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SYNERGISTIC ANTIMIROBIAL AND ANTIOXIDANT ACTIVITIES OF HYDRODISTILLED ESSENTIAL OILS FROM SEEDS OF *NIGELLA SATIVA* AND *CORIANDRUM SATIVUM*

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Abstract

In the present work, we aimed to determine composition and synergistic biological activities of hydro-distilled essential oils from seeds of *Nigella sativa* Linn and *Coriandrum sativum* Linn. The essential oils, obtained by optimized extractions using the hydrodistillation technique, were analyzed for their chemical compositions. Hydro distilled essential oils (EOs) of *Nigella sativa* and *Coriandrum sativum* seeds were analyzed by GC-MS and FT-IR, and their synergistic antimicrobial and antioxidant activities were investigated. According to the GC-MS report, 25 chemical compounds were identified in *Nigella sativa,* with p-cymene and α-pellandrene being the major compounds. Similarly, 23 chemical compounds were identified in *Coriandrum sativum,* with linalool and neryl acetate as the major compounds. Similarly, 23 chemical compounds have been analyzed in *Coriandrum sativum,* and their major compounds are linalool and neryl acetate. The antimicrobial activity was tested using agar well diffusion against several pathogenic microorganisms to study possible synergism when they coexisted. Antioxidant activity was also determined using the DPPH radical scavenging *in vitro* test. The combined essential oils exhibited stronger antimicrobial and antioxidant activities indicating their potential as natural preservatives and therapeutic agents. This study adds to our knowledge of the bioactivity of these plant essential oils and helps justify their application in pharmaceutical and food technologies.The synergistic effect of both plant oils showed maximum antimicrobial activity against *Salmonella typhi* gram-negative bacteria and fungus *Candida albicans*, as well as *Escherichia coli* gram-negative bacteria. The antioxidant activity results showed that the mixed oils of both plants had maximum antioxidant activities compared to the standard antioxidant, ascorbic acid.

Keywords: Essential oils; Synergistic effect; *Nigella sativa*; *Coriandrum sativum*; Antimicrobial activities; Antioxidant activities

1. Introduction

Essential oils extracted from medicinal plants have received significant attention because of their bioactive molecules that may have health benefits, particularly antimicrobial and antioxidant activities. Black cumin (*Nigella sativa* L.) is a well-known medicinal plant, the essential oil of which contains high levels of bioactive substances (such as thymoquinone, p-cymene, and α-thujene) displaying strong antimicrobial, antifungal, anti-inflammatory, and antioxidant activities (Hassanien et al., 2015; Kazemi, 2014). Several extraction techniques, such as microwave-assisted extraction and ultrasound-assisted extraction, have been optimized to maximize the yield and maintain the bioactivity of *N. sativa* essential oil, with thymoquinone identified as a major active compound contributing to its excellent antioxidant and antimicrobial potential (Abedi et al., 2017; Dar et al., 2024).

Furthermore, essential oil of *Nigella sativa* is rich in phenolic compounds, which are of impressive pharmacological importance with thymoquinone (TQ) as the major compound responsible for many of the medicinal properties, such as antioxidant, antimicrobial, antidiabetic and hepatoprotective activities. Other important phenols include dithymoquinone, thymohydroquinone, and thymol. The chemical composition of N. sativa oil varies depending on the extraction method, and the resulting extract contains different quantities of thymoquinone and other antioxidants. The fatty acid composition typically contains palmitic, linoleic, and oleic acid,s and the predominant triacylglycerol is trilinolein. Furthermore, N. sativa oil has demonstrated a broad spectrum of antimicrobial activity against clinically relevant bacteria and fungi, making it relevant to traditional medicine (Abedi et al., 2017; Dubey et al., 2016; Khoddami et al., 2010; Ramadan, 2007; Sicak & Erdoğan Eliuz, 2019; Tiruppur Venkatachallam et al., 2010; Zouirech et al., 2024).

The essential oils of both *Corriandrum sativum* and *Nigella sativa* have different chemical constituents and bioactive properties, highlighting the therapeutic and nutritional potential

of these oils. The essential oil of *Coriandrum sativum* (coriander) contains mainly oxygenated monoterpenes, with linalool as the principal, in addition to α-pinene, γ-terpinene, and geranyl acetate. These components are population/cultivar-dependent and have been grouped into chemotypes depending on their profiles. For example, seed oils have been found to have a strong antibacterial effect against Staphylococcus aureus and Escherichia coli, with some cultivars being more potent. Furthermore, aldehydes such as (E)-2-decenal, decanal, and (E)-2-dodecenal are responsible for the aroma and medium antioxidant capacity of fresh coriander leaves, contributing to its sensory properties (Asgarpanah, 2012; Kumar et al., 2022; Talebi et al., 2024).

Also, *Coriandrum sativum* Linn (coriander) was beneficial for its essential oil, mainly from seeds, which showed a significant antibacterial effect against a wide range of pathogenic bacteria. It has been reported that coriander oil works mainly by disrupting the membranes of bacterial cells, which ultimately contributes to cell death and is therefore promising for food safety and healthcare formulations (Lo Cantore et al., 2004; Silva et al., 2011). Coriander is a bactericidal natural essential oil that can be used to treat bacterial diseases and preserve food. (Abedi et al., 2017; Dubey et al., 2016; Khoddami et al., 2010; Ramadan, 2007; Sicak & Erdoğan Eliuz, 2019; Tiruppur Venkatachallam et al., 2010; Zouirech et al., 2024).

