GC-MS Employed Identification of Phytoconstituents and Antioxidant Activity Study of Coriander Leaf Extract

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "GC-MS Employed Identification of Phytoconstituents and Antioxidant Activity Study of Coriander Leaf Extract" submitted by Oisharja Ghosh Oishi (20346056), of Spring, 2024 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy

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Ethics Statement

This project does not involve any kind of animal and human trial.

Abstract

Coriander is a plant of the Apiaceae family and is very popular in herbal medicine world in and different cuisines. The study was conducted to identify the phytoconstituents that are present in the plant and investigate its antioxidant activity as other studies on the same plant showed therapeutic activities. GC-MS analysis was done in order to identify the phytoconstituents present in the sample and a total of 59 compounds were identified which have several therapeutic benefits. Based on the area % some of the major compounds present in the sample are 13-Docosenamide (Z)(49.82%), Decinamide(4.16%) Stigmasterol(3.45%), Tatetracinamide(3.33%) etc. and majority of these compounds had antioxidant activity. TPC, TFC and DPPH free radical scavenging assay was conducted to measure the antioxidant activity. The average TPC of three sample was 88.595 mg of GAE/g of dry extract at concentration of 88 mg/l of the extract. The average TFC of three sample was 184.5 mg/g RE of dry extract at concentrations of 234.634 mg/l of the extract. Lastly, average the % of inhibition of DPPH free radical by the sample extract was 64.1% at 8320.412 mg/l concentration. Literature review revealed that most of the identified compounds have antioxidant potential thus, it can be said that coriander can be used in treatment of the disease induced by free radicals as it has antioxidant properties.

Keywords: Coriander, Gas chromatography- Mass Spectroscopy, Total Flavonoid Content, DPPH, Free Radicals, Total Phenolic Content,.

Dedication

This work is dedicated to my family and my friends

Acknowledgement

I am thankful to the Almighty for giving me the scope, courage and strength throughout my whole path.

I am deeply indebted to my supervisor Dr. Raushana Akter (Professor, School of Pharmacy, BRAC University) as without her guidance and support it would not have been possible to conduct the research properly.

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Chapter 1

Introduction

1.1 Medicinal Plants

Medicinal plants are the plants in which therapeutic properties have been proven scientifically (Sofowora et al., 2013). From the beginning of ancient times, people used to search for drugs to cure their disease in nature. But at that time enough information was not available regarding the cause of illness or about how the plants could be used to cure the disease, so everything was experience based. The use of medicinal plants slowly moved from an empirical framework to explicatory facts as the use of specified plants for certain diseases was invented. Before the birth of iatrochemistry were the sources of prophylaxis and treatment (Kia et al., 2017). The usage of medicinal plants increased as the efficacy of synthetic drugs decreased and the contradictions of their usage increased. The medicinal plants were given more importance as they had better compatibility and adaptability with the human body and also had fewer side effects. It has been demonstrated that most of the used drugs have plant elements as a main ingredient. Many of them have bioactive components that are extracted from plants as active ingredients. Through various research studies, the medicinal activities of medicinal plants have been discovered through the study of traditional and therapeutic cures. The potential health benefits and the phytochemical composition of many plants are still not studied or discovered, however medicinal plants still have a hopeful future.

1.1.1 Drug discovery from plants

Nowadays, a huge number of medicines (cardiovascular, immunosuppressant, and anticancer) are derived directly from plants or from the active compounds of the plant source. The plant sources comprise many bioactive ingredients which are extensively 3chemical diversity, natural templates

for drug development, and sustainability, providing a vast and renewable source of novel therapeutic compounds. After assuring safety, approval of regulatory body makes the medicinal plant available in market and use by patients (Pan et al., 2013). The combination of traditional knowledge, modern scientific techniques, and sustainable practices holds great promise for discovering novel drugs to treat a wide range of diseases.

1.1.2 Available medicinal plants in Bangladesh and their usage

Bangladesh along with India shares a great portion of South Asia's plant genetic diversity because of its subtropical location. Abundant amounts of medicinal plants are available in the village jungles, forests, and shurbberies of Bangladesh. Recently, as there is an increase of interest of industrial countries and international pharmaceutical countries in complementary medicine thats why peoples interest in traditional medicine has increased (Das et al., 2022). About 25% of drugs in modern pharmacopeia is directly obtained from plants (Rahamatullah et al., 2013). In Bangladesh a good number of companies and industries uses medicinal plants. Digitoxin, vincristine, senna extracted from Digitalis, Catharanthus roseus, and Cassia senna respectively are some of the examples of drugs which are obtained from medicinal plants. The table below contains name of some of the plants that are used for their therapeutic benefits. We can see the plants have therapeutic benefits like antifungal, antiviral. Etc. also they can be used in diseases like mild cold, skin infection, diarrhea, leprosy.

Table 1: Common medicinal plants in Bangladesh used as therapeutic agents (Hasan et al., 2014)

Taxonomic Name	**Family**	**Local Name**	**Part(s) Used**	**Therapeutic Usage**
Ocimum sanctum L.	Labiatae	Tulsi	Leaf	In cold
Nyctanthes arbor-tristis L.	Oleaceae	Shefali	Leaves, Bark, Stem,Seeds	Antifilarial, antifungal, antibactariel
Ixora nigricans L.	Rubiaceae	Rongon	Root, Leaf	Ear infection, Diarrhea
Premna esculenta L.	Verbenaceae	Lalana	Leaf, Root	Fungal and skin infection
Hiptage benghalensis L.	Malphighiaceae	Madhobi lota	Flower, root	Skin infection, leprosy
Holarrhena antidysenterica L.	Apocynaceae	kurchi	Flower, Bark, Roots	Helminthiasis and skin infection
Bauhina purpurea Linn.	Caesalpiniaceae	Kanchan	Root,leaves,barks,fruits,seeds	Carminative, laxative
Carica papaya Linn	Caricaceae	Pepe	Fruits, seeds	Dyspepsia
Dillenia indica Linn.	Dilleniaceae	Chalta	Fruits,leaves	Laxative
Jatropha curcus Linn	Euphorbiaceae	Bagh verenda	Leaves, seeds	Purgative.

1.2Phytotherapy

When medicinal plants are used to treat disease or used as agents that benefit health conditions, it is called phytotherapy. In phytotherapy, for medicinal purpose whole plant or a small amount of slightly adulterated components are used as they preserve the actual composition and the originating plants integrity (Falzon & Balabanova, 2017).

1.3 Phytochemicals as therapeutic agent

Phytochemicals, naturally occurring compounds found in plants, have therapeutic potential due to their vast biological activities, including antioxidant, anticancer, and antimicrobial activity, making them valuable for developing new treatments.

Table 2*:Potential health benefits of some phytochemical compounds (Tyagi et al., 2010)*

Phytochemical	Potential benefit
Isoflavones (genistein and daidzein)	Increases dilation of vessels and reduces blood pressure
ZZmm	Apoptosis induction, vision improvement, and nitric oxide production inhibition
Proanthocyanidins and flavan-3- ols	Cellular oxygenases inhibition
Sulfides	Neutralize cell damaging free radicals
Isothiocyanates (Sulpforaphane)	Neutralize free radicals

1.4 Analytical Techniques for Phytoconstituent Identification:

Today, many pharmaceutical companies have started to process aromatic and medicinal plants extract active ingredients for their formulations. For the extraction of plant components, many processes are available, such as maceration, distillation, expression, enfleurage, fluid and solvent

extraction (Yalavarthi & Thiruvengadarjan, 2013). For the analysis of different types of phytochemicals such as flavonoids, terpenes, and alkaloids, chromatography is a very useful method (Sivagami & Sailaja, 2021). Chromatographic analytical methods like Nuclear Magnetic Resonance (NMR) Spectroscopy, Liquid Chromatography-Mass Spectrometry, High-Performance Liquid Chromatography are used.

1.5 Importance of compound identification

Medicinal plants are used as the central component of traditional herbal medicines worldwide since antiquity to date. In the developing world around 3.4 billion people are dependent on plant-based medicines (Doughari et al., 2009; Yadav et al., 2003). So, the identification of compounds in phytochemistry is crucial as it allows researchers to isolate and characterize active part of the plants, leading to the discovery of revolutionary effective agents with unique mechanisms of action for treating various diseases. The knowledge of phytoconstituents also supports sustainability and conservation efforts by promoting the sustainable use of plant resources.

