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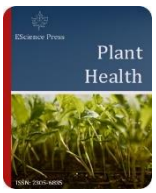

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Exploring the Antibacterial Properties and Chemical Profiles of Essential Oils from *Syzygium aromaticum* and *Nigella sativa* Against MDR Bacteria

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ABSTRACT

For centuries, plants have been studied for their healing properties. Recently, the rise of antibiotic resistance in bacteria has prompted scientists to explore herbal remedies. *Syzygium aromaticum*, from the Myrtaceae family, has been utilized as a histological clearing agent. In contrast, Nigella sativa (black cumin), a member of the Ranunculaceae family, has long been utilized in traditional medicine dating back to ancient times. The current study investigates the chemical composition and antibacterial activity of essential oils from *Syzygium aromaticum* (clove) and *Nigella sativa* (black cumin) against multidrug-resistant (MDR) bacteria. Using the agar well diffusion method, *Syzygium aromaticum* exhibited significant antibacterial activity, particularly against *Salmonella typhi* (30.66 mm inhibition zone), while *Nigella sativa* was effective only against *Staphylococcus aureus* (19 mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for *Syzygium aromaticum* ranged from 3.53 to 7.06 mg/mL, indicating stronger antibacterial properties than *Nigella sativa* (MIC: 12.7 mg/mL, MBC: 22.5 mg/mL). GC-MS analysis revealed that *Syzygium aromaticum* contained bioactive compounds like Diglycolic acid (76.59%) and Eugenol (2.06%), while *Nigella sativa* was dominated by Heptasiloxane (80.83%) and Thymoquinone (2.66%). These findings suggest that *Syzygium aromaticum* essential oil could serve as a potent natural antimicrobial agent against MDR pathogens.

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INTRODUCTION

The rise of multidrug resistance (MDR) in microorganisms poses a major global health challenge, prompting extensive research into plant-derived alternative medicines. In response, the World Health Organization (WHO) has identified key pathogens vulnerable to resistance, underscoring the urgent need for new antimicrobial treatments (Azizi *et al.,* 2021). Essential oils (EOs) have become increasingly popular as natural food preservatives due to their antibacterial properties against foodborne pathogens. Composed of various low-molecular-weight volatile compounds, EOs play a crucial role in plant defense mechanisms and exhibit strong antimicrobial activity. Their bioactive characteristics also make them widely used in the fragrance, cosmetic, and pharmaceutical industries (Meenu *et al.,* 2023). Antimicrobial drugs have been vital in saving lives and improving global life expectancy, but their efficacy is increasingly undermined by the rapid emergence of multidrug-resistant (MDR) bacteria. This resistance has led to more severe infections, longer hospitalizations, higher healthcare costs, and the

development of strains with reduced drug sensitivity (Dahiya & Purkayastha, 2012).

Essential oils, valued for their antibacterial, antiviral, antifungal, and anticancer properties, are widely used in medicine and cosmetics. They can enhance antibiotic effectiveness and offer a natural, often less toxic alternative to synthetic drugs (Naveed *et al.,* 2013; Emeka *et al.,* 2015). *Syzygium aromaticum* (clove), from the Myrtaceae family, produces essential oil rich in bioactive compounds, particularly phenylpropene eugenol, which is responsible for its characteristic aroma (Fagere & Magbou, 2016). This oil is widely used for its medicinal properties, including anti-inflammatory, antioxidant, antiparasitic, and anti-thrombotic effects, and has been traditionally applied to treat ailments such as dyspepsia, anxiety, and infections (Sharma *et al.,* 2014; Fagere & Magbou, 2016).

Nigella sativa, a Ranunculaceae family member, has a long history in traditional medicine across North Africa, the Mediterranean, Asia, and the Middle East for treating ailments like hyperglycemia, respiratory issues, pain, infections, and skin disorders (Dalli *et al.,* 2021; Tiji *et al.,* 2021). Its black seed oil shows strong antibacterial activity against *Staphylococcus* and *Streptococcus* species, alongside antioxidant, antimicrobial, antitumor, and immune-boosting effects (Shafique *et al.,* 2010; Tiji *et al.,* 2021). Essential oils from *Cinnamomum verum, Syzygium aromaticum*, and *Nigella sativa* showed strong antibacterial effects against resistant *E. faecalis* isolates, with *S. aromaticum* fractions exhibiting the lowest MIC and highest zones of inhibition. GC-MS analysis identified eugenic acid as the most effective fatty acid, suggesting these oils may be safe alternatives for treating antibiotic-resistant diarrheal infections in children (Ali *et al.,* 2022).

This study aims to investigate the antibacterial efficacy and chemical composition of *Syzygium aromaticum* and *Nigella sativa* essential oils against multidrug-resistant (MDR) bacteria. The motivation is to identify natural alternatives to antibiotics that can address the growing threat of MDR pathogens. By examining these essential oils, the research seeks to support the development of safer, plant-based antimicrobial therapies.

MATERIALS AND METHODS

Essential Oils Collection

Essential oils of Nigella sativa and Syzygium aromaticum were acquired from the neighborhood market and brought to the University of Haripur's Microbiology Lab.

