VITAMINS, MINERAL COMPOSITION AND IN VITRO ALPHA AMYLASE AND GLUCOSIDASE INHIBITORY ACTIVITY OF ETHANOL EXTRACTS OF *Foeniculum vulgare* SEEDS

BY

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JUNE, 2024.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, IN PARTIAL FUFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

JUNE, 2024.

CERTIFICATION

We the undersigned hereby certify that David Osaro EKAMEZE (BMS1902125) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

Signed

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DEDICATION

This project is dedicated to Almighty God, the way maker, who has seen me through the completion my Bachelor of Science Degree (B.Sc) program in the Department of Medical Biochemistry, also my entire family, particularly my Mother and Uncle for their unwavering support and guidance.

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ABSTRACT

Foeniculum vulgare, a well-known herbaceous plant widely utilized for tis culinary and medicinal properties in rural communities, this suggests the presence of bioactive compounds with therapeutic effects or action. This study aims at elucidating the medicinal properties of the plant by investigating the mineral and vitamin composition and alpha amylase and alpha glucosidase inhibitory properties of ethanol extracts derived from *Foeniculum vulgare.* Ethanol extracts of *Foeniculum vulgare* were prepared using standard extraction techniques, solvent extraction with absolute ethanol as the solvent used, the vitamins and minerals content of the extract were analyzed by high performance liquid chromatography (HPLC) method. The in vitro alpha amylase and alpha glucosidase activity of the plant extracts were assessed by subjecting the extracts to enzyme inhibition assays using acarbose as the control for each assay. The results obtained suggest that ethanol extract of *Foeniculum vulgare* were rich in vitamins, predominantly vitamin C (Ascorbic acid), vitamin B1 (Thiamine) and vitamin B3 (Niacin), also micronutrients like Chromium (Cr), Copper (Cu), Zinc (Zn) and Cadmium (Cd) were found to be present at varying concentrations. Furthermore, the extracts exhibited significant inhibition of alpha-glucosidase enzymes in vitro, indicating their potential as natural inhibitors of the carbohydrate-digesting enzyme. However, the results indicated little in vitro alpha amylase inhibitory activity. These findings uncover the pharmacological potential of F*oeniculum vulgare* extracts as functional food ingredients or dietary supplement for managing conditions associated with abnormal carbohydrate metabolism, **s**uch as diabetes, by controlling postprandial glucose levels through the inhibition of carbohydrate digestion

CHAPTER ONE

1.1 INTRODUCTION

Hyperglycemia and diabetes have been existent since before modern medicine (Yedjou *et al.,* 2023) leading to the use of plant and herb extracts for managing and treating diabetes. In rural places like Ekpon in Edo State, herbs like neem and bitter leaf are used for treating diabetes. Fennel is a plant used in the treatment of diabetes. Fennel has been shown to have hypoglycemic, anti-inflammatory, and antioxidant properties in many studies (Samadi-Noshahr *et al.,* 2020).

Foeniculum vulgare, commonly known as fennel, is a flowering plant which belongs to the carrot family, Apiaceae. It is a hardy biennial or perennial herb of the Apiaceae *(Umbelliferae)* family, native to the Mediterranean region but now cultivated worldwide for its culinary, medicinal and aromatic properties (Singh and Sharma, 2015). In fact, the whole plant is of pharmaceutical interest, while the young leaves and seeds are mainly used for culinary purposes (Malhotra, 2012). In particular, fennel seeds, which are widely used as a spice or for oil extraction (Diao *et al*., 2014), are a rich source of macro and micronutrients. In general, 100 g of FS averagely contains 15.8 g proteins, 14.9 g fats and 36.6 g carbohydrates, as well as numerous micronutrients (e.g. Ca, Fe, K, Mg, Na, P and Zn) (Malhotra, 2012).

Fennel has historically been used for several reasons. Fennel seeds are often ingested post-meals to assist with digestion and relieve gastrointestinal pain (Prakash *et al*., 2018). Fennel tea preparations are used to alleviate coughs and pulmonary congestion. The herb is also used to regulate menstruation, alleviate period cramps, and promote lactation in nursing moms (Srivastava *et al.,* 2010).

Studies have shown that *Foeniculum vulgare* contains a spectrum of bioactive compounds. Notably, fennel seeds are rich in essential oils, primarily composed of trans-anethole, fenchone, and estragole, which contribute to its characteristic aroma and flavour (Prakash *et al.,* 2018). Furthermore, fennel contains phenolic compounds such as flavonoids and phenolic acids, which exhibit antioxidant and anti-inflammatory properties, along with terpenoids like limonene and pinene (Singh and Sharma, 2015).

Blood glucose, often known as blood sugar, is a vital energy source for the body's cells, fueling various metabolic activities such as neurotransmission and other biochemical functions. It is crucial to keep blood glucose levels within a small range for general well-being and health due to its significance (Nakrani *et al.,* 2020). The body maintains blood glucose levels by using hormones, enzymes, and physiological processes. After a carbohydrate- rich meal is ingested, the body breaks it down into its monomeric unit of glucose during digestion. Glucose is then absorbed into the bloodstream thus causing the level of blood glucose to rise and as a result of this increase, the beta cells of the pancreas release the hormone insulin, which facilitates the movement of glucose from the bloodstream into cells, where they are utilised as an energy source or stored as glycogen for future use (Rahman *et al.,* 2021).

Alternatively, when levels of blood glucose drop, which is seen during fasting or in between meals, the alpha cell of the pancreas releases another hormone called glucagon. This hormone; glucagon signals the liver to break down the stored glycogen to yield glucose and release it into the bloodstream, ensuring a steady supply of energy to the body cells and preventing hypoglycemia. The balance of these pancreatic hormones; insulin and glucagon help in the maintenance of blood sugar homeostasis. Interference to this balance can lead to a number of metabolic disorders, including diabetes mellitus (Rix *et al.,* 2015). Blood glucose level as earlier mentioned is also regulated by enzymes which facilitate the digestion of the carbohydrate in the gastrointestinal tract. These enzymes include; Alpha-Amylase and Alpha-Glucosidase (Going *et al.,* 2020).

Alpha-amylase is an enzyme that is produced and secreted by the salivary glands and the pancreas. It plays a vital part in the breakdown of complex carbohydrates i.e. starch, into smaller, simpler polysaccharides, oligosaccharides, and lastly maltose. This process of carbohydrate breakdown starts in the mouth, when the salivary alpha-amylase begins to hydrolyze starch molecules into simpler polysaccharides and maltose during mastication and mixing with saliva. The salivary alpha-amylase ceases acting in the stomach owing to its acidity, then the pancreatic alpha-amylase continues the process of carbohydrate digestion (Akinfemiwa *et al*., 2023).

Alpha-glucosidase is an enzyme that is located in the brush border membrane of the epithelial cells of the small intestine. Its principal role is to further break down disaccharides into their component monomers (monosaccharides), chiefly glucose. This is an essential stage in carbohydrates breakdown as it allows the absorption of glucose into the circulation for energy generation and other metabolic demands (Şöhretoğlu and Sari, 2020).

It is evident that the collective action of the pancreatic hormones; insulin and glucagon and the enzymes; alpha-amylase and alpha-glucosidase, is essential for the regulation of blood glucose level and as such they need to be studied to aid the treatment of metabolic disorder related to blood glucose level which might include; diabetes, hyperglycemia and hypoglycemia.

The aim of this research is to elucidate the vitamins and mineral content as well as to study the in vitro alpha amylase and glucosidase activity of ethanol extracts of *Foeniculum vulgare*.

CHAPTER TWO

LITERATURE REVIEW

2.1 FENNEL SEED (*Foeniculum vulgare*)

2.1.1 OVERVIEW

Foeniculum vulgare also known as fennel is a flowering plant species in the carrot family, it is a biennial medicinal and aromatic plant. It is a hardy perennial herb with yellow flowers and feathery leaves (Anka *et al.,* 2020). It is a medicinal plant that belongs to the Umbelliferae (Apiaceae) family (Pushplata, 2022). The flowers are produced in terminal compound umbels, the fruit is a dry seed 4-10mm long. It is generally considered indigenous to the shores of mediterranean sea but has become widely naturalised in many parts of the world especially on dry soils near the sea coast and on the river banks (Rather *et al.,* 2012).

