

Chemical Profile and *In-Vitro* Antibacterial, Biofilm-Inhibitory, Antioxidant, and Cytotoxic Activities of Cineole-rich Southern Blue Gum Essential Oil

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Eucalyptus globulus Labill essential oil was chemically characterized and evaluated for selected biological activities using integrated *in vitro* assays supported by exploratory *in silico* analysis. GC–MS profiling identified 8 constituents, with 1,8-cineole (eucalyptol) representing 90.51% of the total composition, indicating a cineole-dominant chemical profile. Antibacterial screening was performed against three clinical isolates and three reference strains, representing Gram-positive and Gram-negative bacteria. The essential oil produced inhibition zones ranging from 24.3 to 38.8 mm, with minimum inhibitory concentrations (MICs) of 62.5 to 125 µg/mL, depending on the strain. Biofilm biomass testing demonstrated concentration-dependent inhibition, with the strongest reduction observed for *Pseudomonas aeruginosa* (61.35% at 0.5 MIC). Antioxidant evaluation using DPPH and ABTS assays revealed dose-dependent radical scavenging activity. Cytotoxicity screening in Caco-2 and HepG2 cell lines showed concentration-dependent responses with IC₅₀ values of 24.4 ± 0.15 and 59.52 ± 0.35 µg/mL, respectively. Molecular docking suggested moderate interaction of eucalyptol with bacterial DNA gyrase through hydrophobic contacts and hydrogen bonding. This study provides integrated chemical and functional characterization of *Eucalyptus globulus* Labill oil and supports further investigation of eucalyptus-derived volatile compositions in antimicrobial and biofilm-control research, while indicating that additional pharmacological, selectivity, and *in vivo* studies are required before translational application.

DOI: 10.15376/biores.21.4.10246-10268

Keywords: *Eucalyptus globulus* Labill; Essential oils; Cytotoxicity against cancer-derived cell lines; Antibacterial activity; Antioxidant activity; Molecular docking

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INTRODUCTION

One of the most urgent issues facing global health is antimicrobial resistance (AMR) in pathogenic microorganisms, which seriously compromises the efficacy of traditional antimicrobial treatments (Salam *et al.* 2023; Tang *et al.* 2023; Selim *et al.* 2025). Previously treatable infections are becoming more challenging to control due to the unselective and frequently inappropriate use of antibiotics in clinical, agricultural, and veterinary settings, which has accelerated the evolution of resistant strains (Caneschi *et al.* 2023). The WHO estimates that bacterial AMR was directly responsible for 1.27 million deaths in 2019 and contributed to 4.95 million deaths (Murray *et al.* 2022). Rapid global escalation is largely driven by sustained antimicrobial exposure across human medicine, animal production, and environmental systems (Joshi *et al.* 2025; Selim *et al.* 2026). The persistence and spread of resistance are reinforced by gaps in infection control, sanitation, diagnostics, and equitable access to quality treatment, meaning that reducing antimicrobial use alone cannot fully reverse current trends. What is required is coordinated, one-health strategies that integrate stewardship, surveillance, and continued therapeutic innovation (Holmes *et al.* 2016). Treatment options are limited because AMR bacteria have become resistant to several classes of antibiotics, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. In addition to raising rates of morbidity and mortality, this crisis also raises healthcare expenses and lengthens hospital stays globally (Fareid *et al.* 2025). AMR is especially prevalent in developing nations, where the issue is made worse by a lack of adequate infection control practices, a lack of healthcare infrastructure, and limited access to new antibiotics (Samuel *et al.* 2010). The uncontrolled spread of resistant pathogens in these areas is facilitated by elements such as the sale of over-the-counter antibiotics, a dearth of diagnostic resources, and low public awareness (Elsayed and Eissa 2025).

In the control of AMR bacterial pathogens, natural products have drawn more attention as promising sources for antibacterial compounds. These include essential oils, which are increasingly investigated as sources of antibacterial agents and formulation research, including potential adjuvant roles rather than direct replacement of antibiotics. They have recently shown reduced efficacy against pathogens (Abdallah *et al.* 2023; Joshi *et al.* 2025). These bioactive substances, which are derived from plants, aquatic organisms, and microorganisms, frequently have a variety of chemical structures and distinct modes of action, which enable them to be effective even against drug-resistant strains (Bashar *et al.* 2024).

A promising substitute treatment for AMR is essential oils (EOs), which are complex blends of volatile compounds extracted from aromatic plants. Their various bioactive components, such as terpenes, terpenoids, and phenolic compounds, can damage microbial cell membranes, increase permeability, and result in the leakage of cellular contents. These effects appear to be the source of their antimicrobial activity (Jubair *et al.* 2021; Alhantoobi *et al.* 2025). EOs usually have multi-target effects, which lowers the risk of resistance development, in contrast to traditional antibiotics, which frequently act on specific molecular targets (Aljaafari *et al.* 2021). The potential of EOs in treating infections linked to AMR is highlighted by their broad-spectrum activity against both forms of bacteria (Gram-positive, Gram-negative), as well as bacteria that form biofilms (Touati *et al.* 2025). Moreover, some EOs have anti-biofilm and antioxidant qualities in

addition to their direct antimicrobial effects, which makes them appropriate for both therapeutic and preventive uses.

The selection of *Eucalyptus globulus* Labill oil in the present study was based on its strong and well-documented bioactivity profile, particularly its high content of oxygenated monoterpenes, which are widely recognized for their biological activities (Knezevic *et al.* 2016). There is a distinct research gap in the literature concerning the multi-dimensional assessment of *Eucalyptus globulus* Labill within a cohesive framework. Although the antimicrobial and anticancer properties of essential oils are widely addressed, comprehensive studies that concurrently evaluate antimicrobial, antioxidant, anti-biofilm, cytotoxic, and molecular docking characteristics remain scarce, especially regarding antimicrobial resistance AMR and cancer. Furthermore, the molecular mechanisms that control *Eucalyptus globulus* Labill oil activity are inadequately investigated, particularly its interaction with critical bacterial targets such as DNA gyrase. Furthermore, there is a lack of research linking its cytotoxic effects on human cancer cell lines and its anti-biofilm efficacy to its chemical profile, which creates a significant gap in establishing its multifunctional anticancer activity significance. The present study addresses this gap by offering a comprehensive chemical and biological characterization of *Eucalyptus globulus* Labill oil, utilizing both experimental and *in silico* methodologies to clarify the relationship between composition and biological activity.

The purpose of this study was to assess the broad-spectrum antimicrobial, antioxidant, anti-biofilm, and cytotoxic properties of Southern Blue Gum (SBG) oil and its main component, eucalyptol, against specific human cancer cell lines and clinically relevant AMR. The novelty of this study is multi-dimensional assessment of cineole-rich *Eucalyptus globulus* Labill within a cohesive framework and study the suggested mechanisms. Therefore, the present study aimed to (i) characterize southern *Eucalyptus globulus* Labill essential oil by GC–MS and (ii) evaluate its selected biological activities, including antibacterial effects (diffusion screening and MIC determination), antibiofilm activity, antioxidant capacity (DPPH and ABTS assays), and cytotoxicity (MTT assay), supported by (iii) molecular docking of eucalyptol with bacterial DNA gyrase as an exploratory interaction analysis. This work provides a structured dataset relevant to antimicrobial and biofilm-control research, while acknowledging that further pharmacological investigation and *in vivo* validation are required for translational interpretation.

EXPERIMENTAL

Bacterial Isolates

The Bacteriology Laboratory, Botany and Microbiology Department, Faculty of Science, Al-Azhar University, provided the bacterial isolates and reference strains used in this investigation. The microorganisms that were tested included both Gram-positive *Staphylococcus aureus* (MRSA) and Gram-negative species, such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The isolates were previously identified and their antibiotic susceptibility profiles determined using the VITEK 2 system (BioMérieux, Inc., Durham, NC) (Sharaf *et al.* 2021; El-Sayed *et al.* 2022). In addition, the antimicrobial efficacy and biofilm inhibition capacity of SBG oil were evaluated against standard strains of *Salmonella typhi* (ATCC 14028), *Bacillus cereus* (ATCC 10987), and *Escherichia coli* (ATCC 8739).