Bacterial Strains, Culture Media, and Inoculum Preparation

MDR bacterial strains were obtained from the Microbiology Lab, at the University of Haripur. Antibacterial activity was tested against *Salmonella typhi*, *Pseudomonas aeruginosa*, MRSA (Methicillin-resistant *Staphylococcus aureus)*, and *E. coli*. The experiment was conducted using bacterial cultures as stock cultures, which were kept at 4 °C in suitable agar slants. Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) were utilized (Oulkheir *et al.,* 2017). Bacterial strains stored at -20°C were activated by culturing in nutrient broth for 24 hours at 37 °C. Inoculum preparation involved adjusting the overnight broth culture to cloudiness equivalent to 0.5 McFarland standards by mixing with double-distilled autoclaved water.

Antibacterial Activities

The MH broth dilution method was used to estimate the minimum inhibitory concentration (MIC), and the agar well diffusion technique was utilized to test antibacterial sensitivity. Distributed uniformly on nutrient agar plates, the overnight culture of every microbial strain was adjusted to 0.5 McFarland standard (10^8 CFU/ml). Wells (6 mm in diameter) were formed using a sterile borer, and 100 µl of essential oils were added. The inhibitory zone diameters on inoculated plates were measured in millimeters and compared to controls after they were incubated for 24 hours at 37 °C. Average inhibition zones were computed after three duplicate experiments were carried out (Dalli *et al.,* 2021).

Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined using the broth serial dilution method. Bacteria were preserved on nutrient agar slants and incubated for 24 hours at 37°C. Colonies were transferred to normal saline to achieve a 0.5 McFarland standard, and samples were loaded into a 96-well microplate, and incubated at 37°C for 24 hours. MIC was defined as the lowest concentration that prevented visible growth. A total of 20 µl from MIC clear wells was plated on Mueller-Hinton agar to determine MBC, the lowest concentration resulting in 99.9% bacterial growth control (Oulkheir *et al.,* 2017).

Determination of Synergistic Effects

The FIC was derived from the lowest concentration of tested essential oils that exhibited no growth of bacterial

strains (Dzotam *et al.,* 2015). FIC values were calculated as follows:

FIC (Essential oils) = MIC of essential oils together / MIC of essential oils alone

FIC (Syzygium aromaticum oils) = MIC of Syzygium aromaticum oils together / MIC Syzygium aromaticum oils alone

FIC (Nigella sativa) = MIC of Nigella sativa oils together / MIC of Nigella sativa alone

Synergistic effect: 0.5≥ ∑ FIC; Indifferent effect: 0.5<∑ FIC≤ 0.4; Antagonism effect: 4 >∑ FIC (Dzotam et al., 2015).

Gas Chromatography-Mass Spectrometry (GC–MS)

The chemical composition of both essential oils was assessed using GC-MS on a Perkin Elmer Clarus 600 GC with 600c MS. Two fused silica capillary columns Elite 5-MS (30 m) were used. The oven temperature was initially set to 50 °C for 2 minutes and then programmed to 300°C at a rate of 5 °C/min. The injector temperature was set between 220 and 250 °C. A 1 µl diluted sample (in chloroform) was injected with a 100:1 split ratio. Helium served as the carrier gas (2 mL/min), and analysis utilized the NIST (National Institute of Standards and Technology) library database (Al-Shammari *et al.,* 2017).

RESULTS

Antibacterial Activity of *Syzygium aromaticum* and *Nigella sativa* Essential Oils Against four MDR Strains

According to our research, *Syzygium aromaticum* oil has antimicrobial activity against every bacterial strain that was chosen, with inhibition zones measuring between 24.66 and 30.66 mm. The most significant antimicrobial effect was observed against *Salmonella typhi* (30.66 mm), while the lowest inhibition zone was noted against *Staphylococcus aureus* (24.66 mm) with *Syzygium aromaticum* oil. In contrast, *Nigella sativa* showed the highest inhibition zone against *Staphylococcus aureus* (19 mm) but demonstrated no antibacterial effects against *E. coli*, *Pseudomonas aeruginosa*, or *Salmonella typhi*. Additionally, the negative control exhibited no measurable activity on the culture plates. Overall, *Syzygium aromaticum* essential oil displayed superior antibacterial activity compared to *Nigella sativa* essential oil. Both *Nigella sativa* and *Syzygium aromaticum* oils' antibacterial efficacy against the investigated bacterial strains is shown in Figure 1.

Table 1. *Syzygium aromaticum and Nigella sativa* essential oils' zones of inhibition (mm) against tested bacterial strains.

