

# Antimicrobial Activity and Integrated In Silico Evaluation of Bioactive Constituents from *Trametes versicolor* against Clinically Isolated Pathogens

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## Research Article

**Keywords:** *Trametes versicolor*, antimicrobial activity, mushroom cultivation, GC MS, DNA gyrase B, molecular docking, DFT, molecular dynamics, ADMET, antimicrobial resistance

**Posted Date:** September 7th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-10803454/v1>

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**Additional Declarations:** No competing interests reported.

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# Antimicrobial Activity and Integrated *In Silico* Evaluation of Bioactive Constituents from *Trametes versicolor* against Clinically Isolated Pathogens

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## Abstract

The increasing burden of antimicrobial resistance has intensified the search for structurally diverse antimicrobial agents from natural sources. This study evaluated the cultivation performance, extraction yield, phytochemical profile, antimicrobial activity, and computational properties of bioactive constituents associated with *Trametes versicolor* using an integrated experimental and in silico workflow. Fruiting bodies were cultivated on unsupplemented and supplemented sawdust substrates, extracted with methanol, ethanol, and water, and evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans* by disc diffusion and broth dilution assays. Minimum inhibitory concentration, minimum bactericidal or fungicidal concentration, and dose response analyses were incorporated into the antimicrobial assessment. Qualitative phytochemical screening and gas chromatography mass spectrometry were used for chemical characterization. Candidate ligands were subsequently evaluated by physicochemical and medicinal chemistry screening, ADMET prediction, molecular docking against the ATPase domain of *Staphylococcus aureus* DNA gyrase B, density functional theory, and 200 ns molecular dynamics simulations. Supplemented sawdust increased fresh yield from  $102.60 \pm 3.58$  g to  $373.80 \pm 5.21$  g and biological efficiency from  $11.10 \pm 0.46\%$  to  $40.30 \pm 0.56\%$ . The aqueous extract gave the highest crude extraction yield, whereas methanol produced the highest overall inhibition across the antimicrobial panel. The largest inhibition zone was  $22.33 \pm 2.08$  mm against *S. aureus*. The antimicrobial response depended significantly on extract, concentration, microorganism, and their interactions. GC MS profiling revealed a mixture dominated by terpenoid and oxygenated constituents. Among the computational ligands, CID 5281515 produced the most favourable docking score against GyrB at  $-7.3$  kcal/mol, followed by CID 91457 and CID 3084954 at  $-7.1$  kcal/mol. The four ligands subjected to DFT analysis showed HOMO LUMO gaps of 3.322 to 6.479 eV. Molecular dynamics trajectories distinguished the conformational behaviour of the complexes, with CID 5281515 showing the most consistent overall profile among the selected ligands. The integrated findings support further isolation, structural confirmation, and target based experimental evaluation of *T. versicolor* constituents as potential antimicrobial leads.

**Keywords:** *Trametes versicolor*; antimicrobial activity; mushroom cultivation; GC MS; DNA gyrase B; molecular docking; DFT; molecular dynamics; ADMET; antimicrobial resistance.

## 1. Introduction

Medicinal mushrooms are important sources of chemically diverse metabolites with potential pharmaceutical and biotechnological applications. Polysaccharides, terpenoids, phenolic compounds, sterols, peptides, and other specialized metabolites from medicinal fungi have been associated with antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and cytotoxic activities (Venturella et al., 2021; Hyde et al., 2022; Krupodorova et al., 2024). This chemical diversity is relevant to antimicrobial discovery because natural products provide molecular scaffolds that differ from many conventional synthetic libraries. The continuing global burden of antimicrobial resistance further increases the value of systematic screening of fungal metabolites for antibacterial and antifungal activity (World Health Organization, 2023). *Trametes versicolor*, commonly known as turkey tail, is a white rot basidiomycete widely distributed on lignocellulosic substrates. The species is well known for polysaccharide K and polysaccharide peptide, but chemical investigations have also identified phenolic compounds, sterols, triterpenoids, aromatic constituents, and other secondary metabolites with biological activity (Habibi et al., 2015; Janjušević et al., 2017). Its ligninolytic capacity also supports cultivation on woody residues and agro industrial substrates, which creates an experimental link between biomass production and recovery of bioactive metabolites (Grimm and Wösten, 2018). Previous studies have reported antimicrobial activity of *T. versicolor* extracts, with substantial variation according to extraction solvent, fungal material, target organism, and assay conditions (Bains and Chawla, 2020; Janjušević et al., 2018). A combined experimental and computational strategy can extend extract level observations by characterizing the chemical profile and prioritizing candidate molecules for target based investigation. GC MS provides information on volatile and semi volatile constituents, while physicochemical and ADMET prediction can identify molecular features relevant to early stage drug development. Molecular docking estimates favourable binding poses within a defined protein site, DFT describes electronic features that may influence molecular reactivity, and molecular dynamics examines the time dependent structural behaviour of predicted protein ligand complexes (Daina et al., 2017; Pantsar and Poso, 2018; Case et al., 2023). DNA gyrase B was selected as the bacterial target because its N terminal ATPase domain is essential for DNA supercoiling and is inhibited by established coumarin antibiotics such as novobiocin (Bisacchi and Manchester, 2015; Lu et al., 2014). Accordingly, this study integrated cultivation, solvent extraction, antimicrobial testing, phytochemical screening, GC MS profiling, medicinal chemistry and ADMET assessment, GyrB molecular docking, DFT, and 200 ns molecular dynamics simulation. The experimental stages established extract level activity and chemical composition, while the computational stages provided a structured framework for prioritizing candidate ligands for subsequent biochemical validation.

## 2. Materials and Methods

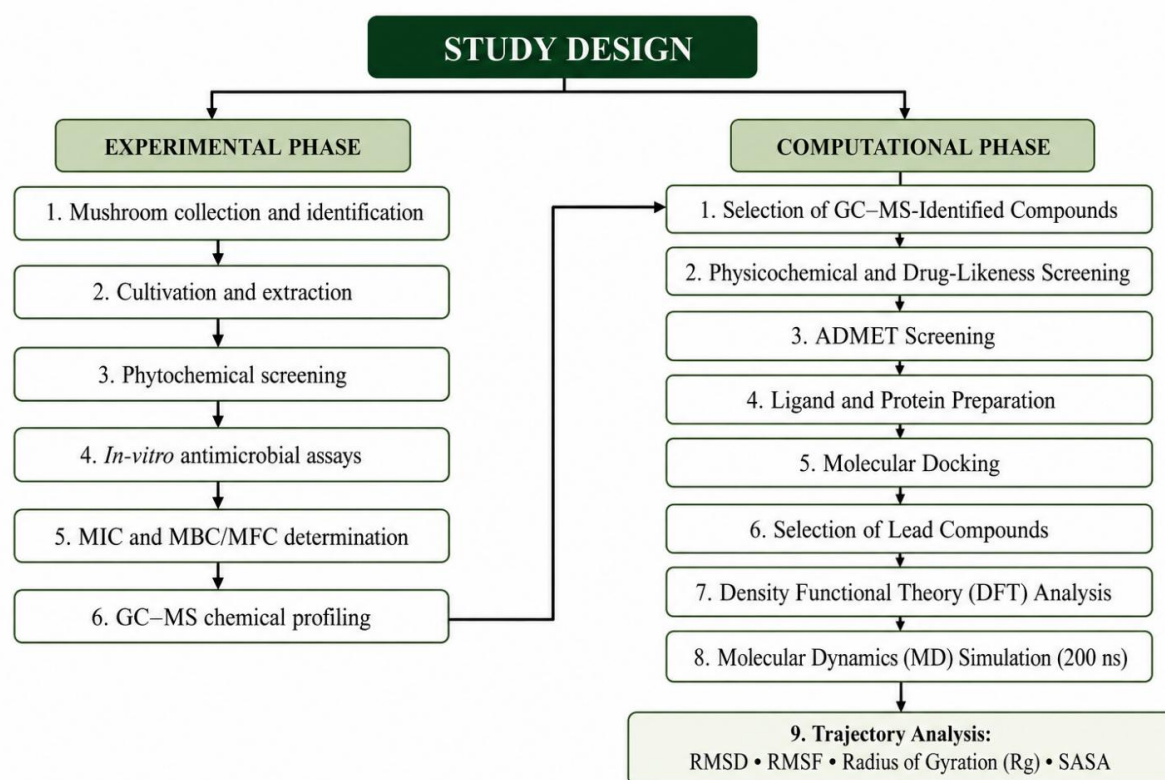


Figure 1. Experimental and computational workflow used to evaluate *T. versicolor* cultivation, extract activity, chemical composition, and selected computational ligands.

## 2.1 Study Design

This study used a sequential experimental and computational design. The experimental component comprised field collection and taxonomic identification of *T. versicolor*, establishment of pure cultures, spawn production, cultivation on two sawdust based substrates, preparation of methanolic, ethanolic, and aqueous extracts, antimicrobial testing, qualitative phytochemical screening, and GC MS profiling. Cultivation performance was evaluated from spawn run duration, fruiting initiation, time to harvest, fresh yield, and biological efficiency. Antimicrobial activity was evaluated by disc diffusion, broth dilution, MIC determination, MBC or MFC testing, and dose response analysis. The chemical profile obtained from the active extract guided selection of candidate structures for the computational component. The computational workflow comprised physicochemical and medicinal chemistry screening, ADMET prediction, preparation of the GyrB ATPase domain, molecular docking, DFT calculations, and 200 ns molecular dynamics simulations. RMSD, RMSF, radius of gyration, and solvent accessible surface area were evaluated from the MD trajectories. This design linked biomass production, extraction efficiency, antimicrobial response, chemical composition, and molecular level target interaction within one analytical framework.

## 2.2 Study Area

The study was conducted at the orchard of Mohamet Lawan College of Agriculture, Maiduguri, northeastern Nigeria, at approximately 11.83° N and 13.15° E (Figure 2). The area lies within the Sudano Sahelian ecological zone and has distinct wet and dry seasons. Sampling was

conducted during the rainy season, when moisture availability favours the development of wood decaying basidiomycetes. The orchard contains naturally decomposing hardwood, fallen branches, and other lignocellulosic materials that provide suitable substrates for *Trametes* species. Environmental and sampling information recorded at collection included date, site, geographic position, host substrate, and observable field conditions.