Preparation of SBG Oil Stock Solution

SBG oil was commercially purchased pharmaceutical-grade. The Trust Chem Company in Cairo, Egypt, provided pharmaceutical-grade SBG oil. To reach the required working concentration, the oil was dissolved in analytical-grade dimethyl sulfoxide (DMSO) to create a stock solution. To prevent any inherent antimicrobial effects, the assay's final DMSO concentration was kept below 1% (v/v). Before being used, the stock solution was kept at 4 °C in the dark in an airtight, sterile container.

Antibacterial Activity

The agar well diffusion method on Mueller Hinton agar (MHA, India) was used to assess the antibacterial activity of SBG oil. The standard strains of *Salmonella typhi* (ATCC 14028), *Bacillus cereus* (ATCC 10987), and *Escherichia coli* (ATCC 8739) were tested with 24-hour-old cultures of clinical isolates, including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and the Gram-positive species *Staphylococcus aureus* (MRSA). Using a sterile cork borer, wells (6 mm in diameter) were aseptically punched into the inoculated agar, and 100 µL of SBG oil was poured into each well. After allowing diffusion for two hours at 4 °C, the plates were incubated for twenty-four hours at 37 °C. The positive control was 30 µg of amikacin. The inhibition zones were measured through the incubation period (El-Sherbiny *et al.* 2022).

MICs were measured for tested pathogens. In sterile microtiter plate wells filled with double-strength Mueller-Hinton broth (MHB), serial dilutions ranging from 1000 to 31.25 µg/mL were made. With the exception of negative controls, which contained broth and sterile distilled water, each well received 20 µL of bacterial suspension 1×10^6 CFU/ml. Only broth containing bacterial suspension was used as a positive control. After 24 hours of incubation at 37 °C, 30 µL of resazurin (0.02% w/v) was added to the plates in order to detect growth. Bacterial growth was indicated by a shift in color from blue to purple or pink. Growth and sterility controls were confirmed. The trials were conducted twice, and the average MIC values were calculated (El-Didamony *et al.* 2024).

Antioxidant Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which was modified slightly from El-Sayed *et al.* (2022), was used to evaluate the antioxidant activity of SBG oil. 8 mg of DPPH was dissolved in 100 mL of methanol to create a DPPH solution (80 µg/mL). 100 µL of this solution and 100 µL of SBG oil at different concentrations (1000 to 7.81 µg/mL) were combined in a 96-well plate, and the mixture was incubated for 30 minutes at room temperature. Using methanol as the control, absorbance was measured at 490 nm (TECAN, Austria). The scavenging activity (%) was computed as follows: $(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$. IC₅₀ values were identified as the concentration causing 50% inhibition. With a few minor adjustments, the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay developed by Lee *et al.* (2015) was also used to assess antioxidant capacity,

$$ASA = \frac{A_{\text{control}} - A_{\text{SBG oil}}}{A_{\text{control}}} \quad (1)$$

where ASA is the ABTS scavenging activity, A_{control} is the absorbance of the control, and $A_{\text{SBG oil}}$ is the absorbance of the SGB oil.

In Vitro Antibiofilm Potential

With a few minor adjustments, sterile, flat-bottom, polystyrene 96-well microtiter plates were used to evaluate the inhibition of biofilm biomass of SBG oil in accordance with Sharaf (2020). 100 μ L of MHB was added to each well, and 10 μ L of an overnight bacterial culture ($OD_{620} = 0.05 \pm 0.02$) was added as an inoculant. After adding SBG oil at concentrations of $\frac{1}{2} \times \text{MIC}$, $\frac{1}{4} \times \text{MIC}$, and $\frac{1}{8} \times \text{MIC}$, the plates were incubated for 48 hours at 37 °C. After being fixed with absolute ethanol, the biofilms were stained for 30 minutes with 0.1% (w/v) crystal violet, allowed to air dry, and then destained with 200 μ L of 33% acetic acid. A microplate reader was used to measure the optical density of the released stain at 630 nm. The percentage of inhibition of biofilm biomass was computed as follows:

$$\text{Biofilm inhibition (\%)} = \frac{1 - OD_{360} \text{ of cells treated with different conc. SBG oil}}{OD_{360} \text{ of non-treated control}} \quad (2)$$

Gas Chromatography-mass Spectrometry (GC-MS)

A Trace GC1310-ISQ single quadrupole mass spectrometer with a 30 m capillary column (0.25 μ m film thickness, 0.25 mm internal diameter) was used for the GC-MS analysis, which covered the mass range of m/z 40 to 1000. It was used in accordance with Hashem *et al.*'s protocol (Hashem *et al.* 2025). To get the oil ready for analysis, it was first dissolved in methanol (CH₃OH). The obtained mass spectra were compared with the WILEY 09 and NIST 11 spectral libraries in order to identify the compound. In order to create a thorough chemical profile of SBG oil, the chemical names, molecular weights, and structural details of the identified constituents were also investigated.

Cytotoxicity against Cancer-derived Cell Lines Activity

The Caco-2 and HepG2 human cell lines were used to test the potential anti-cancer effects of SBG oil. The procedures from Selim *et al.* (2025) were adopted, along with the MTT assay. To assess viability, cells were exposed to different concentrations of SBG oil, and their optical density (OD) was measured at 560 nm. Viability % = (Test OD / Control OD) $\times$ 100 was used to determine the percentage of viable cells, and Inhibition % = 100 – Viability % was used to determine the percentage of inhibition.

The human cancer cell lines used in this study were obtained from the Tissue Culture Unit of VACSERA Co. (Egyptian Vaccine and Serum Authority, Cairo, Egypt). Two authenticated epithelial cancer cell lines were used: Caco-2 (ATCC: HTB-37) and HepG2 (ATCC: HB-8065). The Caco-2 cell line is derived from human colorectal adenocarcinoma and is characterized as an adherent epithelial cell line originating from colon tissue (Organism: *Homo sapiens*; Tissue: colon; Cell type: epithelial; Disease: colorectal adenocarcinoma). The HepG2 cell line is a well-established human hepatocellular carcinoma cell line (Organism: *Homo sapiens*; Tissue: liver; Cell type: epithelial; Culture properties: adherent; Disease: hepatocellular carcinoma). Both cell lines are standard reference cell lines originally deposited in the American Type Culture Collection (ATCC) and were supplied by VACSERA as authenticated cultures for experimental use.

Because the study employed commercially available, established human cancer cell lines and did not involve primary human tissues, patient-derived samples, or animal experiments, specific institutional ethical approval was not required according to standard laboratory research guidelines.

Molecular Docking

Making use of MOE version 2014 to validate the experimental results and better understand the potential interactions underlying the antibacterial properties, eucalyptol was molecularly docked into the active sites of the DNA enzyme *Staphylococcus aureus* (PDB ID: 3G7B). The protein structure, which was prepared by removing the water molecule and valence of atom, was corrected using protonation of the whole molecule. Then energy minimization was carried out by applying MMFF94 force fields. The 3D structures of eucalyptol were obtained from the PubChem database and subjected to energy minimization then prepared for docking. The co-crystallized ligand was used as reference to define the active site which was further confirmed using the site finder tool (Assila *et al.* 2024). To verify molecular docking, the redocking technique was used with of the co-crystallized ligand. The docking process was carried through triangle matcher placement inserted in compute window and the scoring function was London dg. Ten poses for each molecule were generated, and the most ideal pose was selected. When the root mean square deviation (RMSD) between the redocked conformation and the compound's original crystallographic conformation was approximately 1.0 Å, it was confirmed that the docking method was reliable in reproducing the experimentally reported binding mode for the receptor (Sharma *et al.* 2021; Nagare *et al.* 2023).

Statistical Analysis

The data were analyzed using one-way analysis of variance, and differences among treatments were assessed using the LSD test at a significance level of $p < 0.05$. All results are expressed as mean $\pm$ standard error (SE), based on three independent replicates ($n = 3$) except MIC determination that were conducted twice.

