The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 4, Issue 6, 2026

# SYNERGISTIC ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF ESSENTIAL OILS FROM *CORIANDRUM SATIVUM* AND *GLYCYRRHIZA GLABRA*: CHARACTERIZATION AND EFFICACY EVALUATION

**Mehwish Esha Noor[*1], Khalil Ur Rehman[2], Muhammad Iqbal[3], Ramsha Javed[4], Nargis Khan[5], Anam Laraib[6], Rimsha Jabeen[7]**

[*1,2,4]*Department of Biochemistry, Riphah International University, Faisalabad Campus, Faisalabad, Punjab Pakistan, 44000*
[3,5,6,7]*Department of Chemical and Life Sciences, Qurtuba University of Science and Information Technology Dera Ismail Khan, KP, Pkaistan*

[*1]mehwishnoor8608@gmail.com

DOI: https://doi.org/10.5281/zenodo.20699865

**Keywords**
*Coriandrum sativum, Glycyrrhiza glabra, Essential Oils, Synergistic Activity, Antimicrobial, Antioxidant, GC-MS.*

**Article History**
*Received: 13 April 2026*
*Accepted: 25 May 2026*
*Published: 15 June 2026*


**Corresponding Author: ***
**Mehwish Esha Noor**

**Abstract**
*Essential oils from* Coriandrum sativum *(coriander) and* Glycyrrhiza glabra *(licorice) are renowned for their broad-spectrum biological activities, including antimicrobial and antioxidant properties. This study investigates the potential synergistic enhancement of these activities by combining the two oils. The essential oils were extracted via hydrodistillation using a Clevenger apparatus and characterized by Fourier-Transform Infrared (FTIR) spectroscopy and Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analysis identified 23 compounds in* C. sativum *oil, with linalool (80.66%) as the predominant component, while* G. glabra *oil was characterized by three major compounds. The individual and combined oils were evaluated for antimicrobial activity against bacterial pathogens and for antioxidant potential. The combination (25 µL each) demonstrated significantly enhanced efficacy, showing the highest average zone of inhibition (17.5 mm) against* Proteus mirabilis. *Furthermore, the antioxidant activity of the oil mixture surpassed that of each oil alone. These findings confirm a positive synergistic interaction, underscoring the potential of a formulated blend of* C. sativum *and* G. glabra *essential oils as a potent natural alternative for applications in food preservation and therapeutic agents.*

## INTRODUCTION

The extraction of essential oils is a critical process for isolating volatile aromatic compounds from plant materials, utilizing techniques where the desired substances dissolve into a suitable solvent. These complex mixtures, prized for their distinctive flavors, fragrances, and therapeutic properties, find extensive applications in the food, cosmetic, and pharmaceutical industries. Given that extraction is a delicate and time-consuming procedure, careful management is paramount to preserve the bioactive integrity of the resulting oils. This study focuses on the extraction of essential oils from two significant medicinal plants: *Glycyrrhiza glabra* (licorice) and *Coriandrum sativum* (coriander), for a comprehensive evaluation of their biological activities.

Coriandrum sativum, a member of the Apiaceae family, has a long history of use in traditional medicine and as a culinary spice across various

cultures. The essential oils derived from its seeds and leaves are rich in bioactive compounds, most notably linalool, which imparts its characteristic floral aroma. These oils have been extensively investigated for a spectrum of pharmacological properties, including antibacterial, antioxidant, and anticancer effects. Similarly, Glycyrrhiza glabra is renowned for its sweet, moist roots and rhizomes, which contain valuable compounds such as glycyrrhizin and liquiritigenin. Traditionally used for its expectorant, anti-inflammatory, and hepatoprotective qualities, licorice remains a cornerstone of herbal pharmacopeias. Essential oils, in general, are complex blends primarily composed of terpenes and phenylpropanoids, which confer their diverse biological activities. Their lipophilic nature allows them to interact with microbial cell membranes, leading to antibacterial and antifungal effects, while their antioxidant capacity helps mitigate oxidative stress, which is implicated in various degenerative diseases.

A particularly promising area of research involves the synergistic interactions between different essential oils or between oils and conventional antimicrobials. Such combinations can enhance efficacy, reduce required doses, and potentially overcome resistance mechanisms. Therefore, the primary objective of this study is to extract, characterize, and evaluate the individual and combined antibacterial and antioxidant activities of the essential oils from Coriandrum sativum and Glycyrrhiza glabra, with the aim of exploring their potential as natural therapeutic or preservative agents.

## RESEARCH METHODOLOGY

This study was conducted in the Biochemistry Laboratory of RIUF and the Chemistry Laboratory of Qurtaba University, Dera Ismail Khan. The objective was to extract essential oils from the seeds of Coriandrum sativum and the stems of Glycyrrhiza glabra, characterize them, and evaluate their antimicrobial and antioxidant activities.

### 2.1 Sample collection and identification

The Head of Pharmacognosy, Faculty of Pharmacy, Gomal University, D.I. Khan, verified the authenticity of both plants (C. sativum and G. glabra). 500 g of healthy samples were purchased from the Bhakkar local market. These samples' essential oils were extracted, and they were then put through a number of activity tests.

### Extraction of essential oils

500 grams of wholesome seed material were extracted at the solvent's (water) boiling point using the hydro-distillation procedure to extract the coriander essential oil. 500 grams of a healthy stem sample were cut and crushed in order to extract the essential oil of G. glabra using the Soxhlet apparatus. It was observed that the extraction time increased from 4 to 6 hours (Nadeem et al., 2013).

### 1.13.1 Extraction through hydro-distillation method

Samples were properly cleaned with distilled water to get rid of contaminants before extraction. Using a Clevenger apparatus, 500 g of C. sativum seeds were hydro-distilled to extract the essential oil. The oil was collected, moved to a dry vial, carefully sealed, and refrigerated for later usage after the procedure was carried out for six hours until it separated over the water in the side arm (Asilbekova et al., 2021).

### 1.13.1.1Clevenger Apparatus

C. sativum was hydrodistilled using a Clevenger-type device, which is frequently used to extract volatile organic compounds and essential oils (Wahab et al., 2021). After partially filling a round-bottom flask with distilled water, the sample was heated for four to six hours until the oil gathered in the Dean-Stark apparatus's side arm. After transferring the recovered oil into a beaker, a tiny amount of anhydrous $CaCl_2$ was added to eliminate any turbidity. A clean, dry vial was used to decant the resulting pale-yellow essential oil, which was then carefully sealed and kept in the refrigerator until it was needed again.

#### 1.13.1.2 Extraction through Soxhlet apparatus

The sample to be examined is kept in this flask, and the condenser cools and condenses the vapor created during distillation. The purified sample is collected in a collection flask. The distillation process uses heat from the heating source. The sample is heated in the Clevenger apparatus, which causes the volatile substances to evaporate and be transported away by the vapor. The vapors are cooled by the condenser and condense into a pure state, which is then collected in the flask. The composition of volatile organic molecules in a sample can be quickly and effectively ascertained with this apparatus (Ali et al., 2015).

The Soxhlet Apparatus technique was used to extract G. glabra. For this purpose, 500 grams of a healthy sample yielded essential oil. The sample was properly cleaned with distilled water to get rid of any dust particles before to extraction. After being cleaned, the sample was crushed and sliced before being put through a Soxhlet equipment to abstract the essential oil. The procedure was carried out for six hours till the essential oil was gathered over the water in the Soxhlet apparatus's leg. After being extracted from the water, the essential oil was put in a dry vial and sealed tightly. After that, the essential oil was stored in the fridge until it was needed again (Ahmad et al., 2021). This procedure involved placing the 500 grams of finely powdered sample in a thimble into a Soxhlet extractor. A round-bottomed flask that was half full of newly distilled hexane (2.5 L) was fitted with a Soxhlet extractor. Switched on the heating mantle to enable water circulation through the reflux condenser, the water tape attached to the hose was opened. The procedure was carried out for six to eight hours. Hexane and oil mixes were then moved to a rotating evaporating flask after the heat source was switched off. Using a rotary evaporator, the hexane was extracted at low pressure and temperature (Bahrami & fattahi 2021).

### 1.14 Characterization of essential oils

#### 1.14.1 GC-MS analysis of C. sativum

The GC-MS QP2010 plus was used to analyze the essential oil extracted from C. sativum. 23 substances were identified by the GC-MS analysis. The same instrument was used in the same way to perform the GC-MS analysis of G. glabra essential oils. Twelve compounds were obtained from the GC-MS study of G. glabra. GC-MS analysis was performed on hydro-distilled essential oil from C. sativum and Soxhlet extracted oil from G. glabra using a Shimadzu, Japan-made GC-MS Model QP2010 Plus. Twenty-three components of C. sativum essential oil were obtained from the analysis. With an ionization mode of EL (70 eV) and a flow rate of 1 mm/min, helium gas was employed as the carrier. Temperature program: injector and detector temperatures were set to 250 °C, and the temperature was first held static at 40 °C for two minutes before increasing at a rate of 2 °C min-1 to 160 °C and then remaining static at 250 °C for 7.5 minutes as the last step. Samples (essential oils) were inserted into an MS after stable chromatographic conditions were established, and the mass spectra of each peak were ascertained. Essential oils might be qualitatively determined by using the GC-MS G1035A Wiley PBM Library (Probability Based Matching) to find the comparable compound name, molecular weight, and structure (Cary et al., 2018).Pale yellow essential oil attained from C. sativum by hydro distillation technique was exposed to GC-MS analysis over GC-MS Model QP2010 Plus, Shimadzu, Japan. This showed that the essential oil contained 23 different constituents. Each compound's proportion, peak area, and retention time are displayed by the GC-MS of the essential oil extracted from C. sativum. In the GC column, compounds are given in ascending order of retention time. Prior to GC-MS analysis, the oil extracted from G. glabra using the Soxhlet method was transformed into methyl esters. Thirteen methyl esters were obtained from the methyl esters on GC-MS analysis (Ewase & Tawfik 2013).