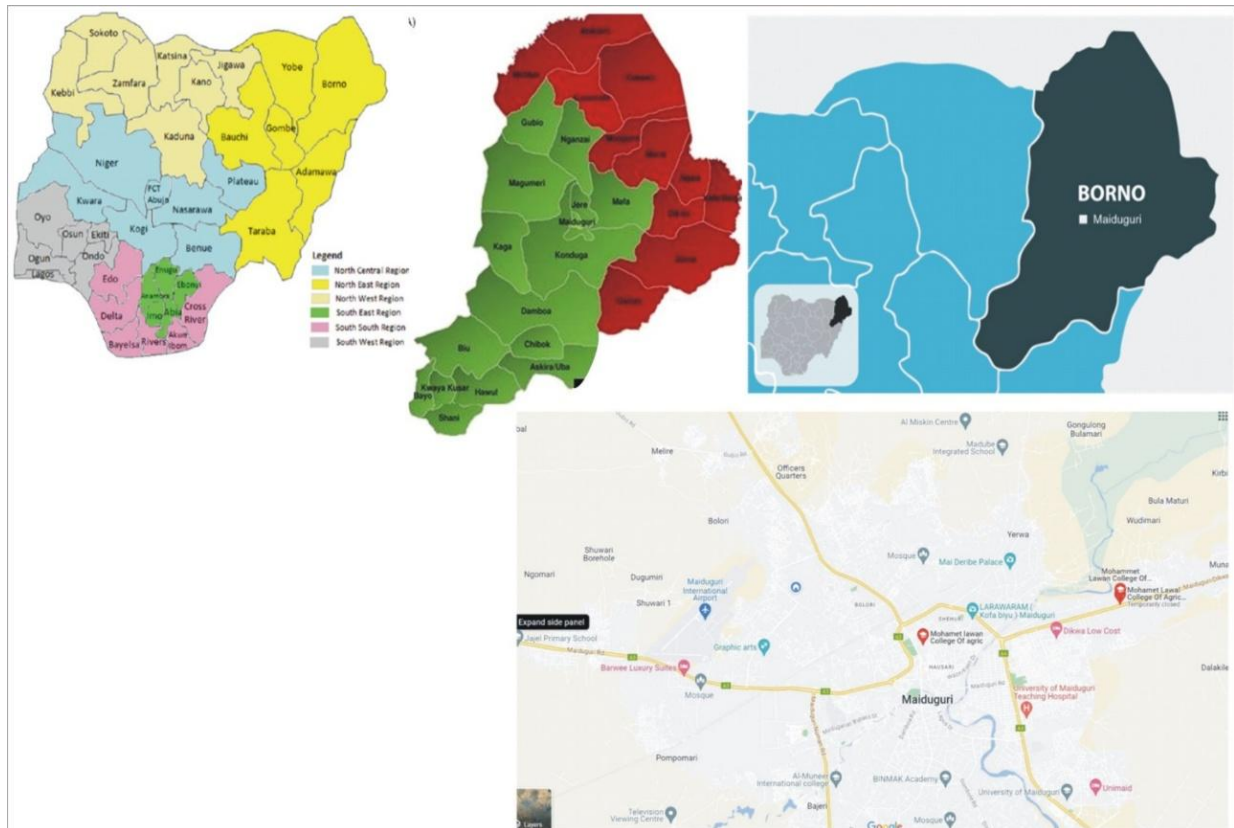


Figure 2. Location of the study area in Maiduguri, Borno State, Nigeria.

### 2.3 Sample Collection and Identification

Fruiting bodies of *Trametes versicolor* were collected during the peak rainy season from naturally decomposing hardwood within the orchard of Mohamet Lawan College of Agriculture, Maiduguri, Borno State, Nigeria. Mature, intact basidiocarps were removed aseptically with sterile scalpels, placed in individually labelled sterile containers, transported to the laboratory in insulated containers, and processed within 24 h. Field records were maintained for sampling location, substrate, collection date, and specimen characteristics.

Taxonomic identification was based on complementary macroscopic and microscopic characters. Macroscopic examination included pileus morphology, concentric zonation, colour pattern, surface texture, basidiocarp consistency, pore characteristics, and the typical fan shaped growth form. Thin basidiocarp sections were examined microscopically for hyphal arrangement, pore structure, and tissue organization. Morphological and microscopic observations were compared with standard basidiomycete taxonomic descriptions, and identification was completed at the Department of Botany, University of Maiduguri. Representative material was retained for traceability of the fungal material used in cultivation and extraction experiments.

## **2.4 Cultivation of *Trametes versicolor***

Pure cultures were established from internal tissue of freshly collected *T. versicolor* basidiocarps. Tissue pieces approximately 2 to 3 mm in diameter were aseptically excised from the inner context and transferred to potato dextrose agar prepared according to the manufacturer's instructions. Approximately 20 mL of sterile medium was dispensed into 90 mm Petri dishes. Inoculated plates were incubated at  $24 \pm 1^\circ\text{C}$  and examined every 48 to 72 h for radial growth, colony morphology, and contamination. Advancing hyphal margins were transferred to fresh PDA until uniform contamination free cultures were obtained. Pure cultures were maintained on PDA and used as inoculum for spawn production.

### **2.4.1 Spawn Production**

Sorghum grain was used as the spawn substrate. Grains were washed thoroughly, soaked in distilled water for 12 to 18 h, drained, and sterilized at  $121^\circ\text{C}$  for 15 min. After cooling under aseptic conditions, the grains were inoculated with actively growing *T. versicolor* mycelium from pure PDA cultures. Spawn containers were incubated at  $24 \pm 1^\circ\text{C}$  until the grain was uniformly colonized. Fully colonized sorghum spawn was then used to inoculate the experimental cultivation substrates.

## **2.5 Substrate Preparation and Mushroom Cultivation**

Two substrate treatments were evaluated: unsupplemented oak sawdust and a supplemented formulation containing 78% oak sawdust, 10% sorghum grain, 10% wheat bran, and 2% gypsum. Each formulation was mixed thoroughly and adjusted to 60 to 70% moisture with distilled water. Substrates were packed into heat resistant polypropylene cultivation bags and sterilized at  $121^\circ\text{C}$  for 2 h. After cooling, each bag was inoculated aseptically with 70 to 80 g of colonized sorghum spawn, corresponding to approximately 7 to 8% of substrate weight. Three independent cultivation bags were prepared for each treatment. Bags were incubated at approximately  $22^\circ\text{C}$  in darkness until complete mycelial colonization, after which they were transferred to fruiting conditions with adequate humidity, aeration, and light. Spawn run duration, fruiting body initiation, time to harvest, and fresh yield were recorded for each replicate.

### **2.5.1 Biological Efficiency**

Cultivation productivity was expressed as biological efficiency (BE), calculated for each replicate from the fresh mass of harvested fruiting bodies relative to the dry substrate mass: BE

(%) = [fresh weight of harvested fruiting bodies (g) / dry weight of substrate (g)] × 100. Biological efficiency was calculated separately for the three replicate bags within each substrate treatment and used together with cultivation time and fresh yield for statistical comparison.

The same replicate structure was maintained for all cultivation measurements, which allowed spawn run, fruiting initiation, harvest time, fresh yield, and biological efficiency to be compared directly between the two substrate formulations.

## 2.6 Preparation of Mushroom Extracts

Harvested *T. versicolor* fruiting bodies were air dried initially and then dried in a hot air oven at 40 to 45°C to constant weight. The dried material was milled and sieved to obtain a uniform powder. Methanol, ethanol, and distilled water were used as extraction solvents to recover constituents across a broad polarity range. For each solvent, 150 g of dried mushroom powder was processed at an overall sample to solvent ratio of 1:10 (w/v) using Soxhlet extraction in successive batches as required by the extractor capacity. Extraction was continued for 6 to 8 h per batch, or until the siphon solvent became substantially clear.

Each solvent treatment was processed independently under the same extraction ratio and temperature control to permit direct comparison of crude extract recovery. The combined extract from each solvent treatment was filtered through Whatman No. 1 filter paper before solvent removal.

Methanolic and ethanolic filtrates were concentrated under reduced pressure at 40 to 45°C using a rotary evaporator. The aqueous filtrate was concentrated under controlled heating and dried to constant recoverable mass. Dried extracts were weighed, transferred into sterile airtight containers, and stored at 4°C until antimicrobial, phytochemical, and GC MS analyses. Extraction yield was calculated as the mass of dried extract relative to the 150 g initial dry mushroom sample.

Extraction yield (%) = [Weight of dried extract (g) / Weight of powdered mushroom sample (g)] × 100

Where:

- Weight of dried extract (g) = mass of extract obtained after solvent removal.
- Weight of powdered mushroom sample (g) = initial mass of mushroom powder used for extraction.

All three crude extracts were handled under the same storage conditions to limit contamination and chemical degradation before biological and chemical analyses.

## 2.7 Microorganism Preparation and Growth Conditions

Five clinically relevant microorganisms were used for antimicrobial testing: *Staphylococcus aureus*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231. The cultures were obtained from the Microbiology Laboratory of the University of Maiduguri Teaching Hospital and maintained

at  $-80^{\circ}\text{C}$  before use. Bacterial strains were revived on organism appropriate bacteriological media and incubated at  $37^{\circ}\text{C}$  for 18 to 24 h. *C. albicans* was cultured on potato dextrose agar at 28 to  $30^{\circ}\text{C}$  for 24 to 48 h. Fresh isolated colonies were suspended in sterile normal saline and adjusted to a 0.5 McFarland standard. For the bacterial cultures, this turbidity corresponded approximately to  $1.5 \times 10^8$  CFU/mL and provided a standardized inoculum for comparative testing.

## **2.8 Antimicrobial Activity Assay**

The antimicrobial activity of the methanolic, ethanolic, and aqueous extracts was evaluated by agar disc diffusion. Bacterial assays were performed on Mueller Hinton agar following the general procedural principles of CLSI M02, while *C. albicans* disk diffusion followed the corresponding yeast testing principles of CLSI M44 (Clinical and Laboratory Standards Institute, 2024, 2018). Standardized 0.5 McFarland inocula were spread uniformly across the agar surface with sterile swabs. Sterile 5 mm filter paper discs were loaded with extract concentrations of 6.25, 12.5, 25, 50, and 100 mg/mL and placed at sufficient distance to prevent overlapping inhibition zones. Organism appropriate antimicrobial agents, including ciprofloxacin, imipenem, amoxicillin clavulanate, and fluconazole, were used as positive controls, while solvent only discs served as negative controls. Bacterial plates were incubated at  $37^{\circ}\text{C}$  for 18 to 24 h and *C. albicans* plates at the yeast specific incubation condition. Inhibition zone diameter was measured in millimetres with a digital caliper. Each extract concentration and microorganism combination was tested in triplicate.

