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RESEARCH ARTICLE
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EXTRACTION AND EVALUATION OF NIGELLA SATIVA AND SYZYGIUM AROMATICUM OILS AND THEIR PHARMACOLOGICAL STANDARDIZATION

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ABSTRACT
Nigella sativa (black seed) and Syzygium aromaticum (clove) have been widely recognized for their medicinal and pharmacological significance, primarily due to their bioactive compounds—thymoquinone and eugenol, respectively. This study focused on the extraction of essential oils by using the cold compression method and to evaluates their antioxidant, anti-inflammatory, and antimicrobial activities. The antimicrobial efficacy of the both extracted oils were assessed against common pathogenic microorganisms including both Gram-positive and Gram-negative bacterial strains against standardized antimicrobial testing methods. The results indicated that both the study samples (oils) exhibited notable antimicrobial properties along with promising antioxidant and anti-inflammatory activities. In addition, both reinforcing the antimicrobial potential as natural therapeutic agents. Standardization processes were conducted to ensure consistency, potency, and safety, to establish their viability for pharmaceutical applications. This research underscores the potential of Nigella sativa and Syzygium aromaticum oils as an effective alternative to synthetic antimicrobial agents, contributing to advancements in natural medicine.

Keywords: Nigella sativa, Syzygium aromaticum, Anti-microbial, Antioxidant and Anti-inflammatory agent.

1.0 INTRODUCTION
Nigella sativa, commonly known as black seed or black cumin, has been widely used in traditional medicine across various cultures. It contains thymoquinone, a bioactive compound known for its antimicrobial, antioxidant, and anti-inflammatory properties. Several studies have claimed that extracts of Nigella sativa exhibit significant antimicrobial activity against a broad spectrum of bacteria and fungi, which promote it as a promising candidate for pharmaceutical applications [(1).] Similarly, Syzygium aromaticum (clove) is a well-known spice with strong medicinal properties, primarily due to its high eugenol content. Eugenol has been extensively also studied for its antimicrobial activity, showing effectiveness against both Gram-positive (aureus & Bacillus subtilus) and Gram-negative (coli & Aeruginosa) bacteria as well as fungal pathogens. Clove extracts are commonly used in dental care and traditional medicine for their antiseptic and analgesic properties [(2).]. Black seed (Nigella sativa) is an annual flowering plant from Ranunculaceae family, native to southwest Asia. This plant

has many food and medicinal uses. The use of its seeds and oil is common for treatment of many diseases, including rheumatoid arthritis, asthma, inflammatory diseases, diabetes and digestive diseases. The purpose of this study was to provide a comprehensive review on the scientific reports that have been published about Nigella sativa [(3)]. The increasing prevalence of bacterial infections and antibiotic resistance poses a significant threat to global health, food security, and development. Without effective antibiotics, critical medical treatments such as organ transplants, chemotherapy, and surgeries become much riskier [(4)]. Simultaneously, noncommunicable diseases (NCDs) have emerged as the leading cause of death worldwide, responsible for 40 million deaths annually, or 70% of all global deaths [(5)]. Many NCDs, including cancer, are linked to oxidative stress, which occurs when free radicals exceed the body's antioxidant capacity. Cancer alone caused 8.8 million deaths in 2015 [(6)].

Recently, Nigella sativa, gained the focus of increasing scientific interest due to its broad therapeutic potential. It is widely recognized for its use in traditional systems of medicine, and its efficacy is largely attributed to thymoquinone, most studied active compound. In various researches, Thymoquinone has demonstrated significant antioxidant capabilities by scavenging free radicals and enhancing the body's enzymatic antioxidant defense mechanisms. These properties contribute to its protective role against cellular damage and chronic inflammation, key contributors to many noncommunicable diseases [(7)]. In addition to antioxidant properties, Nigella sativa exhibits anti-inflammatory effects by downregulating inflammatory mediators such as TNF-α, IL-1β, and NF-κB. Several in vitro and in vivo studies conducted earlier were evident to showed that thymoquinone has an ability to reduce inflammation in disease models of arthritis, respiratory inflammation, and gastrointestinal disorders [(8)]. Moreover, its antimicrobial activity against both Gram-positive and Gram-negative bacteria has been documented, making it a potential natural alternative to conventional antibiotics [(9)]. Moreover, Syzygium aromaticum (clove) is another extensively used Indian spice widely accepted in traditional and modern medicine, primarily due to its high content of eugenol, a phenolic compound with well-documented pharmacological activities. Eugenol has been shown to possess strong antioxidant effects by inhibiting lipid peroxidation and protecting cellular membranes from oxidative damage [(10)]. In pharmacological studies, clove oil has demonstrated anti-inflammatory activity through the suppression of pro-inflammatory cytokines and inhibition of COX enzymes, similar to NSAIDs [(11)].

Despite the widespread uses and proven efficacy of allopathic antimicrobial medicines their administration is often accompanied with notable side effects, particularly during long-term uses. These adverse effects have spurred interest in identifying safer, natural alternatives that offer comparable therapeutic benefits with minimal toxicity. In this context, indigenous medicinal plants such as Nigella sativa and Syzygium aromaticum have garnered considerable attention. Besides, they possess potent bioactive compounds—thymoquinone and eugenol, respectively—that not only demonstrate antimicrobial efficacy but also exert antioxidant and anti-inflammatory actions without the pronounced side effects as associated with synthetic drugs. Therefore, this study was aimed to investigate the antioxidant, anti-inflammatory and antimicrobial properties of Nigella sativa and Syzygium aromaticum oils. Including, efficacy of both these natural oils were standardized against the Tetracycline, a well-known antimicrobial agent.

