https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(3s) (October 2024); 6217-6230
Research Article

"In Vitro Evaluation of Pharmacological and Phytochemical Properties of Coriandrum sativum Microgreens and Their Potential Efficacy Against MCF-7 Breast Cancer Cells"

Disha S Patil[1*], Manoj L[2], Megha M[3], Lakshmi Kanth R N[4]

[1*]Department of Biotechnology and Genetics, M. S. Ramaiah College of Arts, Science and Commerce, Bengaluru, Karnataka, 560054, India.
[2]Department of Biotechnology and Genetics, M. S. Ramaiah College of Arts, Science and Commerce, Bengaluru, Karnataka, 560054, India.
[3]Department of Biotechnology and Genetics, M. S. Ramaiah College of Arts, Science and Commerce, Bengaluru, Karnataka, 560054, India.
[4]Department of Biotechnology and Genetics, M. S. Ramaiah College of Arts, Science and Commerce, Bengaluru, Karnataka, 560054, India.

ABSTRACT:
This Study highlights the key focus areas: in vitro pharmacological and phytochemical analysis, the use of Coriandrum sativum (coriander) microgreens, and the specific investigation into effects on MCF-7 breast cancer cells.
This study characterizes the essential oil derived from edible Coriandrum sativum L. microgreens, cultivated to a height of 2.5 to 7.5 cm with the emergence of true leaves, then harvested, dried, and powdered for analysis. Soxhlet extraction yielded a relatively high percentage of coriander microgreen oil, whose organoleptic and physicochemical properties, including oil globule observation, acid value, ester value, and saponification value, met ISO standards, indicating promising oil quality. GC-MS analysis further elucidated the oil's bioactive chemical profile, while qualitative and quantitative phytochemical assays highlighted significant levels of flavonoids, tannins, and alkaloids, underscoring its pharmacological potential. Notably, the presence of trans-2-decenoic acid—a mono-unsaturated fatty acid—was confirmed through thin-layer chromatography and quantified using high-performance liquid chromatography (HPLC). The health benefits of coriander microgreen oil were evaluated through various assays: antacid testing demonstrated substantial acid-neutralizing capacity and resistance to pH change; anti-cholesterol assays indicated effective lipid inhibition, with a notable half-maximal inhibitory concentration; and alpha-amylase inhibition revealed significant anti-diabetic potential. Antioxidant activity, assessed by DPPH, FRAP, and ABTS assays, was considerable. Furthermore, cytotoxicity testing against MCF-7 cell lines identified the oil as a potent anticancer agent. These findings suggest that C. sativum microgreen essential oil holds promise as a natural source of antacids, anti-diabetic, anti-cholesterol, and anti-carcinogenic agents, advocating its potential role as a nutritional component in daily diets for promoting community health.

Received: 30/09/2024 *Accepted: 04/10/2024*

DOI: https://doi.org/10.53555/AJBR.v27i3S.3524

© 2024 The Author(s).
This article has been published under the terms of Creative Commons Attribution-Noncommercial 4.0 International License (CC BY-NC 4.0), which permits noncommercial unrestricted use, distribution, and reproduction in any medium, provided that the following statement is provided. "This article has been published in the African Journal of Biomedical Research"

1. INTRODUCTION:

Nutrient deficiency is a pressing global health issue, with malnutrition and related diseases impacting children under five and adults over 40, particularly in developing countries. The National Family Health Survey (2019-21) ranked India 5th globally in nutrition challenges, reflecting limited improvement. Indian diets are typically low in essential micro- and macronutrients

crucial for growth, commonly found in green leafy vegetables, fruits, and fermented foods, making nutrient supplementation a priority (Global Nutrition Report 2021). Natural, plant-based products play a critical role in addressing nutrient deficiencies and related health issues, such as obesity, hyperglycemia, and hyperlipidemia. Microgreens young, tender greens harvested at 2.5–7.5 cm height have emerged as nutrient-dense sources rich in vitamins C, E, K, carotenoids, polyphenols, and essential minerals like calcium and magnesium. Studies show microgreens offer a stronger nutraceutical profile than mature greens, with benefits such as cholesterol reduction, improved gut health, anemia prevention, and anticancer properties.

Conveniently grown indoors year-round with minimal resources, microgreens are increasingly popular as both a culinary ingredient and a profitable commercial crop, supporting both nutrition and market potential.

Coriander (Coriandrum sativum), a well-known culinary herb from the Apiaceae family, is widely valued for its flavor and traditional medicinal uses, especially in the Mediterranean region of India. Both the plant and its essential oil serve diverse roles in food, medicine, and perfumery. Modern research highlights coriander's extensive pharmacological benefits, including antioxidant, antimicrobial, anticancer, neuroprotective, hypolipidemic, analgesic, anticonvulsant, and anti-inflammatory properties. Recent findings indicate that coriander microgreens are particularly rich in phytochemicals like Ca, Fe, Mn, and Zn, along with compounds such as monoterpenes, α-pinene, limonene, and linalool. Notably, coriander seed oil contains about 70% linalool, which has significant health effects, while the leaf oil is abundant in aromatic acids, including 2-decenoic acid. This study aims to establish C. sativum microgreens as a valuable culinary and medicinal plant by evaluating their phytochemical, antioxidant, and anticancer properties in vitro.

2. MATERIALS AND METHODS:

2.1 Coriandrum sativum Microgreens Cultivation

Coriander microgreens were grown under ambient conditions with proper nutrients and water supply, harvested at a height of 7.5 cm. Seeds from the multi-cut variety Arka Isha were sourced from ICAR, Bengaluru.

Growth Stages:

1. **Seeding/Sowing (Days 1-4):** Plastic tubs (3-4 inches deep) were filled with a soil mixture of red soil and vermicompost (1:2 ratio). Approximately 20g of seeds were sprinkled on top and covered with a layer of the soil mix. No sprouting was observed until Day 4.
2. **Sprouting (Days 5-7):** Under optimal conditions (24-32˚C, adequate light, and water), seeds began germinating, breaking their outer coats, and producing seed leaves. Sprouting was noted between Days 5 and 7.
3. **Emergence of Microgreens (Days 8-14):** Roots established, and the first pair of true leaves (cotyledons) emerged, marking the growth stage. The microgreens measured between 2.5-7.5 cm during this period.
4. **Harvesting (Days 15-18):** When the microgreens reached 7.5 cm, they were harvested above the soil level using sterile scissors. After washing to remove soil, they were air-dried and then subjected to a hot air oven for complete drying.



Harvest Microgreen Length

2.2 Drying and Powdering:

The harvested coriander microgreen was allowed to dry in hot air oven at 37℃ for approximately 2 days in a petri dish and over a foil paper, till they become completely dry with a brittle texture. After they are completely dried, they are powdered using motor and pestle and even by using mixer grinder and weighed for the dry weight of powder to estimate percentage of the harvest.

2.3 Extraction of Essential Oil

2.3.1 Soxhlet Extraction:

Soxhlet extraction, also known as continuous extraction, is a widely used method for liquid- solid extraction of oils from plant sources using solvents. This efficient technique maximizes analyte extraction while allowing for solvent recycling. The Soxhlet apparatus consists of three main components: a 250 ml round-bottom flask, a 100 ml thimble, and a condenser.

