COMPARISION OF THE ETHANOL AND AQUEOUS EXTRACT OF *Salvia hispanica* (CHIA SEEDS) ON ANTI-PANCREATIC LIPASE ACTIVITY

BY

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CERTIFICATION

We the undersigned certify that Okungbowa Gentle presented this to the Department of Medical Biochemistry, school of Basic Medical Science, university of Benin.

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External examiner Date

DEDICATION

I dedicate this work to God Almighty who sustained me all through my time in the University of Benin and gave me the wisdom and enablement to successfully carry out this research to my late Dad Mr. Festus Okungbowa and my lovely Mum Mrs. Florence Okungbowa, for their immeasurable support and contributions to my academic success so far, and to my brothers, sisters and friends for all their encouragement and advice.

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God bless you all!

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ABSRTACT

Salvia species have been traditionally used in the treatment of colds, aches and pains, infections, wounds, bronchitis, flu, tuberculosis, haemorrhage, insomnia, some cardiovascular and menstrual disorders, stomach, liver, and rheumatism pain. This is due to their bioactive constituents such as terpenoids, phenolics, essential oils, and fatty acids) that give them their medicinal properties. The aim of the study was to evaluate the anti-obesity of *Salvia hispanica* seeds (chia seeds) by investigating the anti-pancreatic lipase activity of the seed. The objective of the study was to determine the anti-pancreatic lipase activity of *Salvia hispanica*. The standard anti-obesity drug (Orlistat) was used as a control. The data obtained was analysed using a Graphpad prisim version 8.0.3.The results showed that the ethanol extract had a greater anti-pancreatic lipase activity (IC_{50}:870.47µg/ml) when compared to the aqueous extract (IC_{50}: 2866.58µg/ml) and the standard (Orlistat) (IC_{50}:2338µg/ml) making it a viable management option for obesity and related metabolic syndrome.

CHAPTER ONE

1.0 INTRODUCTION

A Medicinal plant is defined as any plant species that contain Secondary metabolites that can be used for medicinal uses or can be used as precursors for the manufacture of novel therapeutics (Penso, 2014). Its importance increases with the progress of civilization, the increase in the need for medicine, and the expansion of its uses. And some medicinal plants are also used for other purposes, such as spices, food oils, and essential oils that are used in the manufacture of cosmetics, perfumes, and. pesticides, which has increased interest in these plants in many countries of the world (Otunola, 2021). The greater use of medicinal plants in the treatment of diseases is because plants or their derivatives are safe and effective drugs, in addition to having fewer side effects and low cost (Sofowora *et al*., 2013). *Salvia hispanica* (Chia seeds) has been used medicinally to reduce triglyceride fat (anti-obesity property): An increased amount of fat substances can lead to atherosclerosis which expands to the condition of stroke, cardiac failure, and coronary illness. Increased fat substances can cause acute intensification of the pancreas (Pancreatitis) (Zhang, *et al*., 2019). *Salvia hispanica* (Chia seeds) consist of vital substances including increased fiber, protein and omega-3 fatty acids, which could reduce the risk and development of triglycerides (Enes *et al*., 2020). *Salvia hispanica* (Chia seeds) oil supplementation could modify the lipid profile in the liver and adipose tissue, and decreased amount of leptin, triglycerides, and liver cholesterol levels (Citelli *et al*., 2016).

1.1 AIM OF STUDY

This study aimed to investigate the *in-vitro* anti-obesity potentials of *Salvia hispanica* (chia seeds).

The objective of this study was to:

- investigate the *in-vitro* anti-pancreatic lipase activity of the ethanol extract of *Salvia hispanica* (chia seeds)
- investigate the *in-vitro* anti-pancreatic lipase activity of aqueous extract of *Salvia hispanica* (chia seeds).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OVERVIEW OF MEDICINAL PLANT

There is no disease without medicine. Man has sought to search for plants that reduce this or that pain since ancient times, as historical sources tell us that the history of herbal medicine has played an important role in maintaining the health care system for a large number of the population all over the world (Karunamoorthi *et al*., 2013). In the world, medicinal herbs formed the basis of alternative medicine and led to it being the main path for the conception of new medicines, and until the early nineteenth century (Pan *et al*., 2014). More than 80% of medicines were formulated from plants (World Health Organization, 2013). Especially after the scientific revolution, the field of herbal medicine led to the development of industry (Ullah *et al*., 2020). The greater use of medicinal plants in the treatment of diseases is due to the fact that plants or their derivatives are safe and effective drugs, in addition to having fewer secondary effects and a low cost (Sofowora *et al*., 2013). These plants have been used on a large scale in the form of low - cost products with almost zero side effects that have been manufactured and marketed pharmaceutically. It must be noted here that there are many important medicinal plants, but they are very poisonous plants, as they are toxic and medicinal at the same time. The difference is only in the quantity of the dose given, as the plant may be curative in a small dose and fatal in a larger dose. Here, we must take care, caution, and accuracy in determining the doses when using these plants (Ekor, 2014). Its importance increases with the progress of civilization, the increase in the need for medicine, and the expansion of its uses. And some medicinal plants are also used for other purposes, such as spices, food oils, and essential oils that are used in the manufacture of cosmetics, perfumes, and pesticides, which has increased interest in these plants in many countries of the world (Otunola, 2021). The knowledge of alternative medicine based on the use of plants in treatment represents an inheritance passed from one generation to another over the centuries, whether orally or in writing, bearing in mind that the traditional inheritance may face extinction if it is not passed on to the next generation and is still limited to the previous one using only different plant species (Al Akeel *et al*., 2018) were also aware that a medical plant is defined as a plant that has one or more of its different organs or mutations on one or more chemicals with a low or high concentration and the physiological ability to treat a specific disease. The term "medicinal plant" refers to many different plant species utilized in herbalism ("herbology" or "herbal medicine"). It involves both the study of and use of plants for therapeutic purposes (Khan, 2016).

Medical plants are referred to as Herbs in most scientific sources, which are (small green plants with a strong perfume); However, this concept is far from reality, as the word herbs in the medical and economic conventions indicate an amazing diversity of plants ranging from algae, ears, fungi and prolonged tropical trees. The correct concept of herbs is every plant that evaluates its medicinal and aromatic properties; it is cultivated because of its treatment or general medical properties. Medicinal plants have become an additional material in some food industries' products, and they are used, especially those plants that contain aromatic substances, vitamins, important amino acids, and enzymes that help digest and take their functions. Medicinal plants are also an important source of effective drugs in the treatment of various diseases, especially in traditional medicine (Borokini and Omotayo, 2012). Various parts of the plant are used in traditional medicine, including bark, flowers, fruits, leaves, resins, roots, seeds, and stems (scattered *et al*., 2017).

There is a promising future of medicinal plants as there are about half million plants around the world, and most of them are not investigated yet for their medical activities and their hidden potential of medical activities could be decisive in the treatment of present and future studies (Singh, 2016). In the development of human culture medicinal plants have played an essential role, for example religions and different ceremonies (Hosseinzadeh *et al*., 2015). Among the variety of modern medicines, many of them are produced indirectly from medicinal plants, for example aspirin. Many food crops have medicinal effects, for example garlic. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. The medicinal effects of plants are due to secondary metabolite production of the plants. Keeping this in consideration there have been increased waves of interest in the field of research in natural product chemistry. This interest can be due to several factors, including therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally occurring secondary metabolites, the utility of novel bioactive natural compounds as biochemical probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify, and structurally characterize these active constituents, and advances in solving the demand for supply of complex natural products (clark, 2015). The importance of traditional medicine has also recognized by World Health Organization (WHO) and has created strategies, guidelines and standards for botanical medicines. For the cultivation, processing of medicinal plants and the manufacture of herbal medicines agroindustrial technologies need to be applied (Who, 2014). Medicinal plants are resources of new drugs and many of the modern medicines are produced indirectly from plants.