1.6 Gas Chromatography-Mass Spectrometry (GC-MS)

A popularly used tool to for identifying or quantifying volatile compounds is called gas chromatography mass spectroscopy. Volatile compounds can be separated by using GC (Gas Chromatography) but compounds cannot be identified with it. On the other hand, MS (Mass Spectroscopy) can identify compounds as it has the ability to give structural details of the compound but could not separate compounds. Therefore, in the mid-1950s, GC was invented combining these two techniques. In the GC-MS technique, first the sample is vaporized, and then using an inert gas, it is carried through a chromatographic column. Depending on volatility and interaction with stationary phase of the columns, compounds are separated. After getting separated,

compounds enter the mass spectrometer and there they are ionized and fragmented. Lastly, depending on the mass to charge ratio ions are detected and analyzed which produces a mass spectrum that gives a unique fingerprint for each compound (Karasek & Clement, 2012).

1.6.1 Advantages of GC-MS

Gas chromatography-mass spectrometry has several advantages, which make it an indispensable tool in the identification field. One of its core advantages is its high sensitivity and specificity helps for the identification and detection of compounds at very low concentrations, even in complex mixtures. Additionally, GC-MS is known for its rapid analysis time, making it suitable for high-throughput screening and routine testing in fields such as food safety, forensic science, and pharmaceuticals (Hites, 1997). The technique also provides high resolution and separation of closely related compounds, which is a requirement for accurately analyzing complex samples. This wide range of applications of GCMS makes it a powerful and versatile tool for analyzing compounds.

1.6.2 Comparison of GC-MS with other analytical methods

Other than gas chromatography-mass spectrometry, many powerful analytical techniques such as high-performance liquid chromatography (HPLC), Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy, are used. HPLC is more preferred for non-volatile, thermally unstable, and larger molecules as its mobile phase is liquid (Primpke et al., 2020). On the other hand, LC-MS is similar to GC-MS, as here also chromatography is combined with spectroscopy, but it offers a broader range of analytes, which includes polar and thermally unstable compounds (Kumar & Vijayan, 2014). Although, NMR spectroscopy explains the structural information about molecular frameworks, but dont have the ability to handle complex mixtures as efficiently as GC-MS (Rule & Hitchen, 2006). For

identifying functional groups and bonds FTIR spectroscopy is used but does not offer the same level of sensitivity or specificity, especially in complex matrices, as GC-MS does (Berthomieu & Hienerwadel, 2009). While each technique has its unique advantages, GC-MS stands out for its ability to provide detailed molecular identification of volatile compounds, making it indispensable in the area of separation and identification.

1.7 Free radicals

Molecules that are not stable and have one or more unpaired electrons are called free radicals. They can as oxidants or reductants by donating or accepting electrons from other molecules. Some examples of free radicals which contain oxygen are hydrogen peroxide, nitric oxide radical hypochlorite hydroxyl radical, peroxynitrite radical, etc. These molecules are very reactive with the capability of causing damage of the biomolecules like proteins, DNA, lipid etc. (Lobo, Patil, Phatak, & Chandra, 2010).

1.7.1 Free radical formation in body

Free radicals are formed either naturally in the body through metabolic processes, for example cellular respiration, or can be developed from external sources, such as pollution, cigarette smoke, radiation, and certain chemicals. Both non enzymatic and enzymatic reactions take place while forming free radicals in the body. Nonenzymatic reactions occurs between oxygen and organic compounds. Enzymatic reactions include respiratory chain reactions, phagocytosis, prostaglandin synthesis etc. Some sources of free radicals which are generated internally are mitochondria, inflammation, peroxisomes, Xanthine oxidase etc (Lobo et al., 2010).

1.7.2 Tissue damage by free radicals

Free radicals can cause tissue injury as they have the capability to cause damage to protein, DNA and lipids which results in tissue damage. Free radicals cause tissue damage primarily through a process known as oxidative stress, where their high reactivity leads to the oxidation of essential cellular components.

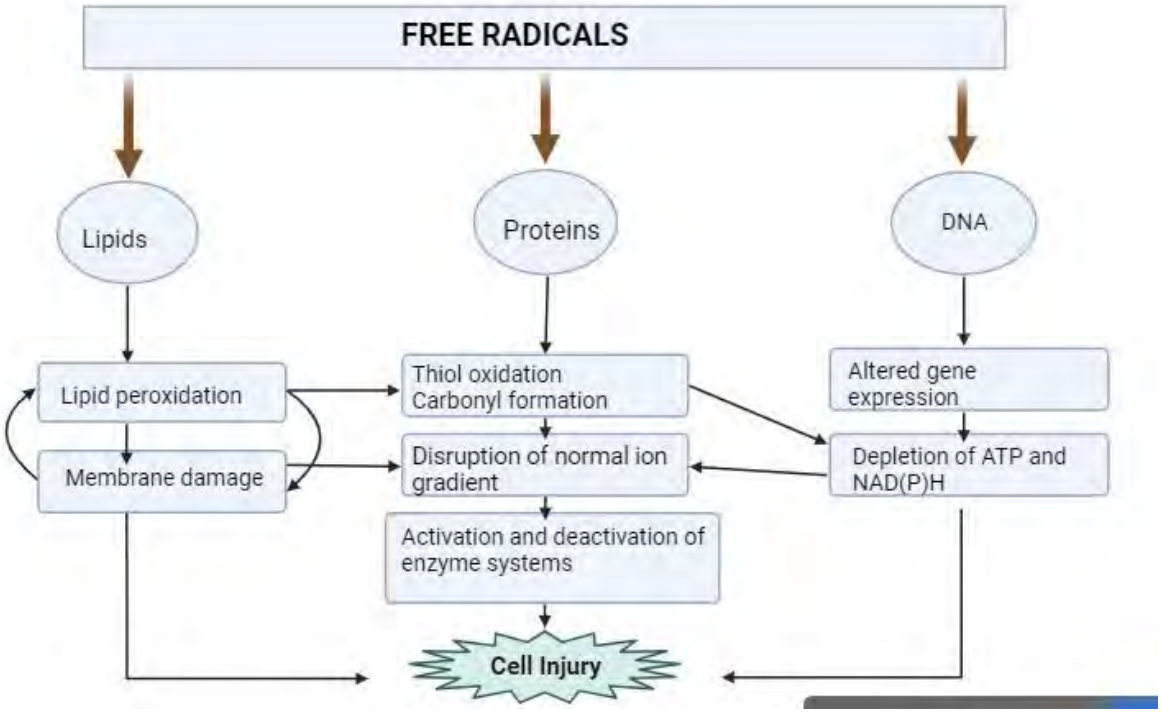

Figure 1:*Cell injury pathway by free radicals*

This figure describes how cell injury occurs when free radicals react with biomolecules. Here, when they react with lipid it causes lipid peroxidation which results into damage of membrane causing cell death. Again, when free radicals react with protein thiol oxidation and carbonyl formation which causes disruption of ion gradient resulting into actication or deactivation of

enzyme system and causes cell injury. Lastly, when they react with DNA and alter gene expression resulting in cell injury (Bhat et al., 2015).

1.8 Oxidative stress and its formation

Oxidative stress is defined by an imbalance between the production of free radicals, (Burton & Juaniaux, 2011). Oxidative stress is formed primarily when excessive production of ROS occurs during normal cellular processes like mitochondrial respiration or through external factors such as exposure to pollution, ultraviolet (UV) radiation and smoking. Also, many serious health conditions like hypertension, sepsis, cancer, diabetes obesity, dyslipidemia, respiratory failure, radiation may also play a role in oxidative stress generation (Pignatelli, Menichelli, Pastori, & Violi, 2018).

1.9 Disease related to oxidative stress

Due to the imbalance between antioxidants and free radicals, oxidative stress occurs in body which deteriorates health. Oxidative stress might give rise to diseases among which some of the major conditions are neurodegenerative disease, cancer generation, arthritis etc. which are fatal to human health and lifestyle (Pizzino et al., 2017).

A type of disease known as neurodegenerative diseases are defined by the degeneration and death of nerve cells in the brain and spinal cord. Neurodegenerative diseases might occur due to oxidative stress. Some examples of these diseases are Alzheimer's disease, multiple sclerosis, Parkinson's disease etc., which occur due to mitochondrial dysfunction. In Parkinson’s disease increased peroxidation of lipids, protein damage and oxidation of DNA in substantia nigra plays a major role in disease formation (Xueping et al., 2011). A toxic compound named ß-amyloid peptide is formed in Alzheimer’s disease play a vital role which is formed due to oxidative stress (Sharma, 2014).

Oxidative stress also contributes to multiple sclerosis by inducing myelin and oligodendrocyte damage, promoting axonal and neuronal loss, exacerbating inflammatory responses, and impairing antioxidant defenses and mitochondrial function.