For this extraction, 70% ethanol was used as the solvent due to its high yield of the mono- unsaturated fatty acid 2-decenoic acid. The procedure involved placing 20 g of coriander microgreen powder in a Whatman filter paper thimble, which was then set in the extractor. The round-bottom flask contained 200 ml of 70% ethanol, heated to approximately 70°C (below ethanol's boiling point of 78.5°C).

As the solvent vapor passed through the thimble, it condensed and dripped onto the packed microgreens, breaking the cells and releasing the oil into the solvent. This cycle continued until the thimble reached 100 ml capacity, completing one cycle. The extraction was performed for about 12 cycles over 50 hours, until the solvent became colorless. The collected solvent, containing essential oil, was then subjected to distillation to remove ethanol, and the Soxhlet

extraction was repeated five times with fresh samples using the same method.

2.3.2 Distillation

The distillation method is used to separate components based on their boiling points, allowing for the partial purification of oil from the solvent. The distillation setup includes:

- a 250 ml round-bottom flask,
- a distillation adapter with a thermo-pocket and 110°C thermometer,
- a condenser, and
- a receiving adapter with a receiving flask.

In this process, the solvent extracted via Soxhlet extraction is poured into the round-bottom flask, which is then heated using a heating mantle. The flask is connected to the distillation adapter, thermometer, and condenser, with a continuous flow of water in the condenser. The temperature is set to approximately 80°C to facilitate the separation of ethanol, which evaporates at 78.5°C.

The ethanol is collected in the receiving flask over 1 hour and 30 minutes, leaving behind grease, oil, and a small amount of ethanol in the round-bottom flask. The remaining mixture is then filtered to obtain the crude oil.

2.3.3 Filtration

Filtration is used to separate components based on size, specifically to remove greasy materials from the oil. The remaining extract in the round-bottom flask after distillation was filtered using Whatman filter paper (grade No. 1, 125 mm) in two layers within a glass funnel, collecting the filtrate in a glass bottle. Filtration continued until the extract turned pale yellow, effectively removing grease. The resulting extract contained essential oil along with small amounts of water and ethanol.

2.3.4 Water Bath Evaporation

Water bath evaporation is employed to eliminate residual impurities (water and ethanol) from the oil. This method is preferred over dry heat, which can convert mono-unsaturated fatty acids to saturated ones. The extract was placed in a china dish on a water bath set at 60°C for approximately 2 hours, allowing for effective evaporation. After this process, the crude oil was extracted and transferred to a glass bottle for storage in the refrigerator.

2.4 Yield of the extract

2.4.1 Percentage of Extraction:

The weight of the packed microgreens powder after the Soxhlet extraction process was taken using the weighing balance to know the percentage of extraction using the formula:

$$\% \text{ of Extraction} = \frac{\text{Weight of the powder (before Soxhlet extraction)} - \text{Weight of the powder (after Soxhlet extraction)}}{\text{Weight of the powder before Soxhlet extraction}} \times 100$$

2.5 Characterization of Oil

2.5.1 Physico-Chemical Characterization:

Physico-chemical characterization of crude coriander microgreen essential oil involves analyzing its physical and chemical properties to understand its behavior and characteristics. Physical characterization includes parameters such as density, viscosity, color, aroma, and oil globules. Chemical characterization encompasses measurements of acid value, saponification value, and ester value.

2.5.1.1 Physical Characterization of Oil

2.5.1.1.1 Observation of Oil Globules:

A drop of crude coriander microgreen essential oil was placed on a clean, grease-free glass slide and examined under a 100X oil immersion microscope. The oil globules appeared spherical and were slightly denser than water.

2.5.1.2 Chemical characterization of oil:

Chemical parameters of the essential oil were analyzed using International Organization of standards (ISO) values as the standard reference.

2.5.1.2.1 Acid Value

To determine the acid value of crude coriander microgreen essential oil, take approximately 0.1 g of the oil in a clean conical flask. Add 10 ml of ethanol and 1-2 drops of phenolphthalein indicator. Titrate the solution with 0.02N KOH until the color changes from colorless to pale pink, maintaining the color for about 30 seconds. Record the volume of KOH used and repeat the titration for accuracy. Perform a blank test using distilled water instead of oil and note the volume of KOH used in the blank. Calculate the acid value of the oil using the titration volumes from the sample and blank.

$$\text{Acid value (mg/g)} = \frac{\text{Mol. weight of KOH} \times \text{Normality of KOH} \times (V_s - V_o) \times F}{W}$$

Molecular weight of KOH = 56.11
Normality of KOH = 0.02
V_s = Titration volume of sample
V_o = Titration volume of blank
F = Factor of 0.02N KOH solution, where

$F = \frac{5}{V_t}$: V_t is the volume of 0.02N KOH required to neutralize 5 ml of the 0.02N H_2SO_4 solutions

W = Weight of oil sample

2.5.1.2.2 Saponification Value

To determine the saponification value of crude coriander microgreen essential oil, take 0.5 g of the oil in a clean conical flask and add 10 ml of 0.5N ethanolic KOH. Mix thoroughly and heat the mixture in a water bath at 80-85°C for 30 minutes. Allow it to cool to room temperature. Titrate the mixture with 0.5N HCl, adding 2-3 drops of phenolphthalein indicator, until the color changes from pink to colorless. Record the volume of HCl used and repeat for accuracy. Prepare a blank using distilled water instead of oil, noting the titer volume. Calculate the saponification value using the titer volumes from both the sample and the blank.

$$\text{Saponification value (mg/g)} = \frac{\text{Half Mol. weight of KOH} \times (A\text{-}B) \times F}{S}$$

Half molecular weight of KOH = 28.05
A = Titration volume of blank (ml)
B = Titration volume of sample (ml)
F = Factor of 0.5N HCl standard solution

$F = \frac{5}{V_t}$: V_t is the volume of 0.5N HCl required to neutralize 5ml of the 0.5N ethanolic KOH solutions.

S = Oil Weight

2.5.1.2.3 Ester value:

The ester value of the crude coriander microgreen essential oil was calculated using the obtained acid and saponification value, using the formula:

Ester value = Saponification value – Acid value

2.5.1.3 Chemical composition of oil:

2.5.1.3.1 Gas Chromatography – Mass Spectroscopy (GC-MS): GC-MS Methodology

The analysis was performed using a Clarus 680 GC equipped with a fused silica column packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane; 30 m × 0.25 mm ID × 250 μm df). Components were separated using helium as the carrier gas at a constant flow rate of 2 ml/min. The injector temperature was set to 220°C, and 1 μL of the extract sample was injected. The oven temperature program was as follows: 50 °C (2 min), ramping to 150 °C at 15 °C/min, holding at 150 °C for 2 min, ramping to 250 °C at 30 °C/min, and holding for 8 min. Mass detector conditions included an inlet line temperature of 250 °C, ion source temperature of 230 °C, and electron impact ionization at 70 eV. The scan time was 0.2 sec with a scan interval of 0.1 sec, covering fragments from 40 to 600 Da. The spectra of the components were compared with the NIST (2014) database of known components.