## **2.9 Determination of Minimum Inhibitory Concentration (MIC)**

Broth dilution was used to characterize growth responses and determine MIC endpoints across the same concentration range used for quantitative analysis. Twofold extract dilutions of 6.25, 12.5, 25, 50, and 100 mg/mL were prepared in sterile peptone water. Each tube was inoculated with a standardized fresh microbial suspension, and matched growth and sterility controls were included. Bacterial cultures were incubated at  $37^{\circ}\text{C}$  for 24 h and *C. albicans* at  $28^{\circ}\text{C}$  for 48 h. Growth was evaluated visually from turbidity and quantitatively by optical density at 600 nm where spectrophotometric measurements were available. The MIC was assigned as the lowest tested extract concentration associated with complete absence of visible growth in a concentration response pattern that remained growth negative at higher concentrations.

## **2.10 Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)**

MBC and MFC testing was performed from broth dilution tubes that showed no visible growth. A 100  $\mu\text{L}$  aliquot from each eligible bacterial tube was subcultured onto nutrient agar, while aliquots from *C. albicans* assays were subcultured onto Sabouraud dextrose agar. Plates were incubated under the organism specific growth conditions and examined for colony recovery. The lowest extract concentration with no detectable regrowth was recorded as the MBC for bacteria or MFC for *C. albicans*. MBC/MIC and MFC/MIC ratios were calculated to characterize killing relative to growth inhibition.

MBC/MIC ratio = MBC (mg/mL) / MIC (mg/mL)

MFC/MIC ratio = MFC (mg/mL) / MIC (mg/mL)

A ratio of  $\leq 4$  was considered indicative of bactericidal or fungicidal activity, whereas a ratio  $>4$  was considered indicative of bacteriostatic or fungistatic activity. The MIC and MBC/MFC values obtained for each extract and test microorganism were recorded and used for subsequent comparative analysis.

## 2.11 Statistical Analysis

Cultivation and antimicrobial data were analysed using IBM SPSS Statistics version 26.0, Microsoft Excel 2019, and GraphPad Prism version 9.0. Cultivation measurements were summarized as mean  $\pm$  standard deviation for three independent bags per substrate treatment, and the two treatments were compared by one way ANOVA for each cultivation outcome. Disc diffusion measurements were obtained in triplicate and are reported as mean  $\pm$  SD, with SEM additionally shown where included in the original data table. A three way fixed effects ANOVA was used for disc diffusion data, with extract type, concentration, and microorganism as factors and inhibition zone diameter as the response variable. Two way and three way interaction terms were included. Partial eta squared ( $\eta^2$ ) was used to describe effect size, and Tukey HSD comparisons were performed for the overall extract main effect. Statistical significance was set at  $p < 0.05$ . Optical density data from the broth dilution experiment were evaluated across the five tested concentrations. Percentage inhibition was calculated relative to the untreated growth control and the assay blank, and dose response curves were fitted by nonlinear regression where the response pattern supported estimation of an IC<sub>50</sub> value.

Percentage inhibition (%) =  $[(A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}} - A_{\text{blank}})] \times 100$ , where  $A_{\text{control}}$  is the absorbance of the untreated growth control,  $A_{\text{sample}}$  is the absorbance at the tested extract concentration, and  $A_{\text{blank}}$  is the corresponding assay blank.

Dose response relationships were modelled with log transformed concentration where the observed inhibition pattern was suitable for nonlinear regression. IC<sub>50</sub> was defined as the concentration associated with 50% inhibition of measured microbial growth. MIC and MBC/MFC findings were summarized from the broth and subculture observations, and MBC/MIC or MFC/MIC ratios were used to classify the relationship between growth inhibition and killing. Results are reported with the statistical precision supported by the underlying triplicate measurements.

## 2.12 Preliminary Phytochemical Screening

Methanolic, ethanolic, and aqueous extracts were reconstituted in their corresponding solvents and screened qualitatively for major classes of secondary metabolites using standard colour, precipitation, and frothing reactions.

### 2.12.1 Qualitative Test Reactions

Alkaloids were assessed with Mayer and Dragendorff reagents; flavonoids by the alkaline reagent test; tannins with ferric chloride; saponins by the frothing test; cardiac glycosides by the Keller Killiani reaction; coumarins following sodium hydroxide treatment and UV examination; and phytosterols with the Salkowski reaction. Additional reactions were used for volatile oils, sterols, phlobatannins, and phenols according to the laboratory screening scheme. Positive and negative responses were recorded as detected (+) or not detected (–), and all extracts were tested under comparable conditions.

### **2.13 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis**

GC MS profiling was performed on the methanolic extract because it produced the highest overall antimicrobial activity in the disc diffusion experiment. The concentrated extract was reconstituted in analytical grade methanol and passed through a 0.22  $\mu\text{m}$  syringe filter. Analysis was carried out on an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass selective detector using an HP 5MS capillary column (30 m  $\times$  0.25 mm internal diameter, 0.25  $\mu\text{m}$  film thickness). Helium was used as the carrier gas at 1.0 mL/min. A 1  $\mu\text{L}$  aliquot was injected at 250°C with a 10:1 split ratio. The oven was held at 60°C for 2 min, increased at 10°C/min to 280°C, and held for 10 min. Electron ionization was performed at 70 eV over  $m/z$  50 to 550, with ion source and quadrupole temperatures of 230°C and 150°C, respectively. Tentative assignments were made by comparison with the NIST mass spectral library using a spectral similarity threshold of at least 90%; retention behaviour and characteristic fragmentation were considered where available. The chromatographic values reported in Table 12 represent relative peak intensity normalized to the base peak, which was assigned 100%. These assignments were used to define the chemical profile and to inform selection of candidate structures for subsequent computational assessment.

### **2.14 Physicochemical, Medicinal Chemistry and Drug-Likeness Analysis**

Candidate ligand structures selected after GC MS profiling were evaluated using DataWarrior version 4.6, SwissADME, and ADMETlab 2.0 (Sander et al., 2015; Daina et al., 2017; Xiong et al., 2021). Ligands were tracked consistently by their computational identifiers throughout the in silico workflow. The evaluated descriptors included molecular weight, hydrogen bond donors and acceptors, MLogP and WLogP, molar refractivity, rotatable bonds, heavy atom count, and topological polar surface area. Medicinal chemistry screening included Lipinski, Veber, Ghose, Egan, and Muegge criteria, PAINS and Brenk alerts, lead likeness, predicted bioavailability score, and synthetic accessibility. The complete descriptor profile was considered during prioritization for ADMET, docking, DFT, and MD analyses.

### **2.15 ADMET Analysis**

The selected computational ligands were evaluated for absorption, distribution, metabolism, excretion, and toxicity using ADMETlab 2.0, SwissADME, DataWarrior, admetSAR, and pkCSM (Cheng et al., 2012; Sander et al., 2015; Daina et al., 2017; Xiong et al., 2021). Predicted pharmacokinetic endpoints included human intestinal or gastrointestinal absorption, blood brain barrier permeability, water solubility, P glycoprotein substrate status, skin permeability, and cytochrome P450 interactions where available. Toxicological screening included AMES mutagenicity, hepatotoxicity, tumorigenicity, reproductive toxicity, and irritant potential according to the outputs supported by each platform. These predictions were integrated with physicochemical and medicinal chemistry descriptors to prioritize ligands for structure based analysis.

### **2.16 Protein Retrieval and Preparation**

The crystal structure of the 24 kDa N terminal ATPase domain of *Staphylococcus aureus* DNA gyrase B in complex with novobiocin was retrieved from the Protein Data Bank as PDB 4URO. The structure was determined by X ray diffraction at 2.59 Å resolution (Lu et al., 2014; Berman et al., 2000). Chain A was retained for receptor preparation. Crystallographic water molecules and non essential heteroatoms were removed, the co crystallized ligand was separated from the receptor, hydrogen atoms were added, and structural integrity was inspected for missing atoms, alternate conformations, side chain geometry, and stereochemistry. The receptor was energy minimized with Swiss PDB Viewer version 4.10 and converted to PDBQT format with AutoDock Tools version 1.5.7.

## 2.17 Molecular Docking Analysis

Molecular docking was used to evaluate the selected ligand set within the ATP binding region of *S. aureus* GyrB. AutoDock 4.2 and AutoDock Tools were used for ligand and receptor preparation and docking calculations. Polar hydrogen atoms and Gasteiger partial charges were assigned before docking. The receptor was held rigid and ligand torsions were allowed to rotate. The Lamarckian Genetic Algorithm was applied with 10 independent runs per ligand, a maximum of 2,500,000 energy evaluations, and 27,000 generations. The search grid was centred at  $x = 3.031$  Å,  $y = 35.614$  Å, and  $z = 14.419$  Å with  $60 \times 60 \times 60$  grid points at 0.375 Å spacing. Novobiocin was docked as the reference ligand. Poses were ranked by predicted binding energy, and the lowest energy representative pose was examined for hydrogen bonding, hydrophobic,  $\pi$  mediated, and other noncovalent contacts within the binding region.