1.2. Synergistic activities of essential oils

The synergistic effect of essential oils (EOs) has emerged as a field of growing interest due to the interaction between several bioactive compounds of EOs, which consequently have a higher antimicrobial activity than single EOs. For example, adding clove bud, star anise, and cinnamon oils to chitosan films improved antimicrobial activity, with the controlled release of cinnamon oil and its compatibility with the film matrix providing the greatest synergistic effect (Wang et al., 2011). The synergistic effect of plant EOs, antibiotics, organic acids, and other preservatives can effectively inhibit seafood spoilage microorganisms and prolong the shelf life of seafood by inhibiting microbial growth (Huang et al., 2021). The use of essential

oils derived from plant sources, such as peppermint, thyme, and lavender, in certain combinations has broadened their antibacterial activity, and these compounds are capable of faster elimination of foodborne pathogens, such as Escherichia coli and Listeria monocytogenes, than when used individually (Angane et al., 2023). However, the composition of the groups (as indicated by the iso-eugenol equivalent 3b) shows that when terpene alcohols, such as linalool, are added to essential oils, the resulting compound is more effective against bacteria and fungi but is not necessarily more toxic. When tested together, this combination lowers the oil concentration required for antimicrobial efficacy (Herman et al., 2015). Additionally, the synergistic or additive antibacterial effects of Lavandula essential oils with compounds such as camphor could benefit the pharmaceutical and food industries (Karaca et al., 2020). Similarly, a blend of essential oils, such as cinnamon and mace or prikhom, has been reported to exhibit strong synergistic antimicrobial and antioxidant activities, further enhancing functional properties such as food safety (Nanasombat & Wimuttigosol, 2011). Systematic reviews have concluded that applying EO mixtures alongside classical antimicrobials enhances therapeutic effects against resistant pathogens owing to the interactive effects of diverse EOCs on multiple microbial targets (Soulaimani, 2025). Finally, mixed EOs can compensate for the limitations of some single EOs and reduce effective doses, sensory effects, and resistance development, making them suitable for natural food preservation or antimicrobial formulations (György et al., 2020; Seow et al., 2013).

2. Materials and methods

2.1. Plant material collection and identification

Healthy seeds of *Nigella sativa* and *Coriandrum sativum* were purchased from a local market of Dera Ismail Khan and authenticated by a taxonomist, Dr. Hameedullah, Head of the Department of Pharmacognosy, Faculty of Pharmacy, Gomal University, Dera Ismail Khan.

2.2. Hydrodistillation oils essential oils

Essential oils (EOs) were obtained through hydrodistillation from *Nigella sativa* and *Coriandrum sativum* seeds. This process employed a Clevenger-type apparatus, and hydrodistillation of essential oils was performed by placing *Coriandrum sativum* seeds (500 g) and distilled water (3000 mL) in a round-bottom flask (5000 mL) of the Clevenger apparatus and fixing it on a heating mantle for heating with the help of iron stands. The heating mantle was then switched on and adjusted to the desired temperature (100 °C). The mixture of seeds and distilled water was boiled to vaporize the volatile components or bioactive compounds. The emerging vapors, consisting of water and essential oil, were gathered in the side arm of the Clevenger apparatus. Water from a chiller was allowed to pass through the condenser to provide it with cold water and keep it cool. After hydrodistillation, the essential oil was extracted for 6h, heat source was switched off. The essential oil (EO) layer, which is less dense than water, was above the aqueous layer. Then, a mixture of water and essential oils from the side arm of the apparatus was transferred into a small clean and dry beaker. A small quantity of anhydrous sodium sulfate (anhydrous) was added to the essential oil to remove cloudiness. This procedure was repeated three times using fresh samples. The outcomes of all three steps were mixed and placed in a dry vial, tightly capped, and stored in a refrigerator at 4 °C until further use. The same hydrodistillation method was used to extract essential oils (EOs) from Nigella sativa seeds by replacing coriander seeds with Nigella sativa seeds (500 g). The essential oils were placed in dry vials, securely sealed, and kept at 4 °C before further use.

2.4. GC-MS and FTIR characterization of essential oils

The hydrodistilled essential oils were analyzed for number of components, concentration and retention time using gas chromatography-mass spectrometry (GC-MS) model QP2010 Plus Shimadzu Japan. A 500 ppm solution of essential oil was prepared in DCM and injected 1µL to GCMS using BDS capillary column (30m × 0.25mm × 0.25µm) using EI +ve ionization mode. Components were detected using mass spectral data base of NIST Library. The

presence of various functional groups in essential oils was Fourier-transform infrared spectroscopy (FT-IR) was used for the characterization of essential oils. The FT-IR spectra were procured on Bruker FT-IR tensor 27.

2.5. Antimicrobial activities

The antimicrobial (antibacterial and antifungal) activities of essential oils hydrodistilled from seed of *Nigella sativa* and *Coriandrum sativum* were determined synergistically using various doses (50µL/well; 1`:1) following a standard procedure (Soukaina *et al*., 2022) employing Agar well diffusion method to assess synergistic inhibition zones of essential oils. The microbes used for the investigation of the synergistic antimicrobial activities of *C. sativum* and *N. sativa* seed essential oils (EOs) were, three gram-positive bacterial strains, five gram-negative bacterial strains, and a single fungal strain. These microorganisms were obtained from the microbiological lab and library complex of the Pakistan Council of Scientific and Industrial Research (PCSIR) in Peshawar. The selected microorganisms were placed in broth at not higher than 4 °C prior to their antimicrobial activities. Table 1 shows microbes and their sources.

Table 1: List microbes and their sources

Species	Type	Source of microbial strain
Bacillus subtilis	Gram positive	Clinical isolate
Staphylococcus aureus	Gram positive	ATCC #6538
Bacillus subtilis	Gram positive	Clinical isolate
Proteus mirabilis	Gram negative	ATCC#12553
Salmonella typhi	Gram negative	Clinical isolate
Pseudomonas aeruginosa	Gram negative	ATCC # 9721
Kleibsiella pneumonia	Gram negative	Clinical isolate
Escherichia coli	Gram negative	ATCC#87381
Candida albicans	Fungus	Clinical isolate

2.5.1. Preparation of culture media

To prepare the nutrient agar medium, a screw-capped bottle of 1litre was taken, filled with 900 mL of distilled water, and 28.1 g of nutrient agar was added (gelatin components: 5.1g/L; beef extract: 1g/L; agar: 15.2g/L; NaCl: 5.2g/L; and yeast extract: 2.1g/L) and filled up to 1litre. The mouth of the bottle was covered with a cap, and the contents of the bottle were mixed thoroughly and heated.

2.5.2. Antimicrobial assay by agar well diffusion method

The antimicrobial activities of different doses of *C. sativum* and *Nigella sativa* seed essential oils (EO) were examined using the well diffusion method. All equipment and nutrient agar media were sterilized in an autoclave at 1.6 pounds of pressure for 21 min at 122 °C. After cooling to 56 °C, the equipment and nutrient agar media were transferred to a biological safety cabinet for further processing. Subsequently, approximately 36 mL of nutrient agar media was poured into each petri plate. The Petri plates were closed with a cap after 30 min of solidification period. The Petri plates were placed in an inverted position for 37 °Cours in an incubator at 37°C (Fazal *et al*., 2025).