Cells growth out of control and their spread in body is known as cancer. Cancer is linked to oxidative stress because it can cause DNA damage, leading to mutations that promote uncontrolled cell growth and tumor development. Oxidative stress also supports cancer progression by activating pathways that enhance cell survival, inflammation, angiogenesis, and metastasis, while inhibiting tumor suppressor functions. Thus, oxidative stress can cause the initiation and progression of cancer (Pizzino et al., 2017).

1.10 Antioxidants

Antioxidants are characterized by stable molecules who have the capability to donate electrons to free radicals, making them neutral which results in reduced damaging capacity of the free radicals. Antioxidants can stop the oxidative chain reaction before the cells get damaged by the interaction of free radicals. Some antioxidants include uric acid, ubiquinol, glutathione etc. which are produced during normal cellular metabolism (Flieger, Flieger, Baj, & Maciejewski, 2021).

1.10.1 Antioxidants defense mechanism

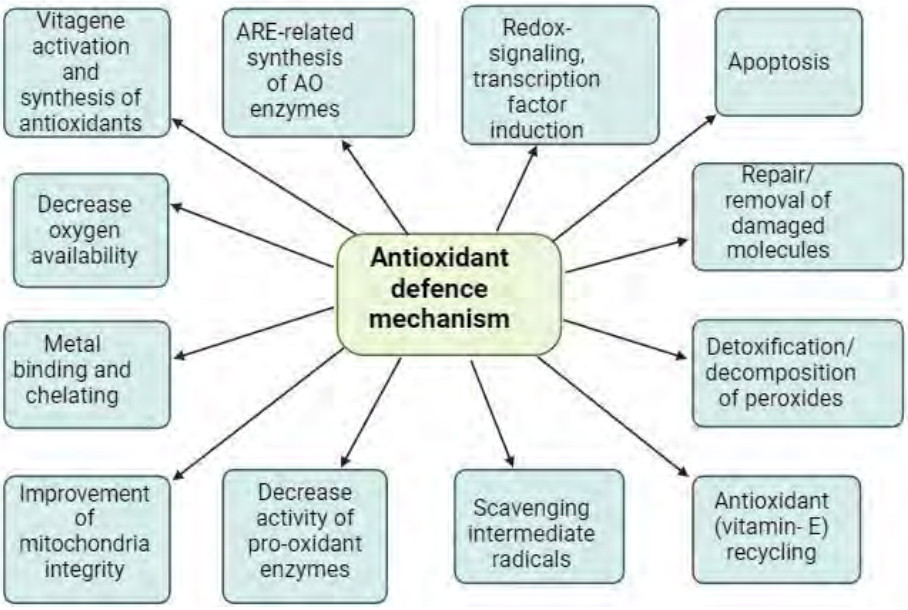

Figure 2*:Defense mechanism of antioxidants in various pathways*

From the figure we can say that antioxidants have several defence mechanisms. Antioxidants decrease production of free radicals by decreasing oxygen availability, which reduces the activities of enzymes responsible for ROS/RNS production. Free radicals are produced by the dysfunction of mitochondria so antioxidants exert their defensive action by maintaining the integrity of mitochondria. Again, scavenging and detoxification/decomposition of the free radicals are useful the antioxidant defense steps. The synthesis of protective molecules who possess antioxidant properties, transcription factor, redox signaling are also part of antioxidants defense mechanism. Lastly, by autophagy, apoptosis and other processes who work to remove damaged cells important elements of the antioxidant defense network (Surai et al., 2017).

1.11 An overview of the selected plant

Coriander belongs to the Apiaceae family and it is generally used throughout the world. Coriander originates from Mediterranean area. Coriander is a major element of many Asian foods (Laribi et al., 2015). Coriander has a height of 30-60 centimeters but its height can vary because of the growing environment for example sunlight, water availability, and soil quality. Its flowers are small and pale pink or white in color. The seeds are round, small and have a citrusy flavour which makes them a popular spice in many cuisines.

Figure 3*:coriander plant*

Taxonomy:

Table 3*:Taxonomy hierarchy of Coriander (Burdock & Carabin, 2009)*

Rank	Scientific Name
Kingdom	Plantae
Class	Magnoliopsida
Order	Apiales
Family	Apiaceae
Genus	Coriandrum L.
Species	Coriandrum sativum L.

1.12 Rationale of this project

The rationale of this project is to identify phytoconstituents by employing Gas Chromatography-Mass Spectrometry and measure the antioxidant activity of coriander leaf extract to explore its potential health benefits, given the rich therapeutic profile observed in similar plants. Coriander (*Coriandrum sativum*) has been traditionally used in various cuisines and herbal medicine, and there is a strong possibility that it contains bioactive compounds with significant antioxidant properties, much like other plants in the Apiaceae family. There has been many research conducted on coriander leaf such identification of bioactive components of coriander leaf has been conducted before (Marzoqi et al., 2015) but identification of phytoconstituents of coriander leaf methanol extract by GC-MS analysis has not been done before. Again, research on antioxidant activity of coriander seeds have been conducted before by measuring polyphenolic content of the seeds and it showed satisfactory results (Deepa & Anuradha, 2011) but antioxidant activity of coriander leaf

methanolic extract by determining total phenolic content and total flavonoid content has not been performed before. Lastly, DPPH free radical scavenging assay of coriander seed and leaf dichloromethane extract has been conducted before and it revealed that the seeds have more antioxidant activity but DPPH free radical scavenging of coriander leaf methanol extract has not been performed yet. By employing GC-MS, a highly sensitive and precise analytical technique, we can identify a wide variety of compounds in coriander leaf extract. This comprehensive analysis allows for the discovery of not only antioxidants but also other bioactive compounds that may have anti-inflammatory, antimicrobial, anti-diabetic, or other health-promoting properties. As other extract and plant part of the coriander has shown satisfactory results so this is why I have selected this plant to explore the phytochemical constituents of coriander and determine its antioxidants properties.

1.13 Aim and Objective of this project

Aim

This project is aimed to identify the phytoconstituents by using GC-MS and determine the antioxidant activity of coriander leaf extract.

Objectives of this project

This study had the following objectives:

- Identification of the phytoconstituents present in the herb using gas chromatography and mass spectroscopy

- Determination of its antioxidant potential using three methods which are determination of total flavonoid content, determination of total phenolic acid and evaluation of DPPH Free Radical Scavenging Assay of the sample.

Chapter 2

Methodology

2.1 Collection and preparation of the plant

2.1.1 Collection of Plants and Extraction Procedure

Coriander was chosen for investigation. It was collected from local market, Dhaka, Bangladesh.

The extraction procedure was done in two parts:

1. Preparation and drying of plant material

2. Extraction

2.1.2 Preparation and drying of plant material

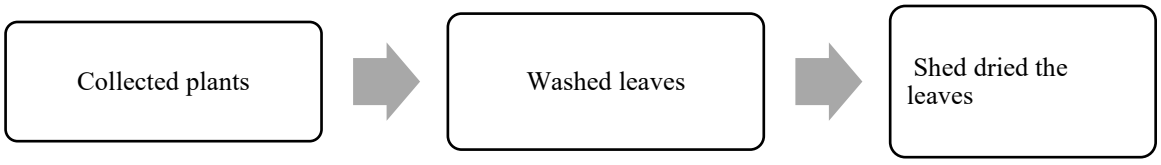

Figure 4*: Process of preparing plant material*

2.1.3 Extraction Process

After shed drying the leaves they were crashed by using a grinding machine to turn them into powder. The powder was kept in airtight glass to avoid any contamination. Then leaf powder extract were soaked in methanol and kept for 20mintues in room temperature (22-25°C). Regular stirring was done so that the powders soaked in methanol solvent properly. After soaking, by using cotton filtration the leaves were filtered. After that using rotary evaporator methanol was removed to concentrate the filtrate. Placing the sample in a petri-dish and with the help of laminar air flow the solvent was evaporated and kept away from any microbial contamination. After drying the petri-dish was covered with aluminum foil paper and kept in refrigerator.

2.2 Identification of phytoconstituents by GC-MS

A method described by Akter et al., 2022 and Chikowe et al., 2024 was followed for the GC-MS analysis of coriander methanol extract. GCMS-TQ8040 instrument (Shimadzu, Japan) was used for identifying compounds that exist in the coriander methanol extract. To perform this test, a DAB-5ms non-polar innowax column was used. The column's film thickness was 0.25 µm and its internal diameter was 30 × 0.25 mm. At the beginning temperature of the column was kept between 50-100°C for 60 seconds. Then the temperature was increased gradually for 20 minutes to 300°C. After that 0.5 µL of the plant extract was injected. Keeping the rate of flow at 1 mL/min, carrier gas helium was used in splitless mode. The split ratio was maintained at 5. The sample injectors temperature was 250°C and temperature of the detector was 300°C. In the electron ionization mass spectrometry, electrons with an energy of +0.50 kV were used. Lastly, it took about 40 minutes to record mass spectra in the 50 m/z to 600 m/z region.