2.2 *Salvia hispanica* (CHIA SEED)

Salvia hispanica L., also known as chia, is an annual herbaceous plant, originally from Southern Mexico and Northern Guatemala. It belongs to the order Lamiales, mint family Labiate, subfamily Nepetoideae, and genus Salvia. The genus Salvia consists of approximately 900 species, which have been widely distributed for thousands of years around several regions of the world, including Southern Africa, Central America, North and South America, and South-East Asia (Grancieri *et al*., 2019). As reported in the literature, chia today is not only cultivated in Mexico and Guatemala, but also in Australia, Bolivia, Columbia, Peru, Argentina, America, and Europe. Nowadays, Mexico is recognized as the words largest chia producer (Grancieri *et al*., 2019). Chia farming is gaining status in East Africa because of its low cost and nutritional benefits (Kibui *et al*., 2018). *Salvia hispanica* (Chia Seeds) is described as "the seed of the 21st century, new gold, superfood or super nutrient" (Segura-Campos *et al*., 2013). Chia seeds are smooth, glossy, and oval, measuring 1 to 2 mm in size, The seed coat can be grey, black-spotted, or white-spotted (Madaan *et al*., 2020). In contrast, *Salvia hispanica* (black chia) seeds are more common, and *Salvia hispanica* (white chia) seeds are slightly larger than *Salvia hispanica (*black chia seeds) (Dinçoğlu and Yeşildemir, 2019). Historical records testify that *Salvia hispanica* .was used beside corn, bean, and amaranth by ancient Mesoamerican cultures Aztecs and Mayas in the preparation of folk medicines and food. In pre-Columbian societies, it was the second main crop after beans (Ullah *et al*., 2016).The latest outcome of various researches shows the result that chia seeds have a extra ordinary nutritional profile and wide health-boosting characteristics (Grancieri *et al*., 2019).

Salvia hispanica is mainly grown for its seeds and oduces white and purple flowers, which are 3 to 4mm small and hermaphrodites. The plant itself is sensitive to day light, it can grow up to 1 m tall, its leaves and 3 to 5 cm wide. *Salvia hispanica* (Chia seeds) are generally very small, oval-shaped, 2 mm long, 1 to 1.5 mm wide, and less than 1 mm thick (Grancieri *et al*., 2019). The color of the seed varies from black, grey, or black spotted to white. As (Knez Hrnčič *et al*., 2018) already reported, there is such a marginal difference between *Salvia hispanica* (black and white Chia seeds) that most consider them equal. Nutritional values are similar—protein content in *Salvia hispanica* (black Chia seeds) is 16.9% and fiber content is 32.6%. In *Salvia hispanica* (white Chia seeds), the protein content is reported to be 16.5% and the fiber content 32.4%. A slight difference is only in morphology white seeds are larger, thicker, and broader compared to black seeds.

Moreover, the plant itself can produce 500 to 600 kg seed/acre under appropriate agronomic conditions (Ullah *et al*., 2016).In recent years, *Salvia hispanica* (Chia seeds) have become one of the world's most recognizable foods based on their nutritional properties and medicinal values (Das, 2018). (Coorey *et al*., 2012) reported that Chia is an excellent ingredient since it contains the highest known amount of α-linolenic acid and can be easily added to commercial food It has been reported in several studies that chia seeds—due to the high percentage of fatty acids present—can be crucial for health, antioxidant, and antimicrobial activity (Ayerza, 2016).

Table 1: Taxonomy of *Salvia hispanica* (Chia seeds)

Kingdom	Plantae
Subdivision	Spermatophyta
Order	Lamiales
Family	Lamiaceae
Genus	*Salvia*
Species	*Hispanica*

Source: Grancieri *et al*., 2019

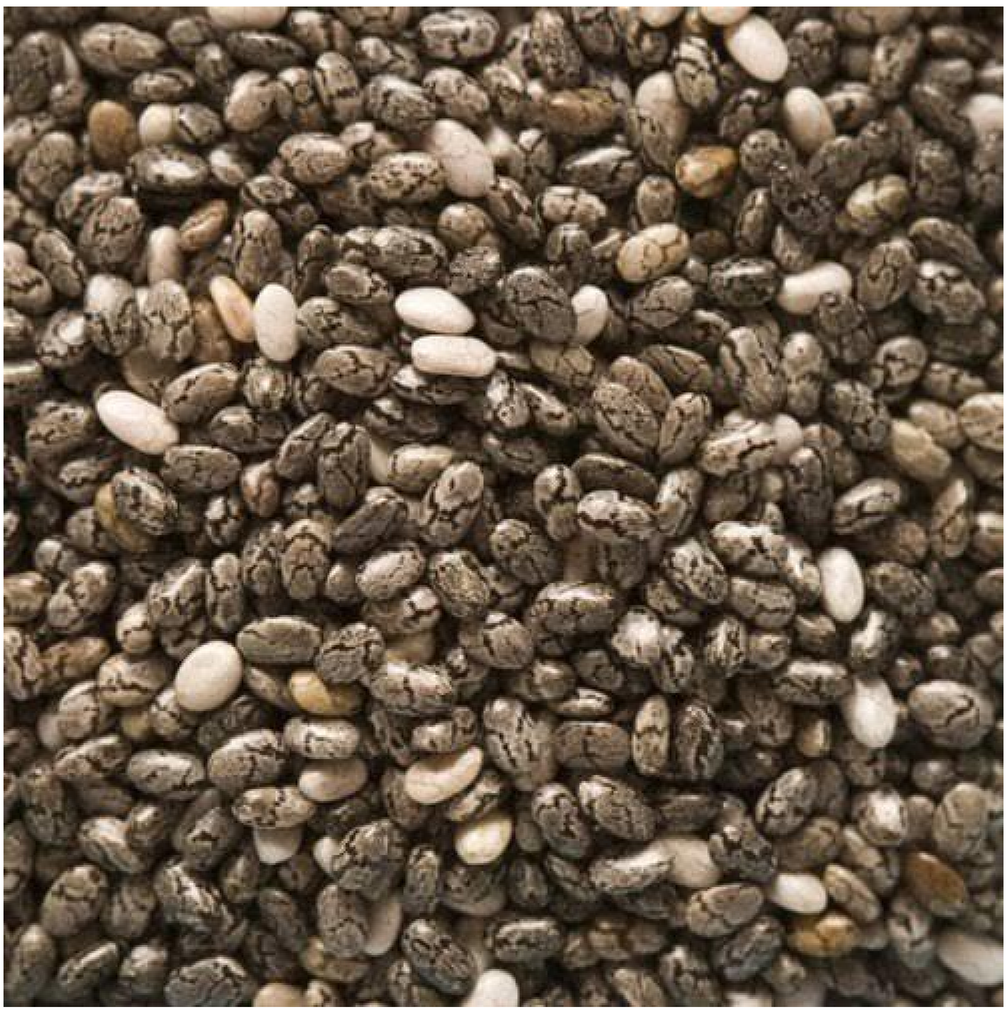

Figure 1: *Salvia hispanica* (Chia seed)