The antimicrobial activity of different doses of *C. sativum* and *Nigella sativa* seed essential oils (EO) was examined using the well diffusion method according to the protocol of (Fazal *et al*. (2025). For the refreshment of the culture media, we added the nutrient broth (2.9 g/101 mL; 1.4 g/101 mL respectively) to hot distilled water and poured it into conical flasks (19–24 mL), Petri plates (21 mL), and test tubes 122 °C mL) and sterilized at 122°C for 21 min by keeping the pressure at 16 37 °C and again incubated at 37°C overnight to check the undesired growth of microbes.

2.5.3. Inoculation of Petri plates

On the next day, the microbial cultures were transferred to broth media in a shaking water bath and kept on shaking for 11hours at 100rpm & 32.1 °C under incubation. The microbial

cultures in the test tubes were standardized by matching with a 0.5 McFarland turbidity standard using a UV spectrophotometer. Subsequently, 0.1 mL of standardized microbial culture was spread on the solid media plates surface with the help of cotton swab sticks that were already impregnated with microbial cultures and then kept in a refrigerator for absorption for 16 min. Two wells were created in each nutrient agar medium in each Petri dish using a sterile cork borer. Then different doses of essential oils (EOs) of *C. sativum* & *Nigella sativa* seeds were subjected to the wells of each petri plate with the help of micropipette. Now in order To compare the antimicrobial activities of *C. sativum* and *Nigella sativa* seed essential oils (EO) with standard antibiotics, we used azithromycin and ciprofloxacin as positive controls against all microbial strains. The Petri plates containing bacterial cultures were incubated at 37℃ for 24hours. However, the fungal cultures were incubated in an incubator for 3-4 days at 37℃. The antimicrobial activities of both standard antibiotics were assessed using the well diffusion method. The antimicrobial activities in terms of inhibition zones (mm) around the wells were measured using the millimeter scale.

2.6. Synergistic antioxidant activities

The individual and synergistic antioxidant activities of the essential oils of *Coriandrum sativum* and *Nigella sativa* were assessed in terms of radical scavenging ability or hydrogen donating ability using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the procedure described by Ahmad *et al.* (2011) with slight modifications. For antioxidant activity, first a 0.1 mM stock solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH), was prepared by dissolving 3.94 mg of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in 100 mL of methanol. Stock solution of *N. sativa* and *C. sativum* essential was prepared by mixing in 10mg/mL of methanol+100 µL DMSO (~10mg/mL). Working solutions of 25µg/mL and 50µg/mL were prepared from the stock. For individual antioxidant values 1mL of working solution of essential oil was mixt with 1mL of DPPH. For synergistic antioxidant activity 1mL containing both oils (0.5 mL of each essential oil; 1:1) was mixed with 1mL of DPPH solution in separate

test tubes. After adding the sample solution to the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution, the test tube was shaken and allowed to stand at 27 °C in the dark for 30 min. Ascorbic acid was used as the standard antioxidant at a concentration of 5µg/mL was prepared. For blank methanol without any desired plant-mixed essential oil (EO) sample, and the blank was used to auto-zero the spectrophotometer. After 30 min of incubation, the absorbance of the solution was measured using UV spectroscopy at 517 nm. Whenever 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable free radical was reduced, its purple color discolored, indicating that the test sample mixture possessed antioxidative properties. Radical scavenging activity was high in cases of lower absorbance of the solution. Each method was performed in triplicate. The radical scavenging activity of the methanolic mixed essential oil (EO) sample was evaluated as the inhibition percentage of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was calculated by the following formula.

Anti-oxidant activity %: (1-AE/AD) x100

Where AE is the absorbance of the methanolic mixed essential oil (EO) sample, and AD is the 2 the 2-diphenyl1-picrylhydrazyl (DPPH) solution, without plant essential oil (EO), which was used as a control.

3. Results & Discussion

The *Nigella sativa* and *Coriandrum sativum* seeds used for extraction of essential oils were obtained from the local market of Dera Ismail Khan. Below Figures 1 and depict seeds of *N. sativa* and *C. sativum*. The pictures have been taken from the internet and URL of the pictures has been provided.

Figure 1: Nigella sativa Seeds

https://www.researchgate.net/profile/Abdurohman-Yessuf/publication/287208063/Figure/fig2/AS:650519487991826@1532107340992/Black-cumin-Nigella-Sativa-Linn-seed.png

Figure 2: Coriandrum sativum Seeds

https://cloudinary.images-iherb.com/image/upload/f_auto,q_auto:eco/images/str/str02551/l/9.jpg

3.1. GC-MS analysis of essential oils

GC-MS results of *Nigella sativa* seed essential oil (EO) were obtained, and the data were analyzed using the retention time (min), peak areas, and percentage concentration of every component present in the essential oil (EO) of *Nigella sativa* seed essential oil. Figure 3 shows GC-MS chromatogram ant Table 2 shows various chemical components Nigella sativa

essential oils. Overall twenty five components were identified from the essential oil of nigella sativa Table 2 shows GC-MS outcome of *N. sativa* essential oil in terms of composition, retention time and concentration.

