. investigate the antimicrobial activities of the Ethanol extract of *A. sativum* at different concentrations
ii. investigate the antimicrobial activities of the Aqueous extract of *A. sativum* at different concentrations
iii. determine the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal concentrations (MBCs/MFCs) of the ethanol and aqueous extract of *A. sativum* against the Test Organisms
iv. investigate the DPPH radical scavenging activity of Garlic extracts determined spectrophotometrically at 517nm
v. investigate the FRAP showing the reducing power of the Garlic extracts by measuring their ability to reduce Fe^{3+} to Fe^{2+}

vi. investigate the phytochemical compounds in the extracts of *A. sativum*

vii. investigate the quantitative analysis of secondary metabolites in the extracts of *A. sativum* [mean ± SD (mg/g DW)]

CHAPTER TWO

LITERATURE REVIEW

2.1 Concept of Medicinal Plants and Phytochemicals

Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plant. The term *medicinal plants* include a various types of plants used in herbalism and some of these plants have a medicinal activities. (Ekor, 2014)These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others (Atanasov *et al.* ,2021)

Other plants their derivatives consider as important source for active ingredients which are used in various pharmaceutical preparations, including aspirin and toothpastes (Fabricant and Fansworth 2001. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published, with more still being identified (Alvarez *et al.,* 2021).

Medicinal plants refer to plant species whose organs—roots, bark, stems, leaves, flowers, seeds, or fruits contain bioactive compounds capable of promoting health, preventing disease, or treating a wide spectrum of ailments. These bioactive constituents, generally classified as phytochemicals, encompass a diverse range of secondary metabolites such as alkaloids, flavonoids, terpenoids, saponins, tannins, glycosides, phenolics, and essential oils. Over centuries, medicinal plants have remained fundamental to the health systems of numerous

cultures, forming the backbone of ethno medicine and serving as the primary therapeutic agents in traditional healing systems including Ayurveda, Traditional Chinese Medicine, and African Traditional Medicine. Their holistic, culturally embedded, and naturally derived therapeutic approaches continue to inspire contemporary research in drug discovery and pharmaceutical development (Li and Wang, 2020).

In contemporary scientific literature, medicinal plants are increasingly defined as biological resources with intrinsic therapeutic potential due to their physiological, pharmacological, and biochemical actions, most of which are derived from their secondary metabolites (Dhami and Mishra, 2021). Unlike primary metabolites such as carbohydrates, proteins, and lipids which are essential for plant growth and development, secondary metabolites serve ecological and defensive functions. They protect the plant against herbivores, microbial pathogens, ultraviolet radiation, and adverse environmental stressors. These same compounds, when consumed by humans, exert profound therapeutic effects ranging from antimicrobial and antioxidant activities to analgesic, anti-inflammatory, antidiabetic, anticancer, and neuroprotective properties Zeng *et al.,* 2022).

The World Health Organization (WHO) defines traditional medicinal plants as natural plant materials that are used with minimal or no industrial processing for the treatment of diseases at local or regional levels (World Health Organization, 2019). Traditional herbal medicine has been utilized for thousands of years in both developing and developed countries, largely because it is natural, culturally acceptable, and associated with comparatively fewer adverse effects (James *et al.,* 2020). Early medical history is deeply intertwined with the history of herbal medicine. Indeed, some of the earliest known medical texts such as the Ebers Papyrus from around 1500 BC document the medicinal use of numerous plant species (Aboelsoud and Metwally, 2021).

Across many regions of Asia, Africa, and Latin America, various forms of traditional herbal preparations remain widely used to satisfy basic health-care needs. The trend is also increasing rapidly in industrialized nations, where these practices are often classified as complementary or alternative medicine. In the United States, for instance, the National Institutes of Health (NIH) uses the term Complementary and Alternative Medicine (CAM) to describe medical practices, systems, and products that are not presently considered part of mainstream conventional medicine (National Center for Complementary and Integrative Health, 2022).

Globally, among the diverse traditional medical systems, Traditional Chinese Medicine (TCM) is currently the most widely practiced, followed closely by Indian Ayurvedic medicine. In many Western nations, “Oriental medicine” typically refers to the combined influence of Chinese, Japanese, and Korean medical practices, often brought through immigration pathways. Meanwhile, the broader term "Asian medicine" may include TCM, Ayurveda, and Tibetan medical systems. Among all treatment modalities within traditional medical systems, medicinal plants constitute the most frequently used therapeutic agents (Zhang *et al.,* 2019).

Recent advancements in photochemistry and molecular pharmacology have expanded the understanding of how medicinal plant compounds interact with human biological systems. For example, phenolic compounds are widely recognized for their strong antioxidant capacities, which help neutralize harmful free radicals and reduce the risk of degenerative diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (Kumar *et al.,* 2022). Alkaloids, another major class of phytochemicals, exhibit potent pharmacological effects and serve as the basis of several clinically important drugs. Morphine, derived from *Papaver somniferum*, is still one of the most effective analgesics

used in modern medicine. Similarly, quinine from *Cinchona* bark revolutionized malaria treatment, while artemisinin from *Artemisia annua* recognized by the 2015 Nobel Prize in Physiology or Medicine continues to be a cornerstone of antimalarial therapy.

The role of medicinal plants in modern drug development cannot be overstated. The pharmaceutical industry consistently draws from plant-based compounds to develop new therapeutic agents. Aspirin, for instance, originated from salicin found in willow bark, and digitalis glycosides from *Digitalis purpurea* are critical in treating heart failure. These examples illustrate how natural products provide lead compounds that inspire synthetic analogues or semi-synthetic drugs with improved efficacy, safety, and pharmacokinetic profiles. Recent research efforts increasingly focus on identifying new plant species with unique medicinal profiles, applying advanced tools such as metabolomics, proteomics, genomic sequencing, and computational drug modeling (Li *et al.,* 2023). These techniques enable researchers to pinpoint active molecules more accurately and evaluate their therapeutic potential with higher precision.

Beyond their medicinal value, medicinal plants hold economic, cultural, and social significance. The global demand for herbal remedies is rising due to a growing preference for natural and holistic treatments, concerns about side effects associated with synthetic drugs, and increasing scientific evidence supporting the efficacy of plant-based therapies. According to the World Health Organization, approximately 80% of the global population especially in developing regions depends on medicinal plants for primary healthcare needs (WHO, 2019). This reliance is attributed not only to the accessibility and affordability of herbal medicine but also to the deep cultural traditions that influence healthcare practices across societies.

However, the therapeutic potential of medicinal plants must be approached with rigorous scientific validation. While traditional knowledge offers invaluable insights, not all medicinal

claims are substantiated by clinical evidence. Issues such as variability in plant phytochemical composition, adulteration, contamination, and misuse remain challenges to the standardized application of herbal medicine (Hariram *et al.,* 2020). Environmental factors, including soil composition, climatic conditions, and harvesting techniques, significantly influence the concentration and stability of bioactive compounds. As a result, inconsistency in herbal formulations persists, making quality control and standardization essential for ensuring safety and efficacy.

Efforts to bridge this gap have led to increased scientific evaluation of medicinal plants through laboratory analyses, preclinical studies, and human clinical trials. Modern techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), and DNA (Deoxyribonucleic Acid) barcoding are now used to authenticate plant materials and quantify their active ingredients. Furthermore, nanotechnology is being explored to improve the delivery, stability, and bioavailability of plant-derived compounds (Zeng *et al.,* 2022), offering promising pathways for future drug development.

In summary, medicinal plants embody a vast reservoir of therapeutic potential rooted in centuries-old traditional knowledge and increasingly validated by modern scientific techniques. Their rich phytochemical profiles provide the basis for numerous medicinal applications, from antimicrobial and anti-inflammatory responses to anticancer and cardiovascular benefits. Continued research, sustainable utilization, and ethical conservation practices are essential to safeguard these valuable natural resources for future generations. As global interest in plant-based medicine grows, the concept of medicinal plants will remain central to biomedical innovation, public health strategies, and the advancement of integrative medicine.

2.2 Historical background of medicinal plant use

Determining the exact period during which humans first began using plants as therapeutic agents is inherently challenging. Archaeobotanical and paleoanthropological evidence indicates that humans may have used plants for medicinal purposes as far back as 60,000 years ago (Smith and Kallio, 2019). Ancient records from early civilizations confirm this long-standing tradition. For instance, documented scripts on medicinal plants date back nearly 5000 years in regions such as India, China, and Egypt, and at least 2500 years in Greece and Central Asia (Huang *et al.,* 2020). Historically, humans relied heavily on their natural environment for healing; just as animals instinctively identify helpful plants, early humans similarly adopted instinctive plant use for survival and treatment (Barker, 2018).

During these ancient periods, limited knowledge existed regarding the etiology of diseases, the specific plants effective for treatments, and the appropriate methods of preparation. Consequently, medicinal plant use was highly empirical. With time, however, discoveries about the physiological effects of specific plants enabled a gradual transition from instinctive or trial-and-error use to more systematic and evidence-based practices. One of the oldest written records of medicinal-plant-based drug preparation was discovered on a Sumerian clay tablet from Nagpur, estimated to be approximately 5000 years old (Alvarez *et al.,* 2021). Furthermore, inscriptions reveal that civilizations such as the Egyptians and Chinese used medicinal plants as early as 2700 BC, making them some of the earliest documented cultures with formalized herbal medical systems (Li and Wang, 2020).

In ancient Greece, knowledge of medicinal plants was also well established. Prominent figures such as Hippocrates considered the father of Greek medicine and his successor Aristotle documented several medicinal plants and their therapeutic significance. Their work was later expanded by Theophrastus, who founded what is regarded as the first scholarly

School of Medicinal Plants. A major milestone in pharmacognosy emerged with Pedanius Dioscorides in the first century A.D., who authored the influential encyclopedia *De Materia Medica*, which catalogued around 600 medicinal plants and remained a core reference for centuries (Klein and Duarte, 2019).

Historical research within Iran similarly demonstrates an ancient tradition of phytotherapy. Records trace the use of medicinal herbs to the Aryan civilization, around 6500–7000 BC, where Zarathustra's writings referenced healing plants. The sacred Zoroastrian plant *Haoma* (Ephedra major) is an example of the religious and medicinal significance of herbs in ancient Persia (Karimi and Ghorbani, 2019). Iran's medical and pharmaceutical knowledge evolved from early Mesopotamian, Babylonian, Assyrian, and Elamite traditions, reflecting a long history of integrating regional scientific insights (Rostami *et al.,* 2020).

Despite the richness of this heritage, major historical tragedies including the burning of the Library of Alexandria and the destruction of libraries during the Mongol invasions led to irreversible loss of documentation on Persian medicinal plants. Nevertheless, between the eighth and ninth centuries, renowned Persian scholars such as Avicenna and Razi made monumental contributions to medicine. Works such as Avicenna's *Canon of Medicine* and Razi's *al-Hawi* represent milestones in medical science. Later, in the 13th century, Ibn al-Baitar further expanded botanical knowledge by describing the properties of over 1400 plants, many of which he studied personally (Sadeghi *et al.,* 2021).

2.3 Medicinal plants and conventional medicine

Today, according to the World Health Organization, more than 80% of the global population relies primarily on traditional medicines especially plant-based remedies as their main source

of health care (World Health Organization, 2019). This estimate includes not only large populations in countries such as China and India, as well as other developing nations, but also a considerable number of people in advanced industrialized countries (Xu *et al.,* 2020). Although many modern diseases are increasingly treated with synthetic drugs developed through advanced laboratory research whose efficacy has significantly promoted their widespread use several of these pharmaceutical agents are associated with adverse effects. Consequently, the therapeutic value of medicinal plants and plant-derived products is gaining increased recognition, and public trust in their use continues to strengthen (Adeyemi and Fadare, 2021).

Contemporary clinical, pharmaceutical, and biochemical studies show that many modern drugs trace their origins to traditional plant-based remedies. Classical examples include Aspirin (derived from willow bark), Digoxin (from foxglove), Morphine (from opium poppy), Quinine (from *Cinchona* bark), and Pilocarpine (from *Pilocarpus jaborandi*). Currently, it is estimated that more than 50% of approved modern pharmaceuticals are directly or indirectly derived from medicinal plants (Newman and Cragg, 2020; Thomford *et al.,* 2018). Phototherapy continues to expand globally, with its adoption rising across continents. As a result, the global shift from synthetic compounds toward herbal remedies is increasingly described as a "return to nature," driven by the search for safer alternatives for disease prevention and management. Indeed, nature remains humanity's primary reservoir of medicinal botanical resources (Ekor *et al.,* 2021).

2.4 Phytochemical Compounds

Phytochemicals (from the Greek word *phyto*, meaning plant) are biologically active, naturally occurring chemical compounds found in plants that provide health benefits beyond those attributed to macronutrients and micronutrients (Olatunde *et al.,* 2021). They protect

plants from disease and environmental damage while also contributing to the plant's color, aroma, and flavor. Generally, the chemical substances that shield plant cells from hazards such as pollution, oxidative stress, drought, ultraviolet radiation, and pathogenic attack are collectively referred to as phytochemicals (Kumar and Pandey, 2020; Sharma *et al.,* 2021). Recent scientific advances confirm that significant dietary intake of these compounds plays a key role in promoting human health.

More than 4,000 phytochemicals have been identified in the plant kingdom (Adefegha, 2018), and they are commonly classified according to protective functions, physical properties, and chemical structures (Farhadi *et al.,* 2019). Although thousands have been cataloged, approximately 150 have been extensively researched for their therapeutic potential (Adefegha, 2018). Phytochemicals are widely distributed in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs, and spices (Sharma *et al.,* 2021). Common dietary sources include broccoli, carrots, cabbage, onions, garlic, tomatoes, grapes, berries, beans, and soy-based foods (Okoduwa *et al.,* 2019). These compounds may accumulate in various plant parts, including roots, stems, leaves, flowers, fruits, and seeds (Singh *et al.,* 2020). Pigment-rich phytochemicals are often concentrated in the outer layers of plant tissues. Their levels vary significantly depending on species, environmental conditions, soil quality, processing, cooking, and agricultural practices (Manzoor *et al.,* 2022). While phytochemicals are also marketed in supplemental form, current evidence does not confirm that such supplements provide the same benefits as whole-food dietary phytochemicals (Adefegha, 2018).