2.2.1 Quantification and identification of individual phytoconstituents Identification of Coriander extract

For identifying and quantifying phytoconstituents of the plant extract, the mass spectra fragmentation pattern and retention indices of each compound were compared with the reference sample available in the Wiley database and National Institute of Standards & Technology (NIST) libraries (ALILOU and AKSSIRA, 2021). Also, by quantifying and relating % peak area the relative proportion of each individual was determined.

2.3 Antioxidant activity determination of Coriander

Among the various assay techniques available for determining antioxidant activity of plant material, three methods were chosen for evaluating the antioxidant activity of coriander leaf extract.

2.3.1 Determination of total phenolic content (TPC)

By slightly modifying the method named Folin-Ciocalteu phenol method, phenolic acid can be determined (Haida & Hakiman, 2019). In this method, samples reducing ability is measured. In the heteropoly phosphotunstates-molybdate compound a series of reduction reactions occurs and forms a complex of blue colour (Ainsworth & Gillespie, 2007). Then at 650nm against blank, the intensity of blue colour is measured (Sukhadiya et al., 2021).

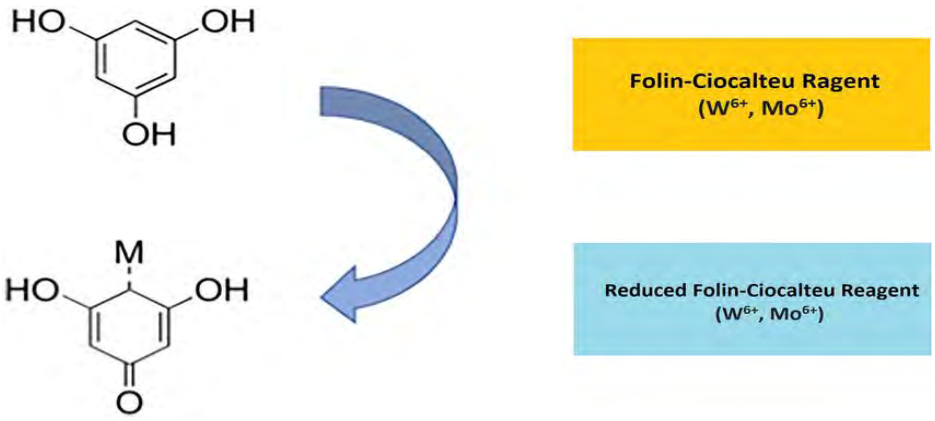

Figure 5: In the presence of phenolics reduction of Folin-Ciocalteu's Reagent

2.3.1.1 Materials and reagents

For the determination of total phenolic acid content of coriander leaf extract following materials and reagents were used

Table 4: *Name of materials and reagents used in TPC experiment*

Materials	Reagents
Test tubes	Gallic acid
Micropipette	Sodium carbonate
Erlenmeyer flasks	Follin-Ciocalteu reagent
UV-visible spectrometer	Distilled water

2.3.1.2 Reagent Preparation

To prepare 2ml of 20% (w/v) sodium carbonate, in 2ml of distilled water 0.4g of sodium carbonate was dissolved.

2.3.1.3 Sample and Standard preparation

Sample preparation: in 1l of methanol solvent 88mg of coriander extract was dissolved, which made a concentration of 88mg/L. 3 samples of 200 microliter of the same concentration were taken and converted to 3ml by adding distilled water.

Standard preparation: Used gallic acid as standard. Here the concentrations of the standard were 50, 100, 200, 400, 500 mg/L.

2.3.1.4 Blank preparation

The blank solution was made by mixing of all the reagents except the plant sample and standard solution. It was prepared by mixing1ml of methanol, 5ml of FCR solution, 4ml of sodium carbonate.

2.3.1.5 Experimental procedure

First 3 samples of the same concentration of the plant sample and 5 samples of different conc. of gallic acid was taken in test tubes. Then added 5 ml of Folin-Ciocalteu reagent and kept for 3min. After that added 2ml (20% w/v) sodium carbonate. They were vortexed for 15min and placed in dark for 60min. Lastly, with the help of a double beam UV-Visible spectrometer absorbance of standard and sample was taken at 650nm against blank. The following equation was used for calculating the total phenolic acid of each of the fragments as Gallic aicd equivalents:

$$C = (c \times V) \div m$$

Here,

C = Total of phenolic acids are expressed as mg of gallic acid per gram of dried plant extract, expressed as gallic acid equivalent (GAE)

c = concentration of gallic acid gained from the calibration curve (mg/l)

V = Volume of sample solution (mL)

m = weight of the sample (g)

2.3.2 Determination of total flavonoid content

A method described by Haida & Hakiman (2019) was employed to measure the total flavonoid content of plant extract. Flavonoids with ortho-hydroxyl groups in the A and B rings form acid-labile complexes with aluminum chloride ($AlCl_3$). Lastly, with a UV-visible spectrophotometer at 510nm, the intensity of yellow complex is measured.

2.3.2.1 Reagents and Materials

The following materials and reagents were used in order to evaluate the TFC of the plant extract

Table 5*:Name of reagents and materials used in TFC experiment*

Materials	Reagents
Test tubes	Sodium Nitrile
Micropipette	Aluminum Chloride
Erlenmeyer flasks	Sodium Hydroxide
UV-visible spectrometer	Rutin
Weighing machine	Distilled water

2.3.2.2 Reagent Preparation

To prepare 0.3 ml of 5% sodium nitrile, 15mg of sodium nitrile was dissolved in 0.3 ml of distilled water. To prepare 0.3 ml of 10% Aluminum chloride, 30 mg of aluminum chloride was dissolved in 0.1ml of distilled water, and then distilled water was added to mark up to 0.3ml. Lastly, to prepare 2ml of 1M Sodium Hydroxide, in 2ml of distilled water 80mg of Sodium Hydroxide was dissolved.

2.3.2.3 Sample and Standard Preparation

Sample preparation: 234.634mg of coriander extract was dissolved in 500ml of methanol and marked up to 1l by adding more methanol, which formed a stock solution having concentration of 234.634mg/l.

Standard preparation: Rutin was used as standard. Here the concentration of the standard were 50, 150, 300, and 500 mg/l.

2.3.2.4 Blank Preparation

The black solution was prepared by mixing all the reagents except the plant extract or standard. It was prepared by adding 200µL of 10% aluminum chloride solution, 200µL of 1M Sodium Hydroxide solution, 200µL of 5% sodium nitrile, 4mL of methanol and 5.4mL of distilled water to make the volume of 10mL.

2.3.2.5 Experimental procedure

Firstly, Three samples of 50 microliters of the same concentration of coriander extract was converted to 1 ml by adding methanol. Then, added 4ml distilled water in both the test tubes of samples and standard. After that 0.3 ml of 5% NaNO was added and then 0.3 ml of 10% AlCl was added after 5 min. Kept standing for 6 min. Then added 2ml 1M NaOH and made it up to 10 ml

by adding distilled water and kept standing for 15 min. Lastly, with a double beam UV-Vis spectrometer at 510 nm absorbance of sample and rutin standard were taken. Total flavonoid content was calculated. Finally, the following equation was used for expressing the total flavonoid content of each of the fragments as Rutin equivalents:

$C = (c \times V) \div m$

Here,

C = Total content of flavonoid compounds, mg of quercetin per gram of dried plant extract, expressed as rutin equivalent (RE)

c = concentration of rutin obtained from calibration curve (mg/l)

V = Volume of sample solution (mL)

m = weight of the sample (g)

2.3.3 Evaluation of DPPH Free Radical Scavenging Assay

Due to the fast analysis time, low cost and easy to use, DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging technique is popularly used for assaying antioxidant capability of plants (Rahman et al., 2015). This method works by assessing the decolorization of DPPH solution when plant extract solution is added. As DPPH is consists of an unpaired electron, which during the assay remains delocalized over the entire atom. The maximum absorbance of DPPH is at 517 nm wavelength while giving violet/purple color in ethanol. Another important factor that needs to be considered is the presence of ethanol as it contributes to antioxidant activity (Liang & Kitts, 2014).