MATERIAL AND METHODS

Fresh seeds of Nigella sativa and Fresh buds of Syzygium aromaticum were obtained from local area Kanke, Ranchi, Jharkhand, India. Both the study samples were authenticated from Botanist at YBN University, Ranchi, Jharkhand. The Reference letter no. of certificate is (Ref. No. YBN/UNIV/BOT/12032025/007). All the study chemicals including tetracycline and ascorbic acid were procured from HiMedia Laboratories Pvt. Ltd. of analytical grade with their purity ≥99%. Also, in this study a standard cold compression technique was used to extract the oils from both the study samples without applying heat.

Preparation of Sample from Extracts

To prepare the sample for the evaluation of antimicrobial activity, 100 µl of sample dissolve in 900 ul of methanol and then mixed well to use in experiment. However, microorganisms were procured from the National Centre for Microbial Resource, National Centre for Cell Science Pune, India. In which, two were Gram-ve {Escherichia coli (ACC-3099) and Pseudomonas aeruginosa (ACC-3973)} and two were Gram+ve bacteria {Bacillus subtilus (ACC-2511) and Staphylococcus aureus (ACC-2408)}. All the bacterial strains used in the investigation were maintained at 4°C on nutrient agar medium.

ANTI-OXIDANT ACTIVITY

DPPH Assay

Preparation of Ascorbic Acid (AA) Standard solution: 0.1 mg of ascorbic acid (SAP Chemical) was dissolved in 1ml methanol to obtain a stock solution of AA.

Preparation of DPPH stock solution: 0.016 g of DPPH was dissolved with little amount of methanol in a 100 mL volumetric flask. After the DPPH fully dissolved, the flask was filled up to the mark using methanol to get a concentration of 160 mg/L solution.

From the fresh stock, take 1000µL of DPPH and mixed it with six different volumes of 1mg/1000 µL concentration (20 µL, 40 µL, and 60 µL, 80 µL, 100 µL, 200 µL) in ascorbic acid solvents, and incubated for 30 min in dark. Absorbance was taken at 517 nm in a spectrophotometer. Triplicate was made of each experiment to calculate the mean/average. 0.1 mM of DPPH (20 µL) was taken as standard. DPPH free radical reducing activity was calculated using the following formula**.**

Inhibition% = Control absorbance – sample absorbance/ control absorbance x100

ABTS Assay

Preparation of ABTS Stock Solution: Weigh the 0.360 g (7mM) of ABTS powder and dissolve in 100ml of water. Weigh the 0.006g (2.45Mm) of potassium persulphate and dissolve in 10 of methanol and both are stored in the dark at room temperature for 12-16 h before use. Then mixed the ABTS solution and potassium persulphate solution in 2:1 ratio. A freshly prepared 1000 µL of the ABTS solution from 7 mM ABTS stock solution was pipetted out and mixed with six different volumes (5 µL, 10 µL, 15 µL, 20 µL, 25 µL, and 30 µL) of each study sample at a concentration of 1 mg/mL and the mixtures were incubated in the dark for 30 minutes.

After incubation, the absorbance was measured at 734 nm using a spectrophotometer. The ABTS radical scavenging activity was determined using the following formula:

ABTS scavenging effect% = $A_B - A_A / A_B \times 100$.

Where, A_B is absorbance of ABTS radical + methanol (control)A_A is absorbance of ABTS radical + sample extract/standard.

ANTI-INFLAMMATORY ACTIVITY

Nitric Oxide (NO) Inhibition Assay

A freshly prepared 100 µl of each sample of Nigella sativa oil & Syzygium aromaticum oil and standard Butylated hydroxytoluene were taken in triplicates. Add 3 µl of sodium nitroprusside (10mM) to the sample in test tubes. Incubate all the test tube at room temperature for 15 minutes. Add 3 µl of Griess reagent (1% sulphanediamine, 2% H3Po4 and 0.1% N(1-napthyl) ethylene diamine dihydrochloride) to all the test tubes. The same reaction mixture except including sample was used as negative control. A test tube with phosphate buffered saline alone was considered as blank. Then absorbance of the chromphore formed at 546nm against the blank was taken and calculate the percent (%) inhibition of NO release by using the following formula.

Inhibition% = Control absorbance – sample absorbance/ control absorbance x100

ANTI-MICROBIAL ACTIVITY

The disc diffusion method was performed to explore the antimicrobial activity. Initially, all study bacterial stains were inoculated in nutrient agar media. 100 µl of microorganism inoculum was spread

(glass rod) uniformly into sterilized petri plates (121°C, 20lbs pressure for 20 min in autoclave). After spreading the inoculum, 6 mm discs were placed into the media with the help of sterilized forceps. Nigella sativa oil and Syzygium aromaticum oil were loaded in different petri-plates using a micropipette. Three different volumes of 0.1 mg/1000 µL (02 µL, 04 µL, 06 µL and 08 uL) were used to test the potential of antimicrobial activity against selected microorganisms and placed inside the incubator for 18-24 hrs to promote the maximum growth of microorganisms. The zones of inhibition observed from both samples were measured, using tetracycline (10 mcg) as the positive control and methanol as the negative control Tetracycline, used as the most effective positive control, demonstrate inhibitory activity against the tested bacteria in the following order: [P. aeruginosa (25-33 ZOI in mm), E. coli (18-25 ZOI in mm) S. aureus (24-30 ZOI in mm) zone size interpretative chart as per EUCAST standard (the European committee on antimicrobial susceptibility testing) [23].