Phytochemicals are categorized as secondary plant metabolites and possess biological activities such as antioxidant effects, antimicrobial action, detoxification enzyme modulation, immune system enhancement, reduction in platelet aggregation, hormone metabolism regulation, and anticancer activities. While plants originally evolved these compounds for

self-defense, modern research demonstrates that many phytochemicals also offer protective effects in humans (Dai *et al.,* 2020). Although they are not essential nutrients and are not required to sustain basic life functions, phytochemicals provide important benefits in preventing and combating several common diseases. Their demonstrated health-promoting properties have led to extensive research examining their potential role in disease prevention and therapy. The purpose of the present review is to provide an overview of the highly diverse phytochemicals found in medicinal plants

2.4.1 Classification of phytochemicals

The exact classification of phytochemicals could not be fully achieved so far because of their wide structural and functional diversity. In recent years, phytochemicals have been classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls, among others. Secondary constituents represent the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, phenolics, and glucosides (Zeb, 2020). Literature surveys indicate that phenolics are the most numerous and structurally diverse group of plant phytoconstituents.

Phenolics

Phenolic phytochemicals constitute the largest category of phytochemicals and are widely distributed across the plant kingdom. The three most important groups of dietary phenolics include flavonoids, phenolic acids, and polyphenols. Phenolics are hydroxyl-group-containing compounds in which the -OH group is directly attached to an aromatic hydrocarbon ring. Phenol is the simplest compound in this category. Phenolic compounds form a large and complex group of chemical constituents found in higher plants (Olatunde and Benjakul, 2018). They are secondary metabolites that play significant defensive roles in

plants. Phenolics exhibit several properties beneficial to humans, particularly their antioxidant activity, which is crucial in protecting against free radical–mediated disease processes. Flavonoids represent the largest and most studied group of phenolic compounds (Alam *et al.,* 2022). Phenolic acids further form a diverse group consisting of hydroxybenzoic and hydroxycinnamic acids, while phenolic polymers (tannins) occur as high-molecular-weight compounds divided into hydrolysable and condensed tannins.

Phenolic Acids

The term "phenolic acids" generally refers to phenolic compounds containing one carboxylic acid functional group. Naturally occurring phenolic acids consist of two major carbon frameworks: hydroxycinnamic acids and hydroxybenzoic acids. Hydroxycinnamic acid derivatives typically occur as esters with glucose or hydroxycarboxylic acids. Plant phenolic compounds differ in molecular structure and are characterized by hydroxylated aromatic rings (Działo *et al*., 2021). These compounds have been extensively studied for their ability to counter oxidative damage linked to degenerative diseases such as cardiovascular disease, inflammatory disorders, and cancer. Tumor cells, including leukemia cells, demonstrate elevated levels of reactive oxygen species (ROS), making them particularly sensitive to oxidative stress caused by phenolic compounds (Szwajgier *et al.,* 2022). Numerous studies also emphasize the bioavailability of phenolic acids, highlighting both direct dietary intake and indirect metabolic conversion through gastric, intestinal, and hepatic processes (Del Rio *et al.,* 2020).

In addition, phenolic acid compounds continue to attract scientific interest in agricultural, biological, chemical, and medical fields. Their antioxidant properties and their use in food industries as natural preservatives have gained increased relevance, with growing evidence that their biological activity is closely related to their antioxidant capacity (Do *et al.,* 2021).

Activity of Phenolic Acids

Phenolic compounds represent a well-studied group of secondary metabolites with extensive pharmacological activities. Many are polymerized into larger molecules such as proanthocyanidins (condensed tannins) and lignins. Phenolic acids may also occur as glycosides or esters with sterols, alcohols, glucosides, and hydroxy-fatty acids. Their biological activities are diverse, including the ability to increase bile secretion, reduce blood cholesterol and lipid levels, and exert antimicrobial activity against pathogens such as *Staphylococcus aureus* (Cheng *et al.,* 2020). Phenolic acids also possess antiulcer, anti-inflammatory, antioxidant, cytotoxic, antitumor, antispasmodic, and antidepressant properties (Alseekh and Fernie, 2018).

2.5 Overview of *Allium sativum* (Garlic)

Allium sativum L. (garlic) belongs to the family Amaryllidaceae, originated in Asia, and is widely cultivated in Egypt, Mexico, China, and Europe (El-Saber Batiha *et al.,* 2020). This plant is extensively consumed in Iran, where its foliage, flowers, and cloves are utilized in traditional medicine for several ailments (El-Saber Batiha *et al*., 2020). All parts of *A. sativum* bulbs, leaves, cloves, and flowers are used to prepare mixtures and decoctions to treat various diseases, and it remains an important spice and food additive.

Phytochemical studies reveal that sulfur-containing compounds such as allicin are the principal bioactive components. Allicin (diallyl-dithiosulfinate) is the most important organosulfur compound responsible for many of its therapeutic effects. Other sulfur-containing phytochemicals found in *A. sativum* include diallyl disulfide (DDS), dialslyl trisulfide (DTS), and S-allyl cysteine (SAC), all of which possess a wide range of pharmacological activities (Borlinghaus *et al.,* 2018).

In India, garlic is traditionally used to manage fever, cough, and various dermatological conditions such as scabies, eczema, and premature graying of hair, as well as inflammatory diseases affecting the lungs and nervous system (Imran *et al.,* 2020). In Pakistan, garlic extract is consumed orally for gastrointestinal ailments, respiratory diseases, and fever. In Nepal, the Middle East, and East Asia, garlic is employed for fever, rheumatism, liver disorders, diabetes, intestinal worms, dysentery, colic, flatulence, tuberculosis, high blood pressure, facial paralysis, and bronchitis. Reports from Africa indicate that the plant exhibits antibiotic, antiviral, hypolipidemic, hypoglycemic, and antithrombotic properties (Imran *et al.,* 2020; Shang *et al.,* 2019; El-Saber Batiha *et al*., 2020).

In this review, the phototherapeutic properties of garlic have been comprehensively examined to present an updated overview of one of the most widely used medicinal and culinary plants globally. Ethnobotanical information is validated through preclinical bioactivities (in vitro and in vivo), with emphasis on underlying mechanisms and signaling pathways. Additionally, recent breakthroughs, research challenges, and future directions in garlic studies are highlighted.

2.5.1 Botanical description and taxonomy

A. sativum L. (Figure 1), commonly known as garlic, belongs to the family Amaryllidaceae (Rahman *et al.,* 2019). The bulb is mostly used to treat ailments, and the perennial herbaceous plant is large, with upright flowering stems that extend up to 1 m (Samarakoon *et al.,* 2020). The leaf blades are linear, flattened, robust, and approximately 0.5–1.0 inch (1.25–2.5 cm) long, with a pointed apex and violet to fuchsia flowers that bloom in the Northern Hemisphere during monsoons. Slender leaves on the exterior of the odoriferous bulb surround an internal sheath containing the cloves, and each bulb contains 10–20 cloves. Its

medicinal benefits have been documented in Sanskrit texts dating back about 5,000 years, and it first appeared in traditional Chinese medicine (TCM) at least 3,000 years ago (Ahmad and Aldred, 2021). Today, garlic is grown almost everywhere and is known to have more than 300 varieties (Sharma and Singh, 2022). At present, *A. sativum* is cultivated around the world. It was first discovered in Central Asia, then spread throughout China, the Near East, and the Mediterranean before making its way to the southern and middle parts of Europe, Mexico, and northern Africa, especially Egypt (Zeng *et al.,* 2021). Garlic is a perennial herb that thrives in mild regions and can be grown all year. Sowing each clove in the ground is a method to propagate the plant asexually in cultivation. Cloves are usually sown six weeks before the land freezes in the cold season. The bulbs only produce roots and have no stems above the surface.

Classification

Taxonomy

Kingdom: Plantae;

Subkingdom: Viridiplantae;

Infrakingdom: Streptophyta;

Division: Tracheophyta;

Subdivision: Spermatophytin;

Class: Magnoliopsida;

Superorder: Lilianae;

Order: Aspargales;

Family: Amaryllidaceae;

Genus: Allium;

Species: *A. sativum*

(Integrated Taxonomic Information System [ITIS], 2020).

2.6 Traditional and modern medicinal uses of *Allium sativum*

Garlic is one of the most important bulb vegetables and is widely utilized as a spice and flavoring agent in culinary practices across the world, owing to its distinctive aroma and pungency (Samarakoon *et al.,* 2020). It is commonly incorporated into diverse dishes globally for its strong flavor and characteristic seasoning properties. Furthermore, contemporary research indicates that garlic remains extensively used in preparing stews, sauces, and various preserved or dried food products due to its sensory attributes and functional benefits (Olatunde and Benjakul, 2018).

The pungency, lachrymatory effects, and spicy aroma of garlic are attributed primarily to organosulfur compounds, particularly allicin, diallyl disulfide, and related derivatives that contribute to both its sensory profile and health-promoting properties (Batiha *et al.,* 2020). Garlic enhances the taste of foods and supports digestibility, making it a key ingredient in many traditional and modern cuisines around the world.

Garlic, whether used fresh or in dehydrated forms, remains a vital spice in the food industry. It is commonly processed into flakes, slices, granules, and powder to support large-scale culinary and industrial applications (Kumar *et al*., 2021). Beyond its culinary value, garlic is nutritionally rich, containing essential minerals and vitamins beneficial to human health. It is a source of proteins, carbohydrates, fats, calcium, potassium, phosphorus, sulfur, iodine, dietary fiber, and silicon, in addition to vital vitamins and bioactive compounds. Its notable

nutritive quality and aromatic strength make both the green tops and bulbs valuable components in seasoning, flavoring, and food formulation.

2.7 Chemical Constituents of Garlic

Bulbs of *Allium sativum* are reported to contain hundreds of phytochemicals, including sulfur-containing compounds such as ajoenes (E-ajoene, Z-ajoene), thiosulfinates (allicin), vinyldithiins (2-vinyl-(4H)-1,3-dithiin, 3-vinyl-(4H)-1,2-dithiin), sulfides (diallyl disulfide [DADS], diallyl trisulfide [DATS]), and others, which account for a substantial portion of garlic's overall sulfur content (Ried *et al.,* 2019). Alliin, the main cysteine sulfoxide, is enzymatically converted to allicin by alliinase after crushing or cutting the garlic tissue, which breaks down the parenchyma (Li *et al*., 2020). S-propyl-cysteine sulfoxide (PCSO), allicin, and S-methyl cysteine sulfoxide (MCSO) are the primary odoriferous compounds in freshly milled garlic homogenates (Salehi *et al*., 2020). PCSO can generate more than fifty metabolites depending on water content, temperature, and enzymatic activity; alliinase acts on the mixture of MCSO, PCSO, and alliin to produce other molecules such as allyl methanethiosulfinates, methyl methanethiosulfonate, and corresponding thiosulfates (R-S-S-R′), where R and R′ are allyl, propyl, and methyl groups (Salehi *et al*., 2020).

S-Alkenyl-L-cysteine sulfoxides are secondary metabolites derived from cysteine that accumulate in plants of the *Allium* genus (Yin *et al.,* 2018). Garlic-based formulations contain multiple organosulfur compounds, including N-acetyl cysteine (NAC) and S-allyl-cysteine (SAC), which contribute to the pharmacological properties of garlic (Zhou *et al*., 2019).

2.8 Pharmacological Activities of Garlic

Due to its biologically active components especially allicin and its derivatives s*Allium sativum* (garlic) has been studied and used for a wide range of diseases and conditions,

particularly those affecting the heart and circulatory system. Numerous recent clinical trials and meta-analyses support garlic's beneficial effects in reducing cardiovascular risk factors. Garlic consumption has been shown to lower blood pressure, improve lipid profiles (reducing total and LDL-cholesterol), decrease markers of oxidative stress and inflammation, and improve vascular function all of which may help prevent atherosclerosis, coronary heart disease, and other cardiovascular diseases (Ried *et al*., 2025; Ahmad *et al.,* 2021).

Beyond cardiovascular health, garlic exhibits potential anticancer effects. Laboratory and epidemiological studies indicate that garlic and its organosulfur compounds can modulate carcinogen metabolism, inhibit DNA damage, and suppress growth of certain cancer cells, thereby reducing risks of colorectal, gastric, and possibly lung and bladder cancers (Xu and Yuan, 2022; Samara Koon *et al*., 2020).

Garlic also demonstrates immunomodulatory, antioxidant, and antithrombotic properties: it suppresses platelet aggregation, enhances fibrinolytic activity, and mitigates oxidative damage — which together may reduce risks of thrombosis, inflammatory conditions, and contribute to general health resilience (Ahmad *et al*., 2021; Samarakoon *et al.,* 2020).

Moreover, some recent evidence suggests that garlic may indirectly support metabolic health beyond cardiovascular disease for example, by improving lipid and inflammatory profiles, which could be relevant for conditions such as metabolic syndrome, diabetes, and obesity (Reid *et al.,* 2025).

Modern research supports that garlic's pharmacological potential is broad: it may contribute to prevention and adjunctive treatment of hypertension, hyperlipidemia, atherosclerosis, cardiovascular disease, certain cancers, and oxidative-stress–related disorders. However, while evidence is growing, many of the strongest findings are from controlled trials or

preclinical studies; long-term clinical trials confirming efficacy for all these conditions are still needed (Ried *et al*., 2025; Xu and Yuan, 2022; Ahmad *et al*., 2021; Samara Koon *et al*., 2020).