FTIR spectra of essential oils from C. sativum and G. glabra were secured on Bruker FTIR tensor 27 model using neat liquid.

### 1.15 Antimicrobial activities

#### 1.15.1 Preparation of Growth medium

Remel Company's dehydrated Mueller-Hinton (MH) agar, which contains (a) 300.0 g of beef, infusion, (b) 1.5 g starch, (c) 17.5 g of technical-grade casamino acid, and (d) 17.0 go to one liter of the double-distilled water, agar agar was added. After meticulously mixing the ingredients, the mixture was heated for one to two minutes to completely dissolve them. The media had a pH of 7.2 to 7.4. The mixture was autoclaved for 15 minutes at 121°C to sterilize it. For each 100 mm Petri plate, 25 ml of the molten media was added, and for each 150 mm Petri plate, 60 ml of the liquid media. In each Petri plate, the medium was 4 mm deep. After cooling the petri plates to 4–8 °C, the medium hardened. The plates were deposited in a freezer at 4-8 $^{0}$C (Bahrami & Fattahi, 2021).

#### 1.15.2 McFarland standard

In this investigation, McFarland standards with barium sulfate suspension from Remel were employed. A Wickerham card was used to visually compare the bacterial density to the normal 0.5 McFarland. There were 1 x 108 and 2 x 108 CFU/ml in a bacterial suspension that visually matched the color of the 0.5 McFarland standard.

#### 1.15.3 Application of inoculum onto Petri plates

To make sure the petri plates were dry before the inoculum was administered, they were either kept at room temperature in an air laminar flow hood for 10 to 30 minutes or placed in an incubator set at 35°C. For every bacterium (E. coli, P. mirabilus, S. mutans, B. subtilis, and C. albicans), a petri plate was meticulously labeled. Four or five colonies of the organism were handled and suspended in two milliliters of sterile saline using a sterile inoculating loop. The tubes were vortexed to provide a smooth suspension. The turbidity was accustomed to 0.5 McFarland either by adding more suspension or by diluting sterile saline. This suspension was used instantaneously (Kaur et al., 2013).

The inoculum suspension was dipped onto a sterilized cotton swab. To get rid of extra suspension, the swab was rubbed up against the vial walls. Three streaks across the entire agar plate were used to inoculate the dry surfaces of the MH agar in Petri plates. During the inoculation process, plates were rotated by 60 degrees each time. The swab was thrown away in a secure container. To allow the plates' surfaces to dry, they were kept at room temperature for five to ten minutes with their lids and jars (Kumar & Sidhu 2011).

### 1.16 Synergistic antibacterial activity

Two 8 mm-diameter wells were excavated in each inoculated Petri plate using a sterile cork borer. 50µl of C. sativum essential oil and 50µl of G. glabra essential oil were combined to create a 100µl stock solution. 50µl of the essential oil mixture from the stock solution was added to each well that had been dug out in the petri plates. Petri plate lids were changed. For 17–20 hours, the plates were kept at 35°C in an air incubator. A Vernier caliper was used to measure the zones of inhibition. The lowest average zone of inhibition against B. subtilis (16 mm) and the highest zone of inhibition against S. mutans (17.5 mm) are both visible. Between these two extremes, several microorganisms had zone of inhibition values. The growth inhibition zone for Candida species is 9.25 ± 0.5, according to the literature. Each microorganism underwent the process twice (Khadam et al., 2019).

### 1.17 Synergistic Antifungal activity

Synergistic antibacterial and antifungal activities (C. albicans) were investigated. 50µl of C. sativum essential oil and 50µl of G. glabra essential oil were combined to create a 100µl stock solution. 50µl of the essential oil mixture from the stock solution was added to each well that had been dug out in the petri plates. Petri plate lids were changed. For 17–20 hours, the plates were kept at 35°C in an air incubator. A Vernier caliper was used to measure the zones of inhibition (Loncar et al., 2024).

### 1.18 Synergistic antioxidant activity

The DPPH (diphenyl picrylhydrazyl) free radical scavenging technique was used to obtain the

synergistic antioxidant activity of essential oils hydro-distilled from C. sativum and G. glabra (Hossain et al., 2019). A DPPH methanolic solution of 0.2 mM was made. After adding 2.5 mL (1:1) of essential oils from C. sativum and G. glabra to 0.5 mL of this solution, it was left for half an hour. The solution's absorbance was then measured at 517 nm. As a reference substance, ascorbic acid was utilized.

Using relation:

$$\text{RSA (\%)} = \frac{A1-Ao}{Ao} \times 100$$

The aforementioned relation was used to compute the radical scavenging activity. Where A1 is the absorbance of the sample solution and Ao is the absorbance of the control.The mixture's synergistic IC50 value, or the concentration required to prevent 50% of oils' radical scavenging action, was determined (Matasyoh et al., 2009).

## RESULTS AND DISCUSSION

Essential oils valued for centuries for their numerous benefits and uses. They are potentially used as pharmaceuticals, natural remedies, natural preservatives, insect's repellents etc. The essential oil extracted synergistically from C. sativum (coriander) and G. glabra (malathi) by using hydro distillation method (Clevenger and Soxhlet apparatus respectively) are very potent and playing indigenous participation in the field of homoeopathic and many others (Ahmad et al., 2021).

### 1.19 Extraction of essential oils

(C. sativum) essential oil was extracted using the hydro-distillation procedure, which yielded about 2.5 mL of essential oil with a 1:6 w/v ratio from 500 grams of seed material extracted at the solvent's (water) boiling point. Using the Soxhlet apparatus, 500 grams of stem material yielded 2 milliliters of essential oil at a 1:5 w/v ratio. This was done in order to extract the essential oil of G. glabra. It was observed that the extraction time increased Coriander from four to six hours (Nadeem et al., 2013).

### 1.20 GC-MS analysis

#### 1.20.1 GC-MS analysis of essential oil from C. sativum

The Pale-yellow essential oil gained from Coriandrum sativum when subjected to GC-MS analysis gave 23 compounds. The compounds are shown in Table 4.1 along with their respective concentrations (%), peak area and retention time.

**Table 4.1: Compounds from GC-MS analysis of essential oil from C. sativum.**

| Compound No. | Name of Compound | R. Time | Area | Conc. (%) |
|---|---|---|---|---|
| 1 | Bicyclo[3.1.1]hept-2-ene,2,6,6-trimethyl-,(+/-)] | 9.072 | 179643 | 0.68 |
| 2 | Camphene | 9.657 | 17324 | 0.07 |
| 3 | beta-Pinene | 10.857 | 24402 | 0.09 |
| 4 | beta-Myrcene | 11.607 | 70075 | 0.26 |
| 5 | p-Cymol | 13.052 | 272021 | 1.02 |
| 6 | Limonene | 13.261 | 107684 | 0.41 |
| 7 | gamma-Terpinene | 14.712 | 231176 | 0.87 |
| 8 | cis-Linalool Oxide | 15.393 | 130767 | 0.49 |
| 9 | 2-(5-Methyl-5-vinyltetrahydro-2-furanyl)-2-propanol | 16.158 | 121872 | 0.46 |
| 10 | 1,6-Octadien-3-ol, 3,7-dimethyl | 16.886 | 21442283 | 80.66 |
| 11 | L-Camphor | 18.421 | 623522 | 2.35 |
| 12 | 6-Octenal,3-7-dimethyl | 18.802 | 34225 | 0.13 |
| 13 | Barneol | 19.169 | 181310 | 0.68 |
| 14 | 4-Carvomenthenol | 19.624 | 47129 | 0.18 |
| 15 | p-Menth-1-en-8-ol | 19.909 | 116510 | 0.44 |
| 16 | 6-Octen-1-ol-3,7-dimethyl | 20.900 | 57594 | 0.22 |

| | | | | |
|---|---|---|---|---|
| 17 | 6-Octen-1-ol-,3,7-dimethyl-(E) | 21.495 | 556420 | 2.09 |
| 18 | Undecanal | 22.563 | 12140 | 0.05 |
| 19 | 2,6-Octadien-1-ol,3,7-dimethyl-,acetate(Z) | 23.921 | 1198291 | 4.51 |
| 20 | Myrtene acid bromide | 27.091 | 51526 | 0.19 |
| 21 | Tetradecanoic acid | 29.061 | 73772 | 0.28 |
| 22 | n-Hexadecanoic acid | 31.281 | 57278 | 0.22 |
| 23 | Nonivamide | 31.476 | 977793 | 3.68 |

The gas chromatogram from C. sativum essential oil is shown as Figure 4.1. The concentration (%), peak areas and retention times in GC-MS chromatogram 0f compounds (1-23) from C. sativum are shown in Figure 4.1.

