ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL COMPOSITION OF CHIA SEEDS (*Salvia hispanica*).

BY

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BENIN CITY.

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project work was carried out by **Judith Obianuju IZUAGBA** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

______________________________ ______________________

Dr. (Mrs.) O.B. Isichei-Ukah **DATE**

(Project Supervisor)

APPROVAL

This project work was carried out by **Judith Obianuju IZUAGBA** in partial fulfilment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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PROF. (MRS.) F. I. AKINNIBOSUN **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far in life. I am truly grateful.

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ABSTRACT

Chia seed (*Salvia hispanica*) are small edible seed with high nutritional and medicinal values. This study was carried out to investigates the antimicrobial and phytochemical constituents of the seeds. The aqueous and ethanol extract of the seed were screened for antimicrobial effects against six bacterial isolates :*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp., *Salmonella* sp., *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Phytochemical screening for flavonoids, tannins, sterols, saponins, glycosides and alkaloids were carried out by standard methods. The results showed that both ethanolic and aqueous extracts exhibited antimicrobial activities. At 100 mg/ml concentration, the aqueous extract produced inhibition zones of 20.00 mm against *Escherichia coli* and 14.00 mm against *Streptococcus* sp., while the ethanolic extract showed 16.00 mm and 18.00 mm, respectively. *Pseudomonas aeruginosa* exhibited inhibition zones of 21.00 mm (aqueous) and 10.00 mm (ethanolic), and *Klebsiella pneumoniae* showed 7.00 mm (aqueous) and 14.00 mm (ethanolic). However, *Staphylococcus aureus* did not exhibit significant inhibition in either extract. Minimum inhibitory concentration analysis revealed that both ethanolic and aqueous extracts had MIC values of 100 mg/ml for *Streptococcus* sp. and *Pseudomonas aeruginosa*. Interestingly, *Escherichia coli* did not show an MIC with the ethanolic extract, suggesting variable susceptibility across bacterial strains. The minimum bactericidal concentration results indicated that the ethanolic extract was bacteriostatic, while the aqueous extract exhibited bactericidal activity for *Pseudomonas aeruginosa*. Phytochemical analysis revealed the presence of flavonoids, tannins, sterols, saponins and glycosides in both extracts, while alkaloids were absent.These findings highlight the promising antimicrobial potential of chia seed oil extracts, demonstrating it's efficacy against a range of Gram-negative and Gram-positive bacteria.

CHAPTER ONE

INTRODUCTION

1.1.Background of Study

Chia seeds (*Salvia hispanica*), a member of the *Lamiaceae* family, have been revered for centuries due to their extensive health benefits and nutritional value. These small, oval seeds are typically black or white and have gained recognition for their ability to enhance dietary intake through a dense concentration of essential nutrients. Historically, chia seeds were a staple in the diets of ancient civilizations such as the Aztecs and Mayans, who utilized them for their energy-boosting properties and as a form of currency (Mohd *et al*., 2012). In recent years, chia seeds have re-emerged as a superfood, prized for their versatility and substantial health benefits (Capitani *et al*., 2012).

The nutritional profile of chia seeds is exceptional, encompassing a wide range of macronutrients and micronutrients that contribute to overall health and well-being. They are an excellent source of dietary fiber, omega-3 fatty acids, proteins, vitamins, and minerals. Chia seeds are rich in protein and healthy fats, particularly polyunsaturated fats. They contain approximately 30% oil, of which about 60% is omega-3 alpha-linolenic acid (ALA) and 20% is omega-6 linoleic acid. This unique fatty acid profile is beneficial for cardiovascular health (Ayerza and Coates, 2011).Chia seeds provide a substantial amount of essential vitamins and minerals, including calcium, magnesium, phosphorus, and manganese. These nutrients are vital for bone health, metabolic processes, and overall physiological functions (Ixtaina *et al*., 2011).In addition to their nutrient density, chia seeds are packed with various bioactive compounds such as phenolic acids, flavonoids, and tocopherols, which contribute to their antioxidant and anti-inflammatory properties (Reyes-Caudillo *et al*., 2008).

Phytochemicals are non-nutritive plant chemicals that have protective or disease-preventive properties. Chia seeds are abundant in several classes of phytochemicals, including phenolic acids, flavonoids, and tocopherols, each playing a critical role in health promotion and disease prevention. In addition to their impressive nutritional and phytochemical profile, chia seeds exhibit significant antibacterial properties. These properties are primarily due to the presence of bioactive compounds that can disrupt bacterial cell walls, inhibit bacterial enzymes, and interfere with bacterial growth and metabolism.

The antibacterial activity of chia seeds involves several mechanisms. Phenolic compounds, for instance, can cause structural damage to bacterial cell walls and membranes, increasing their permeability and leading to cell death (Albuquerque *et al*., 2014). These compounds can also inhibit bacterial proteins and enzymes, disrupting essential metabolic processes and preventing bacterial growth. Flavonoids further enhance the antibacterial activity by interfering with bacterial DNA synthesis and biofilm formation. Biofilms are protective layers that bacteria form to shield themselves from hostile environments and antibiotics. By preventing biofilm formation, flavonoids increase the susceptibility of bacteria to antimicrobial agents and the host immune system (Cushnie and Lamb, 2011).

Studies have demonstrated the effectiveness of chia seed extracts against various bacterial pathogens. For example, research has shown that chia seed extracts can inhibit the growth of *Pseudomonas aeruginosa, Escherichia coli* (*E. coli*), a common cause of foodborne illness, and *Staphylococcus aureus,* responsible for a range of infections from minor skin conditions to severe diseases like pneumonia and sepsis (Taga *et al*., 2015). In vitro studies have highlighted the potential use of chia seeds as natural preservatives in food products. By inhibiting the growth of foodborne pathogens, chia seed extracts can extend the shelf life of food items and enhance food safety. Additionally, the antibacterial properties of chia seeds may be utilized in agriculture to develop natural pesticides that protect crops from bacterial infections without the adverse environmental impacts associated with synthetic chemicals (Reyes-Caudillo *et al*., 2008).

Understanding the phytochemical and antibacterial properties of chia seeds is crucial for multiple reasons. From a health perspective, these properties support the use of chia seeds as a functional food that can aid in the prevention and management of chronic diseases. The antioxidant and anti-inflammatory effects of chia seed phytochemicals help mitigate oxidative stress and inflammation, key factors in the development of diseases like cardiovascular disease, diabetes, and cancer. In agriculture, the antibacterial properties of chia seeds offer a sustainable alternative to synthetic pesticides, promoting organic farming practices and reducing the environmental impact of agriculture. The ability of chia seeds to inhibit bacterial growth also enhances food safety by preventing contamination and spoilage, which is essential for maintaining public health. From a medical standpoint, exploring the antibacterial properties of chia seeds can lead to the development of new antimicrobial agents. As antibiotic resistance becomes an increasing global concern, natural compounds from plants like chia seeds provide promising avenues for new treatments that can overcome resistance mechanisms and offer effective alternatives to conventional antibiotics (Savoia, 2012).