Source: Dekas and Das, 2017

2.3 PHYTOCHEMICAL COMPOSITION OF CHIA SEED

2.3.1 Phenolic compounds

These are the bunch of chemical components having one or more hydroxyl groups (OH) linked to an aromatic hydrocarbon. These are classified on the basis of a count of phenolic groups presented in a compound. These are the major component of plant derivatives and are isolated as phenolic acids and polyphenols. These compounds are presented in the combination with mono and polysaccharides, consisting one or more phenolic groups, which could turn out as ester or methyl esters (Kumar and Goel, 2019).Focusing on phenolic content, dry chia seeds contain 8.8% of phenolic compounds also reported in 2017 (Oliveira-Alves *et al*., 2017) resulted that phenolic compounds from profit-based samples of chia seeds, flour, dietary fiber and oil were isolated using different processing technologies. The fusion of raw and compounded were assessed, major components present were phenolic and caffeic acid, and dhanshesu and their secondary metabolites such as rosmarinic and salvianolic acids. These results updated the knowledge about chia seeds and their phenolic profile, which was majorly phytochemicals and fibers for blocking oxidative stress and disorders caused by it (Scapim *et al*., 2016).some of the examples includes: Rosmarinic acids, Salicylic acid, Protocatechinic acids etc.

2.3.2 Flavonoids

Flavonoids are widely distributed chemicals found in plants. They are part of the polyphenolic subclass and have a 15C chain made up of 2-benzene rings (A & B) linked by a heterocyclic pyrane ring (C).They are mostly responsible for food's colour, flavour, and fat oxidation prevention (Zhu *et al*., 2012).The flavonoids quercetin, chlorogenic acid, and caffeic acid are proven to have anti-cancerogenic, anti-hypertensive, and neuron protective effects (Das, 2018). Both (Mohd Ali *et al*., 2012) and (Ullah *et al.,* 2016) mentioned that chia seeds have no potentially toxic mycotoxins or gluten present. some of the examples includes: Mycertin, Quercetin, Kaempferol, Chlorogenic acid

2.4 PROXIMATE COMPOSITION OF CHIA SEED

The benefits of chia seed as a nutritional supplement and nutraceutical food cannot be inflated (Coelho and Salas-Mellado, 2014).The chemical composition of chia seeds have been analyzed by many researchers (Kulczynski *et al*., 2019). Chia seeds contain a high content of fats (30–33%), carbohydrates (26–41%), dietary fiber (18–30%), proteins (15–25%), vitamins, minerals, and antioxidants (wet basis).

2.4.1 Protein content

These are formed by linking 20-50 amino acids together with peptide bonds. Chia seeds have consisted of around 19% protein. It is recognized to be a great source of proteins. Chia seeds have hoisted levels of proteins and vital amino acids, therefore these are a commendatory source of functional peptides. The structure and beneficial outcomes of protein and peptides in chia seeds have feasible influences on human health. Out of total proteins, 20 are categorized in chia seeds. These are a compatible part of plants basal metabolism (Gomez-Favela *et al*., 2017). The advantageous chia seed effects are linked to humans by consisting the antioxidant ability. anti-hypertensive, anti-hyperglycemic and anti-hypercholesterolemic response. This relatedness could be linked with chia protein along with peptide structures (Grancieri *et al*., 2019). The absence of the protein gluten makes chia seeds highly valued to patients suffering from celiac disease. Moreover, food rich in proteins is highly recommended to people who are fighting to lose weight. (Grancieri *et al*., 2019) was investigating the composition and positive effects of chia seeds, proteins, and peptides, and their effect on the human body. A total of 20 proteins were obtained from chia seeds, eight of them were specially related to the production of the plant lipids, which cause the high concentrations of polyunsaturated fatty acids presented.

2.4.2 Fibre content

It is an insoluble form of carbohydrate which is essential for our body. Its generally presented in food sources such as fruits, vegetables, grains and legumes. Fiber is presented in two forms which includes soluble and insoluble form. Cellulose is also a type of fiber which is being presented in food substances such as grains, fruits, vegetables, nuts and beans which cannot be digested by the body (Gidley and Yakubov, 2019). Fiber is being passed through the body without getting digested and reduces the risk of constipation or deals in maintaining the healthy gastrointestinal tract. Fiber is also aid in splashing out the cholesterol and cancer causing chemicals from the body (Holscher, 2017). Chia seed is consisting about 40% fiber that brings them in the highly considerable fiber providing food category. Soaked chia seeds the increased amount of dietary fiber. Same time while soaking the chia seeds phytic acid content is getting removed from the seeds. It is present in the gel form which helps to escape from the constipation issues and the stool in its movement. Fiber raises the bulkiness and volume of feces which regulates the blood glucose level, body weight, cholesterol level and also maintaining the health of colon by acting as the anti-aging component (Grancieri *et al*., 2019) and (Rahim *et al*., 2021). Due to the mucilage and digestible fiber chia seeds were are sticky in nature. Chia seeds are beneficial in controlling the blood glucose level after consuming and also provide the satiety. Earlier researches declared that consistency of the digestive material can be upgraded because of the fibers presented in the chia seeds. (Lazaro *et al*., 2018) summarized in their study that, they used three quantity of mucilage were used 3, 5, and 8g/kg to assess the differences in the digestion process. This step was carried out because of the mucilage have a tremendous capacity to hold water.

2.4.3 Lipid content

Many researches on the phytochemicals have been reported, highlighting that the major constituents of chia oil are polyunsaturated fatty acids (PUFAs: α-linolenic (ALA, ω-3 fatty acid) and linoleic (LA, ω-6 fatty acid) acids) (Silva *et al*., 2016). Chia seeds contain 39% oil (mass of dry seed), which consists up to 68% of ω-3 and 19% of ω-6 fatty acid (Das, 2018). The ratio between ω-6 and ω-3 fatty acid is 0.3:0.35 (Da luz *et al*., 2013). (Campos et al., 2015) and Coates and Ayerza (Kulczynski *et al*., 2019) stated that the chemical composition of each product can vary due to different factors such as year of cultivation, environment of cultivation, and extraction method used. omega 3 fatty acid These are the key substances in the digestion process of animal fats and also play a essential role in human food and physical functioning (Cholewski *et al*., 2018). One of the peculiar attribute of chia seeds are their high levels of omega-3 fatty acids which are beneficial for the cardiac health. Omega-3 alpha-linolenic acids (ALA) are about 75% and 20% omega-6 fatty acids presented in chia seeds. It is an uncomplicated way to enhance the mental health through consuming chia seeds. Previous researches resulted that eating more Omega-3 than Omega-6 will reduce the infections and inflammations of the body. A small proportion of Omega-3 acid is linked with a reduced chance of plenty of long-lasting diseases like cardiac problem, cancer, inflammation and pre-mature deaths (Saini and Keum, 2018).