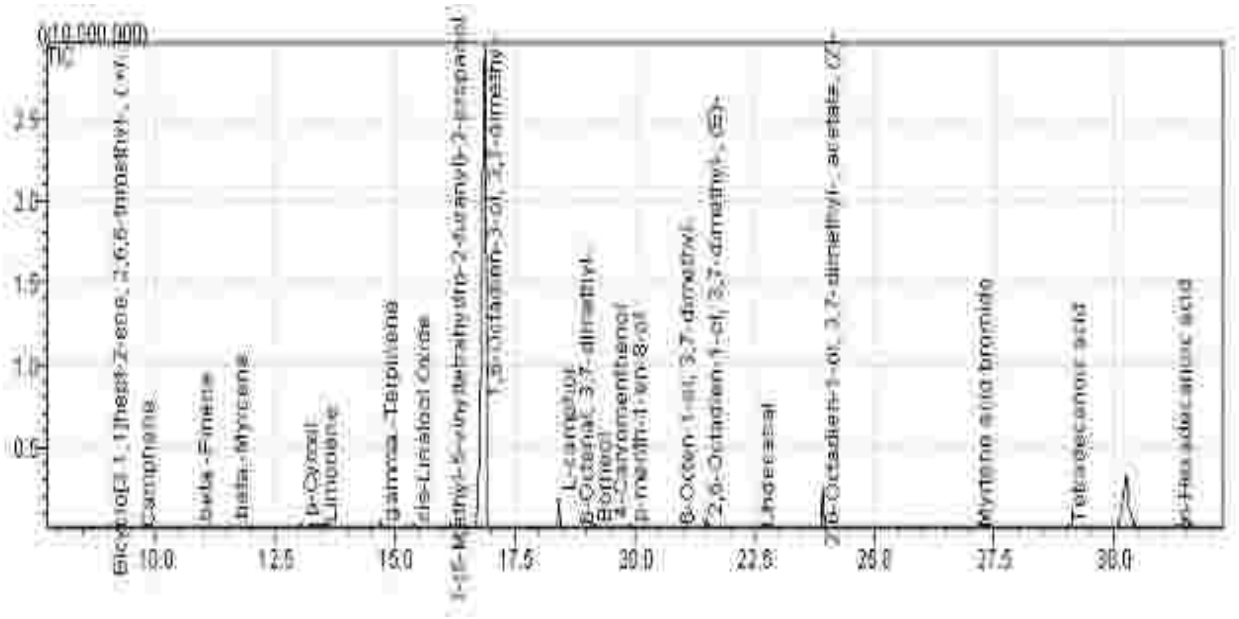


**Figure 4.1: Gas chromatogram (GC) of Coriandrum sativum (coriander) essential oil.**

The retention periods of the different constituents of C. sativum essential oil are displayed in Figure 4.2 below. Compounds exit the GC through the column. Retention time is the amount of time, measured in minutes that a chemical takes to exit the GC column. 9.072 minutes is the retention period for the first chemical found. The final compound had a retention duration of 31.476 minutes. Retention durations for other chemicals ranged between these two extremes.

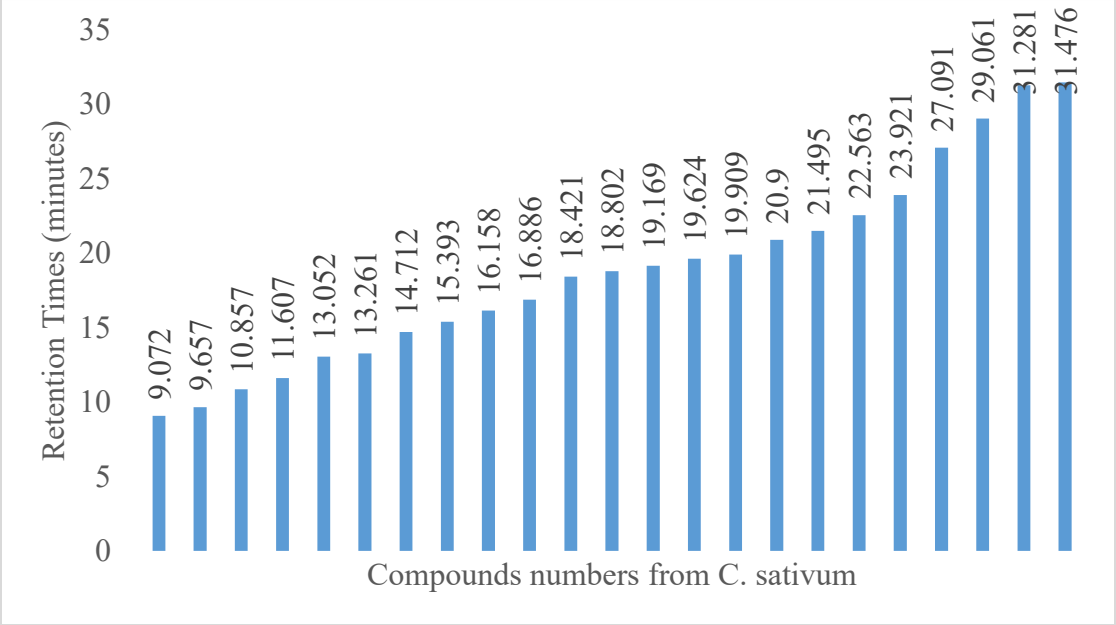


**Figure 4.2: Retention times of compounds from C. sativum in GC column.**

Twenty-three (1-23) compounds discovered by GC-MS analysis of hydro-distilled essential oil from C. sativum are shown in Figure 4.3 below, together with their measured concentration (%). The highest concentration of compound (10) was 80.66 (%). It is represented as 8.066 (%) in Figure 4.3. To make peaks for other concentrations visible in the graph, the concentration of chemical (10) was divided by ten throughout the sketching process. In the C. sativum essential oil, compounds 19 and 23 had the second and third-highest quantities, respectively; Figure 4.3.

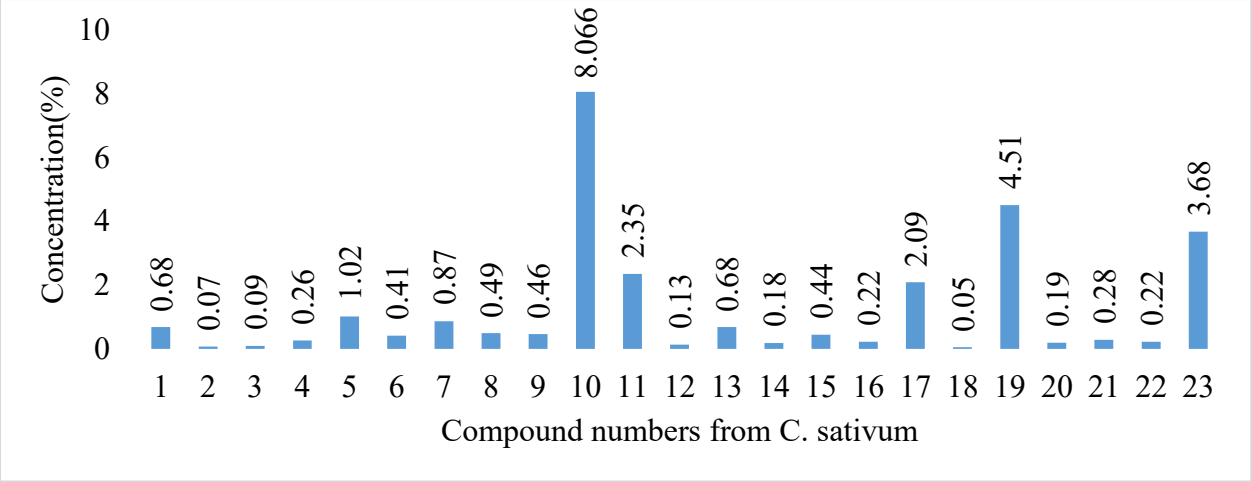


**Figure 4.3: Concentrations of compounds from C. sativum in GC column.**

Peak areas of compounds 1–23 derived from C. sativum essential oils are shown in Figure 4.4. The components' peak areas and concentrations have a nearly linear relationship. In order to make other peak regions visible in the chart, the largest peak area, 21442283, was divided by 100 throughout the design process. Peak areas and concentrations have a linear relationship.

Consequently, compounds 10, 19 and 23 that showed highest concentrations in the essential oil of C. sativum also showed highest values of peak areas.

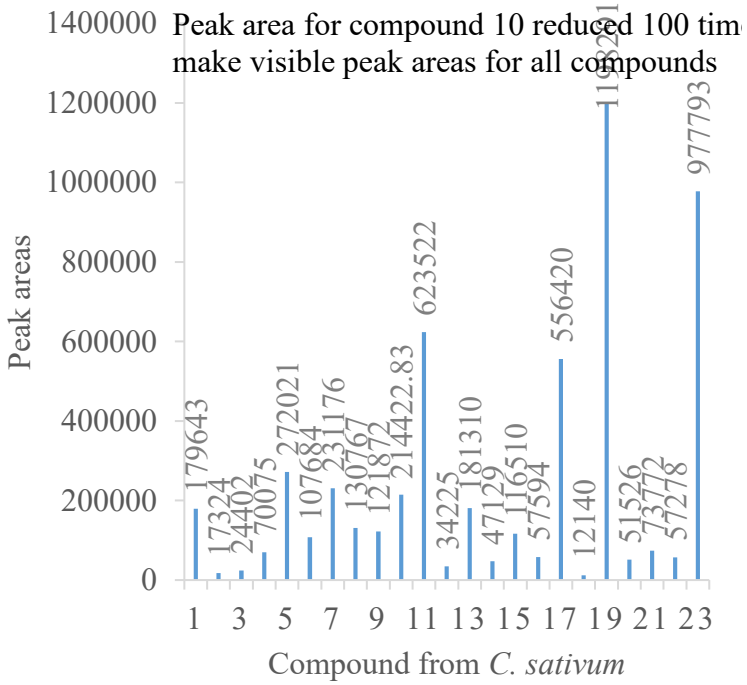


**Figure 4.4: Peak areas of compounds from C. sativum in GC column.**

### 1.21 FTIR spectrum of oil from C. sativum

The presence of several molecules with distinct functional groups was demonstrated by the IR spectra of C. sativum essential oil, which is displayed in Figure 4.5. The existence of -OH groups and hydrogens bonded to sp2 and sp hybridized carbons was indicated by the peaks above 3000 cm-1. The carbonyl groups of aldehydes, ketones, and esters, as well as the C=C of alkenes and aromatics, were indicated by the peaks that were located between 1800 and 1600 cm-1. From 1980 to 1820 cm, saturated CH peaks were visible.