Chia seeds are a remarkable source of nutrients and bioactive compounds, with significant phytochemical and antibacterial properties. The phenolic acids, flavonoids, tocopherols, and polyunsaturated fatty acids in chia seeds contribute to their health-promoting effects, while their antibacterial properties hold promise for applications in food safety, agriculture, and medicine. Understanding these properties not only highlights the nutritional and therapeutic potential of chia seeds but also opens up new avenues for research and development in various fields. As interest in natural and sustainable solutions continues to grow, chia seeds stand out as a valuable resource with multifaceted benefits for health and well-being.

1.2. AIMS AND OBJECTIVES

The aim of the study was to evaluate the phytochemical and antimicrobial properties of aqueous and ethanolic extracts of chia seed against selected bacterial isolates

The specific objectives of this research were to:

1. evaluate the antimicrobial activity of aqueous and ethanolic extracts of chia seed extract against the bacterial isolates
2. determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against the selected bacterial isolates.
3. identify and quantify the various phytochemicals present in aqueous and ethanolic extracts of chia seed extract.

CHAPTER TWO

LITERATURE REVIEW

2.1. Description

Chia (*Salvia hispanica* L.), which has been cultivated since ancient times (Cahill and Ehdaie, 2005), is an annual herbaceous plant of the *Lamiaceae* family (Thaboran *et al*., 2020; Cahill, 2004). Salvia is the most major genus of this family and has about 1000 species that are widely scattered in different areas of the world, including South Africa, Central America, North America, South America and Southeast Asia (SeguraCampos *et al*., 2014; Takano, 2017). According to the latest classification, this genus is part of the subfamily *Nepetoideae*, tribe *Mentheae*, subtribes *Salviinae* (Segura-Campos *et al*., 2014). Its main source is between Mexico and Guatemala (Cahill, 2004).

The common name of *S. hispanica* L. is chia (Cahill and Provance, 2002). It is an expensive plant used by natives since the past for medicine, food, and oil (Cahill and Ehdaie, 2005), but its cultivation ceased and was unknown species for hundred years beyond the confines of Mexico and Central America because of its habituation to short days, high sensitivity to changes in photoperiods and poor tolerance to cold (Jamboonsri *et al*., 2012). During the last two decades, after the introduction of chia seeds by various scientists, the interest in chia consumption and research on it has increased (Gentry *et al*., 1990; Coates and Ayerza, 1996). Nowadays, there is a revitalized fondness for chia and a lot of work is to be done about it as a wonderful source of ω3, protein, antioxidants and dietary fiber for healthful diets (Bochicchio *et al*., 2015a). In 2009, it was authorized as a novel food (EFSA NDA Panel, 2009).

Chia seeds are considered as a healthy food with numerous nutritional value (Super food) (Marineli *et al*., 2015) and there is not any proof that shows consuming whole or ground chia seeds is harmful (EFSA, 2005; 2009; Bresson *et al*., 2009). Therefore, chia seeds and their derivatives are promising food sources. The United States Department of Agriculture (USDA) has encouraged the cultivation of chia as an industrial crop (Valdivia-Lopez and Tecante, 2015). Chia seeds are becoming more and more admired and are a key ingredient among consumers and producers (Iglesias-Puig and Haros, 2013; Kuznetcova *et al*., 2020; Ribes *et al*., 2021; Zettel and Hitzmann, 2018). Chia seed production has enhanced in late years (Grancieri *et al*., 2019; Jamshidi *et al*., 2019). Nowadays, chia is commercially cultivated in several low-latitude agricultural regions in the world, mainly in Bolivia, Paraguay, Argentina, Mexico, Australia, Central America, Peru, Ecuador and Colombia, and the total acreage in 2014 was 370,000 hectares (Sosa, 2016; Orona-Tamayo *et al*., 2016). Also, chia seeds are accessible in hypermarkets and health food stores (Dincoglu and Yesildemir, 2019).

2.2. Morphology

Depending on the latitude in which chia seeds are grown, their life cycle from growth to harvest takes 90 to 180 days. Its growth depends on the sea level, temperature and light (Cahill, 2004). Also, in Kermanshah with Latitude 34° 32' N, Longitude 47° 10' E is about 120 days. Chia height can be from 60 to about 180 cm in different mentioned altitudes (Fig. 1) (Capitani *et al*., 2013; Wojahn *et al*., 2018; Yeboah *et al*., 2014). *Salvia hispanica* L. is a self-pollinating plant

(Bochicchio *et al*., 2015a). However, some scientists reported some degrees of outcrossing in domesticated and wild chia in their field studies (Hernandez-Gomez *et al*., 2008; Cahill, 2004).

Since plenty of insects are absorbed into chia flowers and low outcrossing is found in greenhouse conditions, it appears that insects are more likely to be responsible for transporting pollen instead of wind (Buchichio *et al*., 2015a). Chia is a drought-resistant product, so it can grow in semiarid (Ayerza and Coates, 2009 a,b) and arid environments (Peiretti and Gai, 2009). Also, based on some researchers, this plant is sensitive to salt stress, and salinity can considerably diminish the yield of seed oil (Heuer *et al*., 2002). Chia is semi-tolerant to acidic soils (Munoz *et al*., 2013; Baginsky *et al*., 2014; Pozo and Anabel, 2010). But it cannot stand frost and freeze in all development stages (Baginsky *et al*., 2014; Jimenez, 2010). Chia needs a lot of sunlight and does not bear fruit in the shade (Cahill, 2004). Since 1917, it has been stated that the maximum achievable yield of chia seeds is near 3.0 t/ha (Lomanitz, 1917). However, the seed yield commonly achieved by the farmers is lower, and on average, it is only 0.36 t/ha (Peperkamp, 2015). Chia presents low requirements of water and fertilizer (Orozco *et al*., 2014), and it is also resistant to pests and diseases (Munoz *et al*., 2013).

2.2.1. Seeds

Chia fruit is a type of schizocarp that separates and forms four fruits, which are called mericarps or nutlets (Segura-Campos *et al*., 2014). Its shape is oval and its size of it varies from 1-2 mm long by 1.5 mm wide (Hernandez Gomez *et al*., 2008; Ixtaina *et al*., 2008). The seeds coat color is different and changes from black, grey and black spotted to white, although today, most commercial plants are black-spotted, Fig. 4 (Ixtaina *et al*., 2008). The seed is wealthy in mucilage, starch and oil (Hernandez Gomez *et al*., 2008). Generally, the weight of 100 seeds is about 15 mg (Cahill and Ehdaie, 2005). The mature seeds mucilage, when it comes in contact with water, instantly expands and the seed size increases, so it gives a characteristic gel appearance to chia (Jamboonsri *et al*., 2012).

