THE PHYTOCHEMICALS AND ANTIMICROBIAL PROPERTIES OF *Celosia argentea* LEAVES AGAINST SOME SELECTED BACTERIA ISOLATES

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LSC2007268

(MICROBIOLOGY TECHNIQUES)

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BENIN CITY.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc) IN SCIENCE LABORATORY TECHNOLOGY (MICROBIOLOGY TECHNIQUES).

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work titled THE PHYTOCHEMICALS AND ANTIMICROBIAL PROPERTIES OF *Celosia argentea* LEAVES AGAINST SOME SELECTED BACTERIA ISOLATES was done by Glory Olamiposi AKINNAWONU (Miss) with MAT No LSC2007268 of the Department of Science Laboratory Technology (Microbiology Technique), Faculty of Life Sciences, University of Benin, Benin City, as part of requirement for the award of bachelor of Science (B.Sc.) Degree.

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DEDICATION

I dedicate this project to the Almighty God for His ideas and wisdom while working on this project. I also dedicate this project to my wonderful family for the love and support throughout the course of this project.

ACKNOWLEDGEMENT

I wish to express my heartfelt gratitude to my supervisor, MR Haruna, for his patience, support, and invaluable guidance throughout this work. His dedication, motivation, and commitment to excellence have been a great source of inspiration. I could not have asked for a better mentor.

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I may have forgotten to mention a few name, but I am extremely grateful for everyone of you who I crossed paths with during my time in school. To all those who have supported me and shown me love, all I can say is I’m very grateful and God bless you all abundantly.

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ABSTRACT

Celosia argentea, commonly known as Lagos spinach or “soko yokoto,” is widely used in traditional medicine for the treatment of various ailments such as infections, inflammation, and gastrointestinal disorders.The study investigated the phytochemical constituents and antimicrobial properties of *Celosia argentea* leaves, a medicinal plant widely used in traditional medicine for treating infections, inflammation and other ailments. Growing concerns over antibiotic resistance have driven research into natural sources of antimicrobial agents, particularly from plants rich in bioactive compounds. The study aimed to qualitatively determine the phytochemical components present in *Celosia argentea* leaves and to evaluate their antimicrobial activities against selected pathogenic bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The leaves were collected from Benin City, air-dried, pulverized, and extracted using 50% ethanol. Standard phytochemical tests were performed to identify the presence of secondary metabolites, and antimicrobial sensitivity tests were conducted using the agar disc diffusion method. Results revealed the presence of various phytochemicals such as saponins, terpenoids, flavonoids, alkaloids, glycosides, tannins and phenols, which are known for their pharmacological significance. However, the aqueous extract of Celosia argentea leaves exhibited weak antimicrobial activity, showing mild inhibition zones (5 mm) only at the highest concentration (1000 mg/ml) against Staphylococcus aureus and Escherichia coli, while no activity was observed against Bacillus subtilis and Pseudomonas aeruginosa. The limited antimicrobial response may be attributed to low solubility or concentration of active compounds in water. These findings suggest that although Celosia argentea leaves contain significant bioactive components, solvent optimization and concentration adjustments are essential to enhance antimicrobial efficacy. The study concludes that Celosia argentea has promising therapeutic potential and warrants further investigation using organic solvents and advanced analytical techniques for the development of effective plant-based antimicrobial agents.

CHAPTER ONE

1.0 Introduction

Plants have long been sources of therapeutic agents, particularly in the management of infectious diseases, antioxidants and traditional remedies. As antibiotic resistance becomes an increasing threat to public health globally, research into medicinal plants with antimicrobial and antioxidant properties is becoming more urgent. Among such plants, *Celosia argentea* leaves (family *Amaranthaceae*) has been traditionally utilized in many cultures for treating wounds, diarrhea, inflammation, fever, jaundice and skin diseases, suggesting potential for bioactive compounds that may be effective against microbial pathogens (Sangeetha *et al*., 2023).

Recent phytochemical investigations of *Celosia argentea* have confirmed that it contains multiple classes of secondary metabolites including phenols, flavonoids, tannins, saponins, alkaloids, steroids, triterpenes, glycosides and cyclic peptides (Usunobun and Samuel, 2016). These compounds are well known for their capacity to inhibit microbial growth, scavenge free radicals and protect biological systems from oxidative stress (Usunobun and Samuel, 2016). Moreover, the antioxidant activity of *Celosia argentea* extracts appears to vary by plant part (leaves, stem, inflorescence), solvent used and the developmental (growth) stage, indicating that these variables may influence the potency of its bioactivity (Gupta and Singh, 2023; Niveditha and Rajalakshmi, 2020).

Although some studies have reported antimicrobial activity of various *Celosia argentea* extracts, data are still fragmented. Specific microbial spectrum, minimum inhibitory concentrations (MICs) and the link between phytochemical composition and antimicrobial potency remain incompletely elucidated. There is also limited knowledge about which extracts (solvent types, plant parts,

developmental stage) yield the strongest activity, and whether extracts are safe to use (cytotoxicity) across contexts. Therefore, structured investigations are needed to establish the antimicrobial profile of *Celosia argentea* systematically, correlate it with its phytochemical makeup, and assess potential for therapeutic or preservative applications.

1.1 Background of Study

Celosia argentea is a herbaceous plant widespread in tropical and subtropical regions and is used both as a leafy vegetable and traditional medicine (GlobinMed, 2015; Usunobun and Samuel, 2016). In different cultural phytomedicine systems including Ayurveda, Traditional Chinese Medicine and African traditional medicine the leaves, stems, roots, flowers and seeds are used for a variety of ailments such as fever, wound healing, gastrointestinal disturbances (diarrhea, dysentery), jaundice, urinary disorders, mouth sores and inflammation (Adegbaju and Oladipo, 2018).

Phytochemical studies of *Celosia argentea* reveal that it possesses a rich array of bioactive constituents. For example, the seed extract analysed by UPLC-ESI-Q-TOF-MS identified about 49 compounds including flavonoids, triterpenoids, cyclic peptides, phenols and steroids, many of which had not been previously reported in this species (Suresh and Kumar, 2020). Leaves have been shown to contain phenols, flavonoids, alkaloids, saponins, steroids and glycosides and to have significant antioxidant capacity, assessed via assays such as DPPH radical scavenging and reducing power (Gupta and Singh, 2023; Usunobun and Samuel, 2016). The biopotency (antioxidant) was found to change with maturity and season: flowering stage extracts tend to have higher phenolic and flavonoid content and stronger antioxidant activity than earlier growth stages or leaf-only extracts (Niveditha and Rajalakshmi, 2020).

Some reports also show antimicrobial activity. In particular, ethanol or methanol extracts or inflorescence extracts have been shown to inhibit bacterial growth in *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Proteus vulgaris* among others. Nevertheless, many studies do not report standard metrics (MIC, MBC), use varying extraction methods or do not directly link antimicrobial activity with precise phytochemical profiles. Moreover, cytotoxicity and safety are often under-investigated, limiting practical application. These gaps underscore the need for systematic research that integrates phytochemistry, antimicrobial testing and safety evaluation.

1.2 Aim and Objectives

The aim of the study is to access the phytochemical and antimicrobial properties of *Celosia argentea* leaves.

The objectives of this study were to:

1. determine phytochemical (such as phenols, flavonoids, tannins, saponins, alkaloids, glycosides and tarpenoids) present in *Celosia argentea* leaves.
2. evaluate antimicrobial activity (antibacterial) of Celosia Argentea extracts against some clinical bacteria.
3. to access the potential of *Celosia argentea* as a source of natural antimicrobial agents for pharmaceutical or clinical use.