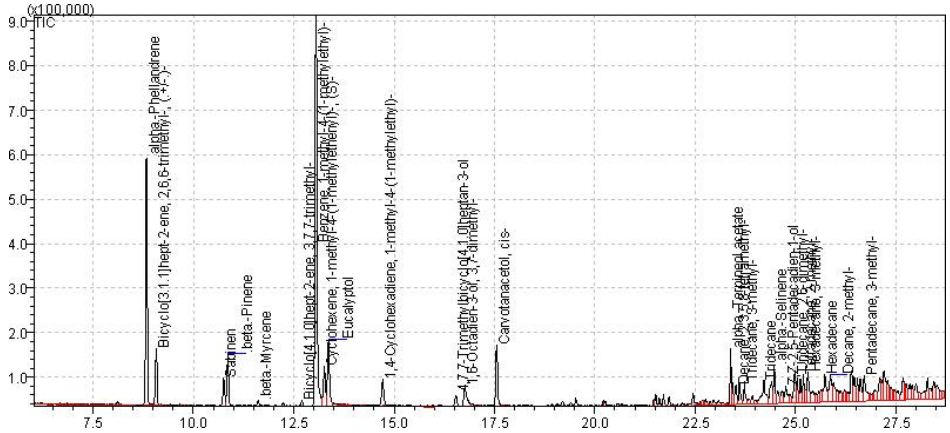

Figure 3: GC-MS chromatogram of *Nigella sativa* seed essential oil

Table 2: GC-MS table essential oil of *Nigella sativa*

QUANTITATIVE RESULT TABLE

ID#	Name	R.Time	Area	Conc. (%)
1	.alpha.-Phellandrene	8.829	367359	18.17
2	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (.+/-.)-	9.075	74324	3.68
3	Sabinen	10.759	42004	2.08
4	.beta.-Pinene	10.861	71761	3.55
5	.beta.-Myrcene	11.612	8218	0.41
6	Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl-	12.702	5029	0.25
7	Benzene, 1-methyl-4-(1-methylethyl)-	13.058	1036440	51.27
8	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	13.266	37807	1.87
9	Eucalyptol	13.369	71125	3.52
10	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	14.714	37572	1.86
11	4,7,7-Trimethylbicyclo[4.1.0]heptan-3-ol	16.543	5625	0.28
12	1,6-Octadien-3-ol, 3,7-dimethyl-	16.765	22347	1.11
13	Carvotanacetol, cis-	17.553	27914	1.38
15	Decane, 2,3,5,8-tetramethyl-	23.519	14469	0.72
16	Tridecane, 3-methyl-	23.725	19468	0.96
17	Tridecane	24.214	19095	0.94
18	.alpha.-Selinene	24.470	11799	0.58
19	Z,Z-2,5-Pentadecadien-1-ol	24.751	6338	0.31
20	Undecane, 2,6-dimethyl-	24.987	36598	1.81
21	Tetradecane, 2-methyl-	25.198	18624	0.92
22	Hexadecane, 3-methyl-	25.307	21947	1.09
23	Hexadecane	25.732	16918	0.84
24	Decane, 2-methyl-	25.866	29401	1.45
25	Pentadecane, 3-methyl-	26.713	19158	0.95

The GC-MS report of *Nigella sativa* seed essential oil (EO) revealed a heterogeneous and complex medical composition, displaying the presence of many volatile constituents with potential biological connections. The complete chromatographic profile reflected a clear dominance of monoterpenes and their oxygenated derivatives, with trace amounts of sesquiterpenes and aliphatic hydrocarbons present. Based on mass spectral matching, retention time, and relative peak areas, 25 chemical compounds were identified, demonstrating the large fraction of the distilled essential oil.

In a recent analysis, benzene, 1-methyl-4-(1-methylethyl), was recognized as the dominant compound, accounting for 51.27 % of the total oil composition. The common name of this compound is p-cymene. P-cymene is widely distributed in nature. Its pleasant aroma makes it a popular ingredient in perfumes and cosmetics products. It is also used as a flavoring agent in food, beverages, and confections. In the industry, it serves as a starting material for the production of pesticides, fungicides, and antioxidants. Scientists have investigated its various pharmacological properties, such as its anti-inflammatory, antioxidant, antimicrobial, and pain-relieving properties, in addition to other potential effects.

The second most prominent compound, α-pellandrene (18.17%), is a monoterpene commonly reported to possess antioxidant, antimicrobial, and anti-inflammatory properties, demonstrating its significant role in the pharmacological efficacy of *Nigella sativa* seed essential oil (EO). The identification of these primary monoterpenes highlights their significance as key bioactive constituents and supports the traditional medicinal use of *Nigella sativa* seeds. In addition to this major compound, many other monoterpenes were identified in adequate concentrations, including bicyclo[3,1,1]hept-2-ene, 2,6,6-trimethyl- (3.68%), β -pinene (3.5%), and the oxygenated monoterpene eucalyptol (3.52%). These compounds are known for their pharmacological activities, including antioxidant, antimicrobial, and anti-inflammatory effects, and their presence further increasing the biological importance of the essential oil.

Many small quantity constituents were also identified, including carvotanacetol, α-selinene, ocimene derivatives, and long-chain aliphatic hydrocarbons, such as tridecane, tetradecane, and pentadecane derivatives. Although these compounds are present in small concentrations, they may provide overall stability, synergistic biological effects, and aroma complexity to essential oils (EO). Minor compounds have been reported to improve the activity of major compounds, thereby increasing the overall pharmacological efficacy of essential oils.

A comparison of the current findings with the existing GC-MS literature on *Nigella sativa* seed essential oil (EO) highlights both consistent compositional trends and significant deviations in the reported profiles. Although many studies have declared monoterpenes as the major constituents, the relative abundance and identity of the dominant compounds sometimes differ, with a few reports highlighting thymoquinone, p-cymene, and α-thujene as the predominant components. Such variability may be attributed to differences in geographical origin, climatic conditions, soil composition, harvesting time, plant maturity, extraction methods, and analytical parameters used during GC-MS analysis.

The relatively high concentration of benzene, 1-methyl-4-(1-methylethyl), observed in this study highlights the influence of location-specific factors and experimental procedures on essential oil composition. Collectively, the GC-MS results demonstrate that the essential oil of *Nigella sativa* seeds possesses a unique and chemically complex profile, dominated by bioactive monoterpenes. This compositional richness provides a solid chemical foundation for traditional therapeutic applications and reinforces its potential use in pharmaceutical, nutraceutical, and essential oil-based formulations.

Moreover, the differences reported among different studies highlight the need for standardized analytical methods to obtain reproducible results and reliable evaluations of the therapeutic value of the plants.

