INVITRO ANTIOXIDANT POTENTIAL OF ETHANOL EXTRACTS OF *Foeniculum vulgare* AND ITS HPLC PROFILE

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BENIN CITY

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

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CERTIFICATION

This is to certify that this project work was carried out by **Aizenose Victory OBOZOKHAE** with matriculation number, **BMS2004998** in the Department of Medical Biochemistry, University of Benin, Benin City in partial fulfilment of the requirements for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

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Date

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(Head of Department)

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Date

DEDICATION

With humility and love I would like to dedicate this project book to a special group of people whom Jehovah God has used to complete and bless me. I dedicate this work to my delightful Parents (Mr. and (Mrs.) Obozokhae), Engr. Ogini-Momodu Charles, Department of Medical biochemistry and National Association of Medical Biochemistry Students (NAMBS), Uniben Chapter.

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ABSTRACT

This study investigates the in vitro antioxidant activity of ethanol extracts derived from *Foeniculum vulgare* seeds, commonly exploited for its medicinal purposes. *Foeniculum vulgare* is rich in bioactive compounds with potential health benefits, including antioxidant properties. High-Performance Liquid Chromatography (HPLC) analysis was employed to identify and quantify the bioactive constituents present in the extracts. The antioxidant potential was evaluated using various in vitro assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP) assays, Total Antioxidant Capacity (TAC), Hydroxyl Radical Scavenging Activity, and Nitric Oxide Scavenging Ability. The results revealed significant a significant difference when compared with the standard ascorbic acid, ethanol extract of Foeniculum vulgare, presence of antioxidant activity with DPPH, FRAP and TAC assays. HPLC profiling provided valuable insights into the chemical composition of the extracts, elucidating the presence of specific antioxidant compounds. This study underscores the potential of *Foeniculum vulgare* seeds as a natural source of antioxidants and highlights the importance of HPLC analysis in characterizing their bioactive constituents. Further research may explore the therapeutic implications of these findings in the context of oxidative stress-related disorder.

CHAPTER ONE

1.1 INTRODUCTION

The presence of free radicals in the body leads to a number of clinical situations, such as aging, cell damage, inflammation, and the progression of chronic diseases like cancer, cardiovascular disorders, and neurodegenerative diseases. Free radicals are highly reactive molecules with unpaired electrons that can damage cellular components, including DNA, proteins, and lipids, thereby impairing normal cellular functions and contributing to various pathological conditions (Noreen *et al*., 2024). These harmful free radicals can be neutralized or scavenged by antioxidants, which are molecules that can donate an electron to free radicals without becoming destabilized themselves. Antioxidants are found in various drugs and plant extracts, and their intake can significantly mitigate oxidative stress and its related damages. An example of plants used for their antioxidant capacities includes *Luffa cylindrica*, *Laginora breviflora* and *Foeniculum vulgare.*

Foeniculum vulgare is an aromatic herb that has been cherished for centuries across various cultures. *Foeniculum vulgare* seeds have also found a place in traditional medicine due to their potential health benefits. *Foeniculum vulgare* is believed to have originated in the Mediterranean region and was cultivated by ancient civilizations such as the Egyptians, Greeks, and Romans. It was highly valued for its culinary and medicinal properties.

Foeniculum vulgare seeds are well-known for their culinary versatility. They impart a distinct anise-like flavor to soups, stews, and baked goods. However, their value extends far beyond the kitchen. *Foeniculum vulgare* has been used as a diuretic, expectorant, and remedy for various dyspeptic disorders (Repajić *et al.,* 2020). What makes these unassuming seeds so remarkable lies in their diverse bioactive constituents such as What makes these unassuming seeds so remarkable lies in their diverse bioactive constituents such as Flavonoids: These natural plant pigments exhibit antioxidant, anti-inflammatory, and antiviral activities. Flavonoids play a crucial role in protecting cells from oxidative damage (Castaldo *et al.,* 2015). Alkaloids: Although present in smaller quantities, alkaloids in fennel seeds may have diverse effects on human health. Their potential contributions warrant further investigation (Ghasemzadeh *et al.,* 2010). Tannins: Known for their astringent properties, tannins contribute to the overall health profile of fennel seeds. They may play a role in gastrointestinal health and beyond (Sultana *et al.,* 2009). Steroids: These compounds participate in various physiological processes. While their specific functions in fennel seeds are still being explored, their presence underscores the complexity of this humble herb (Salehi *et al.,* 2019).

The aim of this study is to investigate the phytochemical composition and antioxidant potential of *Foeniculum vulgare* seed extracts.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW

*Foeniculum vulgare (*Fennel) is a flowering plant belonging to the Apiaceae family, widely cultivated in temperate regions worldwide (Rather *et al.*, 2024). It has been utilized for centuries in traditional medicine and culinary practices due to its various therapeutic properties. Among its diverse applications, *Foeniculum vulgare* is renowned for its potential antioxidant activity attributed to its rich phytochemical composition (Melissa Petruzzello 2024). *Foeniculum vulgare* is a versatile herbaceous plant belonging to the family, commonly known as the carrot or parsley family, *Foeniculum vulgare* is native to the Mediterranean region but is now cultivated in temperate climates worldwide. Its aromatic seeds, feathery leaves, and bulbous root are all utilized for various purposes, making it a valued herb across cultures and generations.

Foeniculum vulgare is a medicinal plant known and used by humans since antiquity. It was cultivated in almost every country (Muckensturm *et al.,* 1997). It is universally known as *Foeniculum vulgare* (Fennel) and is known by more than 100 names. It is a traditional and popular herb with a long history of use as a medicine. A series of studies showed that *Foeniculum vulgare* effectively controls numerous infectious disorders of bacterial, fungal, viral, mycobacterium, and protozoal origin (Kaur and Arora, 2009; Manonmani and Abdul Khadir, 2011; Orhan *et al.,* 2012). It has antioxidant, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic, and oestrogenic activities (Ozbek *et al.,* 2003; Pradham). Some publications stated that *Foeniculum vulgare* has a special kind of memory-enhancing effect and can reduce stress (Koppula and Kumar, 2013). Animal experiments and limited clinical trials suggest that chronic use of *Foeniculum vulgare* is not harmful. *Foeniculum vulgare* may be consumed daily, in the raw form as salads and snacks, stewed, boiled, grilled, or baked in several dishes and even used in the preparation of herbal teas or spirits. A diet with desired quantity of *Foeniculum vulgare* could bring potential health benefits due to its valuable nutritional composition with respect to presence of essential fatty acids (Barros *et al.*, 2010). In recent increased interests in improvement of agricultural yield of *Foeniculum vulgare* due to its medicinal properties and essential oil content has encouraged cultivation of the plant on a large scale (Badgujar *et al.,* 2014).

Fig 2.1: *Foeniculum vulgare* tree
Source: Florence Fennel Herb Seed, 2012

2.1.1 Traditional uses and medicinal properties of Foeniculum vulgare

a. *Foeniculum vulgare* has a long history of traditional use across different cultures for its medicinal properties. *Foeniculum vulgare* has been employed to treat various ailments, including digestive disorders, anemia, flatulence, colic, respiratory issues, and menstrual problems. The medicinal properties of

Foeniculum vulgare are attributed to its rich phytochemical content, which includes volatile compounds, phenolic compounds, flavonoids, terpenoids, and other bioactive constituents. These phytochemicals exhibit diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Elghazaly *et al.*, 2019; Shahrajabian and Sun, 2023).

Foeniculum vulgare has been revered for centuries for its diverse medicinal properties and holds a prominent place as a remedy for a wide range of ailments (Rafieian *et al.*, 2023).

b. One of the most well-known uses of *Foeniculum vulgare* is for promoting digestive health. *Foeniculum vulgare* seeds are often chewed after meals to aid in the alleviation of anemia, flatulence, colic, respiratory issues, and menstrual problems, bloating, and relieve indigestion. They are believed to stimulate the production of digestive enzymes and reduce gastrointestinal discomfort (Rafieian *et al.*, 2023; Medical News Today, 2024).

c. In traditional herbal medicine, *Foeniculum vulgare* has been used to soothe respiratory issues such as coughs, bronchitis, and asthma. It is believed to possess expectorant properties, helping to loosen mucus and ease respiratory congestion (Noreen *et al.*, 2024).

d. *Foeniculum vulgare* has historically been used to alleviate symptoms of menstrual disorders such as menstrual cramps, irregular menstruation, and menopausal symptoms. Its antispasmodic and hormone-regulating properties are thought to contribute to its efficacy in this regard (Al-Okbi *et al*, 2018).

2.2 ANTIOXIDANTS

Antioxidants are the defense system of the body against the damage of reactive oxygen species, which is normally produced during the various physiological processes in the body (Anwar *et al.,* 2018).

Though oxidation reactions are crucial for life, they can also be damaging. Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases (Hamid *et al.,* 2010).

Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson’s dementia, Alzheimer’s disease, inflammatory disease, muscular dystrophy, liver disorder, and even aging (Amit and Priyadarsini 2011). Besides, there are some antioxidants in the form of micronutrients which cannot be manufactured by the body itself such as vitamin E, β-carotene, and vitamin C, and hence these must be supplemented in the normal diet (Teresa *et al.,* 2011).

Antioxidants can be classified into two major types based on their source, i.e., natural and synthetic antioxidants (Mamta *et al.,* 2014).

2.2.1 NATURAL ANTIOXIDANTS

Natural antioxidants either are synthesized in human body through metabolic process or are supplemented from other natural sources, and their activity very much depends upon their physical and chemical properties and mechanism of action. This can be further divided into two categories, enzymatic antioxidants and nonenzymatic antioxidants (Mamta *et al.,* 2014).

a. **Enzymatic Antioxidants**

Enzymatic antioxidants are uniquely produced in the human body and can be subdivided into primary and secondary antioxidant (Mamta *et al.,* 2014).

i. **Primary Antioxidants**: Primary antioxidants mainly include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as described below. Superoxide Dismutase Superoxide dismutase (SOD) enzyme is found in both the dermis and the epidermis. It removes the superoxide radical (O2.−) and repairs the body cells damaged by free radical. SOD catalyzes the reduction of superoxide anions to hydrogen peroxide. SOD is also known to compete with nitric oxide (NO) for superoxide anion, which inactivates NO to form peroxynitrite. Therefore, by scavenging superoxide anions, it promotes the activity of NO (Chakraborty *et al.,* 2009).

Catalase Catalase enzyme (CAT) is found in the blood and most of the living cells and decomposes H_2O_2 into water and oxygen. Catalase along with glucose peroxidase is also used commercially for the preservation of the fruit juices, cream consisting of egg yolk, and salad by removing the oxygen (Chakraborty *et al.,* 2009).

Glutathione Peroxidase Glutathione peroxidase (GPx) is a group of seleniumdependent enzymes, and it consists of cytosolic, plasma, phospholipid hydroperoxide, and gastrointestinal glutathione peroxidase (Chakraborty *et al.,* 2009). GPx (cellular and plasma) catalyzes the reaction of H_2O_2 by reduced glutathione (GSH); as a result, oxidized glutathione (GSSG) is produced and it is again recycled to its reduced form by glutathione reductase (GR) and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

ii. **Secondary Antioxidant**

Secondary antioxidant includes glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH). G6PDH generates NADPH. GR is required to recycle the reduced glutathione (GSH) using secondary enzyme GR and NADPH.

iii. **Non-enzymatic Antioxidants**

They are a class of the antioxidants which are not found in the body naturally but are required to be supplemented for the proper metabolism (Raygani *et al.,* 2007). Some of the known non-enzymatic antioxidants are minerals, vitamins, carotenoids, polyphenols, and other antioxidants such Minerals (Iron, Magnesium, Selenium, Copper), Vitamins (Vitamin A, Vitamin C, Vitamin E, Vitamin B), Carotenoids, Polyphenols,

2.2.2 SYNTHETIC ANTIOXIDANTS

According to Mamta *et al.,* (2014) *Synthetic* antioxidants are artificially produced or synthesized using various techniques. Basically, they are polyphenolic compounds mainly that capture the free radicals and stop the chain reactions. Polyphenolic derivatives usually contain more than one hydroxyl or methoxy group. Ethoxy quinine is the only heterocyclic, N-containing compound reported to be used as antioxidant in the food, especially animal feed. Mostly reported synthetic phenolic antioxidants are p-substituted, whereas the natural phenolic compounds are mostly o-substituted. The p-substituted substances are preferred because of their lower toxicity. Synthetic phenolic antioxidants are always substituted with alkyl groups to improve their solubility in fats and oils and to reduce their toxicity (Wanasundara and Shahidi 2005). These synthetic compounds possessing antioxidant activity are commonly used in pharmaceuticals, as preservatives for cosmetics and to stabilize the fat, oil, and lipid in food (Gupta and Sharma 2006).

2.2.3 ANTIOXIDANT ACTIVITY OF *Foeniculum vulgare*

One of the most remarkable aspects of *Foeniculum vulgare* is its potential antioxidant activity, which is attributed to its rich phytochemical composition. Antioxidants are compounds that neutralize harmful free radicals in the body, thereby protecting cells from

oxidative damage and reducing the risk of chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disorders (Rubio *et al.,* 2024; Milenković *et al.,* 2022).

The antioxidant activity of *Foeniculum vulgare* is of particular interest due to its potential health benefits. Studies have suggested that regular consumption of *Foeniculum vulgare* or its extracts may help mitigate oxidative stress, reduce inflammation, improve immune function, and protect against age-related diseases. Additionally, *Foeniculum vulgare*'s antioxidant properties may contribute to its traditional uses in promoting overall health and well-being (Al-Okbi *et al*, 2018; Medical News Today, 2024).

2.2.4 IN VITRO ASSAYS USED IN EVALUATING ANTIOXIDANT POTENTIAL OF *Foeniculum vulgare*

In vitro assays play a crucial role in evaluating the antioxidant potential of *Foeniculum vulgare*. These laboratory-based experiments colloquially known as “test-tube experiments” and are typically performed with lab wares such as test tubes, flasks, Petri dishes, and micro titer plates.

2.2.4.1 Total Antioxidant Capacity (TAC)

Total Antioxidant Capacity (TAC) assay measures the overall ability of a test solution to scavenge free radicals. It evaluates the antioxidant capacity of biological samples by assessing their ability to counteract oxidative damage caused by free radicals. TAC quantifies how effectively a sample can neutralize these harmful molecules. TAC assays are widely used to assess the overall antioxidant capacity of various materials, including food products. These assays aim to measure the cumulative antioxidant actions of multiple compounds within a complex matrix (Sadowska and Bartosz, 2022; Chelliah *et al.,* 2022).

TAC assays can be classified into two main types which are direct assays and indirect assays. The direct assays directly measure the ability of a sample to inhibit the oxidation of a substance. Trolox Equivalent Antioxidant Capacity (TEAC) is a commonly used direct assay, TEAC assesses antioxidant capacity by comparing it to the synthetic antioxidant Trolox. It involves inhibiting the oxidation of a substance over a specified period of time. Another direct assay is Oxygen Radical Absorbance Capacity (ORAC) that quantifies antioxidant capacity by measuring the inhibition of oxidation (Sadowska and Bartosz, 2022; Chelliah *et al.,* 2022).

The indirect assays indirectly evaluate antioxidant capacity based on the sample's ability to reduce a metal complex. Ferric Reducing Ability of Plasma (FRAP) measures the reducing capacity of a sample by assessing its ability to reduce ferric ions. Cupric Reducing Antioxidant Capacity (CUPRAC) determines antioxidant capacity by evaluating the sample's ability to reduce cupric ions (Sadowska and Bartosz, 2022; Chelliah *et al.,* 2022).

2.2.4.2 Ferric Reducing Antioxidant Power (FRAP)

Ferric Reducing Ability of Plasma (FRAP): This assay measures the antioxidant capacity of plasma. It assesses the ability of antioxidants in the plasma to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), using Trolox as a standard. This assay was first performed by Iris Benzie and J. J. Strain (Benzie and Strain, 1996).

It's based on the reduction of a ferric tripyridyltriazine (Fe^3±TPTZ) complex to a ferrous form (Fe^2±TPTZ) at low pH, which results in an intense blue color that can be measured spectrophotometrically (Benzie and Strain, 1996).

FRAP assay procedure includes:

- Preparation of the FRAP Reagent: This involves mixing acetate buffer, TPTZ solution, and ferric chloride solution.
- Reaction: The sample (such as plasma) is mixed with the FRAP reagent and incubated at room temperature.
- Measurement: After a certain time, the absorbance of the blue complex is measured at a specific wavelength (593 nm). This color change is due to the presence of antioxidants in the sample that reduce Fe^{3+} to Fe^{2+}.
- Quantification: The antioxidant power is quantified by comparing the absorbance change to that of a standard, usually Trolox, which is a vitamin E analog.

The result is often expressed in terms of Trolox equivalents, providing a measure of the sample's antioxidant capacity. This assay is particularly useful for assessing the antioxidant content in foods, beverages, and nutritional supplements, especially those containing polyphenols (Benzie and Strain, 1996; Gonzalez - Rivera *et al.,* 2018)

2.2.4.3 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH assay, or 2,2-diphenyl-1-picrylhydrazyl assay, is a widely used method to assess the antioxidant capacity of substances. It measures the ability of a sample to neutralize free radicals by donating hydrogen atoms (Sirivibulkovit *et al.*, 2018)

DPPH assay involves:

- Preparation: A DPPH solution is prepared in a solvent, usually methanol or ethanol, creating a deep violet-colored solution.