CHAPTER TWO

2.0 Literature Review

Herbal medicines are currently utilized by nearly 80% of the global population, particularly in developing nations where they serve as the primary source of healthcare. The inadequacy and growing limitations of synthetic drugs have highlighted the increasing relevance of herbal medicine. Ayurveda, a traditional Indian medical system practiced for thousands of years, has significantly contributed to modern pharmacological understanding. Extensive studies on pharmacognosy, chemistry, pharmacology, and therapeutic applications have been conducted on Ayurvedic medicinal plants, establishing a scientific foundation for their efficacy (Sachin Parmar *et al*., 2010). The *Celosia* species belongs to Amaranthaceae. The generic name is derived from the Greek word kelos, meaning "burned," and refers to the flame-like flower heads. There are more than seventy different species identified and among all including *Celosia argentea* are routinely used as leafy vegetable (Uusiku *et al*., 2010). Thus we have selected one medicinal plant *Celosia argentea*. This study will give details of phytochemical and phytotherapeutic activities. *Celosia argentea* is classified as a quantitative short-day plant with alternate, entire, or occasionally lobed leaves. It is an erect, coarse, branched annual herb typically growing between 0.5 and 1.5 meters in height, although it can sometimes be taller. The leaves are light green, smooth, and measure approximately 2 × 6 cm, with those on flowering shoots being slightly longer. Even the green foliage may contain substantial amounts of betalain pigments. The plant produces pinkish, white, or purple flowers that are small and densely packed on erect spikes measuring 8–12 mm in length. These spikes may grow 3–30 cm long and 1.5–2 cm thick, without petals, and the sepals are approximately 6 mm long, exceeding the bracts in length. The plant bears membranous fruits containing numerous small, black seeds about 1 mm in diameter.

The flowers bloom from late summer through late fall, and Celosia argentea is categorized as an annual dicotyledon (Jain 2005).

2.1 Taxonomy of *Celosia Argentea*

Division : Magnoliophyta

Kingdom : Plantae

Clade : Angiosperms

Order : Caryophyllates

Family : Amaranthacea

Genus : Celosia

Species : Argentea

2.2 Distribution

Celosia argentea is cultivated and found across tropical and subtropical regions of the world. Although the plant occurs globally, its use as a food and medicinal herb is more common in specific geographical areas. In West Africa, it is widely consumed as a leafy vegetable, especially in Nigeria, Benin, Togo, Cameroon and Gabon, where it is considered an important component of local diets and traditional medicine (Grubben and Denton, 2004). The wild form, sometimes referred to as *Celosia trigyna*, often grows as a weed throughout the savanna zones of tropical Africa and is commonly harvested as a pot herb. *Celosia argentea* thrives particularly well in the humid rainforest belt and is adapted to warm climates with well-drained soils. It frequently appears during the rainy season and can grow both as a cultivated vegetable and as a spontaneous weed in disturbed fields or garden soils. Its adaptability and high seed production

enable it to persist across a wide range of ecological conditions, making it an easily accessible source of food and medicine in many tropical communities (Grubben and Denton, 2004).

2.3 Morphology

Flower : In spikes, dense, cylindraical, pink turning white

Fruit : A Capsule, globose .seeds 12, reticulate

Leaf Apices : Acute

Leaf Arrangement : Alternate spiral

Leaf Bases : Cuneate

Leaf Margins : Entire

Leaf Shapes : Elliptic

Leaf Types : Simple

Habit : An erect, glabrous profusely branched annual herb

2.4 Common Names

Celosia argentea is known by numerous common names worldwide, reflecting its widespread distribution and cultural significance. In English, it is commonly referred to as silver cockscomb, woolflower, quail grass, or Lagos spinach. In India, the plant is locally called sitivara, vitunnaka, sunishannaka, indivara, safed murga and kanne hoo (Jain, 2005). Across West Africa, particularly in Nigeria, it is popularly known as soko yokoto in Yoruba a name that translates to “make your husband fat and happy,” emphasizing its nutritional value. In other local Nigerian

dialects, it is sometimes referred to as farar alayyahu (Hausa) and mgbolodi (Igbo) (Grubben and Denton, 2004).

In Asia, especially in China, *C. argentea* is recognized as Qing xiang zi or *Semen Celosiae*, often used in traditional Chinese medicine for its therapeutic effects on the liver and eyes (Liu *et al*., 2019). Other regional names include plumed cockscomb, silver spiked cockscomb, wheat celosia, kombada, mesor and lagos spinach (Schippers, 2000). The diversity of local names illustrates the plant's integration into various traditional diets and healing systems across continents.

2.5 Medicinal Therapeutic uses of *Celosia Argentea*

Celosia argentea has long been recognized for its diverse therapeutic properties and remains an important component of traditional medicine in Asia and Africa. Almost every part of the plant leaves, flowers, roots, and seeds has been used to treat a range of ailments. In traditional Chinese medicine, *C. argentea* (known as *Semen Celosiae*) is used to manage liver-related disorders, eye inflammation, and bleeding conditions due to its cooling and hemostatic effects (Huang *et al*., 2004; Liu *et al*., 2019). The seeds are also applied as an antipyretic and to relieve gastrointestinal disturbances such as diarrhea and dysentery (Joshi *et al*., 2012).

In African and Indian folk medicine, the leaves are commonly used to reduce inflammation, fever and skin irritation, while poultices prepared from fresh leaves are applied to abscesses, boils, and wounds to relieve pain and promote healing (Santosh *et al*., 2008). The seeds are also reported to act as an aphrodisiac and antidiarrheal when consumed as a decoction or powder (Adegbaju and Oladipo, 2018). Additionally, the plant's root extract is used in managing gonorrhea, eczema, and colic, whereas its flowers and seeds are administered for hemorrhoidal bleeding, leucorrhea, and jaundice (Cheng *et al*., 2013).

In Sri Lankan traditional medicine, leaf preparations are used to treat fever, inflammation, and skin itching (Subba and Basnet, 2014). Studies have also demonstrated that the plant possesses antimicrobial, antioxidant, and hepatoprotective properties, which scientifically justify some of its ethnomedicinal uses (Malomo *et al*., 2011).

Overall, C. argentea is regarded as a multifunctional medicinal plant with promising pharmacological potential. Its rich phytochemical composition including flavonoids, saponins, and triterpenoids supports its use in managing infections, liver disorders and inflammatory diseases..

2.6 Phytochemical Composition of *Celosia argentea*

Celosia argentea has been the subject of increasing phytochemical investigation over the last two decades. Celosia argentea is a chemically diverse plant that has attracted increasing scientific attention because of its rich array of secondary metabolites. Numerous phytochemical studies have confirmed that the species contains a broad range of biologically active compounds such as flavonoids, phenolic acids, tannins, terpenoids, steroids, triterpenoid saponins, glycosides and cyclic peptides (Liu *et al*., 2019; Gupta and Singh, 2023). These compounds account for many of the pharmacological and therapeutic properties associated with the plant, including antioxidant, anti-inflammatory, antimicrobial and hepatoprotective effects (reviews and chemical surveys). These compound classes account for many of the biological properties attributed to the plant, including antioxidant, anti-inflammatory, hepatoprotective and antimicrobial effects.

Recent analytical advancements have improved the characterization of these metabolites. Earlier studies relied mainly on qualitative tests such as thin-layer chromatography (TLC), but modern techniques including high-performance liquid chromatography (HPLC), gas chromatography–

mass spectrometry (GC–MS) and ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC–ESI–Q–TOF–MS) now allow for detailed compound identification (Liu et al., 2019). For instance, UPLC–ESI–Q–TOF–MS profiling of C. argentea seed extract identified more than forty individual compounds, including flavonoid glycosides, triterpenoid saponins, cyclic peptides and various phenolic derivatives (Liu *et al*., 2019).

