Essential Oil (EO) of *Syzgium aromaticum* targeting Biofilm Formation in *Streptococcus pneumoniae*

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Abstract

This study evaluates the anti-biofilm activity of Syzygium aromaticum (clove) essential oil against Streptococcus pneumoniae, a biofilm-forming pathogen commonly associated with the oral cavity. The experimental design included light microscopic 988 to visualize biofilm architecture of S. pneumoniae followed with the treatment of plant extracts. Growth curve analysis used spectrophotometry to assess bacterial proliferation over time. Antibacterial activity assessment used the agar well-diffusion method to evaluate the zone of inhibition and the determination of the minimum inhibitory concentration (MIC) and biofilm inhibitory concentration (BIC) using the microtiter plate method to quantify the oil's inhibitory potential. Light microscopy confirmed that Syzygium aromaticum essential oil effectively inhibited biofilm formation by Streptococcus pneumoniae at sub-MIC levels, as demonstrated by the biofilm inhibition assay. A minimum inhibitory concentration (MIC) of 1 mg/mL was observed against the tested pathogen.

However, at concentrations below the MIC, no significant inhibition was detected in either the growth curve analysis or the antibacterial activity assays. This study highlights Syzygium aromaticum essential oil as a promising natural agent with anti-biofilm properties. The presence of physiologically active compounds in the plant underscores its potential for therapeutic applications. Further identification and characterization of these bioactive constituents could provide a strong foundation for the development of novel treatments in modern medicine.

Keywords: *Syzygium aromaticum*, Essential Oil, Antibiofilm, Biofilm inhibitory concentration, *Streptococcus pneumoniae*.

Introduction

Syzygium aromaticum, commonly known as clove, belongs to the family *Myrtaceae* and is native to the Maluku Islands of eastern Indonesia. It is a tropical evergreen tree that can grow up to 12 meters in height and thrives in coastal regions as well as at higher altitudes. The most well-known and commercially valuable part of the plant is its aromatic flower buds, which are typically harvested before blooming. These buds are produced approximately four years after planting, marking the beginning of the tree's productive phase[3,14]. The essential oil derived from the flower buds of *Syzygium aromaticum* exhibits a chemotype predominantly composed of eugenol, a volatile phenylpropanoid widely recognized for its therapeutic applications in the pharmaceutical industry.

In addition to eugenol, the oil contains other bioactive constituents such as eugenol acetate, β-caryophyllene and humulene, which collectively enhance its biological efficacy[4]. *S. aromaticum* has demonstrated antiviral activity against food-borne viruses, antimicrobial activity with low concentrations, which can inhibit bacterial growth and remains effective in the treatment of bacterial infections[1,5,7]. Many viruses and bacteria can cause upper respiratory tract infections (URTIs). Upper respiratory tract infections (URTIs) are caused by acute infections affecting the nasal passages, sinuses, pharynx, larynx, or trachea. Common conditions include the common cold, nasal congestion, pharyngitis, sore throat, sinusitis, tonsillitis, otitis media and laryngitis. While most URTIs are viral in origin, bacterial pathogens can also be responsible.

Notable bacterial agents include *Streptococcus pneumoniae, Bacillus anthracis, Corynebacterium diphtheriae* and *Bordetella pertussis*. These infections are primarily transmitted through respiratory droplets released when an infected individual coughs or sneezes, or via direct contact with contaminated surfaces or objects[18]. *Streptococcus pneumoniae*, commonly referred to as pneumococcus, is a Gram-positive, lancet-shaped, facultative anaerobic bacterium responsible for a range of serious infections including pneumonia, otitis media (middle ear infections), meningitis and sepsis. Although it often resides asymptomatically in the upper respiratory tract of healthy individuals, it can become pathogenic when it invades other parts of the body. Over 100 serotypes of *S. pneumoniae* have been identified, with a limited number accounting for the majority of pneumococcal diseases.