2.8.1 Cardio protective activity

(Takashima *et al*. 2018) demonstrated that the vasorelaxant effect of *Allium sativum* (garlic) on the rat aorta exhibited significant cardio protective properties, contributing to the reduction of arterial pressure. Endothelium-dependent vasorelaxation induced by aged garlic extract (AGE) is mediated through enhanced nitric oxide (NO) production via endothelial nitric oxide synthase (eNOS), with L-arginine, a precursor of NO, identified as a key contributor to this vasorelaxant effect (Takashima *et al*., 2018). However, the precise mechanism by which AGE reduces arterial pressure in vivo and in drug studies remains under investigation (*Li et al*., 2020).

Meta-analyses and systematic reviews of multiple trials reported that garlic supplementation positively modulates cardiovascular parameters, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), fasting blood glucose (FBG), systolic blood pressure (SBP), and diastolic blood pressure (DBP). Overall, garlic formulations act as a universal cardio protective agent and circulatory tonic, lowering TC, LDL-C, FBG, SBP, and DBP, while maintaining or modestly increasing HDL-C levels (Ried *et al.*, 2019).

In experimental models of pulmonary arterial hypertension induced by monocrotaline (MCT) in rats, fermented garlic extract (FGE) was observed to attenuate right ventricular hypertrophy, reduce arterial stiffness, and decrease atrial natriuretic peptide levels. Additionally, FGE mitigated pulmonary arteriole endothelial dysfunction, medial

hypertrophy, and fibrosis by modulating PKG, eNOS, VCAM-1, and MMP-9 protein expressions. These findings indicate that FGE possesses significant cardio protective effects against pulmonary hypertension and associated cardiac dysfunctions (*Park et al.,* 2021).

2.8.2 Anticancer activity

In rodents with 1,2-dimethylhydrazine (DMH)-induced tumorigenesis and in human colorectal tumor cell lines, the anticancer effects of *Allium sativum* alcohol extracts have been extensively studied. Histopathological analysis demonstrated that garlic extract can reduce the formation of adenocarcinoma and adenoma. Furthermore, aqueous preparations of *A. sativum* were shown to inhibit cell proliferation by slowing cell cycle progression, with downregulation of cyclin B1 and CDK1 expression in human colorectal cancer cells, though apoptosis was not directly induced (Wang *et al.,* 2019).

The effects of garlic extract on the proliferation of human breast cancer (MCF-7), prostate cancer (PC-3), liver cancer (HepG2), and colon cancer (Caco-2) cells, as well as murine macrophage (TIB-71) cells, were evaluated. Crude garlic extract inhibited cell proliferation by 80–90% in HepG2, MCF-7, TIB-71, and PC-3 cells, whereas inhibition in Caco-2 cells was 40–55%. Additionally, garlic extract induced growth arrest and a significant increase in caspase-mediated apoptosis, particularly in PC-3 cells, indicating its potential as a chemopreventive agent (Li *et al.,* 2020).

Diallyl trisulfide (DATS), a bioactive sulfur compound derived from *A. sativum*, has demonstrated strong antitumor properties both in vitro and in vivo. In gastric cancer SGC-7901 cells, DATS treatment suppressed cell proliferation by inducing apoptosis and activating MAPK signaling pathways, including JNK, ERK, and p38 phosphorylation. DATS also inhibited cell invasiveness by modulating MMP9 and E-cadherin expression, and

enhanced host antitumor immune responses by increasing cytokine production, such as TNF-α and IFN-γ (Zhou *et al.,* 2018).

2.8.3 Hepatoprotective activity

Due to its biologically active components, garlic Allium sativum has demonstrated significant liver-protecting potential under various toxic insults. For example, a study showed that fermented garlic extract produced by lactic acid bacteria (LAFGE) protected against acetaminophen (paracetamol)-induced acute liver injury in rats by suppressing hepatocyte apoptosis, inhibiting MAPK phosphorylation, down-regulating p53, and reducing oxidative stress, glutathione and ATP depletion while enhancing antioxidant enzyme activity (Usmani *et al.,* 2019). Similarly, other research found that black garlic extract attenuated carbon-tetrachloride (CCl_4)-induced hepatic injury by lowering malondialdehyde (MDA) levels, increasing glutathione (GSH) content, and boosting activities of antioxidant enzymes such as superoxide dismutase (SOD), GSH-peroxidase, and catalase (Kim et al., 2019).

In addition, a recent study showed that ethanol extract of garlic protected diabetic Wistar albino rats from liver dysfunction: treatment restored elevated liver enzyme levels (ALT, AST, ALP) and normalized bilirubin and other biochemical markers, indicating hepatoprotective effects in metabolic disease contexts (Anyanwu, Onochie and Idama, 2023). Other reports have documented that garlic mitigates heavy-metal and chemical-induced hepatic oxidative stress: for instance, aqueous garlic extract counteracted lead- and nickel-induced ROS generation, lipid peroxidation and liver damage in rodent models (Derbal *et al.,* 2022; Chidinma *et al*., 2018).

2.8.4 Gastric Cancer Chemoprevention by Allicin

Allicin, a bioactive compound derived from *Allium sativum*, has demonstrated significant chemo preventive effects against gastric cancer, one of the most common malignancies worldwide. Multiple studies have shown that allicin selectively inhibits the proliferation of gastric tumor cells while sparing normal gastric epithelial cells by inducing G2/M cell cycle arrest and apoptosis (Chen *et al*., 2019).

Mechanistically, allicin downregulates transforming growth factor-alpha (TGF-α) and its receptor, epidermal growth factor receptor (EGFR), leading to decreased expression of cyclin D1 and cyclin E, thereby preventing cell cycle progression from G2 to M phase (Liu *et al.,* 2020). Additionally, allicin induces reactive oxygen species (ROS) generation, which activates the p53/p21 pathway. This results in inhibition of cyclin-dependent kinase complexes, including CDK4/6-cyclin D and CDK1-cyclin B1, reinforcing G2/M arrest (Zhang *et al*., 2018).

Allicin also promotes mitochondrial-mediated apoptosis by increasing the BAX/BCL2 ratio, which triggers cytochrome c release, caspase-9 activation, and subsequent caspase-3 activation. This cascade leads to poly ADP-ribose polymerase (PARP) cleavage and apoptosis (Wang *et al.,* 2019). Furthermore, p38 mitogen-activated protein kinase (MAPK) is activated, enhancing Fas/FasL expression, which subsequently activates caspase-8, linking the intrinsic and extrinsic apoptotic pathways (Li *et al*., 2021).

Interestingly, allicin can also induce caspase-independent apoptosis. In this pathway, mitochondria release apoptosis-inducing factor (AIF) and endonuclease G (EndoG), which translocate to the nucleus, causing DNA fragmentation and cell death. Allicin additionally increases intracellular Ca^{2+} levels and induces endoplasmic reticulum (ER) stress, further contributing to apoptosis in gastric cancer cells (Zhou *et al.,* 2018).

2.8.5 Anti-inflammatory activity

Due to its biologically active components, *Allium sativum* (garlic) has shown significant anti-inflammatory and immunomodulatory potential. In lipopolysaccharide (LPS)-treated macrophages, garlic extracts and proteins have been shown to suppress the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β) by inhibiting NF-κB nuclear translocation and activating antioxidant proteins such as heme oxygenase-1 (HO-1) and Nrf2 signaling (Zugaro and Liu, 2023; Recinella *et al*., 2022).

Moreover, aged garlic extract (AGE) and other garlic preparations have been demonstrated to exert anti-inflammatory effects in in vivo models, including the reduction of cytokine expression and attenuation of systemic inflammation in mice (Avendaño-Ortiz *et al.,* 2023). These effects are mediated through the suppression of MAPK and NF-κB pathways, as well as the enhancement of antioxidant defenses, confirming garlic's capacity to modulate inflammatory responses (Bouyahya *et al.,* 2025).

Additionally, sulfur-containing compounds in garlic, such as allicin, ajoene, and S-allyl cysteine, contribute substantially to these anti-inflammatory activities. They reduce the transcription of pro-inflammatory cytokines, inhibit oxidative stress, and balance Th1/Th2 cytokine responses, supporting garlic's potential for prevention and adjunct therapy in inflammatory and immune-related disorders (Alm *et al.,* 2023; Zugaro and Liu, 2023).

2.8.6 Antimicrobial Activity of *Allium sativum*

The antibacterial and antifungal activities of *Allium sativum* are well-documented. Garlic essential oil, rich in diallyl monosulfide, diallyl disulfide (DADS), diallyl trisulfide, and dially tetra sulfide, extracted from raw bulbs, exhibits significant antibacterial activity against

Pseudomonas aeruginosa, *Staphylococcus aureus*, and *Escherichia coli*. The presence of allyl groups in these sulfide derivatives is critical for their antimicrobial properties (Zhang *et al.,* 2019).

Additional studies have shown that aqueous garlic bulb extracts display potent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Candida albicans*, primarily by disrupting phospholipid bilayer synthesis through the action of allicin (Kumar et al., 2020). Garlic essential oil also inhibits fungal pathogens such as *Penicillium funiculosum* by penetrating cell membranes and compartments, destabilizing the cytoskeleton, and inducing leakage of cytoplasmic contents and biomolecules. Proteomic analyses indicate that garlic oil modulates the expression of key proteins involved in physiological metabolism, further enhancing its antimicrobial effect (Singh *et al.,* 2021).

Moreover, studies on Australian garlic methanol and aqueous clove extracts revealed antimicrobial activity against *Candida albicans*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Rhodotorula mucilaginosa*. Using ultra-high performance liquid chromatography coupled with mass spectrometry and photodiode array detection (UHPLC-PDA-MS), the bioactive compounds were identified and correlated with both antioxidant and antimicrobial activities, confirming the therapeutic potential of garlic preparations (Patel *et al*., 2020).

2.8 .7 Antidiabetic activity

The antidiabetic effects of *Allium sativum* have been widely studied. The ethyl ether extract of *A. sativum* (at 0.25 mg/kg) has demonstrated enhanced insulin-like efficacy in alloxan-induced diabetic rodents. Oral administration of garlic extract, pulp, and oil stimulates

pancreatic β-cells, enhancing insulin secretion and liver metabolism, thereby improving glycemic control (Alam *et al.,* 2019).

Further investigations have shown that aged garlic extract (AGE) administered at progressive doses exhibits significant hypoglycemic activity in streptozotocin-induced diabetic rats. Diabetic rats treated with 100, 300, and 600 mg/kg of AGE daily for 2 months exhibited dose-dependent reductions in blood glucose levels, serum cholesterol, triglycerides, glycosylated hemoglobin, and kidney and liver fatty acid oxidation compared to untreated diabetic controls (Rahimi *et al.,* 2020).

In human studies, Faroughi et al. (2021) evaluated the effects of *A. sativum* tablets on prediabetic pregnant women. In a triple-blind randomized controlled trial involving 49 women at 24–28 weeks of gestation, garlic supplementation significantly reduced fasting blood sugar (FBS) from 106.6 ± 11.1 mg/dL to 79.4 ± 6.1 mg/dL over 8 weeks. Additionally, diastolic blood pressure and markers of prediabetes were substantially lowered, while systolic pressure remained unchanged, indicating that *A. sativum* can effectively improve glycemic control and mitigate prediabetes symptoms during pregnancy (Faroughi *et al*., 2021).

2.8.8 Antiviral activity

The antiviral properties of *Allium sativum* are largely attributed to its organosulfur compounds, which can interfere with multiple stages of viral infection, including entry, replication, and protein synthesis. Garlic extracts have been shown to inhibit the entry of both enveloped and non-enveloped viruses by interacting with viral surface proteins, thereby blocking or partially preventing virus-host cell binding (Wang *et al.,* 2019).

Aqueous garlic extract has demonstrated potent inhibition of influenza A H1N1 in Madin-Darby canine kidney (MDCK) cells (EC50 = 0.01 mg/mL) (Zhang *et al*., 2020). Similarly,

gold nanoparticles loaded with garlic extract inhibited Measles morbillivirus entry into Vero cells by blocking viral receptors (Patel *et al.,* 2019). Garlic extracts also prevent Newcastle disease virus (NDV) binding to chick embryo cell receptors (Singh *et al*., 2020). Flavonoids in garlic, such as quercetin, along with organosulfur compounds like allice, ajoene, and diallyl disulfide, contribute to antiviral effects by disrupting viral attachment, translation, RNA polymerase activity, and host cell signaling required for viral replication (Al-Kuraishy *et al*., 2021).

Further studies demonstrated that methanoic and ethanoic garlic extracts inhibit influenza A (H1N1) pdm09 viral RNA polymerase and nucleoprotein synthesis, thereby suppressing viral hemagglutinin activity (Sharma *et al.,* 2021). Aqueous garlic extracts have also shown inhibitory effects against avian infectious bronchitis virus (IBV), suggesting potential antiviral activity against coronaviruses (El-Kamash *et al.,* 2020). Hexane garlic extracts exhibited strong inhibition of reverse transcriptase activity in HIV-1 (IC50 ≈ 64.08 ± 1.09 mg/mL), while allicin downregulated the ERK/MAPK pathway to suppress reticuloendotheliosis virus replication (Zhu et al., 2019; Li *et al.,* 2021).

During the COVID-19 pandemic, *A. sativum* was investigated for potential antiviral activity. Organosulfur compounds such as alliin and flavonoids like quercetin showed inhibitory potential against the main protease (Mpro) of SARS-CoV-2 in silico studies (Rahman *et al.,* 2020; Elhassan et al., 2021). Quercetin-3-β-galactoside has previously inhibited SARS-CoV-1 Mpro (IC50 = 42.79 ± 4.97 μM), and due to structural similarity (~96%) between SARS-CoV-1 and SARS-CoV-2 Mpro, garlic compounds may have therapeutic potential (Nguyen *et al.,* 2021). Nevertheless, the World Health Organization clarified that garlic consumption alone does not prevent COVID-19, although its antiviral properties may support general immune defense and aid recovery in viral infections (WHO, 2020; Jayaprakasha *et al*., 2021).