Similarly, HPLC and GC–MS analyses of leaf and stem extracts have revealed numerous phenolic acids and terpenoid derivatives, with mid-polarity solvents such as methanol, acetone and ethyl acetate producing higher yields of these bioactive compounds compared to water (Gupta and Singh, 2023). The phytochemical diversity of *C. argentea* not only explains its wide range of traditional uses but also provides a scientific basis for its pharmacological potential in modern medicine.

2.6.1 Phenols and phenolic acids

Phenols and phenolic acids are vital secondary metabolites synthesized through the shikimic acid pathway in plants. They are known for their structural diversity and contribute to color, flavor, and aroma in plants, as well as their therapeutic potential. These compounds exist either as simple phenolic acids or as complex derivatives such as tannins, lignans, coumarins, anthocyanins, stilbenes and flavonoids (Cheynier *et al*., 2013).

In Celosia argentea, several phenolic glycosides have been isolated and characterized. Notably, compounds such as eugenyl O-β-D-glucopyranoside (citrusin C) and 4-O-β-D-glucopyranosyl-2-hydroxy-6-methoxyacetophenone were found to exhibit significant antioxidant and tyrosinase inhibitory activity, demonstrating their role in skin depigmentation and free radical scavenging

(Joshi *et al*., 2012). The high phenolic content contributes to the plant's strong antioxidant potential and its ability to counter oxidative stress, which is closely linked to inflammatory and degenerative diseases (Gupta and Singh, 2023).

2.6.2 Flavonoids

Flavonoids are one of the most abundant groups of phenolic compounds found in Celosia argentea. They consist of two aromatic rings connected by a three-carbon bridge, often forming a heterocyclic pyran ring (Panche *et al.,* 2016). These compounds are essential in plants for pigmentation, UV protection, and defense against pathogens. In pharmacology, they are valued for their antioxidant, anti-inflammatory, and antimicrobial activities (Cowan, 1999).

Two isoflavones, 5-methoxy-6,7-methylenedioxy-2′-hydroxyisoflavone and its 2′-methoxy derivative, known as tlatlancuayin have been isolated from the aerial parts of C. argentea (Cheng *et al.,* 2013). Flavonoids in the plant also contribute to enzyme inhibition and metal ion chelation, enhancing their role in preventing oxidative damage and supporting cardiovascular health. The presence of these bioactive flavonoids underscores the pharmacological value of C. argentea as a natural therapeutic resource.

2.6.3 Alkaloids

Alkaloids are nitrogen-containing organic compounds with significant physiological and pharmacological properties. They are known for their antimicrobial, analgesic, and anti-inflammatory effects (Wink, 2020). In *Celosia argentea*, the presence of alkaloids has been inconsistently reported, with several qualitative screenings detecting trace levels in the leaves, stems and aerial parts (Usunobun and Samuel, 2016). Variations in alkaloid content are often attributed to environmental conditions, solvent choice and the part of the plant analyzed.

Advanced profiling using UPLC–ESI–Q–TOF–MS by Liu *et al*. (2019) confirmed the presence of alkaloid-like compounds in C. argentea seeds, though structural elucidation remains ongoing. Alkaloids exert antimicrobial effects by interfering with DNA synthesis, inhibiting key bacterial enzymes, and disrupting microbial metabolism (Cowan, 1999). Even in small quantities, these compounds may act synergistically with other metabolites such as phenolics and saponins to enhance the plant's antimicrobial potential.

2.6.4. Terpenoids

Terpenoids, also called isoprenoids, represent one of the largest classes of natural compounds derived from five-carbon isoprene units. They are widely recognized for their pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant and anticancer activities (Brahmachari, 2017). These compounds are synthesized through the mevalonate and methylerythritol phosphate pathways in plants and play significant roles in defense mechanisms, photosynthesis, and growth regulation (Tholl, 2015).

In *Celosia argentea*, several terpenoid compounds particularly triterpenes have been isolated from its seeds and leaves. These include celosian, celosianin, celosin A and celosin B, which exhibit hepatoprotective, immunomodulatory, and cytotoxic properties (Hase et al., 1996; Cheng *et al*., 2013). Studies using chromatographic techniques have confirmed that methanolic and ethanolic extracts of C. argentea contain high concentrations of pentacyclic triterpenoids such as oleanolic acid and betulinic acid (Gupta and Singh, 2023). The biological significance of these terpenoids lies in their ability to modulate oxidative stress and inflammation, offering strong pharmacological potential for drug development.

2.6.5 Saponins

Saponins are glycosidic compounds composed of a sugar moiety linked to a triterpene or steroid aglycone. They are known for their characteristic foaming properties and possess a wide range of biological activities, including hypocholesterolemic, antimicrobial and anti-inflammatory effects (Sparg *et al*., 2004).

In Celosia argentea, several triterpenoid saponins have been identified and structurally characterized. Compounds such as celosin A, celosin B, celosin C, and cristatain have demonstrated immunostimulant and hepatoprotective properties (Cheng *et al*., 2013). Saponins from C. argentea have been shown to stabilize hepatic cell membranes, enhance antioxidant enzyme activity and reduce lipid peroxidation in animal models (Hase *et al*., 1996). These bioactivities confirm their therapeutic potential in managing oxidative liver damage and inflammation.

Furthermore, the amphiphilic nature of saponins allows them to interact with cell membranes, leading to antimicrobial and antifungal effects against a range of pathogens (Usunobun and Samuel, 2016). The presence of saponins, alongside terpenoids and flavonoids, gives *C. argentea* its diverse pharmacological properties, supporting its use in traditional medicine for liver and immune-related conditions.

2.6.6 Glycosides (non-saponin glycosides)

Glycosides are naturally occurring molecules formed by the combination of a sugar unit with a non-sugar aglycone, which may be a phenol, alcohol, or terpenoid. They perform essential

physiological functions in plants and have numerous therapeutic applications in humans, including cardiotonic, antimicrobial, and antioxidant roles (Kamboj and Saluja, 2010).

In Celosia argentea, different classes of glycosides, including flavonoid glycosides and phenolic glycosides, have been isolated. These compounds contribute to the plant's antioxidant potential by scavenging free radicals and protecting cellular membranes from oxidative stress (Gupta and Singh, 2023). Specific glycosides, such as celosianin I and II, have been found in the seeds and exhibit notable antitumor and hepatoprotective activities (Li *et al*., 2005).

The synergistic activity between glycosides, saponins, and flavonoids in *C. argentea* is believed to enhance the plant's pharmacological profile, justifying its extensive use in traditional medicine across Africa and Asia (Liu *et al*., 2019).

2.7 Phytochemistry of *Celosia argentea*

Phytochemical investigations have revealed that *Celosia argentea* contains a wide variety of bioactive compounds, including betalains, nicotinic acid, celogenamide A, celogenin A–D, celogentin H–K and moroidin. These compounds play vital roles in the plant's defense mechanisms and biological activities. Flavonoids act as essential signaling molecules in plant reproduction, growth, and stress response, while also contributing to antimicrobial and antioxidant properties (Cheynier *et al.,* 2013).

Isoflavones such as 5-methoxy-6,7-methylenedioxy-2′-hydroxyisoflavone and its methoxy derivative, tlatlancuayin, have been isolated from the aerial parts of C. argentea (Cheng *et al*., 2013). Other identified compounds include eugenyl O-β-D-glucopyranoside (citrusin C), which exhibits tyrosinase inhibitory and superoxide scavenging activities, and phenolic glycosides like

4-O-β-D-glucopyranosyl-2-hydroxy-6-methoxyacetophenone, known for their potent antioxidant action (Joshi *et al*., 2012).