There is a strong and growing belief in the effectiveness of traditional medicine and natural remedies derived from plant-based extracts and essential oils, particularly those obtained from *Syzygium aromaticum*, *Piper longum*, *Zingiber officinale*, *Piper nigrum and Curcuma longa*. Among these, *Syzygium aromaticum* (commonly known as

clove and synonymously referred to as *Eugenia caryophyllata*) is a medium-sized tree belonging to the Myrtaceae family. Native to the Maluku Islands of eastern Indonesia, it is now cultivated in various parts of the world including Iran. Traditionally, clove has been widely used in herbal medicine for its broad spectrum of therapeutic properties, including antimicrobial, anti-inflammatory, antioxidant and analgesic effects, primarily attributed to its major bioactive constituents such as eugenol, eugenyl acetate and β-caryophyllene[17].

Syzygium aromaticum, commonly known as clove, is a plant-derived spice that has been traditionally used in natural medicine for the treatment of a wide range of ailments. The essential oil extracted from its flower buds is rich in bioactive compounds, particularly eugenol, which is largely responsible for its diverse pharmacological properties. Scientific studies have demonstrated that clove essential oil exhibits antibacterial, antifungal, antiviral, antioxidant, analgesic, anti-inflammatory, anticancer, antidepressant, antiseptic and antispasmodic activities[11]. This analysis explores the antibacterial and anti-biofilm properties of *Syzygium aromaticum* essential oil against *Streptococcus pneumoniae*.

Commonly known as clove, *S. aromaticum* is a fragrant spice extensively used in culinary and traditional medicinal practices. Its essential oil, rich in eugenol and other bioactive compounds, has demonstrated significant antimicrobial activity, including against multidrug-resistant pathogens. In addition to its culinary and therapeutic applications, clove oil has been utilized for food preservation due to its potent pharmacological effects. The use of essential oils as alternatives to conventional antibiotics offers a promising and sustainable strategy to combat bacterial infections and to mitigate the growing threat of antibiotic resistance[6].

Material and Methods

Collection of plant samples: Dried samples of *Syzygium aromaticum* were sourced from local retail markets in Coimbatore, Tamil Nadu and subsequently ground into a fine powder using an electric blender to facilitate further processing.

Collection of *Streptococcus pneumoniae*: *Streptococcus pneumoniae*, a bacterium commonly associated with upper respiratory tract infections, was isolated from oral swab samples collected from patients at Bio Line Laboratory, R.S. Puram, Coimbatore, Tamil Nadu, India. The collected swabs were subsequently subcultured and utilized for further analysis.

Extraction of *Syzygium aromaticum* EO: Essential oil from *Syzygium aromaticum* was extracted using a Clevenger-type apparatus. Finely powdered dried clove buds (18 g) were placed in a round-bottom flask and mixed with 150–200 mL of distilled water. The flask was connected to a condenser via a standard distillation setup. Steam distillation was carried out for approximately two hours, after which 0.5–1 mL of essential oil was collected. A separating funnel was used to isolate the oil from the aqueous phase. The extracted clove oil was stored in a screw-capped container to prevent evaporation and was subsequently used for further analysis[29].

Chemical constituents' analysis of *S. aromaticum* oil by GC-MS: The essential oil extracted from *Syzygium aromaticum* was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) on an Agilent 7890 GC system coupled with an Agilent 5975C Mass Selective Detector. Separation was achieved using an HP-5MS UI capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed to increase at a rate of 6°C/min from 40°C to 200°C, followed by a ramp of 30°C/min from 200°C to 280°C, with a post-run hold at 280°C for 10 minutes.

Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The injector and detector temperatures were maintained at 250°C. 1 µL aliquot of the essential oil sample (diluted to 0.1% in absolute methanol) was injected in splitless mode. The analysis was conducted in scan mode and compound identification was performed by comparing the retention times and mass spectra with entries in the NIST library[10].

FTIR analysis of *S. aromaticum* oil: The functional groups present in the essential oil of Syzygium aromaticum were identified using Fourier Transform Infrared Spectroscopy (FTIR), a technique that analyses molecular vibrations within a sample. To prepare the sample, the extracted oil was homogenized with potassium bromide (KBr) and compressed into a thin, transparent pellet. Before analysis, the FTIR instrument was calibrated using a standard reference, such as a mirror, to ensure accuracy and establish a baseline.