## 2.18 Density Functional Theory (DFT) and Quantum-Chemical Analysis

Density functional theory calculations were performed with Gaussian 16 to characterize the electronic properties of four ligands prioritized from the combined medicinal chemistry, ADMET, and docking analyses (Frisch et al., 2016). Molecular geometries were optimized without constraints using the B3LYP hybrid functional and the 6-31G(d,p) basis set (Becke, 1993; Lee et al., 1988). Frequency calculations were used to confirm optimized minima by the absence of imaginary frequencies. HOMO and LUMO energies, the HOMO–LUMO energy gap, dipole moment, molecular electrostatic potential, ionization potential, electron affinity, electronegativity, chemical potential, hardness, softness, and electrophilicity were calculated from the optimized structures. The conceptual DFT descriptors were obtained using the relationships presented below (Parr and Pearson, 1983; Geerlings et al., 2003; Parr et al., 1999).

$$\Delta E = E_{\text{lumo}} - E_{\text{homo}}$$

The ionization potential (IP) and electron affinity (EA) were estimated from the frontier molecular orbital energies using the orbital-energy approximation:

$$\text{IP} = -E_{\text{homo}}$$

$$\text{EA} = -E_{\text{lumo}}$$

The global electronegativity ( $\chi$ ), chemical potential ( $\mu$ ), chemical hardness ( $\eta$ ), and global softness ( $S$ ) were calculated using the following relationships (Parr and Pearson, 1983; Geerlings et al., 2003):

$$\chi = (\text{IP} + \text{EA}) / 2$$

$$\mu = -\chi = -(\text{IP} + \text{EA}) / 2$$

$$\eta = (\text{IP} - \text{EA}) / 2$$

$$S = 1 / (2\eta)$$

The global electrophilicity index ( $\omega$ ) was calculated according to Parr et al. (1999):

$$\omega = \mu^2 / (2\eta)$$

Molecular electrostatic potential (MEP) analysis was performed using the optimized molecular geometries to characterize the spatial distribution of electrostatic potential across the molecular surfaces. The MEP maps were used to identify regions of relatively negative and positive electrostatic potential and their potential relevance to intermolecular interactions. The dipole moment of each optimized compound was calculated in Debye (D) as an additional descriptor of molecular polarity and charge distribution. The calculated DFT descriptors, including HOMO energy, LUMO energy, HOMO–LUMO gap, ionization potential, electron affinity, electronegativity, chemical potential, chemical hardness, chemical softness, electrophilicity index, dipole moment, and MEP distribution, were compared with the molecular docking results to examine the relationship between the electronic properties of the selected compounds and their predicted interactions with DNA gyrase B.

## 2.19 Molecular Dynamics Simulation

Molecular dynamics simulations were performed with AmberTools23 for GyrB complexes of CID 5281515, CID 91457, CID 3084954, and CID 6432585, with the novobiocin complex included as the reference system (Case et al., 2023). Ligand parameters were generated with Antechamber using the General AMBER Force Field, and the protein was described with ff12SB. Each complex was solvated in an explicit water octahedral box and neutralized with sodium ions. Systems were minimized in two stages. The first stage used 5,000 steepest descent steps followed by 5,000 conjugate gradient steps with solute restraints; the second used 2,500 steepest descent and 2,500 conjugate gradient steps after release of restraints. Systems were heated gradually from 0 to 300 K over 100,000 steps using Langevin temperature control and then equilibrated. Production simulations were conducted for 200 ns at 300 K and 1 atm using a 2 fs integration step, the SHAKE algorithm for bonds involving hydrogen, and the Berendsen barostat for pressure control. Trajectories were processed with CPPTRAJ (Roe and Cheatham, 2013). Protein backbone RMSD, residue RMSF, radius of gyration, and solvent accessible surface area were calculated and compared across the four ligand complexes and novobiocin reference.

### 3. Results and Discussion

The experimental and computational results followed the sequence of the study design. Substrate supplementation first established a more productive source of fungal biomass. Extraction then showed that crude recovery depended on solvent polarity, while antimicrobial assays demonstrated that biological activity depended on extract, concentration, and target microorganism. Phytochemical screening and GC MS profiling characterized the chemical diversity of the active material. The computational analyses subsequently evaluated the molecular and pharmacokinetic properties of selected ligands, their predicted interaction with GyrB, their electronic characteristics, and the dynamic behaviour of selected GyrB ligand complexes. This sequence provides a direct progression from fungal production and extract activity to chemical characterization and molecular prioritization.

#### 3.1 Mushroom Cultivation and Yield Performance

Cultivation performance differed substantially between the two sawdust formulations (Tables 1 and 2; Figure 3). Spawn run was shorter on the supplemented substrate, decreasing from  $46.33 \pm 1.15$  days to  $40.00 \pm 1.00$  days ( $F = 51.57$ ,  $p = 0.002$ ). Fruiting body initiation occurred at  $49.67 \pm 1.53$  days on unsupplemented sawdust and  $40.33 \pm 0.58$  days on supplemented sawdust ( $F = 98.00$ ,  $p < 0.001$ ). Time to harvest was similarly reduced from  $57.33 \pm 1.15$  to  $50.00 \pm 1.00$  days ( $F = 69.14$ ,  $p = 0.001$ ). The supplemented formulation therefore accelerated both vegetative colonization and reproductive development. These effects are consistent with the importance of accessible carbon and nitrogen sources in *T. versicolor* cultivation. Nguyen et al. (2021) showed that substrate composition significantly influenced mycelial growth and biological efficiency, while Woldemariam et al. (2024) also demonstrated marked productivity differences among supplemented agro industrial substrates.

The strongest response to supplementation was observed for fresh yield and biological efficiency. Mean fresh yield increased from  $102.60 \pm 3.58$  g to  $373.80 \pm 5.21$  g, corresponding to approximately 3.64 fold greater production on the supplemented substrate ( $F = 5520.35$ ,  $p < 0.001$ ). Biological efficiency increased from  $11.10 \pm 0.46\%$  to  $40.30 \pm 0.56\%$  ( $F = 4919.08$ ,  $p < 0.001$ ). The value obtained with the supplemented substrate was within the range reported for nutrient enriched *T. versicolor* cultivation systems and approached the productivity reported for supplemented sawdust based formulations (Jahedi et al., 2026; Woldemariam et al., 2024). Variation among studies can reflect strain characteristics, lignocellulosic composition, supplementation level, moisture, aeration, and fruiting conditions. Under the conditions used here, the supplemented sawdust formulation provided a faster cultivation cycle and a markedly larger biomass yield, which supplied the fungal material used in the subsequent extraction experiments.

Table 1. Raw cultivation performance of *T. versicolor* on unsupplemented and supplemented sawdust substrates ( $n = 3$  per treatment).

| Parameter                        | Unsupplemented<br>sawdust<br>Rep 1 | Rep 2 | Rep 3 | Supplemented<br>sawdust<br>Rep 1 | Rep 2 | Rep 3 |
|----------------------------------|------------------------------------|-------|-------|----------------------------------|-------|-------|
| Spawn runs<br>(days)             | 45                                 | 47    | 47    | 39                               | 40    | 41    |
| Fruiting<br>initiation<br>(days) | 48                                 | 50    | 51    | 40                               | 41    | 40    |
| Time to<br>harvest<br>(days)     | 56                                 | 58    | 58    | 49                               | 50    | 51    |
| Fresh yield<br>(g)               | 98.5                               | 104.2 | 105.1 | 368.4                            | 374.2 | 378.8 |
| Biological<br>efficiency<br>(%)  | 10.6                               | 11.2  | 11.5  | 39.7                             | 40.4  | 40.8  |

Table 2. Growth performance and biological efficiency of *T. versicolor* cultivated on unsupplemented and supplemented sawdust substrates. Values are mean  $\pm$  SD from three independent cultivation bags.

| Cultivation<br>Parameter                 | Unsupplemented<br>Sawdust (Mean<br>$\pm$ SD) | Supplemented<br>Sawdust<br>(Mean $\pm$ SD) | SE   | F-value | P-<br>value | Significance |
|------------------------------------------|----------------------------------------------|--------------------------------------------|------|---------|-------------|--------------|
| Initial<br>substrate<br>weight (g)       | 927 $\pm$ 0.00                               | 927 $\pm$ 0.00                             | —    | —       | —           | Standardized |
| Spawn run<br>duration<br>(days)          | 46.33 $\pm$ 1.15                             | 40.00 $\pm$ 1.00                           | 0.63 | 51.57   | 0.002       | **           |
| Fruiting<br>body<br>initiation<br>(days) | 49.67 $\pm$ 1.53                             | 40.33 $\pm$ 0.58                           | 0.67 | 98.00   | <0.001      | ***          |
| Time to<br>harvest<br>(days)             | 57.33 $\pm$ 1.15                             | 50.00 $\pm$ 1.00                           | 0.63 | 69.14   | 0.001       | **           |

| <b>Cultivation<br/>Parameter</b>     | <b>Unsupplemented<br/>Sawdust (Mean<br/>± SD)</b> | <b>Supplemented<br/>Sawdust<br/>(Mean ± SD)</b> | <b>SE</b> | <b>F-value</b> | <b>p-<br/>value</b> | <b>Significance</b> |
|--------------------------------------|---------------------------------------------------|-------------------------------------------------|-----------|----------------|---------------------|---------------------|
| Total fresh<br>mushroom<br>yield (g) | 102.60 ± 3.58                                     | 373.80 ± 5.21                                   | 2.47      | 5520.35        | <0.001              | ***                 |
| Biological<br>efficiency<br>(%)      | 11.10 ± 0.46                                      | 40.30 ± 0.56                                    | 0.24      | 4919.08        | <0.001              | ***                 |

Values are mean ± SD from three independent cultivation bags per treatment (n = 3). The standardized initial substrate mass was identical in both treatments and was not subjected to inferential testing. \*\* p < 0.01; \*\*\* p < 0.001.

### 3.2 Extraction Yield of *T. versicolor* Extracts

Extraction recovery differed modestly among the three solvents (Table 3). Water produced the highest crude yield at 2.69%, followed by ethanol at 2.61% and methanol at 2.19%. The order of crude recovery was therefore aqueous > ethanol > methanol. This pattern reflects the different solubility ranges of water and the two organic solvents and indicates that a larger mass of water soluble material was recovered from the mushroom matrix. Crude extraction yield describes total recovered material and is distinct from biological potency. This distinction was evident in the antimicrobial experiment, where the methanolic extract produced the highest overall inhibition despite having the lowest crude mass yield. The extraction results therefore establish an important transition between biomass production and bioactivity: solvent choice altered both the amount and the chemical composition of recovered material.