- Reaction: The sample being tested for antioxidant activity is added to the DPPH solution.
- Incubation: The mixture is left to stand for a period, during which antioxidants in the sample react with the DPPH.
- Color Change: The DPPH radical is reduced by the antioxidants, leading to a color change from violet to pale yellow or colorless.
- Measurement: The change in color is quantified by measuring the absorbance at 517 nm using a spectrophotometer. The decrease in absorbance indicates the scavenging ability of the antioxidant compounds in the sample.
- Results: The results are often expressed as IC50, which is the concentration of the sample required to inhibit 50% of the DPPH radicals.

The DPPH assay is popular due to its simplicity, speed, and the fact that it does not require complex instrumentation. It's a valuable tool for screening the radical scavenging activity of natural products, food extracts, and new compounds (Sharma and Bhat, 2009)

2.2.4.4 Nitric Oxide Scavenging Ability

The Nitric Oxide Scavenging Ability Assay is a method used to evaluate the antioxidant activity of substances by measuring their capacity to scavenge nitric oxide (NO) radicals. Nitric oxide is a reactive nitrogen species that plays a role in various physiological processes but can also contribute to oxidative stress and cellular damage when produced in excess (Apak *et al.*, 2022)

The assay procedure includes:

- Sodium Nitroprusside Reaction: Sodium Nitroprusside in aqueous solution at physiological pH generates nitric oxide, which reacts with oxygen to form nitrite ions.

- Scavenging Activity: Antioxidants in the test sample compete with oxygen, leading to a reduction in nitrite ion formation.
- Griess Reagent: The nitrite ions produced are then quantified using Griess reagent, which reacts with nitrite to form a pink-colored compound.
- Measurement: The absorbance of this compound is measured at 546 nm using a UV-visible spectrophotometer.
- Calculation: The total scavenging activity is calculated based on the difference in absorbance between the control (without antioxidants) and the test sample.

The assay is significant for screening natural products, food extracts, and pharmaceuticals for their potential to mitigate oxidative stress by neutralizing nitric oxide radicals (Chelliah *et al*., 2022; Gulcin and Alwasel 2023).

2.3 PHYTOCHEMICALS

Phytochemicals are naturally found in plants and have disease-preventing functions. They have an important role in preventing chronic diseases. They act as synergistic agents allowing nutrients to be used more effectively by the body. The beneficial medicinal effect can be used as therapeutic agents against many communicable and non-communicable diseases. In general, these compounds are defined as chemicals produced by vegetable organisms (Varghese *et al.,* 2022).

When consumed in the diet, phytochemicals may reduce the risk of age-related chronic diseases such as coronary heart disease, diabetes, high blood pressure and certain types of cancer. Although, their absence from the diet might not cause deficiency symptoms, such as those found with vitamins and minerals, they are thought to be important for health and wellbeing throughout life, especially in adulthood and in the elderly. (Molyneux *et al*, 2007).

Phytochemicals are thought to act as synergistic agents, allowing nutrients to be used more efficiently by the body. Synergy is the working together of two things (food components, for example) to produce an effect greater than the sum of their individual effects. (Molyneux *et al.*, 2007).

2.3.1 TYPES OF PHYTOCHEMICALS

2.3.1.1 Flavonoids:

Flavonoids are a diverse group of phytochemicals found in various plant-based foods, including fruits, vegetables, grains, herbs, and beverages. They contribute to the vibrant colors of many fruits and vegetables and are associated with numerous health benefits (Kromidas, 2008).

Flavonoids are abundant in a wide range of plant foods. Common sources include: Citrus fruits (oranges, lemons, grapefruits), berries (strawberries, blueberries, raspberries), apples, grapes, cherries, Onions, kale, spinach, broccoli, bell peppers, tomatoes, Whole grains such as buckwheat, barley, and oats, Herbs and Spices such as Parsley, thyme, celery, peppermint, Tea (especially green tea), red wine, cocoa. (Kromidas, 2008; Tsanova-Savova *et al.*, 2018)

According to (Shen *et al.*, 2021), Flavonoids are classified into several subclasses, each with its unique chemical structure and health benefits. Common subclasses include:

- Flavonols: Quercetin, kaempferol, myricetin.
- Flavones: Apigenin, luteolin.
- Flavanones: Hesperidin, naringenin.
- Flavanols (Catechins): Epicatechin, epigallocatechin gallate (EGCG).
- Anthocyanins: Cyanidin, delphinidin, pelargonidin.

The health benefits of Flavonoids include;

- Antioxidant Properties: Flavonoids are potent antioxidants that help neutralize harmful free radicals in the body, reducing oxidative stress and protecting cells from damage (Panche *et al.,* 2016)
- Anti-Inflammatory Effects: Some flavonoids possess anti-inflammatory properties, which can help alleviate inflammation and related conditions. (Panche *et al.,* 2016)
- Cardiovascular Health: Flavonoids may support heart health by improving blood flow, reducing blood pressure, and preventing the oxidation of LDL cholesterol, thus reducing the risk of cardiovascular diseases. (Panche *et al.,* 2016)
- Cancer Prevention: Certain flavonoids have been studied for their potential anti-cancer effects, including inhibiting the growth of cancer cells and promoting apoptosis (cell death) in cancerous cells. (Panche *et al.,* 2016)
- Neuroprotective Effects: Flavonoids may help protect against age-related cognitive decline and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. (Panche *et al.,* 2016)
- Skin Health: Some flavonoids, particularly flavonols and anthocyanins, have been shown to benefit skin health by protecting against UV-induced damage and promoting collagen synthesis. (Panche *et al.,* 2016).

2.3.1.2 Carotenoids

Carotenoids are a group of phytochemicals responsible for the vibrant colors of many fruits and vegetables. They are a subclass of terpenoids and are known for their antioxidant properties and potential health benefits. (Landrum, 2009; Cazzonelli, 2011) Carotenoids are found in various plant-based foods, particularly those with bright red, orange, and yellow hues. Common sources include - Orange and yellow fruits: Carrots, sweet potatoes, apricots, mangoes, oranges; Red fruits and vegetables: Tomatoes, red peppers, watermelon, pink grapefruit; Leafy greens: Spinach, kale, collard greens, Swiss chard; Other vegetables: Pumpkins, squash, corn; Some algae and microorganisms also contain carotenoids. (Zeb and Mehmood, 2004)

There are over 600 different types of carotenoids found in nature, but the most common and well-studied ones include:

- Beta-carotene: Found in carrots, sweet potatoes, and leafy greens. It is converted into vitamin A in the body. (Cazzonelli, 2011)
- Alpha-carotene: Found in carrots, pumpkins, and winter squash. It also converts to vitamin A. (Cazzonelli, 2011)
- Lycopene: Found in tomatoes, watermelon, pink grapefruit. It is responsible for their red color. (Cazzonelli, 2011)
- Lutein and zeaxanthin: Found in leafy greens, corn, and egg yolks. They are essential for eye health. (Cazzonelli, 2011)

Health benefits of Carotenoids include;

- Antioxidant Properties: Carotenoids act as antioxidants, neutralizing harmful free radicals in the body and protecting cells from oxidative damage.
- Vision Health: Lutein and zeaxanthin are particularly important for eye health. They accumulate in the retina and help protect against age-related macular degeneration and cataracts.
- Skin Health: Carotenoids may provide some protection against UV-induced skin damage and contribute to a healthy complexion.
- Immune Support: Some carotenoids, such as beta-carotene, are involved in supporting the immune system and may help reduce the risk of infections.

- Heart Health: Certain carotenoids, such as lycopene, have been associated with a reduced risk of heart disease by helping to lower blood pressure and improve cholesterol levels.
- Cancer Prevention: Some studies suggest that diets rich in carotenoid-containing foods may lower the risk of certain cancers, although more research is needed in this area. (Cazzonelli, 2011; Zeb and Mehmood, 2004)

2.3.1.3 Phenolic Acids:

Phenolic acids are a group of phytochemicals found in various plant-based foods, known for their antioxidant properties and potential health benefits (Hoda *et al.,* 2019).

Phenolic acids are widely distributed in fruits, vegetables, whole grains, nuts, seeds, and beverages such as coffee and tea. Common dietary sources include berries (blueberries, strawberries), citrus fruits (oranges, lemons), apples, grapes, coffee beans, cocoa beans, whole grains (wheat, oats), nuts (almonds, walnuts), and certain herbs and spices (thyme, rosemary) (Hoda *et al.,* 2019; Abdesslem *et al.*, 2022).

Phenolic acids are classified into two main groups: hydroxybenzoic acids and hydroxycinnamic acids, based on their chemical structure (Hamad, 2022).

- Common hydroxybenzoic acids include gallic acid, protocatechuic acid, and ellagic acid
- Common hydroxycinnamic acids include caffeic acid, ferulic acid, and p-coumaric acid. (Hamad, 2022).

The health benefits of Phenolic acid include;

- Antioxidant Activity: Phenolic acids exhibit strong antioxidant properties, helping to neutralize harmful free radicals and reduce oxidative stress in the body. This antioxidant activity may contribute to the prevention of chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders (Kaurinovic and Vastag, 2019).