Sr.	Essential Oils	Zone of Inhibition (mm + standard deviation)				F value	P value
		E. coli	*Pseudomonas aeruginosa*	*S. aureus*	*Salmonella typhi*		
1	*Syzygium aromaticum*	27 ± 2.645	24.66 ± 3.05	29 ± 3.60	30.66 ± 1.15	25.94	0.01
2	*Nigella sativa*	0	0	19 ± 1	0		

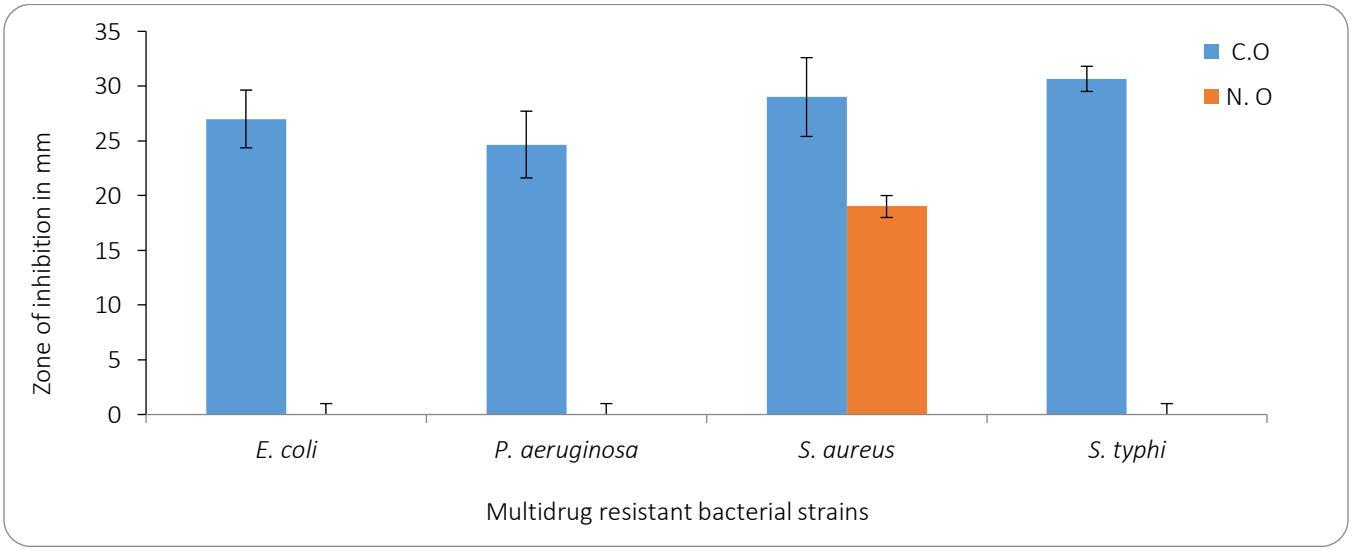

Figure 1. The antibacterial activity of selected essential oils against four MDR bacterial strains.