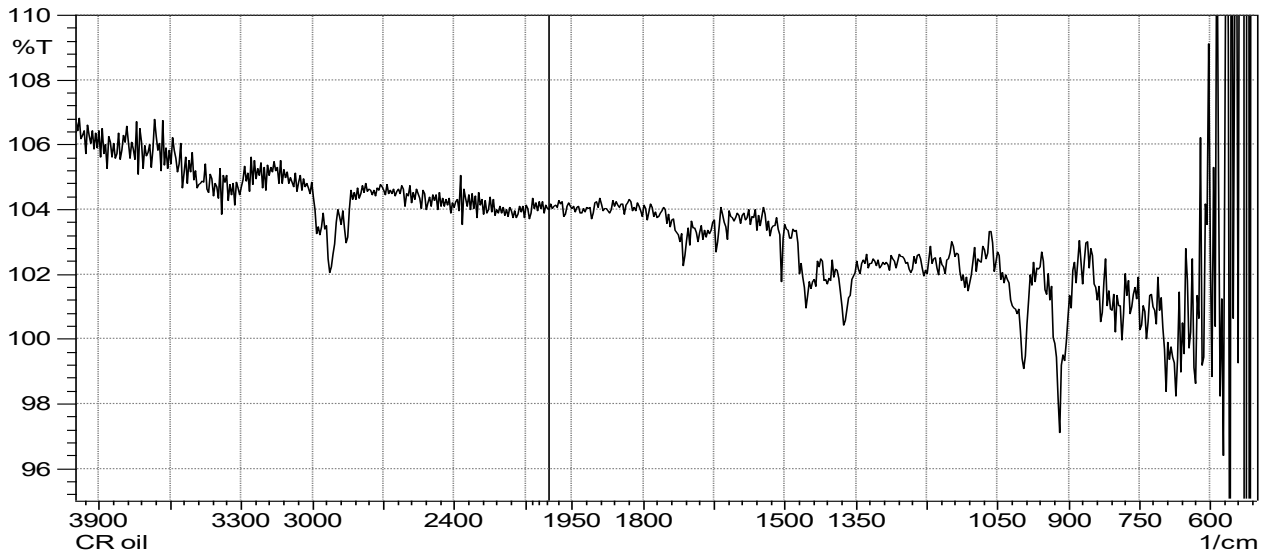


**Figure 5.5: FTIR spectrum of oil from C. sativum**

### 1.22 GC-MS analysis of G. glabra

The oil obtained from G. glabra through Soxhlet extractor when subjected to GC-MS analysis resulted in the identification of twelve (24-35) compounds. The compounds from G. glabra are given in Table 4.2.

**Table 4.2: Compounds from GC-MS analysis of essential oil from G. glabra.**

| Compound No. | Name of Compound | R. Time | Area | Conc. (%) |
|---|---|---|---|---|
| 24 | Caproic acid methyl ester | 4.827 | 20306 | 1.76 |
| 25 | Caprylic acid methyl ester | 10.037 | 3630 | 0.31 |
| 26 | Lauric acid, methyl ester | 20.584 | 6489 | 0.56 |
| 27 | Myristic acid, methyl ester | 26.113 | 17033 | 1.48 |
| 28 | Pentadecanoic acid, methyl ester | 29.547 | 12786 | 1.11 |
| 29 | Palmitic acid, methyl ester | 33.334 | 566689 | 49.23 |
| 30 | Heptadecanoic acid, methyl esters | 37.886 | 24806 | 2.16 |
| 31 | Oleic acid, methyl ester | 40.386 | 11843 | 1.03 |
| 32 | Linoleic acid, methyl ester | 40.087 | 335822 | 29.11 |
| 33 | Stearic acid, methyl ester | 40.900 | 126293 | 10.97 |
| 34 | Docosanoic acid, methyl ester | 42.727 | 13701 | 1.19 |
| 35 | Eicosanoic acid, mthyl ester | 45.847 | 15293 | 1.33 |

The concentration (%), peak areas and retentions times in GC-MS chromatogram of compounds (24-35) from G. glabra are shown in Figure 4.6.

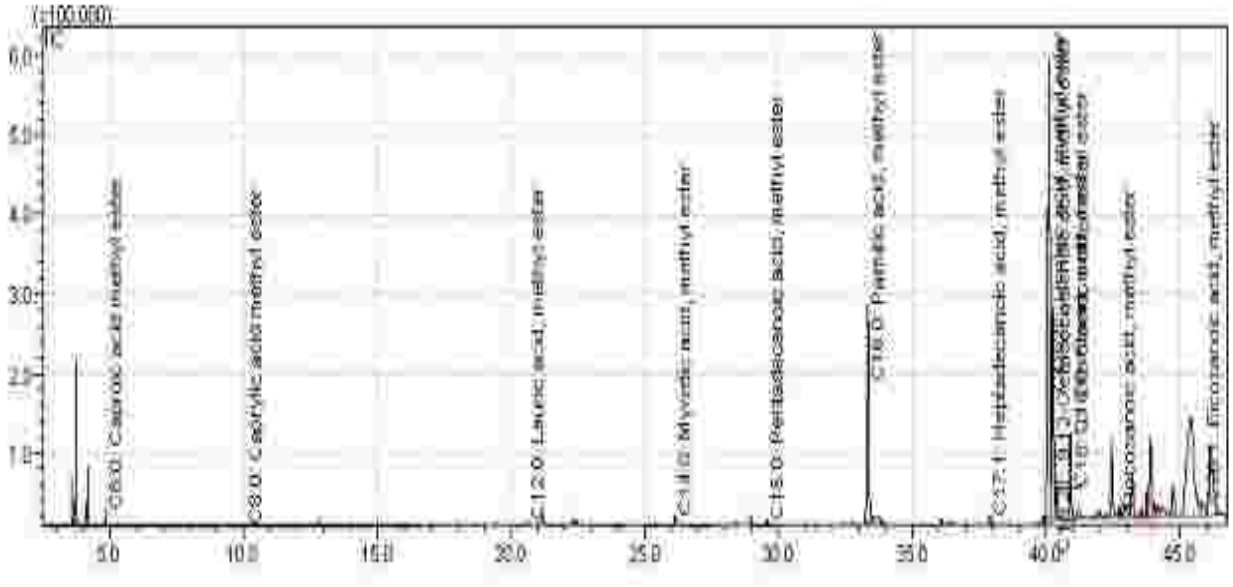

**Figure 4.6: Gas chromatogram (GC) of Glycyrrhiza glabra (Malathi) essential oil.**

The concentrations of twelve (24–35) chemicals from G. glabra oil are displayed in Figures 4.6 and 4.7. In G. glabra oil, component 29 had the highest concentration, while compounds 32 and 33 displayed the second and third highest concentrations, respectively. Compounds (29), (32) and (33) had the greatest concentrations of 49.32, 29.11, and 10.97%, respectively, according to GC-MS analysis of oils extracted from G. glabra. You may see these in Figure 4.6. Substances that were found in significant amounts in G. glabra essential oils.

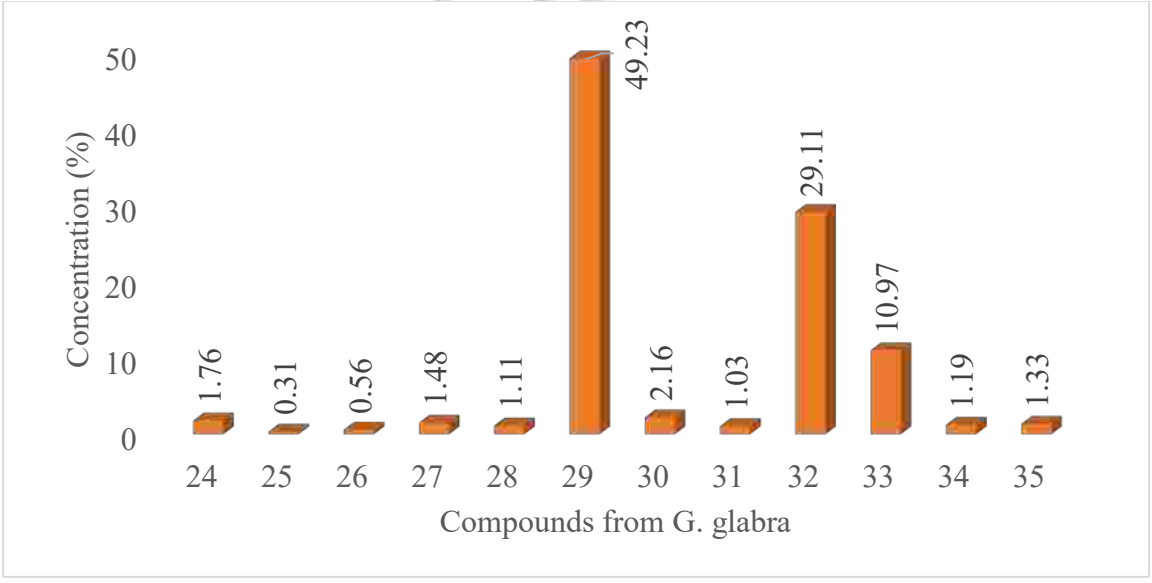


**Figure 4.7: Concentration of compounds from G. glabra.**


**Figure 4.8: Compounds from G. glabra and their retention times in GC column.**

Compounds from G. glabra are identified by their retention durations in GC-MS. The different components of G. glabra oil are listed in Figure 4.8 according to how long they remained in the GC column. In a GC column, the first compound (24) of the twelve (24–35) components of G. glabra compound had a retention time of 4.827 minutes, while the last compound (35) had a retention time of 45.847 minutes. Retention durations for other components ranged between these two extremes. Figure 4.9 shows relative peak areas of the compounds in GC column. The figure 4.9 reveals that the highest peak area was shown by compound 29, while compound 32 and 33 showed 2nd and 3rd high peak areas.