Celosia argentea also contains terpenoids, a group of compounds composed of isoprene units that function as essential metabolites in plants (Tholl, 2015). The plant is additionally rich in amino acids and minerals required for biological and metabolic processes. According to Hase *et al*. (1996), C. argentea contains over eighteen essential minerals, including iron (Fe), manganese (Mn), nickel (Ni), copper (Cu), potassium (K), titanium (Ti) and selenium (Se). A related study showed that Fe, Mn, Cu and zinc (Zn) were present in concentrations of approximately 197, 56, 30 and 160 mg/g, respectively. These elements are crucial for enzyme activation, immune function, and general metabolic balance.

In addition, C. argentea leaves and seeds contain lutein and β-carotene, two carotenoid pigments recognized for their antioxidant and provitamin A activity (Belanger *et al*., 2010). These compounds are known for their protective roles against oxidative damage and for contributing to the plant's distinctive color and pharmacological potential. The chemical complexity of *C. argentea* underscores its medicinal significance and supports further studies on its therapeutic applications.

2.8 Pharmacological Uses of *Celosia Argentea*

With the continuous advancement in phytochemical research, *Celosia argentea* has been recognized for its wide-ranging pharmacological properties. The plant demonstrates antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic and anticancer activities, supporting its ethnomedicinal use in many cultures (Liu *et al*., 2019; Gupta and Singh, 2023).

Its antioxidant capacity is primarily attributed to the high presence of phenolic compounds and flavonoids, which protect biological systems against oxidative stress and free radical-induced damage (Malomo *et al.,* 2011). The antimicrobial activity of *C. argentea* extracts has been reported against common pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, validating its traditional role in treating infections and wounds (Subba and Basnet, 2014). Experimental studies have also demonstrated significant anti-inflammatory and analgesic effects of methanolic extracts, which effectively reduce inflammation and pain (Santosh *et al.,* 2008). The plant's hepatoprotective activity is mainly linked to saponins such as celosin A and celosin B, which stabilize liver cell membranes and improve enzymatic antioxidant functions (Li *et al*., 2005).

2.8.1 Anti-hepatotoxic activity

The water extract of *Celosia argentea* was investigated for hepatoprotective effect in comparison of *Cassia obtusifolia*, *Cucurbita moschata* and *Curcuma aeruginosa*. In the study it was found that among the other species the water extract of *Celosia argentea* is the most effective (Hase *et al*., 1996). These experiments were carried out on the CCl4 induced and D-galactosamine/ lipopolysaccharide, induced liver injury in mice. In same context *Celosian* found in seed are also found to inhibit increase of serum enzymes (GPT, GOT, LTH) and bilirubin levels. The *Celocian* induced the tumor necrosis factor-alpha (TNF-α) production in mice along with production of interleukin-1 ß (IL-1 ß) and nitric oxide (NO) in macrophage cell line J774.1 in a concentration-dependent manner (1 to 1000 micrograms/ml). Significant hepatoprotective effects was implicit due to the antioxidant capability of *Celosia argentea* and proved by CCl4 induced hepatotoxicity in mice, using *celosin* A 32 and *celosin* B 33 *celosin* C 34 and *celosin* D 35 with oral doses 1.0, 2.0 and 4.0 mg kg-1 (Hase *et al*., 1996).

2.8.2 Antibacterial activity

In early 1969, *Celosia argentea* was reported to exhibit antibacterial activity against *Bacillus subtilis, S. aureus, Salmonella typhi, Escherichia coli, Agrobacterium tumefaciens,* and *Mycobacterium tuberculosis*. Further, (Gnanamani *et al*., 2003) researched the antibacterial activity of *Celosia argentea* leaf extracts on eight burn pathogens, finding that the alcohol extract of *Celosia argentea* showed sensitivity in the order *Shigellasp., Pseudomonas sp., Staphylococcus sp., Streptococcus sp., Vibrio sp., Klebsiella sp., E. coli and Salmonella sp.* Regretfully, the promising antibacterial compounds is not clear, and the goal of elucidating their active antibacterial compounds will be part of the focus of this study.

2.8.3 Wound healing activity

The healing efficacy of alcohol extract of *Celosia argentea* in an ointment formulated (10% w/w) using a rat burn wound model. This result confirmed that, a salutary action of the *Celosia argentea* extract on wound healing, and also suggested that this may be due to mitogenic and motogenic promotion of dermal fibroblasts (Priya *et al*., 2004) C. argentea is considered as one of wound healing medicinal plant in India along with various medicinal plants like, *Aloevera, Azadirachta indica, Carica papaya, Cinnamomum zeylanicum, Curcuma longa, Ocimumsanctum, Nelumbo nucifera*, and others (Santosh *et al*., 2012).

2.8.4 Therapeutic effect on eye diseases

For a long time, *Semen celosiae* has been used as an effective herb for treating eye diseases. Compatible with other herbs, it is used to treat ceratitis, epipephysitis, iridocyclitis, and optic atrophy. Huang *et al*. (2004) researched the effects of four Chinese herbs that pass through the liver channel, finding that by improving the anti-oxidant ability of the lens, the water extract of

Semen celosiae could decrease oxidative damage, inhibit lens epithelial cell apoptosis, and reduce lens opacity better than Catalin eye drops. The content of SOD, GSH and GSH-Px in the lenses of the Semen celosiae group were higher than in the Fenton group ($p < 0.01$). Another study focused on their regulation of gene expression related to apoptosis of LEC, finding that the extract modulated Bcl-2 and Bax expressions, making them more approximate to normal ones and more potent than Pirenoxine sodium (Huang *et al*., 2004). Liu *et al*. (2007) observed the treatment of a 20% water extract on senile cataract, finding its therapeutic effect was not significant compared to Catalin, but it caused no side effects.

2.8.5 Anti-inflammatory activity

The in vivo study investigated that, the flavonoid fraction from alcoholic extract of the leaves of *Celosia argentea* for anti-inflammatory activity in animal models like carrageenan induced rat paw edema acute inflammatory and cotton pellet induced chronic inflammatory methods (Santosh *et al*., 2008). Further a study revealed the triterpenoid saponins were isolated from the seeds of *Celosia argentea* and named as *celosin E,F,G,* and *cristatain*. These active constituents are screened for their anti-inflammatory activity by in vitro methods (Wu *et al*., 2011).

2.8.6 Anti-cancer activity

The triterpenoid saponins were isolated from the seeds of *Celosia argentea* and named as *celosin E, celosin F, G,* and *cristatain*. These active constituents are screened for their anti-cancer activity by in vitro methods (Rukshana *et al*., 2013).

2.8.7 Anti-tumor and immunomodulatory activities

Several studies revealed that Celosia argentea is a potent agent for tumor treatment. Hayakawa et al. researched the anti-metastatic effect of Semen Celosiae extracts, finding that administration significantly inhibited liver metastasis by colon 26-L5 carcinoma cells in a dose-dependent manner (Hayakawa *et al.,* 1998). The anti-tumor foundation is immune regulation, including induced IL-12, IL-2, and IFN, resulting in a B dominance state and cell activation. Another study showed significant immunomodulating activity of aerial parts in delayed-type hypersensitivity and neutrophil adhesion tests in mice (Devhare *et al*., 2011). In existing reports, triterpenoid saponins are the most frequently reported class. Celosin A was effective in inducing apoptosis in HeLa and HepG2 cells (Huang *et al*., 2013; Cheng *et al.,* 2013). Wu et al. (2011) tested four triterpenoid saponins for their antitumor activities toward five human cancer cell lines, finding all four had a certain degree of inhibition, with cristatain being the most potent.

2.8.8 Anti-diarrhoeal activity

Celosia argentea could effectively inhibit castor oil-induced diarrhea and charcoal meal-induced diarrhea. Sharma *et al.* (2010) evaluated the anti-diarrhoeal effect of the leaves extract using castor oil-induced diarrhea, charcoal meal test and PGE-induced diarrhea models. Results suggested the extract inhibited diarrhea at doses of 100 to 200 mg/kg and may act centrally and by inhibiting PGE. The extract showed protection against PGE_2-induced enteropooling and decreased propulsive movement, with the 200 mg/kg dose being more efficacious than atropine. For anti-diabetic activity, an alcoholic seed extract significantly reduced blood glucose in alloxan-induced diabetic rats and prevented body weight loss (Vetrichelan *et al*., 2002; Barlocco *et al*., 2002).