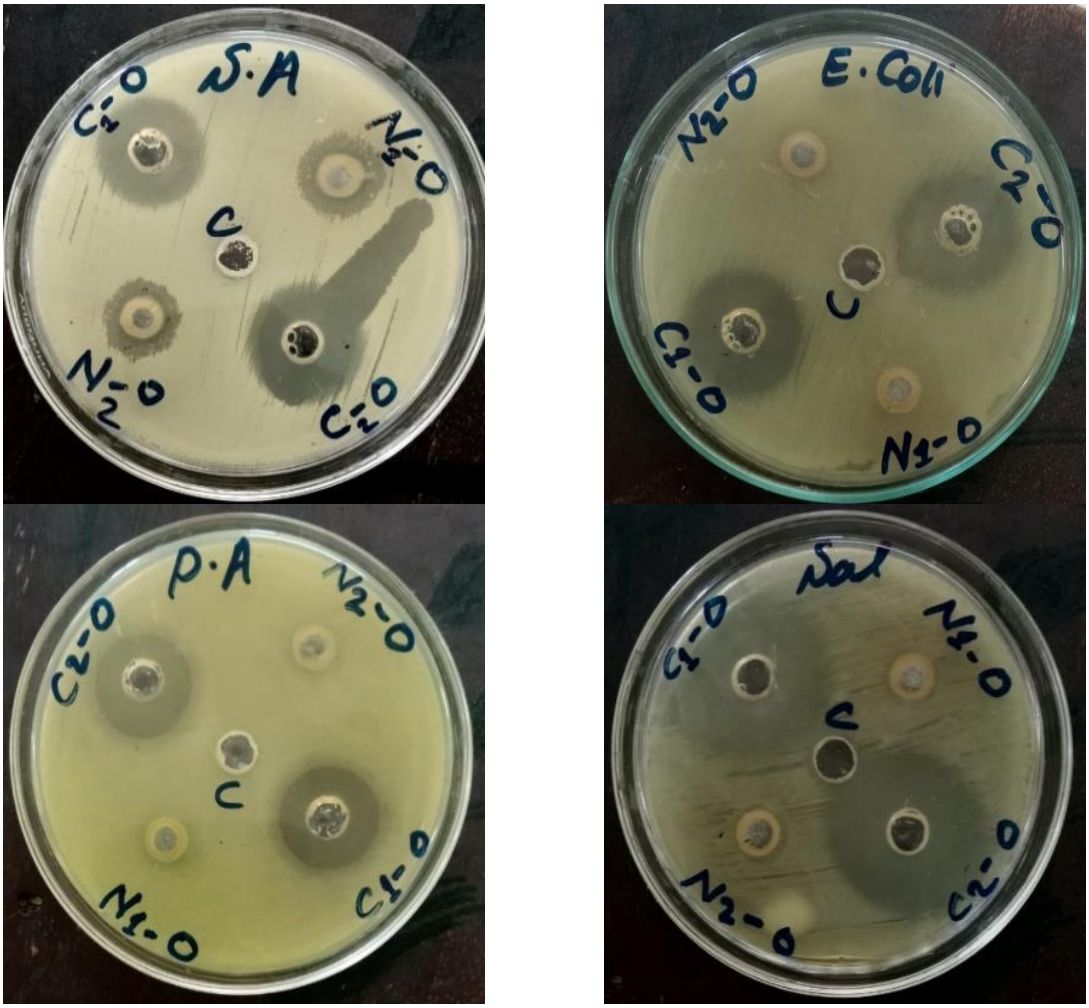

Figure 2. Zone of clearance of *Syzygium aromaticum* and *Nigella sativa* essential oils against all selected bacterial strains.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Essential Oils Against MDR Bacterial Strains

With Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) values ranging from 3.53 to 7.06 mg/mL, *Syzygium aromaticum* oil demonstrated antimicrobial action in this investigation. The highest MIC (3.53 mg/mL) and MBC (7.06 mg/mL) values were observed against all tested bacterial strains, including *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi*. Conversely, the volatile oil from *Nigella sativa* demonstrated a MIC of 12.7 mg/mL and an MBC of 22.5 mg/mL, with these values specifically recorded against *Staphylococcus aureus*. Notably, no MIC was determined for *E. coli*, *Pseudomonas aeruginosa*, or *Salmonella typhi*.

Table 2. *Syzygium aromaticum* and *Nigella sativa* essential oils' MIC and MBC in (mg/ml) against examined bacterial strains.

Se.	Essential Oils	MIC value				F value	P value
		E. coli	*Pseudomonas aeruginosa*	*S. aureus*	*Salmonella typhi*		
1	*S.aromaticum*	3.53	3.53	3.53	3.53	25.94	0.01
2	*Nigella sativa*	0	0	12.7	0		
Sr No.	Essential Oils	MBC value				F value	P value
1	*S.aromaticum*	7.06	7.06	7.06	7.06	0.05	0.82
2	*Nigella sativa*	0	0	22.5	0		

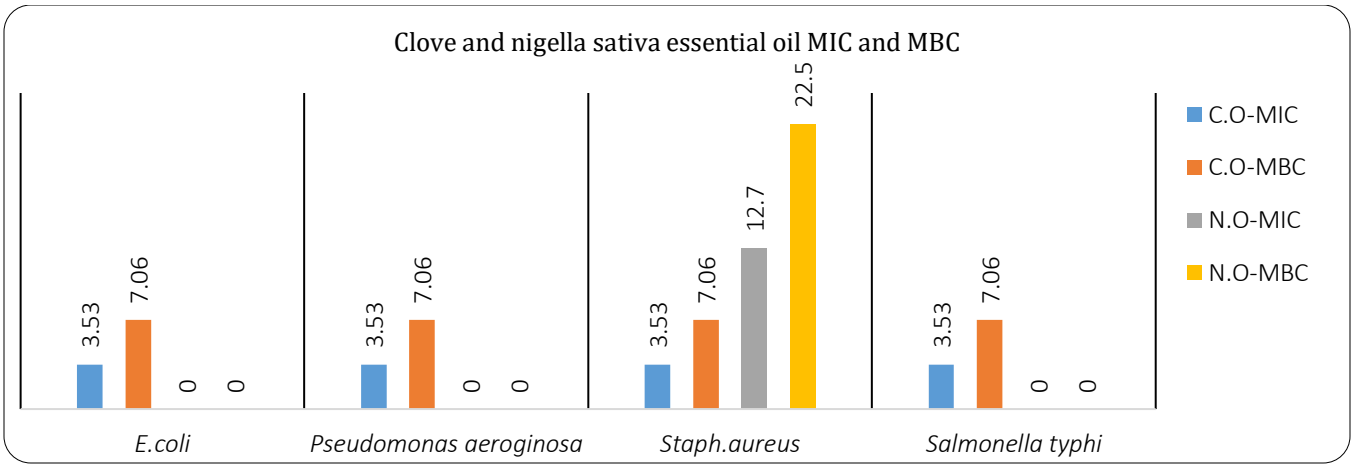

Figure 3. MIC and MBS of selected essential oils against MDR strains.

Combined Antibacterial Activity of *Syzygium aromaticum* and *Nigella sativa* Essential Oils Against MDR Bacterial Strains.

Using a dilution approach, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the essential oils of *Syzygium aromaticum* and *Nigella sativa* were determined to evaluate their antibacterial activity. To test for synergistic effects, equal quantities of each oil were mixed. The combination exhibited MIC values of 1.6 to 3.3 mg/mL and MBC values of 3.3 to 6.7 mg/mL. Specifically, the combined essential oils demonstrated MIC and MBC of 3.3 mg/mL and 6.7 mg/mL, respectively, against *E. coli, Pseudomonas aeruginosa,* and *Salmonella typhi*, while for *Staphylococcus aureus*, the MIC and MBC were 1.6 mg/mL and 3.3 mg/mL, respectively. Results are summarized in Tables 3 and Figure 4.