2.6 Phytochemical Analysis

2.6.1 Qualitative Analysis of Ethanolic Extract of Coriander Microgreens:

Qualitative analysis was conducted to screen for active phytochemical constituents in the crude coriander microgreen essential oil, highlighting its potential health

benefits.

2.6.1.1 Determination of Flavonoids:
To test for flavonoids, add 1 ml of the extract to a clean test tube, followed by 1 ml of 2N NaOH. Then, add a few drops of concentrated HCl and observe for a yellow coloration, which indicates the presence of flavonoids. For the blank, use 1 ml of distilled water instead of the extract.

2.6.1.2 Determination of Alkaloids:
To test for alkaloids, add 2 ml of the extract to a clean test tube and mix in a few drops of 2N HCl. Heat the mixture in a water bath at 50°C for 10 minutes, then cool and filter the solution. Add a few drops of Wagner's reagent and observe for a red precipitate, indicating the presence of alkaloids. For the blank, use 2 ml of distilled water instead of the extract.

2.6.1.3 Determination of Cardiac Glycosides:
To test for cardiac glycosides, add 2ml of the extract to a clean test tube along with 3 ml of glacial acetic acid and a drop of 5% $FeCl_3$. Carefully add 0.5 ml of concentrated H_2SO_4 along the sides of the test tube and observe for a blue color in the acetic acid layer, indicating the presence of cardiac glycosides. For the blank, use 2 ml of distilled water instead of the extract.

2.6.1.4 Determination of Tannins:
To test for tannins, add 1 ml of the extract to a clean test tube, followed by 1 ml of 5% $FeCl_3$. Observe for the formation of a greenish-black pigment, indicating the presence of tannins. For the blank, use 1 ml of distilled water instead of the extract.

2.6.1.5 Determination of Terpenoids:
Add 1 ml of the extract to a clean test tube, followed by 1 ml of chloroform and 4 to 5 drops of concentrated H_2SO_4. Observe for the formation of a reddish-brown interphase, indicating the presence of terpenoids. For the blank, use 1 ml of distilled water instead of the extract.

2.6.1.6 Determination of Quinones:
In a clean test tube, mix 1 ml of the extract with 1 ml of concentrated H_2SO_4. Observe for a red color, indicating the presence of quinones. For the blank, use 1 ml of distilled water instead of the extract.

2.6.1.7 Determination of Saponins:
To a clean test tube, add 5 ml of the extract and 10 ml of water, then shake vigorously. Observe for persistent foam, indicating the presence of saponins. For the blank, use 5 ml of distilled water instead of the extract.

2.6.2 Quantitative Analysis of Ethanolic Extract of Coriander Microgreens:
The concentration of secondary metabolites in the extracted crude coriander microgreen essential oil was estimated.

2.6.2.1 Estimation of Flavonoids (Total Flavonoid Content) by $AlCl_3$ Colorimetric Assay:
Total flavonoid content was measured using the aluminium chloride colorimetric assay. Mix
0.1 ml of extract in a 10 ml volumetric flask and dilute to volume with distilled water. Add
0.30 ml of 5% sodium nitrite, followed by 0.30 ml of 10% aluminium chloride after 5 minutes.
After another 5 minutes, add 2 ml of 1M sodium hydroxide and dilute to 10 ml with distilled water. Prepare a series of quercetin dihydrate standard solutions (20, 40, 60, 80, and 100 µg/ml) similarly. Measure the absorbance of the test and standard solutions against a reagent blank at 510 nm using a UV/Visible spectrophotometer. Determine the total flavonoid content from the standard curve.

2.6.2.2 Estimation of Tanin Content by Folin-Ciocalteau Method:
Tannin content was determined using the Folin-Ciocalteau method. In a test tube, 0.1 ml of extracted crude coriander microgreen essential oil was diluted to 10 ml with distilled water. Then, 0.25 ml of 1N Folin-Ciocalteau reagent and 1.25 ml of 20% sodium carbonate solution were added. The mixture was shaken and allowed to stand at room temperature for 40 minutes. Reference standard solutions of tannic acid (20, 40, 60, 80, and 100 µg/ml) were prepared similarly. The absorbance of the test and standard solutions was measured against a blank at 725 nm using a UV/Visible spectrophotometer. The tannin content was calculated from the standard curve.

2.6.2.3 Estimation of Alkaloids Content by Titration Method:
To estimate alkaloid content, 0.2 g of extracted crude coriander microgreen oil and 0.2 g of coriander microgreen powder were each mixed with 25 ml of distilled water and 10 ml of ammonium solution. The mixtures were stirred and transferred to separating funnels, followed by the addition of 20 ml of chloroform. The chloroform layer, which contains the alkaloids, was collected, and this process was repeated three times.
The combined chloroform layers were evaporated in a water bath at 60°C, leaving a residue. This residue was dissolved in 2 ml of ethanol and evaporated again. The resulting residue was mixed with 20 ml of distilled water and 20 ml of 0.1N HCl, forming the dissolved extract.
In a clean conical flask, 10 ml of the dissolved extract was combined with a few drops of methyl orange indicator and titrated against 0.1N NaOH until the color changed from pink to yellow. The volume of 0.1N NaOH used in the titration was recorded, allowing for the calculation of atropine concentration using the appropriate formula.

Each ml of 0.1N NaOH = 0.03384 µg of Atropine

2.7 Thin Layer Chromatography (TLC):

Thin Layer Chromatography (TLC) is employed to separate components based on molecular weight. A pre-coated TLC sheet with silica gel serves as the stationary phase, while the mobile phase consists of a solvent. A pencil line is drawn 2 cm from the bottom of the TLC sheet, where the extracted crude coriander microgreen essential oil and the standard (2-decenoic acid) are applied. After drying, the sheet is placed in a chromatographic chamber with solvent, allowing the solvent to travel up to three-quarters of the sheet, after which it is removed and air-dried.

To detect the monounsaturated fatty acid, 2-decenoic acid, the chromatogram is sprayed with Chromosulfuric acid reagent and heated at 150°C to char the organic components. The charring positions indicate the presence of the target component, and the Rf value can be calculated using the appropriate formula.

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

2.8 HPLC

Standard solution preparation:

Accurately weighed 10 mg of 2-Decanoic acid standard is transferred into 10 ml standard volumetric flask. 2 ml of DMSO was added and dissolved using sonicator. Made up the volume with the ethanol (10 ml).

Buffer preparation:

Accurately measured Milli Q water is transferred to 1000 ml beaker and 2.5 ml of trimethylamine was added. After dissolution, make up the volume with the same and adjust the pH 3.5 with ortho phosphoric acid. Filtered buffer was used for the analysis.