RESULTS AND DISCUSSION

Antimicrobial Activity

SBG essential oil produced clear growth-inhibition zones against all tested bacterial strains in the agar well diffusion assay (Table 1; Fig. 1). The measured inhibition zones were 38.8 ± 0.4 mm for *Staphylococcus aureus* (MRSA), 31.6 ± 0.3 mm for *Escherichia coli* (ATCC 8739), 30.0 ± 0.5 mm for *Bacillus cereus* (ATCC 10987), 25.8 ± 0.4 mm for *Klebsiella pneumoniae*, and 24.3 ± 0.8 mm for *Pseudomonas aeruginosa*. Because the diffusion of hydrophobic constituents of essential oils may be different from that of classical antibiotics in agar, the inhibition zones were mainly considered here as qualitative screening tools, and the antibacterial potency was therefore evaluated by a parallel MIC determination. The size of the inhibition zone can be determined not only by the antibacterial activity of the oil but also by its solubility, hydrophobicity, volatility, and diffusion capacity in the agar matrix. Thus, the interpretation of inhibition-zone diameters should be performed cautiously, in conjunction with MIC values, which were complementary quantitative evidence of the antibacterial activity of SBG oil.

The reference antibiotic amikacin (30 μ g) produced inhibition zones against most strains but showed no detectable zone against *K. pneumoniae*. Because hydrophobic constituents of essential oils may diffuse differently in agar compared with conventional antibiotics, inhibition zones are interpreted here primarily as qualitative screening indicators, and antibacterial potency is therefore evaluated in parallel using MIC

determination. *K. pneumoniae*'s resistance to amikacin is primarily attributed to the synthesis of aminoglycoside-modifying enzymes and/or 16S rRNA methyltransferases that either deactivate the drug or inhibit its binding to the bacterial ribosome Ramirez and Tolmasky 2017). As indicated in Table 1 and Fig. 1, the results collectively showed that SBG oil exhibits promising *in vitro* antibacterial activity against the tested bacterial isolates, especially in light of the growing prevalence of antibiotic resistance. The lower inhibition seen against *Salmonella typhi* (13 mm) may be because it has a Gram-negative cell structure, especially an outer membrane that is rich in lipopolysaccharides. This makes it harder for hydrophobic compounds like essential oils to get through. Also, efflux mechanisms may make susceptibility even lower.

Due primarily to its high 1,8-cineole content and synergistic phytochemicals, the present results agreed with earlier research showing strong, broad-spectrum antimicrobial qualities of SBG oil (Aldoghaim *et al.* 2018). Differences in bacterial susceptibility were reflected in activity variability, such as decreased efficacy against *Salmonella typhi*. All of these findings pointed to SBG oil's potential as a promising antimicrobial agent in the fight against infections resistant to antibiotics (Knezevic *et al.* 2016).

Table 1. Antimicrobial Activity and MIC of SBG Oil against Bacterial Strains

Microbial strain	Diameter of Inhibition Zone (mm)		MIC (µg/mL)
	SBG oil	Amikacin 30 µg	
<i>Escherichia coli</i> (ATCC8739)	31.6 ± 0.3a	27.6 ± 0.3b	62.5
<i>Klebsiella pneumoniae</i>	25.8 ± 0.4a	0b	125
<i>Salmonella typhi</i> (ATCC14028)	13 ± 0.5b	25.6 ± 0.3a	125
<i>Pseudomonas aeruginosa</i>	24.3 ± 0.8a	15 ± 0.5b	62.5
<i>MRSA clinical isolate</i>	38.8 ± 0.4a	16.1 ± 0.1b	125
<i>Bacillus cereus</i> (ATCC10987)	30 ± 0.5a	21.6 ± 0.3b	125

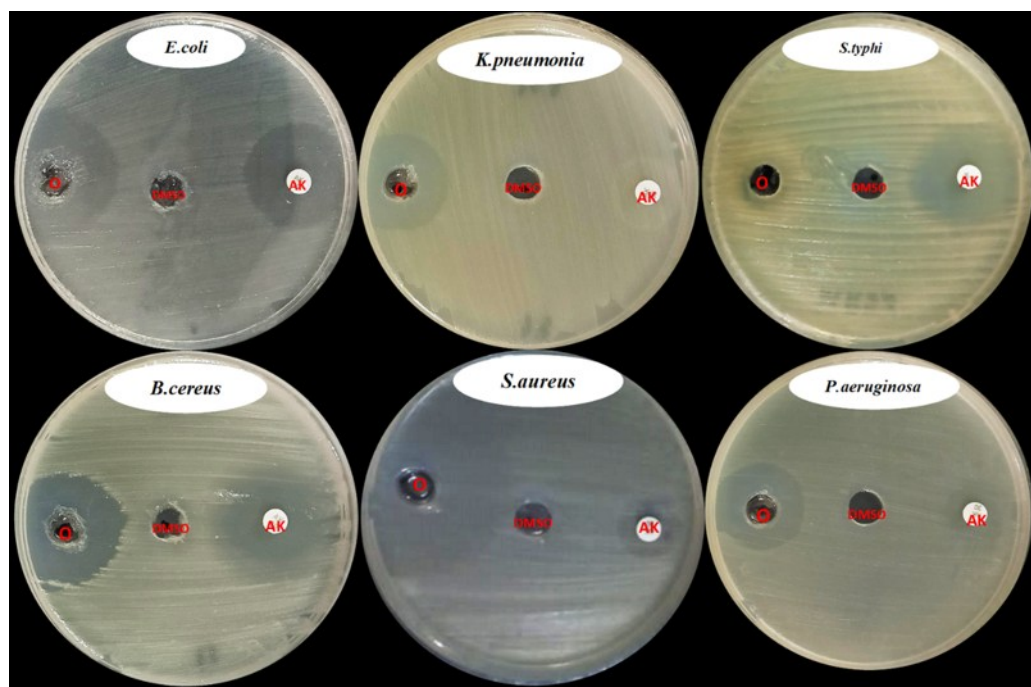


Fig. 1. Antimicrobial activity of SBG oil against microbial strain (o= SBG oil, AK= Amikacin 30 µg)

Although the potency varied by strain, Table 1 demonstrated that SBG oil was able to effectively inhibit all tested bacteria. *Escherichia coli* and *Pseudomonas aeruginosa* had the lowest MIC values (62.5 µg/mL), suggesting that they are more vulnerable to the antibacterial properties of SBG oil. For the same inhibitory effect, the remaining strains of *Bacillus cereus*, *Salmonella typhi*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* needed twice as much (125 µg/mL). This implied that SBG oil had a broad range of activity and was effective even against MDRB. Although the differences in MIC values indicated strain-dependent susceptibility to SBG oil, the observed activity supported its potential as a source of antibacterial compounds. *Escherichia coli* and *Pseudomonas aeruginosa* had lower MIC values (62.5 µg/mL), but other Gram-negative strains such as *Klebsiella pneumoniae* and *Salmonella typhi* were less likely to be affected (125 µg/mL). This difference could be due to differences in the composition of the outer membrane, the structure of the capsule, and the presence of resistance mechanisms like efflux pumps, which caused resistance to essential oil components (Iseppi *et al.* 2020). Although species-specific optimization may be necessary for therapeutic application, these findings point to broad-spectrum efficacy. Differences in membrane permeability, efflux pump activity, and cell wall structure can all be reflected in variations in MIC.