Area of the inhibited zone and percentage of inhibition were calculated as follows: -

- Zone of inhibition (mm^2) = Area of inhibited zone (πr^2 /2) - Area of disc (πr^2 /2).
- The percentage of inhibition of this sample was calculated by using the formula

%inhibition = Zone of sample/Zone of standard ×100.

RESULTS

Antioxidant activity

The study samples (Nigella sativa oil and Syzygium aromaticum oil) was assessed antioxidant potential of the oils by using DPPH and ABTS assays. Nigella sativa oil exhibited moderate free radical scavenging activity, with DPPH inhibition percentages ranging from 3.25% to 59.55% and ABTS inhibition from 4.03% to 93.63%. In contrast, Syzygium aromaticum oil showed stronger antioxidant effects, with DPPH inhibition values of 34.25% to 89.07% and ABTS inhibition of 40.70% to 78.80%. Ascorbic acid, the standard, displayed the highest inhibition (88.54% to 99.58%), serving as a benchmark. The IC50 values for Syzygium aromaticum oil (64.61 µg/mL for DPPH and 28.17 µg/mL for ABTS) were lower than those for Nigella sativa oil (137.58 µg/mL for DPPH and 8.68 µg/mL for ABTS), indicating higher potency. These results suggest that Syzygium aromaticum oil has superior antioxidant capacity, likely due to its high phenolic content.

Table 1.1 Mean of Triplate absorbance reading in DPPH Assay of (Nigella Sativa oil) Concentration of µg/ml.

	Blanks	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	200 µg/ml
	0.308	0.325	0.32	0.294	0.262	0.227	0.135
	0.355	0.331	0.321	0.293	0.272	0.231	0.138
	0.353	0.327	0.32	0.293	0.273	0.227	0.138
Mean	0.338667	0.327667	0.320333	0.293333	0.269	0.228333	0.137

Table 1.2 Percent (%) Inhibition of DPPH by Using Nigella sativa oil.

Concentration (µg/ml)	Control	Samples	%RSA	IC50
20	0.338667	0.327667	3.248028	137.5784
40	0.338667	0.320333	5.413577	
60	0.338667	0.293333	13.38601	
80	0.338667	0.269	20.57094	
100	0.338667	0.228333	32.5789	
200	0.338667	0.137	59.54728	

Graph 1.1: Showing Free Radicals Scavenging Activity of Nigella sativa oil by DPPH Assay.

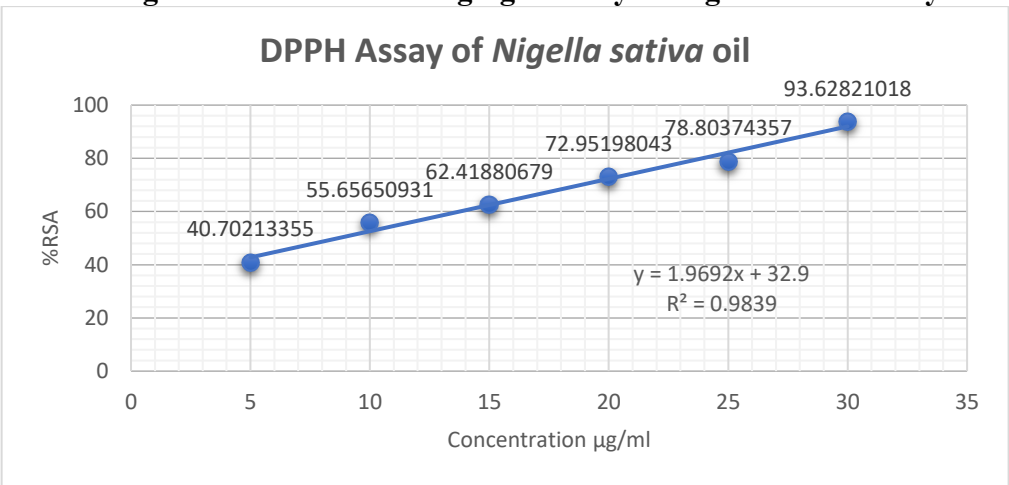


Table 1.3 Mean of Triplate absorbance reading in DPPH Assay of (Syzygium aromaticum oil) Concentration of µg/ml.

	Blanks	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	200 µg/ml
	0.308	0.223	0.202	0.164	0.148	0.118	0.038
	0.355	0.221	0.203	0.167	0.146	0.117	0.037
	0.353	0.224	0.202	0.166	0.146	0.112	0.036
Mean	0.338667	0.222667	0.202333	0.165667	0.146667	0.115667	0.037

Table 1.4 Percent (%) Inhibition of DPPH by Using Syzygium aromaticum oil.

Concentration (µg/ml)	Control	Samples	%RSA	IC50
20	0.338667	0.222667	34.25193	64.61437
40	0.338667	0.202333	40.25606	
60	0.338667	0.165667	51.08263	
80	0.338667	0.146667	56.69286	
100	0.338667	0.115667	65.84639	
200	0.338667	0.037	89.07481	

Graph 1.2 Free radicals scavenging activity of Syzygium aromaticum oil by DPPH assay.