2.9 Aqueous and Ethanolic extracts of *Allium Sativum*

Abdulkarim (2023) evaluated the molluscicidal activities of aqueous and ethanolic extracts of onion bulb (*Allium Sativum*) Against *Bulinus Wrighti.* Snails were exposed to various concentrations of plant preparations in laboratory conditions in a plastic aquarium containing 3L of de-chlorinated water for 96h continuously. Mortality was recorded at every 24hours interval for 96hours. The study shows that, molluscicidal activities are time and dose dependent against snails. The ethanolic extract was more toxic than aqueous extract. Ethanolic extract of *A. sativum* was found highly toxic to *B. wrighti* (24hrs. LC50: 97.07mg/l; 96hrs: 21.70mg/l). Chemical profile of aqueous extracts of *A. sativum* showed the presence of some secondary metabolites. *A. sativum* extracts showed histopathological signs to hermaphrodite glands and the digestive tract of the treated snails.

Table 2.1: Aqueous extracts of Allium sativum

PHYTOCHEMICAL	TEST	OBSERVATION	INDICATION
Flavonoid	Ferric chloride	+ve (green color)	Absent
Tannins	Ferric chloride	+ve (blue-green color)	Absent
Saponins	Frothing test	+ve (frothing)	Present
Glycosides	Fehling's solution	+ve (brick-red precipitate)	Present
Alkaloids	Wagners	+ve (turbidity or precipitate)	Present
Cardiac glycosides	Kellerkilliani	+ve (reddish-brown color)	Present
Steroids	Chloroform	+ve (reddish-brown color)	Present
Volatile oils	Dilute HCL	+ve (white precipitate)	Highly present
Saponin glycosides	Fehling's solution A&B	+ve(bluish-green precipitate)	Absent
Balsams	Alcoholic ferric chloride	+ve (dark green color)	Absent
Anthraquines	Borntrager's test	+ve(pink, red or violet color)	Absent

Source: Abdul Karim (2023)

Atwijukire *et al* (2025). Investigated the antimalarial effects of ethanoic extracts of ndrographis paniculata leaves and Allium sativa bulbs on Plasmodium berghei (NK65)-induced Parasitemia in Albino mice. A controlled laboratory experiment was conducted with 30 mice randomly allocated into six groups: normal control, negative control, positive control (artemether-lumefantrine), A. paniculata monotherapy, *A. sativum* monotherapy, and combination therapy. Extracts were prepared by cold maceration and administered orally at 200 mg/kg. Parasitemia was induced intraperitoneal and monitored microscopically. Antiplasmodial activity was assessed using Rane'scurative test. The combination therapy significantly suppressed parasitemia (43.4% by day 5), showing efficacy comparable to artemether-lumefantrine and A. paniculata monotherapy but more effective than A. sativum alone ($p < 0.0467$). In conclusion, this study demonstrated that the combined ethanoic extracts of Andrographis paniculata leaves and Allium sativum bulbs produced a significant

reduction in Plasmodium Bergheim–induced parasitemia in albino mice, with higher efficacy than either extract alone. These findings provide a scientific basis for the traditional use of herbal combinations in malaria management and highlight the potential of developing phytomedicine formulations as affordable adjuncts or alternatives to conventional antimalarial.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The following materials were used in this study:

3.1.1 Apparatus/ Equipment

Bench autoclave (Gallenkamp, U.K.), Binocular Microscope (Olympus), Incubator (size 2, Gallenkamp, U.K.), Hot air oven (size 2, Gallenkamp, U.K.), Weighing balance (H80, Mettler, Switzerland), Centrifuge (MSE High speed 18), Water bath (Gallenkamp, U.K.), Spectrophotometer (SP8-400 uv/ visible, PYE UNICAM England), Soxhlet apparatus, Glass wares (pyrex burettes, pipettes, beakers, microscopic slides, glass petri diches, measuring cylinders, flasks, separating funnels, bijou, universal and Macartney bottles).

3.1.2 Microbiological Media

Nutrient Agar (BIOTECH, TM 341, India), Mueller Hinton Agar (BIOTECH, TM 339, India), Nutrient Broth (BIOTECH, TM 350, India), Sabouraud Dextrose Broth (BIOTECH, TM 361, India), Potato Dextrose Agar (BIOTECH, TM 387, India),

3.1.3 Chemicals/Reagents

All chemicals used were of analytical grade and they include, Ethanol (99.89%), Distilled water, n-Hexane, Hydrogen peroxide, 1% Tetramethyl-p-phenylenediamine hydrochloride (Oxidase reagent), Phenolphthalene, 0.1N NaOH, Tween-80 (10%), Picric acid, wagner reagent, Dragendroff's reagent, Methylated spirit, Crystal violet(0.5%$^{w}/_{v}$, BEMA), Safranine (BEMA), Grams iodine, Plasma, Kovac's reagent (Merk 6029259559), 1% Barium chloride, 1% sulfuric acid (H_2SO_4), Dettol, Glycerol, Starch, Glycerin, Sodium chloride, Fehling's solution A and B, Ferric chloride, Sodium picrate, dilute ammonia solution.

All media and reagents used were prepared according to the manufacturer's direction.

3.1.4 Antimicrobial agents

Ciprofloxacin (Sigma-Aldrich Biochemika, USA), Nystatin (Sigma-Aldrich Biochemika, USA) and *Alium sativum* - Garlic bulb (ethanol and distilled water extracts).

3.1.5 Source of Test Microorganisms

The microbial isolates used were obtained from stock cultures of clinical isolates from cases of Nosocomial infections from University of Benin teaching Hospital (UBTH) and stored as stock cultures in Pharmaceutical Microbiology and Biotechnology Department of Faculty of Pharmacy, University of Benin (UNIBEN). The selected isolates include *Staphylococcus aureus*, *Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger*.

3.1.6 Sterilization of materials

The oven and autoclave were used to sterilize various materials. Glass-wares such as test tubes, glass rod, pipette, measuring cylinder, beakers and conical flasks used for the research work were soaked and washed using detergent and rinsed several times with distilled water. They were then wrapped with aluminium foil paper and dried in the oven in an inverted position at 160-170^{o}C for 45-60 minutes.

3.2. METHODS

3.2.1 Source, collection and Identification of Plant/Garlic Samples

The Garlic bulbs were sourced from Local Markets in Benin City. After the purchase/acqu sufficient quantity, the samples were transported to the Plant Biology and Biotechnology laboratory, where it was Identified Authenticated by Prof. H. Akinnibosun as well as my supervisor (Prof. Oshomoh) as *Alium sativum* (Garlic bulb).

3.2.2 Preparation and Extraction of the Garlic (*Alium sativum*)

The samples were rinsed with distilled water and oven dried at 55^{o}C for a week before pulverization (milling into fine powder) and Sohxlet extraction using ethanol and distilled water. In the process, specific mass of the powdered sample was weighed and poured into an extraction thimble of a sohxlet apparatus, the solvents (2000 mL) were separately introduced into the round bottom flask, placed in the heating mantle and the apparatus was coupled to the Julabo recirculating cooling system and the apparatus was turned on and the extraction process continued for six (6) hours until the solvent leaving the thimble became clear. After which the liquid extracts collected was evaporated to dryness using rotary evaporator and thermostatically regulated water-bath. The yields upon concentration to dryness were weighed, the percentage yield calculated and the extracts were stored in sterilized sample bottles and kept in the refrigerator at 4°C for subsequent investigation (Alara *et al*., 2012; Dowe *et al*., 2020).

3.2.3 Determination of Percentage yield

The percentage yield of the plant extracts was determined using the formula:

$$Percentage\ (\%)yield = \frac{Weight\ of\ plant\ extract}{Wt.\ of\ pulverised\ powder} x\ \frac{100}{1}$$

1.) Percentage yield of the ethanol extract:

Mass/weight of extract = 10.4g

Mass of pulverized powder = 110.7g

$$Percentage\ (\%)yield = \frac{10.4}{110.7} x\ \frac{100}{1}$$

Percentage yield = 9.31%

2.) Percentage yield of the aqueous extract

Mass/weight of extract = 16.3g

Mass of pulverized powder = 110.7g

Percentage (%) yield = 16/110.7 x 100

Percentage yield = 14.45%

3.2.4 Antimicrobial assay of the extracts

The modified agar well diffusion method described by Cheesbrough, (2006) and CLSI, (2010) was used to determine the antimicrobial sensitivity/potency of the ethanol and aqueous extracts of *Alium sativum* against test organisms. In the process, wells of 6mm in diameter were made into seeded Mueller Hinton agar (antibacterial sensitivity) and Sabouraud dextrose agar (antifungal sensitivity) plates using a flamed cork borer. Prior to seeding, isolated colonies/spores stored in slants were sub-cultured into nutrient broth/sabouraud dextrose broth, vigorously shaken and adjusted to achieve 1:100 dilution of 0.5 Macfarland tubidity standard (containing approximately 10^6 cfu/spores per mL when counted using a cytometer) previously determined using a spectrophotometer. Sterile swab sticks was then dipped into the standardized microbial suspension and gently spread over (seeding) the surface of the agar plates in even strokes to obtain a uniform growth pattern across the entire surface of the plate. This was achieved by rotating the plate 90 degrees followed by 45 degrees with continuous streaking, and finally by streaking round the diameter of the agar. The 6mm wells were filled with equal volumes (100µL) of the stock concentration and lower dilutions of the sample corresponding to 100, 50 and 25 mg/mL concentrations. The same quantity of sterilized normal saline and 1 µg/mL Ciprofloxacin (bacterial plates)/10µg/mL Nystatin served as negative and positive controls respectively. All plates were appropriately incubated i.e 24hrs, 38^{o}C for bacterial plates and ambient temperature (27±2^{o}C) for 48-72hrs for fungal plates in an upright position to allow proper diffusion of extracts. All experiments

were in triplicates. After incubation, the absence or presence of microbial growth around the wells were observed on the plates and the diameter of clear zones were measured using a millimetre (mm) calibrated ruler and the mean Inhibition zone diameters (IZDs) calculated and recorded.

3.2.5 Determination of MICs of the selected antimicrobial agent

The modified broth dilution method described by Firas *et al*. (2008), was used to determine the MICs of the extracts against the test isolates. Varying concentrations of the selected antimicrobial agent ranging from 0.01 -10 mg/mL were constituted in 10 ml of Mueller-Hinton broth in sterile capped tubes from the stock. 100µL of the overnight broth culture of the test standardized microbial suspension. In each round of experiment, a tube without the extract but with same volume of broth and inoculum served as controls. The same experiment was repeated for the fungal isolate but Sabouraud dextrose broth was used in place of Mueller-Hinton. All tubes were appropriately incubated. After incubation, tubes were observed for growth/turbidity. In all cases, the lowest concentration of the extract at which there was no observable bacterial or fungal growth was recorded as the MICs.

3.2.6 Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the Extracts

The broth tubes with no visible growth following MIC determination were inoculated into fresh Nutrient agar/SDA plates using a flamed inoculating loop. Three MIC experimental tubes with concentrations beginning from MIC and progressively higher than the MIC concentrations were considered after which all plates were appropriately incubated (bacterial plates at 38^{o}C for 24 hours and fungal plates at 27±2^{o}C/room temperature for 48 hours). After incubation, all plates were observed for growth and the MBC/MFC was recorded as the lowest concentration of extracts that completely destroyed the microbial cells indicated as no

observable growth of test organisms inoculated from tubes into the fresh agar plates (Lalitha, 2004; CLSI, 2010; Dowe *et al*., 2016).

3.2.8 Qualitative Phytochemical Analyses

Qualitative screening of the phytochemical components of the plant extracts was carried out using the modified method described by sexena *et al*., (2013). Essentially, specific weight of the extracts was made up to 10 ml in a test tube and different reagents were added to specifications. Positive results were indicated by colour change and precipitate formation which were compared against standards. The extracts were tested for the presence of glycosides, alkaloids, tannins, saponins, anthraquinones, phenolics, steroids, resins, terpenoid and flavonoids.

1. Test for saponins

To 1g of the plant extract was added 20 ml of distilled water and heated for 5minutes. 4ml of the solution was measured into a test tube and 2ml of distilled water was added with vigorous shaking, after which it was allowed to stand for 6 minutes. A stable frothing or foaming indicates the presence of saponins.

2. Test for anthraquinone

One gram (1 g) of the extract was shaken vigorously with 10 ml of chloroform. To 4 ml chloroform extract was added 10 % ammonium hydroxide solution (2 ml). Observation of color change to orange indicates the presence of anthraquinones

3. Test for steroids

One gram (1 g) of the extract was extracted with 20 ml methanol, by heating on a water bath. It was filtered and the filtrate evaporated to dryness. A little quantity of the residue obtained

from the filtrate was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added by the side of the test tube to form a lower layer.

4.Test for tannins

One gram (1 g) of the extract dissolved in a tube up to 2ml plus two drops of 5 % ferric chloride. The presence of reddish brown precipitate confirmed the presence of tannins.

5. Test for flavonoids

To 2 ml of the filtrate obtained above, 1 ml of sodium hydroxide was added, and then 1 ml conc. HCl was added. Formation of cloudy precipitate confirms the presence of flavonoids.

6. Phenolics

One milliliter of the extract was added to 1 mL of 10% $FeCl_2$ and mixed together. The presence of blue precipitate confirmed the presence of phenols.