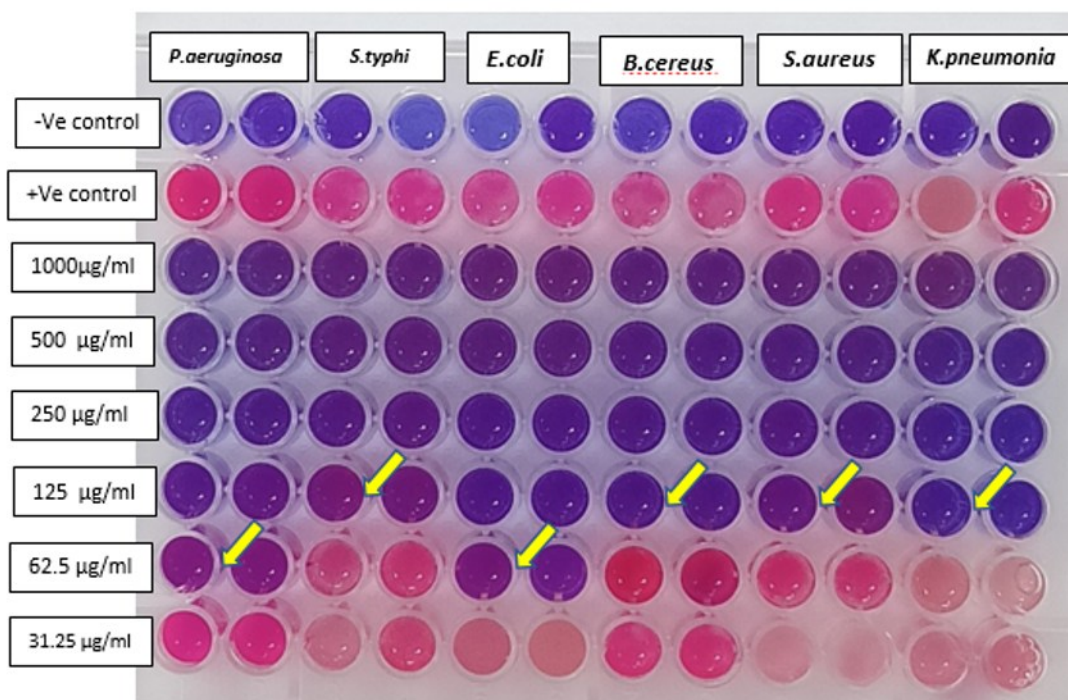


Fig. 2. MIC of SBG oil against *P. aeruginosa*, *S. typhi*, *E. coli*, *B. cereus*, *S. aureus*, and *K. pneumonia*

Antioxidant Activity

Numerous studies have demonstrated that a wide range of chemical and natural substances exhibit strong antioxidant properties (Anaya-Esparza *et al.* 2019). As represented in Fig. 3, two widely used assays, DPPH and ABTS, were applied to compare the antioxidant activities of ascorbic acid and SBG oil at different concentrations. Although sensitivity and reaction conditions varied, both approaches evaluated a substance's capacity to neutralize free radicals. The results clearly demonstrated a dose-dependent

trend, where stronger antioxidant activity was associated with higher concentrations. In both tests, ascorbic acid consistently demonstrated greater antioxidant activity than SBG oil, attaining over 90% radical scavenging at the highest concentrations. As the concentrations were decreased, SBG oil still exhibited notable activity, with values falling from roughly 87.5% to 11.6% (DPPH) and from 79.7% to 8.5% (ABTS). The antioxidant activity of SBG oil differed between the two radical-scavenging assays. The IC_{50} value of SBG oil was 98 $\mu\text{g/mL}$ in the ABTS assay and 58 $\mu\text{g/mL}$ in the DPPH assay. Since a lower IC_{50} value indicates greater radical-scavenging efficiency, SBG oil exhibited higher antioxidant activity in the DPPH assay than in the ABTS assay. This difference may be attributed to the distinct chemical characteristics and reaction mechanisms of the two assays, as DPPH and ABTS radicals differ in their polarity, reaction kinetics, and interactions with the various antioxidant constituents present in the oil. Therefore, the observed antioxidant capacity of SBG oil may vary depending on the radical-scavenging system employed. The IC_{50} values of Ascorbic Acid were 8.9 $\mu\text{g/mL}$ in the ABTS assay and 7.61 $\mu\text{g/mL}$ in the DPPH assay.

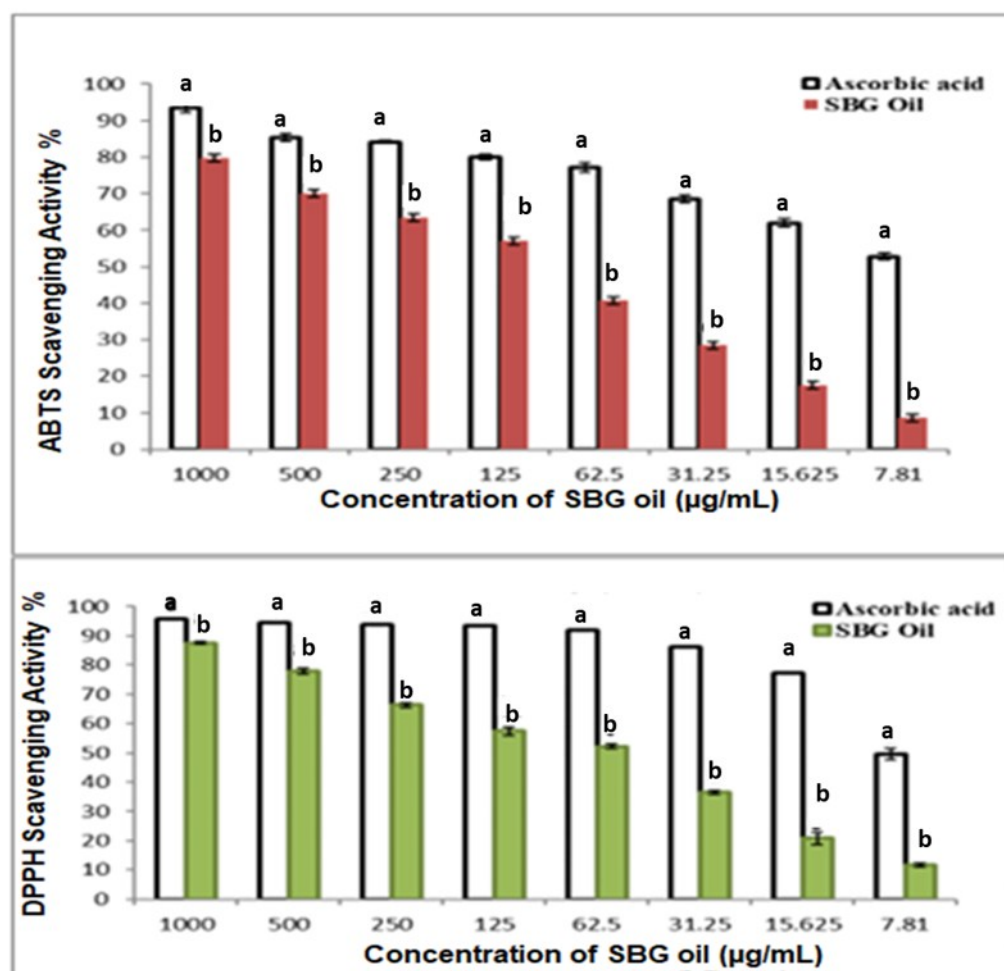


Fig. 3. Antioxidant activity of SBG oil at different concentrations using DPPH and ABTS methods (n=3, $\pm$ standard error)

Because of its solubility and reaction conditions, the ABTS assay frequently exhibited faster and occasionally stronger radical scavenging responses than the DPPH

assay, which is consistent with the generally higher antioxidant values in the ABTS assay for both substances. This indicated that SBG oil exhibits strong antioxidant activity in both kinds of tests, albeit with less potency than ascorbic acid. Although efficacy varies by method and concentration, these results support the potential of SBG oil as a natural antioxidant source (Moges *et al.* 2024).