2.1.2 BOTANICAL DESCRIPTION

Fennel is an ancient seasonal herb. The fennel plant originated in the southern Mediterranean region and through naturalisation and cultivation it grows wild throughout the Northern, Eastern, and Western hemispheres, specifically in Asia, North America, and Europe. Foeniculum vulgare is an upright or erect, branching perennial herb with soft, feathery, almost hairlike foliage growing up to 6.6ft (2m) tall (Badgujar *et al.,* 2014). Its leaves are up to 40 cm long and finely dissected with filiform (threadlike) about 0.5 mm wide. Its bright golden flowers bloom in July and August. It has a green, smooth and slippery stem with straight stiff branches and many divided leaves in linear segments, length is 0.39 -- 2.4 inches (1–6 cm).

Its fruits are oblong, 0.12–0.2 in (3–5 mm) length, and 1.5–2.0 mm broad. The stylopodium persists on its fruit. The fruits feature lengthy and robust ribs. The finest fennel seeds range in length from three to five rows. They are oval, somewhat curved, and significantly thicker at the ends. The fruits of the wild variety are smaller, darker and blunter at the ends, and less fragrant than sweet fennel. Its seeds mature from September to October (Badgujar *et al.,* 2014). The plant may reproduce from root parts or the crown but it reproduces independently from seed. The blooms are produced in terminal compound umbels. The fruit is a dry seed 4–10 mm long. It is typically regarded indigenous to the coasts of Mediterranean Sea but has been extensively naturalised in many regions of the globe particularly on dry soils along the sea shoreline and on the riverbanks (Badgujar *et al.,* 2014).

Figure 2.1 Fennel tree

Source: Toronto botanical garden, 2021

Figure 2.2: Fennel seeds

Source: Medium, 2021

2.1.3 CULINARY USES

Many cultures in India, Afghanistan, Iran, and the Middle East use fennel fruits in cooking (Zafar *et al.,* 2023). The bulb, leaf, and fruits of the fennel plant are utilized in many of the culinary traditions of the globe. The little blossoms of wild fennel are the most powerful kind of fennel, but also the most costly. The leaves are lightly flavored and similar in form to dill. The bulb is a crisp vegetable that may be sautéed, stewed, braised, grilled, or eaten raw. Tender young leaves are used for garnishes, as a salad, to give taste to salads, to flavour sauces to be served with puddings, and in soups and fish sauce. Both the swollen leaf bases and the fragile new shoots may be eaten like celery (Dheebisha and Vishwanath, 2020). Fennel fruits are occasionally mistaken with those of anise, which are similar in flavor and appearance, albeit smaller. Fennel is also a flavor in certain natural toothpastes. The fruits are utilized in cuisine and sweet sweets. On account of its fragrant qualities, fennel fruit constitutes one of the constituents of the well-known compound licorice powder (Dheebisha and Vishwanath, 2020).

2.1.4 PHARMACOLOGICAL PROPERTIES OF FENNEL

Different pharmacological experimenta in a variety of in vitro and in vivo models have effectively proven the capacity of Foeniculum vulgare to display antifungal, antibacterial and antioxidant properties. Phenolic chemicals extracted from F. vulgare are regarded to be responsible for its antioxidant properties while the volatile fragrance components make it a great flavoring agent.

- **Carminative Properties**

Fennel seeds are well-known for their carminative actions, assisting in digestion and easing gastrointestinal pain. Fennel is mostly used medicinally with purgatives to lessen their adverse effects and for this reason constitutes one of the constituents of the well known compound liquorice powder. Fennel water has qualities comparable to those of anise and dill water: combined with sodium bicarbonate and syrup, these liquids comprise the household 'gripe water', used to rectify flatulence of newborns. Fennel tea, also utilized as a carminative, is created by pouring boiling water over a teaspoonful of smashed fennel seeds (Rather *et al.,* 2016). In the Indian Subcontinent, fennel seeds are eaten uncooked, occasionally with added sweetness to enhance vision. Extracts of fennel seeds have been found in animal experiments to have a potential value in the treatment of glaucoma, as a diuretic and a possible medication for the treatment of hypertension. It has been used as a galactagogue boosting the milk production of a breast feeding mother. This is considered to be linked to the presence of phytoestrogens contained in fennel which encourage development of breast tissue (Anka *et al.,* 2020).

- **Antimicrobial properties**

Studies reveal that fennel seeds exhibit antimicrobial activity, suggesting a possible function in combating certain illnesses. Due to the inclusion of chemicals like linoleic acid, 1, 3-benzenediol, oleic acid, and 2,4-undecaprenyl, Fennel has antibacterial characteristics and is used to treat a number of infectious bacterial, fungal, viral, and mycobacterial illnesses. Fennel includes 5-hydroxy-furanocoumarin, a significant component of the plant's antibacterial effect (Kwiatkowski *et al*., 2015). Fennel's aqueous extract showed bactericidal effect against "Escherichia coli, Shigella flexneri, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi and Enterococcus. A research on the antibacterial activity of fennel extract by Moradi *et al. (*2020) focused on Acinetobacter baumannii strains that cause nosocomial infections. The essential oil derived from the fruits of F. vulgare displayed antibacterial activity against foodborne pathogens such as Escherichia coli, Bacillus megaterium and Staphylococcus aureus.. Ethanol and water extracts have showed efficacy against Campylobacter jejuni and Helicobacter pylori. Fennel extract also possesses antifungal characteristics that are effective against a number of fungus, including dermatophytes, Aspergillus species, and Candida albicans.

- **Diuretic Effects**

Fennel seeds may exert modest diuretic effects, encouraging good kidney function and fluid balance. A diuretic is any chemical that stimulates the production of urine. Briefly, it is an agent that promotes diuresis. Diuretics act by encouraging the ejection of urine and urinary sodium (UNa) from the body and thus helps lower the amount of blood moving through the cardiovascular system (Arumugham and Shahin 3023). The ethanolic extract of F. vulgare fruit demonstrates good diuretic properties. In another study, Foeniculum vulgare had no influence on the noradrenalin contractile responses of aortic rings, therefore showing that it operated largely as a diuretic and natriuretic with little effect on arterial vascular tone (Badgujar *et al.,* 2014).

- **Anti-Cancer Properties**

Research has studied the anti-cancer potential of fennel seeds, revealing their possible significance in cancer prevention. TNF-dependent reactions are recognized to play an involvement in both inflammation and cancer. It was demonstrated that the anethole in fennel seed reduces TNF transcription factor NF-activation KBs. Anethole suppresses the cellular responses caused by these cytokines, which may give some insight into how it prevents cancer. Fennel extracts in ethanol were investigated for their capacity to induce apoptosis in leukemia by Kaveh *et al.* (2023). The findings of this investigation demonstrated that the extract strongly triggered apoptosis in cancer cells.

- **Anti-inflammatory**

The seeds include chemicals with anti-inflammatory effects, perhaps helping to the decrease of inflammation in the body. Oral administration of methanol extract of F. vulgare fruit to rat and mice revealed inhibitory effects against acute and subacute inflammatory disorders. The anti-inflammatory activities of methanol extract were tested by employing three screening methods, namely, carrageenan-induced paw edema, arachidonic acid-induced ear edema, and formaldehyde-induced arthritis (Bouyahya *et al.,* 2022). For acute inflammation, methanol extract (200 mg/kg) showed considerable prevention of paw edema (69%) generated by carrageenan injection as compared to the control group of rats. Methanol extract of F. vulgare also reduces ear-edema (70%) produced by arachidonic acid in mice. The level of serum transaminase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) considerably rises in the presence of methanolic extract of F. vulgare on inflammation produced by formaldehyde as compared to control group. The examination of the level of AST and ALT offers an excellent and straightforward instrument to quantify the anti-inflammatory activity of the target substances. (Das *et al.,* 2011).

- **Antiallergic Activity**

Methanolic extract of F. vulgare fruit demonstrated considerable inhibitory impact on DNFB-(2,4-dinitrofluorobenzene-) caused delayed type hypersensitivity following oral administration of 200 mg/kg once a day for 7 days. The inhibitory impact on immunologically induced swelling reveals the putative immunosuppressive features of F. vulgare (Korinek *et al.,* 2021).