2.4.4 Mineral content

Minerals are the components presented as a natural resources which are necessary for natural body functioning and development. Human body is in requirement of certain minerals. These minerals are categorized as essential minerals (Miller, 2017). Chia seeds are an astonishing origin of countless foundational minerals. These are bountiful in manganese, phosphorus, copper, selenium, iron, magnesium and calcium. The body makes use of minerals to carry out several activities, from the construction of strong bones to the itinerant of nerve impulses. However, divergent minerals are used to form hormones or for the normalization of a heat beat (Bailey *et al*., 2011).

2.4.5 Vitamins content

Vitamin is a natural and essential component that is a required micronutrient that an organism needs in low amounts for the pertinent activity of its cellular functions. (Kulczyński *et al*., 2019) resulted that chia seeds consist of vitamin-E as tocopherols: α tocopherol (8 mg/kg of lipids), γ-tocopherol (422 mg/kg of lipids), and δ-tocopherol (15mg/kg of lipids).

2.5 ETHNOMEDICINAL PROPERTIES OF CHIA SEED

2.5.1 Anti-oxidant Property

Several research works have documented the higher antioxidant activity of chia seeds. (Coelho *et al*., 2015) confirmed that the enzymatic oxidation of guaiacol was inhibited by the extracts of chia seeds. (Marineli *et al*., 2015) performed an experiment with obese rats on diet with chia seeds at 133 g/kg for 6 weeks and chia oil at 40 g/kg for 12 weeks. A few days after consumption, the activity of antioxidant enzymes (catalase, glutathione reductase, glutathione, and glutathione peroxidase) was increased significantly in animals fed on seeds or oil compared to animals consuming a high fructose diet without chia supplement. Several studies have found that chia seeds have a strong antioxidant ability. chia seed contains an abundant number of anti-oxidants i.e. kamperfol, quercetin, chlorogenic acid, caffeic acidetc. These antioxidants scavange free radicals that cause cancer or onset aging. Many in vitro studies have done that proved its antioxidant activity. (Reyes-caudillo *et al*., 2008)

2.5.2 Anti-diabetic property

A few reports demonstrated the potentially beneficial physiological effects of Chia against the risk factors for Type 2 diabetes in experimental animals (Creus, 2016) In a 6-month crossover study type, 2 diabetic subjects consuming Chia on a daily basis (37g/d) demonstrated lower blood pressure, lower pro-inflammatory markers and coagulation factor. A recent study on chia seeds compared the effect of black and white chia seeds on the of glucose level of diabetic rats. The results showed that both chia seed types improved the blood glucose level, but here were no significant differences between the white and black chia seeds in terms of their impact on the glucose level of rats (Alamri, 2019).There was noticeable decrease in the glucose concentration of rats fed with chia seeds for two weeks (Silva,2016). A study on humans showed that the glucose level reduced after six-months of treatment, with chia seeds, in patients with type 2 diabetes (Vuskan, 2017).The treatment diabetic rats with chia seeds for 6 or 12 weeks was effective in improving glucose and insulin tolerance (Silva, 2015).Insulin-resistant rats showed a reduction in blood glucose levels after feeding them black chia seeds for three months (Agustina, 2016). Furthermore, white chia seed consumption for 6 and 12 weeks modified blood glucose levels (Rafaela, 2015).

2.5.3 Anti-obesity property

A double-blind, randomized, and controlled trial of 77 overweight or obese patients demonstrated a remarkable reduction in waist circumference and lost weight after six months of consuming chia seeds (36g/1000kcal/day) (Vuksan,2017). (Oliva, 2017) indicated that body weight did not change significantly after three months of white *Salvia hispanica* (white chia seeds) seed intake, whereas body weight was slightly lower than initiated weight, but this was not significantly different from the control group (Oliva, 2017). However, another study showed that the consumption of 50 grams per day of chia seeds had no influence on body mass index of overweight and obese adults.(Nieman, 2009). A study on animals revealed that the treatment of obese rates by chia seeds or oil for 6-12 weeks did not improve the weight of rats or abdominal fat accumulation (Silva, 2015). Recently, using both white and black chia seeds to feed rats showed no favorable changes after 6 weeks of treatment (Alamri, 2019). Several previous studies, with different durations, showed similar results in terms of their being no change in weight (Silva, 2016). It is interesting that one year of chia seed consumption resulted in an increase in body weight (Evelyn, 2018). In addition, a shorter duration of chia seed intake (3 months) resulted in unfavorably elevated body weight (Agustina, 2016).

2.5.4 Anti-cancer property

Nutrition has a significant role in the initiation and progression of cancer (Who, 2018). Dietary polyunsaturated fatty acids were effective in reducing diabetic rats with chia seeds for 6 or 12 weeks was effective in improving glucose and insulin tolerance (Silva, 2015).Insulin-resistant rats showed a reduction in blood glucose levels after feeding them black chia seeds for three months (Agustina, 2016). Furthermore, white chia seed consumption for 6 and 12 weeks modified blood glucose levels (Rafaela, 2015). Chia seeds can be incorporated into bread in order to improve human blood glucose levels (Ho, 2013).This can be attributed to the compositions of chia seeds, such their high content of dietary.

2.5.5 Anti-inflammatory property

The inflammatory disorder is associated with pain, redness, and swelling, severity of which leads to loss of vital functions. An interdependent chain of reactions are mediated by inflammatory molecules released from leukocytes (sustainabilitist, 2020).The key inflammatory mediators inclusive of linoleic and it derive eicosanoids, prostaglandin E2 and leukotriene B4, are derived from arachidonic acid .compete with arachidonic acid for the incorporation into the However, lower risks of pro-inflammatory reactions are demonstrated with Chia seed oil diet. (Adulrashed *et al*., 2016) The n3 PUFA in Chia seed oil are suggested to membrane Hence, generate slightly modified prostaglandins and eicosanoids viz, LTE5, LTB5, and PGE3 which induce lesser extent of inflammation via reduced induction of COX-2.

2.6 THE PANCREAS

The pancreas is an organ that in humans lies in the abdomen, stretching from behind the stomach to the left upper abdomen near the spleen. In adults, it is about 12-15 centimetres (4.7–5.9 in) long, lobulated, and salmon-coloured in appearance.(Gray's, 2016)Anatomically, the pancreas is divided into a head, neck, body, and tail (Atkinson *et al.,* 2021).The head of the pancreas sits within the curvature of the duodenum, and wraps around the superior mesenteric artery and vein(Gray's, 2016).The neck of the pancreas separates the head of the pancreas, located in the curvature of the duodenum, from the body. The neck is about 2 cm (0.79 in) wide, and sits in front of where the portal vein is formed. (Gray's, 2016).The body is the largest part of the pancreas, and mostly lies behind the stomach, tapering along its length. The peritoneum sits on top of the body of the pancreas, and the transverse colon in front of the peritoneum. (Gray's, 2016) .The pancreas narrows towards the tail, which sits near to the spleen.[Gray's, 2016)] It is usually between 1.3–3.5 cm (0.51–1.38 in) long, and sits between the layers of the ligament between the spleen and the left kidney. The pancreas is a mixed or heterocrine gland, i.e., it has both an endocrine and a digestive exocrine function. (Berger's, 2018) 99% of the pancreas is exocrine and 1% is endocrine.(Berger's, 2018).The pancreas has a rich blood supply, with vessels originating as branches of both the coeliac artery and superior mesenteric artery.(Gray's, 2016) The body and neck of the pancreas drain into the splenic vein, which sits behind the pancreas. (Gray's, 2016) the head drains into, and wraps around, the superior mesenteric and portal veins, via the pancreaticoduodenal veins.(Gray's, 2016)].The pancreas drains into lymphatic vessels that travel alongside its arteries, and has a rich lymphatic supply.(Gray's, 2016)] The lymphatic vessels of the body and tail drain into splenic lymph nodes, and eventually into lymph nodes that lie in front of the aorta, between the coeliac and superior mesenteric arteries. The lymphatic vessels of the head and neck drain into intermediate lymphatic vessels around the pancreaticoduodenal, mesenteric and hepatic arteries, and from there into the lymph nodes that lie in front of the aorta (Gray's, 2016).