And the concentration of the target component i.e., 2-Decenoic acid in the extracted crude coriander microgreen essential oil was calculated using the formula:

$$\text{\% of the Target Compound} = \frac{\text{Sample area}}{\text{Standard area}} \times \text{Standard dilution factor} \times \text{Sample dilution factor} \times 100$$

2.9 EVALUATION OF POTENTIAL HEALTH BENEFITS OF CORIANDER MICROGREEN

2.9.1 Antacid Activity:

Antacid activity was assessed using the Rossett-Rice method to evaluate the in vitro acid neutralization capacity of coriander microgreen oil compared to standard $NaHCO_3$. This method measures the time the pH remains between 3.0 and 5.0, as antacid drugs typically maintain a pH of 4.38 ± 0.21.

In the experiment, three 500 ml glass beakers containing 70 ml of 0.1N HCl and 30 ml of distilled water were placed on an electric magnetic stirrer, maintaining a temperature of 37°C. The pH was continuously monitored using a pH meter. Different volumes of coriander microgreen essential oil (12.5–50 mg) were added to the solution. A burette filled with 0.1N HCl delivered acid at a rate of 4 ml/min to simulate normal acid secretion. The pH was recorded, and the Rossett-Rice time was determined, indicating the oil's neutralization capacity. This test was repeated with concentrations of 25 mg and 50 mg of the oil.

2.9.2 Anti-Cholesterol Activity:

The anti-cholesterol activity of extracted crude coriander microgreen essential oil fractions was assessed using the photometric method with the Liebermann-Burchard reagent. The oil and its fractions were tested across a concentration range of 20, 40, 60, 80, and 100 µg/ml.

The procedure involved adding 2.5 ml of 1% cholesterol standard to the sample, shaking for 2 minutes, and then adding 2 ml of glacial acetic acid and 0.1 ml of concentrated H_2SO_4. The mixture was allowed to stand at room temperature for 16 minutes before measurement. Ethanol served as the blank, with 1% cholesterol standard as a negative control and Roseday (5 mg) at the same concentrations as a positive control.

The residual cholesterol was quantified by measuring absorbance at λ_{max} 420 nm using a spectrophotometer. The IC50 concentration of the coriander oil was determined, and the percentage inhibition was calculated using the appropriate formula.

$$\text{\% Inhibition of Cholesterol} = \frac{\mathbf{A0} - \mathbf{As} \times 100}{\mathbf{A0}}$$

Where, **A0**: Absorbance control and **As**: Absorbance sample

2.9.3 Anti-Diabetic Activity:

The anti-diabetic activity of the extracted crude coriander microgreen essential oil was assessed using an in vitro alpha-amylase activity method. A 96-well plate was

prepared with test samples of the oil at concentrations ranging from 20 µg/100 µl to 100 µg/100 µl, along with 15 µL of PBS (pH 6.8), 40 µL of 2% starch solution, and 25 µL of alpha-amylase enzyme (0.14 U/ml). The reaction was halted by adding 20 µL of 1 M HCl to each well, followed by 90 µL of 0.1M iodine reagent. Acarbose (50 mg) served as a positive control, while DMSO was used as a negative control. The absorbance was measured at 620 nm using an ELISA reader, and the IC50 concentration of the coriander oil was determined. The percentage of amylase inhibition was calculated using the appropriate formula.

$$\% \text{ Relative enzyme activity} = \frac{\text{Enzyme activity of test sample}}{\text{Enzyme activity of control}} \times 100$$

$$\% \text{ Inhibition in alpha amylase activity} = (100 - \% \text{ relative enzyme activity})$$

2.10 Anti-Oxidant:

2.10.1 Determination of Antioxidant Activity by DPPH Scavenging Activity:

Add 0.01 mL of various concentrations of the test substance and standard to a 96-well microtiter plate, while using 0.01 mL of DMSO as control and control blank. Add 0.2 mL of DPPH solution to the test and control wells, and 0.2 mL of methanol to the test blank and control blank wells. Repeat for the standard, replacing the test substance with the standard solution. Incubate the plate at 37°C for 30 minutes and measure absorbance at 490 nm using a microplate reader.

2.10.2 Determination of Antioxidant Activity by Ferric Ion Reducing Power Assay:

In the test and test blank, add 0.5 mL of the test oil to 2.5 mL of PBS and 2.5 mL of potassium ferric cyanide. For control and control blank, replace the test oil with 0.5 mL of DMSO and use distilled water instead of potassium ferric cyanide. Incubate the reaction mixture at 50°C for 30 minutes. After incubation, add 2.5 mL of 10% TCA to the test and control, and replace it with distilled water for the test blank and control blank. Centrifuge the mixture at 3000 rpm for 10 minutes and mix 5 mL of the supernatant with an equal volume of distilled water. Then add 1 mL of 0.1% $FeCl_3$ to the test and control, and replace it with distilled water for the blanks.

Incubate the reaction mixture at room temperature for 10 minutes. Transfer 100 µL of the reaction mixture to a microtiter plate. Repeat for the standard, replacing the test sample with the standard solution. Measure absorbance at 700 nm using an ELISA reader; higher absorbance indicates stronger reducing power.

2.10.3 Determination of Antioxidant Activity by ABTS Radical Assay:

For the test, mix 0.2 mL of the test oil or standard with 1.0 mL of PBS and 0.16 mL of ABTS solution to achieve a final volume of 1.36 mL. In the control, replace the test substance with

0.2 mL of methanol. For the test blank and control blank, use 0.16 mL of distilled water instead of the ABTS reagent. Incubate all tubes for 20 minutes. After incubation, pipette 0.1 mL of the reaction mixture into a microtiter plate and measure absorbance at 734 nm using an ELISA reader. Repeat the procedure for the standard by replacing the test sample with the standard solution. Perform the test and control in triplicate, while the blanks are conducted in singlet.

2.11 Anti-Cancer

Anti-Cancer Activity Assessment Using MTT Assay:

The in vitro MTT assay was performed to evaluate the cytotoxicity of extracted crude coriander microgreen essential oil on the Human Breast Cancer (MCF-7) cell line, assessing cell viability.

Preparation of Test Compounds:

1 mL of the test substance was dissolved in 100 mL of DMEM medium with 2% inactivated FBS to create a 1 mg/mL stock solution. Similarly, 1 g of microgreen powder was dissolved in 100 mL of DMEM to obtain another 1 mg/mL stock.

Cell Culture:

MCF-7 cells were obtained from NCCS India and cultured in MEM with 10% FBS, penicillin, streptomycin, and amphotericin B in a humidified atmosphere (5% CO_2, 37 °C). Cells were dissociated with TPVG solution and maintained in 25 cm^2 flasks. Experiments were conducted in 96-well microtiter plates.

Determination of Cell Viability:

Cells were trypsinized, centrifuged, and resuspended to yield approximately 10,000 cells per 200 µL. This suspension was added to each well and incubated for 24 h. After aspirating the medium, different concentrations of the test substance (50, 100, 150, 200, and 250 µg/mL) were added, followed by another 24 h incubation.

Afterwards, 100 µL of medium containing 10% MTT reagent (final concentration 0.5 mg/mL) was added, and the plate was incubated for 3 h. The culture medium was removed, and 100 µL of DMSO was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using a microplate reader. The percentage of growth inhibition was calculated, and the IC50 value was

determined from the dose-response curve.