2.3.3.1 Reagents and Materials

The following materials and reagents were used in the DPPH test of the plant extract

Table 6: *Name of reagents and materials used in the DPPH test*

Materials	Reagents
Test tubes	2,2-diphenyl-1-picrylhydrazyl (DPPH)
Micropipette	Ethanol
Erlenmeyer flasks	Vitamin E
UV-visible spectrometer	Distilled water

2.3.3.2 Reagent preparation

To prepare the DPPH solution, in 50 mL of ethanol solution 1 mg of DPPH powder was dissolved forming a DPPH solution having a concentration of 20 μg/mL.

2.3.3.3 Sample and Standard preparation

To prepare the sample solution, in 1L of methanol, 8320.412 mg of plant extract was dissolved making a solution with a concentration of 8.3 mg/mL.

Standard preparation: Vitamin E was used as standard here. Standards of four different solution were made having concentration of 50, 150, 300 and 500 mg/L.

2.3.3.4 Blank Preparation

The black solution was prepared by mixing all the reagents except the plant extract or standard. Here, blank was prepared by mixing 1ml of ethanol and 3ml of DPPH solution.

2.3.3.5 Experimental procedure

Firstly, 200 microliters of three sample solution and standards of four different concentration were taken in each test tubes. Then added 3.8ml of DPPH solution in each test tubes. Incubated for 60min in dark. Lastly, using a double beam UV-Vis spectrometer absorbance of sample and vit. E standard were taken at 517 nm.

To calculate percent inhibition (% I) of DPPH free radical was calculated using the following equation:

$$\%\text{Inhibition} = (1 - A/A_0) \times 100$$

Where, absorbance of the blank is indicated as A_0 and absorbance of the sample or the standard is indicated as A (Salar et al., 2015).

Chapter 3

Results

3.1 Identification of phytoconstituents by GC-MS

Table 3.1 contains the phytoconstituents identified from the coriander leaf extract by using GC-MS. Individual compounds are listed alongside their respective retention time and area % and it reflects their relative abundance with the plant sample. The figure and the table gives insight into the potential bioactive compounds that are present in the extract**.**

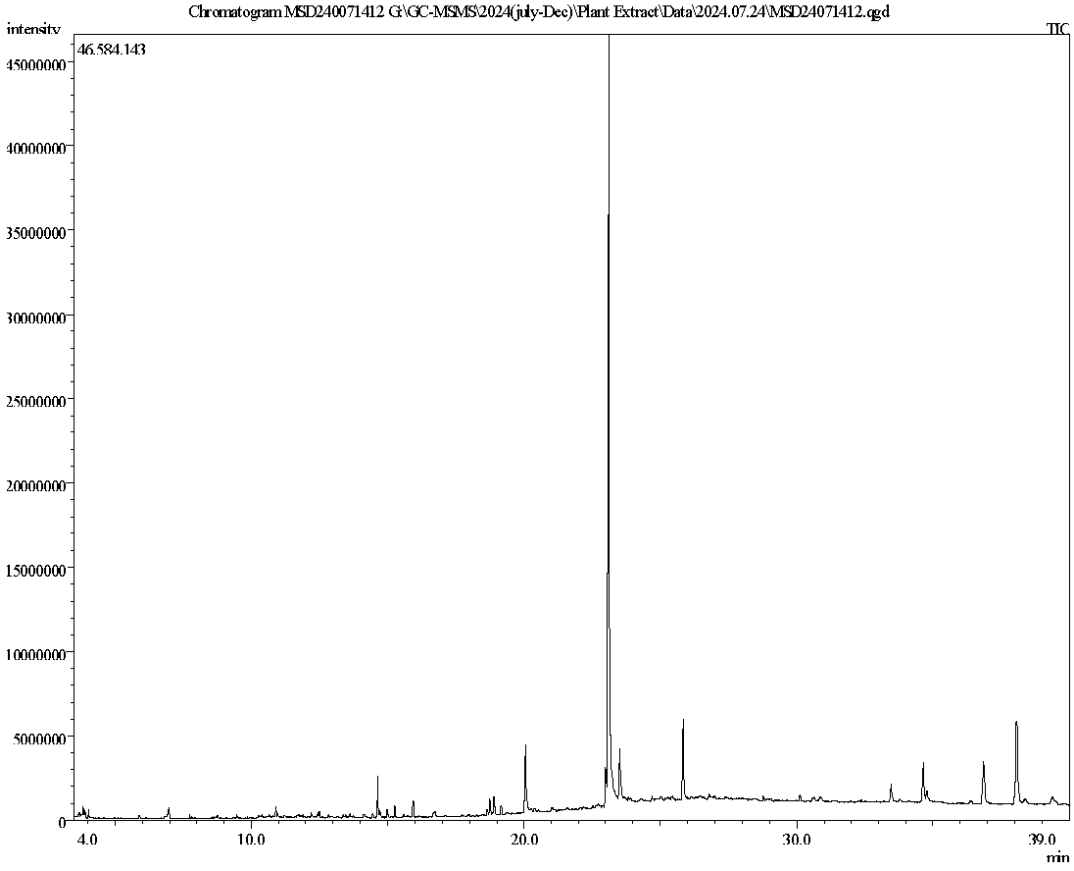

Figure 6: GC-MS chromatogram of coriander methanol extract

Table 7: *Phytoconstituents Identified by GC-MS*

Sl. NO.	Retention Time(min)	Area %	Name
1	3.53	0.22	d-Mannitol, 1-decylsulfonyl
2	3.687	0.15	1,4-Benzenedimethanol
3	5.887	0.15	Arsenous acid, tris(trimethylsilyl) ester
4	10.658	0.08	Phenol, 2,6-bis(1,1-dimethylethyl)-
5	11.222	0.07	Heptadecane, 7-methyl-
6	11.685	0.06	3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide
7	12.198	0.23	Octadecane, 1-chloro-
8	12.446	0.2	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol
9	12.503	0.2	tert-Butyl (2-aminophenyl)carbamate, 2TMS derivative
10	12.84	0.1	tert-Butyl (2-aminophenyl)carbamate, 2TMS derivative
11	8413.174	0.07	tert-Butyl (2-aminophenyl)carbamate, 2TMS derivative
12	13.371	0.12	Eicosane
13	13.628	0.22	Loliolide
14	14.18	14.18	Dodecane, 2,6,11-trimethyl-
15	14.638	1.59	Neophytadiene
16	14.72	0.35	2-Pentadecanone, 6,10,14-trimethyl-
17	15.263	0.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol

18	15.588	0.12	Glucitol, 6-O-nonyl-
19	16.68	0.23	Octadecanamide
20	18.651	0.28	9,11-Octadecadienoic acid, methyl ester, (E,E)-
21	18.756	0.79	8,11,14-Docosatrienoic acid, methyl ester
22	18.904	0.98	Phytol
23	19.174	0.4	Methyl stearate
24	20.06	4.16	Decanamide-
25	20.384	0.2	Eicosyl isopropyl ether
26	21.049	0.25	(.+/-.)-Marmesin
27	22.2	22.2	Tetrapentacontane, 1,54-dibromo-
28	22.66	0.21	Pentatriacontane
29	22.725	0.35	2-[(2S)-7-Oxo-2,3-dihydro-7H-furo[3,2-g]chromen-2-yl]propan-2-yl acetate
30	22.899	0.11	4,8,12,16-Tetramethylheptadecan-4-olide
31	23.002	2.16	6,9-Octadecadienoic acid, methyl ester
32	23.114	49.82	13-Docosenamide, (Z)-
33	23.515	3.33	Tetradecanamide
34	23.663	0.18	Tridecane, 3-cyclohexyl-
35	23.735	0.12	Docosane, 1,22-dibromo-
36	23.833	0.19	2-Methylhexacosane

37	25.174	0.14	Nonyl tetradecyl ether
38	25.274	0.24	2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane
39	25.344	0.11	Octatriacontyl pentafluoropropionate
40	25.838	4.28	Phthalic acid, di(2-propylpentyl) ester
41	27.419	0.12	Dodecyl nonyl ether
42	28.934	0.1	17-Pentatriacontene
43	30.122	0.26	Squalene
44	30.565	0.19	.alpha.-Tocospiro A
45	30.795	0.08	Butylphosphonic acid, di(4-octyl) ester
46	30.876	0.42	.alpha.-Tocospiro B
47	31.984	0.09	.delta.-Tocopherol
48	32.225	0.1	Ginsenol
49	32.533	0.06	Isophthalic acid, allyl isohexyl ester
50	33.467	1.06	.gamma.-Tocopherol
51	33.803	0.17	17.alpha.(H),21.beta.(H)-Homohopane
52	34.648	2.58	Vitamin E
53	34.779	0.7	.alpha.-Tocopherolquinone
54	36.387	0.25	5-Androstenetriol
55	36.862	3.45	Stigmasterol
56	38.068	8.47	.gamma.-Sitosterol