Anti-Biofilm Ability

SBG oil's *in vitro* inhibition of biofilm biomass ability against MDR (Fig. 4) showed that the extract decreased biofilm biomass formation for all tested species in a concentration-dependent manner. *Pseudomonas aeruginosa* exhibited the greatest inhibition, with reductions ranging from $61.35 \pm 1.22\%$ to $22.71 \pm 1.51\%$ at 1/2 MIC and 1/8 MIC, respectively. *Klebsiella pneumoniae*, on the other hand, showed the lowest reduction, with inhibition of biofilm biomass percentages ranging from $33.61 \pm 1.44\%$ to $15.3 \pm 1.61\%$ at 1/2 MIC and 1/8 MIC, respectively. This was in line with earlier research showing that essential oils showed significantly inhibition of biofilm biomass formation in all tested pathogens in a concentration-dependent manner (Nostro *et al.* 2007). The greatest susceptibility was shown by *Pseudomonas aeruginosa*, whose inhibition ranged from $61.35 \pm 1.22\%$ at 1/2 MIC to $22.71 \pm 1.51\%$ at 1/8 MIC. This is probably because SBG oil can disrupt quorum sensing pathways (Luís *et al.* 2016). *Klebsiella pneumoniae* showed the least amount of reduction ($33.61 \pm 1.44\%$ to $15.3 \pm 1.61\%$), which may be due to its strong biofilm matrix and protective capsule (de Oliveira Júnior and Franco 2020).

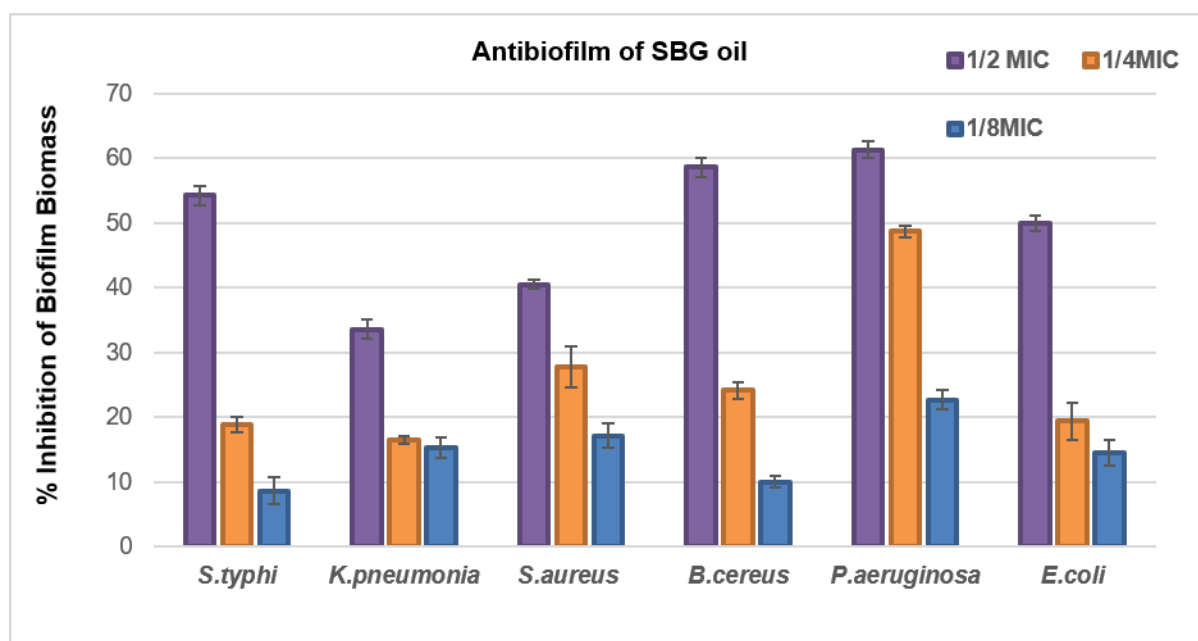


Fig. 4. Inhibition of biofilm biomass activity of SBG oil against *P. aeruginosa*, *S. typhi*, *E. coli*, *B. cereus*, *S. aureus*, and *K. pneumonia* at 1/2MIC, 1/4MIC, and 1/8 MIC. (n=3, ± standard error)

Gas Chromatography Mass Spectrometry

A complex mixture of 8 bioactive compounds with retention times ranging from 8.41 to 29.96 minutes was indicated in the GC-MS analysis of SBG oil, as shown in Table 2 and Fig. 5. 1,8-Cineole was the most prevalent component, according to GC-MS profile (RT and peak-area %) (RT 12.07 min; 90.51% of total peak area). α -Pinene (RT 8.41;

3.92%), γ -terpinene (RT 13.36; 1.67%), l- α -terpineol (RT 19.22; 0.79%), terpinen-4-ol (RT 18.54; 0.31%), p-cymene (RT 11.79; 0.32%), and trace amounts of unsaturated fatty-acid derivatives such as 9-octadecenoic acid (Z) (oleic acid; 0.28%), a docosaheanoic acid, 1,2,3-propanetriyl ester; 0.21%), were the next most prevalent compounds.

Table 2. Gas Chromatography-mass Spectrometry (GC-MS) of SBG Essential Oil

No.	Compound Name	RT (min)	Peak area %	Molecular Formula	Biological Activities in Previous Publications	Reference
1	α -Pinene	8.41	3.92	C ₁₀ H ₁₆	anticoagulant, anti-inflammatory, antileishmanial, antimalarial, antimicrobial, antioxidant, antitumor, analgesic, and resistance-modulating properties against antibiotics	(da Silva Rivas <i>et al.</i> 2012)
2	9-Octa-decenoic acid (z)	9.90	0.28	C ₁₈ H ₃₄ O ₂	Antimicrobial	(Selim <i>et al.</i> 2025)
3	p-Cymene	11.79	0.32	C ₁₀ H ₁₄	Antimicrobial, antioxidant	(Baginska <i>et al.</i> 2023)
4	Eucalyptol	12.07	90.51	C ₁₀ H ₁₈ O	antimicrobial, insecticidal and herbicidal activities	(Barbosa <i>et al.</i> 2016)
5	ζ -Terpinene	13.36	1.67	C ₁₀ H ₁₆	Antimicrobial	(Soliman <i>et al.</i> 2024)
6	Terpinen-4-ol	18.54	0.31	C ₁₀ H ₁₈ O	Broad spectrum antibacterial activity	(Yadav and Rao 2016)
7	L- α -Terpineol	19.22	0.79	C ₁₀ H ₁₈ O	Antioxidant and antimicrobial	(Odoom <i>et al.</i> 2023)
8	Docosaheanoic acid, 1,2,3-propane triyl ester	29.96	0.21	C ₂₂ H ₄₀ O ₂	Antibacterial	(Hamid <i>et al.</i> 2017)