7. Tests for alkaloids

Two grams (2 g) of the extract was dissolved in 5 ml 1 % sulphuric acid and filtered. The filtrate was tested with alkaloidal reagents (Dragendorff, Wagner, Mayer and Hager). In the process, the filtrates are collected in various test tubes. To a tube containing the filterate, a few drops of Wagner's Reagent (Potassium-iodine solution) were added to one part of the filtrate in a test tube. A reddish brown precipitate formation gives a positive result. Generally, the formation of specific precipitate and coloration upon adding drops of Dragendrorff, Wagner, Mayer and Hager's reagent indicates positive results or presence of alkaloids.

8. Test for Resins

To 0.2g of the extract in the test tube was treated with 15 ml of ethanol (98%), vortexes for two minutes and 2ml of the alcoholic extract was then poured into 10 ml of distilled water in test tube, vortexed again for two minutes and allowed to stand for 5minutes undisturbed. The

tube was then observed for precipitate formation. A precipitate occurring indicates the presence of resins.

9. Test for terpenoids

A quantity (9ml) of ethanol was added to 1g each of the extracts and refluxed for a few minute and filtered. Each of the filtrates was concentrated to 2.5ml in a boiling water bath. Distilled water, 5ml was added to each of the concentrated solution, each of the mixtures was allowed to stand for 1 hour and the waxy matter was filtered off. Each of the filtrates was extracted with 2.5ml of chloroform using a separating funnel. To 0.5ml each of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

10. Test for glycosides

To 5ml of the extract in tubes treated with glacial acetic acid containing 1drop of ferric chloride (0.1%) was added to 1ml of concentrated H_2SO_4. A brownish to brick red ring or violet colour at the interphase indicates the presence of glycosides.

3.2.9 Quantitative Phytochemical Composition

After preliminary analysis to determine presence of these phytochemicals, the samples were further subjected to quantitative analysis to determine the percentage of each of these secondary metabolites present the plant extracts. The following procedures were adopted:

1. Determination of total phenolics compounds

The total phenol content was determined using a standard caliberation curve as described by sexena *et al*., (2013). To 1ml of samples/extracts in test tube was mixed with methanol (5 g/L) and further mixed with ethanol solution of gallic acid (1 mL; 0.025-0.400 mg/mL) with 5 mL

of Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 mL, 0.7 M) solution and ultimately the volume was made up to 8 ml with distilled water followed by vigorous shaking and was allowed to stand for 30mins, after which absorbance values were measured at 765 nm using a spectrophotometer and the standard curve was plotted to determine the total phenolic contents. All experiments were carried out in triplicate. The total phenolics components in the extracts in gallic acid equivalents (GAE) were calculated by the formular:

T = CxV/M

Where:

T = total phenolic contents, milligram per gram of sample extract, in GAE

C = the concentration of gallic acid established from the calibration curve, mg/mL

V = the volume of extract, milliliter

M = the weight of sample/extract (g)

Or

Percentage phenol extracted from powdered sample thus:

$$\text{Phenols (\%)} = \frac{100}{W} \times \frac{C}{1000} \times \frac{VF}{VA} \times \frac{D}{1}$$

Where:

W = Weight of sample analysed

C = Concentration of standard in mg/ml

VF = Total filtrate volume

VA = Volume of filtrate analysed

D = Dilution factor where applicable

2. Determination of tannin content

The tannin content was determined by Folin Denis colorimetric method described by Sexena *et al*. (2013). Briefly, Five grams of the powdered sample was measured into a volumetric flask and 50 mL of distilled water was added to the content of the volumetric flask. The mixture was shaken for 30 min at room temperature and filtered to obtain the filtrate. A standard tannic acid solution was prepared, 2 mL of the standard solution and equal volume of distilled water were dispersed into a separate 50 mL volumetric flasks to serve as a standard and reagent blank respectively. Then 2 mL of each of the respective experimental samples were measured into their respective labeled flasks. The content of each flask was mixed with 35 mL distilled water and 1 mL of the Folin reagent . This was followed by 2.5 mL of saturated Na_2CO_3 solution. Therefore, each flask was diluted to the 50 mL mark with distilled water and incubated for 90 min at room temperature. After which their absorbance was measured at 760 nm in a spectrophotometer with the reagent blank at zero. The tannin content was calculated as shown below:

$$\text{Tannin (\%)} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va}$$

Where: W = Weight of sample

au = Absorbance of test sample

as = Absorbance of standard tanning solution

C = Concentration of standard tannin Solution

Vt = Total volume of extract

Va = Volume of extract analyzed

3. Determination of total flavonoids

The method is based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at 415nm. 100µl of the sample/extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorbance at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorbance of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All experiments were carried out in triplicates.

4. Determination of total alkaloids

To 5g of the sample weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed

Percentage alkaloids were computed as follows:

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

Where:

$(W_2 - W_1)$ = Weight of residue

5. Determination of total saponins

The total saponin was done by the double solvent extraction gravimetric method decribed by Sexena *et al*. (2013). Briefly, 5g of sample was mixed with 50 mL of 20% aqueous ethanol solution and incubated for 12 h at a temperature of 55^{o}C with constant agitation. After that, the mixture was filtered through Whatman No. 42 grades of filter paper. The residue was re-extracted with 50 mL of the ethanol solution for 30 min and the extracts weighed together. The combined extract was reduced to about 40 mL by evaporation and then transferred to a separating funnel and equal volume (40 mL) of diethyl ether was added to it. After mixing well, there was a partition and the other layer was discarded while the aqueous layer was reserved. This aqueous layer was re-extracted with the ether after which its pH was adjusted with drop-wise addition of dilute NaOH solution. Saponin in the extract was taken up in successive extraction with 60 and 30 mL portion of normal butanol. The combine extract was washed with 5% NaCl solution and evaporated to dryness in a previously weighted evaporating dish. The saponin was then dried in the oven at 60^{o}C (to remove any residual solvent) cooled in a desiccators and re-weighed. The saponin was determined and calculated as a percentage of the original samples.

Saponin (%) = (W2-W1/W) x 100

Where: W = Weight of sample used

W1 = Weight of empty evaporation dish

W2 = Weight of dish + saponin extract

6. Determination of total glycosides

The digested glycoside content of the sample was determined using the method described by Gilliani *et al*., 2007 and Sexena *et al*., 2013. In the process, 5g of the sample was dissolved in

250 ml of distilled water and treated with glacial acetic acid containing 1drop of ferric chloride (0.1%) and introduced into a beaker containing 1ml of concentrated H_2SO_4 with continuous agitated for 3hours using a shaker, followed by filtration. After which 10 ml of freshly prepared 0.10% Anthrone reagent was added, stoppered and mixed thoroughly by gently shaking. The experiment was repeated to obtain a blank using distilled water in place of sample. After which samples obtained were transferred to spectrophotometer and absorbance read at 630 nm against the blank. The total available glycosides were then calculated accordingly:

$$\text{Glycoside (\%)} = \frac{25\ A_1 \times 100}{W \times A_2}$$

Where: W = weight of sample

25 = Constant

A_1 = Absorbance of diluted sample

A_2 = Absorbance of diluted standard

3.2.8 Invitro Antioxidant assay

1.) DPPH radical scavenging assay

Free radical scavenging ability of the sample/extracts was tested by DPPH radical scavenging assay as described by Jha*et al*., (2018). Summarily, a solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol making a whole volume of 3mL in per test-tubes of different concentrations (15–960 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark or incubated with complete foil masking in the dark at ambient trmperature (27±2^{o}C) for 30 min. The hydrogen atom donating ability of the sample was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants which indicates a positive result and characterized by decrease in absorbance readings. The absorbance of the

mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference or positive control while tubes with reagents without sample served as (negative control). The blank correction was a preparation of the extract concentration in the reference solvent (without DPPH reagents). Percentage DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity (% RSA) = {(A0− A1)/A0}×100

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard.

Or % Inhibition = $\frac{A_{control}-(A_{sample}-A_{sample\ blank})}{A_{control}}$ x 100

Where $A_{control}$ = Absorbance of DPPH in methanol (negative control)

Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. IC_{50} estimation is given by IC_{50} = concentration giving 50% inhibition. Determine by plotting % inhibition vs log(concentration) and interpolate, or rather from a linear interpolation between the two points that straddle 50%. All experiment was done in triplicates for each concentration.

2.) Ferrous reducing antioxidant Potential (FRAP) assay

The ferrous reducing antioxidant Potential (FRAP) of samples was evaluated by the method described by Baydar and Baydar (2013). Accordingly, the freshly prepared stock solution contains 300 mM acetate buffer (3.1g C2H3NaO2.3H2O and 16 M C2H4O2), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 nMHCl, and 20 mM FeCl3.H2O solution. The extracts (1.5 ml) were allowed to react separately with 2.85 ml of the FRAP solution incubated for 5-30 min in the dark in a water bath at 37^{o}C and readings (absorbance) of the coloured product (ferrous tripyridyltriazine complex) were then taken at 593 nm.

The standard curve of FeSO4 (absorbance vs [Fe^{2+}] µM) was made after conversion of sample absorbance to µmol Fe^{2+} equivalent per gram of extract) according to the following:

$$\text{FRAP } (\mu\text{mol}Fe^{2+}/\text{g}) = \frac{\text{X } \mu\text{mol}Fe^{2+}/\text{mL}}{\text{mg sample/mL}} \times 1000$$

3.3 Data Analyses

Data analysis was carried out using Microsoft excel, Spss and Graphpad prism applications. All data were summarised by descriptive (mean, mean ± standard error of mean, etc.) into table charts and graphs and inferential (ANOVA, Tukeys multiple comparison) statistics at 0.05 significance levels.

CHAPTER FOUR

4.0 RESULTS

4.1 Yields of garlic extracts

Table 4.1: Yield of the ethanol and aqueous extracts of *A. sativum*

Extraction Solvent	Weight of plant material (g)	Weight of extract (g)	Percentage yield (%)
Ethanol	81.6	11.5	14.09
Aqueous	80.9	18.5	22.87

The percentage yield of the ethanol and aqueous extracts of *A. sativum* pulverized plant, the yield was 11.5(14%) and 18.5(22.87) respectively. (Table 4.1).

Table 4.2: Antimicrobial activities of the Ethanol extract of *A. sativum* at different concentrations

Organisms	Zones of Inhibition (mean ± S.E.M mm)					
	Concentrations (mg/mL)			CIP	Nystatin	Sterilized
	25	50	100	1µg/mL	10µg/mL	D.H_2O
S. aureus	18.3±1.7	26.6±2.4	33.5±2.6	31.0±1.1	0.0±0.0	0.0±0.0
B. subtilis	21.4±1.6	28.5±1.5	35.7±3.8	33.5±1.5	0.0±0.0	0.0±0.0
K. pneumonia	17.1±2.7	24.7±2.1	31.1±1.3	30.3±2.6	0.0±0.0	0.0±0.0
P. aeruginosa	14.1±1.3	21.5±2.5	26.3±3.6	33.1±3.4	0.0±0.0	0.0±0.0
C. albicans	15.7±2.9	22.3±1.5	25.8±2.3	0.0±0.0	31.5±2.5	0.0±0.0
A. niger	11.5±2.5	14.5±2.5	19.3±1.7	0.0±0.0	34.1±1.9	0.0±0.0

Key: S.E.M = Standard Error of Mean, 0.0 = No activity, CIP = ciprofloxacin, D.H_2O = Distilled water

The antimicrobial activities (zones of Inhibition (mean ± S.E.M mm)) of the Ethanol extract of *A. sativum* at different concentrations 25mg/mL, 50mg/mL and 100mg/mL which was tested against bacteria; *S. aureus, B. subtilis, K. pneumoniae,* and *P. aeruginosa,* fungi; *C. albicans* and *A. niger*, for 25mg/mL zone of inhibition ranges from *P. aeruginosa* (14.1±1.3) to *B. subtilis* (21.4±1.6), 50mg/mL ranges from *P. aeruginosa* (30.3±2.6) to *B. subtilis* (28.5±1.5) and 100mg/mL ranges from *K. pneumoniae* (19.3±1.7) to *B. subtilis* (35.7±3.8). The zones of Inhibition against the test fungi ranges from 25mg/mL *A. niger* (11.5±2.5) to *C. albicans* (15.7±2.9), for 50mg/mL *A. niger* (14.5±2.5) to *C. albicans* (22.3±1.5) and 100mg/mL *A. niger* (19.3±1.7) to *C. albicans* (25.8±2.3).

For ciprofloxacin (1µg/mL) test ranges from *K. pneumonia* (30.3±2.6) to *B. subtilis* (33.5±1.5), Nystatin (10µg/mL) ranges from *C. albicans* (31.5±2.5) to *A. niger* (34.1±1.9) and Sterilized D.H_2O against all the test organisms were (0.00±0.00) as indicated in Table 4.2

Table 4.3: Antimicrobial activities of the Aqueous extract of *A. sativum* at different concentrations

Organisms	Zones of Inhibition (mean ± S.E.M mm)					
	Concentrations (mg/mL)			CIP	Nystatin	Sterilized
	25	50	100	1µg/mL	10µg/mL	D.H_2O
S. aureus	15.5±2.5	21.±3.3	26.8±2.2	31.0±3.1	0.0±0.0	0.0±0.0
B. subtilis	18.8±1.3	23.5±2.5	33.7±3.6	29.5±3.5	0.0±0.0	0.0±0.0
K. pneumonia	13.1±3.0	17.7±1.6	25.2±1.6	34.3±2.7	0.0±0.0	0.0±0.0
P. aeruginosa	10.1±1.7	14.5±2.5	21.3±1.3	33.1±3.0	0.0±0.0	0.0±0.0
C. albicans	10.7±2.3	15.9±1.1	21.8±1.2	0.0±0.0	32.3±2.1	0.0±0.0
A. niger	7.5±1.5	13.5±1.6	17.3±1.7	0.0±0.0	30.5±1.5	0.0±0.0

Key: S.E.M = Standard Error of Mean, 0.0 = No activity, CIP = ciprofloxacin, D.H_2O = Distilled water

The antimicrobial activities (zones of Inhibition (mean ± S.E.M mm)) of the Aqueous extract of *A. sativum* at different concentrations 25mg/mL, 50mg/mL and 100mg/mL which was tested against bacteria; *S. aureus, B. subtilis, K. pneumoniae,* and *P. aeruginosa,* fungi; *C. albicans* and *A. niger*, for 25mg/mL zone of inhibition ranges from *P. aeruginosa* (13.1±3.0) to *B. subtilis* (18.8±1.3), 50mg/mL ranges from *P. aeruginosa* (14.5±2.5) to *B. subtilis* (23.5±2.5) and 100mg/mL ranges from *P. aeruginosa* (21.3±1.3) to *B. subtilis* (33.7±3.6). The zones of Inhibition against the test fungi ranges from 25mg/mL *A. niger* (7.5±1.5) to *C. albicans* (10.7±2.3), for 50mg/mL *A. niger* (13.5±1.6) to *C. albicans* (15.9±1.1) and 100mg/mL *A. niger* (17.3±1.7) to *C. albicans* (21.8±1.2). For ciprofloxacin (1µg/mL) test ranges from *B. subtilis* (29.5±3.5) to *K. pneumonia* (34.3±2.7), Nystatin (10µg/mL) ranges from *A. niger* (30.5±1.5) to *C. albicans* (32.3±2.1) and Sterilized D.H_2O against all the test organisms were (0.00±0.00) as indicated in Table 4.3.