During the analysis, an infrared beam was passed through the sample and the resulting absorption of energy was recorded. The raw time-domain signal was processed using a Fourier Transform algorithm to generate a frequency-domain infrared (IR) spectrum. This spectrum was then interpreted to identify characteristic absorption bands corresponding to specific functional groups, providing insights into the chemical composition and structural features of the essential oil[24].

Total antioxidant capacity analysis of *S. aromaticum* oil: The total antioxidant capacity of *Syzygium aromaticum* essential oil was evaluated using the phosphomolybdenum assay. A sample weighing 5–10 g was dissolved in an appropriate solvent and 0.3 mL of the resulting extract was mixed with 3 mL of reagent solution containing 1 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The reaction mixture was incubated at 95°C for 90 minutes. After cooling to room temperature, the

absorbance of the solution was measured at 695 nm using a UV-Visible spectrophotometer, with a blank containing 0.3 mL of the solvent used for baseline correction. The total antioxidant capacity was quantified by comparing the absorbance values to a standard calibration curve prepared using ascorbic acid at concentrations of 1000, 500, 250, 125, 62.5 and 31.25 µg/mL. Results were expressed in terms of ascorbic acid equivalents (AAE)[12].

FRAP analysis of *Syzygium aromaticum* oil: The antioxidant potential of *Syzygium aromaticum* oil was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay. The FRAP reagent was prepared by mixing 25 mL of 30 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ and 2.5 mL of 20 mM ferric chloride and then incubating at 37°C for 15 minutes. For the assay, 2.85 mL of FRAP reagent was mixed with varying volumes of the ethanol-dissolved sample or ascorbic acid standard (10–50 µg/mL). After 30 minutes of dark incubation, absorbance was measured at 593 nm. Methanol served as the blank. Results were expressed as µg of ascorbic acid equivalents (AAE) per gram[28].

DPPH radical scavenging analysis of *S. aromaticum* oil: The antioxidant activity of *Syzygium aromaticum* oil was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, a widely used method for assessing free radical scavenging ability in plant extracts. 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of ethanol. The oil was diluted in DMSO at varying concentrations (up to 4 µL) and mixed with 2.96 mL of DPPH solution. The mixture was incubated in the dark at room temperature for 20 minutes. Absorbance was then measured at 517 nm using a UV-Vis spectrophotometer. A control sample containing 3 mL of DPPH solution without oil was used for comparison.

Determination of total flavonoid content of *S. aromaticum* oil: The total flavonoid content (TFC) of *Syzygium aromaticum* oil was determined using the aluminium chloride colourimetric method. A reaction mixture was prepared by combining 1 mg of the sample with 4 mL of distilled water in a 10 mL volumetric flask. Then, 0.3 mL of 5% sodium nitrite was added followed by 0.3 mL of 10% aluminum chloride after 5 minutes. After another 5 minutes, 2 mL of 1 M sodium hydroxide was added and the volume was adjusted to 10 mL with distilled water. Standard solutions of quercetin, kaempferol and isorhamnetin (20–100 µg/mL) were prepared similarly. Absorbance was measured at 510 nm using a UV-Vis spectrophotometer. TFC was expressed as µg of each flavonoid equivalent per gram of sample[25].

Determination of total phenolic content of *S. aromaticum* oil: The total phenolic content (TPC) of *Syzygium aromaticum* oil was determined using the Folin–Ciocalteu method. A reaction mixture was prepared by combining 1 mL of alcoholic extract with 9 mL of distilled water in a 25 mL volumetric flask. Then, 1 mL of Folin–Ciocalteu reagent was added and mixed thoroughly. After 5 minutes, 10 mL of 7% sodium carbonate solution was added and the volume was adjusted to 25 mL with distilled water. Standard solutions of gallic acid (20–100 µg/mL) were prepared similarly. The mixtures were incubated at room temperature for 90 minutes and absorbance was measured at 550 nm using a UV-Vis spectrophotometer. TPC was expressed as mg of gallic acid equivalents (GAE) per gram of sample[21].

Cytotoxicity analysis of *S. aromaticum* oil by brine shrimp assay: The cytotoxicity of *Syzygium aromaticum* oil was assessed using the brine shrimp lethality assay. Various concentrations of the oil (100, 250, 500, 1000 and 1500 µL) were prepared in saline solution. Approximately 30 brine shrimp larvae were introduced into each concentration (in 25 mL saline) observed at 1, 2, 4, 6 and 24-hour intervals. A blank control (saline with shrimp only) and a positive control (1 mg/mL potassium dichromate) were included. After 24 hours, shrimp mortality was recorded to evaluate the cytotoxic potential of the oil[27].