- **Antioxidant Activity**

The antioxidant activity of wild, edible and medicinal fennels from various Mediterranean nations has been studied. Wild fennel has been reported to have a radical scavenging activity greater than that of both therapeutic and edible fennels. Fennel seeds display antioxidant capabilities, helping to alleviate oxidative stress and battle free radicals. Natural antioxidants may be found in abundance in fennel. Because it contains a lot of polyphenols and flavonoids, this plant may inhibit the development of free radicals. This plant had phenolic components that demonstrated antioxidant effect, including "caffeoylquinic acid, rosmarinic acid, eriodictyol-7-o'rutinoside, quercetin 3-O-galactoside, and kaempferol-3-O-glucoside." Additionally, fennel volatile oil has powerful antioxidant capabilities. Plant ethanolic and aqueous extracts in compared to their essence have reduced antioxidant activity (Tungmunnithum *et al.,* 2021).

- **Antithrombotic action**

The essential oil of F. vulgare and its primary component, anethole has been demonstrated to have a safe antithrombotic activity that arises owing to its broad-spectrum antiplatelet activity, clot destabilising impact and vasorelaxant action. Anethole, the major component of fennel oil studied in guinea pig plasma, was equally powerful as the fennel oil in preventing arachidonic acid, collagen-ADP and U46619 induced aggregation. Anethole also reduced thrombin induced clot response at doses equivalent to fennel oil. Furthermore, both F. vulgare essential oil and anethole (100 mg/kg oral treatment) offered considerable protection against ethanol induced stomach ulcers in rats (Tognolini *et al.,* 2007).

- **Oestrogenic action**

F. vulgare has been utilized as an oestrogenic agent for ages. It has been claimed to stimulate milk supply, induce menstruation, assist delivery, reduce the symptoms of the male climacteric and boost libido. The major ingredient of fennel essential oil, anethole has been suggested to be the active oestrogenic agent. Some additional research have shown that the true pharmacologically active substances are polymers of anethole, such as dianethole and photoanethole. Other applications include its usage in helping to stimulate and control the menstrual cycle (Mahboubi, 2019).

Fennel seeds also provide different ayurvedic qualities that assist heal skin disorders including rashes and dryness. In addition, using fennel paste is particularly useful for the skin since the minerals inside are absorbed by the skin, keeping it moist, clean and healthy. Studies also reveal that fennel seeds have anti-ageing benefits. Fennel seed water is an element of many skincare regimes. It cleanses blocked pores caused to dirt, bacteria, dead skin cells and grime (Purwanjani *et al.,* 2021).

2.2 VITAMINS IN FENNEL SEEDS

Previous research studies have shown that fennel seeds may contain vitamins such as A B6, C, E and K. These vitamins help to contribute to the antioxidant and antiinflammatory properties as the are known to have multiple benefits to human health (Mehra *et al.,* 2021).

2.2.1 VITAMIN A

Vitamin A (Retinol) is a lipophilic vitamin that occurs naturally in several dietary sources. Vitamin A is a collective designation for a collection of chemically related organic substances, which include retinol, retinal (also known as retinaldehyde), retinoic acid, and several provitamin carotenoids, with beta-carotene being the most notable. Vitamin A is a crucial micronutrient that is necessary for the body, along with other vitamins, minerals, and compounds. Consequently, our bodies lack the ability to produce it endogenously, necessitating its inclusion in our dietary intake. Food-derived Vitamin A is stored in the liver until it is needed by the body and is attached to protein before being sent to its desired destination (Carazo *et al.,* 2021). Vitamin A is present in animal sources as retinol, which is the biologically active form of the vitamin. The liver, notably fish liver, is an excellent source. Additional animal sources include egg yolk (excluding the white) and dairy products like milk (including human breast milk), cheese, and butter. Animal-derived muscle tissue, often known as meat, is not considered a reliable source of nutrition. Plant sources include vitamin A in the form of carotenoids which have to be transformed during digestion into retinol before the body can utilize it. Plant sources of vitamin A include: mangos, papaya, several of the squashes, carrots, sweet potatoes and maize (Carazo *et al.,* 2021).

- **Health Benefits**

Vitamin A is a critical ingredient that plays a significant role in preserving good eyesight, guaranteeing the appropriate operation of the immune system, and supporting skin health. Its relevance extends to encouraging the development and repair of bodily tissues, making it crucial for wound healing and the preservation of healthy bones and teeth. Vitamin A also serves as an antioxidant, countering oxidative stress by neutralising damaging free radicals in the body, hence possibly lowering the risk of chronic illnesses such as cancer and heart disease (Carazo *et al.,* 2021).

2.2.2 VITAMIN B6

Vitamin B6 is a water-soluble organic molecule that is a vital vitamin for microbes and mammals. It occurs in three forms: pyridoxine (or pyridoxol), pyridoxal, and pyridoxamine. Its active form, pyridoxal 5′-phosphate, functions as a coenzyme in more than 140 enzyme processes in amino acid, carbohydrate, and lipid metabolism. It is also involved in the creation of the neurotransmitters serotonin and norepinephrine and of heme (a molecular ingredient of hemoglobin) and in the conversion of the amino acid tryptophan to the vitamin niacin (Para *et al.,* 2018).

Plants synthesise pyridoxine as a way of protection from the UV-B radiation present in sunshine and for the function it plays in the synthesis of chlorophyll. Animals cannot synthesise any of the different forms of the vitamin, and consequently must receive it from food, either of plants, or of other animals. There is some absorption of the vitamin generated by gut bacteria, but this is not adequate to fulfill dietary demands (Shi and Lui, 2021).

- **Health Benefits**

One of its key advantages is its critical involvement in amino acid metabolism, which is required for the manufacture of neurotransmitters such as serotonin and dopamine, hence aiding brain health and mood control. Vitamin B6 is also important in haemoglobin formation, which is required for delivering oxygen throughout the body, adding to energy levels and preventing exhaustion. Moreover, Vitamin B6 contributes in maintaining appropriate blood sugar levels by participating in glucose metabolism. It has been demonstrated to have a good influence on immune function by promoting the development of antibodies required to combat numerous illnesses. Additionally, it may help lower the risk of heart disease by aiding in the management of homocysteine levels, an amino acid that, at high levels, is linked with an elevated risk of cardiovascular issues (Tardy *et al.,* 2020).

2.2.3 VITAMIN C

Vitamin C (also known as ascorbic acid and ascorbate) is a water-soluble vitamin found in citrus and other fruits, berries and vegetables. It is also a generic prescription drug and in certain countries is available as a non-prescription nutritional supplement. As a treatment, it is used to prevent and cure scurvy, a condition caused by vitamin C deficiency. Vitamin C may be given by mouth or by intramuscular, subcutaneous or intravenous injection (Doseděl *et al.,* 2021).

- **Health Benefits**

It serves a critical function in supporting the immune system, making it one of the body's essential antioxidants (Carr and Rowe, 2020). It assists in the protection against environmental oxidative stress and decreases the risk of chronic illnesses, including heart disease, by neutralising damaging free radicals. Beyond its immune-boosting powers, vitamin C is vital for the manufacture of collagen, a protein that is needed for the preservation of healthy skin, blood vessels, bones, and cartilage, contributing to wound healing and the general integrity of human tissues. Its participation in the creation of neurotransmitters further highlights its relevance in mental well-being, possibly impacting mood and cognitive performance. Moreover, vitamin C increases the absorption of nonheme iron from plant-based meals, treating iron deficiency and improving blood iron levels, which is especially advantageous for persons at risk of anaemia. The antioxidant capabilities of vitamin C also have a role in eye health, perhaps decreasing the advancement of age-related macular degeneration and lowering the likelihood of cataract formation (Carr and Rowe, 2020).

2.2.4 VITAMIN E

Vitamin E is a collection of eight fat soluble molecules that contain four tocopherols and four tocotrienols. Vitamin E deficiency, which is unusual and typically related to an underlying difficulty with processing dietary fat rather than from a diet low in vitamin E, may induce nerve difficulties. Vitamin E is a fat-soluble antioxidant which may help protect cell membranes from reactive oxygen species (Rizvi *et al.,* 2014). There are eight naturally occurring forms of vitamin E; notably, the alpha, beta, gamma and delta classes of tocopherol and tocotrienol, which are synthesised by plants from homogentisic acid. Alpha-and gamma-tocopherols are the two principal forms of the vitamin, with the relative quantities of these depending on the source. The richest dietary sources of vitamin E are edible vegetable oils since they include all the various homologues in variable quantities. Among the tocopherols, the alpha- and gamma-tocopherols are found in the serum and the red blood cells, with alpha-tocopherol present in the greatest quantity (Jiang, 2014).