57	38.375	0.52	Ergosta-5,24(28)-dien-3-ol, (3.beta.)-
58	39.372	0.85	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-
59	39.52	0.37	1,2-Bis(trimethylsilyl)benzene

3.1.1 Bioactivity of compounds identified by GC-MS

The table below includes chemical formula, classification and bioactivity of the phytoconstituents identified by GC-MS. This information gives idea about the chemical nature and their biological roles**.**

Table 8*:Bioactivity of compounds identified by GC-MS*

Sl. No.	**Name**	**Chemical formula**	**Class**	**Bioactivity**	**Reference**
1	d-Mannitol, 1-decyl sulfonyl	$C_{16}H_{34}O_7S$	Sulfones	N/A	
2	1,4-Benzenedimethanol	$C_8H_{10}O2$	Aromatic alcohol	Antimicrobial	Liu et al. 2019
3	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	Organosilicon	N/A	
4	Phenol, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	Alkylated phenols	Antioxidant, preservative	Octarya et al. 2021
5	Heptadecane, 7-methyl-	$C_{18}H_{38}$	Alkanes	N/A	

6	3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1dioxide	$C_{14}H_{16}O_2S_2$	Alkene	N/A	
7	Octadecane, 1-chloro-	$C_{18}H_{37}Cl$	Haloalkane	N/A	
8	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol	$C_{14}H_{22}O$	Cycloalkene	Antimicrobial, Antioxidant	Taher et al. 2023
9	tert-Butyl (2-aminophenyl)carbamate, 2TMS derivative	$C17H_{34}N_2O_2Si_2$	Carbamates, aromatic amine	N/A	
10	Nonadecane	$C_{19}H_{40}$	Hydrocarbons	N/A	
11	1-dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	Alcohol	N/A	
12	Eicosane	$C_{20}H_{42}$	Alkane	N/A	
13	Loliolide	$C_{13}H_{18}O_3$	Alcohol	Antioxidant, Anti-inflamatory	Grabarczyk et al., 2015
14	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	Alkane	Antimicrobial, antifungal, antioxidant	ALRaddadi et al. 2024
15	Neophytadiene	$C_{20}H_{34}$	Alkene	Sedative, Anti-depressant	Bhardwaj et al. 2020
16	2-Pentadecanone, 6,10,14-trimethyl-	$C_{20}H_{40}O$	Ketone	Antibacterial, anti-inflamatory	Fahem et al. 2020
17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Alcohol, Alkenes	Antioxidant, Emollient	Suganandm et al. 2022

18	Glucitol, 6-O-nonyl-	$C_{15}H_{32}O_6$	Polyols	N/A	
19	Octadecanamide	$C_{18}H_{37}NO$	Amide	Antimicrobial	Islam et al., 2021
20	9,11-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	Ester	Antioxidant, Anti-inflamatory	Sianipar et al. 2021
21	8,11,14-Docosatrienoic acid, methyl ester	$C_{23}H_{42}O_2$	Ester	Antioxidant, Anti-inflamatory	Amee et al. 2024
22	Phytol	$C_{20}H_{40}O$	Terpenoids	Antioxidant	Islam et al. 2018
23	Methyl stearate	$C_{19}H_{38}O_2$	Ester	Emollient, lubricant	Lu et al., 2020
24	Decanamide-	$C_{10}H_{21}NO$	Amides	Emollient	Ali et al. 2023
25	Eicosyl isopropyl ether	$C_{23}H_{50}O$	Ether	Emollient	Lykholat et al., 2021
26	(.+/-.)-Marmesin	$C_{12}H_{10}O_4$	Coumarin	Antimicrobial, Anti-inflamatory	Trumble & Millar, 1996
27	Tetrapentacontane, 1,54-dibromo-	$C_{45}H_{92}Br_2$	Alkane	Antimicrobial	Enema et al., 2019
28	Pentatriacontane	$C_{35}H_{72}$	Alkanes	N/A	
29	2-[(2S)-7-Oxo-2,3-dihydro-7H-furo[3,2-g]chromen-2-yl]propan-2-yl acetate	$C_{15}H_{16}O$	Furochoromoe	N/A	

30	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O$	Lactone	Flavor, Fragrance	Elwekeel, 2021
31	6,9-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	Fatty acid methyl ester	Antioxidant, Anti-inflamatory	Abdel-Rahman, 2020
32	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	Fatty amide	Emollient, Anti-microbial	Islam et al., 2021
33	Tetradecanamide	$C_{14}H_{29}NO$	Fatty amide	N/A	
34	Tridecane, 3-cyclohexyl-	$C_{19}H_{38}$	Alkane	N/A	
35	Docosane, 1,22-dibromo-	$C_{22}H_{44}Br_2$	Halogenated alkanes	N/A	
36	2-Methylhexacosane	$C_{27}H_{56}$	Alkane	N/A	
37	Nonyl tetradecyl ether	$C_{23}H_{50}O$	Alkyl ether	Emollient	Joss & Xavier et al., 2021
38	2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane	$C_{25}H_{50}$	Terpene	N/A	
39	Octatriacontyl pentafluoropropionate	$C_{38}H_{77}F_5O_2$	Fluorinated ester	Antimicrobial	Murniasih et al., 2022
40	Phthalic acid, di(2-propylpentyl) ester	$C_{20}H_{34}O_4$	Phthalate ester	N/A	
41	Dodecyl nonyl ether	$C_{21}H_{44}O$	Alkyl ester	N/A	
42	17-Pentatriacontene	$C_{35}H_{70}$	Alkene	N/A	

43	Squalene	$C_{30}H_{50}$	Lipids	Antioxidant, Anti-inflamatory	Lou-Bonafonte et al., 2018
44	α -Tocospiro A	$C_{28}H_{50}O_2$	Tocopherols	N/A	
45	Butylphosphonic acid, di(4-octyl) ester	$C_{24}H_{51}O_4P$	Phosphonic ester	N/A	
46	α -Tocospiro B	$C_{28}H_{50}O_2$	Tocopherols	N/A	
47	δ -Tocopherol	$C_{27}H_{46}O_2$	Tocopherols	N/A	
48	Ginsenol	$C_{30}H_{50}O_5$	Ginsenosides	Antioxidant	Volobueva et al., 2021
49	Isophthalic acid, allyl isohexyl ester	$C_{16}H_{26}O_4$	Ester	N/A	
50	γ-Tocopherol	$C_{27}H_{46}O_2$	Tocopherols	Antioxidant, Anti-inflamatory	Jiang et al., 2001
51	17α(H),21β(H)-Homohopane	$C_{30}H_{52}$	Hopanes	N/A	
52	Vitamin E	$C_{27}H_{46}O_2$	α-tocopherol	Immunity, Antioxidant, Anti-inflamatory	Brigelius-Flohé, 2006
53	α-Tocopherolquinone	$C_{27}H_{42}O_3$	α-tocopherol	Cellular signaling, gene expression	Zhan et al. 2020
54	5-Androstenetriol	$C_{18}H_{26}O_3$	Androgen	Hormonal balance	Uspenskaya et al., 2024

55	Stigmasterol	$C_{29}H_{48}O$	Sterols	Cholesterol lowering effect	Islam et al., 2021
56	γ-Sitosterol	$C_{29}H_{50}O$	Sterols	Cholesterol lowering effect, Antioxidant	Saeidina et al., 2014
57	Ergosta-5,24(28)-dien-3-ol, (3.beta.)-	$C_{28}H_{44}O$	Sterols	Vitamin D precursor	Metwalley et al., 2020
58	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	$C_{30}H_{50}O_2$	Sterols	Cholesterol lowering effect, Antioxidant	Penduka et al., 2014
59	1,2Bis(trimethylsilyl)benzene	$C_{12}H_{18}Si_2$	Organosilicon	N/A	

3.2 Antioxidant activity of Coriander

3.2.1 Determination of Total Phenolic content

Total phenolic acid content (TPC) is a tool to measure the phenolic content. In this study, TPC of the coriander leaves extract was calculated from gallic acids standard curve which had peak absorbance at a wavelength of 650 nm. Phenolic compounds are attributed to antioxidant ability as they can remove free radicals.