Antibacterial activity of *S. aromaticum* EO by well diffusion assay: The antibacterial activity of *Syzygium aromaticum* oil was evaluated using the agar well diffusion method. Nutrient agar plates were prepared, solidified and inoculated with *Streptococcus pneumoniae* using the spread plate technique. Sterile wells were created in the agar using a well cutter and each was filled with a fixed volume of the oil sample and a blank control using a sterile pipette. Plates were incubated at 37°C for 24–48 hours. Post-incubation, zones of inhibition around the wells were measured to assess the bactericidal effect of the oil[9].

Minimal inhibitory concentration (MIC) of *S. aromaticum* oil by the broth dilution method: The minimum inhibitory concentration (MIC) of *Syzygium aromaticum* oil was determined using the broth dilution method. Serial dilutions of the oil (8 to 0.0625 mg/mL) were prepared in a 96-well plate. Each well received 10 µL of *Streptococcus pneumoniae* MTCC 1924 (1.0×10^6 CFU/mL). Plates were covered with aluminium foil and incubated at 37°C for 24 hours. To assess bacterial growth, 40 µL of p-iodonitrotetrazolium violet (0.04 mg/mL) was added to each well and incubated at room temperature for 3 hours. Absorbance was measured using a UV-Vis spectrophotometer to evaluate microbial inhibition[2].

Growth curve analysis of *S. pneumoniae* treated with *S. aromaticum* oil: A growth curve analysis was conducted to evaluate the effect of *Syzygium aromaticum* oil on *Streptococcus pneumoniae*. Overnight cultures of the bacterium were adjusted to an OD_{600} of 0.5 and 1% inoculum was added to 50 mL of lysogeny broth containing 1 mg/mL of the essential oil (sub-MIC concentration). Cultures were incubated at 37°C with shaking at 170 rpm. Cell density was measured hourly for 8 hours using a UV-Vis spectrophotometer at 600 nm[19].

Anti-biofilm analysis of *S. aromaticum* oil: The anti-biofilm activity of *Syzygium aromaticum* essential oil was evaluated using a crystal violet staining assay, following the method described by Kang et al[13] with slight modifications. The assay was performed in a 24-well microtiter plate. After incubation, planktonic cells were carefully aspirated and the wells were gently rinsed with 1X phosphate-buffered saline (PBS, pH 6.8) to remove non-adherent cells. The biofilm was fixed by allowing the plate to stand at room temperature for one hour.

Subsequently, 0.4% crystal violet solution prepared in a mixture of isopropanol, methanol and PBS (1:18) was added to each well and incubated for 15 minutes. Excess stain was removed by washing the wells with PBS until the solution ran clear, followed by two gentle rinses with deionised water. The stained biofilm was then solubilised in 1 mL of 80% ethanol and absorbance was measured at 620 nm using a UV-Visible spectrophotometer to quantify biofilm biomass[13].

Microscopic Analysis of *S. pneumoniae* Biofilms Treated with *S. aromaticum* Oil: Light microscopic analysis of biofilms formed by *Streptococcus pneumoniae* in the presence of *Syzygium aromaticum* essential oil was conducted following the protocol described by Fathi et al[8]. Biofilms were developed on sterile glass coverslips (1 cm × 1 cm) placed in 24-well polystyrene plates containing varying concentrations of the essential oil (8–0.0625 mg/mL). The plates were incubated at 37°C for 24 hours. After incubation, the biofilms were stained with crystal violet and the coverslips were mounted with the biofilm side facing upward. The structural characteristics of the biofilms were examined under a light microscope at 400× magnification[8].