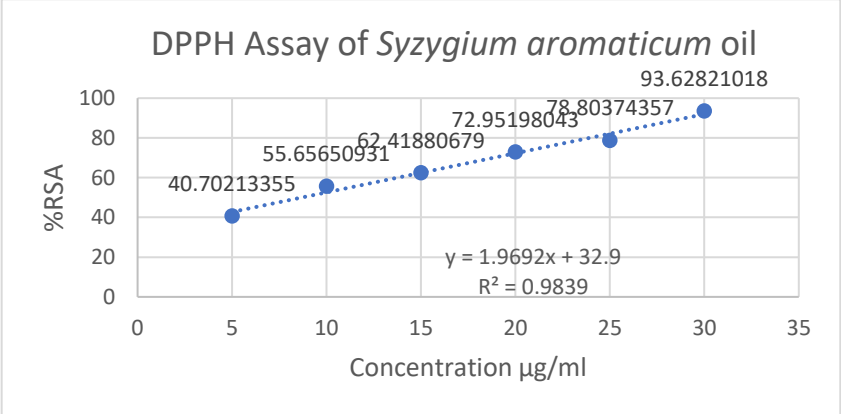


Table 1.5 Mean of Triplate absorbance reading in ABTS Assay of (Nigella Sativa oil) Concentration of µg/ml.

	Blanks	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	25 µg/ml	30 µg/ml
	0.256	0.146	0.114	0.094	0.06	0.051	0.025
	0.256	0.149	0.115	0.099	0.076	0.051	0.028
	0.257	0.141	0.112	0.096	0.072	0.061	0.026
Mean	0.2563	0.1453	0.1136	0.0963	0.0693	0.0543	0.0263

Table 1.6 Percent (%) Inhibition of ABTS by Using Nigella sativa oil

Concentration (µg/ml)	Control	Samples	%RSA	IC50
5	0.256333	0.152	40.70213	
10	0.256333	0.113667	55.65651	
15	0.256333	0.096333	62.41881	
20	0.256333	0.069333	72.95198	
25	0.256333	0.054333	78.80374	
30	0.256333	0.016333	93.62821	8.683729

Graph 1.3 Free radicals scavenging activity of Nigella sativa oil by ABTS assay.

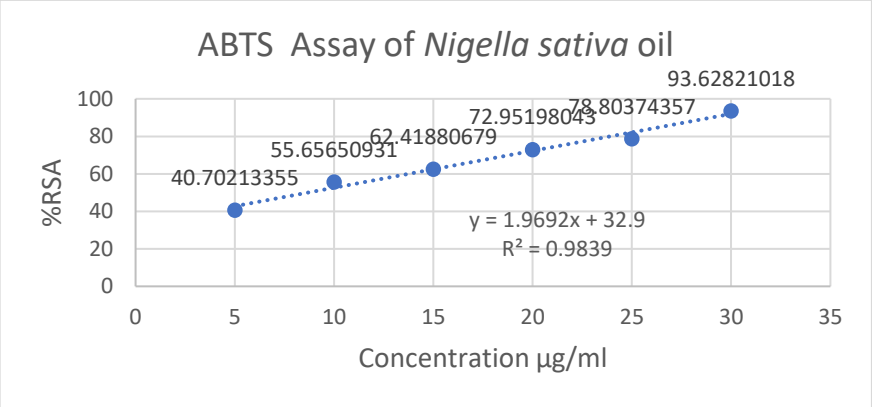


Table 1.7 Mean of Triplate absorbance reading in ABTS Assay of (Syzygium aromaticum oil) Concentration of µg/ml.

	Blanks	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	25 µg/ml	30 µg/ml
	0.256	0.243	0.231	0.212	0.188	0.168	0.15
	0.256	0.249	0.231	0.213	0.188	0.169	0.145
	0.257	0.246	0.236	0.213	0.189	0.169	0.147
Mean	0.2563	0.2466	0.2326	0.2126	0.1883	0.1686	0.1473

Table 1.8 Percent (%) Inhibition of ABTS by Using Syzygium aromaticum oil.

Concentration (µg/ml)	Control	Samples	%RSA	IC50
5	0.256333	0.246	4.031085	
10	0.256333	0.232667	9.232522	
15	0.256333	0.212667	17.03487	
20	0.256333	0.188333	26.52799	
25	0.256333	0.168667	34.20004	
30	0.256333	0.147333	42.52281	28.17497

Graph 1.4 Free radicals scavenging activity of Syzygium aromaticum oil by ABTS assay.