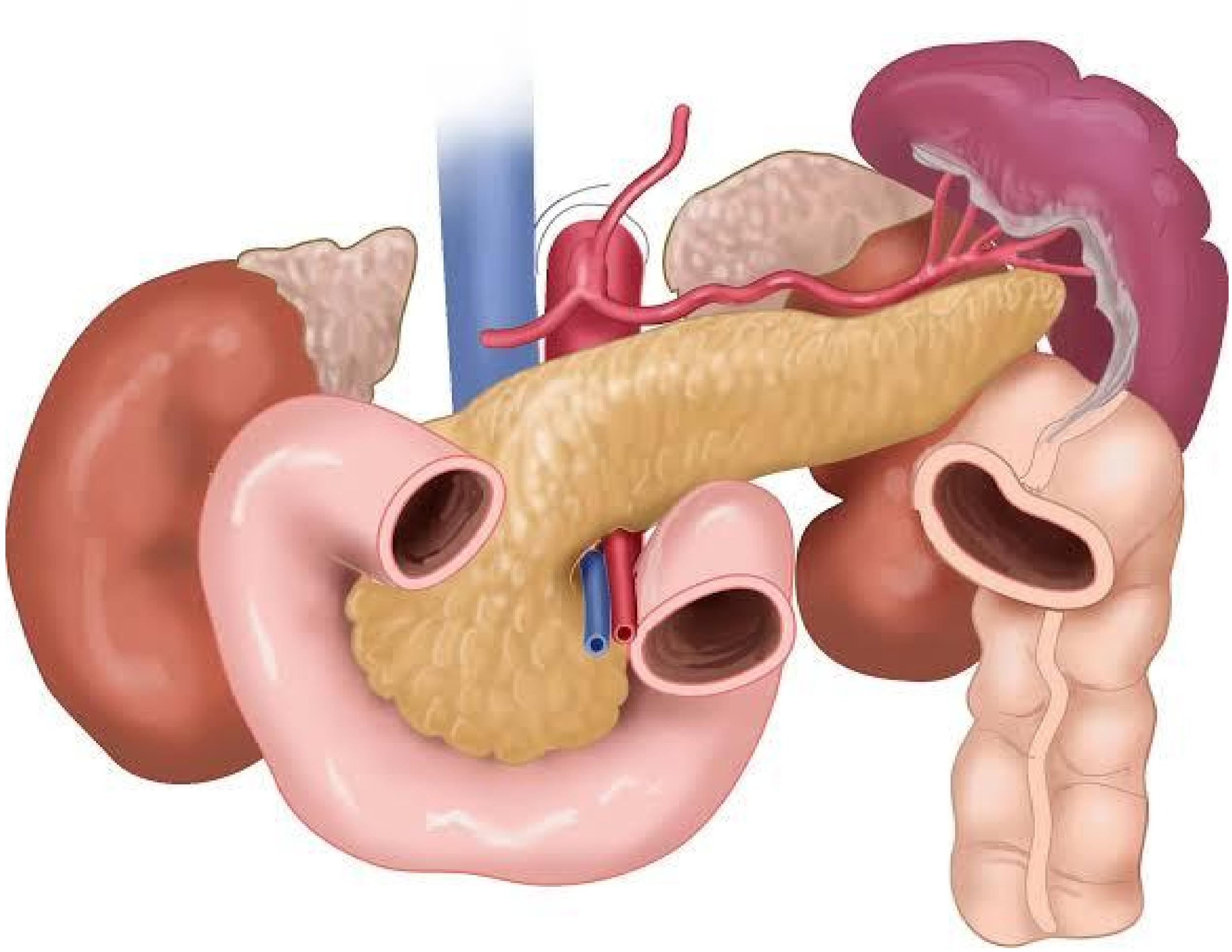

Figure 2: The pancreas

Source: Bang, 2021

2.6.1 Functions of the pancreas

The pancreas is involved in blood sugar control and metabolism within the body, and also in the secretion of substances (collectively pancreatic juice) that help digestion. These are divided into an "endocrine" role, relating to the secretion of insulin and other substances within pancreatic islets that help control blood sugar levels and metabolism within the body, and an "exocrine" role, relating to the secretion of enzymes involved in digesting substances in the digestive tract. (Wheater's, 2013).

2.6.1.1 Endocrine function of the pancreas.

Cells within the pancreas help to maintain blood glucose levels (homeostasis). The cells that do this are located within the pancreatic islets that are present throughout the pancreas. When blood glucose levels are low, alpha cells secrete glucagon, which increases blood glucose levels. When blood glucose levels are high beta cells secrete insulin to decrease glucose in blood. Delta cells in the islet also secrete somatostatin which decreases the release of insulin and glucagon. (Wheater's, 2013) Glucagon acts to increase glucose levels by promoting the creation of glucose and the breakdown of glycogen to glucose in the liver. It also decreases the uptake of glucose in fat and muscle. Glucagon release is stimulated by low blood glucose or insulin levels, and during exercise. (Harrison's, 2015) Insulin acts to decrease blood glucose levels by facilitating uptake by cells (particularly skeletal muscle), and promoting its use in the creation of proteins, fats and carbohydrates. Insulin is initially created as a precursor form called preproinsulin. This is converted to proinsulin and cleaved by C-peptide to insulin which is then stored in granules in beta cells. Glucose is taken into the beta cells and degraded. The end effect of this is to cause depolarisation of the cell membrane which stimulates the release of the insulin. (Harrison's, 2015) Somatostatin acts as an inhibitor of both insulin and glucagon

2.6.1.2 Exocrine function of the pancreas.

The pancreas plays a vital role in the digestive system. It does this by secreting a fluid that contains digestive enzymes into the duodenum, the first part of the small intestine that receives food from the stomach. These enzymes help to break down carbohydrates, proteins and lipids (fats). This role is called the "exocrine" role of the pancreas. The cells that do this are arranged in clusters called acini. Secretions into the middle of the acinus accumulate in intralobular ducts, which drain to the main pancreatic duct, which drains directly into the duodenum. About 1.5 - 3 liters of fluid are secreted in this manner every day. (Harrison's, 2015) The cells in each acinus are filled with granules containing the digestive enzymes. These are secreted in an inactive form termed zymogens or proenzymes. When released into the duodenum, they are activated by the enzyme enterokinase present in the lining of the duodenum. The proenzymes are cleaved, creating a cascade of activating enzymes. (Harrison's, 2015). Enzymes that break down proteins begin with activation of trypsinogen to trypsin. The free trypsin then cleaves the rest of the trypsinogen, as well as chymotrypsinogen to its active form chymotrypsin. (Harrison's, 2015) Enzymes secreted involved in the digestion of fats include lipase, phospholipase A2, lysophospholipase, and cholesterol esterase.(Harrison's, 2015)Enzymes that break down starch and other carbohydrates include amylase.(Harrison's, 2015).These enzymes are secreted in a fluid rich in bicarbonate. Bicarbonate helps maintain an alkaline pH for the fluid, a pH in which most of the enzymes act most efficiently, and also helps to neutralise the stomach acids that enter the duodenum.(Harrison's, 2015) Secretion is influenced by hormones including secretin, cholecystokinin, and VIP, as well as acetylcholine stimulation from the vagus nerve.