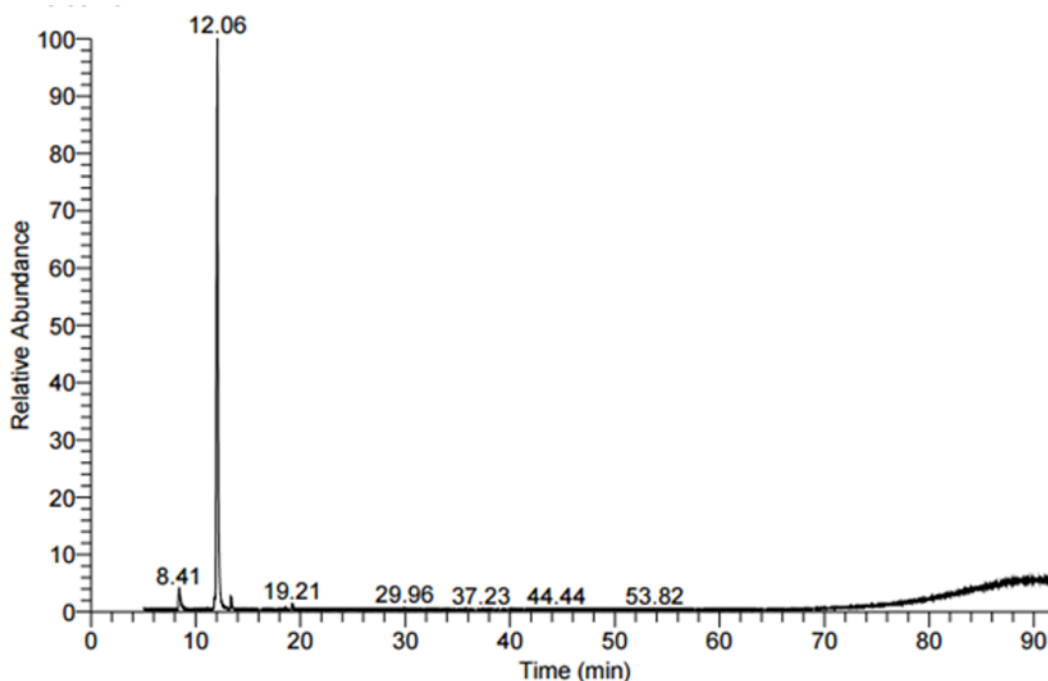


Fig. 5. Gas chromatography mass spectrometry of SBG essential oil

Since eucalyptol makes up more than 90% of the oil, it had a significant influence on the biological profile of the oil. Mechanistic studies demonstrated that 1,8-cineole can cause ROS and impair cell viability in MDR, disrupt microbial membrane integrity, and contribute to fumigant/insecticidal effects in pest assays. Its antimicrobial, anti-inflammatory, and insecticidal properties are well-established. These oxidative and membrane-targeting processes align with the wide-ranging antimicrobial properties anticipated from an oil containing a high concentration of eucalyptol (Merghni *et al.* 2023). Common co-constituents that frequently work in concert with cineole included α -pinene (3.92%) and p-cymene/ γ -terpinene (trace levels). α -Pinene exhibits anti-inflammatory, antimicrobial, and antibiotic-resistance-modulating properties; p-cymene had antimicrobial and antioxidant properties, partly through proton leakage and membrane energetics disruption. These substances can alter efficacy through additive or synergistic interactions as well as by altering volatility and penetration, even at low relative abundances (Park *et al.* 2021). As the main active ingredients, terpinen-4-ol (0.31%) and α -terpineol (0.79%) were known antibacterial and antibiofilm agents. They had linked to synergistic antimicrobial formulations and had the ability to inhibit vegetative growth and biofilm formation; in multiple studies, combinations of terpinen-4-ol and α -terpineol exhibit enhanced antibacterial effects. Even though they are small, these ingredients probably contribute to the oil's antibacterial and anti-biofilm properties, especially when it is applied on species of Gram-positive bacteria (Cordeiro *et al.* 2020). Trace fatty acid derivatives, such as trilinolein and DHA glyceride, can have antimicrobial properties in certain situations due to the disruption of microbial membranes caused by free fatty acids and mono-glycerides. They may stabilize radicals in assays (improve DPPH/ABTS results) or provide mild antibacterial action when released or hydrolyzed, despite their low abundance here (Zhao *et al.* 2022). Some components, including fatty acids, 1,8-cineole, α -pinene, and p-cymene, damage microbial cell membranes, resulting in ion leakage, the

breakdown of the proton motive force, and ultimately cell death. Together with other monoterpenes, eucalyptol intensifies this effect by generating reactive oxygen species, which causes oxidative stress and overwhelms the antioxidant defenses of microorganisms. Terpinen-4-ol also adds to the antimicrobial spectrum by preventing the formation of biofilms and possibly interacting with proteins associated with cell walls or membranes to interfere with vital enzymatic functions (Cordeiro *et al.* 2020).

Cytotoxicity Against Cancer-derived Cell Lines Activity

SBG oil had a potent dose-dependent effect on Caco-2 cells, according to the results of the cytotoxicity assay (Fig. 6). Cell viability fell precipitously to 2.42% at the highest concentration tested (250 µg/mL), which corresponded to 97.57% toxicity. While moderate concentrations (62.5 µg/mL and 31.2 µg/mL) led to decreased cell viability of 12.8% and 27.4%. Viability significantly increased to 81.3% at 15.6 µg/mL, whereas the lowest tested dose (7.81 µg/mL) had virtually no effect (99.8% viability). Strong antiproliferative potential against Caco-2 cells is suggested by the calculated IC₅₀ value of 24.4 ± 0.15 µg/mL. Untreated Caco-2 cells exhibited the distinctive elongated epithelial morphology in a dense, confluent monolayer. High concentrations of SBG oil (250 and 125 µg/mL) caused a sharp decline in cell density along with noticeable membrane disruption, cell rounding, and detachment, all of which point to almost total cell death. There were noticeable gaps in the monolayer and a partial recovery at moderate concentrations (31.2 µg/mL). While the lowest concentration (7.81 µg/mL) closely resembles the control, most cells maintain their normal shape at 15.62 µg/mL. Viability drops from 99.8% at 7.81 µg/mL to just 2.42% at 250 µg/mL, indicating a dramatic dose-dependent decline in the viability curve.

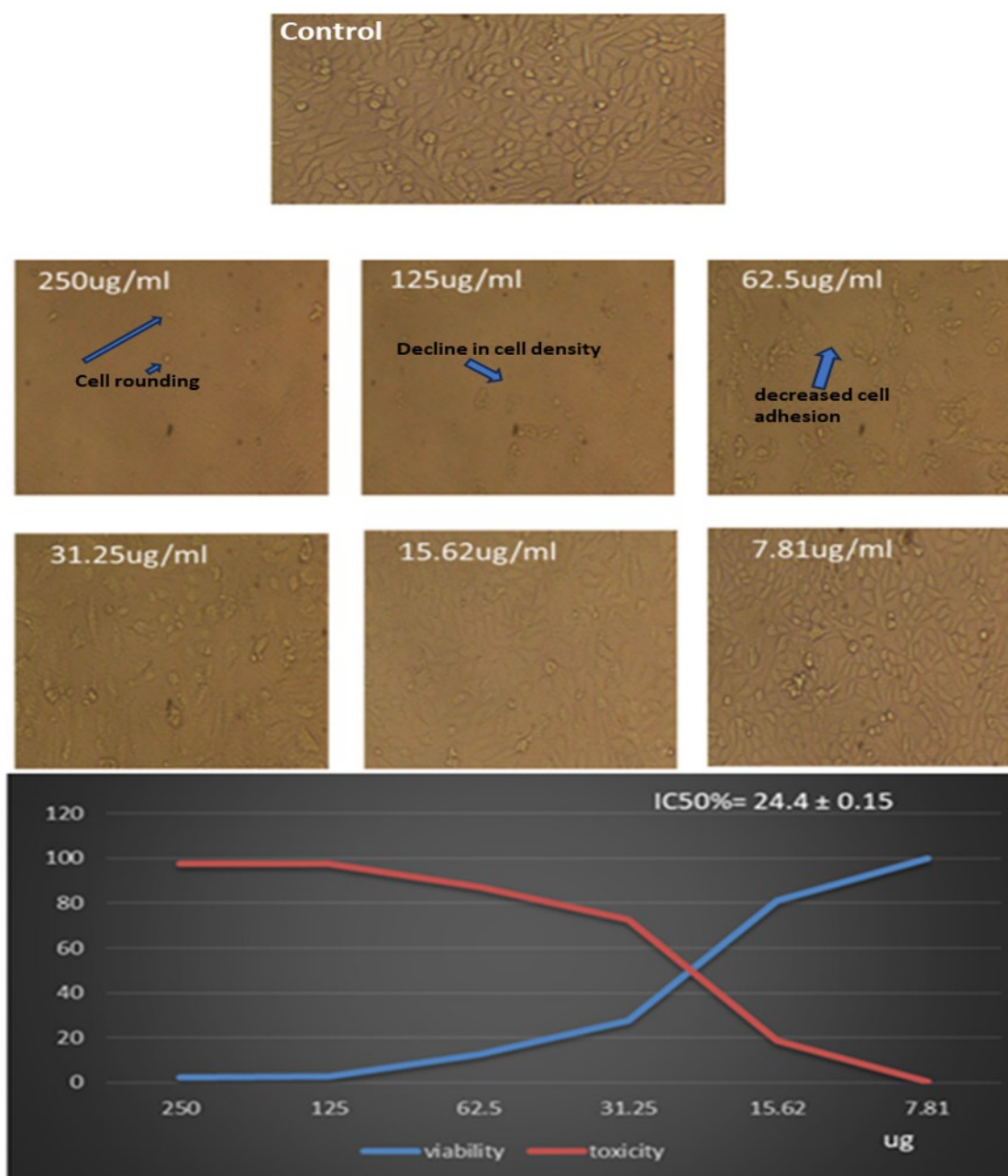


Fig. 6. Cytotoxicity against cancer-derived cell lines activity of SBG oil against Caco-2 cancer cell at concentrations 250 µg/mL: 7.81 µg/mL (blue curve = viability, red curve = toxicity). Magnification at 15 x, Scale bar: 100 µm

HepG2 cells were significantly cytotoxically affected by SBG oil in a dose-dependent manner, as shown in Fig. 7. Cell viability decreased to 3.80% (96.20% toxicity) at the maximum concentration (250 µg/mL) and to 6.56% (93.44% toxicity) at 125 µg/mL. Viability dropped to 50.6% at 62.5 µg/mL and 66.5% at 31.2 µg/mL, indicated moderate inhibition. 99.91% viability was maintained at low concentrations (15.6 and 7.8 µg/mL), which had minimal effects.