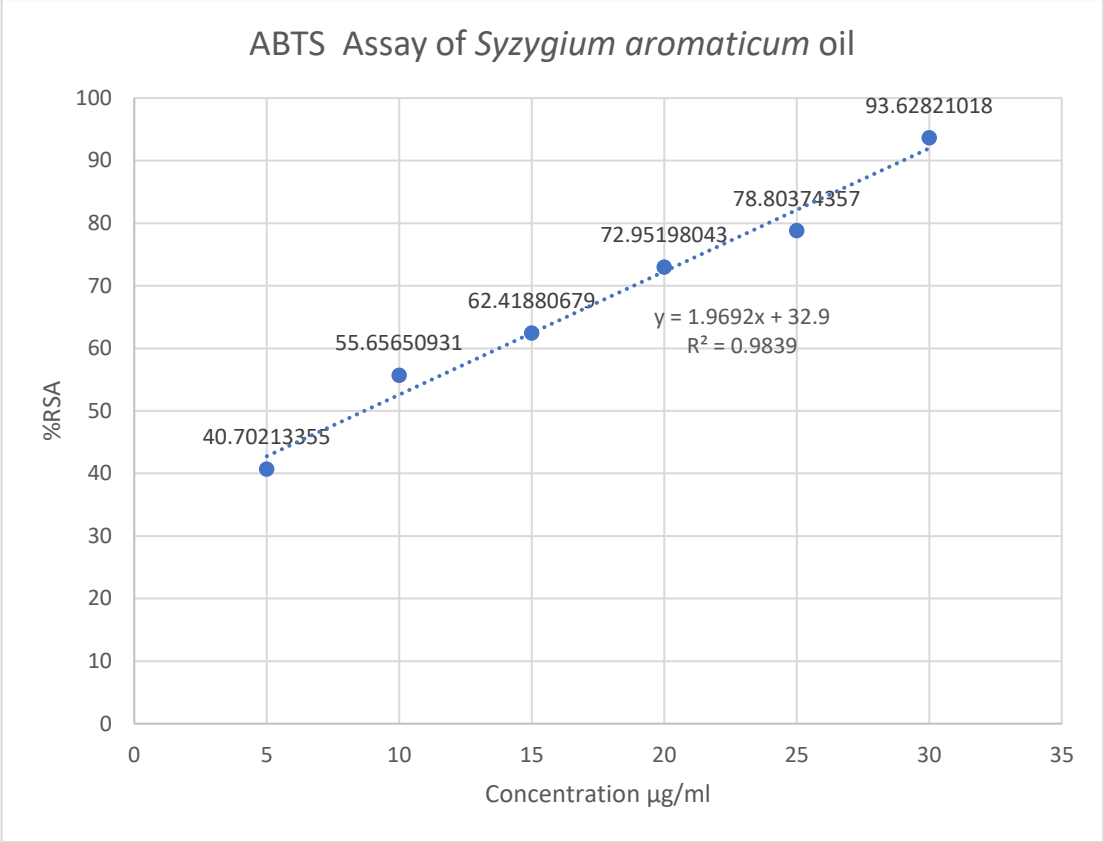


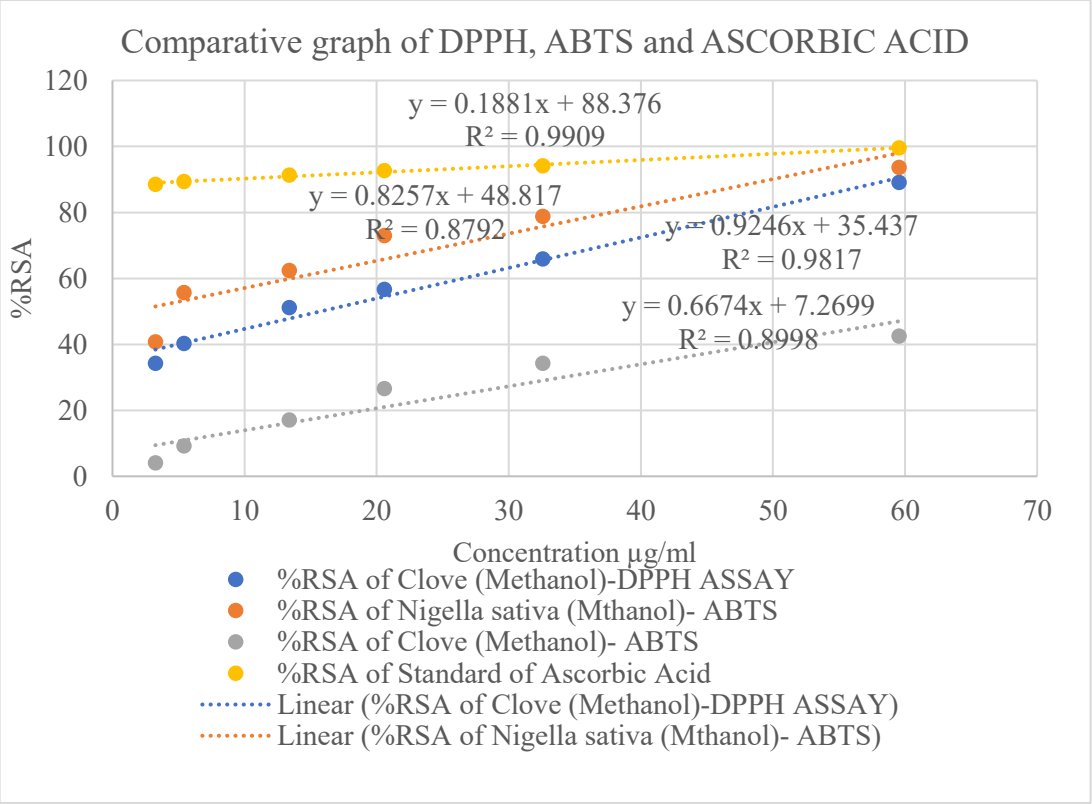
Table 1.9 Mean of Triplate absorbance reading in DPPH Assay of Ascorbic Acid.

	Blanks	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	200 µg/ml
	0.398	0.044	0.041	0.033	0.029	0.024	0.001
	0.395	0.046	0.043	0.037	0.029	0.023	0.002
	0.394	0.046	0.042	0.033	0.029	0.023	0.002
Mean	0.3956	0.0453	0.0422	0.0343	0.029	0.0233	0.0016

Table 1.10 Percent (%) Inhibition of DPPH by Using Ascorbic Acid.