Table 4.4: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal concentrations (MBCs/MFCs) of the ethanol and aqueous extract of *A. sativum* against the Test Organisms

Organisms	**Aqueous**		**Ethanol**	
	MIC	**MBC**	**MIC**	**MBC**
		(mg/mL)		
S. aureus	0.8	2	0.4	0.6
B. subtilis	0.3	0.5	0.08	0.1
K. pneumoniae	2	2	0.8	2
P. aeruginosa	4	7	2	5
	MIC	**MFC**	**MIC**	**MFC**
C. albicans	4	6	2	2
A. niger	8	10	4	6

Key: MIC = Minimum Inhibitory Concentration, MBC= Minimum Bactericidal concentrations, MFC= Minimum Fungicidal concentrations, Mg/Ml = milligrams/milliliter

The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal and Fungicidal concentrations (MBCs) and (MFCs) of the ethanol and aqueous extract of *A. sativum* against the Test Organisms. For aqueous extracts, MIC ranges from B. *subtilis* (0.3) to *P. aeruginosa* (4) and MBC ranges from *B. subtilis* (0.5) to *P. aeruginosa* (7). The ethanol extract treatment MIC against the test organisms ranges from *B. subtilis* (0.08) to *P. aeruginosa* (2) and MBC range from *B. subtilis* (0.1) to *P. aeruginosa* (5). The Minimum Inhibitory Concentration (MIC) for the fungi using aqueous extract ranges from *C. albicans* (4) to *A. niger* (8) and MFC ranges from *C. albicans* (6) to *A. niger* (10). For Ethanol extract of *A. sativum* against

the test fungi, the MIC ranges from *C. albicans* (2) to *A. niger* (4) and MFC value ranges from *C. albicans* (2) to *A. niger* (6) (Table 4.4).

Table 4.5: DPPH radical scavenging activity of Garlic extracts determined spectrophotometrically at 517nm

Concentration (µg/mL)	Aqueous Extract	Ethanol Extract	Ascorbic acid (Standard Antiox.)
10	0.507±0.31	0.421±0.12	0.362±0.11
25	0.473±0.25	0.416±0.23	0.331±0.01
50	0.416±0.05	0.374±0.01	0.287±0.05
100	0.377±0.13	0.331±0.05	0.251±0.07
200	0.366±0.16	0.311±0.14	0.185±0.01

Key:
Mg/dl = milligrams /deciliter, Antiox = antioxidant, DPPH = 2,2-diphenyl-1-picrylhydrazyl, Nm = nanometer

The DPPH radical scavenging activity of Garlic extracts determined spectrophotometrically at 517nm of aqueous and ethanol extracts at different concentrations (mg/mL) 10, 25, 50, 100 and 200. For the aqueous extracts DPPH radical scavenging activity ranges from 200mg/mL (0.366±0.16) to 10mg/mL (0.507±0.31), the ethanol extract DPPH radical scavenging activity ranges from 200mg/mL (0.311±0.14) to 10mg/mL (0.421±0.12). For the Ascorbic acd (Standard antioxidant) DPPH radical scavenging activity ranges from 200mg/mL (0.185±0.01) to 10mg/mL (0.362±0.11) (Table 4.5).

Table 4.6: Percentage inhibition of DPPH radical/Radical Scavenging activity (RSA) of the Aqueous and Ethanol extracts of *Alium sativum* at different concentrations

Concentration (µg/mL)	Aqueous Extract	Ethanol Extract	Ascorbic acid (Standard Antiox.)
10	11.53±0.41	17.65±0.41	31.81±0.11
25	17.99±0.66	31.54±0.63	49.59±0.31
50	27.61±0.85	44.77±0.75	67.95±0.56
100	49.87±0.63	70.51±0.58	88.61±0.53
200	71.52±0.66	85.92±0.66	98.85i±0.35

The percentage inhibition of DPPH radical/Radical Scavenging activity (RSA) of the Aqueous and Ethanol extracts of *Alium sativum* at different concentrations indicated that for the aqueous, ethanol and Ascorbic acid (standard antioxidant) extracts, as the concentration (µg/mL) increases 10µg/mL to 200µg/mL the DPPH/RSA increases 11.53±0.41 to 71.52±0.66 for ethanol 17.65±0.41 to 85.92±0.66 and ascorbic acid 31.81±0.11 to 98.85±0.35 respectively (Table 4.6 Figure 4.1).

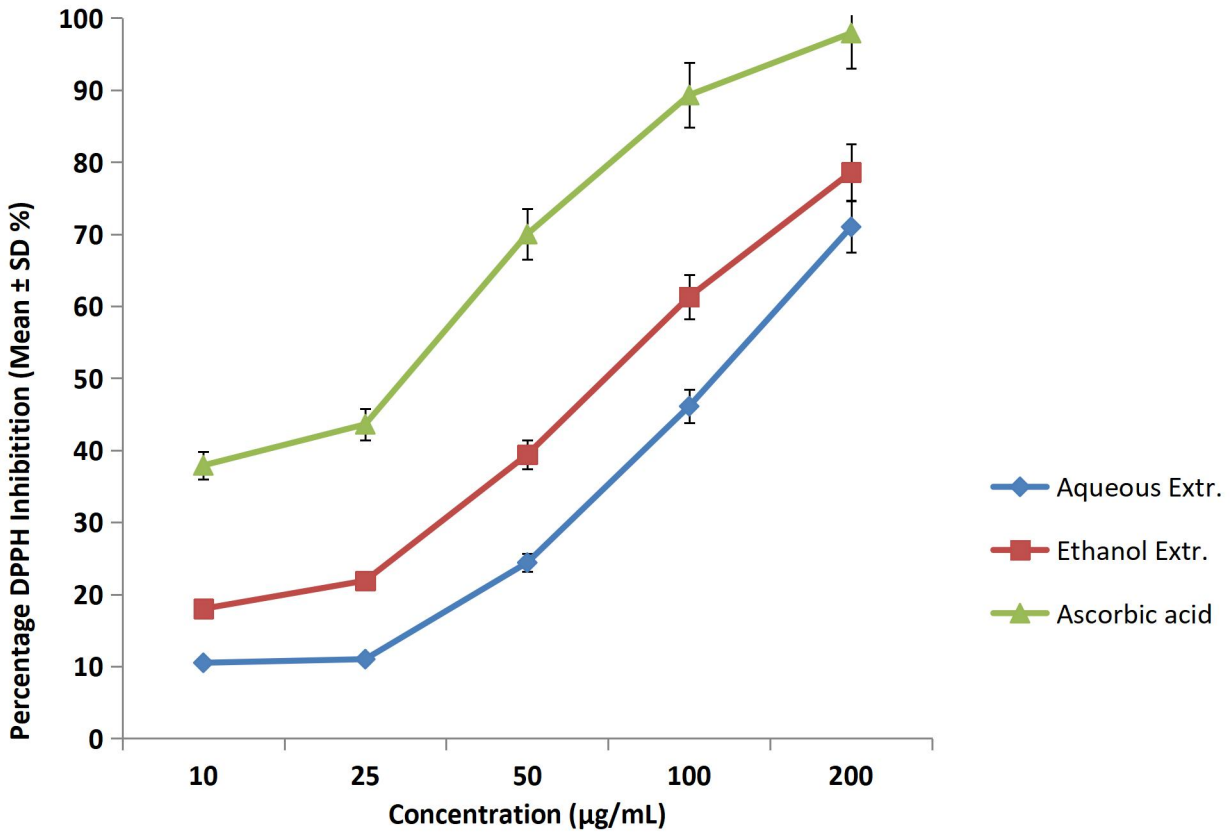

Figure 4.1: Percentage inhibition of DPPH radical/Radical Scavenging activity (RSA) of the Aqueous and Ethanol extracts of *Alium sativum* at different concentrations

Key

DPPH = 2,2-diphenyl-1-picrylhydrazyl

Mg/ml = milligram/milliliter

Aqueous extr = aqueous extracts

Ethanol extr = ethanol extracts

Mean ± SD: = Average value plus or minus standard deviation

Table 4.7: IC50 of the various *Alium sativum* extracts and positive control for DPPH assay

Extract	IC50 (µg/mL)
Aqueous	69.37±6.61
Ethanol	48.51±3.15
Ascorbic acid	31.16±3.13

The half-maximal inhibitory concentration (IC^{50}) of *Allium sativum* (garlic) extracts in a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay indicated that IC50 (µg/mL) for aqueous extract was 69.37±6.61, ethanol was 48.51±3.15 and the ascorbic acid standard was 31.16±3.13.

Table 4.8: FRAP showing the reducing power of the Garlic extracts by measuring their ability to reduce Fe^{3+} to Fe^{2+}

Sample	FRAP (µmol Fe^{2+}/g extract ± SD)
Ethanol extract	512.5 ± 11.6
Aqueous extract	351.2 ± 8.4
Ascorbic acid (standard)	626.6 ± 12.8

Key : Fe^{2+} = Ferous ion ,Fe^{3+} = Ferric ion ,FRAP = Ferric reducing antioxidant power,µmol = Micromole , g extract = gram of extract , SD = Standard deviation

Ferric Reducing Antioxidant Power (FRAP) indicates the reducing power of the Garlic extracts by measuring their ability to reduce Fe^{3+} to Fe^{2+}, such as a of *Alium sativum* aqueous and ethanol extracts act as an electron donor in a critical mechanism through which antioxidants neutralize oxidative agents and free radicals by measuring their ability to reduce Fe^{3+} to Fe^{2+}. The FRAP (µmol Fe^{2+}/g extract ± SD) for ethanol extract was 512.5 ± 11.6, aqueous extract was 351.2 ± 8.4 and the Ascorbic acid (standard) was 626.6 ± 12.8. The ethanol extract exhibited stronger antioxidant potential than the aqueous extract, though both were less active than Ascorbic acid (Standard antioxidant) (Table 4.8).

Table 4.9: Phytochemical compounds in the extracts of *A. sativum*

Plant constituents	Aqueous	Ethanol
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	+
Glycosides	+	+
Steroids	+	+

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

The use of ethanol and aqueous extract of *Alium sativum* indicated the presence of the following phytochemical compounds which are; alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, glycosides and steroids (Table 4.9).

Table 4.10: Quantitative analysis of secondary metabolites in the extracts of *A. sativum* [mean ± SD (mg/g DW)]

Plant constituents	Aqueous	Ethanol
Alkaloids	6.71 ±1.56	11.06± 1.03
Glycosides	4.35±1.63	4.21±1.36
Tannins	10.03±2.51	13.15±2.63
Saponins	13.45± 1.55	12.36±0.31
Phenolics	27.65±3.81	33.25±2.73
Steroids	5.61±0.73	7.13±1.58
Terpenoids	3.03±1.01	6.31±0.17
Flavonoids	23.21±2.03	31.31±1.05

Key: Mean ± SD = Mean plus or minus standard deviation, mg/g DW = milligram / gram Dry weight

The quantitative analysis of secondary metabolites in the extracts of *A. sativum* [mean ± SD (mg/g DW)] using aqueous and ethanol; Alkaloids (6.71 ±1.56), (11.06± 1.03) Glycosides (4.35±1.63), (4.21±1.36), Tannins (10.03±2.51) , (13.15±2.63), Saponins (13.45± 1.55), (12.36±0.31), Phenolics (27.65±3.81), (33.25±2.73), Steroids (5.61±0.73), (7.13±1.58), Terpenoids (3.03±1.01), (6.31±0.17) and Flavonoids (23.21±2.03), (31.31±1.05) respectively (Table 3.10).

CHAPTER FIVE

RESULTS, CONTRIBUTION TO KNOWLEDGE, CONCLUSION AND RECOMMENDATIONS

5.1 DISCISSIONS

Yield of the ethanol and aqueous extracts of *A. sativum*

The percentage yield of plant extracts is an important early indicator of extraction effectiveness and solvent compatibility for separating bioactive components. In the current study, the ethanol and aqueous extracts of crushed *Allium sativum* yielded 11.5 (14%) and 18.5 (22.87%), respectively. The observed variance in extract yield can be plausibly attributed to changes in solvent polarity, phytochemical ingredient solubility, and plant matrix-solvent interactions.