- Anti-Inflammatory Effects: Some phenolic acids possess anti-inflammatory properties, which can help alleviate inflammation and related conditions such as arthritis and inflammatory bowel diseases (Robbins, 2003).
- Cardiovascular Health: Certain phenolic acids, such as caffeic acid and ferulic acid, have been linked to improved heart health by reducing inflammation, improving blood vessel function, and lowering blood pressure and cholesterol levels (Rahman *et al.,* 2022).
- Cancer Prevention: Phenolic acids, particularly ellagic acid and ferulic acid, have been studied for their potential anti-cancer effects, including inhibiting the growth of cancer cells and inducing apoptosis (cell death) in cancerous cells (Lin *et al.,* 2016).
- Neuroprotective Effects: Some phenolic acids may help protect against age-related cognitive decline and neurodegenerative diseases by reducing oxidative stress and inflammation in the brain (Kiokias and Oreopoulou, 2021).

2.3.1.4 Isoflavones

Isoflavones are a subgroup of flavonoids, phytochemicals found predominantly in soybeans and soy products. They have gained attention due to their potential health benefits, particularly in promoting hormonal balance and reducing the risk of certain chronic diseases (Harborne, 1989).

Isoflavones are primarily found in soybeans and soy-based products, including tofu, tempeh, soy milk, soy protein isolates, and edamame (Harborne, 1989). Other legumes such as chickpeas, lentils, and peanuts also contain smaller amounts of isoflavones, although soybeans are the richest dietary source (Harborne, 1989).

The main isoflavones found in soybeans are genistein, daidzein, and glycitein. Genistein is the most abundant isoflavone in soy products, followed by daidzein (Manayi, 2021).

The health benefits of isoflavones includes;

- Phytoestrogenic Effects: Isoflavones are classified as phytoestrogens due to their structural similarity to the hormone estrogen. They can mimic and modulate estrogenic activity in the body, leading to potential health effects (Harborne, 1989).
- Menopausal Symptom Relief: Isoflavones have been studied for their potential to alleviate menopausal symptoms such as hot flashes, night sweats, and mood swings. They may act as weak estrogen agonists in postmenopausal women, helping to compensate for declining endogenous estrogen levels (Harborne, 1989).
- Bone Health: Some studies suggest that isoflavones may have a protective effect on bone health, potentially reducing the risk of osteoporosis by promoting bone formation and inhibiting bone resorption (Harborne, 1989).
- Cardiovascular Health: Isoflavones may contribute to cardiovascular health by improving lipid profiles, reducing LDL cholesterol levels, and inhibiting platelet aggregation, thus lowering the risk of heart disease (Harborne, 1989).
- Cancer Prevention: Isoflavones have been investigated for their potential anti-cancer effects, particularly in hormone-related cancers such as breast and prostate cancer. They may exert anti-cancer properties through various mechanisms,
- including estrogen receptor modulation, antioxidant activity, and inhibition of cancer cell proliferation (Harborne, 1989).

2.3.1.5 Sulfur Compounds

Sulfur compounds are a group of phytochemicals found in various plant foods, particularly in members of the Allium genus (such as garlic, onions, leeks, and chives) and cruciferous vegetables (such as broccoli, cabbage, cauliflower, and Brussels sprouts). These compounds are responsible for the pungent odor and taste characteristic of these foods (Ramirez *et al.,* 2017; Khandagale *et al.*, 2020; Fike *et al.,* 2015).

Sulfur compounds are abundant in allium vegetables, including garlic, onions, leeks, shallots, and chives. These vegetables contain organosulfur compounds such as allicin, diallyl sulfide, diallyl disulfide, and allyl mercaptan (Upadhyay, 2017).

Cruciferous vegetables like broccoli, cabbage, cauliflower, Brussels sprouts, kale, and collard greens also contain sulfur compounds, including glucosinolates and isothiocyanates (Upadhyay, 2017).

The various types include;

- Allium Compounds: Allium vegetables contain sulfur-containing compounds such as allicin, which is responsible for the characteristic odor and taste of garlic. Other sulfur compounds found in allium vegetables include diallyl sulfide, diallyl disulfide, and allyl mercaptan (Xiuxiu *et al.,* 2021).
- Glucosinolates and Isothiocyanates: Cruciferous vegetables contain glucosinolates, which are sulfur-containing compounds that give these vegetables their distinctive flavor. When cruciferous vegetables are chopped, chewed, or digested, glucosinolates are broken down into biologically active compounds called isothiocyanates, such as sulforaphane and indole-3-carbinol (Xiuxiu *et al.,* 2021).

The various health benefits of Sulfur compounds include;

- Antioxidant Properties: Sulfur compounds, particularly those found in allium and cruciferous vegetables, exhibit antioxidant properties, helping to neutralize free radicals and reduce oxidative stress in the body (Bastaki *et al.,* 2021).
- Anti-Inflammatory Effects: Some sulfur compounds have been shown to possess anti-inflammatory properties, which may help reduce inflammation and inflammation-related diseases such as arthritis and inflammatory bowel diseases (Caroline *et al.,* 2023).
- Cardiovascular Health: Certain sulfur compounds, such as allicin in garlic, have been studied for their potential cardiovascular benefits, including lowering blood pressure, reducing cholesterol levels, and improving blood vessel function (Bastaki *et al.,* 2021).
- Immune Support: Sulfur compounds may support immune function and help defend against infections due to their antimicrobial properties (Caroline *et al.,* 2023).
- Cancer Prevention: Some sulfur compounds, particularly those found in cruciferous vegetables like sulforaphane, have been investigated for their potential anti-cancer effects. They may help inhibit the growth of cancer cells, induce apoptosis (cell death) in cancerous cells, and prevent tumor formation (Bastaki *et al.,* 2021).

2.3.1.6 Terpenes

Terpenes are a large and diverse group of organic compounds found in many plants, particularly aromatic plants and herbs. They are responsible for the characteristic aromas and flavors of various botanicals and are widely used in perfumery, flavoring, and alternative medicine (Brahmkshatriya and Brahmkshatriya, 2013; Cock, 2023).

Terpenes are found in abundance in the essential oils of many plants, including herbs, spices, fruits, flowers, and certain trees.

Common dietary sources of terpenes include aromatic herbs and spices such as basil, rosemary, thyme, oregano, mint, cinnamon, and ginger. Fruits like citrus fruits (lemons, oranges), mangoes, and pineapples also contain terpenes, as do flowers like lavender and chamomile (Noriega, 2021).

Terpenes are classified based on the number of isoprene units they contain, with isoprene being a basic building block of these compounds (Noriega, 2021; Vedantu, 2024). Common types of terpenes include:

- Monoterpenes: Contain two isoprene units and are found in citrus fruits, conifers, and many herbs and spices (Noriega, 2021; Vedantu, 2024).
- Sesquiterpenes: Contain three isoprene units and are found in herbs, spices, and certain flowers (Noriega, 2021; Vedantu, 2024).
- Diterpenes: Contain four isoprene units and are found in certain trees, resins, and herbs (Noriega, 2021; Vedantu, 2024).
- Triterpenes: Contain six isoprene units and are found in some fruits, herbs, and medicinal plants (Noriega, 2021; Vedantu, 2024).
- Tetraterpenes: Contain eight isoprene units and are primarily found in pigmented plants like tomatoes and carrots (Noriega, 2021; Vedantu, 2024).

The various health benefits of terpenes include;

- Antioxidant Properties: Many terpenes exhibit antioxidant activity, helping to neutralize free radicals and protect cells from oxidative damage (Del Prado-Audelo *et al.,* 2021; Baccouri and Rajhi, 2021).
- Anti-Inflammatory Effects: Some terpenes possess anti-inflammatory properties, which can help reduce inflammation and related conditions such as arthritis and

inflammatory bowel diseases (Del Prado-Audelo *et al.,* 2021; Baccouri and Rajhi 2021).

- Antimicrobial Activity: Certain terpenes have antimicrobial properties, helping to inhibit the growth of bacteria, fungi, and other microorganisms (Del Prado-Audelo *et al.,* 2021; Baccouri and Rajhi 2021).
- Mood Enhancement: Some terpenes, particularly those found in aromatic plants like lavender and citrus fruits, have mood-enhancing effects and may help reduce stress, anxiety, and depression (Del Prado-Audelo *et al.,* 2021; Baccouri and Rajhi 2021).
- Potential Anti-Cancer Effects: Certain terpenes, such as limonene found in citrus fruits, have been studied for their potential anti-cancer effects, including inhibiting the growth of cancer cells and inducing apoptosis (cell death) in cancerous cells (Del Prado-Audelo *et al.,* 2021; Baccouri and Rajhi 2021).

2.3.2. PHYTOCHEMICAL ACTIVITIES IN THE HUMAN BODY

Antioxidant - Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals with antioxidant activity includes allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes) (Phytochemicals, 2024).

Hormonal action - Isoflavones, found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis (Phytochemicals, 2024).

Stimulation of enzymes - Indoles, which are found in cabbages, stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries) (Phytochemicals, 2024).

Interference with DNA replication - Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens (Phytochemicals, 2024).