- **Health Benefits**

Vitamin E is the principal lipid-soluble component in the cell antioxidant defense system and is entirely derived from the food. It has various critical functions inside the body due of its antioxidant action. It has various critical functions inside the body due of its antioxidant action (Rizvi *et al.,* 2014). Oxidation has been connected to various different ailments and diseases, including cancer, aging, arthritis and cataracts; vitamin E has been found to be beneficial against them. Platelet hyperaggregation, which may contribute to atherosclerosis, may also be reduced by vitamin E; moreover, it also helps to inhibit the formation of prostaglandins such as thromboxane, which induce platelet clumping. Diets richer in vitamin

E may include other substances that impart health advantages, or be eaten by individuals who adopt non-diet lifestyle choices that decrease disease risk, so that the observed impact may not be attributable to the vitamin E content (Rizvi *et al.,* 2014).

2.3 MINERAL COMPOSITION OF FENNEL SEEDS

Several research have been conducted out to identify the various phytoconstituents and mineral content of F. vulgare. In a study by Bukhari *et al. (*2014) it was discovered that fennel seeds may include potassium, sodium, manganese and calcium in fennel as 504.6, 29.45, 23.55 and 20.95 mg/100 g and trace quantity of iron and zinc, accordingly. Sodium, potassium and calcium are the primary micronutrients found in plants as an important activity. These minerals are essential for the human body in considerable proportions. However, its shortage leads in arthritis, bone and teeth associated problems.

2.3.1 COPPER

Copper is an essential trace mineral necessary for survival. It is found in all body tissues and plays a role in making red blood cells and maintaining nerve cells and the immune system.

It also helps the body form collagen and absorb iron, and plays a role in energy production.

Most copper in the body is found in the liver, brain, heart, kidneys, and skeletal muscle.

Both too much and too little copper can affect how the brain works. Impairments have been linked to Menkes, Wilson’s, and Alzheimer’s disease (Ware, 2017).

Copper is associated with bone health, immune function and increased frequency of infections, cardiovascular risk and alterations in cholesterol metabolism. Its metabolism is tightly intertwined with other microminerals and its deficiency is known to impair iron mobilisation, resulting in secondary iron deficiency (Danzeisen *et al*., 2007).

2.3.2 ZINC

Zinc is a multipurpose trace element for the human body, as it plays a crucial part in various physiological processes, such as cell growth and development, metabolism, cognitive, reproductive, and immune system function. One notable area where zinc has shown beneficial effects is in the prevention and treatment of various diseases, including cancer (Kiouri *et al*., 2023).

Zn deficiency is observed almost in 17% of the global population and affects many organ systems, leading to dysfunction of both humoral and cell-mediated immunity, thus increasing the susceptibility to infection (Chasapis *et al*., 2020)

2.3.3 CHROMIUM

Chromium, one of the most common elements in the earth's crust and seawater, exists in our environment in several oxidation states, principally as metallic (Cr^0), trivalent (+3), and hexavalent (+6) chromium. The latter is largely synthesized by the oxidation of the more common and naturally occurring trivalent chromium and is highly toxic. Trivalent chromium, found in most foods and nutrient supplements, is an essential nutrient with very low toxicity (Cefalu and Hu, 2004).

Chromium is one of the vital minerals involved in the regulation of insulin action. According to abundant evidences this mineral seems to be an essential factor involved in the reduction of insulin resistance and decreasing the risk of type 2 diabetes mellitus (T2DM) and cardiovascular diseases (Gossa Al-Saadde *et al*., 2023).

2.3.4 CADMIUM

Cadmium (Cd) is a ductile metal in the form of a blueish or silvery-white powder. It is naturally found in soil (about 0.2 mg/kg), minerals, and water. Cd belongs to the group of toxic, carcinogenic, and stimulating elements. Some lung diseases (such as emphysema, asthma, and bronchitis) and high blood pressure are thought to be related to slow poisoning (Charkiewicz *et al*., 2023)

2.4 ALPHA-AMYLASE ENZYME

Glucose is the most significant carbohydrate fuel in the body. In the fed state, the bulk of circulatory glucose originates from the food; in the fasting state, gluconeogenesis and glycogenolysis sustain glucose concentrations. Very little glucose is present in the diet as glucose; most is contained in more complex carbs that are broken down to monosaccharides throughout the digestive process. Abnormal blood glucose levels might suggest several health concerns (Nakrani *et al.,* 2020)

Following a carbohydrate heavy meal, the amount of glucose in the body is controlled by two key hormones: Insulin and glucagon. The digestion of most starch molecules happens in the upper gastrointestinal tract where they become broken into smaller molecules (monosaccharides) which are absorbed by glucose transporters (GLUT) into the blood stream (Koepsell, 2021). The GLUT-family is encoded by SLC2 genes and is responsible to transport monosaccharide, polysaccharide, and other tiny molecules across the membrane. Fourteen GLUT proteins are expressed in human; GLUT 1-12, GLUT 14, and a myo-inositol transporter (HMIT). GLUT 2 is responsible to carry glucose from the blood to pancreatic β cells where it becomes oxidized and leads to the production of insulin (Koepsell, 2021). The reduction of blood glucose levels occurs through three main mechanisms: The enhancement of a glucose uptake by peripheral tissues through the translocation of GLUT 4; (ii) the inhibition of lipolysis and the promotion of lipogenesis; and (iii) the promotion of glucose storage and utilization in the liver. One the other side, when the glucose level in the body is low, the amount of glucagon secretion rises owing to two mechanisms: The encouragement of glucose generation and release in the liver and (ii) the enhancement of lipolysis and releasing free fatty acids from adipose tissue (Colson *et al.,* 2021).

In diabetes and other blood glucose related disorders, the enzymes alpha-amylase and alpha-glucosidase play key roles in carbohydrate metabolism, impacting blood glucose levels (Gong *et al.,* 2020). Alpha-amylase, prevalent in saliva and pancreatic juice, breaks down complex carbs into simpler sugars. Elevated levels of alpha-amylase may be related with insulin resistance in diabetes. On the other hand, alpha-glucosidase, present in the small intestine, assists in the absorption of glucose. Medications that block alpha-glucosidase are employed in diabetes care, since they impede the digestion of complex carbohydrates, limiting fast rises in blood glucose after meals (Shah *et al.,* 2021).

2.4.1 Alpha-Amylase (α-Amylase)

α-Amylase is an enzyme (EC 3.2.1.1; systematic name 4-α-D-glucan glucanohydrolase) that hydrolyses α bonds of big, α-linked polysaccharides, such as starch and glycogen, releasing shorter chains thereof, dextrins, and maltose. α-Amylases may be derived from plants, animals and microbes (Gong *et al.,* 2020). The α-amylase belongs to a family of endo-amylases that catalyses the first hydrolysis of starch into shorter oligosaccharides by the cleavage of α-D-(1–4) glycosidic linkages. Neither terminal glucose residues nor α-1,6-linkages can be broken by α-amylase. The end products of α-amylase activity include oligosaccharides of varied length with an α-configuration and α-limit dextrins, which form a combination of maltose, maltotriose, and branched oligosaccharides of 6–8 glucose units that contain both α-1,4 and α-1,6 links (de Souza and de Oliveira Magalhães, 2010). Others amylolytic enzymes contribute in the process of starch breakdown, but the contribution of α-amylase is the mos significant for the commencement of this process.