The higher percentage yield of the aqueous extract compared to the ethanol extract indicates that *A. sativum* includes a significant amount of water-soluble chemicals. Garlic is well proven to contain polar substances such as phenolic acids, flavonoid glycosides, carbohydrates, proteins, amino acids, and some organosulfur compounds, many of which dissolve more readily in water than in organic solvents (Kim *et al*., 2018; Martins *et al*., 2021). This finding is consistent with other research that indicate greater extraction yields using aqueous solvents when working with garlic and other medicinal plants strong in hydrophilic phytochemicals (Batiha *et al*., 2020; El-Saber Batiha *et al*., 2021).

Although less polar than water, ethanol is known for its capacity to extract both moderately polar and non-polar molecules, such as phenolics, flavonoids, and sulfur-containing compounds like allicin and ajoene (Shang *et al*., 2019). The lower yield obtained with ethanol

in this study suggests that, while ethanol is excellent in selectively extracting bioactive chemicals, it may solubilize a narrower spectrum of constituents than water, resulting in a smaller total mass of extract. This conclusion is congruent with the findings of Borlinghaus *et al*. (2021), who noted that ethanol frequently provides extracts with lower yields but higher biological potency due to selective extraction.

The use of crushed garlic promotes solvent penetration and mass transfer, hence increasing extraction efficiency. However, solvent selection remains the primary predictor of yield. Recent literature supports the idea that higher yield does not always imply higher bioactivity, as aqueous extracts may contain a higher proportion of inert or nutritionally relevant compounds, whereas ethanol extracts may contain more pharmacologically active molecules (Dhanalakshmi *et al*., 2019; Moutia *et al*., 2020). As a result, the lower yield of the ethanol extract should not be viewed as inferior, but rather as indicative of its selectivity.

Antimicrobial activities of the ethanol extract of *A. sativum* at different concentrations

The results show that the ethanol extract of *Allium sativum* has significant antibacterial activity against the tested bacterial and fungal isolates, with the degree of inhibition changing depending on the microbial type and extract concentration. The zones of inhibition, presented as mean ± S.E.M (mm), show a clear concentration-dependent antimicrobial action, which is consistent with recent results on garlic-derived bioactive chemicals.

At the lowest dose (25 mg/mL), the ethanol extract demonstrated significant antibacterial activity, with inhibition zones ranging from 14.1 ± 1.3 mm in *Pseudomonas aeruginosa* to 21.4 ± 1.6 mm in *Bacillus subtilis*. This finding indicates that *B. subtilis* is more sensitive to garlic ethanol extracts than *P. aeruginosa*. *P. aeruginosa's* lower susceptibility can be linked to its intrinsic resistance mechanisms. Low outer membrane permeability and the presence of

multidrug efflux pumps have been frequently reported to impair plant-derived antibacterial activity (Pang *et al*., 2019; Moradali *et al*., 2020).

Increasing extract concentration to 50 mg/mL significantly improved antibacterial activity, with inhibition zones ranging from 28.5 ± 1.5 mm in *B. subtilis* to 30.3 ± 2.6 mm in *P. aeruginosa*. This significant increase emphasizes the dose-dependent nature of garlic's antimicrobial effect and backs up the claim that larger concentrations of ethanol-extracted phytochemicals promote membrane disruption and metabolic interference in bacterial cells (Batiha *et al*., 2020). At 100 mg/mL, the maximum antibacterial activity was reported, particularly against *B. subtilis* (35.7 ± 3.8 mm), while *Klebsiella pneumoniae* showed the least inhibition (19.3 ± 1.7 mm). *K. pneumoniae* decreased susceptibility may be due to its polysaccharide capsule, which functions as a physical barrier against antimicrobial drugs (Wyres *et al*., 2020).

The ethanol extract displayed significant antifungal activity against *Candida albicans* and *Aspergillus niger* at all doses tested. At 25 mg/mL, zones of inhibition varied from 11.5 ± 2.5 mm in *A. niger* to 15.7 ± 2.9 mm in *C. albicans*. This suggests that *C. albicans* is more sensitive to garlic ethanol extracts than *A. niger*, probably because of variations in cell wall composition and ergosterol concentration (Perlin *et al*., 2019). At concentrations of 50 mg/mL and 100 mg/mL, antifungal activity gradually increased, reaching 25.8 ± 2.3 mm against *C. albicans*. These findings are consistent with recent studies revealing substantial antifungal effects of garlic extracts, particularly against *Candida* species, owing to allicin's inhibitory effects on thiol-containing enzymes required for fungal metabolism (Borlinghaus *et al*., 2021).

This study found that Gram-positive bacteria (*S. aureus* and *B. subtilis*) were more susceptible than Gram-negative bacteria (*K. pneumoniae* and *P. aeruginosa*), which is

consistent with documented antibiotic trends. Gram-positive bacteria have a simpler cell wall structure without an outer membrane, allowing bioactive substances to penetrate more easily (Silva *et al*., 2021). Ethanol, as an extraction solvent, most likely increased the recovery of organosulfur compounds, phenolics, and flavonoids, which are principally responsible for garlic's antibacterial activity (Shang *et al*., 2019).

Ciprofloxacin (1 µg/mL) and nystatin (10 µg/mL) showed wider inhibitory zones than garlic extract at most doses, indicating purified and greater specific action. However, at 100 mg/mL, the ethanol extract generated inhibitory zones similar to ciprofloxacin against *B. subtilis*. Sterilized distilled water showed no inhibition, indicating that the observed antibacterial properties were primarily due to the garlic extract. Similar findings were observed in recent research comparing crude plant extracts to conventional antibiotics (Dhanalakshmi *et al*., 2019; Batiha *et al*., 2020).

Antimicrobial activities of the aqueous extract of *A. sativum* at different concentrations

The current study found that the aqueous extract of *Allium sativum* had measurable antimicrobial activity against the tested bacterial (*Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) and fungal (*Candida albicans* and *Aspergillus niger*) isolates, with the magnitude of inhibition increasing as the extract concentration increased from 25 mg/mL to 100 mg/mL. This clearly reveals a concentration-dependent antibacterial activity, which is widely reported for crude plant extracts and is due to increased availability of bioactive chemicals at higher concentrations (Shang *et al*., 2019; Batiha *et al*., 2020).

At 25 mg/mL, zones of inhibition against bacteria varied from 13.1 ± 3.0 mm in *P. aeruginosa* to 18.8 ± 1.3 mm in *B. subtilis*. At 100 mg/mL, inhibition increased significantly, reaching 33.7 ± 3.6 mm in *B. subtilis*. The increased susceptibility of *B. subtilis* and *S. aureus*

compared to *P. aeruginosa* and *K. pneumoniae* is consistent with documented antimicrobial patterns, which show that Gram-positive bacteria are often more sensitive to plant-derived antimicrobials than Gram-negative bacteria. Gram-negative bacteria have an exterior membrane that prevents them from absorbing hydrophilic and high-molecular-weight chemicals from aqueous extracts (Silva & Fernandes Júnior, 2021; Wyres & Holt, 2020).

The antibacterial action detected in the aqueous extract could be attributed to water-soluble phytochemicals found in garlic, such as phenolic acids, flavonoids, saponins, glycosides, and some sulfur-containing compounds. Although organic solvents are more efficient for extracting allicin, aqueous solutions have been demonstrated to contain substantial levels of bioactive elements capable of suppressing microbial growth (Kim *et al*., 2018; Martins *et al*., 2021). The relatively small zones of inhibition found at lower concentrations indicate that these water-soluble chemicals have mild antimicrobial properties that become more prominent as concentration increases.

The antifungal results showed that *Candida albicans* was more sensitive than *Aspergillus niger* at all doses tested. At 25 mg/mL, inhibitory zones varied from 7.5 ± 1.5 mm in *A. niger* to 10.7 ± 2.3 mm in *C. albicans*, up to 21.8 ± 1.2 mm against *C. albicans* at 100 mg/mL. This differential susceptibility may be due to structural and physiological variations between yeast and filamentous fungus. *Candida* species have cell membranes rich in ergosterol and thiol-containing enzymes that are particularly susceptible to garlic-derived chemicals; however, Aspergillus species have more sophisticated cell walls that may hinder drug penetration (Perlin *et al*., 2019; El-Saber Batiha *et al*., 2021).

Ciprofloxacin (1 µg/mL) and nystatin (10 µg/mL) inhibited bacteria more effectively than aqueous garlic extract at all concentrations. Standard pharmaceuticals are purified molecules with well-defined modes of action and increased specific activity, thus this conclusion is to

be expected. Nonetheless, the ability of the aqueous extract at 100 mg/mL to produce zones larger than 30 mm against *B. subtilis* indicates significant antibacterial activity. The absence of inhibition by sterile distilled water suggests that the antibacterial effects seen were caused purely by the garlic extract, not the solvent. Recent antimicrobial screening tests have shown similar comparison trends between crude plant extracts and conventional antibiotics (Dhanalakshmi *et al*., 2019; Batiha *et al*., 2020).

Minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentrations (MBCS/MFCS) of the ethanol and aqueous extract of *A. sativum* against the test organisms

The MIC, MBC, and MFC results show that both ethanol and aqueous extracts of *Allium sativum* have antimicrobial activity against a panel of bacteria (*S. aureus*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*) and fungi (*C. albicans*, *A. niger*), but the ethanol extract was consistently more potent (lower MIC and MBC/MFC values) than the aqueous extract. For example, ethanol MICs for bacteria ranged from 0.08 mg/mL (*B. subtilis*) to 2 mg/mL (*P. aeruginosa*), but water MICs varied from 0.3 to 4 mg/mL for the same organisms. Fungi exhibit a similar pattern (ethanol MICs 2-4 mg/mL versus aqueous MICs 4-8 mg/mL). These distinctions are physiologically and methodologically feasible, and agree with current studies on Allium extracts and solvent effects (Martins *et al*., 2021; Barbu *et al*., 2023).

Potency varies depending on the solvent. Ethanol is a relatively polar organic solvent that extracts both polar and moderately non-polar phytochemicals, such as biologically active organosulfur compounds (allicin, diallyl disulfide, ajoene) and numerous polyphenols that contribute significantly to antimicrobial activity (Martins *et al*., 2021; Bar *et al*., 2022). Water extracts highly polar substances (sugars, glycosides, proteins) that may have less direct microbicidal activity. Thus, the lower MICs/MBCs observed with ethanol are likely due to improved extraction of allicin and other thiosulfinates, as well as hydrophobic antimicrobials,

which disrupt microbial targets more effectively than the predominantly hydrophilic constituents in aqueous extracts (Borlinghaus *et al*., 2021; Barbu *et al*., 2023).

Spectrum and organismal distinctions. The findings indicate that Gram-positive bacteria (e.g., *B. subtilis*) were suppressed at lower concentrations than Gram-negative bacteria (e.g., *P. aeruginosa*, *K. pneumoniae*), and *Candida albicans* was more susceptible than *Aspergillus niger*. Gram-negative bacteria have an outer membrane rich in lipopolysaccharide and active efflux systems that inhibit phytochemical penetration, resulting in higher MIC/MBC values (Magryś *et al*., 2021; Pang *et al*., 2019). Filamentous fungus, such as Aspergillus, frequently require higher concentrations due to thicker cell walls and different membrane/sterol structures than yeasts like *Candida* (Perlin *et al*., 2019; Sasi *et al*., 2021).

Garlic's antibacterial activity is mostly ascribed to allicin and similar sulfur compounds, which react with thiol groups in enzymes and cellular thiols (e.g., glutathione), inactivate key metabolic enzymes, disrupt redox equilibrium, and damage membranes. These processes are consistent with the rapid bactericidal and fungicidal activity seen in other experimental systems (Nakamoto *et al*., 2019; Borlinghaus *et al*., 2021; Tao *et al*., 2023).

Interpretation of MIC versus MBC/MFC. The published MBC and MFC values (e.g., MBC aqueous *P. aeruginosa* = 7 mg/mL; ethanol MBC *P. aeruginosa* = 5 mg/mL) reveal that bactericidal/fungicidal concentrations are higher than MICs, as expected, and the ethanol extract required lower concentrations to kill. This difference between MIC and MBC/MFC is informative: Extracts with a small MIC-MBC difference are likely bactericidal at near-inhibitory concentrations, while wider gaps may imply bacteriostatic activity at lower doses and the need for higher dosages to kill (Magryś *et al*., 2021; Corbu *et al*., 2021).

Multiple studies of *Allium* spp. and other medicinal plants have confirmed that ethanol extracts are more potent than water extracts (Martins *et al*., 2021; Barbu *et al*., 2023). Allicin

or concentrated Allium fractions have reported MICs against *Candida* spp. and many Gram-positives in the low µg•mL^{-1} to sub-mg•mL^{-1} range in purified preparations, but crude extracts show higher MICs because active constituents are present at lower concentrations and are mixed with inert material (Sasi *et al*., 2021; Hasan *et al*., 2023). The current results (mg•mL^{-1} range) are consistent with expectations for crude ethanol and aqueous extracts, supporting further separation and chemical characterization (Barbu *et al*., 2023; Corbu *et al*., 2021).

DPPH Radical Scavenging Activity Of Garlic Extracts

The findings show that both aqueous and ethanol extracts of *Allium sativum* have significant DPPH radical scavenging activity, as measured spectrophotometrically at 517 nm, indicating their antioxidant potential. The DPPH assay is a well-known method for determining the hydrogen- or electron-donating ability of plant-derived antioxidants, with the decrease in absorbance at 517 nm reflecting test samples' ability to neutralize the stable DPPH free radical (Shang *et al*., 2019; Martins *et al*., 2021).

Across the dosage range of 10-200 mg/mL, both extracts showed a dose-dependent trend, with higher concentrations showing bigger reductions in DPPH absorbance (lower absorbance values), indicating better radical scavenging action. The aqueous extract's DPPH absorbance dropped from 0.507 ± 0.31 at 10 mg/mL to 0.366 ± 0.16 at 200 mg/mL.