***Table 9**:Determination of Regression Equation from Gallic Acid Absorbance*

Concentration(mg/l)	Absorbance	Regression Equation	R^2 Value
50	0.537	y=0.0094x+0.0652	0.9996
100	0.978		
200	1.990		
400	3.818		

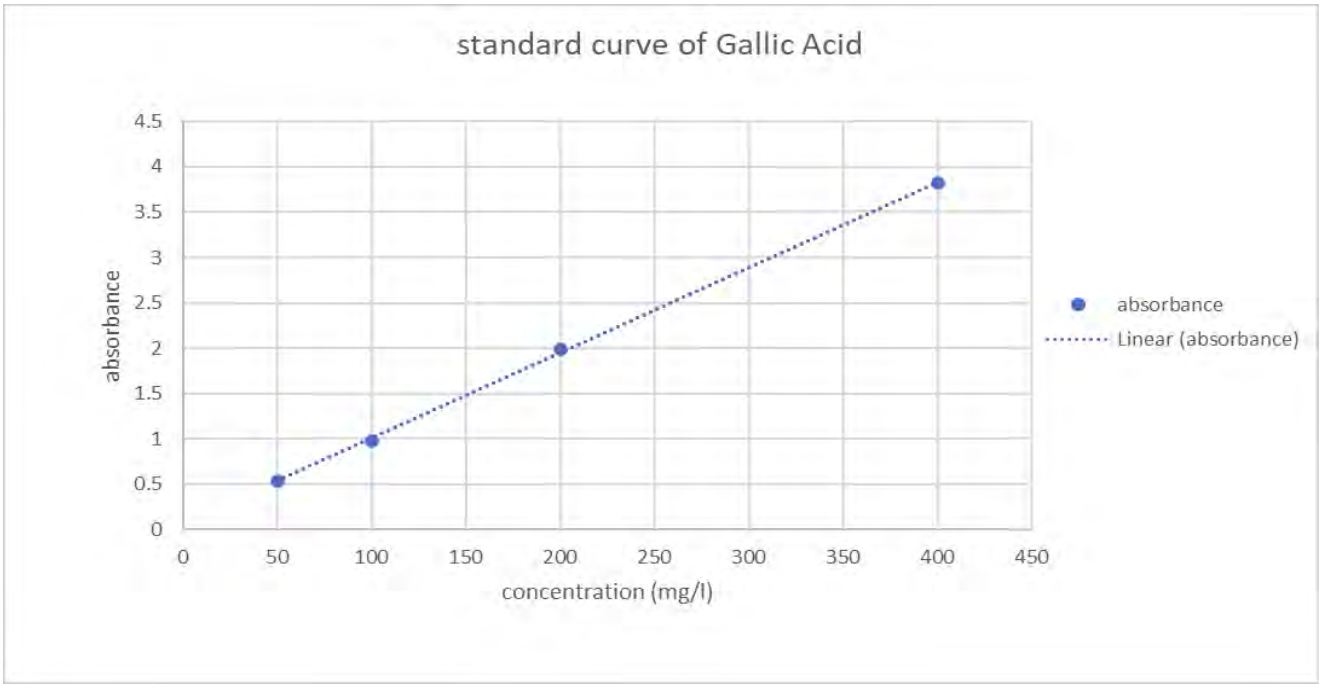

***Figure 3.1:** Standard Curve of Gallic Acid for TPC Test*

Interpretation: The gallic acid calibration curve gave the equation y=0.0094x+0.0652, total phenolic content of coriander will be calculated from this equation. A very strong relationship between the absorbance and concentration is indicated by regression coefficient of 0.9996.

***Table 10**: Determination of Total Phenolic Content of Test Sample*

Concentration(mg/l)	Absorbance of Extract (Mean±SD)	Total Phenolic Content of sample (mg/g) GAE of dry extract (Mean±SD)
88.432	0.891 ± 0.0037	88.593 ± 0.0027
88.432	0.895 ± 0.0003	88.595 ± 0.0007
88.432	0.898 ± 0.0033	88.599 ± 0.0033

Explanation: By using the regression equation of table 3.4 above, the average concentration of phenolics in the methanol extract of leaves was 88.595 mg/g GAE of dry extract and the phenolic content is same for all the three sample as the concentration was same for the three samples. So, coriander leaves extract contains a great of phenolic compounds which means it has great antioxidant activity.

3.2.2 Determination of Total Flavonoid Content

The Total Flavonoid Content test is done to determine the flavonoid concentration. The greater the concentration of flavonoids, the greater will be the antioxidant potency of the plant extract. In this study, TFC of coriander extract was detected spectrophotometrically using a standard curve of rutin, which shows peak absorbance at 510 nm. Flavonoids exert their antioxidant ability scavenging free radicals.

***Table 11**: Determination of Regression Equation from Rutin Absorbance*

Concentration(mg/l)	Absorbance	Regression Equation	R^2 Value
50	0.005	y=0.0002x+0.0001	0.9807
150	0.024		
300	0.053		
500	0.075		

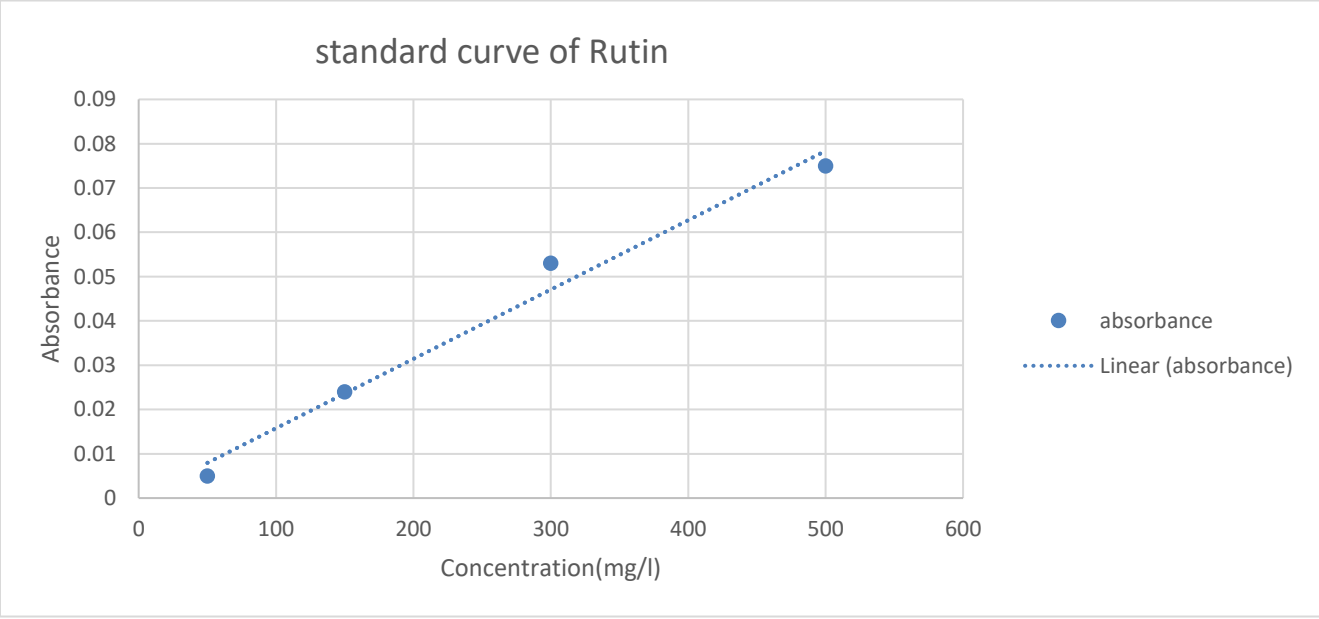

***Figure 3.2**: Standard Curve of Rutin for TFC Test*

Interpretation: The gallic acid calibration curve gave the equation y=0.0002x+0.0001, and total flavonoid content will be measured using this equation. A good relationship between the absorbance and concentration is indicated by regression coefficient of 0.9807.

***Table 12**: Determination of Total Flavonoid Content of Test Sample*

Concentration(mg/l)	Absorbance of Extract (Mean±SD)	Total Flavonoid Content of sample (mg/g) RE of dry extract (Mean±SD)
234.634	0.032 ± 0.0026	184.2 ± 0.2666
234.634	0.035 ± 0.0003	184.5 ± 0.0333
234.634	0.038 ± 0.0033	184.7 ± 0.2333

Explanation: Using the regression equation of table 3.6 above, the average concentration of flavonoid in the methanol extract of leaves was determined to be 184.5 mg/g RE of dry extract. All the three samples had almost the same flavonoid content as the concentration of all the three sample were same. As the coriander leaves extract exerted a noteworthy amount of flavonoid compounds so it can be said that coriander leaf has antioxidant activity.