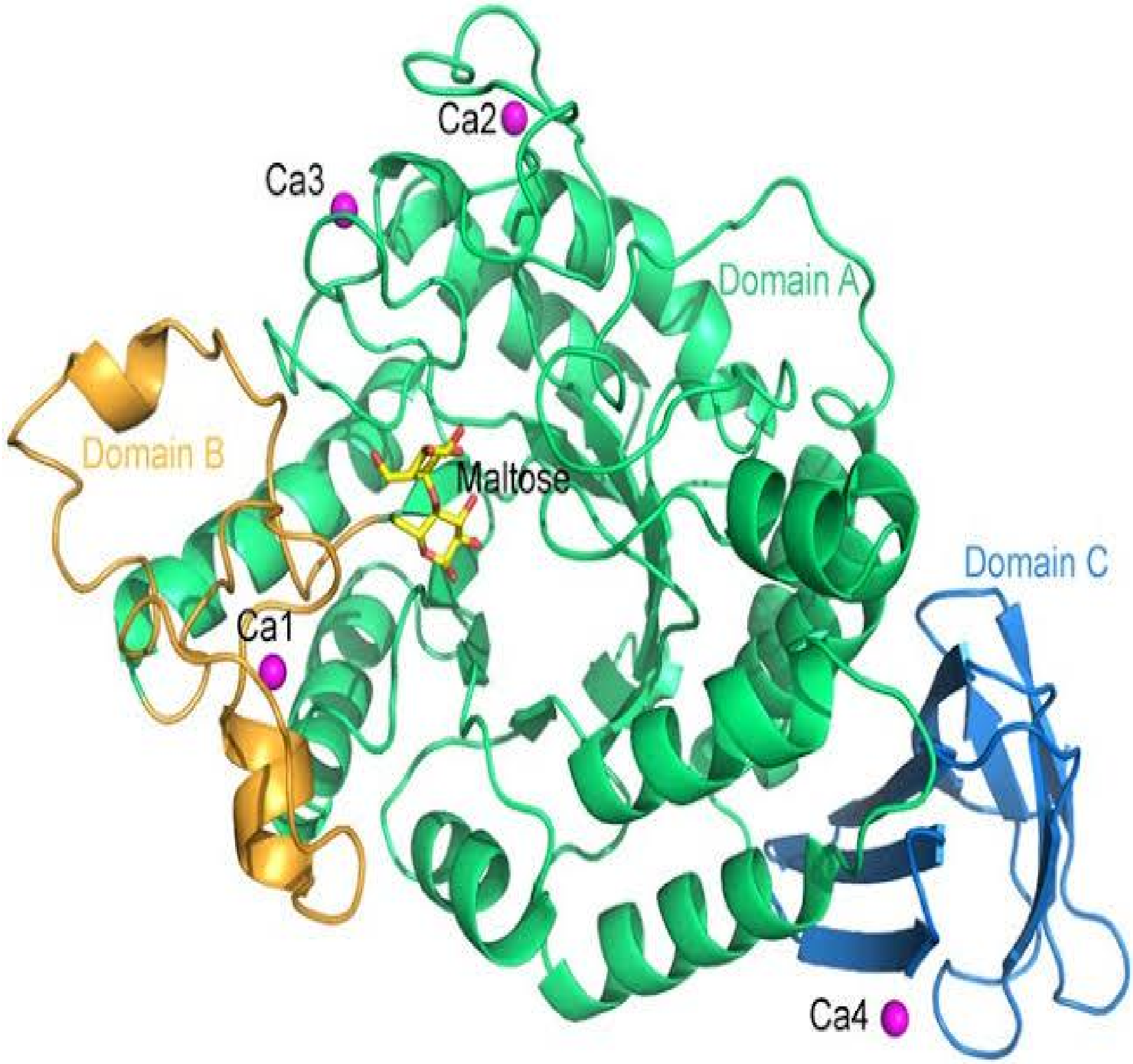

Figure 2.3: Three-Dimensional Structure of Alpha amylase

Source: Chai et al., (2016)

The amylase has a three-dimensional structure capable of attaching to substrate and, by the action of highly specialized catalytic groups, facilitate the breakdown of the glycoside linkages. The human α-amylase is a typical calcium-containing enzyme comprised of 512 amino acids in a single oligosaccharide chain with a molecular weight of 57.6 kDa (Kaur *et al.*, 2021). The protein has 3 domains. The A domain is the biggest, showing a typical barrel shaped (β/α)8 super structure. The B domain is placed between the A and C domains and is connected to the A domain via disulphide bond. The C domain has a β sheet structure connected to the A domain by a simple polypeptide chain and looks to be a separate domain with unknown function. The active site (substrate-binding) of the α-amylase is found in a lengthy cleft located between the carboxyl terminus of the A and B domains (Kaur *et al.,* 2021).

In the human body, Alpha-amylase initiates the process of starch digestion. It takes starch chains and splits them into smaller fragments containing two or three glucose units. Two comparable forms of amylase are created in your body one is released in saliva, where it begins to break down starch granules while you chew, and the other is secreted by the pancreas, where it continues its task (Peyrot des Gachons and Breslin, 2016). Then, these small fragments are split into individual glucose units by a group of enzymes that are anchored to the walls of the gut (Peyrot des Gachons and Breslin, 2016). The salivary amylase is generally named after a Swedish chemical, called ptyalin. This ptyalin can only function on linear alpha (1,4) glycosidic bonds. However, conditions like increasing gastric acid in the stomach might deactivate the salivary amylase and it is unable to break down starch into maltose. It transforms big, insoluble starch molecules into soluble starches (amylodextrin, erythrodextrin, and achrodextrin) creating gradually smaller starches and finally maltose. Salivary amylase is inactivated in the stomach by gastric acid (Gupta and Patil, 2023).

Pancreatic α-amylase randomly cleaves the α(1-4) glycosidic bonds of amylose to produce dextrin, maltose, or maltotriose. It utilizes a twofold displacement mechanism with retention of anomeric structure. In humans, the salivary amylase developed from a duplicate of it. Pancreatic α-amylase is generated by pancreatic acinar cells and delivered into the intestinal tract via the pancreatic duct system. The enzymatic activity of P-type amylase is optimum in the somewhat alkaline environment of the duodenum (Paul *et al.,* 2021).

α-Amylase has been obtained from numerous fungi, yeasts, plants and bacteria. However, enzymes from fungal and bacterial origins have dominated usage in industrial industries. It has potential uses in a large range of industrial processes such as food, fermentation, textile, paper, detergent, and pharmaceutical sectors. Fungal and bacterial amylases might be potentially valuable in the pharmaceutical and fine-chemical industries. It is used in ethanol production to break starches in grains into fermentable sugars (Gupta and Patil, 2023).

2.5 ALPHA-GLUCOSIDASE ENZYME

The enzyme α-glucosidase performs a crucial function to raise blood glucose level in the body. α-Glucosidase enzyme is located in the brush border small intestine, which is important in the digestion and absorption of carbohydrates. It induces the hydrolysis of polysaccharides and oligosaccharides into monomers and thereby raises glucose content in the body. α-Glucosidase is the primary enzyme catalyzing the last stage in the digestive process of carbohydrates (Sohrabi *et al.,* 2020).

α-Glucosidase hydrolyzes terminal non-reducing (1→4)-linked α-glucose residues to release a single α-glucose molecule. α-Glucosidase is a carbohydrate-hydrolase that releases α-glucose as opposed to β-glucose. β-Glucose residues may be released by glucoamylase, a functionally identical enzyme. The substrate selectivity of α-glucosidase is attributed to subsite affinities of the enzyme's active site (Assefa *et al.,* 2019).

The enzyme consists of many subunits, each contributing to its overall structure and activity. Crystallography investigations have offered extensive insights into the three-dimensional configuration of these subunits. The catalytic site, where the enzymatic process takes place, is well-defined within the structure (Kashtoh and Baek, 2022). It may be categorized into two families based on its fundamental structure: glycoside hydrolase family 13 (GH13) and family 31 (GH31). The GH13 family comprises enzymes with a distinctive (β/α)8 barrel fold. Within this family, α-amylase stands out as a noteworthy member, playing a major role in breaking down starch and glycogen. On the other hand, the GH31 family has a (α/α)6 barrel fold and comprises α-glucosidases that contribute to the last stages of carbohydrate digestion. Maltase and isomaltase are examples of α-glucosidases within GH31, responsible for breaking down maltose and isomaltose into individual glucose molecules (da Costa-Latgé *et al*., 2021).

2.6 IN VITRO ALPHA AMYLASE AND GLUCOSIDASE ACTIVITY

Previous study on the in vitro alpha-amylase and glucosidase activity of fennel seeds shows possible advantages in blood sugar control. Studies have indicated that fennel seeds may exhibit inhibitory effects on these enzymes, slowing down the digestion and absorption of carbs. The reduction of alpha-amylase and alpha-glucosidase activity might thus postpone glucose release from complex carbohydrates modifying the start of postprandial hyperglycemia, so giving it an excellent target for the control of type 2 diabetes (Kashtoh and Baek, 2022). Alpha-amylase inhibitors are mostly prevalent in plants. One technique that has been explored to treat type-2 diabetes is suppression of the activity of alpha-glucosidases using synthetic medicines. However, these inhibitors are frequently accompanied with gastrointestinal adverse effects. Therefore, the creation of inhibitors from natural ingredients provides an additional approach for the treatment of hyperglycemia. In recent years, different investigations have been undertaken to uncover alpha-glucosidases inhibitors from natural sources such as plants, and several candidates have proven to be secondary metabolites including alkaloids, flavonoids, phenols, and terpenoids (Kalinovskii *et al.,* 2023).