GC-MS results of *Coriandrum sativum* seeds (EO) were obtained, and the data were analyzed using the retention time (min), peak areas, and percentage concentration of every component present in the essential oil (EO) of *C. sativum* seeds. Figure 4 shows GC-MS chromatogram of *C. sativum* essential oil. The components of essential oil identified by GC-MS are shown in table 3:

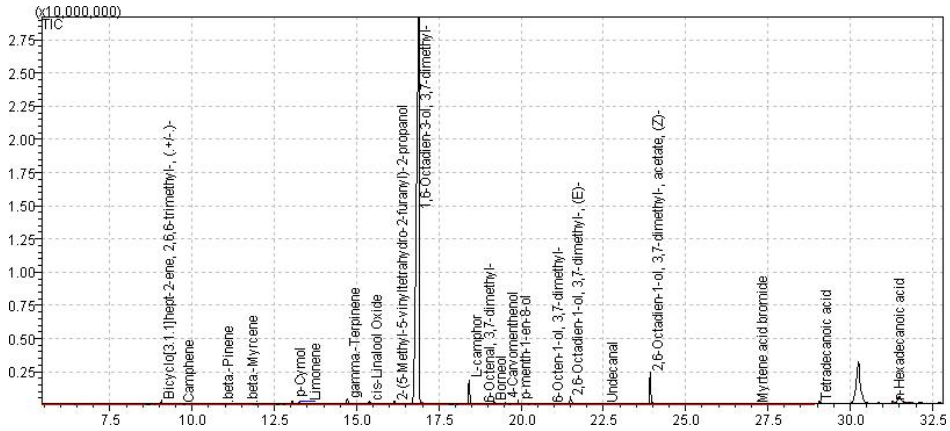

Figure 4: GC-MS chromatogram of *Coriandrum sativum* seed essential oil

Table 3: GC-MS analysis of coriander essential oil showing composition, retention time and peak area

QUANTITATIVE RESULT TABLE

ID#	Name	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	Borneol	19.169	181310	0.68
14	4-Carvomenthenol	19.524	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	2,6-Octadien-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

GC-MS analysis for chemical profiling was performed using GC-MS analysis Figure 4. GC-MS analysis demonstrated that *Coriandrum sativum* seed essential oil (EO) has a diverse and rich chemical composition (table 3). The oil exhibited a broad spectrum of volatile constituents.

The presence of these constituents demonstrates the biological efficacy and functional importance of essential oils. In total, 23 chemical compounds were detected in the essential oil samples. Compound identification was based on the retention time, mass spectral data, and relative peak areas. Together, these compounds represent the dominant portion of the oil. Analysis of the essential oil revealed a clear predominance of oxygenated monoterpenes in its chemical profile. Smaller amounts of monoterpene hydrocarbons, esters, aldehydes, fatty acids, and other smaller compounds were also identified. This characteristic compositional arrangement is a defining feature of *the essential oil (EO) of Coriandrum sativum seeds*. The dominance of these compounds is closely associated with the chemical stability, characteristic fragrance, and therapeutic potential of the oil.

Linalool (6-octadiene-3-ol, 3,7-dimethyl) was the dominant compound among all the identified compounds in the essential oil. This compound constituted 80.66 % of the overall oil content. The pronounced dominance of this compound influences the overall chemical and aromatic profiles of *Coriandrum sativum* seed essential oil (EO). Linalool is a well-known oxygenated monoterpene alcohol with antioxidant, antimicrobial, anti-inflammatory, and anxiolytic properties. In addition, its predominance may reflect favorable environmental conditions and extraction processes that enhance the retention and biosynthesis of oxygenated monoterpenes in seeds.

In addition to linalool, which is the major compound, the GC-MS report of the essential oil revealed many other oxygenated monoterpene hydrocarbons in moderate amounts. The prominent compound was 2,6-octadiene-1-ol, 3,7-dimethyl-, acetate (Z) (4.5 %). nonivamide (3.68 %), camphor (2.35 %) & limonene (1.02 %). These compounds are widely known for their antimicrobial, analgesic, digestive, and aromatic activities. Together, these compounds contribute significantly to the therapeutic and functional efficacy of this oil. 2, 6-octadiene-1-ol, 3, 7-dimethyl-, acetate (Z) is the second major compound, and its common names are neryl acetate, neryl acetate, and cis-geranyl acetate.

Many monoterpene hydrocarbons were identified in the essential oil. The identified compounds were γ -terpinene, α-pinene, β-pinene, and β-myrc. Although present at lower concentrations, these compounds further enhanced the oil's aromatic richness and biological potential through synergistic effects.

Several minor constituents, including borneol, p-cymenol, octanal, tetradecanoic acid, and hexadecanoic acid, were detected in trace amounts. Despite their lower abundance, these compounds may alter the biological efficacy of major compounds through synergistic interactions with them. Such interactions can contribute to improving the overall therapeutic effectiveness, stability, and aroma longevity of essential oils. GC-MS analysis detected fatty acids and aldehydes in the essential oil. These identifications further enhance the chemical complexity of the essential oil. The detection of these compounds indicates the presence of a complex mixture of compounds in the oil. This complexity extends beyond the dominant monoterpene components of the essential oils.

When we compared the GC-MS report with previously published GC-MS investigations, the present investigation demonstrated both similarities and prominent differences in the results. In many studies, linalool has been identified as the dominant component of *Coriandrum sativum* seed essential oil (EO). Although its relative abundance varies widely, reflecting the influence of environmental conditions, geographical origin, cultivar selection, seed maturity, and extraction techniques.

The elevated linalool content detected in this study aligns with the values reported for *Coriandrum sativum* seed essential oils (EOs) enriched with oxygenated monoterpenes. However, it surpassed the levels documented in studies where linalool was reported at moderate concentrations. In addition to linalool, many other constituents have been identified in essential oils. These include limonene, camphor, borneol, γ -terpinene, and acetate derivatives of monoterpene alcohols. These compounds have been previously reported, although their relative proportions differ widely between studies.

The variations in chemical composition identified in different studies are attributed to many influencing factors. These include soil composition, agro-climatic conditions, harvest timing, post-harvest handling, extraction methods and GC-MS analytical settings. Additionally, the frequent identification of minor compounds, including aldehydes, fatty acids, and long-chain hydrocarbons, supports the view that *Coriandrum sativum* seed essential oils (EOs) have a multifaceted chemical profile.

The observed chemical diversity of *Coriandrum sativum* seed essential oil (EO) may partially account for its wide range of pharmacological properties. These activities have been well documented in traditional medicinal systems and modern scientific studies. The interplay among many bioactive constituents may synergistically contribute to these outcomes.

The present results not only verified the monoterpene-rich nature of *Coriandrum sativum* seed essential oil (EO), but also provided region-specific valuable chemical data that increase our knowledge of their composition. Such information supports its utilization in the pharmaceutical, nutraceutical, food, and flavoring sectors.