3.2.3 Evaluation of DPPH Free Radical Scavenging Assay

For DPPH free radical scavenging assay, absorbance of standard and sample was taken with a UV-Visible spectrophotometer. Then % of inhibition was determined using the equation mentioned in 2.3.3.5 section. The blank solution had an absorbance (A_0) of 0.621 at 517 nm wavelength.

Table 13: *Estimation of % of Inhibition of Vit. E*

Concentration (mg/l)	Absorbance of Vit. E (Standard)	% of Inhibition
50	-0.598	196.3
150	-0.621	196.9
300	-0.598	196.2
500	-0.592	195.4

Table 14*: Estimation of % of Inhibition of Coriander*

Concentration (mg/L)	Absorbance of Coriander (Mean±SD)	% of Inhibition(Mean±SD)
8320.412	0.221 ± 0.0023	64.11
8320.412	0.223 ± 0.0003	64.3
8320.412	0.226 ± 0.0026	64.16

Explanation: The % of inhibition of Vit. E for 50, 150, 300, 500 (mg/L) concentration were ranging between 195.4% and 196.9% which is remarkably consistent. On the other hand, the average % of inhibition of coriander extracts for three samples of concentration of 8320.412 (mg/L) was 64.1% which is quite low compared to the inhibition of standard. So, it can be said that the inhibitory activity of coriander is quite lower than standard.

Chapter 4

Discussion

Coriander (*Coriandrum sativum*) is a plant of the Apiaceae family which is widely used in various cuisines and in preparing herbal medicine. Coriander and other plants of its family has been widely studied for its therapeutic activity. Different parts of this plant such as seeds have shown many pharmacological activity such as anti-inflamatory, antioxidant, antimicrobial, hypoglycemic, antidiabetic effects (Mandal & Mandal, 2015). Presence of essential oils, phenols, polyphenols, flavonoids are responsible for these properties. For its numerous therapeutic benefits, Coriander is an excellent choice for further exploration in medicinal research. Depending on this, present research was conducted to determine the therapeutic properties of the plant and it includes determining the phytoconstituents of the plant sample by employing GC-MS, its antioxidant activities using total flavonoid content total phenolic content and evaluating DPPH free radical scavenging assay.

The GC-MS analysis revealed that 59 phytoconstituents were present in the methanol extract of coriander plant. Some of the compounds are d-Mannitol, 1-decyl sulfonyl, tert-Butyl (2-aminophenyl)carbamate, 2TMS derivative, Loliolide, Nonadecane etc. Among them, 13-Docosenamide, (Z) is present in the highest concentration, as its area% is the highest, which is 49.82. This compound has antioxidant activity (Islam et al., 2021). Most of the compounds had antioxidant, antimicrobial, and anti-inflammatory effects. 8,11,14-Docosatrienoic acid, methyl ester, phytol, and squalene these compounds had antioxidant activity. Compounds like stigmasterol and γ-sitosterol are classified as sterols that have a cholesterol lowering effect and are

antidiabetic (Kaur et al., 2011; Balamurugan et al., 2011). Previous research on coriander hexane seeds extract revealed that it has antidiabetic effects (Zainab et al., 2022). Also, studies are done on the aqueous extract of coriander and results claimed that it has phenolic content which exerts antioxidant effect (Melo et al., 2005). GC-MS analysis of coriander essential oil revealed that linalool, γ-Teroinene and Pinene were present in high concentrations. Again, in-vitro antioxidant assay results stated that coriander has great antioxidant activity as its scavenging rates for BCBT is 9.02 mg/mL and CS-EO is 1.52 mg/mL (Nouioura et al., 2024). This indicates the importance of solvent and plant part choice in determining the effects of the plant extract as different solvents had different effects.

Total Phenolic Content (TPC) test was performed for analyzing antioxidant properties of the sample. As phenolic acids are part of polyphenols, they play their antioxidant role by reacting with free radicals; thus, they have antimicrobial, antiallergic, antiviral, and anticancer activity. The experiment was done by taking a sample (coriander extract) of 88 mg/l of concentration where the total phenolic content was 88.595 mg of GAE/g of dry extract, which indicates the presence of a good amount of phenolic acid. Oliveria decumbens, which is a plant of the same family (Apiaceae) as coriander, had a total phenolic content of 16.7 mg GAE/g plant extract (Esmaeili et al., 2018), which is very low compared to the coriander sample. Also, the polyphenolic content of coriander seeds was 12.2 gallic acid equivalents (GAE)/g, (Depa & Anuradha, 2011), which is also quite low compared to our sample. So, it can be said that the coriander sample contains a noteworthy amount of phenolic acid, thus it has antioxidant activity.

As flavonoid has several therapeutic properties such as antimicrobial, antioxidant, antiarthiric, and anticancer, to identify its presence, the total flavonoid content of the plant was measured. This antioxidant property plays an important role in preventing cardiovascular disease, cancer, and

deterioration of cell components caused by aging (Sulaiman & Balachandran, 2012). So this is why the total flavonoid content of the sample was measured to determine the antioxidant activity of the coriander extract. The experiment was done by taking a sample (coriander extract) of 234,634 mg/l concentration, where the total flavonoid content was 184.5 mg/g RE of dry extract, which indicates the presence of a generous amount of flavonoid. Other studies on coriander seed methanol extract showed that it had a flavonoid content of 1.114 ± 0.0005 mg quercetin equivalents per gram of samples (mg QE/g) (Tibebe et al., 2024), which is very low compared to our sample. Oliveria decumbens, which is a plant of the same family (Apiaceae) as coriander, had a total flavonoid content of 5.4 mg QE/g plant extract (Esmaeili et al., 2018), which is also very low compared to our coriander sample. As the coriander sample has a great amount of flavonoid content, it will be enough for free radical scavenging; thus, we conclude that the plant possesses antioxidant activity by this method.

Lastly, DPPH free radical scavenging method was also performed. As free radicals are unstable molecules with unpaired electrons, this assay measures the antioxidants ability to donate hydrogen, thus neutralizing DPPH radicals. In this study, Vitamin E was used as standard which exhibited very high % of inhibition ranging from 195.4% to 196.9% whereas coriander plant extract tested at a concentration of 8320.412 mg/L, had a % of inhibition of 64.1, which indicates moderate antioxidant activity. Although the % inhibition of coriander plant extract is very less than the standard, its significant free radical scavenging indicates that bioactive compounds with antioxidant properties are present. Previous study of DPPH scavenging assay on the methanolic extract of coriander leaf showed that it has antioxidant effect, the leaf extract had % inhibition of 95.95, 92.32, 89.77, and 87.63 at 1.0, 0.5, 0.25, and 0.125 mg/mL concentrations, respectively (L.

Joji Reddy et al., 2011) which is very high compared to our samples % inhibtion. We can say that as the concentration of extract increased, the % inhibition also increased. Again, the DPPH assay on coriander petroleum ether extract exhibited % inhibition of 76.75, 73.13, 53.30, and 49.25 at 1.0, 0.5, 0.25, and 0.125 mg/mL concentrations, respectively (L. Joji Reddy et al., 2011) which is also quite higher than our samples % inhibition.

Conclusion and Future Perspective

Conclusion

Since ancient times medicinal plants have been used in the treatment of various disease. But now a days medicinal plants are also researched so that they can be used for the development of modern medicine. For this the project work was based on identifying the phytoconstituents of coriander and determining the antioxidant activity of coriander as previous studies showed it had several therapeutic effects. Here, the GC-MS analysis revealed that coriander had about 59 compounds and the compounds had several therapeutic activities like antioxidant, antimicrobial, anti-inflammatory etc. Also, TPC, TFC and DPPH free radical scavenging assay were conducted to estimate the antioxidant property of the plant. The TPC test revealed that amount of phenolic content was 88.595 mg of GAE/g of dry extract. Also, the flavonoid content of the sample was 184.5 mg/g RE of dry extract which proves that the flavonoid content of the sample is great, so it has quite strong antioxidant activity. Lastly, evaluation of DPPH free radical scavenging assay revealed % of inhibition of was 64.1% of the sample and it indicates a moderate antioxidant activity. To sum up it can be said that the coriander plant extract has a strong antioxidant activity.

Future Perspective

As the research findings suggested that coriander extract had strong antioxidant activity and had diverse phytoconstituents so this plant can be suggested for future research. future studies can use advanced techniques like NMR or HPLC for identifying and isolating key components responsible for therapeutic benefits. Also different solvent and extraction methos coulde be utilized to maximize the yield of antioxidant compounds. Other properties of coriander like antimicrobial,

anti-inflammatory properties could be examined. Lastly, to assess acceptability the antioxidant effect of coriander extract coulde be tested in in-vivo models.

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