Anti-bacterial effect - The phytochemical allicin from garlic has anti-bacterial properties (Phytochemicals, 2024).

Physical action - Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health (Phytochemicals, 2024).

2.3.3 *PHYTOCHEMICAL COMPOSITION OF* Foeniculum vulgare *SEEDS*

Foeniculum vulgare is a veritable treasure trove of phytochemicals, including volatile compounds, phenolic compounds, flavonoids, terpenoids, and other bioactive constituents. These phytochemicals possess diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Šunić *et al.,* 2023).

Foeniculum vulgare seeds are particularly rich in phytochemicals, which contribute to their therapeutic properties. The major phytochemical constituents found in *Foeniculum vulgare* seeds include:

Volatile compounds: *Foeniculum vulgare* seeds contain volatile oils rich in compounds such as trans-anethole, fenchone, estragole, limonene, and α-pinene. These volatile compounds contribute to the characteristic aroma and flavor of *Foeniculum vulgare* and possess various pharmacological properties.

Phenolic compounds: *Foeniculum vulgare* seeds are a rich source of phenolic compounds, including flavonoids (e.g., quercetin, kaempferol) and phenolic acids (e.g., caffeic acid, chlorogenic acid). These phenolic compounds exhibit antioxidant activity and contribute to its overall health benefits.

Terpenoids: *Foeniculum vulgare* seeds contain terpenoid compounds such as α-pinene, β-pinene, myrcene, and limonene. These terpenoids possess antioxidant, anti-inflammatory, and antimicrobial properties.

Other bioactive constituents: *Foeniculum vulgare* seeds also contain other bioactive compounds, including coumarins, saponins, and sterols, which contribute to their medicinal properties.

The phytochemical composition of *Foeniculum vulgare* seeds can vary depending on factors such as cultivar, growing conditions, and extraction method. Analytical techniques such as HPLC play a crucial role in identifying and quantifying these phytochemicals (Milenković *et al.,* 2022; Barakat *et al.,* 2022; Hamada *et al.,* 2023).

2.4 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC, formerly known as high-pressure liquid chromatography, is a versatile technique widely employed in analytical chemistry. It allows scientists to separate, detect, and quantify individual components in various sample mixtures, including those from food, chemicals, pharmaceuticals, biological, environmental, and agricultural sources. The method relies on the interaction between a mobile phase (a solvent mixture) and a stationary phase (solid particles within a column) to achieve separation (Lozano-Sánchez *et al.,* 2018).

HPLC key principle exploits the partitioning behavior of sample components between the mobile and stationary phases. The column contains particles coated with the stationary phase material (e.g., silica or polymers). Each component interacts differently with these particles, leading to distinct migration rates. Components with higher affinity for the stationary phase exhibit longer retention times within the column. Conversely, those more attracted to the mobile phase flow out faster. The resulting output of an HPLC analysis is a chromatogram—a graphical representation of signal intensity versus time or volume. Peaks in the chromatogram correspond to individual sample components, with each peak's area proportional to its concentration (Chemistry Views, 2016; Lozano-Sánchez, 2018).

The instrumentation of HPLC includes High-pressure pump (delivers the mobile phase at uniform pressure typically 500-5000 psi), Column (packed with solid particles coated with the stationary phase), Detector (monitors the eluting components e.g UV detectors), Data Acquisition System (Records the chromatogram). HPLC is used during the production of pharmaceuticals and biological products, it helps detect performance-enhancing drugs in urine samples, for separating complex biological samples or synthetic chemicals, and also to determine vitamin D levels in blood serum (Horváth, 2013; Malviya *et al.*, 2022)

2.4.1 Principles and Applications of HPLC in Phytochemical Analysis

High-performance liquid chromatography (HPLC) is a powerful analytical technique widely used for the separation, identification, and quantification of phytochemicals in plant extracts. HPLC operates based on the principles of liquid chromatography, wherein a liquid mobile phase is passed through a stationary phase packed into a column. The components in the sample interact differently with the stationary phase, leading to separation based on their physicochemical properties.

HPLC offers several advantages for phytochemical analysis, including high sensitivity, selectivity, accuracy, and reproducibility. It allows for the simultaneous analysis of multiple phytochemicals in complex plant extracts, making it an indispensable tool for studying the phytochemical composition of medicinal plants such as *Foeniculum vulgare*. (Odeh and Abdul, 2017)

2.4.2. HPLC Analysis of Phytochemicals in Foeniculum vulgare

Numerous studies have employed HPLC techniques to analyze the phytochemical composition of *Foeniculum vulgare* seeds. These studies have identified and quantified various classes of phytochemicals present in *Foeniculum vulgare* seeds, including volatile compounds, phenolic compounds, terpenoids, and other bioactive constituents.

For example, a study by Noreen *et al.,* (2023) compared flaxseed and *Foeniculum vulgare* seeds for their nutritional composition, bioactive moieties, and antioxidant activity. HPLC was used to analyze the phytochemical composition of the *Foeniculum vulgare* seeds. Notable findings included the identification of trans-anethole as the major volatile compound in *Foeniculum vulgare* seeds, along with other minor components such as fenchone and estragole.

Another study by Barakat *et al.,* (2022) investigated the phenolic and volatile composition of *Foeniculum vulgare* seeds using HPLC and identified the presence of compounds like trans-anethole, 2-pentanone, and benzaldehyde-4-methoxy. Other studies include (Bukhari *et al.,* 2014; Noreen *et al.,* 2023).

These studies highlight the versatility of HPLC in analyzing the phytochemical composition of *Foeniculum vulgare* seeds and provide valuable insights into their chemical constituents.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 MATERIALS

- *Foeniculum vulgare* (Fennel) seeds
- Distilled water
- Ethanol
- Chemical reagents (DPPH, FRAP reagents, Griess reagent, phosphate buffer saline, Folin-Ciocalteu reagent, hydrogen peroxide, sulfuric acid, adrenaline solution, pyrogallol, thiobarbituric acid)
- Solvents for HPLC analysis (acetonitrile, methanol, water)
- Glassware (volumetric flasks, beakers, graduated cylinders, syringes, vials, test tubes, pipettes, conical flasks, HPLC vials, inserts)
- Laboratory equipment (spectrophotometer, HPLC system, centrifuge, water bath)
- Standard reference compounds (ascorbic acid, quercetin)

3.1.1 Collection of plant sample

The seeds of *Foeniculum vulgare* were obtained 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 then deposited at the Department Herbarium with a voucher number UBH – F665.

3.2 ANTIOXIDANT PROCEDURE

3.2.1 Nitric oxide radical scavenging assay

Nitric oxide (NO) scavenging ability was determined using Griessllosvoy reagent and is based on the principle that at physiological pH sodium nitroprusside produces nitric oxide in aqueous solution and this interact with O_2 to produce NO_3^-. Scavengers of NO compete with O_2 resulting in decreased production of NO (Marcocci *et al*., 1994). Sodium nitroprusside (10 millimole per liter) in phosphate buffer saline was then mixed with varying concentrations of the extract and incubated at 25 °C for 150 minutes. The samples were added to Griess reagent (1percent sulphanilamide, 2 percent H_3PO_4 and 0.1 percent napthylethylenediamine dihydrochloride). The absorbance was determined at 540 nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Griess reagent as a positive control. The percentage of inhibition was calculated using the formula below:

$$Radical\ scavenging\ activity(\%) = \left(\frac{A\ control - A\ test}{Acontrol}\right) \times 100$$

Where $A_{control}$ is the absorbance of the control (without extract) and A_{test} is the absorbance in the presence of the extract/standard.

3.2.2 DPPH radical scavenging assay

A modified procedure as described by Jain *et al*. (2008) was used to estimate the DPPH radical scavenging ability of the extracts. A solution of 0.1 millimolar DPPH in methanol was prepared and 0.1milliliter of this solution was mixed with 3.0 milliliter of extracts in methanol containing 0.01-0.2 milligram/milliliter of the extract. The reaction mixture was thoroughly mixed and left for 30 minutes in a dark area. The absorbance of the mixture was read at 517 nm using a spectrophotometer. Ascorbic acid served as the standard.

$$DPPH\ radical\ scavenging\ activity(\%) = \left[\left(\frac{A0 - A1}{A1}\right)\right] \times 100$$

Where A_0 was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract or standard.

3.2.3 Ferric ion Reducing Antioxidant Power (FRAP) Assay

A modified procedure of Benzie and Strain (1996) was utilized for the ferric reducing antioxidant power (FRAP) assay and the principle is based on the ability of the extracts to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)- TPTZ) at low pH. The deep blue colour of Fe (II)-TPTZ is read spectrophometrically at 593 nm. 1.5 milliliter of freshly prepared FRAP solution (25 milliliter of 300 millimolar acetate buffer pH 3.6, 2.5 milliliter of 10 millimolar 2,4,6-tripyridyls- triazine (TPTZ) in 40 millimolar Hydrochloric acid, and 2.5 milliliter of 20 millimolar ferric chloride solution was mixed with 1milliliter of the extracts at concentration of 1.0 milligram per ml. The reaction mixtures were incubated at 37 °C for 30 minutes and absorbance was measured at 593 nm. FeSO4 was used for the calibration curve and ascorbic acid was used as the positive control. FRAP values (expressed as mg Fe (II) per gram of the extract) and those of the extracts were then extrapolated from the standard curve.