3.3. FT-IR analysis of essential oils

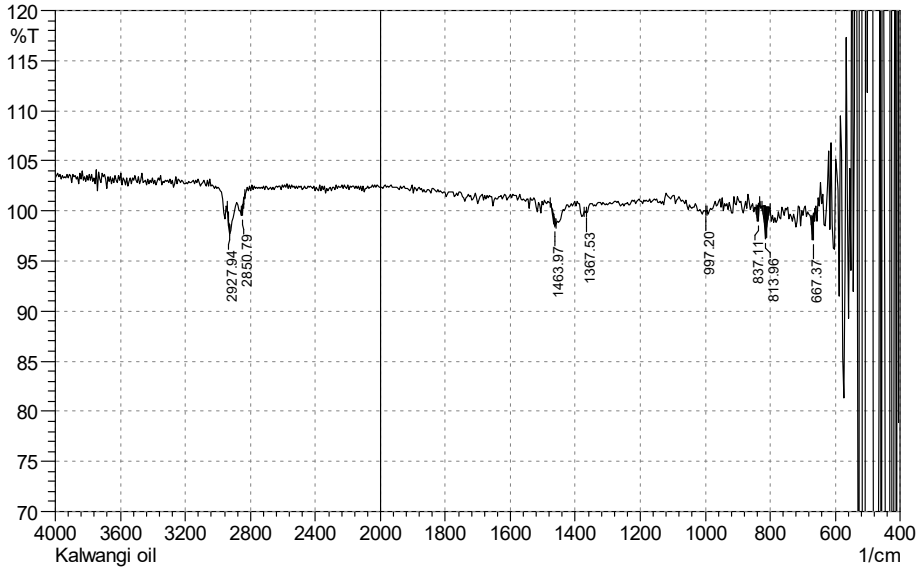

Figure 5: FT-IR Spectra of the essential oil (EO) of *Nigella sativa* seeds.

The FT-IR analysis of the essential oil (EO) of the seeds of *Nigella sativa* is given as Figure 5 which provided the following findings. The aliphatic hydrocarbons included in the essential oil (EO) of *Nigella sativa* seeds have C-H stretching vibrations, represented by the peaks at 2927.94 and 2850.79 cm^{-1}, respectively. These peaks, typically observed in the FTIR spectra of the essential oil (EO) of *Nigella sativa* seeds, indicate the presence of alkanes or alkenes. Similarly, the peak at 1463.97 cm^{-1} is likely caused by the bending of the C-H bonds in the aliphatic hydrocarbons found in the essential oil (EO) of Nigella sativa seeds. Additionally, the peak at 997.20 cm^{-1} may be related to the stretching vibrations of C-O bonds in ethers, which may be present in the essential oil (EO) of *Nigella sativa* seeds, while the peak at 1367.53 cm^{-1} may be related to the stretching vibrations of C-H bonds in the aromatic ring of compounds such as thymoquinone, which is a significant component. Additionally, the peak at 667.37 cm^{-1} may be caused by a C-halogen impurity in the essential oil (EO) of *Nigella sativa* seeds, while the peak at 837.21 cm^{-1} may be related to the bending vibrations of the C-H bonds in the aromatic ring.

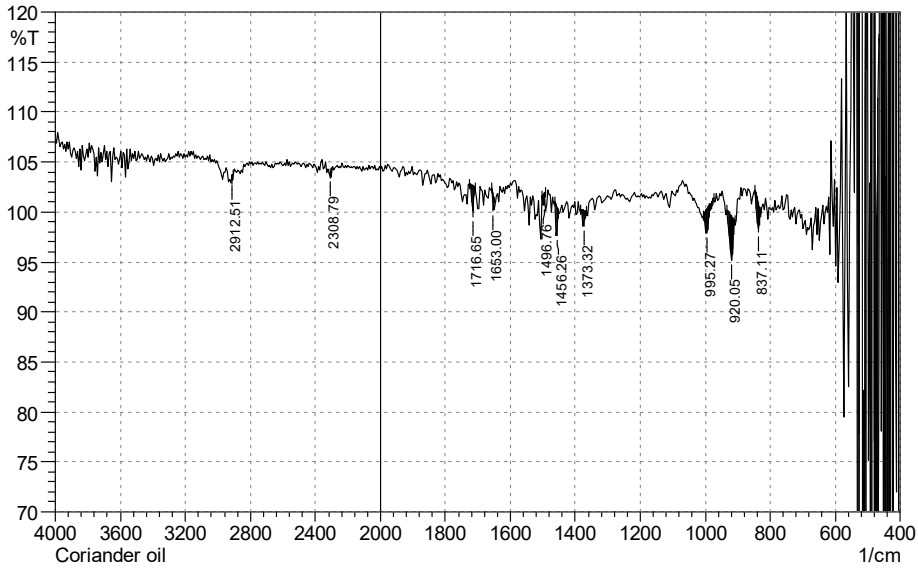

Figure 6: FT-IR Spectra of the essential oil (EO) of *Coriandrum sativum* seeds

The FT-IR analysis of the essential oil (EO) extracted from *Coriandrum sativum* seeds is shown as Figure 6. The study showed that, the stretching vibration of the C-H bonds in the methyl and methylene groups of the aliphatic chains is responsible for the peak at 2912.51 cm^{-1}. The presence of contaminants or ambient gases, such as nitrogen or carbon dioxide, is most likely to cause the peak at 2308.79 cm^{-1}. The peak at 1716.65 cm^{-1}, which is also likely to be present in the essential oil (EO) of *Coriandrum sativum* seeds as an ester, is related to the stretching vibration of the C=O bond in a carbonyl group. The peak at 1653.00 cm-1 is attributed to the stretching vibration of the C=C bond in an unsaturated carbonyl molecule, such as an aldehyde or a ketone. The peak at 1456.20 cm^{-1} is attributed to the bending vibration of the C-H bonds in an aliphatic chain, whereas the peak at 1496.76 cm^{-1} is associated with the bending vibration of the C-H bonds in an aromatic ring, suggesting the existence of an aromatic component in the essential oil (EO) of *Coriandrum sativum* seeds. The bending vibration of the C-H bonds in the aromatic ring caused a peak at 1373.32 cm^{-1}. The stretching vibration of the C-O bond in an ether group, which may be present in the essential oil (EO) of *Coriandrum sativum* seeds as a terpene or other oxygenated molecule, is also thought to be the cause of the peak at 995.27 cm^{-1}. The bending vibration of the C-H bonds in the out-of-plane deformation of an aromatic ring is responsible for the peak at 920.05 cm^{-1}, whereas the bending vibration of the C-H bonds in the in-plane deformation of an aromatic ring is responsible for the peak at 837.12 cm^{-1}.