Figure 1. Different colors of chia seeds; a) Black spotted, b) White.

2.2.2. Leaves

Its leaves are arranged alternately or opposite (Ixtaina *et al*., 2008). The leaves are oval, with a sharp point and jagged edge. It grows on the point and branches (Cahill and Ehdaie, 2005). The leaves have different degrees of pubescence (Capitani *et al*., 2013). The leaves' length are 4-8 cm and their width is 3-5 cm (Baginsky *et al*., 2014).

2.2.3. Stems

Chia stems height is about 1-2 m and it presents a quadrate branching stem (Jamboonsri, 2010; Capitani *et al*., 2013).

2.2.4. Flower

Chia has a perfect flower (hermaphrodite flower) (Cahill and Ehdaie, 2005). Its flowers are small and usually purple, blue or white, Fig. 3 (Capitani *et al*., 2013) and their small size reflect an extremely selfpollinating breeding system (Haque and Ghoshal, 1980). The corolla is bilabiate monopetalous (Ramamoorthy, 1985). It has 4 ovaries and 4 stamens. There will be 1-4 seeds in one flower (Cahill and Ehdaie, 2005). The flowers are arranged in groups of six or more on the axis of the inflorescence (Baginsky *et al*., 2016).

2.3. Nutritional Composition of Chia Seed

There is scientific proof that dietary phytochemicals can play a critical role in the therapy and prevention of many diseases. By growing the universal health consciousness, people request functional foods with countless health advantages. As all foods supply various amounts of nutrients that are important to grow or support vital processes, it can be said that all foods are functional. Functional foods are common foods that contain different kinds of benefits that may improve optimal health or reduce disease hazards (Hasler *et al*., 2000). Over the years, people have turned to unhealthy foods and become addicted to artificial and carbonated beverages. Therefore, they suffered from various heart diseases at a young age. Many texts and literatures emphasize the health benefits of chia seeds and their nutritional value (Coates and Ayerza, 1996).

2.3.1. Oil

Chia has the highest content of ω3 among all-natural sources, so its fatty acids have been highly regarded by researchers (Palma *et al*., 1947; Ayerza, 1995; Ayerza and Coates, 2011; Segura-Campos *et al*., 2014). In general, the mature seeds have 25–40% oil, of which omega (ω)-3 alpha-linolenic acid makes up 60% of it and 20% of it is omega (ω)-6 linoleic acid (Ayerza and Coates, 2004; 2009a,b; Rocha Uribe *et al*., 2011; Silveira Coelho and de las Mercedes Salas-Mellado, 2014). Ecosystem effects, various climatic conditions, different geographical areas (Ayerza, 1995; Coates and Ayerza, 1996, 1998; Ayerza and Coates, 2004), extraction methods (Ixtaina *et al*., 2011), genotype and environmental factors (Bochicchio *et al*., 2015a), date of sowing (Coates and Ayerza, 1996; Baginsky, 2016) and salinity of irrigation water (Heuer *et al*., 2002) can affect the oil percentage and fatty acids composition.

α linolenic acid (ALA) content of chia is higher than the other ALA rich oils seeds such as flax (57%) (Ayerza and Coates, 2004), camelina (48.4%) (Peiretti and Meineri, 2007), and similar or slightly lower than perilla (Ciftci *et al*., 2012). Both of the essential fatty acids are crucial for the human body health, but humans and animal bodies can not synthesize them, so they must obtain them through food. The highly unsaturated metabolites can be constructed from these fatty acids; arachidonic acid and γ-linolenic acid (n-6 PUFA) from linoleic acid (LA) and the most important metabolites: eicosapentaenoic acid and docosahexaenoic acid (n-3 PUFA) from α linolenic acid (ALA) (Gorjao *et al*., 2009). Therefore, it is recommended to eat foods that are rich in α-linolenic acid. Marine fish are the best-known sources of n-3 PUFAs (Gorjao *et al*., 2009), but flax seeds and chia seeds are also important plant sources with the highest α linolenic acid concentrations (Ayerza, 1995; Coates and Ayerza, 1998; Oomah *et al*., 1995).

Also, the omega-6/omega-3 fatty acid ratio in the human diet is an important ratio of unsaturated fats. The proper ratio of it varies from 1:1 to 3:1, but commonly it is much higher in the human diet. This ratio in chia seed oil is lower than 1 (Ixtaina *et al*., 2011; Silveira Coelho and de las Mercedes Salas-Mellado, 2014); therefore, it can be used for balancing the unsaturated fatty acid in the diets.

2.3.2. Protein

Animal-derived proteins have good quality, but they are expensive and can cause allergies in some people. Plant proteins can be a good source of essential amino acids, supplementing or even substituting animal sources (Montoya-Rodrıguez *et al*., 2015; Sandoval-Oliveros and Paredes-Lopez, 2013). Chia seeds have high levels of protein compared to cereal seeds or other oilseeds, 16-26% depending on the environment (Ayerza and Coates, 2004, 2009, 2001), though it does not plant as a protein crop commercially around the World.

The amount of seed protein strongly depends on environmental and agronomic factors. Ayerza and Coates (2009a, b, 2011) reported that the amounts of proteins change with the environment of production. For example, the protein content significantly decreases when elevation increases. Its protein content (20%) is higher than that reported for other crops, like *Triticum aestivum* (14%), Hordum vulgare (9.2%), Avena sativa (15.3%), Zea mays (14%), and Oryza sativa (8.5%) (Monroy-Torres *et al*., 2008). Moreover, the digestibility of chia protein is good (78.9%) (Sandoval-Oliveros and Paredes-Lopez, 2013) and it is higher than beans (77.5%), corn (66.6%), *Oryza sativa* (59.4%), and *Triticum aestivum* (52.7%) proteins (Betancur-Ancona *et al*., 2004). Chia seeds have all amino acids that are essential for human nutrition such as glutamine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine and valine (Sandoval-Oliveros and Paredes- Lopez, 2013). Among them, glutamine has the highest quantity and histidine has the lowest amounts (FAO/WHO/UNU, 2008; Sandoval-Oliveros and Paredes-

Lopez, 2013). The amino acid profile of chia is appropriate for the adult diet (Weber *et al*., 1991). The seed is also free from mycotoxins (Jamboonsri *et al*., 2012) and there is no proof of allergic reactions (EFSA, 2005, 2009) to chia seeds consumption.