2.7 PANCREATIC LIPASES

Pancreatic lipase is an enzyme which mediates the complete digestion and absorption of fats in the body. In humans the enzyme is majorly released from the pancreatic acinar cells and convert the dietary triglycerides into products such as fatty acids and monoglycerides. (Tian-Tian *et al*., 2020).Bile salts secreted from the liver and stored in gallbladder are released into the duodenum, where they coat and emulsify large fat droplets into smaller droplets, thus increasing the overall surface area of the fat, which allows the lipase to break apart the fat more effectively. Unlike some pancreatic enzymes that are activated by proteolytic cleavage (e.g trypsinogen), pancreatic lipase is secreted in its final form. However, it becomes efficient only in the presence of colipase in the duodenum (Johnson *et al*., 2013).The enzyme classification number of the pancreatic lipase or triglyceride lipase is 3.1.1.3. The classification tree of the enzyme tells that 3 represent hydrolases class of enzymes, 3.1 is subclass and 3.1.1 is sub, sub class of the enzyme they both representing the reaction performed by the lipase such as it acts on the ester bonds present in the acylglycerols and triglycerides and it catalyze the hydrolysis of carboxylic ester groups, respectively while the whole number which is 3.1.1.3 is representing triacylglycerol lipase. Pancreatic enzymes, also known as pancreases or pancrelipase and pancreatin, are commercial mixtures of amylase, lipase, and protease. The pancreatic lipase gene family consists of 3 lipase subfamilies of pancreatic origin, namely, pancreatic lipase, PLRP1, and PLRP2. (De Caro *et al*., 2008). Although pancreatic lipase is expressed in all mammals, the expression of PLRP1 and PLRP2 differs between vertebrate species. (De Caro *et al*., 2008). Unlike the family other members, PNLIPRP2 plays an elemental role in lipid digestion, especially for newborns (Andersson *et al*., 2011). Pancreatic lipase has 2 distinct domains, an N-terminal domain consisting of amino acid residues 1 to 336 and a C-terminal domain consisting of amino acid residues 337 to 449).(winkler *et al*., 2016) The N-terminal domain functions as the catalytic domain, and the C-terminal domain is important for noncatalytic functions, such as binding of cofactors, lipids, and heparin (wong and schotz 2014) The N-terminal domain contains the α/β-hydrolase fold, which is common to esterases and thioesterases.(Belle *et al*., 2007).This domain also contains the catalytic triad, consisting of 3 amino acid residues: serine 152, aspartic acid 176, and histidine 263 (in human pancrease) (Breton *et al*., 2007.)This catalytic triad contains the active site where substrate hydrolysis occurs. It is common to all mammalian lipases of the pancreatic lipase gene family, carboxyl ester lipase, and gastric lipase. (Belle *et al*., 2007) Pancreatic lipase may be present in the inactive or active form, based on the position of a mobile lid structure originating from the N-terminal domain. (lome, 2014) In the inactive form, the lid covers the catalytic triad, blocking access of substrates to the active site, When in the active form, the lid opens to expose the catalytic triad (Xiao and Lowe, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 CHEMICALS AND REAGENTS

Pancreatic lipase from porcine pancreas was obtained from Sigma Aldrich Co. USA. Methanol AR (CH,OH) was obtained from Guangdong Guanghua Sci-Tech Co., Ltd (GHTECH), China. Hydrochloric acid was obtained from Sigma Aldrich Co. USA. Hexane (CHi) was obtained from Guangdong Guanghua Sci-Tech Co., Ltd (GHTECH), China. Para nitrophenyl butyrate was obtained from Sigma Aldrich Co. USA. Ecoslim orlistat capsules 120mg was obtained from (St peter's Pharmaceuticals, Nigeria). Ethanol Absolute AR (CH,CH OH) was obtained from Guangdong Guanghua Sci-Tech Co., Ltd (GHTECH), China. Tris-buffer was obtained from Molychem Co. Ltd, India. Acetonitrile (Methyl cyanide - CH,CN) was obtained from Loba Chemie Ltd. Mumbai, India. Sodium hydroxide was obtained from Loba Chemie Ltd. Mumbai, India. Sodium chloride was obtained from Loba Chemie Ltd. Mumbai, India.

3.2 EQUIPMENT

The major equipment used are sensitive electronic balance (TYPE: LAC214C, 704010), UV/VIS- spectrophotometer-A&E lab UK (Model: AE-560-20), HISENSE refrigerator (Model: REFH2DR), Haier THERMOCOOL CHEST FREEZER (Model HTF-31911), rotary evaporator

(Model: RE-201D), freeze dryer (Model: LGJ-10(-80C)), thermostat oven (Model: DHG-9053A), Digital Thermostatic Water bath (Search tech Instruments, model no: HH-S6). Electronic Weighing balance (S-Mettler, model no: 704010). Freeze Dryer (Search tech Instruments, model no: LGJ-10). Laboratory Oven (Search tech Instruments, model no: DHG-9053A). Electronic pH meter (PHS- 25).

3.3 PLANT COLLECTION AND IDENTIFICATION

The plant material or sample of *salvia hispanica* (chia seeds) was obtained from wuse 2 market, local government, Abuja.which was further identified in the department of plant biology and botany, university of benin. An herbarium specimen was deposited; UBH-D632 Herbarium number was given to the plant.

3.4 PLANT EXTRACTION

The plant material, *Salvia hispanica* (the chia seeds) was thoroughly washed under clean running tap water and was air dried. At the end of air drying the plant material was grounded to fine powder. After grinding, the plant extract was weighed using and electronic weighing balance. The weight obtained was 89.7g which was used for the experiment .For the extraction proper an extractant (ethanol and distilled water) was used for the study. Before the extraction the plant material was first defated using the reagent; n-hexane; the plant material was placed in a 2000ml beaker containing n-hexane and was allowed to stand for 72 hours at the end, the defated portion was carefully separated from the plant material, the plant material was further subjected for defating for 48 hours and 24 hours respectively, this was done in order to obtain proper defatation of the plant. The plant residue was air dried and placed in a clean wide disposable bag that was spread. At the end of air drying the plant material was extracted using absolute ethanol and distilled water. The ethanol extract was placed in a 2000ml beaker and soaked with ethanol and covered with a foil paper and placed in a dark corner, while the Aqueous extract was placed in a 2000ml beaker and soaked with distilled water and covered with a foil paper and placed in a dark corner. At the end of 3 days the ethanol and aqueous extract was filtered using a double layered cheese cloth, a thick cotton wool was placed on this cheese cloth used as absorbent for the extraction. This process was repeated 5-6 times for exhaustive extraction. At the final extraction, the cheese cloth and clean white handkerchief was placed in a funnel to get the final extract. The ethanol extract was concentrated using a rotary evaporator at 40 degree celcius and freeze dried using a freeze dryer, after freeze drying it was placed in an air tight container and stored in a freezer, same as the aqueous extract it was freeze dried in a freeze dryer placed in an air tight container and stored in a freezer.