Concentration (µg/ml)	Control	Samples	%RSA
20	0.395667	0.045333	88.54264
40	0.395667	0.042	89.38501
60	0.395667	0.034333	91.32275
80	0.395667	0.029	92.6706
100	0.395667	0.023333	94.10287
200	0.395667	0.001667	99.57869

Graph 1.5 showing Comparison of percentage (%) inhibition of Nigella sativa oil and syzygium aromaticum oil against absorbic acid.



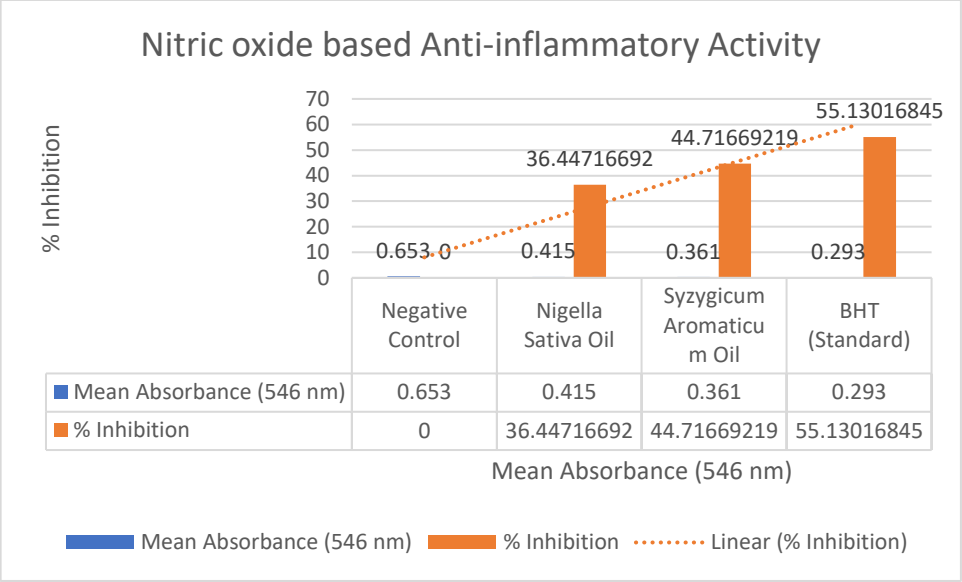
Anti-inflammatory activity

The nitric oxide (NO) inhibition assay revealed that both oils significantly reduced NO production, a marker of inflammation. Syzygium aromaticum oil showed 44.72% inhibition, outperforming Nigella sativa oil (36.45%) and approaching the efficacy of the standard Butylated hydroxytoluene (55.13%). This anti-inflammatory effect is attributed to bioactive compounds like eugenol and thymoquinone, which modulate inflammatory pathways. The results corroborate existing literature on the anti-inflammatory properties of these oils, supporting their potential use in managing inflammatory conditions.

Table 1.11 Mean of Triplate absorbance reading Nitric oxide based Anti-inflammatory Activity of Nigella sativa Oil and Syzygium aromaticum Oil.

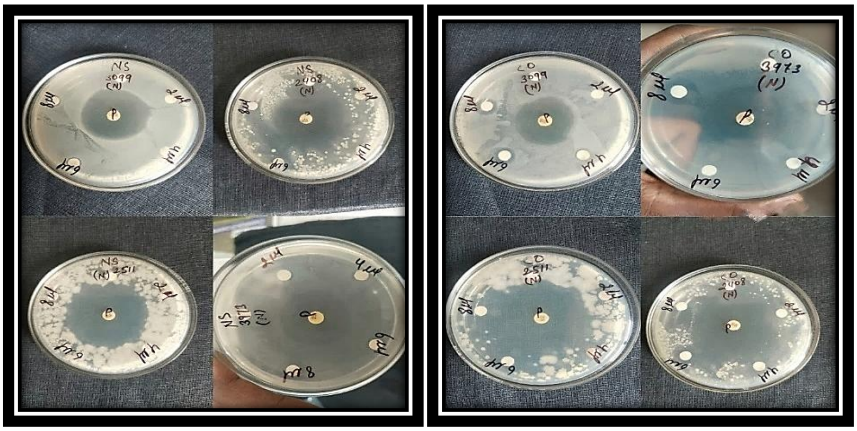
Sample	Replicate 1	Replicate 2	Replicate 3	Mean Absorbance (546 nm)	% Inhibition
Negative Control	0.651	0.653	0.655	0.653	0
Nigella Sativa Oil	0.415	0.417	0.414	0.415	36.44716692
Syzygium Aromaticum Oil	0.358	0.361	0.363	0.361	44.71669219
BHT (Standard)	0.291	0.295	0.293	0.293	55.13016845

Graph 1.6 Showing the % Inhibition of NO (Nitric oxide) Release of Nigella sativa Oil and Syzygium aromaticum Oil.



Antimicrobial Activity

The study samples (Nigella sativa oil and Syzygium aromaticum oil) exhibited diverse antimicrobial activities against the pathogenic bacterial strains, of varying degrees of effectiveness. The antimicrobial efficacy of both study samples was evaluated and the outcome of study has been presented in Fig. 1.1 The study results demonstrated that both oils exhibited inhibitory effects, whereas in comparison, Syzygium aromaticum oil shows higher activity against all tested strains. The zones of inhibition (ZOI) for Syzygium aromaticum oil was found in a ranged from 5 to 20 mm, while Nigella sativa oil showed ZOIs between 0 to 15 mm. Tetracycline, the positive control, presented ZOIs limited between 18 to 33 mm, confirming the sensitivity of the bacterial strains. Notably, Syzygium aromaticum oil was more effective against Bacillus subtilis (ACC-2511) and Staphylococcus aureus (ACC-2408), with ZOIs of 20 mm and 15 mm, respectively, at the highest concentration (8 µL). These findings align with previous studies highlighting the antimicrobial properties of these oils due to their bioactive compounds, such as thymoquinone in Nigella sativa and eugenol in Syzygium aromaticum.