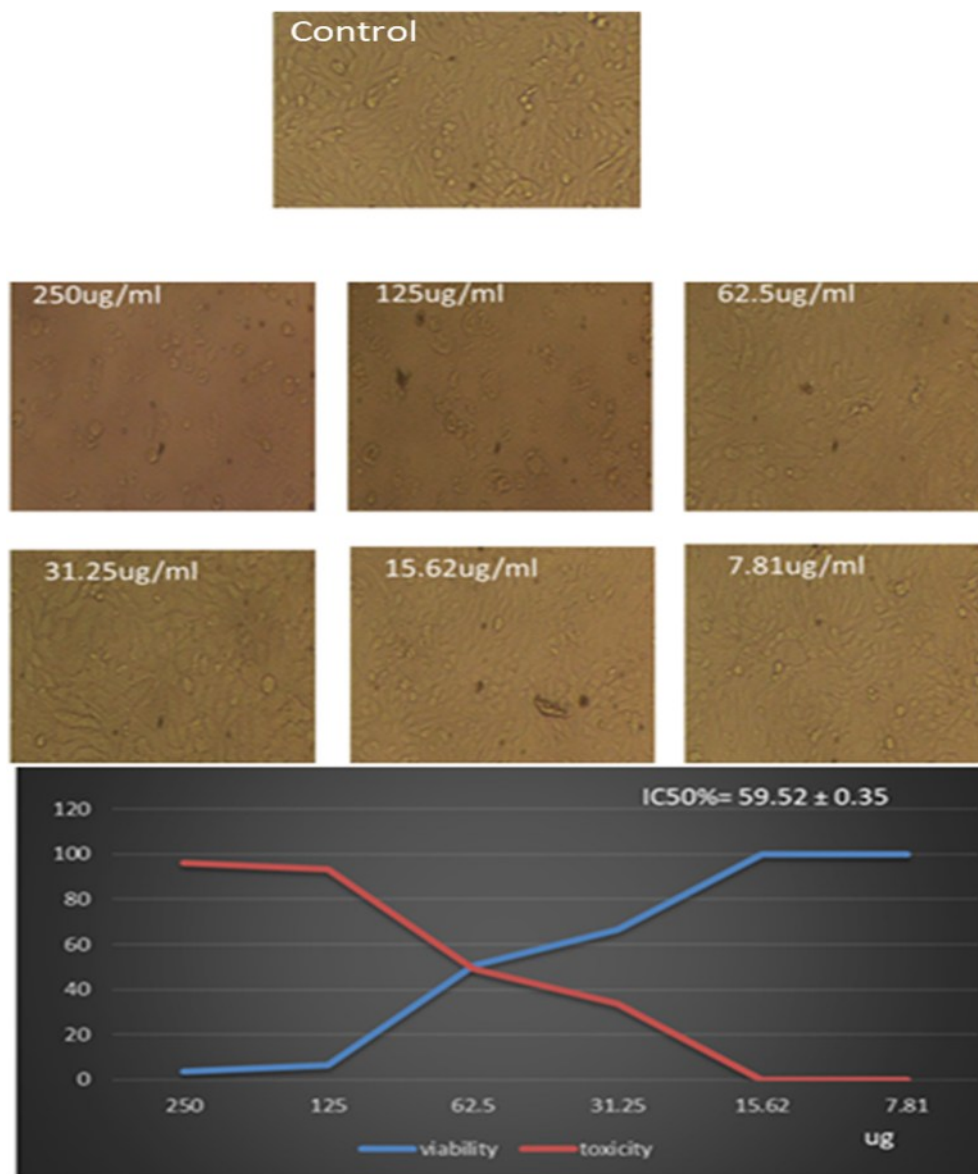


Fig. 7. Cytotoxicity against cancer-derived cell lines activity of SBG oil against HepG2 cancer cell at concentrations 250 µg/mL: 7.81 µg/mL) (blue curve = viability, red curve = toxicity). Scale bar: 100 µm, Magnification at 15 x

Strong cytotoxic potential at relatively low doses was indicated by the calculated IC₅₀ of 59.52 ± 0.35 µg/mL. Untreated control cells had intact cell-to-cell connections and a typical polygonal shape. Cells showed significant detachment, rounding, and shrinkage at 250 µg/mL and 125 µg/mL, which corresponded to viability rates of 3.80% and 6.56%, respectively. Viability dropped to 50.7% at 62.5 µg/mL, indicating partial monolayer disruption. At 31.2 µg/mL there was 66.53% viability. With viability above 99%, cells closely resembled controls at the lowest concentrations (15.62 and 7.81 µg/mL). An IC₅₀ of 59.52 ± 0.35 µg/mL was obtained through quantitative analysis.

Strong antiproliferative action at low concentrations was confirmed by the IC₅₀ value of 24.4 ± 0.15 µg/mL. High doses resulted in severe membrane disruption, cell rounding, detachment, and monolayer collapse, while untreated cells morphologically formed dense monolayers. While the lowest dose maintained normal morphology,

moderate doses resulted in a partial recovery. These outcomes are consistent with previous research on the apoptosis of cancer cells induced by essential oils (Dhakad *et al.* 2018; Al-Bachir 2020).

SBG oil's observed dose-dependent cytotoxicity toward HepG2 cells is in line with earlier studies showing the cytotoxicity against cancer-derived cell lines potential of essential oils high in phenolic constituents and monoterpenes (Al-Bachir 2020). When compared to certain plant-derived oils, the IC₅₀ value of 59.5 µg/mL showed a strong effect, indicating that bioactive compounds like 1,8-cineole and α-pinene may be involved in oxidative stress, mitochondrial membrane depolarization, and apoptosis induction (Wu *et al.* 2021). Essential oils have been shown to cause DNA fragmentation, caspase activation, and cell cycle arrest in HepG2 cells, exhibiting similar cytotoxicity patterns (Bayala *et al.* 2020). Following earlier research on the cytotoxicity against cancer-derived cell lines effects of essential oils, the results show that SBG oil exhibits strong, concentration-dependent cytotoxicity against HepG2 cells. The bioactive components, especially 1,8-cineole and α-pinene, may cause apoptosis through oxidative stress, mitochondrial depolarization, and caspase activation, according to the IC₅₀ value of 59.5 µg/mL. Apoptosis is supported as the main mechanism by morphological changes such as rounding and detachment (Miguel 2010). This cytotoxicity against cancer-derived cell lines activity may be caused by mitochondrial dysfunction, disruption of cellular redox balance, and modulation of ROS. Membrane disruption is another well-recognized mode of action, as the lipophilic nature of essential oil constituents allows them to interact with and destabilize phospholipid bilayers, increasing membrane permeability and leading to loss of cellular integrity (Alhantooob *et al.* 2025). The major limitation in the present study was the fact that normal cell lines were not included in this study. This issue would be considered as future study to determine the selective toxicity of this essential oil against cancer cell lines.