3.5 PREPRATION OF PLANT EXTRACT

Salvia hispanica (chia seeds) (ethanol and aqueous extract) was reconstituted appropriately in ethanol and distilled water, which was used for in-vitro analysis.

3.6 BIOCHEMICAL ANALYSIS

In vitro Anti-pancreatic Lipase Assays

3.6.1 Pancreatic Lipase Inhibition Assay

For this assay, the procedure described by Chedda *et al*. (2016) was employed with a few modifications. To prepare the standard solution, 120 mg of orlistat was dissolved in 120 ml of methanol (1 mg/ml). The ethanol extract was made by dissolving 0.02 gof extract in 20 ml of methanol (1 mg/ml), whilst the aqueous extract was prepared by dissolving 0.02 g in 20 ml of distilled water (1 mg/ml). The standard and extracts were then successively diluted to various concentrations of 12.5, 25, 50, 100, 200, 300, 400, and 500 µg/ml. To prepare the porcine pancreatic lipase enzyme solution, 6 milligram of the enzyme was dissolved in 10 ml of Tris buffer solution (100 mM, pH 7.4). Para- nitrophenyl butyrate (pNPB) was employed as the substrate, 8.403 µl of pNPB stock solution was made up to 10 ml by acetonitrile in a vial. All reagents were freshly prepared. A total of 500 ml of porcine pancreatic lipase enzyme solution, 1000 ml of Tris buffer solution, and 250 ml of pNPB solution were added to 250 µl of standard or extracts at various concentrations. The absorbance was monitored at 400 nm after 30 minutes of incubation at 37^{o}C. Each assay was carried out three times. Both the IC_{50} and the percentage of pancreatic lipase inhibition were calculated.

3.7 STATISTICAL ANALYSIS

The data were expressed as means of 4-7 determination +/- SEM. The differences among groups were analysed using the one-way analysis of variance (ANOVA). Inter-group comparison was done by the Duncan's Post hoc test. Graphpad prism version 8.0.3 was used to plot the graph for this analysis.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 The IC_{50} values of the ethanolic and aqueous extract of *Salvia hispanica* (chia seeds) and the standard (Orlistat)

Inhibitory assay	**Standard**	**Standard IC_{50} (µg/ml)**	**Ethanol extract IC_{50} (µg/ml)**	**Aqueous extract IC_{50} (µg/ml)**
Pancreatic lipase	Orlistat	2338	870.45	2866.58

The table shows that the pancreatic lipase inhibitory activity of ethanol, aqueous extract, and standard (Orlistat) with their IC_{50} which was observed to be 870.45µg/ml, 2866.58µg/ml, and 2338 µg/ml respectively.

4.1 *In vitro* ANTI-PANCREATIC LIPASE ACTIVITY OF ETHANOL EXTRACT OF *Salvia hispancia* (CHIA SEEDS).

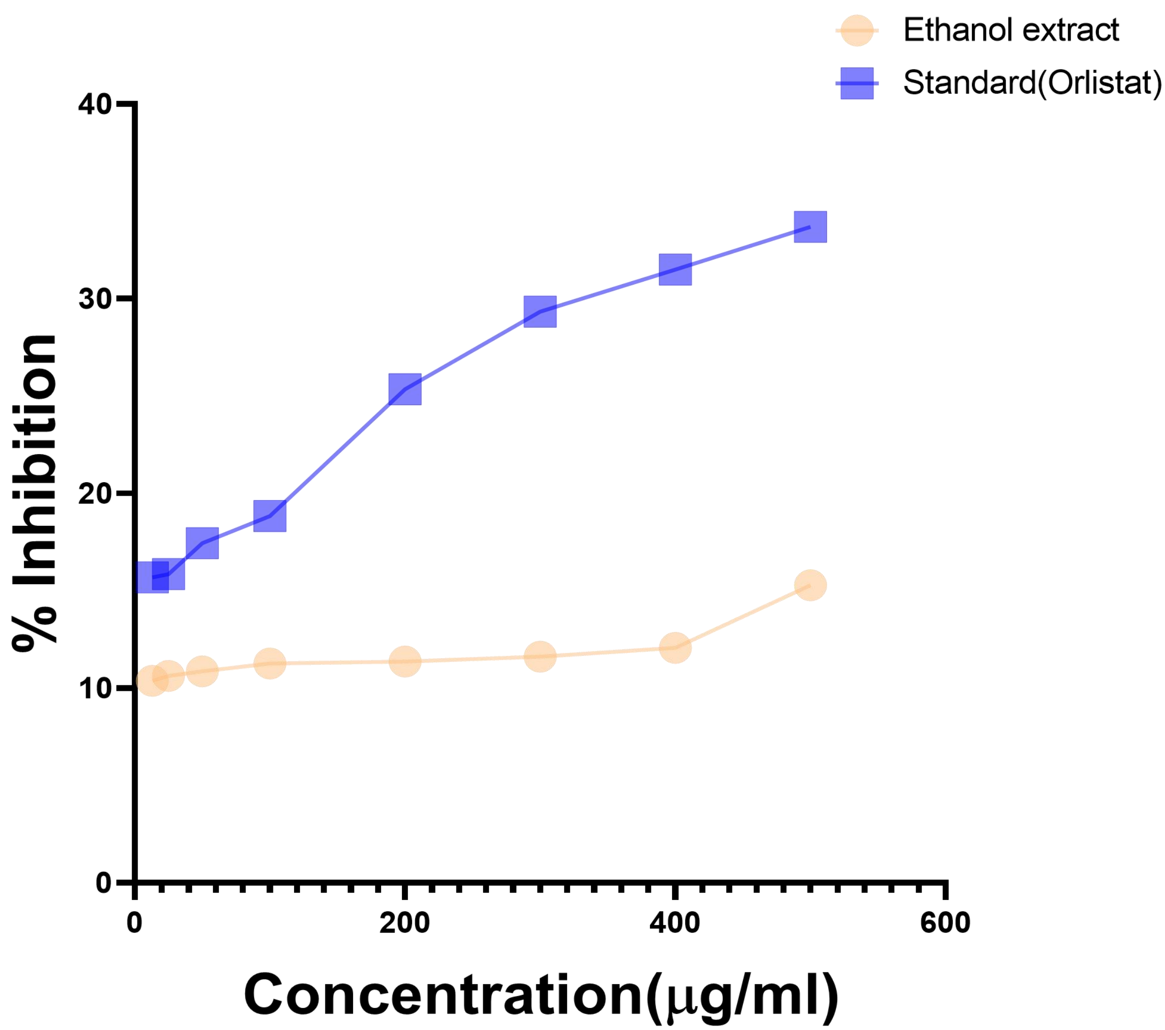

Figure 4.1: Pancreatic lipase inhibitory activity of different concentration of standard (Orlistat) and ethanol extract of *Salvia hispanica. Data* are Mean +/- SEM of triplicate

determinants. The figure 4.1 shows that the standard (Orlistat) has a relatively higher percentage inhibition (33.69 %) of pancreatic lipase activity than ethanol extract of *Salvia hispanica* (chia seeds) (15.28 %).

4.2 *In vitro* ANTI-PANCREATIC LIPASE ACTIVITY OF AQUEOUS EXTRACT OF *Salvia hispancia* (CHIA SEEDS).