3. RESULT AND DISCUSSION:

3.1 Growth and Yield of the extract

The harvested coriander microgreen had the wet weight as 234.41g after the drying process in the hot air oven the dry weight obtained was 20g. The extraction yield of coriander microgreen essential oil, obtained via Soxhlet extraction, was 35.2% with a relative concentration of 0.1g/mL on a dry weight basis. This yield, though variable due to climatic and geographic factors, is considered relatively high.

3.2 Characterization of oil

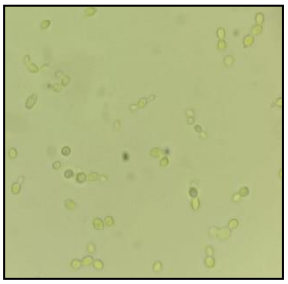
The organoleptic and physicochemical properties of coriander microgreen essential oil, including oil globule visualization under oil immersion microscopy, acid value, ester value, and saponification index, were assessed as quality indicators. These tests, aligned with ISO standards, serve as preliminary evaluations of the oil's quality (Table 1).

3.2.1 Physical Characterization of Oil

3.2.1.1 Observation of Oil Globules:

Using 100X oil immersion microscopy, the oil globules in extracted crude coriander oil appeared ovular and denser than water.

Observation of Oil Globules under Microscope



3.2.2 Chemical Characterization of Oil

3.2.2.1 Acid Value:

The acid value reflects the amount of free acids, with a lower value indicating higher oil quality. The acid values for the ISO standard, reference, and our sample were 3, 2, and 3.43 mg/g, respectively, suggesting minimal oxidation in our sample.

3.2.2.2 Saponification Value:

The saponification value indicates the amount of potassium hydroxide needed to saponify one gram of fat, pointing to the presence of short- and medium-chain fatty acids. The saponification values for our sample, reference, and ISO standard were 25.245, 23.51, and 25 mg/g, respectively, confirming these fatty acids in the coriander oil.

3.2.2.3 Ester Value:

Based on acid and saponification values, the ester content was calculated. Higher ester values suggest greater ester content, with our sample and reference at 21.809 and 21.51 mg KOH, and the ISO standard at 22 mg KOH. These values indicate the presence of low molecular weight fatty acids, such as tetradecenoic acid, in the coriander oil.

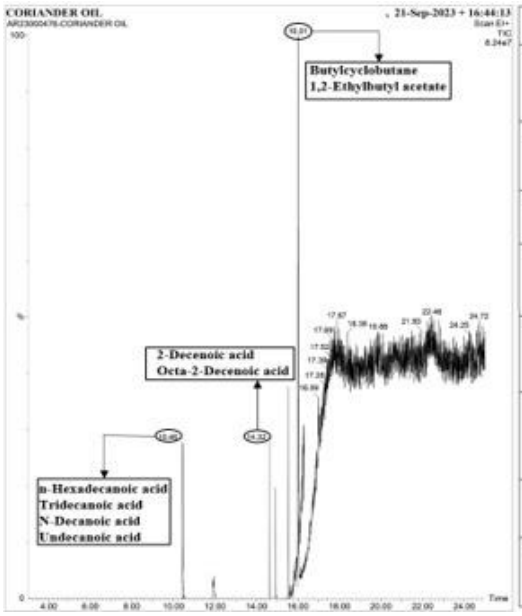
Table 1: Physico-chemical characteristics

Extracted crude coriander microgreen essential oil				
Sl No.	**Parameters studies**	**Reference paper**	**ISO**	**Our study**
1	**Acid value**	2	3	3.4352
2	**Saponification value**	23.51	25	25.245
3	**Ester value**	21.51	22	21.8098

3.2.3 Chemical Composition of oil:

Gas Chromatography – Mass Spectroscopy (GC-MS):

Physico-chemical characterization alone is insufficient to fully assess the quality and composition of coriander microgreen oil. To achieve a comprehensive analysis, it is essential to establish the chromatographic profile of its aromatic compounds. GC/MS analysis was performed on the ethanolic extract of coriander microgreen oil to identify its primary biochemical constituents. Gas Chromatography (GC) separates the mixture's components, while Mass Spectrometry (MS) analyzes and identifies them. The GC/MS chromatogram displays retention time on the X-axis (or mass-to-charge ratio in the spectrum) and intensity counts on the Y-axis, indicating component quantity. Key peaks for the crude extract were observed at 16.01, 14.32, and 10.46. Table 2 provides detailed constituent information, including data from the NIST library.



Name	Structure	MoLwt g/mol & Mol. formula
n-Hexadecanoic acid		256.24023 $C_{16}H_{32}O_2$
Tridecanoic acid		214.19328 $C_{13}H_{26}O_2$
n-Decanoic acid		172.14633 $C_{10}H_{20}O_2$
Undecanoic acid		186.16198 $C_{11}H_{22}O_2$
Butylcyclobutane		112.1252007 C_8H_{16}
1,2-Ethylbutyl acetate		144.115029 $C_8H_{16}O_2$
2-Decenoic acid		170.250019 $C_{10}H_{18}O_2$
Octa-2-Decenoic acid		282.500262 $C_{18}H_{34}O_2$

Table 2: NIST Library data of GCMS Result

3.3 Phytochemical analysis

3.3.1 Qualitative analysis of ethanolic extract of coriander oil:

Phytochemicals, produced through primary or secondary plant metabolism, were analyzed in the ethanolic extract of coriander microgreen oil to identify secondary metabolites. Tests confirmed the presence of flavonoids (sodium hydroxide test), alkaloids (Wagner's reagent), tannins (ferric chloride), and saponins (foam test), while cardiac glycosides, terpenoids, and quinones tested negative (Table3). This phytochemical profile highlights the medicinal potential of coriander microgreens. Flavonoids function as antioxidants, hypolipidemic, and anticancer agents; alkaloids act as anesthetics and anti-inflammatory agents; tannins serve as antioxidants; and saponins help reduce blood lipids, cancer risk, and blood glucose, thus making coriander microgreens pharmacologically valuable.

Table 3: Qualitative analysis of ethanolic extract of coriander oil

Sl No.	Metabolites	Present/Absent
1	Flavonoids	+
2	Alkaloids	+
3	Cardiac Glycoside	-
4	Tannin	+
5	Terpenoid	+
6	Quinones	-
7	Saponin	+

Where, '+': Present and '-': Absent

3.4.2 Quantitative analysis of ethanolic extract of coriander oil:

The concentration of secondary metabolites Flavonoids, Alkaloids and tannins were carried out.

3.4.2.1 Estimation of Flavonoids [Total Flavonoids Content] by $AlCl_3$ colorimetric assay:

The total flavonoid content in coriander microgreen essential oil was assessed using the $AlCl_3$ colorimetric assay, with quercetin dihydrate as the standard. Flavonoids, known for their antioxidant and chelating properties, help inhibit lipid peroxidation and interact with reactive oxygen species. The assay involved forming stable complexes between aluminum chloride and flavonoids. A concentration range of quercetin (20-100 µg/ml) was used, and the absorbance of the oil sample was measured at 510 nm. The flavonoid concentration in the coriander microgreen oil was determined to be 22.20 mg/ml.