Table 3. Extraction yield of *T. versicolor* obtained with methanol, ethanol, and water.

| Extract  | Initial sample weight (g) | Dried extract weight (g) | Extraction yield (%) |
|----------|---------------------------|--------------------------|----------------------|
| Methanol | 150                       | 3.29                     | 2.19                 |
| Aqueous  | 150                       | 4.03                     | 2.69                 |
| Ethanol  | 150                       | 3.92                     | 2.61                 |

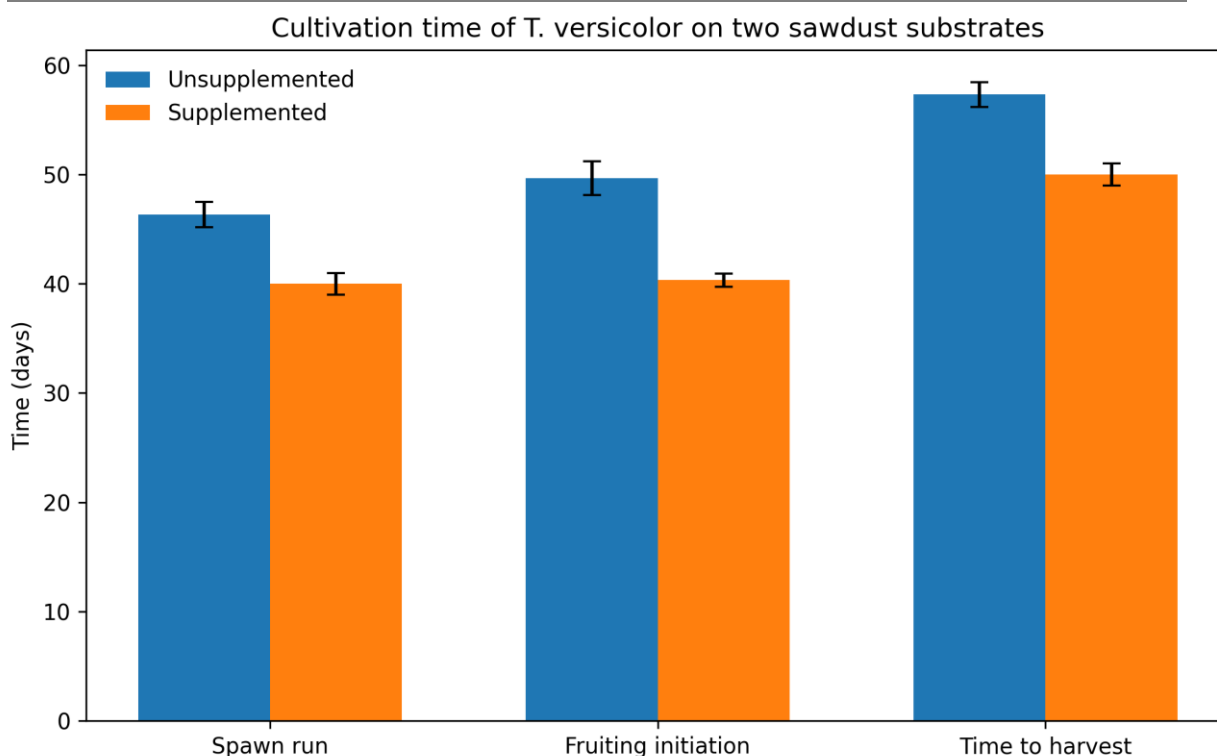


Figure 3. Cultivation time of *T. versicolor* on unsupplemented and supplemented sawdust substrates. Bars show mean  $\pm$  SD from three cultivation bags per treatment.

### 3.3 Disc Diffusion Antimicrobial Activity

Disc diffusion activity varied across extract, concentration, and microorganism combinations (Table 4; Figures 4 and 5). Averaged across the five concentrations and five test organisms, methanol produced the highest mean inhibition zone (13.72 mm), followed by ethanol (11.37 mm) and water (10.92 mm). The largest individual response was observed for the methanolic extract against *S. aureus* at 50 mg/mL ( $22.33 \pm 2.08$  mm). Across all extract and concentration combinations, *E. coli* showed the highest overall mean inhibition, followed by *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, and *C. albicans*. The concentration effect was positive overall, with mean inhibition increasing from 8.42 mm at 6.25 mg/mL to 14.20 mm at 100 mg/mL, although individual extract organism combinations were not uniformly monotonic. This heterogeneous response is expected for crude natural product mixtures in which the concentration of multiple constituents changes simultaneously. The three way ANOVA confirmed strong effects of extract type, concentration, and microorganism, as well as significant interaction terms (Table 5). Concentration showed a large partial eta squared value ( $\eta^2 = 0.883$ ), and the extract by microorganism and three way interactions were also large, indicating that the magnitude and direction of concentration effects depended strongly on both solvent and target organism. Tukey analysis of the overall extract main effect showed higher mean activity for methanol than for both ethanol and water, while ethanol also differed from water (Table 6). The broader qualitative phytochemical profile of methanol and the terpenoid rich GC MS profile provide a chemical context for its higher overall activity. Comparable solvent dependent antimicrobial activity has been reported for *T. versicolor* and other medicinal mushroom extracts (Bains and Chawla, 2020; Elkhateeb et al., 2022).

Table 4. Disc diffusion inhibition zones of *T. versicolor* extracts against the test microorganisms. Values are mean  $\pm$  SD; values in parentheses are SEM from triplicate measurements.

| Microorganisms                | Extract  | 6.25 mg/mL                              | 12.5 mg/mL                           | 25 mg/mL                                | 50 mg/mL                             | 100 mg/mL                            |
|-------------------------------|----------|-----------------------------------------|--------------------------------------|-----------------------------------------|--------------------------------------|--------------------------------------|
| <i>Candida albicans</i>       | Ethanol  | 8.00 $\pm$ 1.00<br>(0.58) <sup>b</sup>  | 7.67 $\pm$ 1.53 (0.88) <sup>b</sup>  | 9.67 $\pm$ 0.58 (0.33) <sup>a</sup>     | 2.67 $\pm$ 0.58 (0.33) <sup>c</sup>  | 5.33 $\pm$ 0.58 (0.33) <sup>c</sup>  |
|                               | Methanol | 2.67 $\pm$ 0.58<br>(0.33) <sup>c</sup>  | 8.33 $\pm$ 0.58 (0.33) <sup>b</sup>  | 10.67 $\pm$ 0.58 (0.33)<br><sup>a</sup> | 7.00 $\pm$ 1.00 (0.58) <sup>b</sup>  | 7.00 $\pm$ 1.00 (0.58) <sup>b</sup>  |
|                               | Aqueous  | 8.00 $\pm$ 2.00<br>(1.15) <sup>b</sup>  | 9.67 $\pm$ 1.53 (0.88) <sup>b</sup>  | 7.67 $\pm$ 1.53 (0.88)<br><sup>b</sup>  | 11.00 $\pm$ 1.00 (0.58) <sup>a</sup> | 17.00 $\pm$ 1.00 (0.58) <sup>a</sup> |
| <i>Staphylococcus aureus</i>  | Ethanol  | 8.00 $\pm$ 1.00<br>(0.58) <sup>c</sup>  | 8.33 $\pm$ 1.15 (0.67) <sup>c</sup>  | 10.33 $\pm$ 0.58 (0.33)<br><sup>b</sup> | 20.67 $\pm$ 0.58 (0.33) <sup>a</sup> | 20.67 $\pm$ 0.58 (0.33) <sup>a</sup> |
|                               | Methanol | 12.33 $\pm$ 1.15<br>(0.67) <sup>b</sup> | 16.67 $\pm$ 0.58 (0.33) <sup>b</sup> | 17.00 $\pm$ 2.00 (1.15)<br><sup>b</sup> | 22.33 $\pm$ 2.08 (1.20) <sup>a</sup> | 21.67 $\pm$ 1.53 (0.88) <sup>a</sup> |
|                               | Aqueous  | 0.00 $\pm$ 0.00<br>(0.00) <sup>c</sup>  | 3.00 $\pm$ 1.00 (0.58) <sup>c</sup>  | 10.33 $\pm$ 0.58 (0.33)<br><sup>b</sup> | 8.33 $\pm$ 0.58 (0.33) <sup>b</sup>  | 8.33 $\pm$ 0.58 (0.33) <sup>b</sup>  |
| <i>Escherichia coli</i>       | Ethanol  | 9.33 $\pm$ 0.58<br>(0.33) <sup>c</sup>  | 16.67 $\pm$ 1.15 (0.67) <sup>b</sup> | 13.00 $\pm$ 1.00 (0.58)<br><sup>b</sup> | 19.33 $\pm$ 0.58 (0.33) <sup>a</sup> | 20.00 $\pm$ 0.00 (0.00) <sup>a</sup> |
|                               | Methanol | 10.67 $\pm$ 0.58<br>(0.33) <sup>c</sup> | 13.33 $\pm$ 1.53 (0.88) <sup>b</sup> | 17.67 $\pm$ 0.58 (0.33)<br><sup>a</sup> | 14.67 $\pm$ 0.58 (0.33) <sup>b</sup> | 15.00 $\pm$ 1.00 (0.58) <sup>b</sup> |
|                               | Aqueous  | 13.00 $\pm$ 2.00<br>(1.15) <sup>b</sup> | 7.67 $\pm$ 1.15 (0.67) <sup>c</sup>  | 14.67 $\pm$ 0.58 (0.33)<br><sup>b</sup> | 12.67 $\pm$ 0.58 (0.33) <sup>b</sup> | 12.67 $\pm$ 0.58 (0.33) <sup>b</sup> |
| <i>Pseudomonas aeruginosa</i> | Ethanol  | 13.67 $\pm$ 1.15<br>(0.67) <sup>a</sup> | 10.00 $\pm$ 1.00 (0.58) <sup>b</sup> | 4.67 $\pm$ 0.58 (0.33) <sup>c</sup>     | 7.00 $\pm$ 1.00 (0.58) <sup>c</sup>  | 7.00 $\pm$ 1.00 (0.58) <sup>c</sup>  |
|                               | Methanol | 11.00 $\pm$ 1.00<br>(0.58) <sup>c</sup> | 12.67 $\pm$ 0.58 (0.33) <sup>b</sup> | 14.67 $\pm$ 0.58 (0.33)<br><sup>b</sup> | 18.67 $\pm$ 0.58 (0.33) <sup>a</sup> | 18.67 $\pm$ 0.58 (0.33) <sup>a</sup> |
|                               | Aqueous  | 9.67 $\pm$ 0.58<br>(0.33) <sup>c</sup>  | 13.33 $\pm$ 1.53 (0.88) <sup>b</sup> | 20.00 $\pm$ 1.00 (0.58)<br><sup>a</sup> | 7.67 $\pm$ 0.58 (0.33) <sup>c</sup>  | 7.67 $\pm$ 0.58 (0.33) <sup>c</sup>  |
| <i>Klebsiella pneumoniae</i>  | Ethanol  | 1.67 $\pm$ 0.58<br>(0.33) <sup>c</sup>  | 8.33 $\pm$ 1.15 (0.67) <sup>b</sup>  | 14.33 $\pm$ 0.58 (0.33)<br><sup>b</sup> | 19.00 $\pm$ 1.00 (0.58) <sup>a</sup> | 19.00 $\pm$ 1.00 (0.58) <sup>a</sup> |

| Microorganisms | Extract  | 6.25 mg/mL                          | 12.5 mg/mL                       | 25 mg/mL                            | 50 mg/mL                         | 100 mg/mL                        |
|----------------|----------|-------------------------------------|----------------------------------|-------------------------------------|----------------------------------|----------------------------------|
|                | Methanol | 12.33 ± 1.15<br>(0.67) <sup>b</sup> | 10.67 ± 1.15 (0.67) <sup>b</sup> | 17.33 ± 0.58 (0.33)<br><sub>a</sub> | 15.00 ± 1.00 (0.58) <sup>b</sup> | 15.00 ± 1.00 (0.58) <sup>b</sup> |
|                | Aqueous  | 6.00 ± 1.00<br>(0.58) <sup>c</sup>  | 8.67 ± 1.53 (0.88) <sup>b</sup>  | 20.00 ± 0.00 (0.00)<br><sub>a</sub> | 18.00 ± 1.00 (0.58) <sup>a</sup> | 18.00 ± 1.00 (0.58) <sup>a</sup> |