Table 3. Combination of *Syzygium aromaticum* and *Nigella sativa* essential oils MIC and MBC in (mg/ml) against MDR strains.

Sr. No	Bacterial Strains	*S.aromaticum* and *Nigella sativa* essential oil (mg/ml)	
		MIC	MBC
1	*E. coli*	3.3	6.7
2	*P. aeruginosa*	3.3	6.7
3	*S. aureus*	1.6	3.3
4	*Salmonella typhi*	3.3	6.7

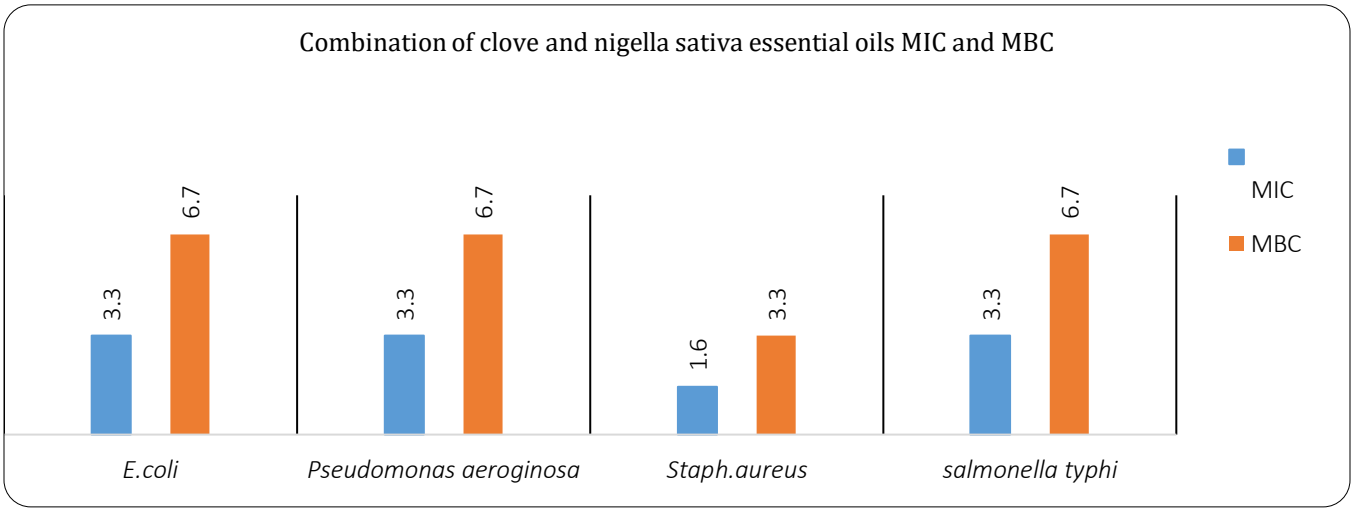

Figure 4. Combination MIC and MBC of selected essential oil against MDR strains.

Determination of Combined Effects of Selected Essential oils

Fractional inhibitory concentration (FIC) was also determined in order to examine the combined effects of volatile oils against MDR strains of bacteria. The level of cumulative impact of essential oil shows various impacts with FIC values ranging from 3.53-12.7. The results are shown in Table 5.

Table 4. Selected essential oils (FIC) fractional inhibitory concentration index and (MIC) minimum inhibitory concentration.

Sr.	Bacterial Strains	*S. aromaticum* oil			*Nigella sativa* oil		
		MIC (mg/mL)	FIC (Index)	Type of interaction	MIC (mg/mL)	FIC (Index)	Type of interaction
1	*E. coli*	3.53	0.934	Additive	0	0	Not determined
2	*P. aeruginosa*	3.53	0.934	Additive	0	0	Not determined
3	*S. aureus*	3.53	0.5	Additive	12.7	0.126	Indifferent
4	*Salmonella typhi*	3.53	0.934	Additive	0	0	Not determined

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis

The chemical constituents of *Syzygium aromaticum* and *Nigella sativa* essential oils were analyzed using gas chromatography-mass spectrometry (GC–MS).

GC-MS analysis of *Syzygium aromaticum* Oil

The GC-MS chromatogram of *Syzygium aromaticum* oil displayed 16 peaks (Fig. 5), identified based on their retention times on a fused silica capillary column. The major components were identified as follows: Diglycolic acid (76.59%), 2,3-Dichloropropene (7.44%), Benzyl Alcohol (2.06%), Eugenol (2.06%), 2-Methoxy-4-1-propenylphenol (2.88%), 2,3-Dihydroindole (3.20%), Allyl-6-Methoxyphenol (2.92%), Bromodichloromethane (0.16%), Dichloroacetic acid methyl ester (0.80%), and 1,4-Dihydroxybenzene (0.73%). Diglycolic acid and 2,3-dichloropropene were the predominant compounds in the *Syzygium aromaticum* essential oil.