2.8.9 Antiurolithiatic activity

Antiurolithiatic activity of ethanolic extract of *Celosia argentea* (seed) in rats was evaluated by (Joshi *et al*., 2012) The result of the study showed that groups treated with 250 kg and 500 kg of *Celosia argentea* extract showed significant anti urolithiatic activity compared with the standard and *Celosia argentea* demonstrated a potent prophylactic effect on formation of kidney stone confirming the folklore about its antiurolithiasis activity

2.8.10 Immunological activity

The *Celosian*, one of the chemical constituent of *Celosia argentea* shows immunostimulating activity. *Celosian* is an acidic polysaccharide from the seeds of this plant. *Celosian* found to be a potent antihepatotoxic agent for chemical and immunological liver injury models in animals. *Celosian* is an immunostimulating agentbecause a study shows that it induced production of tumor necrosis factor-alpha (TNF-alpha), interleukin-1 β, nitric oxide (NO) and γ interferon on various in-vitro experimental methods (Hase *et al*., 1997).

2.8.11 Antifungal activity

Fungal study using plant seed oil n- hexane extract of *Celosia argentea* was studied by (Diéméléou *et al*., 2013) who observed that *Celosia argentea* seed oil showed antifungal activity against Aspergillus fumigatus, Candida tropicalis and Trichophyton mentagrophytes with minimal inhibitory concentration of 50% and therefore concluded that these characteristics should be exploited for possible applications in the food supplement, pharmaceutical and cosmetic industries.

2.8.12 IgE antibody suppression activity

The work of (Imaoka *et al*., 1994) on effects of *Celosia argentea* and Cucurbit amoschata extracts on anti-DNP IgE antibody production in mice showed that Anti-DNP IgE production was markedly suppressed but IgG responses were not affected. It was also found that mitogenic activity occurred in *Celosia argentea* extract dose dependently in vitro. They concluded that these results suggest that *Celosia argentea* extract may be more useful than Perilla frutescens extract (PFE) for the suppression of IgE antibody in certain allergic disorders.

2.8.13 Anti-oxidant activity

Research conducted by Subba and Basnet (2014) on indigenous plants demonstrated that the ethanol extract of *Celosia argentea* exhibited considerable antimicrobial efficacy against pathogens including S. aureus, K. pneumonia, P. vulgaris, and E. coli, along with moderate antioxidant activity. In vitro analysis of the methanolic leaf extract further confirmed its antioxidant properties through significant radical scavenging activity in DPPH, nitric oxide and hydrogen peroxide assays, indicating that phytochemical constituents are likely responsible for these effects (Malomo *et al*., 2011). The same study reported that the aqueous leaf extract inhibited linoleic acid oxidation at 10 mg/ml, comparable to ascorbic acid, and exhibited membrane-stabilizing effects at 2 mg/ml. Phytochemical screening identified alkaloids, saponins, cardiac glycosides, cardenolides, phenolics, and flavonoids. Additionally, the aqueous extract reduced cadmium-induced oxidative stress in animal models, with optimal results observed at 400 mg/kg body weight. While tannins were absent in the aqueous extract, they were detected in ethanolic extracts (Demla and Verma, 2012). In a comparative study of Nigerian leafy vegetables, Odukoya *et al*. (2007) found that Celosia argentea possessed the highest antioxidant activity, attributed to its high ascorbic acid and phenolic content, suggesting that regular consumption may help delay degenerative diseases. Furthermore, novel saponins, designated *Celosia argentea*

C and D, demonstrated potential in animal studies for the development of treatments targeting hepatic, cardiovascular, cerebrovascular, metabolic and neurological disorders.

2.8.14 Anti-mitotic activity

Anti-mitosis properties have been reported for compounds in the moroidin and celogentin families isolated from Semen Celosia. Morita *et al.* (2000) demonstrated that moroidin strongly inhibits tubulin polymerization, showing greater potency than colchicine. Subsequent research indicated that celogentins A–H, J and moroidin all exhibit varying degrees of anti-mitotic activity, with some compounds rivalling or exceeding the potency of vinblastine (Kobayashi et al., 2003). This difference of bioactivity among celogentins and moroidin might be related to the ring size and conformation suitable for interaction with tubulin.

2.9 Other bioactivities

Celosia argentea has other pharmacological activities. The alcohol extracts of *Celosia argentea* promote cell motility and proliferation of primary dermal fibroblasts at 0.1 1g/ml but did not alter these responses in primary keratinocytes. In an initial examination of molecular mechanisms, the *Celosia argentea* extract did not alter fibroblast and keratinocyte responses to the wound repair-associated epidermal growth factor receptor ligands. This may be due to mitogenic and motogenic promotion of dermal fibroblasts (Kobayashi *et al*., 2003)

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials Used

The reagent used for this study is Water (H2O) and Ethanol the equipment/apparatus used are. Thermostatic drying oven, Conical flask, Beaker, Sample bottles, Water bath, Refrigerator, Analytical Weighing balance, Filter paper, Cheese cloth, Electric blender, Petri dish, Micropipette, test tube, cock borer, foil paper, cotton wool, gas cylinder, burner, beakers, measuring cylinder, steel pot, Bama bottles, universal bottles, weighing balance, electronic blender, electric oven, electronic blender, incubator, autoclave, coloured markers, universal bottles

3.2 Sample Collection and Preparation

The leaves of Celosia Argentea was collected on July, 2025 from New Benin market , Benin City, Edo state and taken to the laboratory. The leaf sample were washed to remove debris. The leave sample were air dried for 23 days at room temperature (25-30°C). After air drying, the leaves were further dried in a thermostatic drying oven that was set at 45°C. The sample grounded into a fine powder and macerated using solvent (ethanol). 50gm of the powdered leaves was weighed and dissolved in 500ml of 50% ethanol .The mixture was allowed to stay for 72 hours and was filtered through a cheese cloth, the filtrate was concentrated using water bath set at 40°C until totally evaporated and stored for further use. The extract was taken for phytochemical screening and Antimicrobial sensitivity.

3.3 Phytochemical Screening

Preparation for Alkaloids Reagent

Wagner's Reagent:

0.4g of Potassium Iodide (KI) was dissolved in 0.254g of Iodine. This is done because iodine dissolves better in KI than in water. Once fully dissolved, 20ml of distilled water was added and stirred properly until completely dissolved.

Test Procedure Using Wagner's Reagent:

Add a few drops of Wagner's reagent to 2ml of the plant extract in a test tube. Mix gently and observe. The formation of a reddish-brown or brown precipitate indicates the presence of alkaloids.

Mayer's Reagent:

1g of Potassium Iodide (KI) was dissolved in 0.272g of Mercuric Chloride ($HgCl_2$). Once fully dissolved, 20ml of distilled water was added and stirred until it dissolved properly.

Test Procedure Using Mayer's Reagent:

Add a few drops of Mayer's reagent to 2ml of the plant extract in a test tube and observe. The appearance of a creamy white or pale yellow precipitate confirms the presence of alkaloids.

Preparation for Terpenoids Reagent (Salkowski Test)

2ml of the plant extract was mixed with 2ml of chloroform in a test tube. Then, 1–2ml of concentrated H_2SO_4 was carefully added along the sides of the test tube to form a layer below the

extract (without mixing). A reddish-brown coloration at the interface indicates the presence of terpenoids.