Results and Discussion

Yield of EO and Phytochemical analysis of *S. aromaticum* by GC-MS: The essential oil yield from *Syzygium aromaticum* was approximately 0.8 mL per 20 g of finely powdered clove buds. Gas Chromatography–Mass Spectrometry (GC-MS) analysis revealed the presence of around 15 phytochemical constituents in the oil. Eugenol was identified as the major component, accounting for 56.51% of the total peak area (Figure 1). Other notable compounds included caryophyllene (9.87%), phenol (9.12%) and 1,4,7-cycloundecatriene (5.97%). Several of these constituents have been previously reported to exhibit antibacterial and antioxidant properties (Table 1).

Functional group analysis of *Syzygium aromaticum* oil by FTIR: The FTIR analysis confirmed that *S. aromaticum* oil showed the presence of aromatic, nitro, alkyl/aryl and alkenes types of functional groups (Figure 2 and table 2).

Total phenol and flavonoid content: As shown in table 3, the essential oil of *Syzygium aromaticum* exhibited a total phenolic content of 105 mg GAE/g and a total flavonoid content of 81 mg QE/g. Phenolic and flavonoid compounds represent a significant class of phytochemicals, widely recognised for their potent antioxidant properties which contribute to the therapeutic potential of the oil.

Antioxidant assay

DPPH assay: Figure 3 illustrates the concentration-dependent antioxidant activity of *Syzygium aromaticum* essential oil, with an IC_{50} value determined to be 68 µL. This value is notably higher than those reported in previous studies such as 0.2 µg/mL by Selles et al[23], 4.5 µg/mL by Saeed and Shahwar[22], 23.17 µg/mL by Sokamte et al[26] and 26 µg/mL by Mahboubi and Mahboubi[16]. According to Kennouche et al[15], the potent antioxidant activity of clove oil is primarily attributed to high content of eugenol and eugenol acetate, along with synergistic interactions among its minor constituents. The antiradical efficiency of eugenol is believed to operate through several mechanisms, including hydrogen atom donation, delocalisation of substituents at the para-position, dimerisation of phenoxyl radicals and complexation of DPPH• with aryl radicals[26].

Table 1
Phytocompounds identification of *Syzygium aromaticum* essential oil

S.N.	#PK	RT	Compound Name	Area	Quality
1	18	8.575	Eugenol	56.51	98
2	19	9.486	Caryophyllene	9.87	99
3	24	10.131	Phenol	9.12	93
4	20	9.831	1,4,7 Cycloundexatriene	5.97	98
5	22	9.975	Farnesene	1.91	87
6	21	9.875	Naphthsalene	1.88	90
7	30	10.73	Carophylleneoxide	1.53	91
8	5	5.309	Bicyclo (3.1.0) hex-2-ene	1.47	93
9	26	10.331	Naphthalene	1.41	93
10	25	10.264	Naphthalene	1.38	96
11	16	7.886	Benzene	1.08	97
12	23	10.019	Naphthalene	1	96
13	2	4.453	Camphene	0.97	97
14	11	7.009	Methyl Salicylate	0.73	96
15	35	11.064	Aromadendrene	0.53	60

Table 2
Functional groups identified in *S. aromaticum* essential oil

S.N.	Frequency	Group	Compound Class
1	1512.19	C=C Stretch	Aromatic
2	1458.18	NO_2 Stretch	Nitro
3	1427.32	NO_2 Stretch	Nitro
4	1265.3	C-F Stretch	Alkyl/Aryl
5	1234.44	C-F Stretch	Alkyl/Aryl
6	1203.58	C-F Stretch	Alkyl/Aryl
7	1149.57	C-F Stretch	Alkyl/Aryl
8	1033.85	C-F Stretch	Alkyl/Aryl
9	995.27	C-H Bend	Alkenes
10	910.45	C-H Bend	Alkenes
11	848.68	C-H Bend	Alkenes
12	817.82	C-Cl Stretch	Alkyl/Aryl
13	794.67	C-Cl Stretch	Alkyl/Aryl
14	748.38	C-Cl Stretch	Alkyl/Aryl
15	648.09	C-Cl Stretch	Alkyl/Aryl