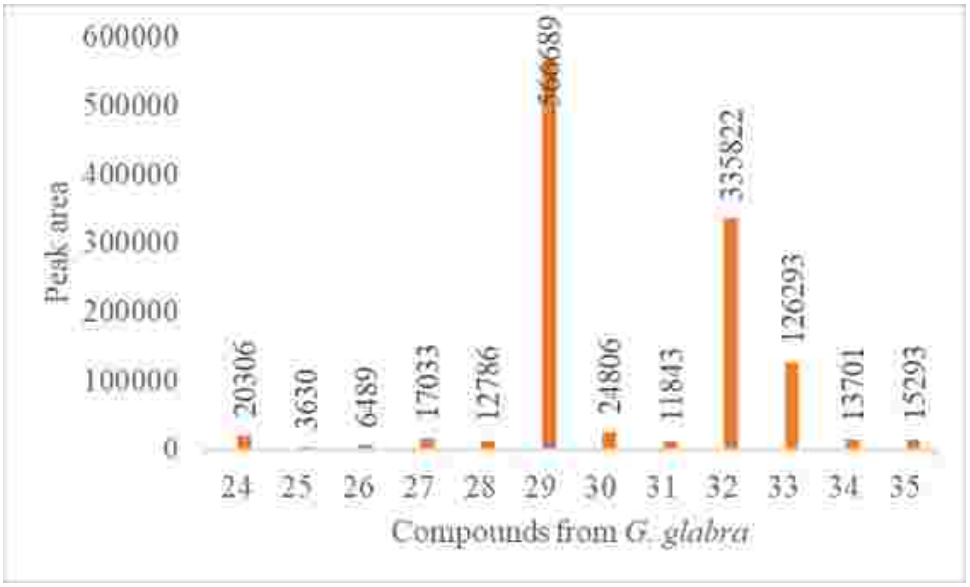


**Figure 4.9: Compounds from G. glabra and their peak areas in GC column.**

### 1.23 FTIR spectrum of essential oil from G. glabra

The IR spectrum of essential oil of G. glabra shown in figure 4.10 indicated esters functional groups. No peaks above 3000 cm-1 were found, indicating the absence of -OH groups, hydrogens bonded to sp2, or sp hybridized carbons. The carbonyl group of esters was indicated by the peaks that occurred between 1750 and 1700. Between 1970 and 1810 cm-1, saturated CH peaks were visible.

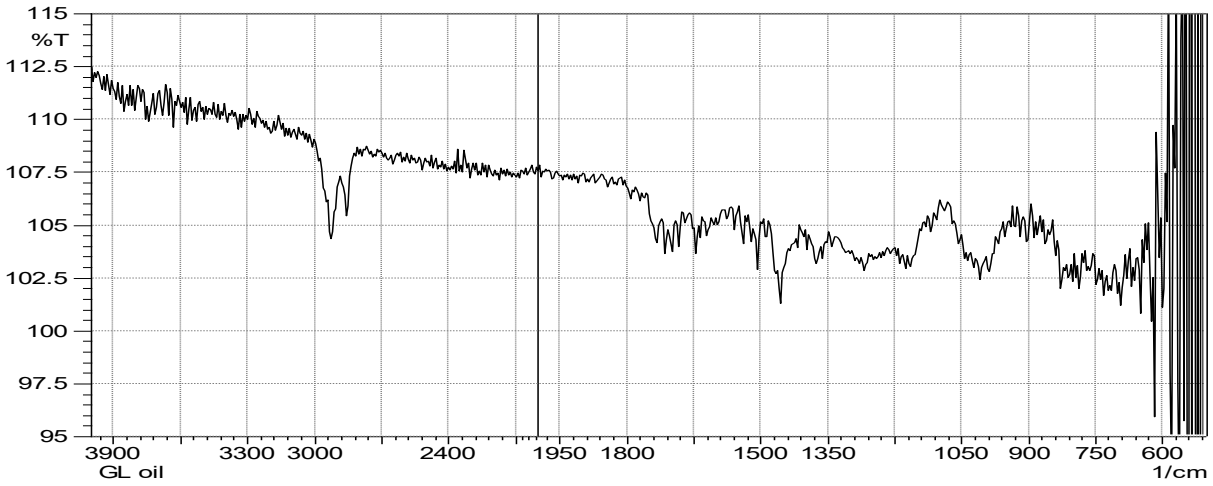


**Figure 4.10: FTIR spectrum of oil from G. glabra.**

**Table 4.3: Synergistic antibacterial and antifungal activities of EOs from C. sativum & G. glabra.**

| Microbe | Synergistic zones of inhibitions (mm) | | | Individual zones literature values | | Zone of inhibition of Standard drugs |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | Average | C. sativum | G. glabra | |
| E. Coli | 17 | 16 | 16.5 | 10, 14 | 10,12.5 | Cipro (100µg/ml)28.3 |
| P. mirabilus | 18 | 17 | 17.5 | 10 | 25 | Azithro (100µg/ml)19.7 |
| S. mutans | 16 | 17 | 16.5 | Variable | Variable | |
| B. subtilis | 17 | 15 | 16 | 10-12 | 12 | |
| C. albicans | 16 | 17 | 16.5 | 8 | 10.5 | |

### 1.24 Synergistic antimicrobial activities

#### 1.24.1 Synergistic antibacterial activities

4.11 compares the synergistic zones of inhibitions against different bacteria and the fungus Candida albicans. As can be shown, the largest zone of inhibition was recorded against S. mutans (17.5 mm), while the minimum average zone of inhibition was observed against B. subtilis (16 mm). Zones with inhibitory values in between these two extremes were seen in other microorganisms. The growth inhibition zone for Candida species is 9.25 ± 0.5, Figure according to the literature (Soares et al., 2012).

#### 1.24.2 Synergistic antifungal activities

The observed values of synergistic antifungal activities of essential oils from C. sativum and G. glabra against C. albicans are shown in Figures 4.11 and 4.12.

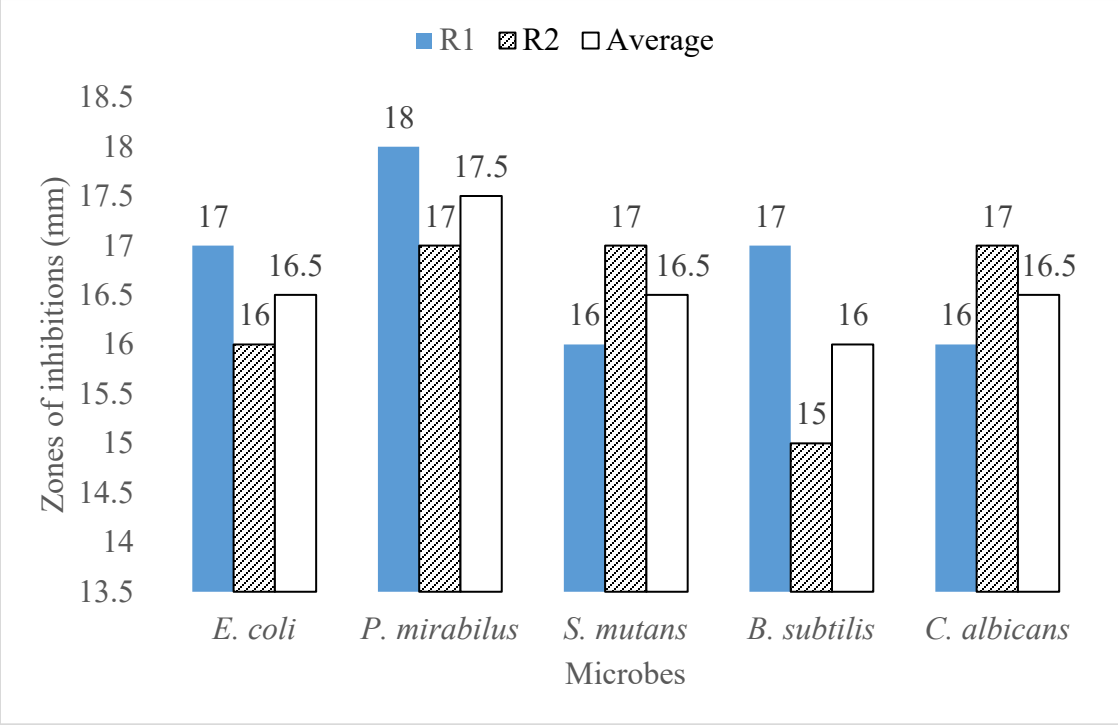


**Figure 4.11: Synergistic zones of inhibitions of essential oils.**

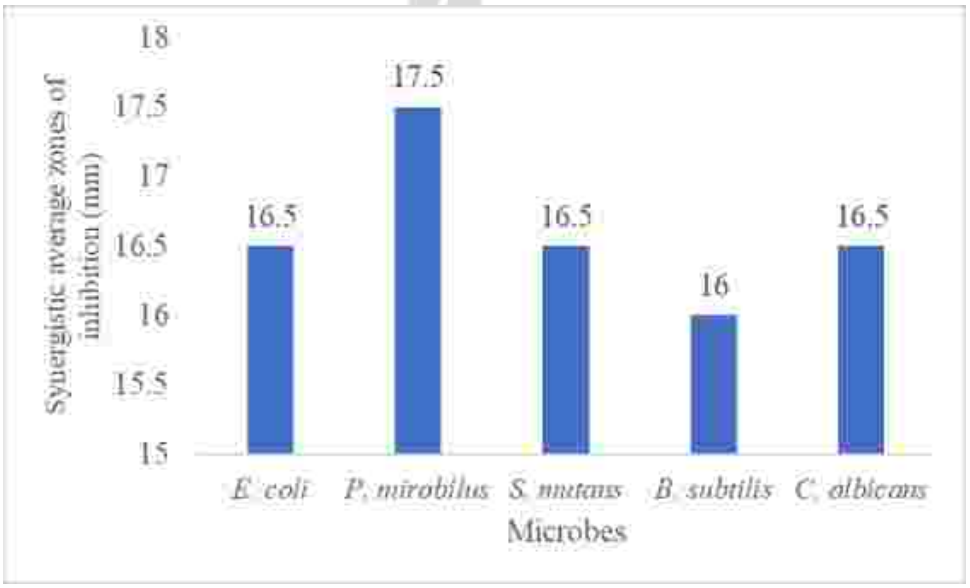


**Figure 4.12: Average synergistic zones of inhibition.**

To measure zones of inhibition of essential oil against numerous microbes, petri plates used in the study are shown in figure 4.13. Zones of inhibitions measured using these petri dishes are shown in Table 4.3.