2.3.3. Fiber

Another favorable characteristic of chia seeds for researchers is that it contains high fiber (5-6%), which can be used as dietary fiber (Ayerza and Coates, 2001; Reyes-Caudillo *et al*., 2008) and is also very interesting for drug and industrial uses because this high content of soluble fiber can form a very hydrophilic mucilage. As well as chia meal has 33.9–39.9 % of dietary fiber (Capitani *et al*., 2012). So it is a great crop for being consumed in human and animal nutrition (Ting *et al*., 1990). In some studies, have been determined that chia seed total dietary fiber content ranges between 32.4 and 37.50 g/100 g, which most of them being insoluble (>93%) and the remainder (>7%) being soluble (ReyesCaudillo, 2008).

2.3.4. Vitamin and antioxidants

chia seeds and oil also possess a lot of useful compounds which are good for human health, like vitamin B (B6 and B1), A, C, K and E (Bushway *et al*., 2006; Mehta *et al*., 2020) and natural antioxidants (Reyes-Caudillo *et al*., 2008; Amato *et al*., 2015), carbohydrates, magnesium, zinc, iron, calcium and phosphorous (USDA, 2004) and it does not have any danger for human health (Bresson *et al*., 2009).

2.4. Phytochemicals Composition of Chia Seed

2.4.1. Flavonoids

Flavonoids are an essential natural product class; they are commonly secondary metabolites with a polyphenolic structure (Panche *et al.,* 2016). The polyphenol group consists of a fifteen-carbon element with two benzene rings. Flavonoids contain biochemical functions like antiviral, antioxidant, hepatoprotective, antibacterial, anti-inflammatory, and anticancer (Das, 2017). These compound are present in chia seeds, and microbial infection effects increase their synthesis. The most common flavonoids in Chia seeds are Myricetin, Quercetin, and Kaempferol (Capitani *et al.,* 2012),

Naturally, flavonoids compounds are found in several parts of the plant and can be extracted. Flavonoids are mainly used by plants for growth and protection against plaques (Havsteen, 2002). The flavonoids can be categorized into different subgroups depending on the carbon of the C ring on which the B ring is linked and the level of oxidation and saturation of the C ring. The flavonoids subgroups are isoflavones, neoflavanoids, flavonols, Chalcones, Anthocyanins, and flavones (Panche *et al.,* 2016).The subgroup flavones are the most present flavonoid in Chia, and Its subclasses consist of Quercetin, Myricetin, Kaempferol, Rutin, and Morin(Panche *et al.,* 2016).

2.4.2. Sterols

Chia seeds contain a variety of sterols, including β-sitosterol, campesterol, and stigmasterol, which are among the most biologically active forms (Ixtaina *et al*., 2011). These sterols are naturally present in plant cell membranes and contribute to various physiological processes. Studies have reported that the sterol content in chia seeds ranges between 200–400 mg per 100 g, depending on factors such as seed variety, cultivation conditions, and processing methods

(Borneo *et al*., 2010). This significant sterol content makes chia seeds a valuable source of phytosterols, which have been extensively studied for their cholesterol-lowering effects.

One of the most well-documented benefits of sterols in chia seeds is their role in reducing cholesterol levels. Phytosterols compete with dietary cholesterol for absorption in the intestine, thereby reducing overall cholesterol uptake and subsequently lowering LDL (low-density lipoprotein) cholesterol while maintaining or increasing HDL (high-density lipoprotein) cholesterol levels (Ostlund, 2002). This mechanism is particularly beneficial for individuals at risk of cardiovascular diseases. Furthermore, β-sitosterol, the predominant sterol in chia seeds, has been shown to have anti-inflammatory and antioxidant properties, which help mitigate oxidative stress—a key factor in the development of chronic diseases such as diabetes and atherosclerosis (Calpe-Berdiel *et al*., 2009).

Beyond their cardiovascular benefits, sterols also contribute to immune system modulation. Certain phytosterols, including those found in chia seeds, have been reported to enhance immune responses by influencing cytokine production and reducing inflammation (Bouic, 2001). This immunomodulatory effect makes phytosterols valuable in managing conditions such as autoimmune diseases and inflammatory disorders. Additionally, emerging research suggests that sterols, particularly β-sitosterol, may have protective effects against hormone-related cancers such as prostate and breast cancer by inhibiting cancer cell proliferation and inducing apoptosis (Von Holtz *et al*., 1998). The functional applications of sterols from chia seeds extend beyond their health benefits. These compounds are widely used in the food industry, particularly in the formulation of cholesterol-lowering products such as margarine, dairy products, and dietary supplements (Gylling *et al*., 2014). Additionally, sterols possess emulsifying properties, making them useful in food processing and cosmetic formulations. Their ability to stabilize emulsions and improve the texture of food products has made them an essential ingredient in functional food development.

2.4.3. Tannins

Tannins in chia seeds are polyphenolic compounds that contribute to their astringent taste and have potent antioxidant and antimicrobial properties. These tannins are capable of hydrolyzing into simpler phenolic compounds, which exhibit strong antioxidant activity. They can inhibit the growth of bacteria, fungi, and viruses, making them effective in preventing infections and promoting overall health (Haslam, 1998). Also known as proanthocyanidins, these compounds are known for their role in reducing the risk of cardiovascular diseases by inhibiting the oxidation of low-density lipoprotein (LDL) cholesterol. They also exhibit anti-cancer properties by inducing apoptosis in cancer cells and inhibiting tumor growth (Cos *et al*., 2004).

2.4.4. Saponins

Saponins are glycosides found in chia seeds that have soap-like properties and contribute to their health benefits. Saponins have been shown to lower cholesterol levels by binding to bile acids and preventing their reabsorption. This action helps in reducing the risk of cardiovascular diseases (Francis *et al*., 2002).Saponins enhance the immune system by stimulating the production of antibodies and activating immune cells. This makes chia seeds beneficial for improving resistance against infections and diseases (Makkar *et al*., 2007). Saponins exhibit antimicrobial properties by disrupting the cell membranes of pathogens, leading to their lysis and death. This makes chia seeds effective in preventing bacterial and fungal infections (Sparg, Light, and van Staden, 2004).

2.5. Bioactive Properties of Chia Seeds

2.5.1. Antioxidant Properties

The antioxidant properties of chia seeds are primarily attributed to their high levels of phenolic compounds, flavonoids, and essential fatty acids. These antioxidants play a crucial role in neutralizing free radicals and protecting cells from oxidative stress, thereby preventing cellular damage and reducing the risk of chronic diseases. Chia seeds are rich in phenolic acids such as caffeic acid, chlorogenic acid, and gallic acid. These compounds are known for their ability to scavenge reactive oxygen species (ROS) and inhibit lipid peroxidation, thus protecting cellular components from oxidative damage (Reyes-Caudillo *et al*., 2008). Flavonoids like quercetin, kaempferol, and myricetin present in chia seeds exhibit strong antioxidant activities. They help in reducing inflammation and protecting against oxidative stress-related diseases such as cardiovascular diseases and cancer (Menga *et al*., 2010). Chia seeds are one of the richest plant sources of alpha-linolenic acid (ALA), an omega-3 fatty acid. ALA has been shown to reduce oxidative stress by enhancing the activity of antioxidant enzymes and reducing the production of ROS (Ayerza, 2011).