3.2.4 Reducing power assay

The method of Lai *et al*. (2001) was used to determine the reducing power of the extracts. 1 milliliter of varying concentrations of extracts (0.1-1.0 milligram per milliliter) in water was mixed with 2.5 milliliter of 0.2molar phosphate buffer, pH 6.6 and 2.5 milliliter of 1 percent potassium ferricyanide. After incubating at 50^{O}C for 20 minutes, 2.5 milliliter of trichloroacetic acid (10 percent) was added to stop the reaction. 2.5 milliliter of distilled water and 0.5 milliliter of 0.1percent FeCl3 were then added and the absorbance determined spectrometrically at 700 nm. Higher absorbance values indicated higher reducing power. Vitamin C was used as a positive control.

3.2.5 Hydroxyl free radical scavenging activity

The modified procedure of Chen *et al*. (2013) was utilized to determine the hydroxyl radical scavenging ability of the extracts. A reaction mixture containing 1 milliliter of 1, 10-phenanthroline (0.75 millimolar), 1.5 milliliter of 0.75 millimolar $FeSO_4$ and 3.8 milliliter of 0.2 molar Phosphate buffered solution (PH 7.4) was mixed with 1milliliter of sample extracts (100-500microgram per ml) and 1milliliter of 0.01 percent (volume per volume) H_20_2 and the volume was made up to 10 milliliter with distilled water., and the absorbance was measured at 536 nm after incubation at 37 ºC for 60 minutes. The scavenging effect was calculated using the following equation:

$$Scavenging\ effect(\%) = \left(\frac{A2 - A1}{A0 - A1}\right) \times 100$$

Where A_2 and A_1 are the absorbance with or without sample and A_0 is the absorbance without sample and H_2O_2.

CHAPTER FOUR

4.1 RESULTS

The below charts and tables represent the results on the analysis carried out on *Foeniculum vulgare*. The charts and tables provided shows the FRAP, TAC, DPPH, Hydroxyl and Nitric Acid values of *Foeniculum vulgare* (sample E), and Ascorbic acid (ASC) which was used as a control and analysed under different wavelength in triplicates (Abs1, Abs2, Abs3).

Figure 4.1 below shows the DPPH value of the *Foeniculum vulgare* sample and its control. The provided data includes values at various concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/mL) and corresponding values for two parameters: ‘E’ (*Foeniculum vulgare*) and ‘ASC’ (Ascorbic acid used as a standard).

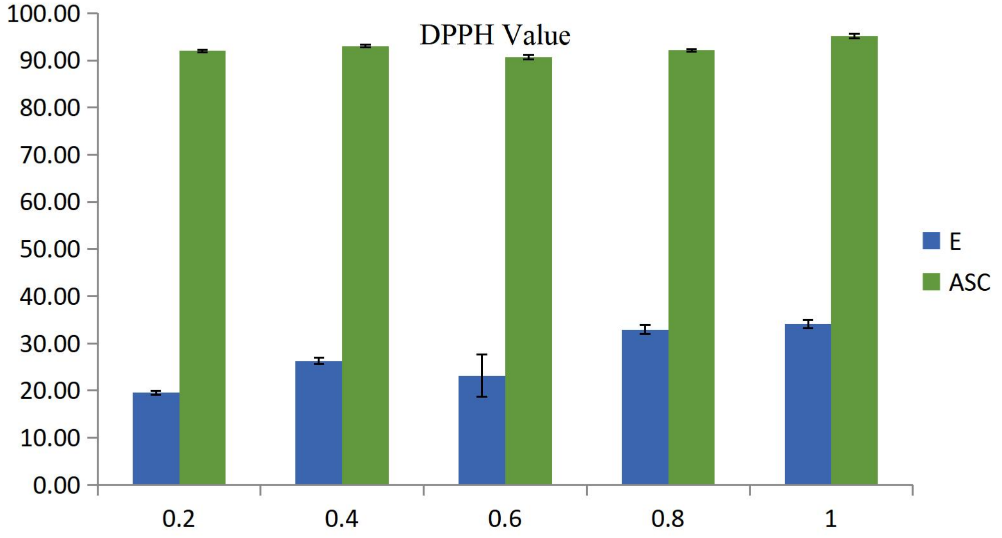

Figure 4.1: Chart showing the DPPH value of samples E and ASC. The samples were tested at different concentrations (0.2, 0.4, 0.6, 0.8, 1).

Table 4.1 below shows the IC50 values, which represent the concentration of the extract required to inhibit 50% of DPPH radicals.

The sample E shows an IC50 value of 0.72 μM, indicating potent antioxidant activity and this suggests that the fennel seed extract contains a diverse array of antioxidants with varying scavenging capacities.

Sample ASC shows an IC50 value of 1.27 μM, indicating considerable antioxidant activity. While slightly higher than samples E, it still falls within a range suggesting significant scavenging capacity.

The analysis shows that fennel extracts exhibits good DPPH radical scavenging activity and this agrees with the findings of Bano *et al.*, (2016). and Tanveer *et al.,* 2024.

Sample	**IC50 Values**
E	0.72
ASC (Control)	1.27

Table 4.1: Table showing the DPPH IC50 values of sample E and ASC

Figure 4.2 below shows the hydroxyl radical scavenging activity of sample E and the standard antioxidant (ASC) at different concentrations (0.2, 0.4, 0.6, 0.8, and 1) showing increase in scavenging activity as concentration increases. Sample E is shown to exhibit higher absorbance values than the ASC. This suggests that sample E has stronger hydroxyl radical scavenging activity compared to the standard antioxidant.

These findings agree with the study on Assessment of Antioxidant Activity of *Foeniculum vulgare* Seed Extract Using Fenton Reaction carried out by Ankita Singh and Wasim Raja (2020) which demonstrated that *Foeniculum vulgare* extract possess hydroxyl radical scavenging activity.

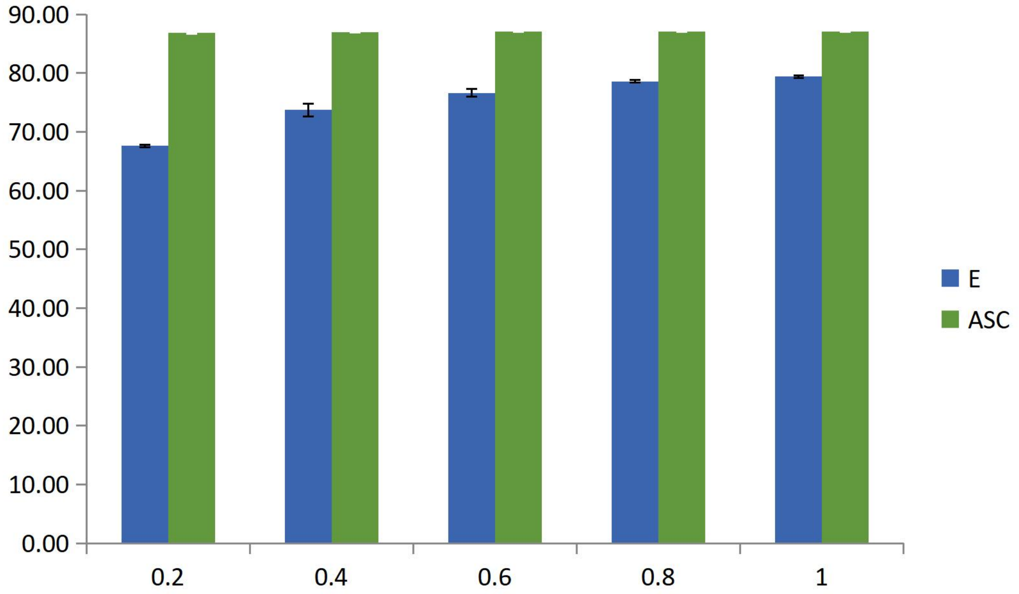

Figure 4.2: Chart showing the Hydroxyl value of sample E and control ASC

Table 4.2 below shows sample E exhibits a lower IC50 value than the standard antioxidant, suggesting that it has stronger inhibitory activity against the relevant biological process.

Table 4.2: Table showing Hydroxyl IC50 Value of sample E and ASC

Sample	IC50 VALUES
E	0.29
ASC	0.40

Figure 4.3 below shows the Nitric acid values of sample E and ASC. The figure shows the Nitric Acid assay and evaluates the extract's ability to inhibit nitric oxide production, which is associated with inflammation and oxidative stress. The provided data includes absorbance values for E and ASC at different concentrations (0.2, 0.4, 0.6, 0.8, and 1).

As the concentration increases, the absorbance generally decreases. This suggests that sample E has nitric acid scavenging activity, and its effectiveness decreases as the concentration rises.