Table 5. Chemical composition of *Syzygium aromaticum* essential oil.

Components name	Retention time	Peaks Area	Percentage %
Methane, Bromodichloro	3.039	46146036	0.16
Acetic Acid, Dichloro-, Methyl	3.159	223080896	0.80
Diglycolic Acid, 2,3-Dichlorop	8.136	21251708928	76.59
Benzyl Alcohol	12.362	2065998592	7.44
Eugenol	13.783	572385024	2.06
Phenol, 2-Methoxy	14.738	800382080	2.88
Allyl-6-Methoxyphenol	16.309	812415488	2.92
Eugenol	17.229	707559616	2.55
4,7-Methano-1h-Indenol, Hexahy	18.015	172566272	0.62
Indole, 2,3-Dihydro	19.835	890120896	3.20
(18s,19s)-18,19-Dihydroxy	28.259	203121536	0.73
Diacetin, 1-Trimethylsilyl Eth	32.08	382524.813	0.01
Tripropylene Glycol Monopropyl	32.176	204875.656	0.07
4-Phenyl-2-Butanol, Tms Deriva	32.251	176932.469	0.06
3,6,9,12,15-Pentaoxahexadecano	32.321	102919.727	0.03
18,21-Heptaoxadoco	32.361	54732.367	0.01

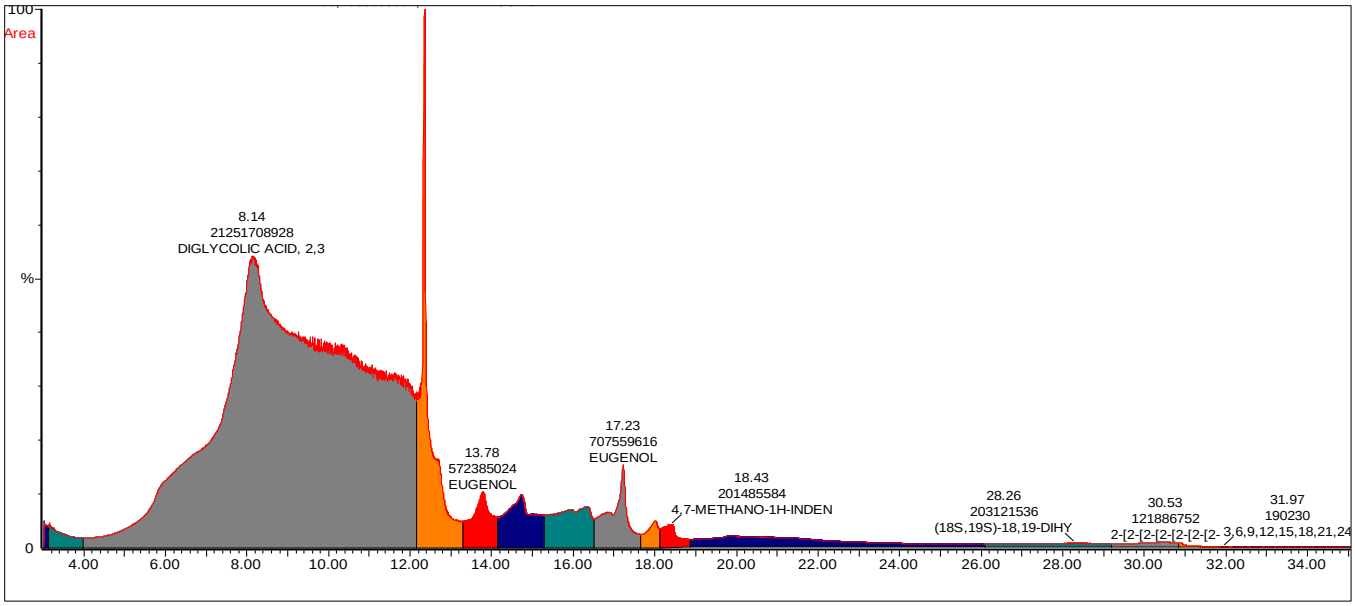

Figure 5. GC-MS chromatogram of *Syzygium aromaticum* oil.

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis of *Nigella sativa* Oil

The GC-MS chromatogram of *Nigella sativa* oil displayed 28 peaks (Fig. 6), identified based on their retention times on a fused silica capillary column. The major components were as follows: Heptasiloxane (80.836%), Dichloronitromethane (2.22%), Bicyclo[3.1.0]hex-2-ene (1.67%), 7-Methylene-bicyclo Nona (0.54%), Glycol Salicylate (0.08%), 3-Phenoxypropionic Acid (0.267%), O-Cymene (3.616%), Thymoquinone (2.669%), Limonene (0.24%), Glycol Salicylate (0.03%), 3,4-Dimethylbenzyl Alcohol (0.13%), 7-Octen-2-ol (0.43%), Ethylphenylmalonic Acid Diethyl (0.07%), Glycerol, 1-Tert-Butyl 3-Trimethyl (0.14%), Guaiol (0.48%), and Indan-1,3-Diol Monoacetate (0.67%). These findings indicate a rich abundance of phytochemicals in *Nigella sativa* essential oil.