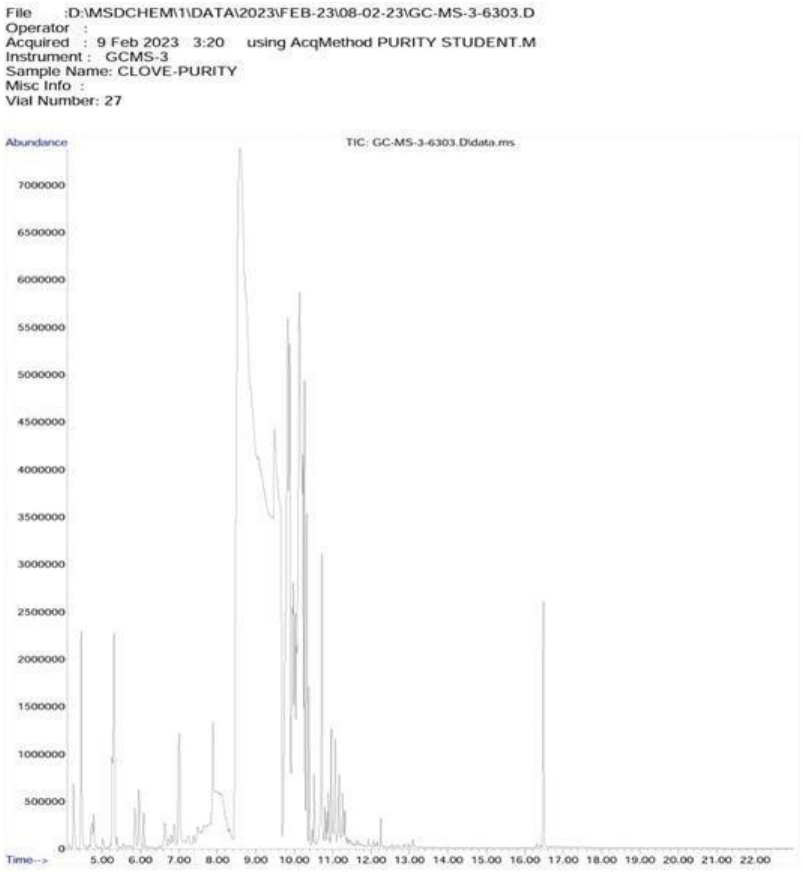


Fig. 1: GC-MS Chromatogram of *S. aromaticum* oil

FRAP and total antioxidant capacity: The antioxidant properties of *Syzygium aromaticum* essential oil were assessed using the FRAP (Ferric Reducing Antioxidant Power) and TAC (Total Antioxidant Capacity) assays. The FRAP analysis yielded a value of 175 µL of ascorbic acid equivalents (AAE) per mL of essential oil, indicating a strong capacity to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. This reflects the oil's potential as an effective electron donor in redox reactions.

In the TAC assay, the essential oil demonstrated a value of 400 µL/mL, highlighting its substantial ability to neutralize free radicals and mitigate oxidative stress. These findings, presented in tables 4 and 5, underscore the potent antioxidant

activity of *S. aromaticum* essential oil, suggesting its potential role in protecting biological systems from oxidative damage and contributing to overall health benefits.

Antibacterial activity: The essential oil extracted from *Syzygium aromaticum* exhibited significant antibacterial activity against the tested bacterial strains. Among them, *Streptococcus pneumoniae* demonstrated the highest sensitivity, with the largest zone of inhibition observed at a concentration of 50 µL (Table 6). The antibacterial efficacy of the essential oil was compared to streptomycin, which served as the positive control, revealing notable differences in susceptibility patterns.

These findings align with previous research by Radunz et al[20] who reported that a eugenol-rich clove essential oil produced inhibition zones of 28.3 mm and 28.1 mm against *Staphylococcus aureus* and *Escherichia coli* respectively. This supports the notion that clove essential oil, particularly due to its high eugenol content, possesses broad-spectrum antibacterial properties.

Growth curve analysis: The growth curve analysis demonstrated a clear distinction between the control and treatment groups. In the control group, bacterial growth increased steadily over time when cultured in nutrient-rich media, indicating normal proliferation. In contrast, treatment with *Syzygium aromaticum* (clove) essential oil resulted in a gradual reduction in growth rate. This suggests that the essential oil effectively inhibits bacterial proliferation, likely due to its bioactive constituents interfering with cellular processes essential for growth.