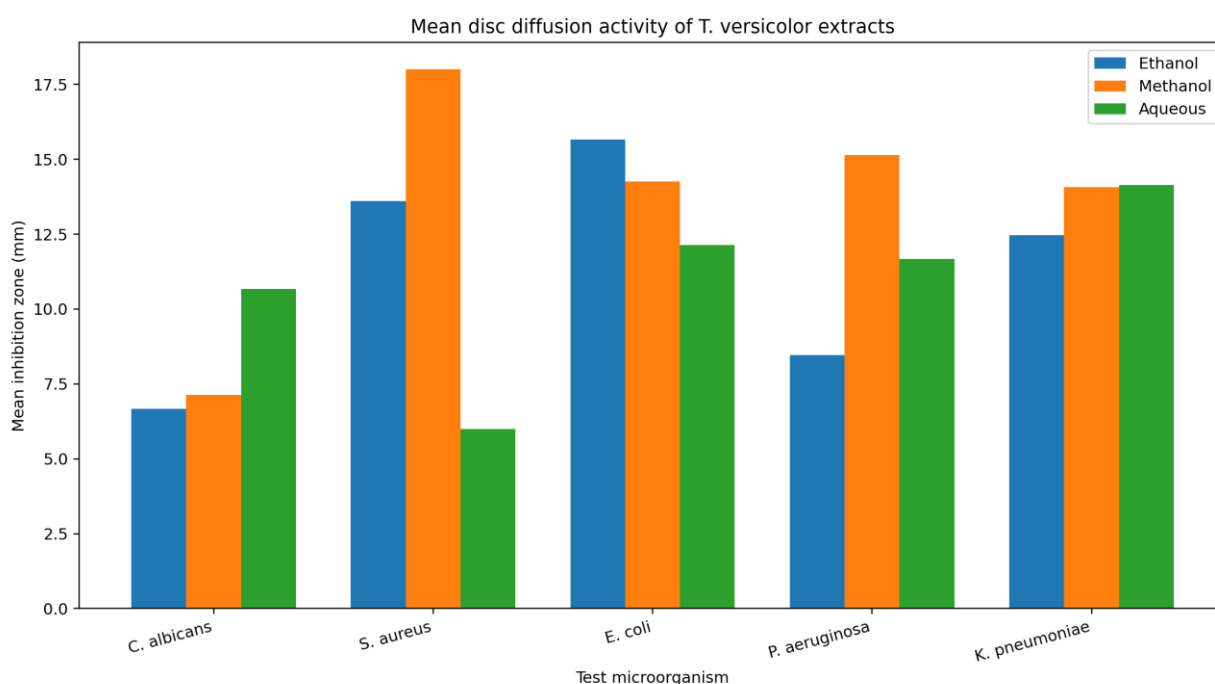


Figure 4. Comparative disc diffusion activity of aqueous, methanolic, and ethanolic *T. versicolor* extracts against the test microorganisms. Bars represent mean inhibition zone  $\pm$  SD from triplicate assays.

Table 5. Three way ANOVA for disc diffusion antimicrobial activity of *T. versicolor* extracts.

| Source of Variation                  | DF | Sum of Squares | Mean Square | F-value | p-value | $\eta^2$ | Significance |
|--------------------------------------|----|----------------|-------------|---------|---------|----------|--------------|
| Extract Type                         | 2  | 338.86         | 169.43      | 169.30  | <0.001  | 0.693    | ***          |
| Concentration                        | 4  | 1130.89        | 282.72      | 282.51  | <0.001  | 0.883    | ***          |
| Microorganism                        | 4  | 973.20         | 243.30      | 243.11  | <0.001  | 0.866    | ***          |
| Extract $\times$ Concentration       | 8  | 216.71         | 27.09       | 27.07   | <0.001  | 0.591    | ***          |
| Extract $\times$ Microorganism       | 8  | 1365.85        | 170.73      | 170.60  | <0.001  | 0.901    | ***          |
| Concentration $\times$ Microorganism | 16 | 868.55         | 54.28       | 54.24   | <0.001  | 0.853    | ***          |

| Source of Variation                                         | DF  | Sum of Squares | Mean Square | F-value | p-value | $\eta p^2$ | Significance |
|-------------------------------------------------------------|-----|----------------|-------------|---------|---------|------------|--------------|
| Extract $\times$<br>Concentration $\times$<br>Microorganism | 32  | 1326.87        | 41.46       | 41.43   | <0.001  | 0.898      | ***          |
| Error                                                       | 150 | 150.12         | 1.00        | —       | —       | —          | —            |
| Total                                                       | 224 | 6370.24        | —           | —       | —       | —          | —            |

Key: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ;  $\eta p^2$  = partial eta squared.

- Factors: extract (ethanol, methanol, aqueous), concentration (five levels), and microorganism (five levels).

*Table 6. Tukey HSD comparisons for the overall extract main effect in the disc diffusion experiment.*

| Comparison           | Mean Difference (mm) | q-value | p-value | Significance |
|----------------------|----------------------|---------|---------|--------------|
| Methanol vs. Aqueous | 2.80                 | 24.24   | <0.001  | ***          |
| Ethanol vs. Aqueous  | 0.45                 | 3.92    | 0.017   | *            |
| Methanol vs. Ethanol | 2.35                 | 20.32   | <0.001  | ***          |

Key: \*\*\*  $p < 0.001$ ; \*  $p < 0.05$ .

Relative contribution to overall disc diffusion activity

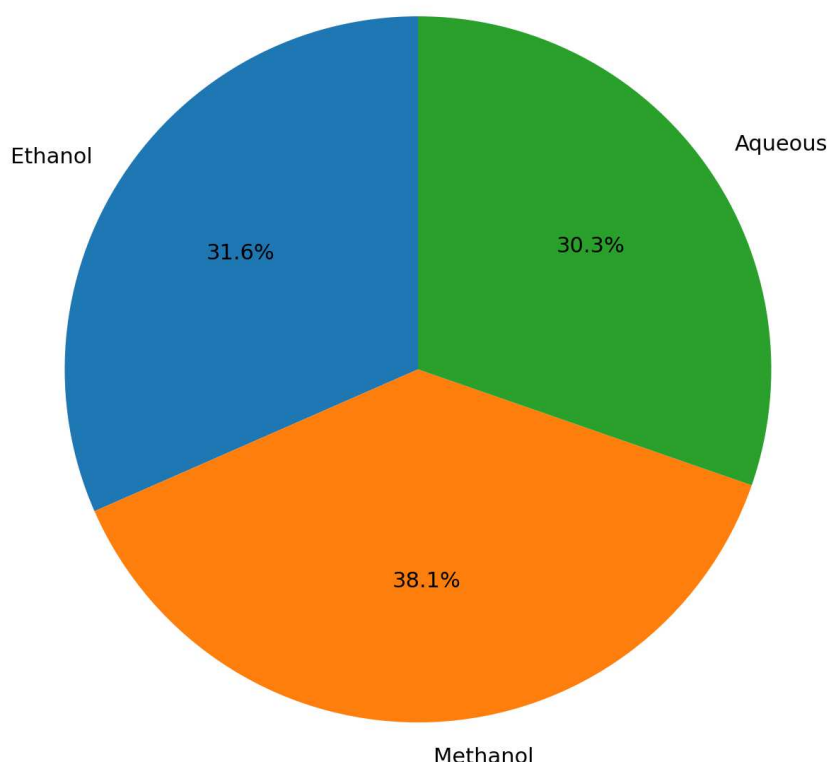


*Figure 5. Relative contribution of ethanol, methanol, and aqueous extracts to overall disc diffusion antimicrobial activity.*

### **3.4 Broth Dilution, MIC, MBC/MFC, and IC<sub>50</sub> Analysis**

The broth dilution experiment extended the disc diffusion findings by measuring growth directly in liquid culture (Tables 7 and 8). Optical density responses varied across extract, concentration, and microorganism, and the visible growth table showed that several extract organism combinations produced clear concentration dependent loss of growth. Interpretable MIC endpoints included 50 mg/mL for methanol against *C. albicans*, 50 mg/mL for ethanol and methanol against *S. aureus*, 12.5 mg/mL for ethanol against *E. coli*, 100 mg/mL for methanol against *E. coli*, and 25 mg/mL for ethanol against *K. pneumoniae*. Growth remained detectable through 100 mg/mL in several aqueous or less responsive combinations, placing their MIC above the tested range. Other combinations showed nonmonotonic visible growth patterns across the dilution series and were therefore characterized from the complete broth response rather than from a single concentration endpoint.

Dose response modelling provided a second quantitative description of broth activity. The fitted IC<sub>50</sub> values varied among extracts and organisms, and estimates could not be defined for combinations that did not generate an adequate sigmoidal response over the tested concentration range (Table 10). The methanolic extract showed the strongest overall activity across the integrated agar and broth experiments, while ethanol produced strong organism

specific responses and the aqueous extract remained active in selected combinations. These patterns agree with the qualitative phytochemical results, in which methanol and ethanol contained a broader range of detectable secondary metabolite classes than water. The combined disc diffusion, broth dilution, MIC, MBC/MFC, and dose response analyses therefore show that antimicrobial performance was governed by both solvent dependent chemical composition and organism specific susceptibility. The nonmonotonic responses observed in several crude extract combinations are compatible with concentration dependent interactions among multiple constituents and reinforce the value of subsequent chemical profiling and compound level analysis.