3.4.2.2 Estimation of Tannin content by Folin-Ciocalteau method:

Tannins, complex phenolic compounds, possess antioxidant properties that can help lower cholesterol, reduce blood pressure, and stimulate the immune system. Their concentration in coriander microgreen essential oil was quantified using the Folin-Ciocalteau method, with tannic acid as the standard. This assay

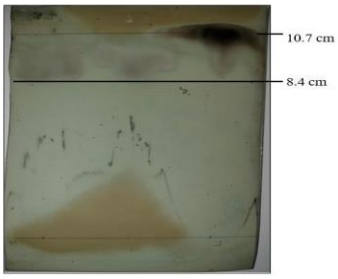
measures the reduction of Folin-Ciocalteau reagent by phenolics, resulting in a blue complex that is quantified spectrophotometrically at 725 nm. Tannic acid concentrations ranged from 20-100 µg/ml, and the tannin content in the coriander microgreen oil was found to be 0.564 mg/ml.

3.4.2.3 Estimation of Alkaloids content by Titration method:

Alkaloids are active organic compounds with significant pharmacological properties. The total alkaloid content in the ethanolic extract of coriander microgreen oil was determined using a titrimetric method, with titration indicated by the formation of a yellow color. The titration utilized 0.1N NaOH, where each milliliter was equivalent to 0.03384 µg of standard atropine. The concentration of alkaloids in coriander microgreen oil was found to be 0.089 µg/g, while the powder contained a slightly higher concentration of 0.101 µg/g.

3.5 Thin Layer Chromatography (TLC):

Thin-layer chromatography (TLC) was used to separate lipids based on their retention time, specifically targeting trans-2-decenoic acid. The procedure was conducted in one dimension using different solvent systems for dissolution. The resolved lipid species were visualized by charring with chromosulphuric acid, revealing three brown bands on the silica gel plate: the ethanolic extract of the sample, the ethanolic standard, and the methanolic standard of trans-2- decenoic acid. The Rf values for both the standard and sample were measured to be 0.7850, confirming the presence and purification of trans-2-decenoic acid in coriander microgreens.



TLC Bands

3.6 High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) was used to quantify 2-decenoic acid in coriander microgreen oil extracts. The oil sample, dissolved in methanol, was analyzed with a mobile phase of 0.25% triethylamine and acetonitrile (60:40) on a C18 column at 254 nm. A sharp peak at 2.707 minutes indicated that the 2-decenoic acid content was 15.8%.

3.7 EVALUATION OF POTENTIAL HEALTH BENEFITS OF CORIANDER MICROGREEN

3.7.1 Antacid:

Coriander microgreen oil was evaluated for its antacid properties through the Rosette-Rise test, measuring its acid neutralization capacity. Different concentrations (12.5 mg, 25 mg, and 50 mg) of the oil were tested, showing increased antacid activity with higher concentrations. The oil-maintained pH levels between 3.0 and 5.0 for 3.47 minutes, 8.25 minutes, and 15.24 minutes, respectively, compared to 10.8 minutes for the standard sodium bicarbonate (2.5 mg). Results indicated that coriander microgreen oil has significant acid neutralizing capacity, suggesting its potential use as an antacid to inhibit gastric secretion.

3.7.2 Anti-Cholesterol:

The hypolipidemic activity of coriander microgreens was assessed through in vitro anti- cholesterol activity using the Lieberman-Burchard reagent, which visually indicates cholesterol degradation. Cholesterol was oxidized with acetic anhydride and chloroform, forming a greenish complex. As the concentration of coriander microgreen oil increased (20-100 µg/ml), the percentage of cholesterol inhibition rose significantly, ranging from 23.35% to 64.26%. The standard drug, Roseday, showed inhibition percentages of 35.47% to 73.21% (Table 4). The IC50 values for coriander oil and Roseday were 74.66 µg/ml and 55.07 µg/ml, respectively, indicating significant anti-cholesterol activity. The presence of flavonoids in coriander microgreens suggests their role in reducing LDL and increasing HDL. While the IC50 of coriander microgreen oil is higher than Roseday, it demonstrates potential as a natural aid for hyperlipidemia.

Table 4: Percentage of inhibition of cholesterol

Sl. No.	Concentration (µg/ml)	The Oil Sample	Std. Roseday Drug
1	20	23.35025%	35.47053%
2	40	35.32995%	41.88211%
3	60	42.43655%	50.15512%
4	80	49.44162%	60.91003%
5	100	64.26396%	73.21613%

3.7.3 Anti-Diabetic:

The enzyme α-Amylase plays a crucial role in polysaccharide breakdown during digestion, primarily found in pancreatic juice and saliva. To evaluate the anti-diabetic activity of coriander microgreen essential oil, the percentage of α-Amylase inhibition was measured using concentrations ranging from 20-100 µg/ml. The breakdown of starch to glucose and maltose was indicated by color changes: dark blue for starch presence, yellow for absence, and brownish for degradation. As the concentration of coriander oil increased, the brownish color intensity decreased, showing significant enzyme inhibition in a dose-dependent manner. The percentage of inhibition ranged from 16.17% to 61.03% for the oil, compared to 19.72% to 72.03% for the standard drug Acarbose (Table 5).

The calculated IC50 values were 78.11 µg/100 µl for coriander oil and 65.71 µg/100 µl for Acarbose, indicating that while coriander oil's IC50 is slightly higher, it shows promise as an effective anti-diabetic agent.

Table 5: Percentage of inhibition of alpha amylase

Sl. No.	Concentration (µg/100µl)	The Oil Sample	Std. Acarbose Drug
1	20	16.17933%	19.72354%
2	40	27.51078%	34.17654%
3	60	43.35611%	45.20340%
4	80	50.76345%	60.18957%
5	100	61.03180%	72.03594%

3.8 Anti-Oxidant:

Free radicals, which have an unpaired electron, are generated during oxidative stress. Primary antioxidants neutralize these radicals through hydrogen atom transfer (HAT) or single electrontransfer (ET) mechanisms. The in vitro antioxidant capacity of coriander microgreen essential oil was assessed using radical scavenging assays (DPPH and ABTS) and the reducing antioxidant power assay (FRAP), with ascorbic acid serving as the standard in all assays.

3.8.1 Determination of Antioxidant Activity by DPPH Scavenging Activity:

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay measures antioxidant potential through an electron transfer method. In this assay, the odd electron of the nitrogen atom in DPPH is reduced when antioxidants donate a hydrogen atom, resulting in the violet complex turning colorless. The degree of discoloration indicates the radical scavenging potential of the sample. The coriander microgreen oil demonstrated antioxidant activity, with an IC50 value of 980.71 µg/ml, reflecting its effectiveness against DPPH.

3.8.2 Determination of Antioxidant Activity for Ferric Ion Reducing Power Assay:

The Ferric Reducing Antioxidant Power (FRAP) assay evaluates the reducing capacity of antioxidants by measuring their ability to convert ferric ions to ferrous ions through an electron transfer reaction, resulting in a blue-colored complex. The ethanolic extract of coriander microgreen oil demonstrated significant ferric reducing power, with an IC50 value of 738.36 µg/ml.