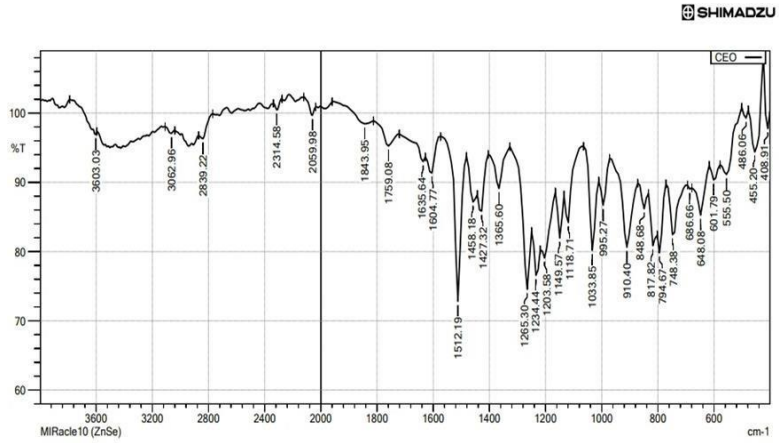


Fig. 2: FTIR Chromatogram of *Syzygium aromaticum* oil

Table 3
The total phenolic and flavonoid content contributes to oxidative damage and contributes to overall health benefits.

S.N.	Name of the Assay	Result	Remarks
1	Total phenolic content	105 mg of GAE/g	-
2	Total flavonoid content	81mg QE/g	-

Table 4
DPPH assay of EO

S.N.	Name of the Assay	10µL	25µL	50µL	75µL	100µL	IC_{50}
1	DPPH	8.0%	19.2%	37.5%	55.1%	72.8%	68µL

Table 5
FRAP of *S. aromaticum* essential oil

S.N.	Name of the Assay	Result	Remarks
1	FRAP	175µL of AAE/mL	-
2	Total antioxidant capacity	400 µL/mL	-

Table 6
Antibacterial activity of *S. aromaticum*

S.N.	Pathogen	EO (25 µL)
1	*Streptococcus pneumoniae*	+
2	Streptomycin	+++
3	H_2O	-

+: minimal inhibition, ++:moderateinhibition, +++: high inhibition, -: no effect

Minimal inhibitory concentration: The essential oil of *Syzygium aromaticum* exhibited notable antibacterial activity against *Streptococcus pneumoniae*, with a minimum inhibitory concentration (MIC) determined to be 1 mg/mL. This indicates the concentration at which the oil effectively inhibits visible bacterial growth. In comparison, eugenol, the primary bioactive component of clove oil, demonstrated a more potent antimicrobial effect, with a reported MIC of 0.06% against *S. pneumoniae*. These findings highlight the concentration-dependent efficacy of both the whole essential oil and its major constituent, suggesting that eugenol plays a key role in the antimicrobial properties of *S. aromaticum*[30].

Light micrograph of biofilm inhibition: *Syzygium aromaticum* essential oil (EO) demonstrated significant inhibitory effects on biofilm formation by *Streptococcus pneumoniae*. Notably, the essential oil was effective at sub-MIC concentrations ranging from 0.25 to 4.0 mg/mL, indicating its potential to prevent biofilm development even at doses below those required for bacterial growth inhibition. Eugenol, the principal active compound in clove oil, exhibited potent bactericidal activity, with 0.12% concentration achieving complete bacterial kill within 60 minutes and the most pronounced effect occurring within the first 15 minutes of exposure.