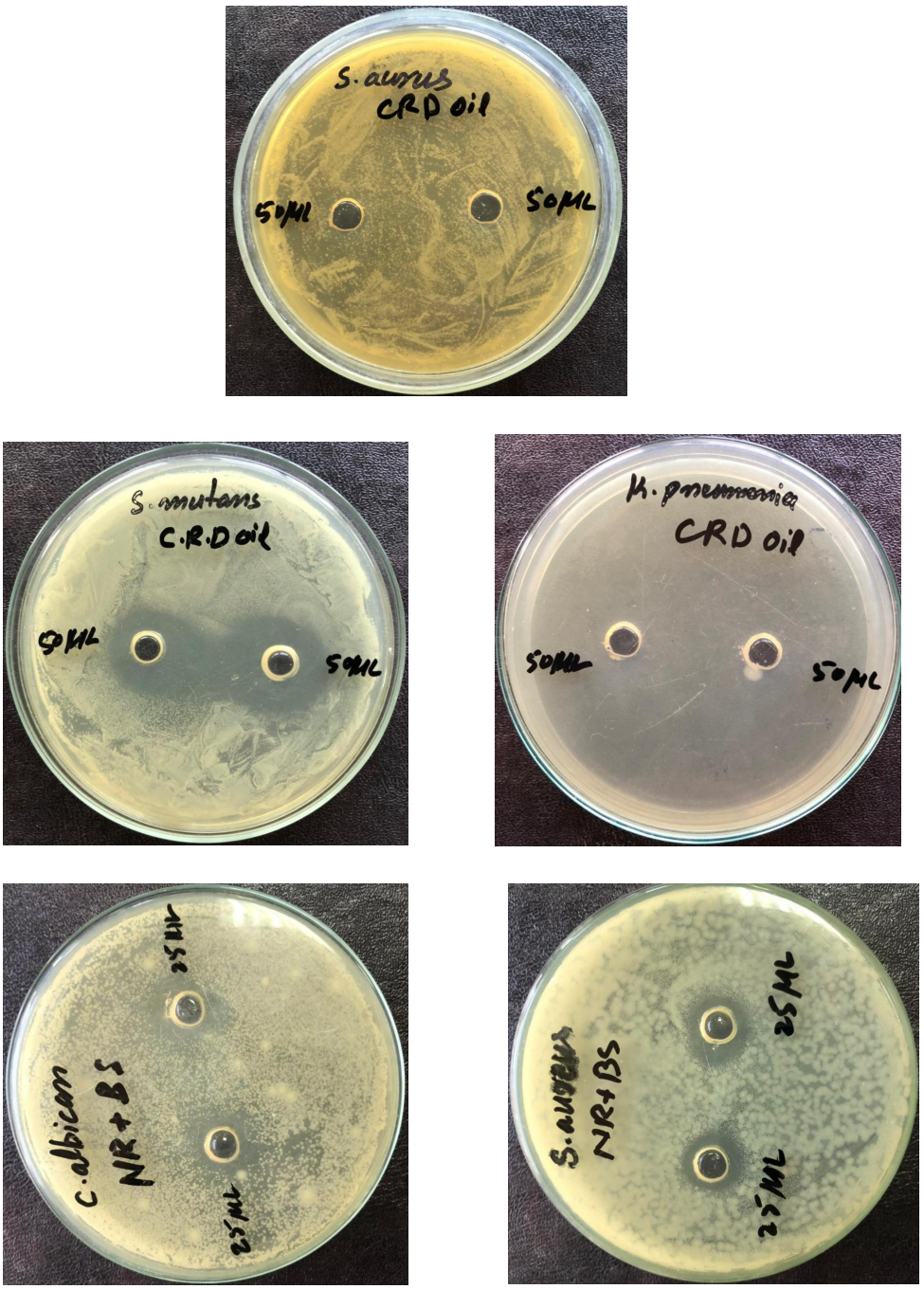

Figure 7: Petri plates used in antimicrobial activities

3.2. Synergistic antimicrobial activities of *C. sativum* and *Nigella sativa* seed essential oils (EO) samples.

Sample ID: CR+BS (CR = Coriander and BS = Black seed)

Strains used	**Concentration/ Zone of inhibition (mm)**		**Standards drugs**	
	CR+BS (25 µL+25 µL)		**Azith.**	**Cipro.**
	R_1	**R_2**	**(25µg)**	**(25µg)**
Escherichia coli	17	17	20	35
Pseudomonas aeruginosa	15	14	35	63
Klebsiella penumonie	15	14	23	NA
Salmonella typhi	22	19	32	42
Proteus mirabilis	14	15	41	42
Staphylococcus aureus	13	14	31	50
Streptococcus mutans	15	15	39	43
Bacillus subtilis	14	12	26	44
Candida albicans	17	18	30	50

*****_Escherichia coli_ American type culture collection (ATCC)# 8738, *Pseudomonas aeruginosa* ATCC#9721, *Staphylococcus aureus* American type culture collection (ATCC)# 6538, *Streptococcus mutans* American type culture collection (ATCC)# 35668, *Proteus mirabilis* American type culture collection (ATCC)# 12553 { *Klebsiella penumonie, Bacillus subtilis, Salmonella typhi*, *Candida albicans* (Clinical isolates)}, Azithromycin (Azithro), Ciprofloxacin (Cipro), Not applied (NA)