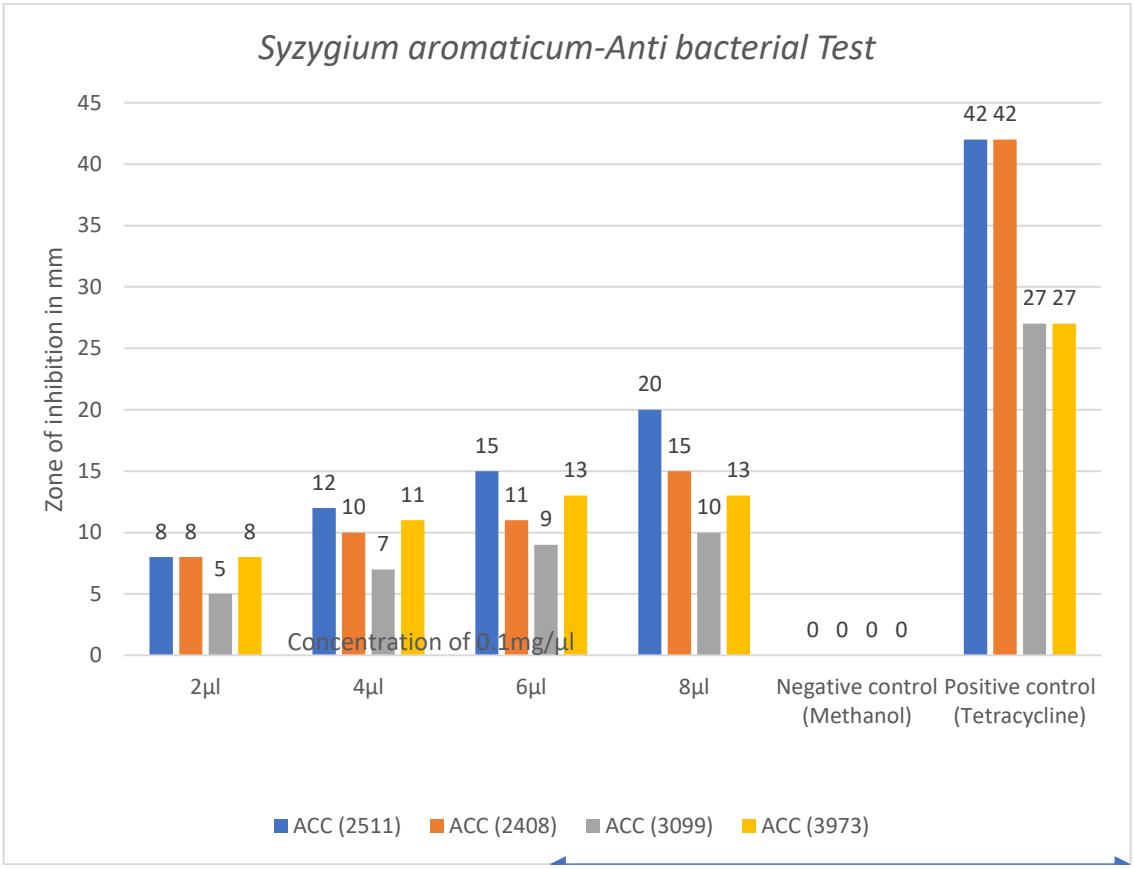


(A) **(B)**

Fig. 1.1 Showing Zone of Inhibition Against Different Bacterial Strain by Using Disc Diffusion Method in (A) Syzygium aromaticum & (B) Nigella sativa.

Table 1.12 ZOI for Syzygium aromaticum oil (Disc diffusion method) Concentration of 0.1 mg/µl.

Bacterial Strain	Zone of inhibition in mm				Negative control (Methanol)	Positive control (Tetracycline)
	2µl	4µl	6µl	8µl		
ACC (2511)	8	12	15	20	0	42
ACC (2408)	8	10	11	15	0	42
ACC (3099)	5	7	9	10	0	27
ACC (3973)	8	11	13	13	0	27