2.5.2. Anti-inflammatory Properties

Chia seeds possess significant anti-inflammatory properties due to the presence of bioactive compounds that modulate inflammatory pathways and reduce the production of pro-inflammatory mediators. The α linolenic acid in chia seeds is converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the body. These long-chian omega-3 fatty acids are known to inhibit the production of inflammatory cytokines and prostaglandins, thereby reducing inflammation (Ursoniu *et al*., 2017). Quercetin, kaempferol, and chlorogenic acid in chia seeds have been shown to suppress the activity of nuclear factor-kappa B (NF-κB) and other inflammatory signaling pathways, thus reducing the expression of inflammatory markers (Li *et al*., 2016). The high fiber content in chia seeds contributes to their anti-inflammatory effects by promoting gut health and reducing the production of endotoxins that can trigger systemic inflammation (Vuksan *et al*., 2010).

2.5.3. Antimicrobial Properties of Chia Seeds

Chia seeds (*Salvia hispanica* L.) are rich in bioactive compounds that exhibit significant antimicrobial properties. These properties are primarily attributed to phenolic acids, flavonoids, and saponins found in chia seeds. These compounds can effectively inhibit the growth of a variety of bacteria, fungi, and viruses by disrupting their cellular structures and interfering with their metabolic processes. Phenolic acids, such as gallic acid and caffeic acid, are abundant in chia seeds and play a crucial role in their antimicrobial activity. These compounds have been shown to exhibit broad-spectrum antimicrobial effects against various pathogens. Studies have demonstrated that phenolic acids from chia seeds are effective against a range of bacteria, including *Staphylococcus aureus, Escherichia coli,* and *Salmonella typhimurium*. Gallic acid and caffeic acid disrupt bacterial cell membranes, leading to cell lysis and death. They also interfere with bacterial enzyme activity and DNA synthesis, inhibiting bacterial growth and reproduction (Grancieri *et al*., 2019).

Phenolic acids exhibit antifungal properties against fungi such as *Candida albicans* and *Aspergillus niger.* These compounds disrupt the fungal cell membrane integrity and interfere with ergosterol synthesis, which is essential for fungal cell membrane structure and function. This results in the inhibition of fungal growth and proliferation (De Souza *et al*., 2015). Flavonoids, including quercetin and kaempferol, are another group of bioactive compounds in chia seeds that contribute to their antimicrobial properties. These flavonoids have been shown to possess antibacterial, antifungal, and antiviral activities. Flavonoids like quercetin and kaempferol inhibit the growth of bacteria such as *Pseudomonas aeruginosa, Bacillus cereus,* and *Listeria monocytogenes.* They achieve this by targeting bacterial cell walls and membranes, disrupting their integrity and permeability. Flavonoids also inhibit bacterial enzymes and interfere with nucleic acid synthesis, preventing bacterial replication and survival (Ramos *et al*., 2017). Flavonoids are effective against fungal pathogens such as *Penicillium expansum* and *Fusarium oxysporum.* They inhibit fungal growth by disrupting the cell wall and membrane integrity, leading to cell leakage and death. Additionally, flavonoids can inhibit the synthesis of essential fungal components like chitin, further impairing fungal viability (Hassan *et al*., 2019).

Saponins have been shown to be effective against bacteria such as *Streptococcus mutans* and Helicobacter pylori. They bind to the bacterial cell membrane, causing increased permeability and leakage of cellular contents, ultimately leading to bacterial cell death. Saponins also inhibit bacterial adhesion and biofilm formation, further reducing bacterial virulence (Guzmán-

Maldonado *et al*., 2019). Saponins exhibit antifungal effects against fungi like *Trichophyton mentagrophytes* and *Cryptococcus neoformans*. They disrupt the fungal cell membrane by forming complexes with sterols, leading to increased membrane permeability and cell lysis. This disrupts the fungal cell's osmotic balance and results in cell death (Sparg *et al*., 2004).

2.5.4. Antidiabetic Properties

Chia seeds have been shown to have beneficial effects on blood glucose regulation and insulin sensitivity, making them a valuable food for managing diabetes. The soluble fiber in chia seeds forms a gel-like substance in the gut, which slows down the digestion and absorption of carbohydrates. This helps in preventing rapid spikes in blood glucose levels post-meal (Ulbricht *et al*., 2009). Phenolic acids such as caffeic acid and chlorogenic acid in chia seeds have been shown to inhibit carbohydrate-hydrolyzing enzymes like alpha-amylase and alpha-glucosidase, thereby reducing glucose absorption and postprandial blood glucose levels (Grancieri *et al*., 2019). Quercetin and other flavonoids in chia seeds improve insulin sensitivity and enhance glucose uptake by peripheral tissues, thus helping in better glycemic control (Jankovic *et al*., 2012).

2.5.5. Anticancer Properties

Chia seeds exhibit anticancer properties due to the presence of bioactive compounds that inhibit cancer cell proliferation and induce apoptosis. Quercetin and kaempferol in chia seeds have been shown to inhibit the growth of cancer cells and induce apoptosis. These flavonoids modulate signaling pathways involved in cell cycle regulation and apoptosis, thereby exerting anticancer effects (Mabasa and Ijeh, 2012).Phenolic acids such as caffeic acid and chlorogenic acid have been found to possess anticancer properties by inhibiting the proliferation of cancer cells and inducing cell death through various mechanisms (Kim *et al*., 2016). Saponins in chia seeds exhibit cytotoxic effects against cancer cells by disrupting their cell membranes and inducing apoptosis. These compounds also inhibit the growth of tumors and metastasis (Zhao *et al*., 2016).

2.6. Applications of Chia Seeds And Derived Products

Chia seeds (*Salvia hispanica* L.) have garnered significant attention for their exceptional nutritional profile and versatility. Native to Central and South America, these tiny seeds are rich in essential nutrients and have found applications across various industries, including food, cosmetics, pharmaceuticals, and agriculture.

2.6.1. Nutritional and Culinary Uses

Chia seeds are used as a functional food or nutritional supplement. The seeds can be eaten whole, after oil extraction (consumption of oil and meals) or ground as an additive to other foods. Chia seeds have some advantages, including the higher amount of ω-3 (Ayerza and Coates, 2004), the long shelf-life of whole seeds (Ahmed *et al*., 1994; Amato *et al*., 2015), and the lack of fishy flavors (Coates and Ayerza, 1998). Ayerza and Coates (2011) stated that an adult who needs 2,700 calories a day should consume 22.5 to 26.5 grams of chia seeds or 9.6 to 9.7 grams of oil per day to get the daily recommended amounts of omega-3 fatty acids. As chia has high amounts of protein compared to other grains, it can be included in the human di*et al*one or as a food ingredient to create a richer source of protein than other grains. Chia oil extracted may be used as a condiment or in beauty products (Munoz *et al*., 2013). Also, it has been used for eye infections in racial medicine (Lu and Foo, 2002; Reyes-Caudillo *et al*., 2008).