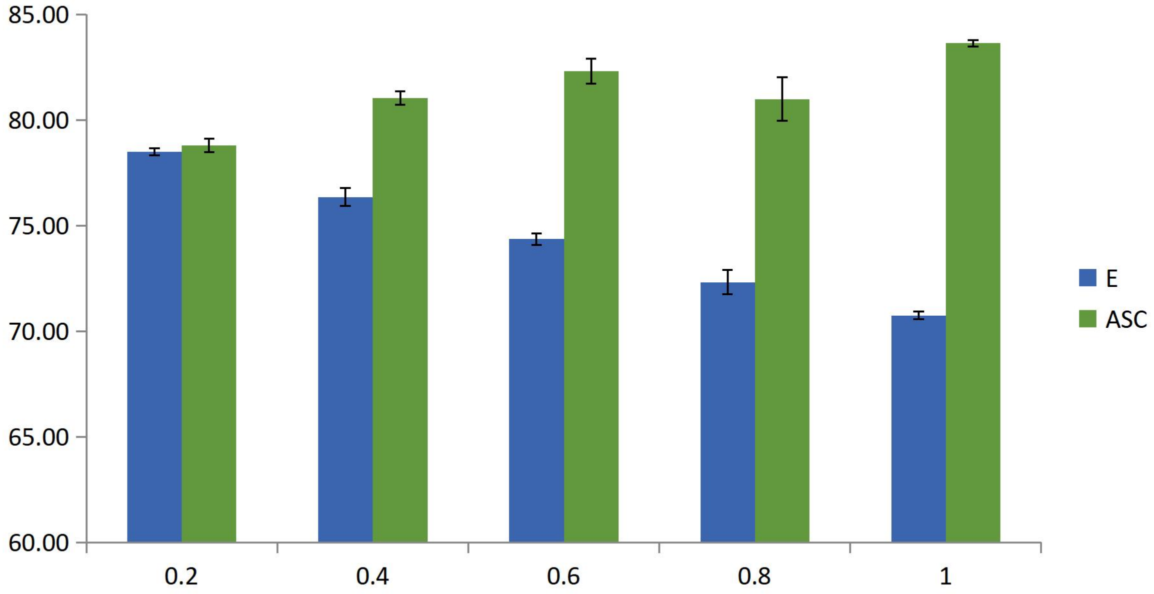

Figure 4.3: Chart showing Nitric acid value of sample E and ASC

Table 4.3: The IC50 represents the Nitric Acid concentration required to inhibit 50% of a specific biological activity (in this case, scavenging of DPPH radicals by nitric acid is 1.16). The findings align with those reported by Hamada *et al.,* (2023), suggesting that the fennel seed extract has significant antioxidant potential in terms of inhibiting nitric oxide production.

Table 4.3: Table showing Nitric acid IC50 value of sample E and ASC

Sample	**IC50 VALUES**
E	1.16
ASC	0.06

Figure 4.4 shows the Ferric Reducing Antioxidant Power (FRAP) values for sample *Foeniculum vulgare* (E) and its control (ASC). Sample E was tested at various concentrations (50, 100, 200, 300, 400, and 500 μM) using a FRAP standard. The y-axis of Figure 4.4 represents the FRAP value, which indicates the antioxidant capacity of the sample.

Sample E exhibited a remarkably high FRAP value of 1781.67, suggesting robust antioxidant potential. This finding aligns with a recent study by Rubio *et al.* (2024), which investigated the influence of genotype on the antioxidant activity and phenolic profile of fennel bulbs.

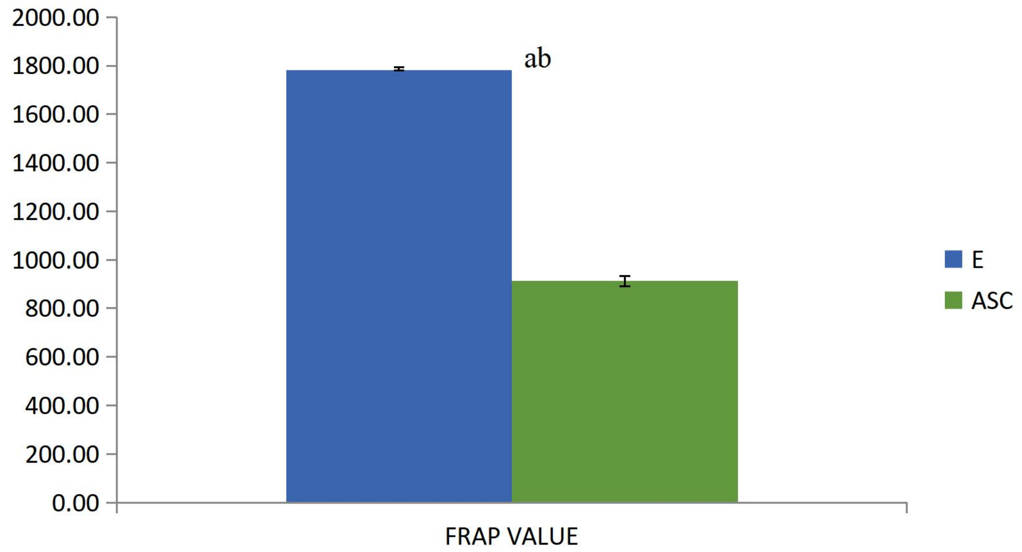

Figure 4.4: Chart showing FRAP Value of sample E and ASC

Figure 4.5 shows Total Antioxidant Capacity (TAC) of the *Foeniculum vulgare* sample. The TAC assay result indicate that the extract exhibits antioxidant activity across different concentrations, as evidenced by the reduction of ferric ions. Our TAC value of 142.24 agrees with a study done by Apak *et al.,* (2022) titled "Methods to evaluate the scavenging activity of antioxidants toward reactive oxygen and nitrogen species (IUPAC Technical Report)" which reported TAC values of 87-144.

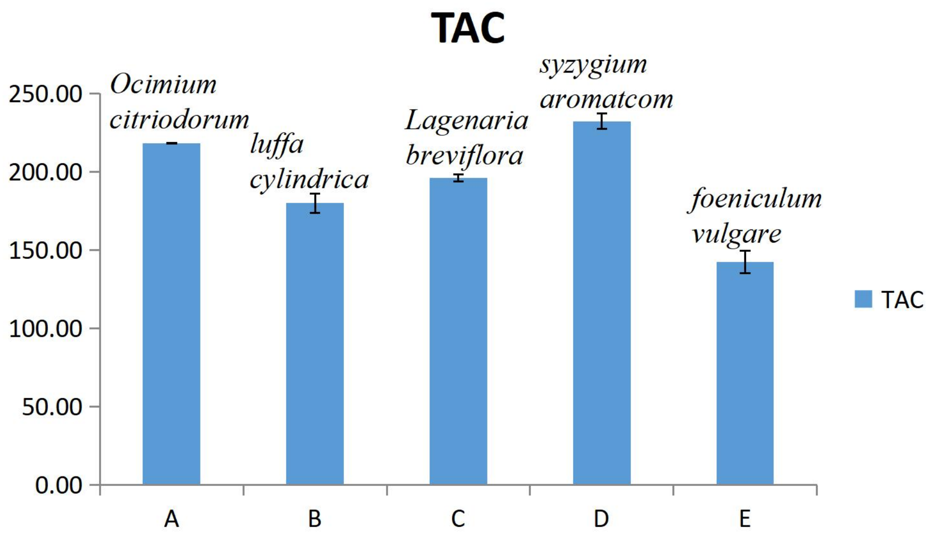

Figure 4.5: Chart showing TAC Value of sample E

Figure 4.6 shows a chromatogram obtained from the HPLC Phytochemical profile from ethanol extract of *Foeniculum vulgare*. This simply shows a significant spike in Quercetin, which indicates it is present in high concentrations.