3.8.3 Determination of Antioxidant Activity from ABTS Radical:

The ABTS assay utilizes a single electron transfer (SET) method, where the oxidized ABTS cation reacts with antioxidants, reducing the bluish-green ABTS radical to a colorless form. The IC50 value of coriander microgreen essential oil in this assay was found to be 251.63µg/ml.

Variations in antioxidant activity may stem from different growing conditions, which influence the biosynthesis of secondary metabolites. The antioxidant capacity of coriander microgreens is largely linked to ascorbic acid content, a potent antioxidant in both plants and humans. Additionally, differences in antioxidant activity among Apiaceae species can be attributed to genotypes, growth conditions, and fertilization practices.

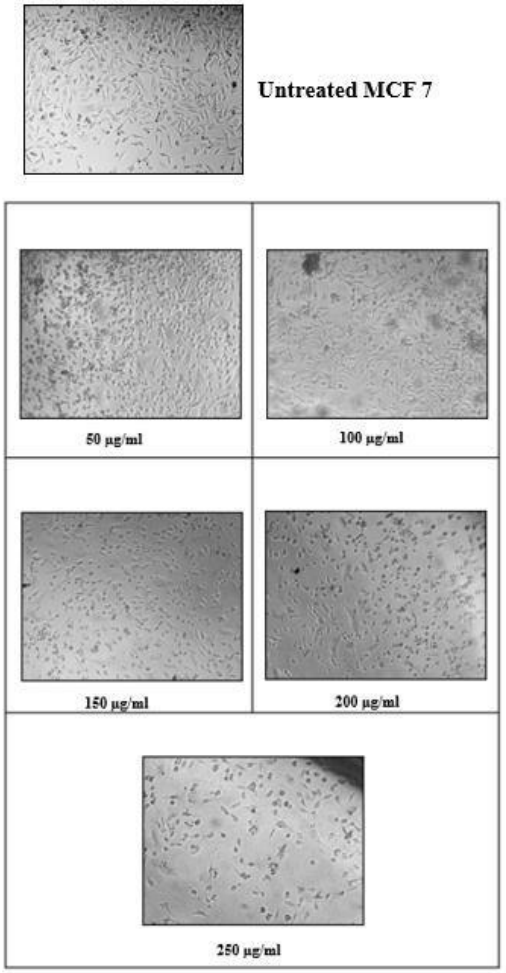
3.9 Anti-Cancer Activity of Coriander Microgreens

Coriandrum sativum demonstrates potential anti-cancer properties, inducing cytotoxicity in cancer cells due to its bioactive constituents. The cytotoxic effect of coriander oil was evaluated using the MTT assay against MCF-7 human breast cancer cell lines. This colorimetric assay measures cell viability by the reduction of yellow MTT to purple formazan crystals by viable cells.

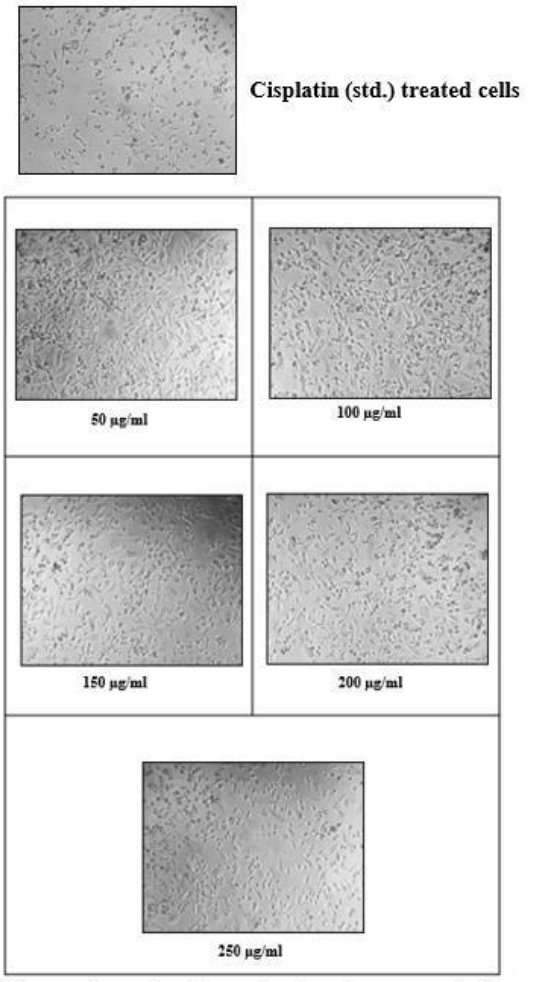
Both lyophilized powdered and oil extracts were tested at concentrations ranging from 50 to 250 µg/ml, with cisplatin (15 µg/ml) as the standard. The percentage of cell viability for the standard was 16.85%, while for the powdered and oil samples, it ranged from 83.94% to 26.87% and 78.58% to 17.88%, respectively (Table 6). The IC50 values for the powdered and oil samples were calculated as 167.12 µg/ml and 131.45 µg/ml, respectively, indicating the oil sample's superior efficacy in reducing cell viability. The observed anti-cancer potential is attributed to the high concentrations of bioactive unsaturated fatty acids in the coriander microgreen. These findings support the potential of coriander microgreen as a therapeutic agent against MCF-7 cell lines.

Table 6: Percentage Viability of MCF-7 Cancer Cell Line

Sl. No.	Concentration (µg/ml)	Powder Sample	Oil Sample
1	Untreated	100%	100%
2	Std. Cisplatin 15 µg/ml	16.85649%	16.85649%
3	50	83.94077%	78.58770%
4	100	70.72893%	56.94761%
5	150	54.89749%	43.16629%
6	200	38.61048%	24.94305%
7	250	26.87927%	17.88155%



MTT Assay of oil sample at various concentrations



MTT Assay of powdered sample at various concentrations

4. REFERENCE:

1. Alley, M. C., Scudiere, D. A., Monks, A., Czerwinski, M., & Shoemaker, R. II. and Boyd, M. R.(1986) Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines. In Proc. Am. Assoc. Cancer Res (Vol. 27, p. 389).
2. Alley, M. C., Scudiero, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., ... & Boyd, M. R. (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer research, 48(3), 589-601.
3. Alves, A. Q., da Silva Jr, V. A., Góes, A. J. S., Silva, M. S., de Oliveira, G. G., Bastos, I. V. G. A., ... & Alves, A. J. (2019). The fatty acid composition of vegetable oils and their potential use in wound care. Advances in skin & wound care, 32(8), 1-8.
4. Asgarpanah, J., & Kazemivash, N. (2012). Phytochemistry, pharmacology and medicinal properties of Coriandrum sativum L. African Journal of Pharmacy and Pharmacology, 6(31), 2340-2345.
5. Barbouchi, M., Elidrissi, M., Amechrouq, A., & Chokrad, M. (2019). Chemical composition and physicochemical properties of the essential oil of coriander (Coriandrum sativum L.) grown in Morocco. RHAZES: Green and Applied Chemistry, 4(4), 35-50.
6. Bhuiyan, M. N. I., Begum, J., & Sultana, M. (2009). Chemical composition of leaf and seed essential oil