By adding chia seeds and oil as additives to food products, we can achieve functional foods as tested in bakery products (Pizarro *et al*., 2013; Silveira Coelho and de las Mercedes Salas-Mellado, 2014). Chia seeds are gluten-free, so it is very suitable for celiac disease. Celiac disease has become one of the most important gastrointestinal diseases today, and a gluten-free diet is often prescribed for these patients (Steffolani *et al*., 2014). So, using chia seeds in the food industry for the production of bread, bars, cookies, and breakfast products has increased especially in the USA, Latin America, and Australia (Cerna *et al*., 2014). According to studies on making bread, it is better to use whole or pre-soaked chia grains compared to ground grains because it has less specific volume, higher firmness of bread and better color (Steffolani *et al*., 2014).

Cerna *et al*. (2014) found that using 3% chia seeds to make bread is very suitable and ideal, and they also concluded that the concentration and amount of seeds used in bread preparation only affect its texture and color and it has no effect on its taste. When chia fruits hydrated, they produced a gel surrounding the seeds. It absorbs water 27 times its weight (Munoz *et al*., 2012a, b). Ahmed *et al*. (1994) stated hydrated chia seeds are traditionally used in beverages called “agua fresca” or “chia Fresca” in Mexico. Polysaccharides of chia can be used in many applications, from food to the pharmaceutical industry (de la Paz Salgado-Cruz *et al*., 2013).

2.6.2. Agricultural Applications

Chia seeds also contribute to agricultural practices, primarily as a source of animal feed and soil enhancement. Because chia is rich in health-promoting compounds, it can be used in animal feed to enhance the concentration of linolenic acid and reduce cholesterol levels in meat and eggs (Mohd Ali *et al*., 2012). The main purpose of using chia in the animal diet was to increase the omega-3 fatty acid content of animal products. So far, only chia seeds (raw or processed) and their oil, also other products such as seed meal, have been used in animal feed. There is no report on how whole chia plants can affect the diet of animals (Ahmed *et al*., 1994; Peiretti and Gai, 2009; Peiretti, 2010; Amato *et al*., 2015).

Ayerza and Coates (1999, 2000, 2001) and Ayerza *et al*. (2002) stated that chia seeds do not have some detriments, such as fishy flavor and digestive problems of other sources of polyunsaturated fatty acids in the animal diet. In the study of poultry diets with chia seeds, Ayerza and Coates (2000) found that their egg yolks are rich in polyunsaturated fatty acids and have a low level of cholesterol and saturated fats. In a study, In a study Ayerza and Coates (2006) feeding dairy cows with chia seeds, their outcomes showed that the percentage of omega-3 fatty acids increased without affecting the production or content of total fatty acids and cholesterol. Meineri and Peiretti (2007) used chia seeds for rabbit feed and reported that the regime containing 10 % of chia seeds enhances the digestibility of acid detergent fiber, dry matter, organic matter, crude protein, crude fiber and gross energy.

In another study, Peiretti and Meineri (2008) enhanced the chia seed ratio in the rabbit diet. Their results showed polyunsaturated fatty acids in the meat significantly increased. Peiretti and Gai (2009) with respect to chia leaves and whole plants, reported that the quality of chia forage depends on the time of harvest and is optimal before shooting. Peiretti (2010), in an experiment, showed that chia is a good plant for silage because by observing the deficiency of lactic acid and the presence of alcohols and volatile fatty acids, it was determined that it had been fermented.

Also, The residues from chia seed processing can be used as organic soil amendments. These residues improve soil structure, water retention, and nutrient content, promoting sustainable agricultural practices (Ixtaina *et al*., 2011).

2.6.3. Ethnobotanical and medical applications

Cahill (2003) stated that the leaves and vegetative parts of S. hispanica L. can be used for pharmaceutical goals. According to Ahmed *et al*. (1994), chia leaves can be used to extract flavors and aromas. Medical studies have shown that ω-3 fatty acids are essential nutrients and play an important role in human health for the prevention of various diseases, including cardiovascular disease, anti-thrombotic, antiinflammatory, anti-arrhythmic and plaque stabilization (Galli and Marangoni, 2006).

In the point of nutritional value, vegetable oil composition is important. The n-3 fatty acids (FAs) have a very vital role in body physiology and development, particularly during fetal and infant growth (Bowen and Clandinin, 2005). Therefore, the health administration of many countries has independently encouraged people to consume foods having high amounts of n-3 FAs and a favorable n-3/n6 fatty acid (FA) ratio. With respect to this specific FA composition, the food industry has to seek out peculiar fats and oils having these compounds to optimize the ‘‘fat profile’’ of the end products (Dubois *et al*., 2007). Although traditionally, using oils with high amounts of n-3 is the limited cause of their inconsistency and flavor reversion, the availability of stabilized products allows manufacturers to provide different kinds of products (Shahidi, 2008).

Three out of every five people in the world die from diseases such as cardiovascular disease, cancer and diabetes (Wang *et al*., 2016). These diseases are caused by high blood pressure, high cholesterol and ultimately, overweight and obesity (World Health Organization, 2017).

According to epidemiological studies, obesity is directly related to the consumption of sugar, fructose and products containing high amounts of saturated fats. This dietary pattern, in which people consume large amounts of fats, animal foods, refined carbohydrates and added sugars, is often referred to as the "western diet" (Popkin *et al*., 2011; Popkin *et al*., 2004).

This type of diet is remarked as an unbalanced diet due to insufficient distribution of macronutrients to meet human needs (Institute of Medicine, 2005). A steady and inactive lifestyle and an unbalanced diet have led to obesity (World Health Organization, 2017), which led to some events that are related to the development of some diseases like insulin resistance (IR), type 2 diabetes mellitus, and cardiovascular diseases (Pozza and Isidori, 2018). Plant foods contain bioactive compounds that can be used to prevent and treat disease (Borowska and Brzoska, 2016). Chia seed is one of the plant foods known for its high concentration of beneficial nutritional compositions (da Silva *et al*., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Collection of sample

The seeds of *Salvia hispanica* were procured from Uselu Market in Benin City, Edo State, Nigeria, in July 2024. Upon acquisition, the seeds were authenticated in the Department of Plant Biology and Biotechnology at the University of Benin, Benin City. Following identification, the seeds were air-dried at a controlled room temperature of 25°C for approximately two weeks. This drying process was essential to reduce moisture content and prevent microbial growth, ensuring the seeds were in optimal condition for further processing. Once adequately dried, the seeds were milled into a fine powder using a Thomas Wiley milling machine, which is designed to produce a consistent particle size. The powdered seed samples were then carefully stored in sterile containers to maintain their integrity and prevent contamination, pending subsequent analyses.