Alpha-glucosidase inhibitors are saccharides that serve as competitive inhibitors of enzymes required to digest carbohydrates: especially alpha-glucosidase enzymes in the brush border of the small intestines (Akmal and Wadhwa, 2020). The membrane-bound intestinal alpha-glucosidases hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the small intestine. Acarbose, a kind of Alpha-glucosidase inhibitor also inhibits pancreatic alpha-amylase in addition to blocking membrane-bound alpha-glucosidases. Pancreatic alpha-amylase hydrolyzes complicated starches to oligosaccharides in the lumen of the small intestine (Hossain *et al.,* 2021).

Alpha-amylase inhibitor is a protein family which inhibits mammalian alpha-amylases selectively, by creating a tight stoichiometric 1:1 complex with alpha-amylase. This class of inhibitors has no influence on plant and microbial alpha amylases. Inhibition of these enzyme systems lowers the rate of digestion of carbohydrates. Less glucose is taken because the carbs are not broken down into glucose molecules. In diabetic patients, the short-term impact of these pharmacological therapy is to lower current blood glucose levels: the long-term effect is a slight drop in hemoglobin A1c level (Gong *et al.,* 2021).

In a previous research done by Godavari *et al*. (2018) on fennel seeds, it found that the benzene extract of Foeniculum vulgare Mill. seeds exhibited significant alpha-glucosidase inhibitory influence than the alpha-amylase inhibition when compared with the standard acarbose values. In another study done by Palukuri and Mythily in 2022 also found that the ethanolic extract of Foeniculum vulgare displayed inhibition of α-amylase and α-glucosidase by 87.14% in and 85.20% at 1000 μg/ml. They ascribed this response to the phytochemical features of the plant. The findings from these research show that Foeniculum vulgare a possible beneficial agent in the treatment of diabetes.

CHAPTER THREE

MATERIALS AND METHOD

3.1MATERIALS AND APPARATUS

- F*oeniculum vulgare*
- Masking tape
- Aluminum foil
- Filter paper
- Measuring cylinder
- Conical flasks
- Bowls
- Handkerchief
- Funnels
- Beakers
- Weigh balance
- Plain bottles
- Glass jar
- Stirring rod
- Test tubes
- Magnetic stirrer
- Micropipette
- Cuvette
- Tissue paper
- Acarbose

3.2 REAGENTS USED

- Ethanol
- Distilled water
- Sodium phosphate buffer
- Di-nitro-salicylic acid (DNSA)
- P-Nitrophenyl-α-D-glucopyranoside (P-NPG)
- Sodium hydroxide

3.3 EQUIPMENT USED

- Refrigerator
- Rotary evaporator
- Spectrophotometer
- Water bath
- Blender

3.4 COLLECTION AND PREPARATION OF PLANT SAMPLE

The seeds of *Foeniculum vulgare* were acquired from forestry located in Oredo Local Government Area of Edo State. The seeds of the plant were then vouched by Prof. Akinnibosun H.A., at the Herbarium in the Department of Plant Biology and Biotechnology, University of Benin. The specimen samples were thereafter deposited at the department's Herbarium with voucher number UBH-F665.

The already dried seeds were then blended with an electric blender and then weighed with a weigh balance. The blended sample was then soaked into a jar of absolute ethanol and the mixture stirred properly with a stirring rod and allowed to stand for 3 days where it was stirred each day. After the third day, the extracts were filtered using basket sieves, sieve cloth and then handkerchief and filter paper with funnel were used for ultrafiltration. The extracts gotten were placed in a bottle and labelled properly with a masking tape, then refrigerated.

The crude extracts were taken to the Department of Pharmaceutical Chemistry, University of Benin, to be concentrated using rotary evaporator and the concentrated plant extracts obtained were kept in an airtight container.

3.5 DETERMINATION OF α-AMYLASE ACTIVITY

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the plant extracts in concentration range 20-100 mg/mL were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNSA (Di-nitro-salicyclic acid) reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. Acarbose was used as the control. The % inhibitions were then calculated according to the formula as illustrated below (Sindhu *et al*., 2013).

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)}}{\text{Abs 540(control)}} * 100$$

3.6 DETERMINATION OF α-GLUCOSIDASE ACTIVITY

P-Nitrophenyl-α-D-glucopyranoside (P-NPG), Acarbose, Baker's Yeast alpha glucosidase were purchased from Sigma (USA).The yeast alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside (P-NPG) was used as the substrate. Plant extracts were used in the concentration ranging from 20-100 mg/mL. Different concentrations of plant extracts were mixed with 320 µl of 100 mM phosphate buffer pH 6.8 and incubated in a boiling water bath at 30 °C for 5 minutes. 3 ml of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. Acarbose was used as the control. The % inhibition was calculated according to the formulas illustrated below (Sindhu *et al*., 3013)

$$\text{Inhibition (\%)} = \frac{\text{Abs 410 (control)} - \text{Abs 410 (extract)}}{\text{Abs 410 (control)}} * 100$$

CHAPTER FOUR

RESULTS

4.1 VITAMIN COMPOSITION OF *Foeniculum vulgare mill*

The data in table 4.1 demonstrate the retention duration, amounts, peak height in parts per million of several vitamins evaluated using chromatography. The study of the indicated variable quantities of various vitamins in the sample. Vitamin C (Ascorbic Acid) displayed the greatest concentration. Vitamin B3 (Niacin) was also detected in substantial concentrations. However, Vitamins B1 (Thiamine), A (Retinol), and B2 (Riboflavin) were discovered in modest levels. Pantothenic Acid was either missing or present in insignificant levels, with Retinol and Riboflavin.

Figure 4.1 shows the chromatogram obtained from the HPLC vitamins profile of ethanol extract of *Foeniculum vulgare,* with showing a greater spike indicating it is resent in highest concentration the sample.

Table 4.1: HPLC active test on vitamin profile of *Foeniculum vulgare mill*

COMPONENT	RETENTION	AMOUNT (ppm)	HEIGHT
Vit C (Ascorbic Acid)	1.116	185.7600	20.094
Vit B1 (Thiamine)	1.850	36.0710	2.394
Vit B3 (Niacin)	4.250	95.5080	6.767
Vit B5 (Pantothenic Acid)	8.800	120.4990	2.656
Vit A (Retinol)	10.466	45.2790	3.638
Vit B2 (Riboflavin)	11.850	45.4210	3.191
		528.5380	