Preparation for Flavonoids Reagent Aluminum Chloride Colorimetric Method:

Dissolve 0.2g of Aluminum Chloride ($AlCl_3$) in 20ml of distilled water.

Test Procedure for Flavonoids:

Add 1ml of 1% Aluminum Chloride ($AlCl_3$) to 1ml of the plant extract, shake, and observe. The development of a yellow coloration indicates the presence of flavonoids.

Preparation for Glycosides Reagent

Baljet Test:

Dissolve 0.1g of Picric Acid in 10ml of distilled water. Separately, dissolve 1g of Sodium Hydroxide (NaOH) in 10ml of distilled water. Mix equal volumes of the 1% Picric Acid and 10% NaOH solutions just before use.

Test Procedure Using Baljet Reagent:

Take 2ml of the plant extract in a test tube and add a few drops of Baljet reagent. An orange to reddish coloration indicates the presence of glycosides.

Keller-Killiani Test:

Take 2ml of the plant extract in a test tube and add 2ml of glacial acetic acid containing one drop of 5% Ferric Chloride ($FeCl_3$) solution. Then, carefully add 1ml of concentrated H_2SO_4 down the side of the test tube to form a separate layer at the bottom. A reddish-brown ring or bluish-green coloration indicates the presence of glycosides.

Test Procedure for Saponins

Foam Test:

Add 1ml of the plant extract to 10ml of distilled water in a test tube. Shake vigorously for 30 seconds and allow the tube to stand undisturbed for 10–15 minutes. Observe for the formation of stable foam, which indicates the presence of saponins.

Emulsion Test:

Mix 2ml of the plant extract with a small amount of oil and ethanol. Shake vigorously to dissolve any lipid content. Add 2–3ml of distilled water, shake gently, and observe. The formation of a white, cloudy emulsion (milky appearance) indicates the presence of lipids.

Preparation for Tannins Reagent

Ferric Chloride Test:

Dissolve 0.2g of Ferric Chloride crystals in 5ml of distilled water while stirring. Once dissolved, make up the volume to 20ml.

Test Procedure Using Ferric Chloride Solution:

Dissolve 1ml of the plant extract in 5ml of distilled water and add 1–2 drops of 1% $FeCl_3$. A blue-black or greenish-black coloration indicates the presence of tannins.

Lead Acetate Test:

Weigh 2g of Lead Acetate and dissolve in 5ml of distilled water. Once dissolved, make up the volume to 20ml.

Test Procedure Using Lead Acetate Solution:

Mix 2ml of extract with 2ml of 10% lead acetate solution in a test tube. A white or cream-colored precipitate indicates the presence of tannins.

Preparation for Phenolics Reagent

Ferric Chloride Test:

Dissolve 0.2g of Ferric Chloride crystals in 5ml of distilled water while stirring. Once dissolved, make up the volume to 20ml.

Test Procedure Using Ferric Chloride Solution:

Dissolve 1ml of plant extract in 5ml of distilled water and add 1–2 drops of 1% $FeCl_3$. A blue, green, or purple coloration indicates the presence of phenols.

Lead Acetate Test:

Weigh 2g of Lead Acetate and dissolve in 5ml of distilled water. Once dissolved, make up the volume to 20ml.

Test Procedure Using Lead Acetate Solution:

Mix 2ml of extract with 2ml of 10% lead acetate in a test tube. A white or cream-colored precipitate indicates the presence of phenols.

3.4 Preparation of Media

The media for microbiological analysis were weighed according to the manufacturer's specifications.

3.4.1 Nutrient Agar

Thirty-nine grammes (28 g) of nutrient agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was placed in an autoclave to sterilize it for 15 min at 121 C at a pressure of 15 psi. After sterilization, the flask was allowed to cool before being aseptically poured into Petri dishes. The composition of this medium is as shown in the appendix

3.4.2 Mueller Hinton Agar

Thirty-nine grammes (39 g) of Mueller Hilton agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was the placed in an autoclave to sterilize it for 15 minutes at 121 Cat a pressure of 15psi. After sterilization, the flask was allowed to cool before it was poured into Petri dishes. aseptically. The composition of this medium is as shown in the appendix

3.5 Micro Organisms Used

The organisms used for this study were, *staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.*

3.6 Preparation of Test Organism

The clinical bacterial isolates were sub-cultured on nutrient Agar and incubated at 37°C for 24 hrs. The bacterial inoculum was then standardized to 0.5 McFarland standard.

3.7 Antimicrobial susceptibility testing

This was carried out with Mueller Hinton Agar and agar well diffusion method. Using the pour plate method, the agar was poured into sterile petri dishes and 1ml of standardized inoculum was introduced. A sterile glass spreader was used to evenly distribute the isolate. Wells were bored using a 8mm cork borer in the isolate inoculated Mueller Hinton Agar plate and then impregnated with different concentrations of the extract (1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml). The plates were allowed to diffuse and then incubated at 37°c for 24hours after the zones of inhibition were measured.

3.8 Antimicrobial sensitivity bioassay

The antibiotic susceptibility of the bacterial isolates was determined using the Kuby Bauer disk diffusion technique on Mueller Hipton agar. Using the pour plate method, the agar was poured into sterile petri dishes and 1ml of standardized inoculum was introduced. A sterile glass spreader was used to evenly distribute the isolate. After the agar solidified, Gram-positive and Gram-negative antibiotics sensitivity disc were aspectically placed on the surface using sterile forceps. Antibiotic disc used and their concentrations were as follows: Cram positive discs contained; Pefloxacin (10 µg), Gentamycin (10 µg), Ampiclox (30 µg), Zinnacef (20 µg), Amoxacillin (30 µg), Rocephin (25 µg), Ciprofloxacin (10 µg), Azithromycin (12 µg), Levofloxacin (20 µg), Erythromycin (10 µg). The Gram negative disc contain; Levofloxacin (20µg), Cefotaxim (10µg), Sparfloxacin (10µg), Ciprofloxacin (30µg), Amoxacillin (30µg), Augmentin (10µg), Gentamycin (30µg), Pefloxacin (30µg), Tarlvid (10µg), Azithromycin (12µg). Plates were incubated in an inverted position at 30-31°C for 18-24 hours. Following incubation, the diameter of the inhibition zones around each antibiotic disc was measured in millimeters using a transparent ruler (Jawetz *et al*., 2004) .

A gram-positive disc was used on the culture of Staphylococcus aureus and Bacillus subtilis while a gram-negative disc was used on Escherichia coli and Pseudomonas aeruginosa.Antibiotic discs were used as positive controls.

CHAPTER FOUR

4.0 PRESENTATION OF RESULTS

4.1 Quanlitative Phytochemical Screening

This study was conducted to qualitatively determine the bioactive constituents present in Celosia Argentea leaves. The result represented in table 1 indicates the presence of various secondary metabolites in an aqueous extract of Alstonia boonei leaves, each of which plays a crucial role in the plant's biochemical and pharmacological properties, with their respective quantities and are reported in the table 1. It was observed that Alkaloids, saponins, terpenoids,flavonoids and glycosides were present in high quantities, while phenols and tannins were present in moderate quantities. The presence of these secondary metabolites in the 50%ethanol extract, particularly in high and moderate quantities, suggests that the extract could possess a broad spectrum of bioactive properties.

Table 1: Quanlitative phytochemical screening of solvet extract of *Celosia argentea* leaves

Phytochemicals screened for	Result
Saponins	+
Terpenoids	+
Flavonoids	+
Alkaloids	+
Glycosides	+
Tannins	+
Phenols	+

KEYS

- Negative

+ Positive

4.2 Antimicrobial Analysis Result

The antimicrobial activity of the aqueous extract of Celosia argentea leaves was evaluated in comparison with standard antibiotic discs against selected bacterial isolates. The results presented in Table 2 show the zones of inhibition produced by the extract and the antibiotic discs, which serve as controls for determining the sensitivity of the test organisms.