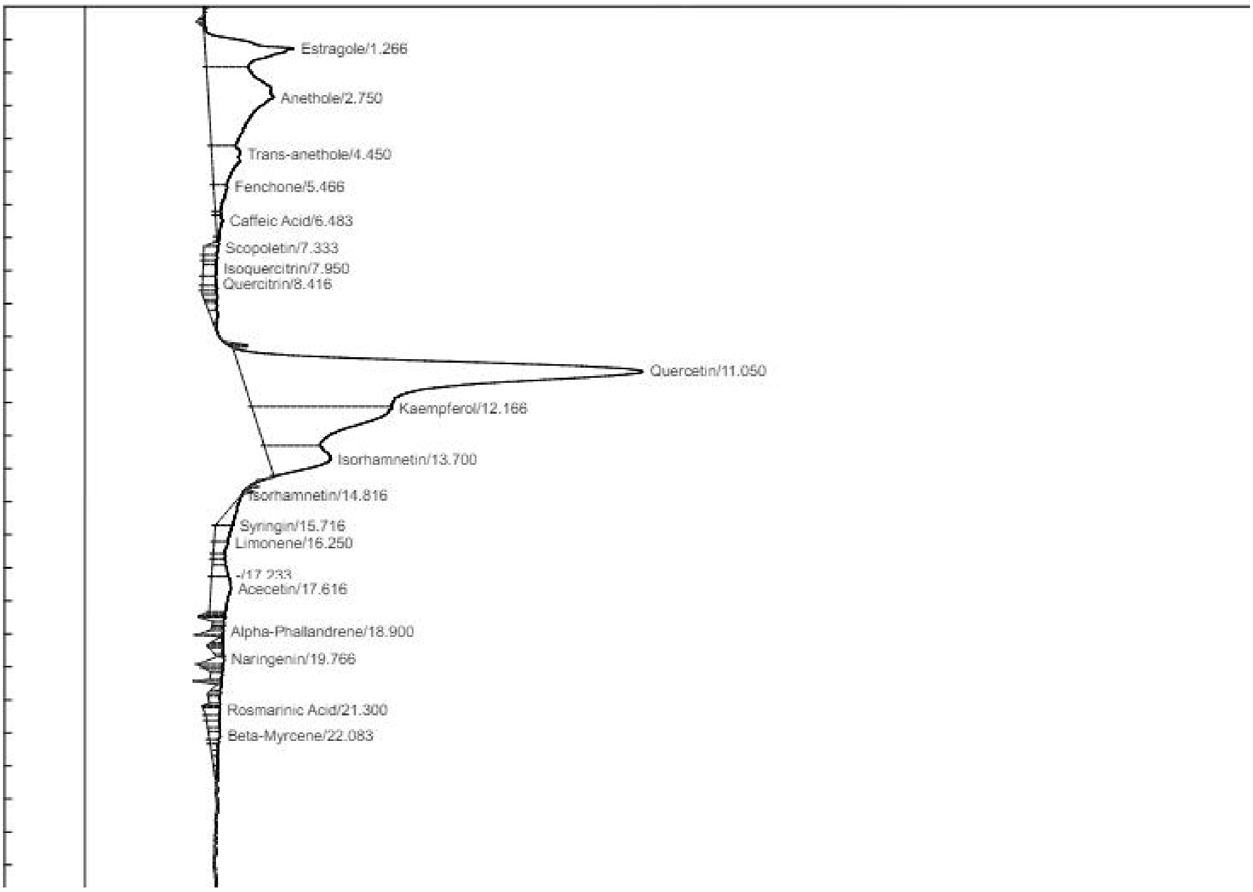

Figure 4.6: HPLC Phytochemical profile chromatogram.

Table 4.4 shows the retention time, peak height, the amount in ppm of the phytochemicals present in ethanol extract of *Foeniculum vulgare* with quercetin having the highest amount.

Table 4.4: HPLC Phytochemical Profile

COMPONENT	RETENTION	HEIGHT	AMOUNT (ppm)
Estragole	1.266	33.328	1162.6710
Anethole	2.750	24.546	2445.0705
Trans-anethole	4.450	10.695	591.3700
Fenchone	5.466	5.168	172.4650
Caffeic acid	6.483	2.719	66.4685
Scopoletin	7.333	5.385	81.9950
Isoquercitrin	7.950	5.087	102.3055
Quercitrin	8.416	5.048	82.3060
Quercetin	11.050	150.738	8228.2380
Kaempferol	12.166	52.347	2592.5880
Isorhamnetin	13.700	23.542	985.4310
Isoharmnetin	14.816	0.439	175.3440
Syringin	15.712	6.464	164.7435
Limonene	16.250	5.271	103.2780
Acecetin	17.616	7.511	435.2880
Alpha-Phallandrene	18.900	5.403	65.2410
Naringenin	19.766	4.859	77.7860
Rosmarinic acid	21.300	6.247	81.9340
Beta-Myrcene	22.038	4.371	61.6335

CHAPTER FIVE

5.1 DISCUSSION

The purpose of this analysis is to examine the invitro antioxidant potential and HPLC phytochemical profile of ethanol extracts of *Foeniculum vulgare* seeds. The Figures and Tables above show the antioxidant activity of the *Foeniculum vulgare* samples using various assays (Ferric Reducing Antioxidant Power, Total Antioxidant Capacity, 2,2-diphenyl-1-picrylhydrazyl, Hydroxyl Radical Scavenging Activity, and Nitric Oxide Scavenging Ability).

Figure 4.1 shows the DPPH value of the *Foeniculum vulgare* sample and its control. The provided data includes values at various concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/mL) and corresponding values for two parameters: 'E' (*Foeniculum vulgare*) and 'ASC' (Ascorbic acid used as a standard).

In a recent study done by Rubio *et al.,* (2024) a lower DPPH value was reported than those of the *Foeniculum vulgare* which suggests that the antioxidant activity of *Foeniculum vulgare* varies significantly.

Tables 4.1 shows the IC50 values, which represent the concentration of the extract required to inhibit 50% of DPPH radicals.

The sample E shows an IC50 value of 0.72 µM, indicating potent antioxidant activity and this suggests that the fennel seed extract contains a diverse array of antioxidants with varying scavenging capacities.

Sample ASC shows an IC50 value of 1.27 µM, indicating considerable antioxidant activity. While slightly higher than samples E, it still falls within a range suggesting significant scavenging capacity.

The analysis shows that fennel extracts exhibits good DPPH radical scavenging activity and this agrees with the findings of Bano *et al.*, (2016). and Tanveer *et al.,* 2024.

Figure 4.2 shows the hydroxyl radical scavenging activity of sample E and the standard antioxidant (ASC) at different concentrations (0.2, 0.4, 0.6, 0.8, and 1) showing increase in scavenging activity as concentration increases. Sample E is shown to exhibit higher absorbance values than the ASC. This suggests that sample E has stronger hydroxyl radical scavenging activity compared to the standard antioxidant.

These findings agree with the study on Assessment of Antioxidant Activity of *Foeniculum vulgare* Seed Extract Using Fenton Reaction carried out by Ankita Singh and Wasim Raja (2020) which demonstrated that *Foeniculum vulgare* extract possess hydroxyl radical scavenging activity.

Table 4.2 shows sample E exhibits a lower IC50 value than the standard antioxidant, suggesting that it has stronger inhibitory activity against the relevant biological process.

Figure 4.3 Shows the Nitric acid values of sample E and ASC. The figure shows the Nitric Acid assay and evaluates the extract's ability to inhibit nitric oxide production, which is associated with inflammation and oxidative stress. The provided data includes absorbance values for E and ASC at different concentrations (0.2, 0.4, 0.6, 0.8, and 1).

As the concentration increases, the absorbance generally decreases. This suggests that sample E has nitric acid scavenging activity, and its effectiveness decreases as the concentration rises.

Table 4.3 The IC50 represents the Nitric Acid concentration required to inhibit 50% of a specific biological activity (in this case, scavenging of DPPH radicals by nitric acid is 1.16). The findings align with those reported by Hamada *et al.,* (2023), suggesting that the fennel seed extract has significant antioxidant potential in terms of inhibiting nitric oxide production.

Figure 4.4 shows the Ferric Reducing Antioxidant Power (FRAP) values for sample *Foeniculum vulgare* (E) and its control (ASC). Sample E was tested at various concentrations (50, 100, 200, 300, 400, and 500 μM) using a FRAP standard. The y-axis of Figure 4.4 represents the FRAP value, which indicates the antioxidant capacity of the sample.

Sample E exhibited a remarkably high FRAP value of 1781.67, suggesting robust antioxidant potential. This finding aligns with a recent study by Rubio *et al.* (2024), which investigated the influence of genotype on the antioxidant activity and phenolic profile of fennel bulbs.

Figure 4.5 shows Total Antioxidant Capacity (TAC) of the *Foeniculum vulgare* sample. The TAC assay result indicate that the extract exhibits antioxidant activity across different concentrations, as evidenced by the reduction of ferric ions. Our TAC value of 142.24 agrees with a study done by Apak *et al.,* (2022) titled "Methods to evaluate the scavenging activity of antioxidants toward reactive oxygen and nitrogen species (IUPAC Technical Report)" which reported TAC values of 87-144.

In Figure 4.6 and Table 4.4, the results for the HPLC profiling of Foeniculum Vulgare showed high levels of quercetin which is correspondent with the work by (Rather *et al.,* 2016).

5.2 CONCLUSION

From the results, the in vitro antioxidant assays demonstrated significant antioxidant activity exhibited by the ethanol extract of *Foeniculum vulgare* seeds. Although it has a lesser Total Antioxidant Capacity (TAC) as compared to other plants like *luffa cylindrica*, *lageneria breviflora.* The extract exhibited strong scavenging activity against free radicals, as depicted by their ability to reduce DPPH radicals, as well as their ferric reducing antioxidant power (FRAP) and total antioxidant capacity (TAC). The HPLC analysis also revealed the presence of several bioactive compounds, with Quercetin being the highest phytochemical present, including anethole, estragole, and other phenolic compounds, known for their antioxidant properties.

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