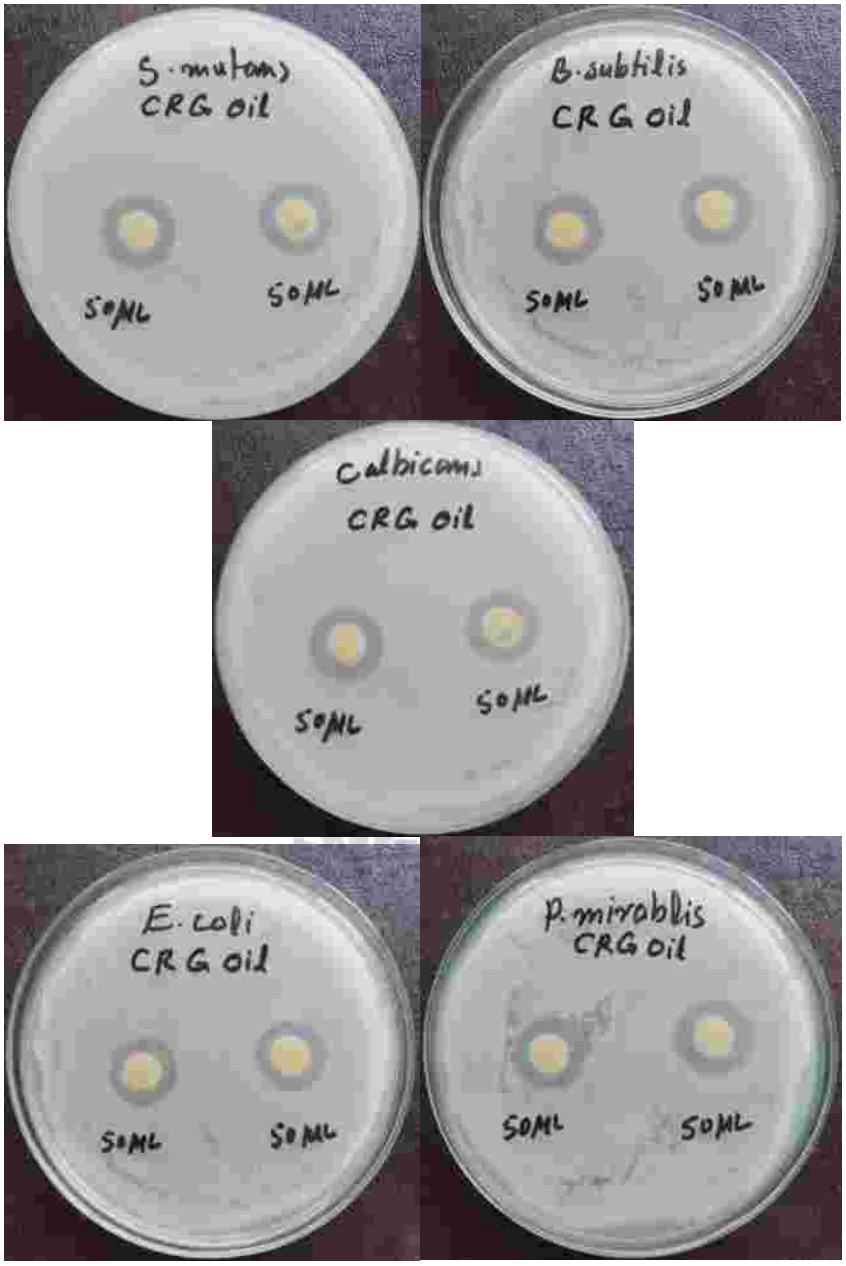


**Figure 4.13: Petri plates showing synergistic zones of inhibition of essential oils from C. sativum & G. glabra**

To measure zones of inhibition of standard drugs against numerous microbes petri plates used in the study are shown in Figure 4.14. Standard drugs were used in larger quantities so these showed larger inhibition zones than essential oils.

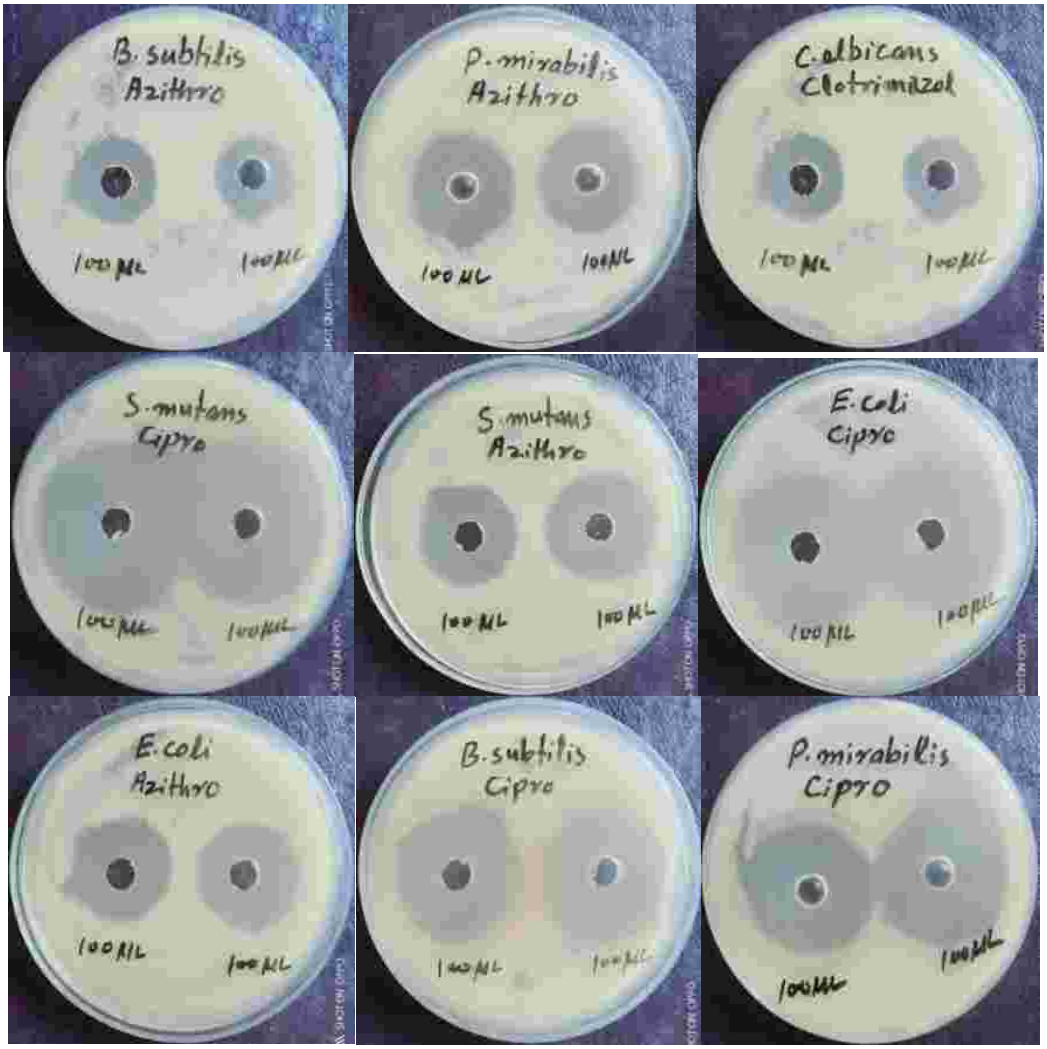


**Figure 4.14: Zones of inhibition standard drugs against microbes.**

### 1.25 Synergistic Antioxidant activity

Using the DPPH technique, the synergistic antioxidant activity of essential oils from C. sativum and G. glabra was assessed. In this investigation, ascorbic acid served as the standard. Table 4.4 displays the results of the antioxidant activity. Figure 4.14 reproduces the same findings. The antioxidant activity of synergistic compounds is nearly identical to that of ascorbic acid. Figure 4.14 displays the in vitro synergistic antioxidant activity (89.87138%) of essential oils from C. sativum and G. glabra in comparison to the standard ascorbic acid's radical scavenging activity (94.39085%). The synergistic effect was similar to that of regular ascorbic acid. Conversely, synergistic activities outperformed trivial antioxidant activities. Synergistic antioxidant activities were shown to be higher than solo activity.

**Table 4.4: Synergistic antioxidant activity of essential oils from C. sativum and G. glabra**
Wave length = 517nm
DPPH Absorbance = 1.1196

| Sample code | % A-O |
|---|---|
| CRG oil | 89.87138 |
| ascorbic acid | 94.39085 |

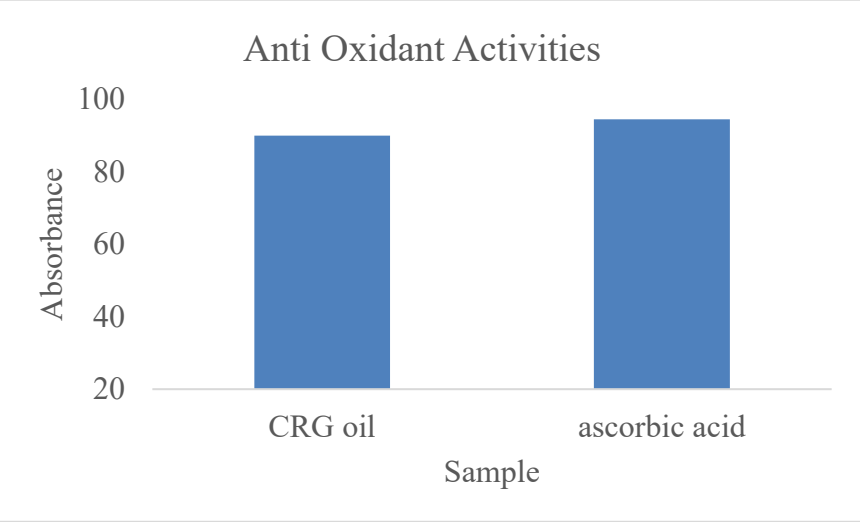


**Figure 4.15: Synergistic antioxidant activity of EOs from C. sativum and G glabra.**

**1.**
**26 Discussion**

By using Clevenger apparatus and Soxhlet apparatus correspondingly essential oils of C. sativum and G. glabra were extracted. The synergistic extraction of essential oils from these two plants could potentially yield a unique blend with enhanced bioactive properties. Essential oil from coriander and licorice root have been reported to possess antimicrobial and antioxidant activities among others. The synergistic extraction of essential oils from these two plants could potentially yield a unique blend with enhanced bioactive properties. The activities performed include the antiviral, antibacterial activities and antioxidant activity. The combination of the coriander and licorice essential oil exhibited enhanced inhibitory effects against various microorganisms, including bacteria and fungi. They also show increased antioxidant potential, scavenging free radical and reducing oxidative stress. These findings suggest that the synergistic extraction of essential oils from coriander and licorice may central to the development of the natural products with enhanced antimicrobial and antioxidant properties. These properties could be useful in various applications, such as food preservation, pharmaceuticals, and cosmetics etc (Ramos et al., 2023)