Table 2: Antimicrobial Analysis Result Using Antibiotic Discs

Gram Positive Organism	*Staphylococcus aureus*	*Bacillus subtillis*
Pefloxacin (10 µg)	9(R)	11(I)
Gentamycin (10 µg)	8(R)	7(R)
Ampiclox (30 µg)	10(R)	4(R)
Zinnacef (20 µg)	0(R)	4(R)
Amoxacillin (30 µg)	0(R)	0(R)
Rocephin (25 µg)	6(R)	7(R)
Ciprofloxacin (10 µg)	10(R)	12(I)
Azithromycin (12 µg)	9(R)	7(R)
Levofloxacin (20 µg)	10(R)	10(R)
Erythromycin (10 µg)	8(R)	7(R)

KEYS

R – Resistance (0-10)

I - Intermediate (11-16)

S – Susceptibility(17 and above)

Table 3: Antimicrobial Analysis Result

Gram Negative Organism	*Escherichia coli*	*Pseudomonas aeruginosa*
Pefloxacin (10 µg)	4(R)	7(R)
Gentamycin (10 µg)	5(R)	11(I)
Sparfloxacin (10µg)	0(R)	0(R)
Augmentin (10µg)	0(R)	0(R)
Amoxacillin (30 µg)	0(R)	0(R)
Cefotaxim (10µg)	0(R)	20(S)
Ciprofloxacin (10 µg)	0(R)	13(I)
Azithromycin (12 µg)	6(R)	14(I)
Levofloxacin (20 µg)	0(R)	11(I)
Tarlvid (10µg)	10(R)	8(R)

KEYS

R – Resistance (0-10)

I - Intermediate (11-16)

S – Susceptibility (17 and above)

Table 4: Antimicrobial Analysis Result Using Extract of *Celosia argentea* leaves

Organism	*Staphylococcus aureus*	*Bacillus subtillis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1000mg/ml	5(R)	0(R)	5(R)	0(R)
500mg/ml	0(R)	0(R)	0(R)	0(R)
250mg/ml	0(R)	0(R)	0(R)	0(R)
125mg/ml	0(R)	0(R)	0(R)	0(R)
62.5mg/ml	0(R)	0(R)	0(R)	0(R)

KEYS

R – Resistance (0-10)

I - Intermediate (11-16)

S – Susceptibility (17 and above)

CHAPTER FIVE

5.0 Discussion and Conclusion

5.1 Discussion

The phytochemical screening of the aqueous extract of *Celosia argentea* leaves revealed the presence of saponins, terpenoids, flavonoids, alkaloids, glycosides, tannins, and phenols. These phytochemicals are secondary metabolites that play crucial roles in plant defense mechanisms and pharmacological activities. The detection of these compounds is consistent with previous findings by Usunobun and Samuel (2016) and Gupta and Singh (2023), who reported that *Celosia argentea* contains a diverse range of bioactive components. Phenolic compounds and flavonoids, in particular, are well-known for their antimicrobial and antioxidant potential, while saponins and alkaloids contribute to the disruption of microbial membranes and inhibition of nucleic acid synthesis (Cowan, 1999; Mandal *et al*., 2010). The presence of these phytochemicals confirms that *Celosia argentea* is a pharmacologically rich plant with possible therapeutic applications.

However, despite this phytochemical richness, the antimicrobial results of the aqueous extract were generally low when compared with standard antibiotic discs. From Table 4.2.3, the extract showed only mild inhibitory activity at the highest concentration (1000 mg/ml) against *Staphylococcus aureus* and *Escherichia coli*, both showing 5 mm zones of inhibition. No inhibition was observed against *Bacillus subtilis* and *Pseudomonas aeruginosa*, even at the highest concentration. All lower concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml) showed no inhibition. This suggests that the antimicrobial constituents in the aqueous extract may be present in low concentrations or poorly extracted into water. According to

Harborne (1998), solvent polarity greatly affects phytochemical solubility and extraction efficiency, with ethanol and methanol often extracting higher concentrations of bioactive compounds compared to water.

When compared with the antibiotic control (Tables 4.2.1 and 4.2.2), the standard antibiotic discs exhibited varying degrees of inhibition against the tested organisms. For the Gram-positive isolates (*Staphylococcus aureus* and *Bacillus subtilis*), antibiotics such as Ciprofloxacin, Pefloxacin, and Levofloxacin showed intermediate (I) to resistant (R) responses, while Amoxicillin, Ampiclox, and Rocephin recorded no significant inhibition. In the Gram-negative group (E. coli and P. aeruginosa), resistance was even more pronounced; however, Cefotaxime (20 mm) and Ciprofloxacin (13 mm) produced notable inhibition against *Pseudomonas aeruginosa*, which was classified as sensitive (S) and intermediate (I) respectively. These results indicate the presence of multidrug-resistant (MDR) strains among the tested isolates, which mirrors the global pattern of antimicrobial resistance reported by the World Health Organization (WHO, 2022).

The weak inhibitory action of *Celosia argentea* extract compared to the antibiotics could also be due to the nature of the test organisms. Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* possess a complex cell wall structure with an outer membrane rich in lipopolysaccharides that restricts the entry of many hydrophilic compounds, including some plant-derived phytochemicals (Tortora et al., 2019). On the other hand, Gram-positive bacteria like *Staphylococcus aureus* have a thick peptidoglycan layer that allows easier penetration of such compounds. Despite this, the extract only showed minimal inhibition, implying that the concentration of active phytochemicals was not sufficient to elicit strong bactericidal or bacteriostatic effects.

The presence of saponins and terpenoids in the extract could explain the slight inhibition zones observed against *Staphylococcus aureus* and *Escherichia coli*. Saponins possess surfactant properties that disrupt bacterial cell membranes, leading to leakage of cell contents, while terpenoids interfere with bacterial cell wall synthesis and protein metabolism. Alkaloids, which were also detected, have been associated with the inhibition of bacterial DNA gyrase, thereby preventing replication and transcription. However, the extract's overall effectiveness likely depends on the combined action and concentration of these compounds. The low activity recorded suggests that either the active molecules were insufficiently extracted or degraded during the aqueous extraction process due to heat or oxidation.

Interestingly, previous studies using organic solvents have demonstrated significantly higher antimicrobial potency of *Celosia argentea*. Gnanamani et al. (2003) reported that ethanolic extracts of *Celosia argentea* leaves exhibited strong activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while Malomo et al. (2011) found that methanolic extracts inhibited E. coli and Klebsiella pneumoniae with zones up to 14 mm. Similarly, Subba and Basnet (2014) observed moderate antimicrobial activity of *Celosia argentea* against several clinical pathogens, attributing it to phenolic and flavonoid content. These findings reinforce that solvent selection plays a critical role in the extraction of antimicrobial compounds from medicinal plants.

The resistance observed among most antibiotic discs also carries clinical implications. The fact that both *Staphylococcus aureus* and *Escherichia coli* were resistant to several commonly used antibiotics such as Ampiclox, Amoxicillin, and Augmentin underscores the increasing trend of antimicrobial resistance (AMR) among pathogenic bacteria. The intermediate responses of *Bacillus subtilis* and *Pseudomonas aeruginosa* to Ciprofloxacin and Cefotaxime suggest partial susceptibility, but this pattern indicates a narrowing spectrum of effective antibiotics. This

growing resistance crisis emphasizes the need for novel antimicrobial agents from plant sources, and *Celosia argentea* may be a potential candidate for such development if extracted and formulated optimally.