3.2 Test Organisms

The microorganisms selected for this study included *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus* sp., and *Pseudomonas aeruginosa*. These bacterial strains were obtained from a stock culture maintained in the Microbiology Laboratory at the University of Benin, Benin City, Edo State. To ensure viability and consistency throughout the experiment, all bacterial strains were routinely subcultured and maintained on freshly prepared nutrient agar medium. This practice helped preserve the purity and potency of the test organisms, making them suitable for the antimicrobial assays conducted during the study.

3.3 Sample Preparation

The *Salvia hispanica* seeds were first sorted to remove any debris or foreign material. Once clean, the seeds were air-dried at room temperature (25°C) for about two weeks to reduce their moisture content. After drying, the seeds were milled into a fine powder using a Thomas Wiley milling machine. The milled seed powder was then sieved to obtain a uniform particle size, ensuring consistency in subsequent analyses. Finally, the powdered *Salvia hispanica* seeds were stored in sterile, airtight containers, ready for use in the experimental procedures.

3.4 Preparation of Muller Hinton Agar (MHA).

Muller Hinton agar medium was prepared by dissolving 38g of Muller Hinton agar medium in 1000 ml of distilled water according to manufacturer's instructions. It was heated with frequent agitation and boiled to dissolve the medium completely. The agar medium was sterilized by autoclaving at 121^{o}C for 15 minutes and then cooled at 45-50^{o}C. The agar was poured into sterile Petri-dish inside the laminar air flow chamber in order to prevent contamination of the medium.

3.5. Preparation of Chia Seed Extracts

40 grams of the finely milled *Salvia hispanica* seed powder were weighed. The seed powder was soaked in 100 ml of ethanol for 24 hours to facilitate the extraction of bioactive compounds. The mixture was then filtered to separate the liquid extract from the solid residue. The residue was washed with an additional 50 ml of ethanol to ensure maximum extraction. The combined filtrate was concentrated under reduced pressure. The resulting concentrated extract was redissolved in ethanol to achieve the desired concentration for further analysis. For the aqueous extraction, 40 grams of *Salvia hispanica* seed powder were weighed. The seed powder was soaked in 200 ml of distilled water and allowed to stand at room temperature for 24 hours. After maceration, the mixture was blended with an additional 100 ml of water to create a slurry. The slurry was then filtered to obtain the aqueous extract. The filtrate was concentrated using an oven set at 60°C until a thicker consistency was achieved.

3.6. Antibiotic susceptibility test

Test organisms were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion on prepared media. The antibiotic discs were carefully and firmly placed on the inoculated plates using a sterile pair of forceps. The plates were inverted and incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured in millimeters (mm) using a meter rule. The experiments were carried out in triplicates to minimize probability of error.

3.7. Antibacterial activity

The antibacterial activity against the test isolates was checked by agar well diffusion method. Cultures of the isolates were aseptically swabbed on Muller Hinton agar plates (standardized inoculums of the test bacteria adjusted to 0.5 MCFARLAND turbidity standards). Wells of 5 mm diameter was made aseptically by cork-borer on the Inoculated plates and different concentrations of the oil extract were introduced into the labeled wells. The plates were incubated at 37 °C for 24 h in an upright position. The zone of inhibition in millimeter was recorded with the help of meter rule. The experiment was carried out in triplicates to minimize probability of error.

3.7.1. Minimum Inhibitory Concentration

The lowest concentration of the oil extract which prevents visible growths of the test isolates on the sterile medium was also determined by agar well diffusion method.

3.7.2. Minimum Bactericidal Concentrations

1 ml of the sample of known concentration was transferred into a test tubes, 1ml of the test organisms previously diluted to 0.5 MCFARLAND turbidity standard was also introduced into the test tubes and incubated for 24 hours. A loopful of the innoculum was aseptically introduced on a sterile agar medium and incubated for 24 hours.

3.8 Phytochemical Screening

Detection of Flavonoids

Sulfuric acid test: A few drops of Sulfuric acid are added to the extracts and the formation of orange colour indicates the presence of flavonoids.

Detection of Alkaloids

Add a few drops of Dragendorff’s reagent to the extract and the formation of a colored precipitate indicates the presence of alkaloids.

Detection of Tannins

A few millilitres of the extract are mixed with a few millilitres of water and heated in a water bath and the mixture is filtered. Ferric chloride is added to the filtrate. The dark green colour indicates the presence of tannins.

Detection of Glycoside

A few millilitres of the extract are mixed with ammonia and heated in a water bath and the formation of pink coloration indicates the presence of glycoside .

Detection of Saponins

0.5mg of the extract is mixed vigorously with 5ml of Distilled water. The formation of frothing indicates the presence of saponins.

Detection of Sterols

A few drops of chloroform are added to the extract, followed by concentrated sulfuric acid along the sides of the test tube. The formation of a reddish-brown ring at the junction of the two layers indicates the presence of sterols.

CHAPTER FOUR

RESULTS

In this study, the antimicrobial potential of Chia seed oil extract was assessed on some bacterial isolates namely *Escherichia coli, Staphylococcus aureus*, *Streptococcus* sp, *Salmonella, Pseudomonas aeruginosa. and Klebsiella pneumoniae*. In this study,

Table 1 shows the phytochemical test result, flavonoid, tanins, sterol, saponin, and glycoside was present in both ethanolic and aqueous extract of the sample while alkaloid was absent from all sample extract of the Chia seed oil extract.

Table 2 shows the zones of inhibition of aqueous and ethanolic extracts of Chia seed oil extract against clinical isolates. The zones of inhibitions were measured in millimeters and the control (ciprofloxacin) used in the study was also represented in millimeters.

Table 3 represent the minimum inhibitory concentration of the Chia seed oil extract against the isolates. *Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* had the minimum inhibitory concentration at 100mg/ml for the ethanolic extracts, while *Escherichia coli* and *Streptococcus* sp had no MIC, and *Streptococcus* sp, *Salmonella, Pseudomonas aeruginosa. and Klebsiella pneumonia* and *Escherichia coli* had the minimum inhibitory concentration at 100mg/ml for aqueous extract.

Table 4 represents the minimum bacteriocidal concentration for both aqueous and ethanolic Chia seed oil. The minimum bactericidal concentration in this study was obtained to be static for *Escherichia coli, Staphylococcus aureus*, *Streptococcus* sp, *Salmonella, and Klebsiella pneumoniae*. *Pseudomonas aeruginosa was bactericidal at* 100mg/ml for aqueous extract.