The hydro distillation method (Clevenger apparatus) for coriander and the Soxhlet apparatus for G. glabra were used to extract the essential oils. The sample was put into the round-bottomed flask of the Clevenger equipment in order to extract the oil from the coriander. Distilled water filled half of the flask. Two to three milliliters of essential oil were gathered in the side arm of the Dean Stark apparatus when the heat source was switched on and the essential oil was distilled for four to six hours. The heat source was then switched off, and Dean Stark's arm was used to extract essential oil into a little beaker. By adding a modest amount of anhydrous CaCl2, the essential oil's turbid hue was released. The

essential oil, which had a pale yellow hue, was decanted into a dry and clean vial. After being securely sealed, the vial was kept in a refrigerator until it was needed again (Husain et al., 2021). A 500-gram sample that had been finely pulverized was put in a Soxhlet extractor thimble to extract oil from G.glabra. A round-bottomed flask that was half full of newly distilled hexane (2.5 L) was fitted with a Soxhlet extractor. They switched on the heating mantle. To enable water circulation through the reflux condenser, the water tap attached to the condenser's hose was opened. The procedure was carried out for six to eight hours. The heat source was then switched off, and the hexane and oil mixture was moved to a rotating evaporation flask. Using a rotary evaporator, the hexane was extracted at low pressure and temperature. After being cleaned and dried, the oil was put into a carefully sealed vial and kept in the refrigerator until it was needed again. Following the acquisition of the required essential oils, additional actions were taken (Bahrami and Fattahi, 2021).

**GC-MS and FTIR, both techniques were used to characterize the both samples.**

The GC-MS analysis of coriander gave 23 compounds. Twelve compounds were obtained from the GC-MS study of G. glabra. GC-MS analysis was performed on hydro-distilled essential oil from C. sativum and Soxhlet extracted oil from G. glabra using a Shimadzu, Japan-made GC-MS Model QP2010 Plus. Twenty-three C. sativum essential oil components were obtained from the analysis. The essential oil analysis was conducted using a fused silica capillary column of DBI with preset size (30 m × 0.53 mm x 1.5 µm). With an ionization mode of EL (70 eV) and a flow rate of 1 mm/min, helium gas was employed as the carrier. Temperature program: injector and detector temperatures were set to 250 °C, and the temperature was first held static at 40 °C for two minutes before increasing at a rate of 2 °C min-1 to 160 °C and then remaining static at 250 °C for 7.5 minutes as the last step. Samples (essential oils) were inserted into an MS after stable chromatographic conditions were established, and the mass spectra of each peak were ascertained. Essential oils might be qualitatively determined by using the GC-MS G1035A Wiley PBM Library (Probability Based Matching) to find the comparable compound name, molecular weight, and structure (Cary et al., 2018).

Muhammadi et al. (2023), the oil that Soxhlet extracted from G. glabra produced twelve compounds, or fatty acids. These chemicals underwent a conversion process to methyl esters before being subjected to GC-MS analysis. In the oil of G. glabra, thirteen methyl esters compounds were identified using GC-MS analysis. Table 4.5 displays the 13 methyl esters that were afforded and identified. In GC-MS analysis, these substances had varying retention durations, peak regions, and concentrations. To create methyl esters, these were transformed. Figure 4.5 displays the GC chromatogram of (Malathi) essential oil, which includes these components. The peak areas, percent composition and retention times of the compounds obtained from G. glabra are shown in figures. Compounds 29 (49.23%), 32 (29.11%) and 33 (10.97%) were the three top concentration compounds in the oil of G. glabra, Table 4.6(Kumar et al., 2011).

The methods utilized for susceptibility evaluation, the type of drug, the amount of the drug and the quantity of the pathogen used, as well as the sanitation of the equipment used in the susceptibility assessment procedures, all affect the antimicrobial and antioxidant activities in terms of zone of inhibitions (Ramos et al., 2011). Accordingly, the zones of inhibition of pure natural products, extracts, and essential oils rarely match the values reported in the literature. According to published research, the individual zone of inhibition of S. mutans in 10% and 50% ethanol extracts from G. glabra was 16 and 19 mm, respectively At 50 µl, coriander essential oil demonstrated a 25.00 mm zone of inhibition against S. mutans.

A 10.73 mm zone of inhibition against E. coli was provided by coriander essential oil (Sambasivaraju & Fazeel, 2016). Standard Cipro demonstrated inhibition zones of 8-11 mm, while 100% pure essential oil from coriander habitant

of various regions generated inhibition zones of 9-18 mm when administered to E. Coli (Loncar et al., 2024).

By employing the Agar well diffusion method to measure the zones of inhibition (mm) of essential oils, natural products, and medications, it is simple to evaluate both their synergistic and individual antibacterial capabilities. The antibacterial activity are directly correlated with the zones of inhibition values. A higher zone of inhibition value indicates that the essential oil or medication has more antibacterial power. To assess their synergistic effect on bacteria and fungi, essential oils from C. sativum and G. glabra were tested for their synergistic antibacterial properties against two strains of Gram-negative bacteria, two strains of Gram-positive bacteria, and one strain of fungus. The individual zones of inhibition values of essential oils from C. sativum and G. glabra that are available in the literature were contrasted with the synergistic values of zones of inhibition. In this investigation, predeveloped petri plates that were commercially accessible were employed. Using a Vernier caliper, the zones of inhibition were measured and reported in millimeters (mm). In comparison to the individual activity levels of zones of inhibition, this investigation demonstrated a synergistic improvement of antibacterial and antifungal activities. In this work, synergistic activity was measured using equal quantities of essential oils from G. glabra and C. sativum. To guarantee a decent level of accuracy in the results, the process was done twice for every microorganism.

The results of the antifungal and antibacterial activities are shown in Table 4.3. The 50µL essential oil blend used in the study (25µL of C. sativum + 25µL of G. glabra) showed the strongest synergistic activity against S. mutans. 18 mm was the value of its first zone of inhibition (R1), and 17 mm was the value of its second zone. The average of the two measured zones of inhibition was found to be 17.5 mm. According to individual literature figures, the zone of inhibition for essential oils from C. sativum and G. glabra against P. mirabilus is 10 mm and 25 mm, respectively. This study shows that the essential oils from C. sativum have increased in activity whereas the individual activity of G. glabra essential has decreased. When compared to typical medications, the zone of inhibition for Ciprofloxacin (100µg) against P. mirabilus was 28.3 mm, while the zone of inhibition for Azithromycin (100µg) was 19.7 mm. The synergistic value of the essential oils from C. sativum and G. glabra is near the zone of inhibition (activity) of azithromycin (19.7mm). The synergistic values of the zones of inhibition for the essential oils of C. sativum and G. glabra are shown in the table (Zhai et al., 2019).

With an average zone of inhibition value of 16 mm, the essential oils from C. sativum and G. glabra showed the least amount of synergistic action against B. subtilis, with a first zone of inhibition value (R1) of 17 mm and a second zone of inhibition value (R2) of 15 mm. According to published reports, the essentials' zones of inhibition were 12 mm for G. glabra and 10–12 mm for C. sativum. The synergistic zones of inhibition are larger than the individual zones. We can conclude that the synergistic action against

**B. subtilis has been enhanced.**

When compared to their solo actions, these essential oils demonstrated an increase in synergistic activity. The same explanation that applies to S. mutans and B. subtilis also applies to the synergistic actions of these essential oils against the other two bacteria, E. coli and S. mutans (Table 4.3).

Essential oils from C. sativum and G. glabra were tested for their synergistic antifungal efficacy using agar well diffusion methods. An average of 16.5 mm was found in the results, with the first zone of inhibition value (R1) measuring 16 mm and the second zone of inhibition measuring 17 mm. The literature has documented antibacterial activity values for these essential oils that indicate an increase in synergistic actions: C. sativum (8 mm) and G. glabra (10.5 mm). Table 4.3 shows the results. Comparing the antibacterial and antifungal properties of these essential oils separately, this study revealed an enrichment in both. Therefore, a blend of these essential oils

would have greater potential as negotiators of antifungal and antibacterial properties (Ramos et al., 2023).

The synergistic zones of essential oil inhibitions against several bacteria and the fungus Candida albicans are contrasted in Figure 4.11. The average zones of inhibition for the oils from G. glabra and C. sativum that were found to function in concert are shown in Figure 4.12. Figure 4.12 displays the highest zone of inhibition against S. mutans (17.5 mm) and the lowest average zone of inhibition against B. subtilis (16 mm). Other bacteria showed zones with inhibitory values in the middle of these two extremes. The literature states that the growth inhibition zone for Candida species is 9.25 ± 0.5 (Soares et al., 2012).

## FINDINGS, CONCLUSION AND RECOMMENDATION

### 1.27 Findings

The synergistic extraction of essential oils from these two plants could potentially yield a unique blend with enhanced bioactive properties. Essential oil from coriander and licorice root have been reported to possess antimicrobial and antioxidant activities among others.

Among the activities performed include antioxidant, antifungal, and antibacterial ones. The 50µL essential oil blend used in the study (25µL of C. sativum + 25µL of G. glabra) showed the strongest synergistic activity against P. mirabilis. 18 mm was the value of its first zone of inhibition (R1), and 17 mm was the value of its second zone. The average of the two measured zones of inhibition was found to be 17.5 mm. Since individual antioxidant activities were lower, it was determined that synergistic antioxidant activities were higher. GC-MS examination of oils isolated from G. glabra revealed that compounds (29), (32) and (33) had the highest concentrations, at 49.32, 29.11, and 10.97%, respectively.