Table 7. Optical density pattern of *T. versicolor* extracts in the broth dilution experiment.

| <i>T. versicolor</i><br>Extract conc.<br>(mg/mL) | Ethanol |      |      |      |      |      |      | Methanol |      |      |      |      |      |      | Aqueous |      |      |      |      |      |      |
|--------------------------------------------------|---------|------|------|------|------|------|------|----------|------|------|------|------|------|------|---------|------|------|------|------|------|------|
|                                                  | 6.25    | 12.5 | 25   | 50   | 100  | AA   | TE   | 6.25     | 12.5 | 25   | 50   | 100  | AA   | TE   | 6.25    | 12.5 | 25   | 50   | 100  | AA   | TE   |
| <b>Microorganisms</b>                            |         |      |      |      |      |      |      |          |      |      |      |      |      |      |         |      |      |      |      |      |      |
| <i>C. albicans</i>                               | 0.72    | 1.21 | 1.4  | 1.9  | 2.03 | 0.14 | 2.09 | 1.09     | 1.05 | 0.79 | 0.63 | 0.75 | 0.14 | 2.09 | 0.94    | 1.09 | 1.08 | 1.36 | 1.93 | 0.12 | 2.09 |
| <i>S. aureus</i>                                 | 0.8     | 0.69 | 0.96 | 1.96 | 2.08 | 0.21 | 2.09 | 1.04     | 0.44 | 0.53 | 0.54 | 0.98 | 0.17 | 2.09 | 0.53    | 0.64 | 0.94 | 1.28 | 1.89 | 0.13 | 2.09 |
| <i>E. coli</i>                                   | 0.89    | 1.15 | 1.08 | 1.66 | 1.5  | 0.49 | 2.09 | 1.03     | 0.95 | 1.25 | 0.45 | 0.65 | 0.28 | 2.09 | 0.81    | 0.83 | 0.92 | 1.23 | 1.98 | 0.17 | 2.09 |
| <i>P. aeruginosa</i>                             | 0.91    | 1.14 | 1.5  | 1.97 | 2.07 | 1.62 | 2.09 | 1.05     | 1.24 | 1.46 | 1.83 | 1.89 | 1.08 | 2.09 | 0.48    | 1.09 | 1.28 | 1.54 | 1.98 | 0.29 | 2.09 |
| <i>K. pneumoniae</i>                             | 1.38    | 1.12 | 1.4  | 1.81 | 2.01 | 1.99 | 2.09 | 1.17     | 1.29 | 1.34 | 1.37 | 1.15 | 1.89 | 2.09 | 1.39    | 1.43 | 1.49 | 1.43 | 1.39 | 1.14 | 2.09 |

Key: AA = baseline absorbance; TE = untreated growth control.

Table 8. Visible growth pattern of *T. versicolor* extracts in the broth dilution experiment.

| <i>T. versicolor</i><br>Extract conc.<br>(mg/mL) | Ethanol |      |    |    |     | Methanol |      |    |    |     | Aqueous |      |    |    |     | Control |     |
|--------------------------------------------------|---------|------|----|----|-----|----------|------|----|----|-----|---------|------|----|----|-----|---------|-----|
|                                                  | 6.25    | 12.5 | 25 | 50 | 100 | 6.25     | 12.5 | 25 | 50 | 100 | 6.25    | 12.5 | 25 | 50 | 100 | (+)     | (-) |
| <b>Microorganisms</b>                            |         |      |    |    |     |          |      |    |    |     |         |      |    |    |     |         |     |
| <i>C. albicans</i>                               | +       | +    | +  | -  | +   | +        | +    | +  | -  | -   | +       | +    | +  | +  | +   | -       | +   |
| <i>S. aureus</i>                                 | +       | +    | +  | -  | -   | +        | +    | +  | -  | -   | +       | +    | +  | +  | +   | -       | +   |
| <i>E. coli</i>                                   | +       | -    | -  | -  | -   | +        | +    | +  | +  | -   | +       | +    | +  | +  | +   | -       | +   |
| <i>P. aeruginosa</i>                             | -       | -    | +  | +  | +   | +        | +    | +  | +  | +   | +       | +    | +  | +  | +   | -       | +   |
| <i>K. pneumoniae</i>                             | +       | +    | -  | -  | -   | +        | +    | -  | -  | +   | -       | +    | -  | +  | +   | -       | +   |

*Table 9. Main effect summary from the three way ANOVA of disc diffusion antimicrobial activity.*

| <b>Factor/Organism</b> | <b><math>\eta p^2</math></b> | <b>p-value</b> | <b>Significance</b> |
|------------------------|------------------------------|----------------|---------------------|
| Concentration          | 0.883                        | <0.001         | ***                 |
| Extract Type           | 0.693                        | <0.001         | ***                 |
| Microorganism          | 0.866                        | <0.001         | ***                 |

Key: effect sizes are partial eta squared ( $\eta p^2$ ); \*\*\*  $p < 0.001$ .

Table 10. Dose response IC50 estimates for *T. versicolor* extracts against the test microorganisms.

| Organism                      | Methanol (mg/mL) | Ethanol (mg/mL) | Aqueous (mg/mL) |
|-------------------------------|------------------|-----------------|-----------------|
| <i>Staphylococcus aureus</i>  | ~10–15           | ~1.20           | >1.5            |
| <i>Escherichia coli</i>       | ~0.75–0.85       | ~1.26           | >1.5            |
| <i>Candida albicans</i>       | ~0.80–0.90       | ~1.26           | >1.5            |
| <i>Pseudomonas aeruginosa</i> | Not defined      | Not defined     | Not defined     |
| <i>Klebsiella pneumoniae</i>  | Not defined      | Not defined     | Not defined     |

Key: IC50 values are model estimates from the broth dilution dose response analysis; “not defined” indicates that a 50% response could not be estimated from the tested concentration range.

### 3.5 Preliminary Phytochemical Screening

Qualitative phytochemical screening demonstrated clear solvent dependent differences in the classes detected in *T. versicolor* extracts (Table 11). Flavonoids and sterols were detected in all three extracts. Saponins, tannins, volatile oils, and phlobatannins were detected in methanol and ethanol but not in the aqueous extract. Cardiac glycosides were detected in ethanol and water, while coumarins and phytosterols were detected in methanol and water. Alkaloids were not detected in any extract. Methanol produced the broadest overall qualitative profile, which was consistent with its

higher mean antimicrobial activity. Solvent polarity and extraction conditions determine the recovery of chemically distinct metabolite classes, and previous studies have similarly reported substantial variation in *T. versicolor* phenolic and related constituents across extraction systems (Janjušević et al., 2017; Kıvrak et al., 2020). Bains and Chawla (2020) also reported phenolic and flavonoid constituents in methanolic *T. versicolor* preparations together with antimicrobial activity. The present qualitative results establish the major metabolite classes present in each crude extract and provide the chemical basis for selecting the methanolic extract for GC MS profiling. Because the tests are qualitative, compound identity and relative chromatographic intensity were determined in the subsequent GC MS experiment rather than inferred from colour reactions alone.

*Table 11. Preliminary phytochemical profile of T. versicolor extracts.*

| <b>Phytochemical</b> | <b>Ethanol</b> | <b>Methanol</b> | <b>Aqueous</b> |
|----------------------|----------------|-----------------|----------------|
| Flavonoids           | +              | +               | +              |
| Saponins             | +              | +               | –              |
| Tannins              | +              | +               | –              |
| Alkaloids            | –              | –               | –              |
| Cardiac glycosides   | +              | –               | +              |
| Volatile oils        | +              | +               | –              |
| Sterols              | +              | +               | +              |
| Phlobatannins        | +              | +               | –              |
| Coumarins            | –              | +               | +              |
| Phytosterols         | –              | +               | +              |
| Phenols              | +              | +               | –              |

Note: + = detected; – = not detected. The qualitative tests indicate phytochemical class presence and do not quantify individual compounds.

### 3.6 GC MS Profiling of the Methanolic Extract

GC MS profiling of the methanolic extract revealed 12 major volatile and semi volatile constituents (Table 12). The chromatogram was dominated by the signal tentatively assigned to 6-(3-methyl-3-cyclohexenyl)-2-methyl-2,6-heptadienol at 7.287 min, which was normalized to a relative peak intensity of 100%. Other prominent signals were assigned to a methanoazulene derivative (11.33%), a bicyclohexenone derivative (8.54%), (Z,Z)-

$\alpha$ -farnesene (5.62%), 4-ethylbenzoic acid, 2-methylphenyl ester (5.58%), caryophyllene (5.41%), and the bicyclo[7.2.0]undecene derivative (5.17%). Benzyl alcohol, butylphthalide, 1,4-cyclohexadiene, and the sulfur containing thiatricycloheptane derivative occurred at lower relative intensities. The profile was therefore dominated by sesquiterpenoid and oxygenated constituents with additional aromatic, cyclic, and sulfur containing signals. This chemical diversity is compatible with previous reports of terpenoids, sterols, aromatic compounds, phenolic constituents, and other secondary metabolites in *T. versicolor* (Habibi et al., 2015; Janjušević et al., 2017). The occurrence of caryophyllene and other terpenoid related signals is relevant to the antimicrobial results because terpenoid scaffolds are widely associated with membrane active and enzyme interacting properties. The GC MS assignments were treated as tentative library based identifications, and the selected chemical candidates were advanced to computational characterization to compare their physicochemical properties and potential interaction with GyrB.