Similarly, the ethanol extract decreased from 0.421 ± 0.12 to 0.311 ± 0.14 in the same concentration range. This dose-response relationship is consistent with the idea that increasing extract concentration enhances the availability of antioxidant compounds that can donate hydrogen atoms or electrons to DPPH radicals (Batiha *et al*., 2020).

A comparison of the two solvents demonstrates that the ethanol extract had higher DPPH radical scavenging action than the aqueous extract at the same doses, as evidenced by

consistently lower absorbance values. This observation is consistent with recent research revealing that ethanol is more efficient than water in extracting phenolic chemicals, flavonoids, and some organosulfur compounds, which are mostly responsible for garlic's antioxidant properties (Kim *et al*., 2018; Martins *et al*., 2021).

Phenolic compounds, in particular, are renowned for their high hydrogen-donating properties, which improve free radical scavenging in DPPH tests (Shang *et al*., 2019). Despite this difference, the aqueous extract showed significant antioxidant activity, indicating the presence of water-soluble antioxidant elements in garlic, such as phenolic acids, vitamin C, and some sulfur-containing compounds. This study supports studies that traditional aqueous garlic preparations, such as infusions and decoctions, have significant antioxidant capacity, albeit often lower than that of organic solvent extracts (El-Saber Batiha *et al*., 2021; Moutia *et al*., 2020).

When compared to ascorbic acid, the standard antioxidant, both garlic extracts demonstrated lower radical scavenging ability, as seen by greater absorbance values at all doses. Ascorbic acid has the lowest absorption values. The concentration decreased from 0.362 ± 0.11 at 10 mg/mL to 0.185 ± 0.01 at 200 mg/mL, indicating its effectiveness as a pure antioxidant. This result is predicted given that crude plant extracts are complex mixtures including both active and inert constituents, whereas ascorbic acid is a purified substance with a defined antioxidant mechanism (Dhanalakshmi *et al*., 2019).

Percentage inhibition of DPPH radical/Radical Scavenging activity (RSA) of the Aqueous and Ethanol extracts of *Alium sativum* at different concentrations

The ethanol extract showed higher levels of phenolic and flavonoid compounds, correlating with its greater antioxidant potential observed in the DPPH and FRAP assays. Conversely, the aqueous extract was richer in saponins and reducing sugars, suggesting a difference in solvent polarity influencing phytochemical solubility. These findings support the antioxidant role of garlic extracts and align with previous reports on the correlation between phenolic contents and radical scavenging activities (Chang *et al*., 2002).

IC_{50} of the various *Alium sativum* extracts and positive control for DPPH assay

The results of the DPPH radical scavenging test show that Allium sativum extracts have significant antioxidant activity, which clearly varies based on the extraction solvent. Compared to the water extract (69.37 ± 6.61 µg/mL), the ethanol extract showed a lower IC_{20} value (48.51 ± 3.15 µg/mL), suggesting a greater ability to scavenge free radicals. This finding supports the idea that stronger antioxidant efficacy is correlated with lower IC_{50} values. As anticipated, the ascorbic acid standard demonstrated the highest antioxidant activity (IC_{50} = 31.16 ± 3.13 µg/mL), demonstrating its purity and proven effectiveness as a reference antioxidant.

The capacity of ethanol to solubilize a wider range of bioactive phytochemicals, especially phenolic compounds and flavonoids, which are recognized contributors to antioxidant activity in garlic, may be the reason for the ethanol extract's improved effectiveness. Comparing ethanolic or hydroethanolic extracts of A. sativum to aqueous extracts, recent research has consistently shown higher total phenolic content and stronger DPPH scavenging action. On the other hand, water alone may be less successful in extracting some antioxidant

ingredients, even though it is effective in dissolving highly polar molecules, as indicated by the aqueous extract's comparatively larger IC_{50} value.

FRAP showing the reducing power of the Garlic extracts by measuring their ability to reduce Fe^{3+} to Fe^{2+}

The Ferric Reducing Antioxidant Power (FRAP) assay employed in this study gives important information about the electron-donating ability of *Allium sativum* extracts, which is a key method by which antioxidants combat oxidative stress. The FRAP method assesses antioxidant capacity by measuring the ability of bioactive substances to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), more FRAP values indicate stronger reducing power and thus more antioxidant activity (Shang *et al*., 2019; Martins *et al*., 2021).

The results show that the ethanol extract of garlic had a much higher FRAP value (512.5 ± 11.6 µmol Fe^{2+}/g extract) than the water extract (351.2 ± 8.4 µmol Fe^{2+}/g extract). This finding shows that the ethanol extract possesses a higher ability to operate as an electron donor.

This results in more effective neutralization of oxidized intermediates and reactive oxygen radicals. The observed difference between the two extracts is mostly due to the solvent-dependent extraction efficiency of antioxidant chemicals. Ethanol is frequently recognized to be more successful than water in extracting phenolic chemicals, flavonoids, and some organosulfur elements, all of which contribute to garlic's reducing activity (Kim *et al*. 2018; Batiha *et al*. 2020).

The significant FRAP value found for the aqueous extract, while lower than that of the ethanol extract, suggests that water-soluble antioxidants are also present in garlic. These include hydrophilic phenolics, vitamin C, and several sulfur-containing compounds that keep their reducing capacity in aqueous conditions. This discovery supports earlier and current reports that aqueous garlic preparations, frequently used in traditional medicine, continue to offer considerable antioxidant effects despite their considerably reduced potency (El-Saber Batiha *et al*., 2021; Moutia *et al*., 2020). Compared to ascorbic acid, which had the highest FRAP value (626.6 ± 12.8 μmol Fe^{2+}/g), both garlic extracts demonstrated decreased reducing power. This result is predicted given that ascorbic acid is a pure, well-characterized antioxidant with a strong and direct electron-donating action. In contrast, crude plant extracts are complex combinations with changing proportions of active and inactive ingredients, which can reduce overall antioxidant efficacy (Dhanalakshmi *et al*., 2019). Nonetheless, the comparatively high FRAP value of the ethanol extract indicates that garlic includes a significant quantity of redox-active chemicals.

Phytochemical Compounds In The Extracts F *A. sativum*

The phytochemical analysis of *Allium sativum* ethanol and aqueous extracts revealed the presence of a variety of bioactive compounds, including alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, glycosides, and steroids. These findings are consistent with recent research demonstrating garlic's abundance of different secondary metabolites, which contribute to its broad range of pharmacological characteristics (Batiha *et al*., 2020; Martins *et al*., 2021).

The presence of these compounds in both ethanol and aqueous extracts demonstrates the solvent-dependent extraction of phytochemicals. Ethanol, a moderately polar organic solvent, extracts both polar and non-polar constituents such as flavonoids, phenols, terpenoids, and

steroids, whereas water preferentially extracts highly polar compounds like saponins, glycosides, and certain phenolics (Martins *et al*., 2021; Kim *et al*., 2018). The existence of these bioactive chemicals in the aqueous extract explains why garlic infusions and decoctions have been used in ethnomedicine for centuries, despite the fact that ethanol extracts have higher bioactivity.

Quantitative Analysis Of Secondary Metabolites In The Extracts of *A. sativum* [mean ± SD (mg/g DW)]

The quantitative phytochemical examination of *Allium sativum* extracts indicated significant levels of various bioactive secondary metabolites, with ethanol extracts typically holding higher concentrations of most compounds than aqueous extracts. This trend highlights the effect of solvent polarity and extraction efficiency on phytochemical yield, which is consistent with recent studies on garlic and other medicinal plants (Martins *et al*., 2021; Batiha *et al*., 2020).

The most abundant chemicals examined were phenolics (27.65 ± 3.81 mg/g DW in aqueous; 33.25 ± 2.73 mg/g DW in ethanol) and flavonoids (23.21 ± 2.03 mg/g DW in aqueous; 31.31 ± 1.05 mg/g DW in ethanol). Phenolics and flavonoids are well-known for their antioxidant qualities, serving as electron donors to neutralize free radicals and chelate metal ions and safeguard biological macromolecules against oxidative damage (Shang *et al*., 2019; El-Saber Batiha *et al*., 2021). The higher concentrations observed in ethanol extracts most likely explain the superior radical scavenging (DPPH) and ferric reducing (FRAP) activities previously reported for these extracts, as phenolic and flavonoid content is directly proportional to antioxidant capacity (Kim *et al*., 2018; Martins *et al*., 2021).

Tannins (10.03 ± 2.51 mg/g DW in aqueous; 13.15 ± 2.63 mg/g DW in ethanol) and saponins (13.45 ± 1.55 mg/g DW in aqueous; 12.36 ± 0.31 mg/g DW in ethanol) were also detected in substantial concentrations. Tannins have antibacterial activity by forming complexes with microbial cell wall proteins and enzymes, whereas saponins can increase membrane permeability, leading to microbial cell lysis (Barbu *et al*., 2023; Batiha *et al*., 2020). These results support the antibacterial and antifungal properties of both ethanol and aqueous extracts, with ethanol extracts exhibiting somewhat greater activity, perhaps as a result of their higher tannin content.

Although present in comparatively lower concentrations, alkaloids (6.71 ± 1.56 mg/g DW in aqueous; 11.06 ± 1.03 mg/g DW in ethanol), steroids (5.61 ± 0.73 mg/g DW in aqueous; 7.13 ± 1.58 mg/g DW in ethanol), and terpenoids (3.03 ± 1.01 mg/g DW in aqueous; El-Saber Batiha *et al*., 2021). The reported increased antibacterial efficacy of ethanol extracts in comparison to aqueous extracts, especially against Gram-positive bacteria, is further supported by the higher quantities of alkaloids and terpenoids in ethanol extracts.

Glycosides demonstrated similar concentrations in both extracts (4.35 ± 1.63 mg/g DW in aqueous; 4.21 ± 1.36 mg/g DW in ethanol), indicating that both polar solvents effectively extract these water-soluble substances. The effects of various secondary metabolites in *A. sativum* extracts may be enhanced by the antioxidative, cardioprotective, and immunomodulatory properties of glycosides (Shang *et al*., 2019; Moutia *et al*., 2020).

5.2 Contribution to Knowledge

This study contributes to existing scientific knowledge in the following ways:

1. It offers experimental proof that ethanol extraction produces biologically more potent extracts of *A. sativum*, despite water extraction producing a larger amount of extract.

This highlights the significance of solvent selection based on expected bioactivity rather than yield alone.

2. It strengthens knowledge of garlic's range of antimicrobial activity by providing a thorough comparison of zones of inhibition, MIC, MBC, and MFC values for both ethanol and aqueous garlic extracts against a variety of bacterial and fungal pathogens.
3. By highlighting the radical scavenging and electron-donating processes of garlic extracts, the DPPH and FRAP experiments help explain their antioxidant properties.
4. The results show that ethanol extracts are better suited for pharmaceutical and nutraceutical applications while also providing scientific support for the ongoing use of aqueous garlic preparations in traditional medicine.

5.3 Conclusion

The extraction yield, phytochemical content, antibacterial activity, and antioxidant potential of ethanol and aqueous extracts of *Allium sativum* were all thoroughly assessed in this work. The results show that *A. sativum* is a rich source of physiologically active substances with important antioxidant and antibacterial qualities.

Garlic contains a lot of water-soluble components, as seen by the extraction yield data, which revealed that the aqueous extract provided a larger percentage yield than the ethanol extract. Nevertheless, the ethanol extract continuously shown higher antibacterial and antioxidant properties despite its lower yield, demonstrating that extraction efficiency in terms of yield does not necessarily correspond to biological potency.

Both extracts shown broad-spectrum antibiotic action against fungus (*Candida albicans* and *Aspergillus niger*), Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), and Gram-negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Larger zones of inhibition and lower MIC, MBC, and MFC values showed that the ethanol extract was

more effective. In general, Gram-positive bacteria were more vulnerable than Gram-negative ones, and *Aspergillus niger* was less sensitive than *Candida albicans*. The structural changes in microbial cell walls and membranes are responsible for these variations.

The antioxidant tests confirmed the garlic extracts' ability to reduce and scavenge free radicals. Although both were less effective than ascorbic acid, the DPPH and FRAP results showed concentration-dependent antioxidant activity, with the ethanol extract exhibiting greater activity than the aqueous extract.

This demonstrates that garlic extracts can reduce oxidative stress by acting as efficient donors of hydrogen and electrons.

The existence and significant amounts of phenolics, flavonoids, tannins, saponins, alkaloids, terpenoids, steroids, and glycosides were confirmed by phytochemical screening and quantitative analysis; ethanol extracts often included greater levels of the majority of secondary metabolites. The significance of phytochemicals, especially phenolics, flavonoids, tannins, and organosulfur elements, in the bioactivity of *A. sativum* is highlighted by the substantial association between phytochemical content and documented antibacterial and antioxidant activities.

Overall, the results provide scientific support for the long-standing use of *Allium sativum* in traditional medicine and establish its therapeutic value as a natural antibacterial and antioxidant agent.

5.4. Recommendations

In view of the findings of this study, the following recommendations are proposed:

1. To isolate, identify, and characterize the particular bioactive molecules responsible for the reported antibacterial and antioxidant activity, sophisticated chromatographic and spectroscopic techniques should be used.
2. Prior to clinical or industrial use, it is advised to conduct in vivo investigations and toxicity evaluations to assess the safety, effectiveness, and pharmacokinetic characteristics of garlic extracts, especially the ethanol extract.
3. To clarify the specific mechanisms of antimicrobial action, such as membrane rupture, enzyme inhibition, and redox regulation, molecular and cellular research should be carried out.
4. The ethanol extract should be investigated for development into antibacterial and antioxidant formulations such as topical treatments, preservatives, or nutraceutical supplements due to its enhanced biological potency.
5. It is advised to look into how garlic extracts work in concert with traditional antibiotics and antifungal medications, particularly when battling antimicrobial resistance.

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