Table 6. Chemical composition of *Nigella sativa* essential oil.

Components name	Retention time	Peaks Area	Percentage %
Methane, Dichloronitro-	3.028	4014116.25	2.22
1,1,3,3,5,5,7,7,Heptasiloxane	3.299	145799680	80.83
Bicyclo[3.1.0]Hex-2-Ene, 2-Met	5.209	3026434.25	1.67
7-Methylene-Bicyclo[3.3.1]Nona	5.364	988736.188	0.54
1,1,3,3,5,5,7,7,Octasiloxane	6.265	105628.391	0.05
3-Phenoxypropionic Acid	7.4	481743.125	0.26
O-Cymene	7.505	6523705	3.61
Limonene	7.58	446963.844	0.24
2-Propanol, 1-2-Hydroxyethyl	7.825	7957.818	0.04
3,4-Dimethylbenzyl Alcohol	8.321	241889.156	0.13
1,1,3,3,5,5,7,7,Tetrasiloxane	9.041	35312.758	0.01
3,7,11-Trimethyl-3-Hydroxy	9.611	401374.969	0.22
Salicylic Acid, 2tms Derivative	9.991	203165.047	0.11
Thymoquinone	13.713	4814831.5	2.66
Ethylphenylmalonic Acid Diethy	16.094	135061.344	0.07

3,4-Dimethylbenzyl Alcohol	16.164	528477.5	0.29
Trimethylsilyl-Di(Timethylsilo	16.449	110111.367	0.06
Phenol, 2-Methoxy-4-(1-Propeny	16.819	256525.484	0.14
Silane, Diethylethoxy(2-Ethoxy	17.104	208369.953	0.11
Trimethylsilyl-Di(Timethylsilo	17.184	180517.047	0.10
Silane, Diethylethoxy(2-Ethoxy	17.459	178889.453	0.09
Guaiol	17.664	871112.75	0.48
Ethylphenylmalonic Acid Diethy	17.78	354545.469	0.19
Silane, Dimethyl(2-Methoxyetho	17.855	271448.219	0.15
Indan-1,3-Diol Monoacetate	18.175	1222617.125	0.67
3,6,9,12,15-Pentaoxahexadecano	18.575	271301.5	0.15
Glycerol, 1-Tert-Butyl 3-Trime	18.915	1489551.875	0.82
3,6,9,12,15,18,21-Heptaoxadoco	19.38	1030159.938	0.57
1,1,1,3,5,5,5-Heptamethyltrisi	19.54	958723.063	0.53