The limited antimicrobial action of the aqueous extract might also result from the synergistic nature of phytochemicals in their natural state. In traditional medicine, decoctions and infusions are typically administered in combination with other plants, which may potentiate overall efficacy (Fabricant & Farnsworth, 2001). The loss of synergy during isolation or concentration processes may reduce effectiveness when the extract is tested singly. Furthermore, some phytochemicals require enzymatic activation or metabolic transformation in vivo to express their full antimicrobial potential (Harborne, 1998), which may not occur under in vitro laboratory conditions.

Another explanation for the minimal inhibition is the possible antagonistic effects of some phytochemical constituents. For example, high levels of tannins can form insoluble complexes with proteins, reducing the availability of active compounds to interact with microbial cells (Aiyegoro and Okoh, 2009). This may have contributed to the overall low zones of inhibition recorded in this study despite the confirmed presence of multiple bioactive metabolites. Similarly, glycosides, although pharmacologically active, may exist as inactive precursors that require enzymatic hydrolysis to release the aglycone component responsible for antimicrobial activity (Mujeeb *et al*., 2014).

5.2 Conclusion

Overall, while the aqueous extract of *Celosia argentea* leaves demonstrated minimal antimicrobial activity, the presence of diverse phytochemicals suggests strong therapeutic potential. The weak inhibition observed in this study does not negate the plant's ethnomedicinal relevance; rather, it highlights the need for optimized extraction methods, higher concentrations, and perhaps synergistic formulations. Plants like *Celosia argentea* could serve as valuable sources of novel antimicrobial agents if their active components are isolated, purified, and evaluated further (Eloff, 2019). Future research should also assess the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for different solvent extracts to establish quantitative antimicrobial profiles.

In conclusion, the study confirms that *Celosia argentea* leaves are rich in bioactive compounds but that their aqueous extract, as tessted, was not potent enough against the selected bacterial isolates. The results align with earlier research indicating that organic solvent extracts generally show superior antimicrobial activity compared to aqueous extracts. Therefore, further studies should focus on fractionation, solvent variation, and combinational studies with antibiotics to fully exploit the antimicrobial potential of *Celosia argentea* leaves.

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APPENDIX

Plate 1: Experimental Procedures (Field Work, 2025)

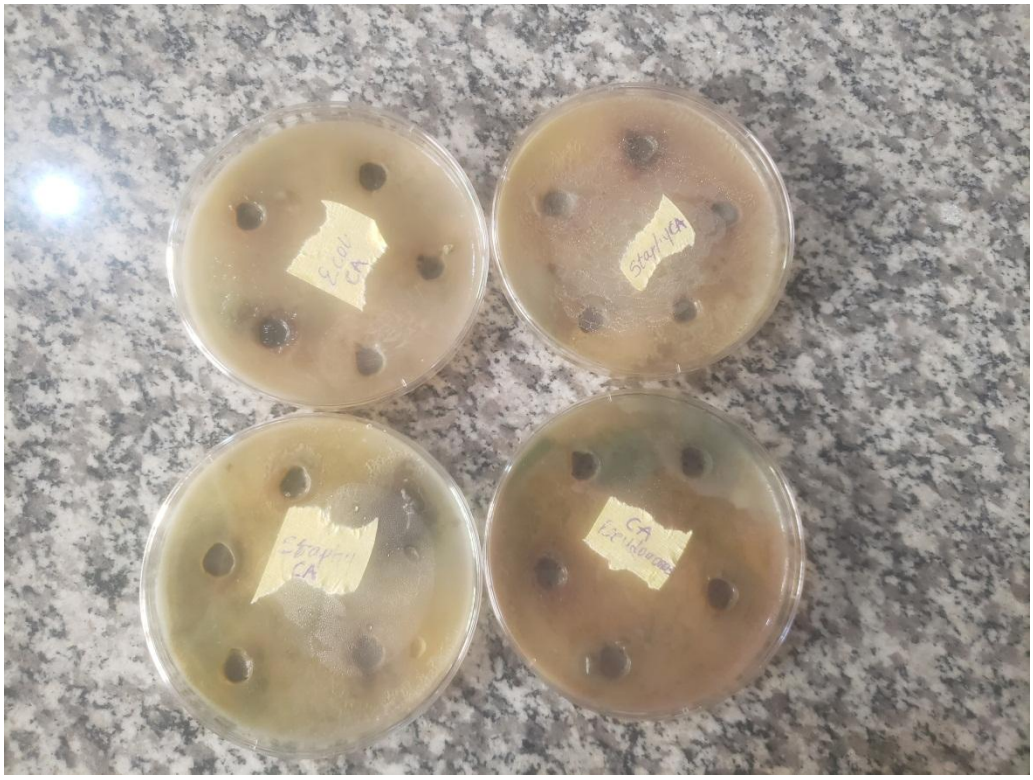

Plate 2: Agar well plate (Field Work, 2025)

Plate 3: Antibiotics Disc plate (Field Work, 2025)

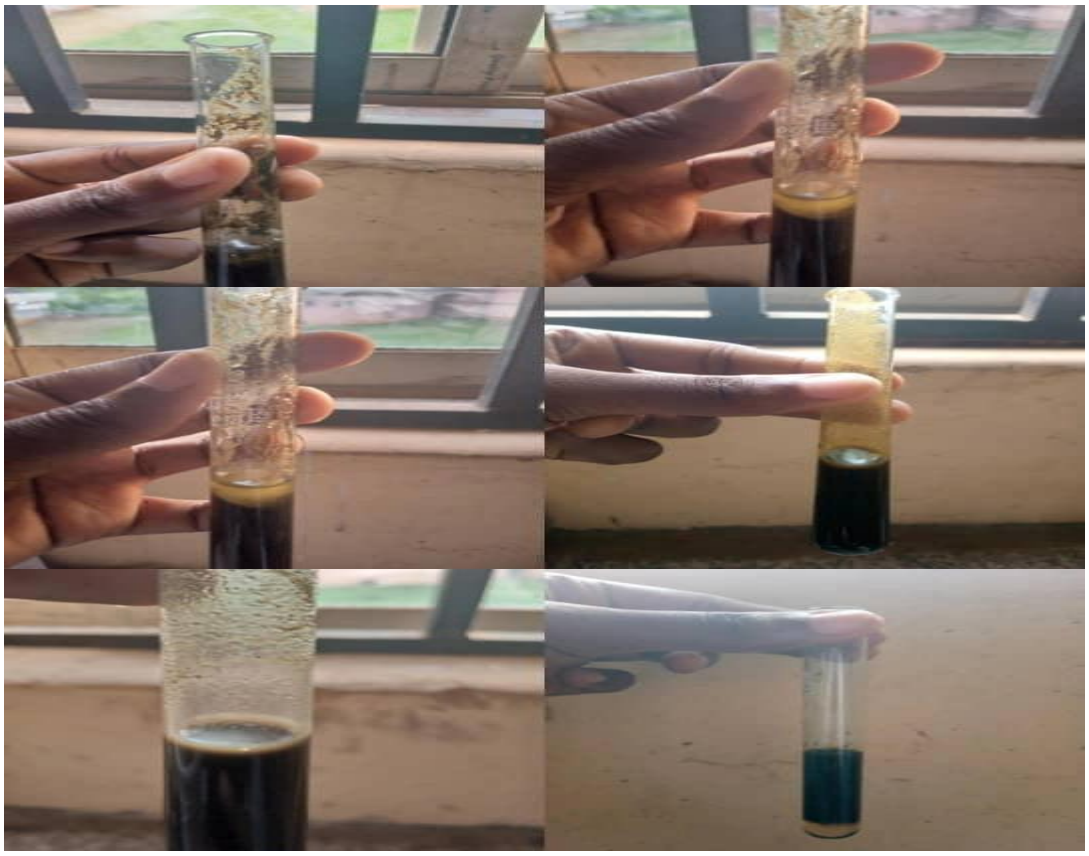

Plate 4: Phytochemical Screening (Field Work,