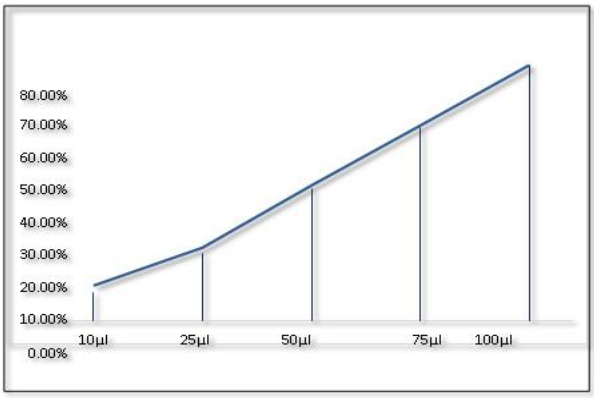


Fig. 3: Graphical representation of DPPH assay

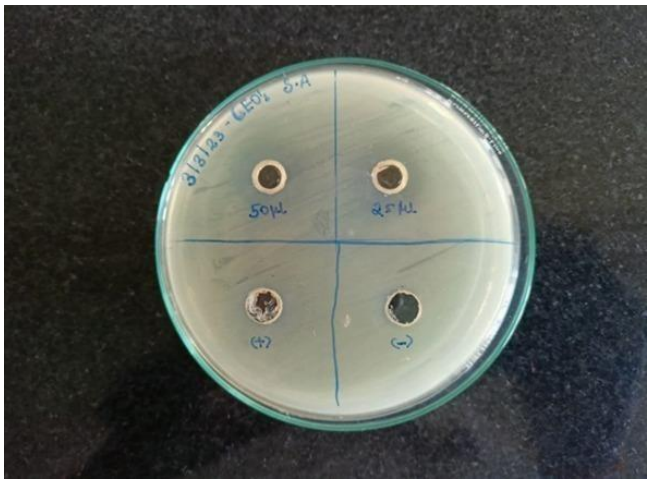


Fig. 4: The zone of inhibition indicating the efficacy of *Syzygium aromaticum*

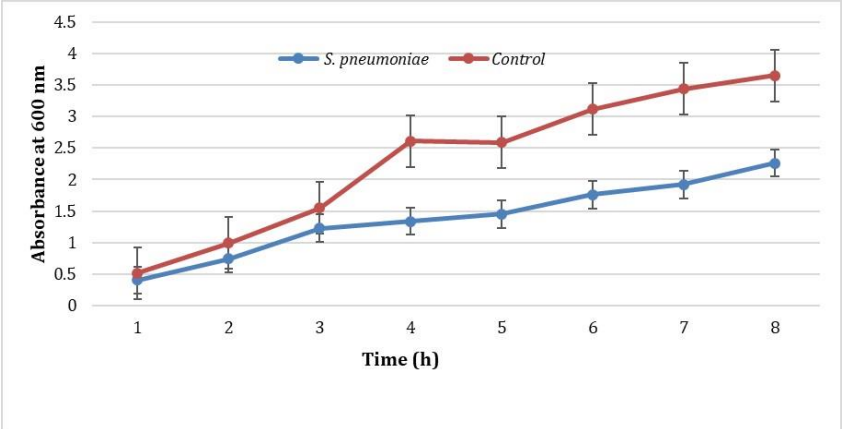


Fig. 5: Effect of *Syzygium aromaticum* oil on the growth of *Streptococcus pneumoniae*

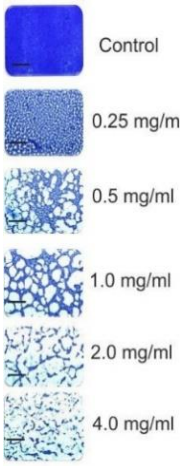


Fig. 6: Light Micrographs (x400) of Biofilm inhibition of clove oil on *S. pneumoniae* at various concentrations

In addition to its bactericidal properties, eugenol effectively inhibited biofilm formation *in vitro*. While its impact on mature biofilms was less pronounced in terms of matrix disruption, it significantly reduced cell viability within the biofilm structure. This suggests that eugenol primarily exerts its anti-biofilm effects through bactericidal action rather than mechanical disruption of the biofilm matrix[30].

Conclusion

The essential oil of *Syzygium aromaticum* was successfully isolated and subjected to comprehensive screening and characterization. Phytochemical analysis, along with Gas Chromatography–Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FT-IR), confirmed the presence of key bioactive constituents. Quantitative assays revealed notable levels of total flavonoids and phenolic compounds, which are often associated with strong biological activity. The essential oil was further evaluated for its antibacterial, antioxidant and anti-biofilm properties using established *in vitro* methodologies.

Among the identified constituents, eugenol and caryophyllene emerged as the primary compounds contributing to the oil's potent anti-biofilm activity. While these findings underscore the therapeutic potential of *S. aromaticum* essential oil, particularly in combating microbial infections and oxidative stress, further *in vivo* studies are warranted to validate its efficacy and safety for medicinal applications.

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