of Coriandrum sativum L. from Bangladesh. ||| Bangladesh Journal of Pharmacology|||, 4(2), 150-153.
7. DeliveReD, G. (2012). ATCC Ū ANIMAL CELL CULTURE GUIDE.
8. Dhakshayani, G. M., & Alias, P. S. J. (2022). A comparative study of phytochemical, antioxidant, anticarcinogenic, and antidiabetic potential of coriander (Coriandrum sativum L.): Microgreen and mature plant. Foods and Raw materials, 10(2), 283-294.
9. Elmosallamy, A., Eltawil, N., Ibrahim, S., & Hussein, S. A. A. (2021). Phenolic Profile: Antimicrobial Activity and Antioxidant Capacity of Colocasia esculenta (L.) Schott. Egyptian Journal of Chemistry, 64(4), 2165-2172.
10. Fokwen, V. F., Tsafack, H. D., Touko, B. A. H., Djopnang, J. D., Afeanyi, T. A., Kong, A. T., ... & Womeni, H. M. (2018). Nutrients composition, phenolic content and antioxidant activity of green and yellow Moringa (Moringa oleifera) leaves. Journal of food Stability, 1(1), 46-56.
11. Gerlier, D., & Thomasset, N. (1986). Use of MTT colorimetric assay to measure cell activation. Journal of immunological methods, 94(1-2), 57-63.
12. Ghoora, M. D., Haldipur, A. C., & Srividya, N. (2020). Comparative evaluation of phytochemical content, antioxidant capacities and overall antioxidant potential of select culinary microgreens. Journal of Agriculture and Food Research, 2, 100046.
13. Giordano, M., Petropoulos, S. A., Kyriacou, M. C., Graziani, G., Zarrelli, A., Rouphael, Y., & El-Nakhel, C. (2022). Nutritive and phytochemical composition of aromatic microgreen herbs and spices belonging to the apiaceae family. Plants, 11(22), 3057.
14. Golubkina, N., Kharchenko, V., Moldovan, A., Zayachkovsky, V., Stepanov, V., Pivovarov, V., ... & Caruso, G. (2021). Nutritional value of Apiaceae seeds as affected by 11 species and 43 cultivars. Horticulturae, 7(3), 57.
15. Gomez-Flores, R., Hernández-Martínez, H., Tamez-Guerra, P., Tamez-Guerra, R., Quintanilla-Licea, R., Monreal-Cuevas, E., & Rodríguez-Padilla, C. (2010). Antitumor and immunomodulating potential of Coriandrum sativum. Journal of Natural Products, 3, 54- 63.
16. Kanti Das, S., i Sengupta, P., Mustapha, M. S., Kifayatudullah, M., & Gousuddin, M. (2015) Phytochemical Investigation and Antioxidant Screening of Crude Leaves Extract from Epipremnum aureum.
17. Kumari, S., Deori, M., Elancheran, R., Kotoky, J., & Devi, R. (2016). In vitro and in vivo antioxidant, anti-hyperlipidemic properties and chemical characterization of Centella asiatica (L.) extract. Frontiers in pharmacology, 7, 400.
18. Lirazan, M., Cua, S. J., & Alvarez, M. R. (2018). In vitro Antacid Screening of the Aqueous and Ethanolic Leaf extracts of Triticum aestivum (Linn.) and Hordeum vulgare (Linn.). Oriental Journal of Chemistry, 34(1).
19. Mechchate, H., Es-Safi, I., Conte, R., Hano, C., Amaghnouje, A., Jawhari, F. Z., ... & Bousta, D. (2021). In vivo and in vitro antidiabetic and anti-inflammatory properties of Flax (Linum usitatissimum L.) seed polyphenols. Nutrients, 13(8), 2759.
20. Muharni, M., Julinar, J., Yohandini, H., Fitrya, F., & Melati, R. (2021, March). In vitro anti-cholesterol and anti-hypertensive activity of stem bark the Flacourtia rukam. In IOP Conference Series: Earth and Environmental Science (Vol. 713, No. 1, p. 012056). IOP Publishing.
21. Nithya, T. G., & Sumalatha, D. (2014). Evaluation of in vitro anti-oxidant and anticancer activity of Coriandrum sativum against human colon cancer HT-29 cell lines. International Journal of Pharmacy and Pharmaceutical Sciences, 6(2), 421-424.
22. Pant, G., Simaria, C., Varsi, R. A., Bhan, P., & Sibi, G. (2015). In vitro anti-cholesterol and antioxidant activity of methanolic extracts from flax seeds (Linum usitatissimum L.). Research Journal of Medicinal Plant, 9(6), 300-306.
23. Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. International journal of biomedical science: IJBS, 4(2), 89.
24. Rahardhian, M. R. R., Yuniarti, N., Ariani, L. W., & Suharsanti, R. (2020). In Vitro Determination of Antioxidant Activity, Total Phenolics, Total Flavonoid, Anti-cholesterol of Extracts Saffron (Crocus sativus). J. Glob. Pharma Technol, 12(9), 223-230.
25. Ranjith Santhosh Kumar, D. S., Elango, N., Selvaraju, G. D., Matthew, P. A., Palanisamy, S., Cho, H., ... & Roy, A. D. (2022). Mycosynthesis of Zinc Oxide Nanoparticles Coated with Silver using Ganoderma lucidum (Curtis) P. Karst and Its Evaluation of In Vitro Antidiabetic and Anticancer Potential. Journal of Nanomaterials, 2022.
26. Reddy, L. J., Reshma, D. J., Jose, B., & Gopu, S. (2012). Evaluation of antibacterial and DPPH radical scavenging activities of the leaf extracts and leaf essential oil of Coriandrum sativum Linn. World J Pharm Res, 1(3), 705-716.
27. Scudiero, D. A., Shoemaker, R. H., Paull, K. D., Monks, A., Tierney, S., Nofziger, T. H., ... & Boyd, M. R. (1988). Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer research, 48(17), 4827-4833.
28. Sidhu, A. K., Wani, S. J., Tamboli, P. S., & Patil, S. N. (2014). In vitro evaluation of anti- diabetic activity of leaf and callus extracts of Costus pictus. Int. J. Recent Sci. Res, 3, 1622- 1625.
29. Singh, S., Agrawal, N., & Mishra, I. (2022). Pharmacology and Phytochemistry of Coriander. In Research Anthology on Recent Advancements in Ethnopharmacology and Nutraceuticals (pp. 711-734). IGI Global.
30. Vaijanathappa, J., Badami, S., & Bhojraj, S. (2008). In vitro antioxidant activity of Enicostemma

axillare. Journal of health science, 54(5), 524-528.
31. Vani and Rajashree Mashru. “To Study the Antacid Property of Classically Known Edibles”. Acta Scientific Pharmaceutical Sciences 2.10 (2018): 81-89.