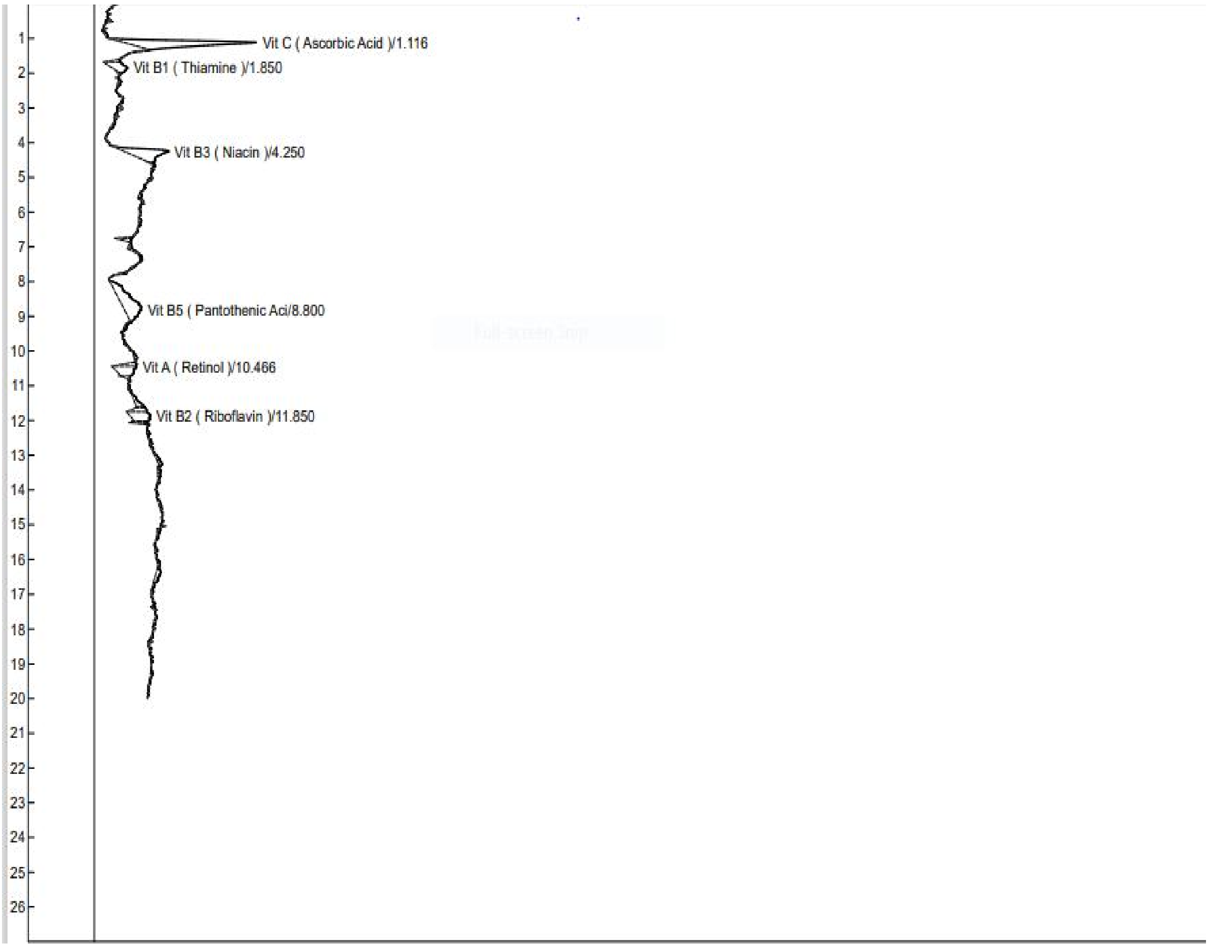

Figure 4.1: Chromatogram of the HPLC active test on vitamin profile of *Foeniculum vulgare mill*

4.2 ALPHA AMYLASE ACTIVITY OF *Foeniculum vulgare mill*

The findings in figure 4.2 and table 4.2 demonstrate theα-amylase activity of Foeniculum vulgare mill. Acarbose was utilized as the standard in the test. It indicated that the IC_{50} value of Foeniculum vulgare mill is higher than that of Acarbose, suggesting that Acarbose is more effective in suppressing α-amylase activity compared to Foeniculum vulgare mill under the circumstances examined. There was a significant difference ($p > 0.05$) in their values.

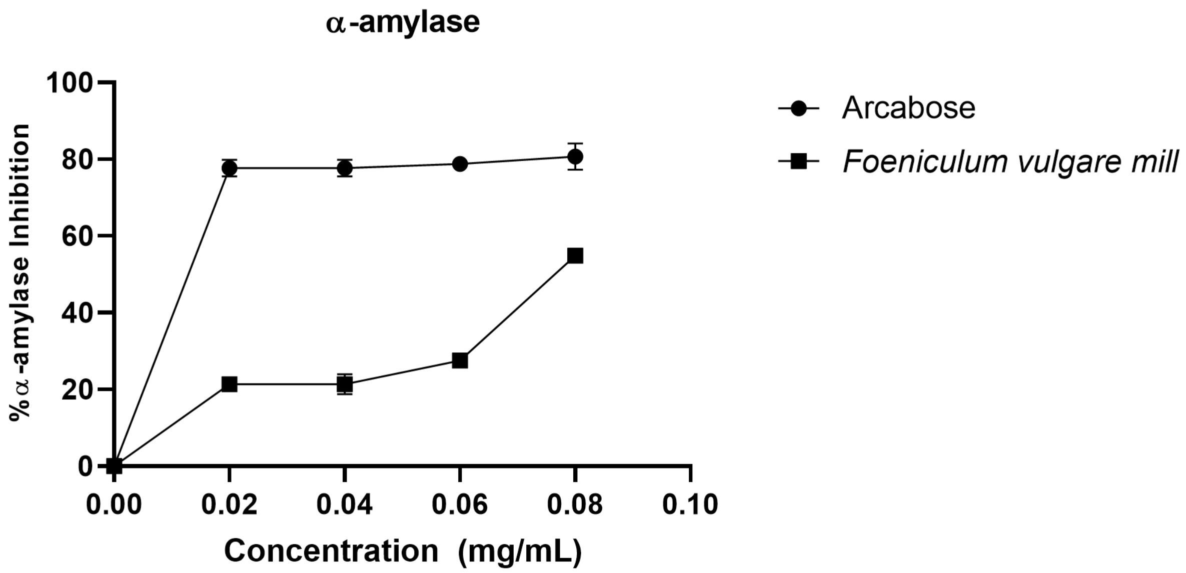

Figure 4.2: α-amylase activity of *Foeniculum vulgare mill* at varying concentration

Table 4.2: IC$_{50}$ of α-amylase activity of *Foeniculum vulgare mill* (mg/ml)

	Acarbose	*Foeniculum vulgare mill*
α-amylase	0.038 ± 0.005[a]	0.081 ± 0.003[b]

Values represent means of triplicate. Values with the same alphab*et al*ong the same column are not significantly different ($p > 0.05$).

4.3 ALPHA GLUCOSIDASE ACTIVITY OF *Foeniculum vulgare mill*

The findings from figure 4.3 and table 4.3 reveal the α-glucosidase activity of *Foeniculum vulgare mill*. The findings demonstrate that there was no significant difference ($p \leq 0.05$) in the IC_{50} value of *Foeniculum vulgare mill* when compared to Acarbose. The graph in figure 4.3 further demonstrated that *Foeniculum vulgare mill* displayed stronger α-glucosidase inhibition, up to 90 percent at a concentration of 0.08 mg/ml and above.

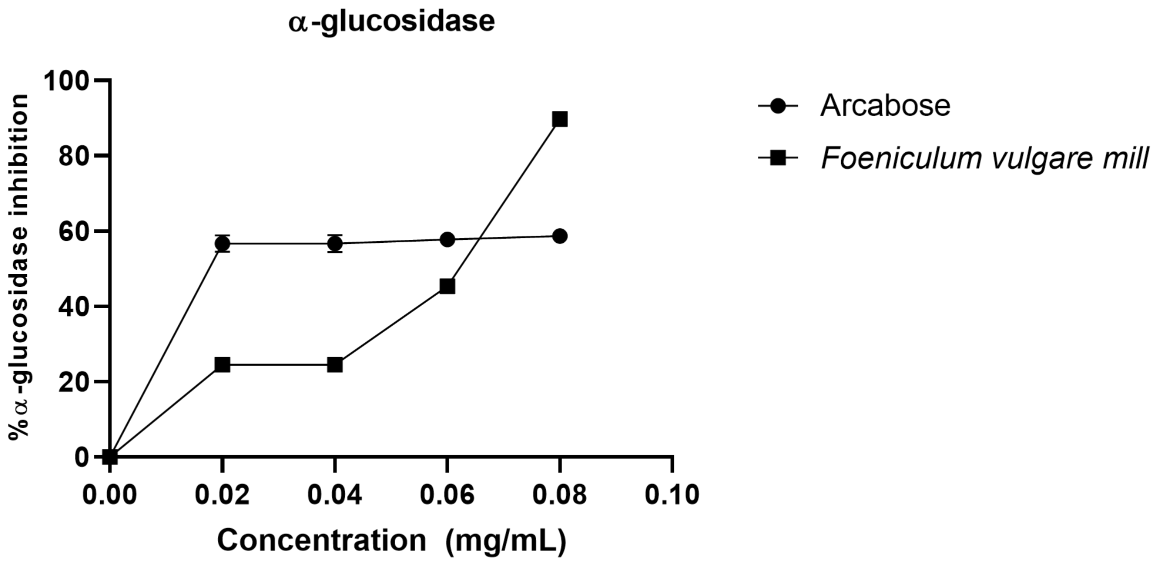

Figure 4.3: α-glucosidase activity of *Foeniculum vulgare mill* at varying concentration

Table 4.3: IC_{50} of α-glucosidase activity of *Foeniculum vulgare mill* (mg/ml)

	Acarbose	*Foeniculum vulgare mill*
α-glucosidase	0.052 ± 0.002[a]	0.053 ± 0.002[a]

Values represent means of triplicate. Values with the same alphab*et al*ong the same column are not significantly different ($p \leq 0.05$).