Table 4.1: Antibiotic potential of aqueous and ethanolic extracts of Chia seed oil extract on the bacterial isolates.

		Zones of Inhibitions (mm)				
Test organism	Extract	100%	50%	25%	12.50%	CPX (control)
Escherichia coli	Ethanolic	16.00	0.00	0.00	0.00	24
	Aqueous	20.00	11.00	0.00	0.00	
Staphylococcus aureus	Ethanolic	0.00	0.00	0.00	0.00	20
	Aqueous	0.00	0.00	0.00	0.00	
Streptococcus sp.	Ethanolic	18.00	8.00	0.00	0.00	
	Aqueous	14.00	0.00	0.00	0.00	28
Pseudomonas aeruginosa	Ethanolic	10.00	4.00	0.00	0.00	
	Aqueous	21.00	13.00	0.00	0.00	16
Klebsiella pneumoniae	Ethanolic	14.00	10.00	3.00	0.00	
	Aqueous	7.00	4.00	0.00	0.00	22

Key: CPX = Ciprofloxacin

Table 4.2: Minimum inhibitory concentration (MIC) of Chia seed oil extract in (mg/ml)

	Ethanol L (extract)	Aqueous (extract)
Escherichia coli	-	100
Klebsiella pneumoniae	100	100
Staphylococcus aureus	100	-
Streptococcus sp.	-	100
Pseudomonas aeruginosa	100	100

Table 4.3: Minimum bactericidal concentration (MBC) of Chia seed extract oil on bacterial isolates (mg/ml)

	Ethanol (extract)	Aqueous (extract)
Escherichia coli	Static	Static
Klebsiella pneumonia	Static	Static
Staphylococcus aureus	Static	Static
Streptococcus sp	Static	Static
Pseudomonas sp.	Static	100

Table 4.4: Phytochemical screening of Chia seed oil

PHYTOCHEMICALS	INFERENCE
Alkaloid	-
Flavonoid	+
Glycoside	+
Tanins	+
Sterol	+
Saponin	+

Key: + = present

- = absent

CHAPTER FIVE

DISCUSSION AND CONCLUSSION

5.1. DISCUSSION

Chia seed oil, derived from the seeds of *Salvia hispanica*, is increasingly recognized for its potential medicinal benefits. The oil is rich in essential fatty acids, well as various bioactive compounds, including antioxidants, vitamins, and minerals. Its medicinal properties have been the subject of growing interest in both traditional and modern medicine. This study seek to evaluate the phytochemical and antibacterial activity of chia seed oil on selected pathogenic bacterial.

The phytochemical analysis of Chia seed extracts, as summarized in Table 1, reveals the presence of flavonoids, tannins, sterols, saponins, and glycosides in both ethanolic and aqueous extracts. Alkaloids were notably absent. The presence of these phytochemicals aligns with findings from other studies. For instance, a study by Moreno *et al*. (2013) demonstrated that flavonoids and saponins contribute to the antimicrobial properties of various plant extracts. Similarly, Pandey *et al*. (2017) reported that tannins and phenols are significant in mediating antimicrobial effects, which corroborates our findings regarding the potential mechanisms of Chia seed extracts.

The presence of flavonoids, tannins, sterols, saponins, phenols, and glycosides in both ethanolic and aqueous extracts of Chia seed oil (Table 1) highlights its rich phytochemical profile. These compounds are known for their antimicrobial properties, which can be attributed to their ability to disrupt microbial cell membranes, inhibit enzyme activity, or chelate essential metal ions required for microbial growth (Nostro *et al*., 2000; Kumar *et al*., 2012).

The effectiveness of Chia seeds oil in inhibiting bacterial growth can be compared to other plant extracts. For instance, Kumar *et al*. (2020) studied the antimicrobial activity of flaxseed extracts and found comparable inhibition zones, suggesting that Chia seeds, which are closely related to flaxseeds, may share similar antimicrobial properties. This finding is consistent with the observed inhibition zones for *Streptococcus sp*., *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* in our study. Moreover, Chia seed extracts showed varying inhibition across bacterial strains, which aligns with the results from the study by El-Mahmood *et al*. (2009) on the antimicrobial activity of different plant extracts. The variations in effectiveness highlight the complexity of antimicrobial activity, where certain phytochemicals may be more effective against specific bacterial strains. Ciprofloxacin, used as a control, provided a benchmark for comparison. The relative effectiveness of Chia seed oil extract compared to ciprofloxacin underscores the potential of Chia seed oil as a natural antimicrobial agent, though its efficacy may not match that of standard antibiotics

Table 3 details the MIC values of Chia seed oil extracts. The ethanolic extract exhibited an MIC of 100 mg/ml for *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. This result is consistent with the findings of Singh *et al*. (2012), who reported similar MIC values for ethanolic extracts of other medicinal plants. The MIC values for aqueous extracts against *Streptococcus* spp., *Salmonella*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* also align with the results of studies by Nascimento *et al*. (2000), which found that aqueous extracts of various plants demonstrated comparable antimicrobial activity.

The absence of detectable MIC for *Escherichia coli* and *Streptococcus* spp. in ethanolic extracts suggests a differential sensitivity, which may be attributed to the specific composition of the extracts. Similar differential sensitivity was observed by Okwu and Morah (2006), who found variable MIC values for different bacterial strains treated with plant extracts.

As shown in Table 4, the MBC values indicate that Chia seed oil extracts were generally bacteriostatic, with *Pseudomonas aeruginosa* being bactericidal at 100 mg/ml for the aqueous extract. This result is consistent with the findings of Sultana *et al*. (2016), who reported similar MBC values for aqueous extracts of various seeds. The static nature of MBC for most isolates in this study is in agreement with the results of studies by Ahmad *et al*. (2015), which demonstrated that certain plant extracts exhibit bacteriostatic rather than bactericidal properties.

The antimicrobial activity observed in Chia seed oil extract is consistent with findings from studies on chia seeds and other plant extracts. Chia seed oil, for instance, has been reported to exhibit antimicrobial properties due to its phytochemical composition, which includes flavonoids and phenolic compounds (Gonzalez-Aguilar *et al*., 2014). The absence of alkaloids in Chia seed oil, while not unusual, contrasts with other plant oils where alkaloids contribute to antimicrobial activity (Rangari, 2004). The variability in antimicrobial efficacy among different bacterial strains and extracts is a common theme in the literature, highlighting the need for targeted research to optimize the use of plant extracts in antimicrobial applications.

5.2. CONCLUSION

Chia seeds offer a promising source of antimicrobial agents, and their bioactive compounds warrant further investigation. This study contributes to the growing body of evidence supporting the use of natural plant extracts in combating microbial infections, potentially paving the way for new strategies in public health and medicine particularly in the face of rising antibiotic resistance.

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