### 1.28 Conclusion

Clevenger and Soxhlet apparatuses were used to extract the essential oils of C. sativum and G. glabra, respectively. These essential oils are vital components of medications. Their activity tests provide powerful insights into their potential for treating various microbial (bacterial, fungal) illnesses. Antimicrobials (both antibacterial and antifungal) and antioxidants are important in the treatment of various oxidizing agents that pose a threat to human health. They primarily demonstrate a crucial role as antiaging agents. Both plants' essential oils are important in the fields of odontology and cosmetics.

The combination of the coriander and licorice essential oil exhibited enhanced inhibitory effects against various microorganisms, including bacteria and fungi. They also show increased antioxidant potential, scavenging free radical and reducing oxidative stress.

### 1.29 Recommendations

These findings suggest that the synergistic extraction of essential oils from coriander and licorice may lead to the development of the natural products with enhanced antimicrobial and antioxidant properties. These properties could be useful in various applications, such as food preservation, pharmaceuticals, and cosmetics etc.

## REFERENCES

Abhilasha, S., Deepak. R., Devendra. J., Sunil. K. K., & Narendra. K. G., (2019). Biochemical characterization of coriander (coriander sativum L) genotypes. Indian journal of pure and applied biosciences. 7(6), 85-90.

Ahmed, S. F., Sajid, M., Ahmad, W., Zeenat, F., & Shakir, M. (2021). A comprehensive review on an important Unani drug mulethi (Root of *Glycyrrhiza glabra* Linn). *Journal of Pharmacognosy and Phytochemistry*, *10*(3), 488-493.

Ali, B., Al-Wabel, N. A., Shams, S., Ahamad, A., Khan, S. A., & Anwar, F. (2015). Essential oils used in aromatherapy: A systemic review. *Asian Pacific Journal of Tropical Biomedicine/Asian Pacific Journal of Tropical Biomedicine*, *5*(8), 601–611.

Asilbekova, D. T., Bobakulov, K. M., Sotimov, G. B., & Sagdullaev, S. S. (2021). Lipids and Essential Oil from Roots of *Glycyrrhiza glabra* growing in Uzbekistan. *Chemistry of Natural Compounds*, *57*, 238-241.

Bahrami, A., & Fattahi, R. (2021). Biodegradable carboxymethyl cellulose–polyvinyl alcohol composite incorporated with *Glycyrrhiza glabra* L. essential oil: Physicochemical and antibacterial features. *Food science & nutrition*, *9*(9), 4974-4985.

Bakhane, Y., Yadav, A. S., Bajaj, A., Sharma, A. K., & Raghuwanshi, D. K. (2014). Glycyrrhizaglabra L.: A Miracle Medicinal Herb. *Indo American Journal of Pharmaceutical Research*, *4*(2), 5880-5816.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods For In Vitro Evaluating Antimicrobial Activity: A Review. *Journal of Pharmaceutical Analysis*, *6*(2), 71-79.

Bhattarai, A. K., & Dixit, S. M. (2020). Ethnomedicinal and Pharmacological Importance of *Glycyrrhiza glabra* L. Wild Plants, *1*, 444-56.

Cary, J. W., Gilbert, M. K., Lebar, M. D., Majumdar, R., & Calvo, A. M. (2018). Aspergillus flavus secondary metabolites: more than just aflatoxins. *Food Safety*, *6*(1), 7-32.

Dastagir, G., & Rizvi, M. A. (2016). *Glycyrrhiza glabra* L. (Liquorice). *Pakistan Journal of Pharmaceutical Sciences*, *29*(5), 1727-1733.

Dettmer, K., Aronov, P. A., & Hammock, B. D. (2007). Mass Spectrometry-Based Metabolomics. *Mass Spectrometry Reviews*, *26*(1), 51-78

Ewase, A. E., El-Sherif, S., & Tawfik, N. (2013). Effect of Salinity Stress on Coriander (Coriandrum sativum) Seeds Germination and Plant Growth. *Egyptian Academic Journal of Biological Sciences, H. Botany/Egyptian Academic Journal of Biological Sciences, H. Botany*, *4*(1), 1–7.

Farooqui, A., Khan, A., Borghetto, I., Kazmi, S. U., Rubino, S., & Paglietti, B. (2015). Synergistic Antimicrobial Activity of Camellia sinensis and Juglans regia against Multidrug-Resistant Bacteria. *PloS One*, *10*(2), e0118431.

Freires, I. A., Denny, C., Benso, B., Alencar, S. M. D., & Rosalen, P. L. (2015). Antibacterial Activity of Essential Oils and Their Isolated Constituents against Cariogenic Bacteria: A Systematic Review. Molecules, 20(4), 7329-7358.

Kaur, R., Kaur, H., & Dhindsa, A. S. (2013). *Glycyrrhiza glabra*: a phytopharmacological review. I*nternational journal of pharmaceutical Sciences and Research*, *4*(7), 2470.

Khadam, S., Afzal, U., Gul, H., Hira, S., Satti, M., Yaqub, A., & Gulfraz, M. (2019). Phytochemical screening and bioactivity assessment of leaves and fruits extract of Carica papaya. *Pakistan journal of pharmaceutical sciences*, *32*(5). Pakistan Journal Pharmacetical Sciences, *32*(5), 1941-1948.

Kumar, N. D., & Sidhu, P. (2011). The antimicrobial activity of azardirachta indica, *Glycyrrhiza glabra*, cinnamum zeylanicum, syzygium aromaticum, accacia nilotica on streptococcus mutans and enterococcus faecalis - An in vitro study. *Endodontology*, *23*(1), 18.

Laribi, B., Kouki, K., M'Hamdi, M., & Bettaieb, T. (2015). Coriander (*Coriandrum sativum* L.) and its bioactive constituents. *Fitoterapia*, *103*, 9–26.

Loncar, B., Pezo, L., Pezo, M., Jovanović, A., Šuput, D., Radosavljević, M., & Aćimović, M. (2024). Do climate conditions affect the quality of the Apiaceae fruits' essential oils? *Horticulturae*, *10*(6), 577.

Mahajan, M., Kuiry, R., & Pal, P. K. (2020). Understanding the consequence of environmental stress for accumulation of secondary metabolites in medicinal and aromatic plants. *Journal of Applied Research on Medicinal and Aromatic Plants*, *18*, 100255.

Mahleyuddin, N. N., Moshawih, S., Ming, L. C., Zulkifly, H. H., Kifli, N., Loy, M. J., Sarker, M. M. R., Al-Worafi, Y. M., Goh, B. H., Thuraisingam, S., & Goh, H. P. (2021). *Coriandrum sativum* L.: A Review on Ethnopharmacology, Phytochemistry, and Cardiovascular Benefits. *Molecules, 27*(1), 209.

Matasyoh, J., Maiyo, Z., Ngure, R., & Chepkorir, R. (2009). Chemical composition and antimicrobial activity of the essential oil of Coriandrum sativum. *Food Chemistry*, *113*(2), 526–529.

Nadeem, M., Anjum, F, M. A., Khan, M. I. K., Tehseen, S., El-Ghorab, A., & Iqbal Sultan, I. J. (2013). Nutritional and Medicinal Aspects of Coriander (*Coriandrum sativum* L.) A Review. *British Food Journal*, *115*(5), 743-755.

Nazzaro, F., Fratianni, F., Coppola, R., & De Feo, V. (2017). Essential Oils and Antifungal Activity. *Pharmaceuticals*, *10*(4), 86.

Olalere, O. A., Gan, C., Taiwo, A. E., Adeyi, O., & Olaiya, F. G. (2024). Essential Oils: Sustainable Extraction Techniques and Nutraceuticals Perspectives. In *Methods and protocols in food science* (pp. 373–389).

Pathak Nimish, L., Kasture Sanjay, B., Bhatt Nayna, M., & Rathod Jaimik, D. (2011). Phytopharmacological Properties of Coriander Sativum as A Potential Medicinal Tree: An Overview. *Journal of Applied and Pharmaceutical Sciences*, *1*(4), 20-25.

Rajeshwari, U., & Andallu, B. (2011). Medicinal Benefits Of Coriander (*Coriandrum sativum* L). *Spatula DD*, *1*(1), 51-58.

Ramos, L. F. S., De Siqueira Amorim, R., Dantas, M. K. G., Marques, M. B., Costa, M. J. F., & De Souza, P. H. S. (2023). Atividade antimicrobiana do Sanativo® frente a microrganismos de interesse odontológico. *Peer Review*, *5*(19), 259–269.

Soares, B. V., Morais, S. M., Fontenelle, R. O. D. S., Queiroz, V. A., Vila-Nova, N. S., Pereira, C. M. C., Brito, E. S., Neto, M. a. S., Brito, E. H. S., Cavalcante, C. S. P., Castelo-Branco, D. S. C. M., & Rocha, M. F. G. (2012). Antifungal Activity, Toxicity and Chemical Composition of the Essential Oil of *Coriandrum sativum* L. Fruits. *Molecules/Molecules Online/Molecules Annual*, *17*(7), 8439–8448.

Wahab, S., Annadurai, S., Abullais, S. S., Das, G., Ahmad, W., Ahmad, M. F., Kandasamy, G., Vasudevan, R., Ali, M. S., & Amir, M. (2021). *Glycyrrhiza glabra* (Licorice): A Comprehensive Review on Its Phytochemistry, Biological Activities, Clinical Evidence and Toxicology. *Plants*, *10*(12), 2751.

Zhai, K. F., Duan, H., Cui, C. Y., Cao, Y. Y., Si, J. L., Yang, H. J., & Wei, Z. J. (2019). Liquiritin From Glycyrrhiza Uralensis Attenuating Rheumatoid Arthritis Via Reducing Inflammation, Suppressing Angiogenesis, And Inhibiting MAPK Signaling Pathway. *Journal of Agricultural and Food Chemistry*, *67*(10), 2856-2864.