4.4 MINERAL COMPOSITION OF *Foeniculum vulgare mill*

Table 4.4 shows the composition of trace elements present in ethanolic extract of Foeniculum vulgare after being subjected to HPLC profiling. Minerals such as Copper, Chromium, Zinc and Cadmium were found to be present, with Chromium showing the highest concentration and cadmium being lowest.

Table 4.4: HPLC active test on micronutrients profile of *Foeniculum vulgare mill*

MINERALS	CONCENTRATION (mg/kg)
COPPER	0.240 ± 0.000
ZINC	0.370 ± 0.001
CADMIUM	0.190 ± 0.000
CHROMIUM	0.910 ± 0.001

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Herbal medicine has been identified and used in traditional medicinal techniques since ancient times. Plants produce several chemical substances for different purposes, such as defense and safeguarding against insects, fungus, illnesses, and herbivorous animals (Chaachouay and Zidane, 2024). Medicinal plants are used for the purpose of preserving health, administering treatment for a particular ailment, or both, whether in contemporary medicine or traditional medicine. The use of plant-derived substances, such as herbal or natural health products, believed to possess advantageous effects on health, is on the rise in industrialized nations (Well *et al*., 2018). Despite the perceived safety of herbal treatments, there are inherent dangers of poisoning and other adverse consequences on human health.

Fennel (*Foeniculum vulgare*) is reported to provide phenolic compounds and vitamins that have advantageous effects on human health. Trans-anethole, estragole, fenchone, and quercetin have been discovered as bioactive compounds derived from this plant (Barakat *et al*., 2022). Several of these substances have been associated with possible impacts on various human body systems. Fennel has several pharmacological properties and its bioactive components are essential for preserving human health, making it a valuable ingredient in the production of various medications. Additionally, the herb is said to possess antidiabetic and antioxidant properties (El-Soud *et al*., 2011).

Results in table 4.1 from this investigation indicated that *Foeniculum vulgare* possesses an abundance of Vitamin C (Ascorbic acid), with considerable levels of Vitamin B3 (Niacin) also present. Vitamins B1 (Thiamine), A (Retinol), and B2 (Riboflavin) were also discovered in modest levels. Pantothenic Acid was either missing or present in insignificant levels. This conclusion is in alignment with a recent research done by Salama *et al*. (2015), who showed a high amount of vitamin C in two cultivars of fennel. Some pharmacological properties of fennel extracts, including anti-allergic, analgesic, anti-inflammatory and antioxidant activity might be in part ascribed to the availability of these vitamins in the plant. Ascorbic acid is generally recognized for its high antioxidant and anti-inflammatory qualities whereas niacin also has antioxidant characteristics and plays a function in cell signaling and DNA repair (Gęgotek and Skrzydlewska, 2022).

The findings from figure 4.2 and 4.3, and table 4.2 and 4.3 reveal the α-amylase and α-glucosidase activity of *Foeniculum vulgare*. Diabetes mellitus is a chronic metabolic illness, characterized by hyperglycemia and carbohydrate, protein, and fat metabolic abnormalities. It causes failure of insulin synthesis or insulin action or both (Dilworth *et al*., 2021). The use of herbal remedies as alternative methods in current treatments for the treatment of diabetes and its complications is rising globally and various plants in different countries are known to have antidiabetic properties. Two primary carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) are responsible for postprandial hyperglycemia. α-amylase commences the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic bonds of polysaccharides (starch, glycogen) to disaccharides and α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia (Gong *et al*., 2020). Hence, inhibitors of α-amylase and α-glucosidase are effective in the treatment of hyperglycemia since they delay carbohydrate digestion, and subsequently lower the postprandial plasma glucose level (Alqahtani *et al*., 2019). Acarbose is one of the major inhibitors of carbohydrate metabolic enzymes and is utilized as a standard in this investigation. The findings demonstrated that Foeniculum vulgare mill had high inhibitory potential against α-glucosidase. When compared to Acarbose there was no noticeable change. It demonstrated a greater % inhibition at 0.08mg/ml when compared to Acarbose. However, for α-amylase, the IC_{50} value of Foeniculum vulgare mill was greater than that of Acarbose, showing that

Acarbose is more efficient in suppressing α-amylase. There was a big difference between their values. *Foeniculum vulgare* has been previously observed to have hypoglycemic and antidiabetic effects. Results from earlier research done by El-Ouady *et al*. (2020), El-Soud *et al*. (2011) and Mehra *et al*. (2023) all revealed that various *Foeniculum vulgare* displays hypoglycemic effects in streptozotocin (STZ)- caused diabetic rats. The cause for this hypoglycemic feature may be related to its inhibitory potential against α-glucosidase and a-amylase. The results are in accordance with a study conducted by Sayah *et al*. in 2020, their results showed the antidiabetic potential of *Foeniculum vulgare,* however, their results further revealed that the rootstock extract exhibited a more remarkable inhibitory capacity of α-amylase and α-glucosidase when compared with *Foeniculum vulgare* leaf and seed extract. A proposed reason for this inhibitory action is the presence of polyphenolic substances such as flavonoids and phenolic acids in fennel. These substances have been demonstrated to block alpha-amylase and alpha-glucosidase activity by binding to the active sites of these enzymes, hence inhibiting the breakdown of complex carbohydrates into simpler sugars like glucose. Additionally, fennel contains essential oils, notably anethole, which may potentially contribute to its inhibitory effects on these enzymes. Anethole has been shown to display anti-diabetic characteristics and may interfere with carbohydrate digestion and absorption by blocking alpha-amylase and alpha-glucosidase activity.

The findings from Table 4.4, obtained using High-Performance Liquid Chromatography (HPLC) analysis, reveal the presence of many minerals in Foeniculum vulgare mill. These minerals include Copper, Chromium, Zinc, and Cadmium, with Chromium exhibiting the greatest concentration and Cadmium the lowest. Chromium is a vital mineral for the human body. It contributes to the mechanism by which insulin assists in the regulation of blood sugar levels in the body, as well as its impact on the metabolism of carbohydrates, proteins, and lipids (NIH, 2022). Several studies have hypothesized that chromium supplements augment the metabolic function of insulin and reduce certain risk factors associated with cardiovascular disease, especially in persons who are overweight (Gossa Al-Saadde *et al.,* 2023). A separate research discovered that the addition of chromium to the diet had a notable positive impact on blood sugar levels in those diagnosed with diabetes (Zhao *et al*. 2022). The elevated content of Chromium in Foeniculum vulgare mill may have a role in its capacity to sustain and control blood glucose levels. The presence of copper and zinc in Foeniculum Vulgare enhances its nutritional value. Copper is essential for proper growth, cardiovascular health, lung elasticity, neovascularization, neuroendocrine function, and iron metabolism (Balk *et al*., 2007). Meanwhile, zinc, the most crucial trace element in the body, supports a healthy immune system and safeguards cells against oxidative stress. Additionally, it plays a crucial role in DNA synthesis, cellular proliferation, protein synthesis, and tissue regeneration(Jarosz *et al*., 2017). Cadmium is known for its capacity to induce renal damage, bone demineralization, and heightened susceptibility to cancer when exposed for extended periods (Rafati Rahimzadeh *et al.,* 2017). However, the study's findings indicate that the low quantity of cadmium observed poses no immediate hazards in the therapeutic use of Foeniculum vulgare. The findings align with previous research conducted by Endalamaw and Chandravanshi (2015) and more recently by Uslu *et al.* (2021), they both reported the presence of the minerals in extracts of Foeniculum vulgare mill.

5.2 CONCLUSION

The investigation on ethanol extracts of fennel seeds indicated that fennel is rich in Vitamin C and B3, as well as micronutrients such as chromium, copper, zinc and cadmium. It also displayed strong inhibition against α-glucosidase, an enzyme connected to postprandial hyperglycemia in diabetes mellitus, equivalent to the medication Acarbose. This highlights the possibility of fennel as a natural therapy for treating postprandial hyperglycemia, attributable to its polyphenolic components and essential oils

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