To determine the synergistic antimicrobial activities of *C. sativum* and *Nigella sativa* seed essential oils (EO). First, we mixed the essential oils (EOs) from both plant seeds. Then, we

put a 25µl dose of this mixed essential oil (EOs) to each of the two wells of each petri dish with the help of a micropipette. We had already cultured nutrient agar medium on these petri-plates and then cultured these petri plates with five gram-negative bacterial strains, three gram-positive bacterial strains, and a single fungal strain against these mixed essential oils (EOs) of both plant seeds. Now in order to compare the antimicrobial activities of these mixed essential oils (EOs) we used the standard antibiotics azithromycin & ciprofloxacin as positive controls & filled each of their wells with 25µg dose of this mixed essential oils (EOs) with the help of a micropipette. We incubated the petri plate of bacteria for 24 h at 37℃. Similarly, we incubated the petri plates of th37 ℃*andida albicans* fungus for 3-4 days at 37℃ in an incubator. The overall study showed that the synergistic effect of *C. sativum* and *Nigella sativa* seed essential oils (EOs) enhanced their antimicrobial activities. Figure 7 shows Petri plates used in this study. One well of 50µl dose of mixed essential oils in 1:1 of *C. sativum* and *Nigella sativa* seeds in these Petri plates showed a zone of inhibition (ZOI) of 17 mm, and the other well of the same amount of dose showed a zone of inhibition (ZOI) of 18 mm against *Candida albicans*. Both standard antibiotics, azithromycin and ciprofloxacin at a 25µg dose showed a zone of inhibition (ZOI) of 30 mm and 50 mm, respectively. So the zone of inhibition of mixed essential oil (EO) was close to the zone of inhibition (ZOI) of standard azithromycin antibiotic. However, the zone of inhibition (ZOI) of the standard ciprofloxacin antibiotic was much larger than the zone of inhibition (ZOI) area of both wells of mixed essential oils (EOs). In this study, we used a lower dose of mixed essential oil (EO) than the 50µl dose of individual *C. sativum* seed essential oil (EO) that we used in the antimicrobial test. However, the synergistic effect of both plant seed essential oils (EO) showed efficient results against *Candida albicans*.

The cells were incubated for 24 h at 37℃ in an incubator. One well with a 50 µL dose of mixed essential oil (EO) of *C. sativum* and *Nigella sativa* seeds in a petri plate showed a zone of inhibition (ZOI) of 13 mm, and the other well with the same amount of dose showed zone

of inhibition (ZOI) of 14 mm against *Staphylococcus aureus* bacteria. Both standard antibiotics, azithromycin and ciprofloxacin at a 25µg dose showed a zone of inhibition (ZOI) of 31 mm and 50 mm, respectively. The zone of inhibition (ZOI) of the mixed essential oil (EO) of both wells was close to the zone of inhibition (ZOI) of standard azithromycin antibiotic, but the diameter of zone of inhibition (ZOI) of ciprofloxacin antibiotic was very large compared to the diameter of zone of inhibition (ZOI) of both wells of mixed essential oil (EO). Instead of using a lower dose of mixed essential oil (EO) as compared to the dose that was used in the case of individual *C. sativum* seeds essential oil (EO) antimicrobial test. However, the synergistic effect of the desired plant seed essential oils (EOs) showed good results against *Staphylococcus aureus*.

Overall, we observed that the synergistic effect of the mixed essential oils (EO) of the desired plant seeds showed efficacious results against all the selected microbial strains. In conclusion, the synergistic effect of the mixed essential oils (EO) of the desired plant seeds showed more efficient results than their individual effects.

3.3. Synergistic antioxidant activity results

Wave length = 517nm, DPPH

Anti-oxidant activity %: (A°-A/A°) x100

Table 4: Synergistic antioxidant activity results

Sample code	%AO
CRBS	46.9209
Ascorbic acid	95.1087

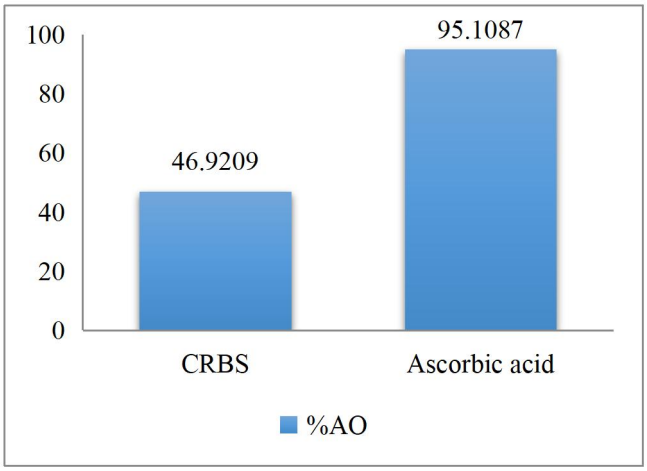

Figure 8: Graph of synergistic antioxidant activities of essential oils (EOs) of *N. sativa* and *C. sativum*

3.4. Synergistic antioxidant capacity

The synergistic antioxidant activities of methanolic mixed essential oil (EO) from the seeds of *Nigella sativa* and *Coriandrum sativum* showed a maximum antioxidant effect of 47% compared with the standard antioxidant ascorbic acid, which possessed 95% antioxidant activity. The absorbance of the methanolic mixed essential oil (EO) gradually increased with increasing concentrations of the organic mixed essential oil (EO) from the seeds of *Nigella sativa* and *Coriandrum sativum*. The methanolic mixed essential oil (EO) showed a significant percentage of inhibition comparable to that of ascorbic acid. The synergistic antioxidant activity, expressed as the percentage of inhibition, is presented in Table 4 and Fig. 6.

4. Conclusion

The antimicrobial and antioxidant activities of *Nigella sativa* and *Coriandrum sativum* seed essential oils (EO) were investigated individually and synergistically, and their chemical compositions were identified through GC-MS & FT-IR analyses. Most compounds in the essential oils (EO) of these medicinal plant seeds possess potent antimicrobial and antioxidant activities. The essential oils (EOs) of these medicinal plant seeds possess significant antimicrobial and antioxidant activities individually, but their synergistic effect

possesses great antimicrobial and antioxidant activities owing to their effectiveness against different gram-positive and gram-negative bacterial strains, as well as a fungal strain. The results of the investigation showed the significance of these medicinal plant seed essential oils (EO) in the treatment of many diseases owing to their therapeutic potential against different microbial strains and their increased synergistic antioxidant effect compared to their individual effects. Both plant seed essential oils (EO) can be used for food preservation and many other purposes for the welfare of humans and animals owing to their potent antioxidant and antimicrobial activities.

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Conflict of interest statement

The authors declare no conflict of interest.

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