Molecular Docking

According to molecular docking analysis, eucalyptol, which accounts for 90% of the extract, maintains its anti-microbial activity and takes on a binding mode akin to that of the reference ligand even though it does not entirely fill all of the active site's pockets because the small molecular size of eucalyptol compared to the crystalized ligand. Specifically, eucalyptol interacts with the Asn54 residue *via* an acceptor hydrogen bond, which is reinforced by other hydrophobic contacts that help stabilize the ligand inside the binding cavity (Figs. 8 and 9). These interactions imply that when important anchoring residues are engaged, functional binding can be maintained even with partial pocket occupancy. Every eucalyptol derivative examined in this investigation exhibited detectable affinity for the DNA gyrase enzyme (PDB ID: 3G7B). The redocked co-crystallized ligand obtained a higher docking score of −6.49, indicating its optimal fit within the binding site and stronger interaction, whereas eucalyptol's docking score of −3.643 indicated a moderate binding potential. The comparison demonstrates that eucalyptol can still form significant and stable protein–ligand interactions in spite of its smaller size and less extensive pocket coverage.

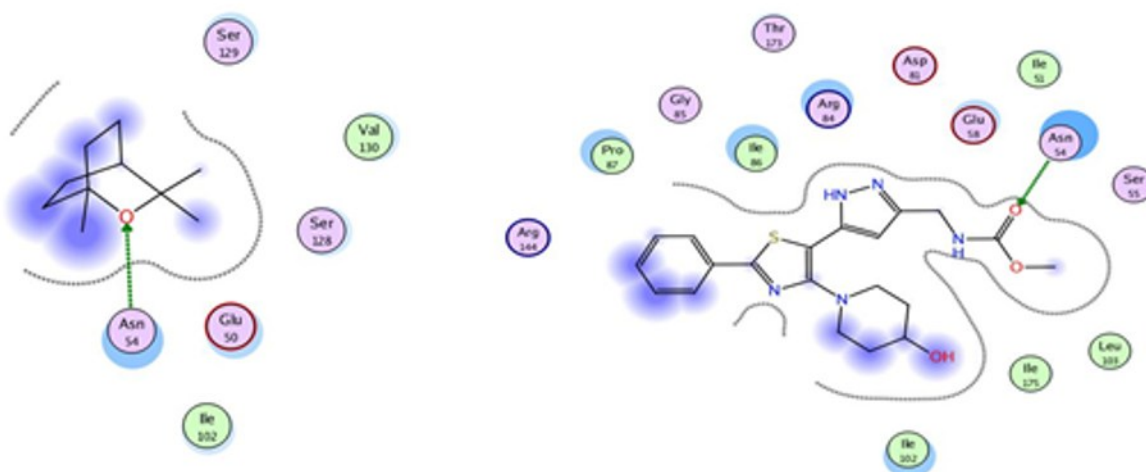


Fig. 8. 2D diagram of the interaction between DNA Gyrase PDB ID: 3G7B with original ligand and Eucalyptol

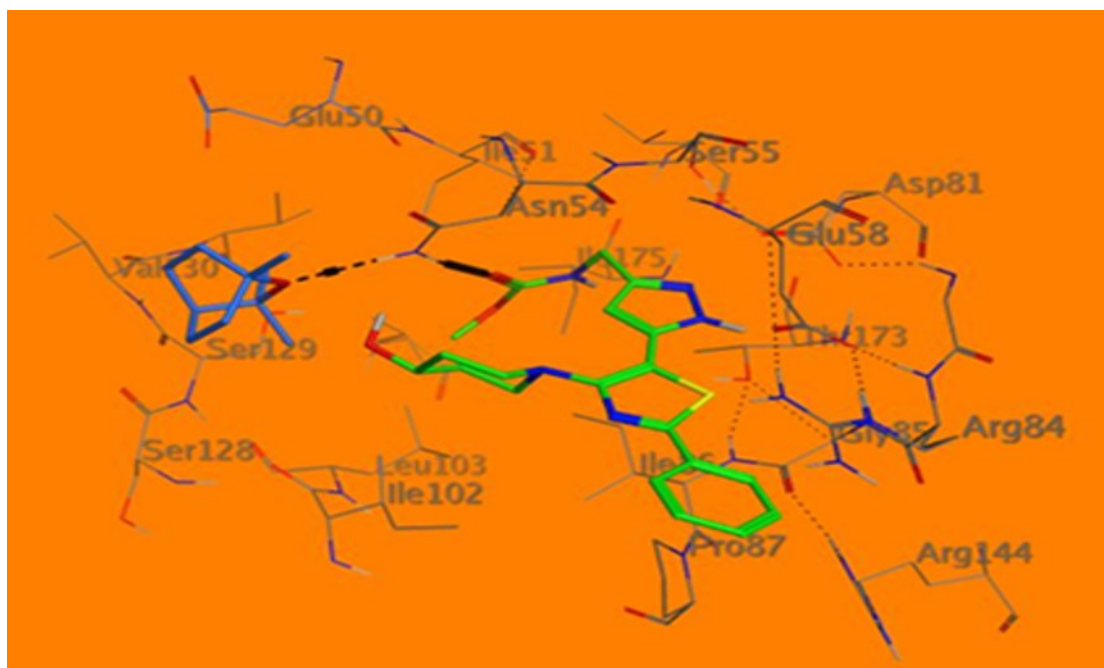


Fig. 9. 3D diagram overlaying between DNA Gyrase PDB ID: 3G7B with original ligand (green color) and Eucalyptol (blue color)

CONCLUSIONS

The study evaluated a chemical and functional *Eucalyptus globulus* Labill essential oil. *In vitro*, antibacterial, biofilm-biomass-reducing, antioxidant, and cancer-cell cytotoxic effects were found under the tested conditions. Exploratory *in silico* docking analyses were done. The cineole-dominant composition showed significant antibacterial, antibiofilm, antioxidant, and cytotoxic activities against cancer-derived cell lines activities under the tested experimental conditions, while docking analysis provides supportive structure. However, these biological activities need broader microbial panels, isolates characterization, selectivity assessment using normal cell lines, and evaluation of formulation strategies to improve stability and delivery of volatile compounds. The present findings can be regarded as a promising window after complete identification of bioactive compounds, the biosafety, selective toxicity, formulation, *in vivo* studies, in controlling multidrug-resistant bacteria as a natural agent.

ACKNOWLEDGEMENTS

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R158), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Conflicts of Interest

The authors declare there are no conflicts of interest.

Competing Interest

The authors declare that they have no known competing interests.

Author Contributions (CRediT Taxonomy)

Samy Selim: Conceptualization (lead); Methodology (lead); Supervision (lead); Investigation (supporting); Writing – original draft (lead); Writing – review and editing (lead); Project administration (lead). **Samiah Hamad Al-Mijalli:** Funding acquisition (lead); Resources (lead); Methodology (supporting); Writing – review and editing (equal). **Mohammed Aladhadh:** Methodology (supporting); Data curation (supporting); Writing – review and editing (equal). **Emad M. Abdallah:** Formal analysis (supporting); Interpretation of antimicrobial and bioactivity data (supporting); Writing – review and editing (equal). **Mohammed S. Almuhayawi:** Microbiological assays (lead); Antibacterial and antibiofilm experimentation (lead); Data validation (supporting); Writing – review and editing (equal). **Mohammed H. Alruhaili:** Antimicrobial testing (supporting); MIC determination (supporting); Data curation (supporting); Writing – review and editing (equal). **Hattan S. Gattan:** Cytotoxicity assays (lead); Cell culture experiments (lead); Data analysis (supporting); Writing – review and editing (equal). **Abdullah Alsrhani:** Molecular docking analysis (lead); Computational modeling and validation (lead); Visualization of docking results (lead); Writing – review and editing (equal). **Abdullah A. Saber:** Conceptualization (lead), GC–MS analysis (lead); Chemical profiling and interpretation (lead); Formal analysis (equal); Writing – review and editing (equal). **Amer M. Abdelaziz:** Conceptualization (lead), GC–MS analysis (lead); Chemical profiling and

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Funding

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R158), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Facilities

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R158), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Availability of Data and Materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request

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Article submitted: June 2, 2026; Peer review completed: July 31, 2026; Revised version received and accepted: August 16, 2026; Published: September 1, 2026.

DOI: 10.15376/biores.21.4.10246-10268