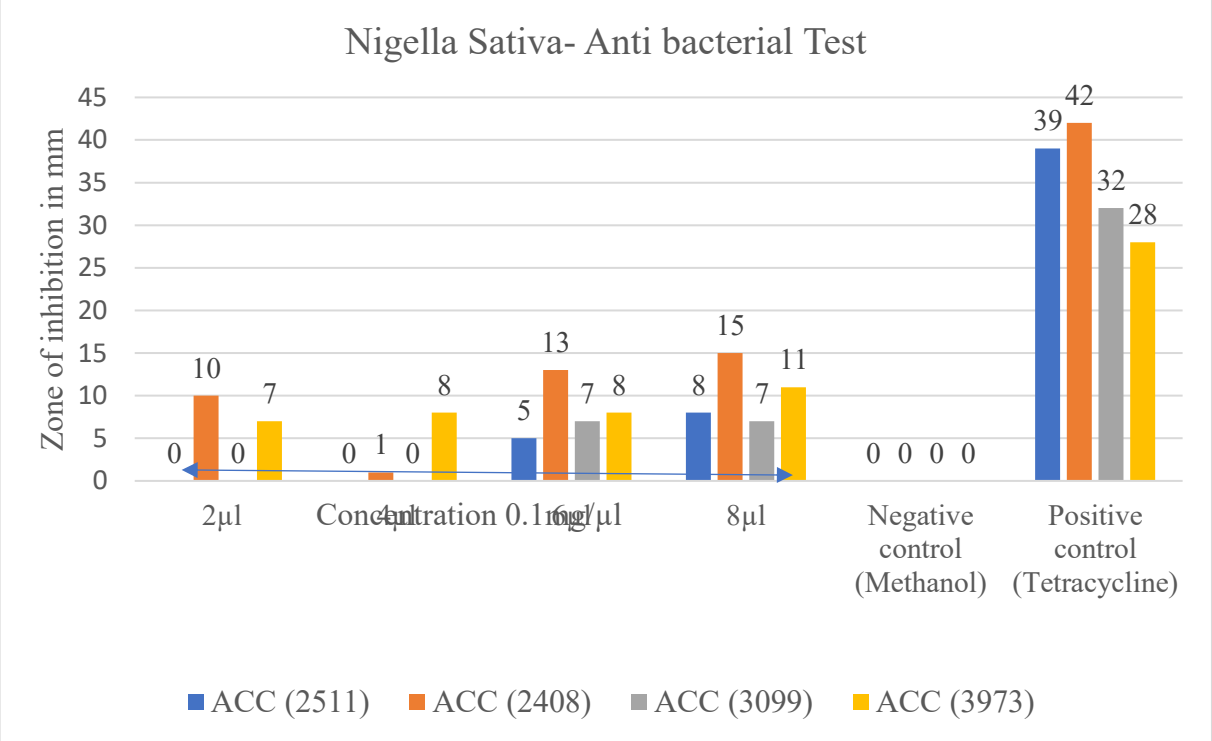


Graph 1.7 Showing Zone of Inhibition Against Different Bacterial Strain by Using Disc Diffusion Method in Syzygium aromaticum test sample.

Table 1.13 ZOI for Nigella Sativa oil (Disc diffusion method) Concentration of 0.1 mg/µl.

Bacterial Strain	Zone of inhibition in mm				Negative control (Methanol)	Positive control (Tetracycline)
	2µl	4µl	6µl	8µl		
ACC (2511)	0	0	5	8	0	39
ACC (2408)	10	1	13	15	0	42
ACC (3099)	0	0	7	7	0	32
ACC (3973)	7	8	8	11	0	28

Graph 1.8 Showing Zone of Inhibition Against Different Bacterial Strain by Using Disc Diffusion Method in Nigella Sativa test sample.



DISCUSSION

Results revealed that both Nigella sativa oil particularly due to its active compound thymoquinone and clove oil, rich in eugenol exhibited significant protective activity. They effectively reduced oxidative stress, enhanced antioxidant defences, and modulated inflammatory and microbial responses, suggesting their potential as natural alternatives or complementary agents in the treatment of oxidative stress associated illness and infectious conditions.

The DPPH and ABTS assays confirmed the antioxidant potential of both oils. Syzygium aromaticum oil exhibited stronger free radical scavenging activity (DPPH: 34.25–89.07%; ABTS: 40.70–78.80%) compared to Nigella sativa oil (DPPH: 3.25–59.55%; ABTS: 4.03–93.63%). The lower IC50 values for clove oil (64.61 µg/mL for DPPH, 28.17 µg/mL for ABTS) indicate higher antioxidant efficiency, likely due to its high phenolic content. Although Nigella sativa oil showed variable activity, its ABTS results suggest strong radical scavenging at higher concentrations. Both oils, however, were less potent than ascorbic acid (standard), which exhibited near-complete inhibition (88.54–99.58%).

The nitric oxide (NO) inhibition assay revealed that Syzygium aromaticum oil (44.72% inhibition) was more effective than Nigella sativa oil (36.45%) in suppressing inflammation, though both were less potent than BHT (55.13%). The anti-inflammatory effects can be attributed to eugenol (in clove oil) and thymoquinone (in black seed oil), which are known to inhibit pro-inflammatory mediators like NO. These findings support traditional uses of these oils in treating inflammatory conditions.

Including both Nigella sativa (black seed) and Syzygium aromaticum (clove) oils exhibited significant antimicrobial activity against Gram-positive (Bacillus subtilis, Staphylococcus aureus) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria. Syzygium aromaticum oil demonstrated stronger inhibition, particularly against B. subtilis (20 mm ZOI) and S. aureus (15 mm ZOI), likely due to its high eugenol content, a well-known antimicrobial compound. Nigella sativa oil showed moderate activity, with thymoquinone as its primary bioactive agent. The higher efficacy of clove oil aligns with previous studies, suggesting its potential as a natural antibacterial agent, possibly as an alternative to conventional antibiotics like tetracycline (positive control).

CONCLUSION

The study demonstrated that Nigella sativa and Syzygium aromaticum oils possess significant antimicrobial, antioxidant, and anti-inflammatory properties. Syzygium aromaticum oil exhibited superior antimicrobial and antioxidant activities, while both oils showed notable anti-inflammatory effects. These findings highlight the potential of these oils as natural alternatives for therapeutic and pharmaceutical applications. Further research is recommended to isolate and characterize the active compounds responsible for these bioactivities and to explore their mechanisms of action in vivo.

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