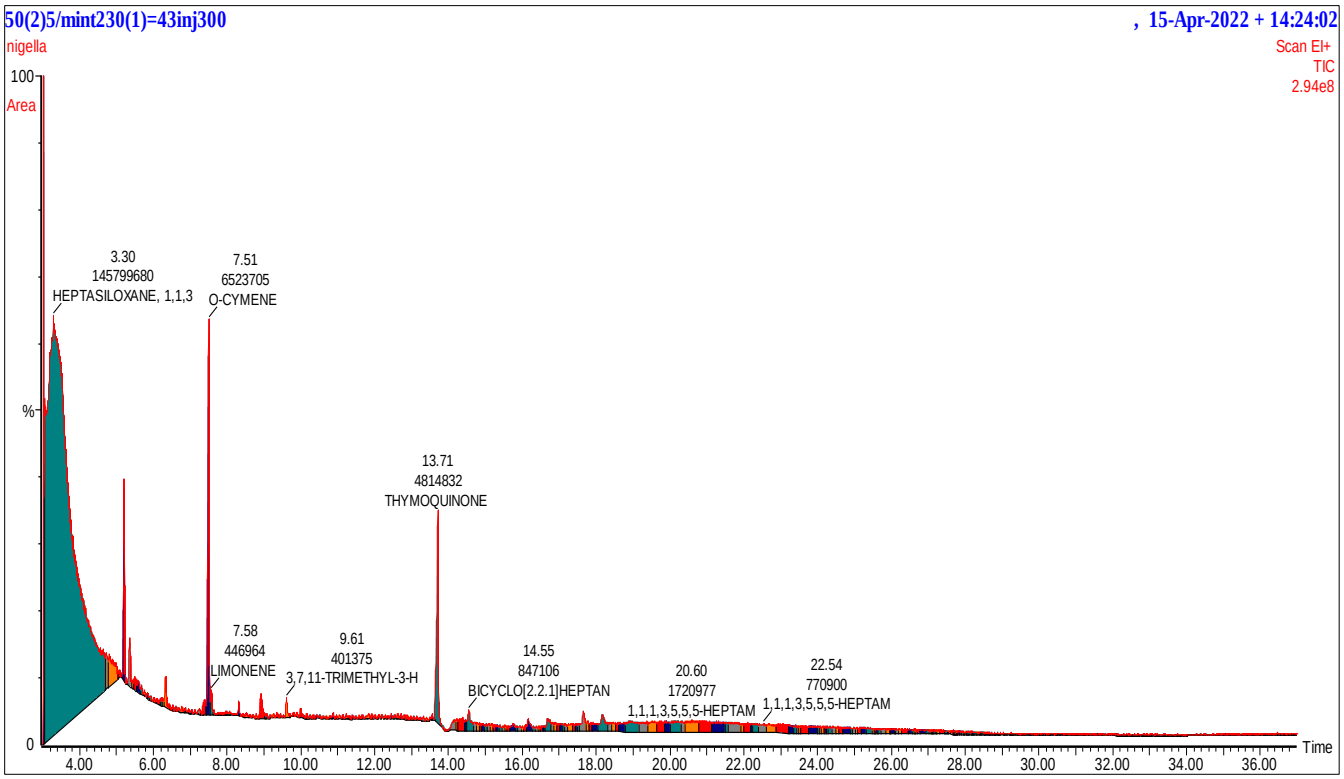

Figure 6. GC-MS chromatogram of *Nigella sativa oil.*

DISCUSSION

The present study assessed the antibacterial activity of *Syzygium aromaticum* and *Nigella sativa* essential oils, identifying their chemical components using gas chromatography. While both oils exhibited antibacterial effects, *Syzygium aromaticum* showed stronger inhibition across a wider range of bacterial species, with inhibition zones reaching 30.66 mm, particularly against *Salmonella typhi*. These findings align with prior studies, such as those by Saeed et al., (2014), which reported significant activity of *S. aromaticum* against *E. coli*, and El-Darra et al. (2018), which noted the efficacy of *N. sativa* against MRSA. However, variations in efficacy compared to other reports (Aghrouch *et al.,* 2017)

suggest differences in oil composition and strain susceptibility.

A study conducted on ginger essential oil (GEO), rich in zingiberene and α-curcumene, exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. SDS-PAGE analysis showed a loss of bacterial protein bands with higher GEO concentrations (Wang *et al.,* 2020).

Our study demonstrated that *Syzygium aromaticum* oil exhibited the strongest antibacterial activity across all tested strains, with inhibition zones ranging from 19 mm to 30.66 mm, particularly against *Salmonella typhi* (30.66 mm). In contrast, *Nigella sativa* oil showed inhibition zones between 0 and 19 mm. Additionally, *S. aromaticum* oil was highly effective against multi-drug-resistant strains such as *E. coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, corroborating previous findings (Saeed *et al.,* 2014; El-Darra *et al.,* 2018).

Previous studies have reported the MIC and MBC values for *Syzygium aromaticum* oil to range between 2.5-5 mg/mL (MIC) and 5-10 mg/mL (MBC), with lower values observed against *E. coli, Salmonella spp*., and *Streptococcus spp.,* and higher values against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Oulkheir & Moukale, 2017). Consistent with these findings, our study identified MIC and MBC values of 3.53-7.06 mg/mL and 12.7-22.5 mg/mL, respectively, highlighting the significant antibacterial potential *of S. aromaticum* oil. GC-MS analysis revealed 16 chemical components, with Diglycolic acid, 2,3-Dichlorop (76.592%) as the major compound, followed by Benzyl Alcohol (7.446%) and Eugenol (2.062%), contributing to the oil's potent antibacterial properties.

A study on *Nigella sativa* seeds essential oils demonstrated significant antibacterial activity, likely attributed to various bioactive compounds, supporting the current findings (Dalli *et al.,* 2021). These findings align with previous studies, though variations in major components have been noted. For example, Ahmed *et al.,* (2021) identified 19 peaks in Syzygium aromaticum oil, with eugenol (22.94%), caryophyllene (10.37%), and eugenyl acetate (16.65%) as the major compounds. Selles and Kouidri (2020) reported 64 peaks, with eugenol (78.72%), eugenyl acetate (8.74%), and β-caryophyllene (8.82%) as dominant constituents. Such differences may stem from variations in extraction methods, plant origins, and environmental factors. Despite these variations, most studies agree on eugenol, β-caryophyllene, and eugenyl acetate as key components (Selles et al., 2020). GC-MS analysis of *Nigella sativa* essential oil revealed several phytochemical components, with Heptasiloxane (80.836%), Methane, Dichloronitro (2.225%), Bicyclo Hex-2-Ene, 2-methyl (1.677%), and O-Cymene (3.616%) being the most abundant. Other notable components included Thymoquinone (2.669%), Limonene (0.247%), and Guaiol (0.482%). These findings are consistent with previous studies, such as Srinivasan (2018), which identified key active compounds in *Nigella sativa*, including thymohydroquinone (30-48%) and p-cymene (7-15%). The presence of these bioactive phytochemicals in both Syzygium aromaticum and *Nigella sativa* essential oils highlights their potential as natural antimicrobial agents, supporting their further research in combating pathogenic bacteria.

CONCLUSION

This study reveals that *Syzygium aromaticum* essential oil has superior antibacterial properties compared to *Nigella sativa* oil, supported by GC-MS analysis of its diverse phytochemical composition. These findings highlight its potential as an effective natural antimicrobial agent.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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