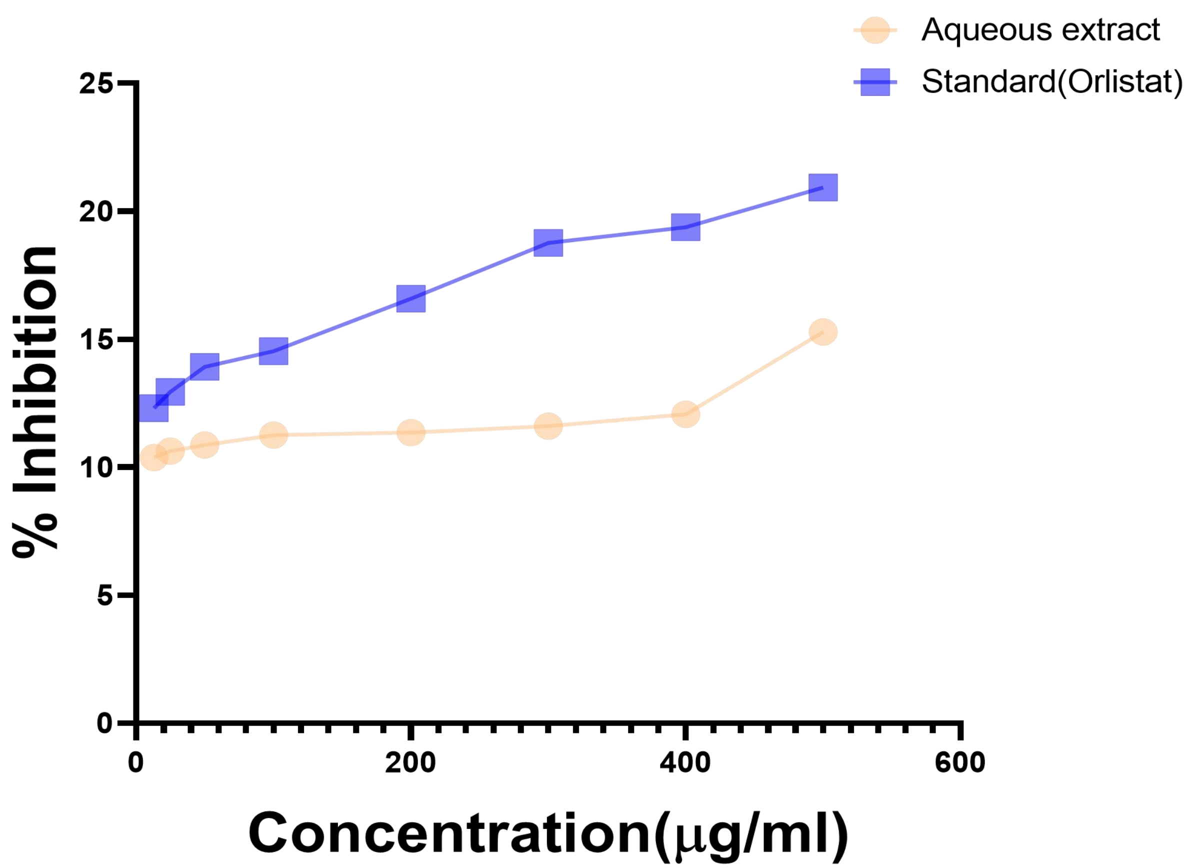

Figure 4.2: Pancreatic lipase inhibitory activity of different concentration of standard (Orlistat) and ethanolic extract of *Salvia hispanica*. Data are Mean +/-SEM of triplicate determinants. The

figure 4.2 shows that the standard (orlistat) has a relatively higher percentage inhibition (20.93 %) of pancreatic lipase activity than aqueous extract of *Salvia hispanica* (chia seeds) (15.28 %).

CHAPTER FIVE

5.0 DISCUSSION

The World Health Organization (WHO) defines obesity as an "abnormal or excessive fat accumulation that may impair health," further clarifying that "the fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended" (Salvador and Andreas, 2017). The unit "Body Mass Index" (BMI), which is measured by calculating (weight in kg)/ (height in m^2), is a simple index intended to classify adults into one of three categories: "underweight," "overweight," or "obese.", BMI is still widely used as a measurement of obesity and obesity rates. This WHO classification is beneficial in distinguishing individuals who may be at increased risk of morbidity and mortality due to obesity. Over the last two decades, the rates of obesity (calculated as adults having a BMI over 30 kg/m2) have rapidly increased across the developing world. Researchers have also noted that various forms of obesity, including abdominal obesity, are related to increased risk of several chronic conditions and diseases, which include asthma, cancer, diabetes, hypercholesterolemia, and, cardiovascular diseases (lihuan *et al*., 2017) Thus, while obesity is undoubtedly a condition, it also exacerbates pre-existing conditions and instigates new ones (Hülya, 2020). More specifically, (Stephan *et al*., 2017) maintained that obesity can affect nearly every organ system, from the cardiovascular (CV) system to the endocrine system, central nervous system, and the gastrointestinal (GI) system. The seed of S*alvia hispanica* (chia seeds) have been used in the treatment of obesity by inhibiting pancreatic lipase activity, the consumption of *Salvia hispanica* (chia seeds) can be useful in dietary strategy in the prevention of overweight /obesity among health individuals (Ayazetal, 2017).(Toscano *et al.,* 2015) showed that ingestion of 35g/day CF for 12 weeks, reduced body weight (BW) and waist circumference significantly, but with a greater reduction among obese than overweight individuals.

The IC_{50} of the plant extract was statistically determined. IC_{50} is a quantitative measure that indicates how much of a particular inhibitory substance (e.g., drug) is needed to inhibit in-vitro, a given biological process, or biological component by 50% (Aykul *et al*., 2016).

In Table 4.1 the result of the pancreatic lipase inhibitory activity of the aqueous extract of *Salvia hispanica* (chia seed) shows that aqueous extract gave a lower inhibitory effect with an IC_{50} of 2866.58µg/ml compared with the standard (Orlistat) with $1C_{50}$ of 2338 µg/ml. The result of the pancreatic lipase inhibitory activity of the ethanol extract of *Salvia hispanica* (chia seeds) shows that the ethanol extract gave a higher inhibitory effect with IC_{50} of 870.45µg/ml when compared with the standard (Orlistat) with IC_{50} of 2338 µg/ml.

Pancreatic lipase is an enzyme that mediates the complete digestion and absorption of fats in the body. In humans, the enzyme is majorly released from the pancreatic acinar cells and converts the dietary triglycerides into products such as fatty acids and monoglycerides. (Tian-Tian *et al.,* 2020).The standard (Orlistat) inhibited (33.69 %) of the enzyme activity when compared with the ethanol extract which inhibited (15.28 %) of the enzyme activity. The standard (Orlistat) inhibited (20.93%) of the enzyme activity when compared with that of the aqueous extract which inhibited (15.28%) of the enzyme activity.

CONCLUSION

Considering the result obtained it was concluded that the ethanol extract of *Salvia hispanica* (chia) has a higher potential of inhibiting pancreatic lipase activity compared to the aqueous extract. It can be said that *Salvia hispanica* can potentially inhibit pancreatic lipase activity. This suggests that *Salvia hispanica* is very helpful in the management of obesity.

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