Table 12. GC MS constituents tentatively assigned in the methanolic *T. versicolor* extract.

| Peak | Tentatively identified compound | Molecular formula               | Molecular weight (g/mol) | Retention time (min) | Relative peak intensity (%) |
|------|---------------------------------|---------------------------------|--------------------------|----------------------|-----------------------------|
| 1    | Caryophyllene                   | C <sub>15</sub> H <sub>24</sub> | 204                      | 5.708                | 5.41                        |

| Peak | Tentatively identified compound                                            | Molecular formula                              | Molecular weight (g/mol) | Retention time (min) | Relative peak intensity (%) |
|------|----------------------------------------------------------------------------|------------------------------------------------|--------------------------|----------------------|-----------------------------|
| 2    | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene                   | C <sub>15</sub> H <sub>24</sub>                | 204                      | 6.063                | 5.17                        |
| 3    | Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl) | C <sub>15</sub> H <sub>24</sub>                | 204                      | 6.572                | 1.13                        |
| 4    | 1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene              | C <sub>15</sub> H <sub>24</sub>                | 204                      | 6.100                | 11.33                       |
| 5    | (Z,Z)- $\alpha$ -Farnesene                                                 | C <sub>15</sub> H <sub>24</sub>                | 204                      | 7.030                | 5.62                        |
| 6    | 6-(3-Methyl-3-cyclohexenyl)-2-methyl-2,6-heptadienol                       | C <sub>15</sub> H <sub>24</sub> O              | 220                      | 7.287                | 100.00                      |
| 7    | Butylphthalide                                                             | C <sub>12</sub> H <sub>14</sub> O <sub>2</sub> | 190                      | 7.591                | 2.28                        |
| 8    | 4-Ethylbenzoic acid, 2-methylphenyl ester                                  | C <sub>16</sub> H <sub>16</sub> O <sub>2</sub> | 240                      | 8.060                | 5.58                        |
| 9    | Benzyl alcohol                                                             | C <sub>7</sub> H <sub>8</sub> O                | 108                      | 8.323                | 2.51                        |
| 10   | Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-                  | C <sub>10</sub> H <sub>14</sub> O              | 150                      | 9.324                | 8.54                        |
| 11   | 1,4-Cyclohexadiene                                                         | C <sub>6</sub> H <sub>8</sub>                  | 80                       | 9.416                | 1.27                        |
| 12   | 3-Thiatricyclo[3.1.1.0(2,4)]heptane                                        | C <sub>6</sub> H <sub>8</sub> S                | 112                      | 9.479                | 1.45                        |

Key: RT = retention time; MW = molecular weight. Relative peak intensity is normalized to the base peak at 100%.

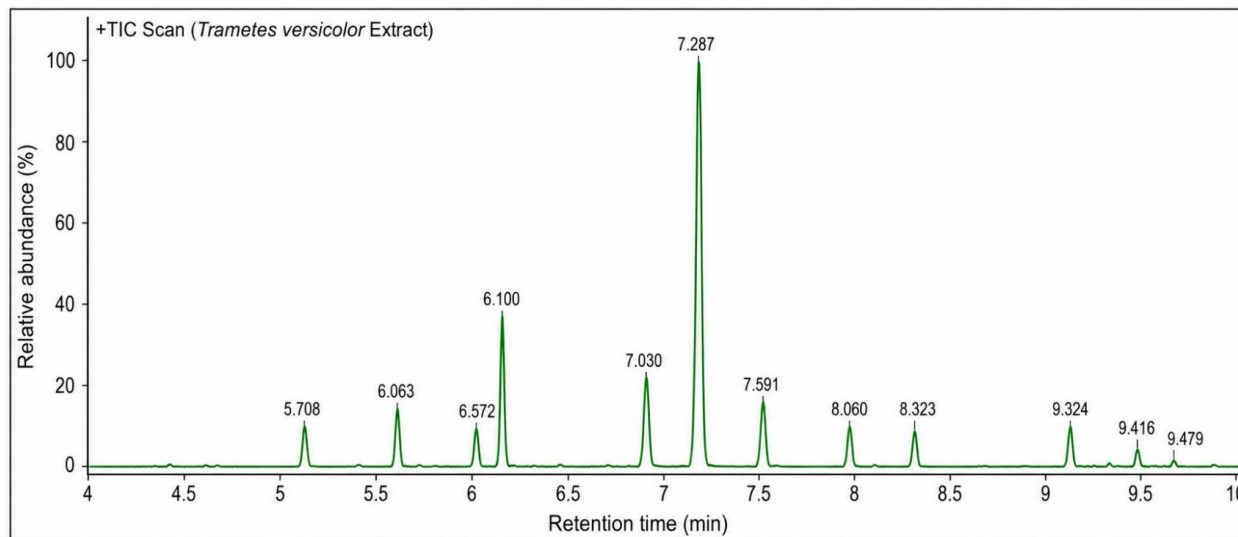


Figure 6. Total ion chromatogram of the methanolic *T. versicolor* extract. Peak labels correspond to the retention times summarized in Table 12.

### 3.7 Physicochemical Properties of the Computational Ligands

The computational ligand set showed substantial variation in molecular size, lipophilicity, hydrogen bonding capacity, polarity, and conformational flexibility (Table 13). Molecular weight values ranged from 108.14 to 240.30 g/mol in the computational descriptor set. Hydrogen bond acceptor and donor counts were low across the series, while MLogP and WLogP distinguished the more hydrophobic ligands from the oxygen containing candidates. TPSA ranged from 0 to 26.30 Å<sup>2</sup>, and rotatable bond counts ranged from 0 to 7. These features place the selected ligands within a compact, predominantly low polarity chemical space. All ligands remained below the conventional Lipinski molecular weight threshold and below the Veber thresholds for rotatable bonds and polar surface area (Lipinski et al., 2001; Veber et al., 2002). The variation in lipophilicity and polarity was carried forward into the drug likeness and pharmacokinetic analyses because these properties influence membrane partitioning, solubility, and predicted distribution. The physicochemical stage therefore provided the first computational filter between chemical profiling and target based docking.

Table 13. Physicochemical descriptors of the computational ligand set selected for in silico analysis.

| Computational ligand ID | MW<br>(g/mol) | HBA | HBD | MLogP | WLogP | Heavy<br>atoms | MR | NRB | TPSA |
|-------------------------|---------------|-----|-----|-------|-------|----------------|----|-----|------|
| CID 5281515             | 230.39        | 0   | 0   | 5.12  | 4.97  | 17             | 2  | 2   | 32.0 |
| CID 6432585             | 204.35        | 0   | 0   | 4.63  | 4.73  | 15             | 0  | 0   | 32.0 |
| CID 91457               | 190.32        | 0   | 0   | 4.37  | 4.34  | 14             | 1  | 1   | 32.0 |
| CID 12315492            | 204.35        | 0   | 0   | 5.65  | 4.42  | 15             | 0  | 0   | 32.0 |
| CID 5281516             | 232.40        | 0   | 0   | 5.33  | 5.98  | 17             | 7  | 7   | 32.0 |
| CID 5318042             | 220.35        | 1   | 1   | 3.46  | 4.01  | 16             | 5  | 5   | 32.0 |
| CID 119838              | 190.24        | 2   | 0   | 2.73  | 2.76  | 14             | 3  | 3   | 32.0 |
| CID 3084954             | 240.30        | 2   | 0   | 4.19  | 3.78  | 18             | 4  | 4   | 32.0 |
| CID 244                 | 108.14        | 1   | 1   | 1.54  | 1.03  | 8              | 1  | 1   | 32.0 |
| CID 6453554             | 150.22        | 1   | 0   | 2.20  | 2.18  | 11             | 1  | 1   | 32.0 |

Key: MW = molecular weight; HBA = hydrogen bond acceptors; HBD = hydrogen bond donors; MLogP = Moriguchi logP; WLogP = Wildman–Crippen logP; MR = molar refractivity; NRB = number of rotatable bonds; TPSA = topological polar surface area.

### 3.8 Drug Likeness and Medicinal Chemistry

Drug likeness and medicinal chemistry screening showed a heterogeneous but generally tractable profile across the ten computational ligands (Table 14). Six ligands had one recorded Lipinski violation and four had none. All ten satisfied the Veber criteria and none generated a PAINS alert. Ghose, Egan, and Muegge filters differentiated individual ligands further, while Brenk alerts identified structural motifs that may require attention during later optimization. These filters were interpreted collectively because each rule samples a different region of physicochemical and medicinal chemistry space (Daina et al., 2017).

The Ghose filter produced no violations for most ligands, whereas a smaller subset showed one or more departures from the preferred descriptor range. Egan violations were uncommon, and Muegge filtering produced zero to two violations across the set. The bioavailability score was 0.55 for all ten ligands. Synthetic accessibility values ranged from 1.00 to 4.57, indicating appreciable variation in predicted synthetic complexity. The absence of PAINS alerts across the entire set was favourable for subsequent target based analysis, while the Brenk and lead likeness outputs provided additional prioritization information. Taken together, the screening did not identify a single universal lead profile; instead, it separated ligands according to complementary strengths in size, polarity, flexibility, structural alerts, and synthetic accessibility.

The combined medicinal chemistry output identified several ligands with balanced profiles. Candidate 8 showed no Veber, Ghose, Egan, or Muegge violation in the screening results, while candidates 6 and 7 also performed favourably across several filters. Other ligands retained acceptable profiles despite isolated Lipinski, Muegge, Brenk, or lead likeness alerts. This ranking strategy preserved chemical diversity while reducing dependence on any single empirical rule. The selected ligands were therefore advanced to pharmacokinetic prediction and molecular docking on the basis of their integrated descriptor profiles.

The medicinal chemistry stage connected the chemical descriptor analysis to the subsequent ADMET and docking experiments. Compounds with complementary physicochemical properties were retained so that favourable GyrB binding could be evaluated alongside predicted absorption and distribution. This approach is particularly useful for natural product derived chemical space, where strict application of a single drug likeness rule can remove structurally distinctive scaffolds before their target interactions have been examined.

Table 14. Drug likeness and medicinal chemistry descriptors of the selected computational ligands.

| Computational<br>ligand ID | Lipinski<br>RO5 | Veber | Ghose | Egan | Muegge | Bioavailability<br>score | PAINS | Brenk | Lead-<br>likeness | Sy<br>acce |
|----------------------------|-----------------|-------|-------|------|--------|--------------------------|-------|-------|-------------------|------------|
| ID 5281515                 | 1               | 0     | 0     | 0    | 2      | 0.55                     | 0     | 1     | 2                 |            |
| ID 6432585                 | 1               | 0     | 0     | 0    | 1      | 0.55                     | 0     | 1     | 2                 |            |
| CID 91457                  | 1               | 0     | 0     | 0    | 2      | 0.55                     | 0     | 1     | 2                 |            |
| ID 12315492                | 1               | 0     | 0     | 0    | 1      | 0.55                     | 0     | 1     | 2                 |            |
| ID 5281516                 | 1               | 0     | 1     | 1    | 2      | 0.55                     | 0     | 2     | 2                 |            |
| ID 5318042                 | 0               | 0     | 0     | 0    | 1      | 0.55                     | 0     | 1     | 2                 |            |
| ID 119838                  | 0               | 0     | 0     | 0    | 1      | 0.55                     | 0     | 0     | 1                 |            |
| ID 3084954                 | 1               | 0     | 0     | 0    | 0      | 0.55                     | 0     | 1     | 2                 |            |
| CID 244                    | 0               | 0     | 3     | 0    | 2      | 0.55                     | 0     | 0     | 1                 |            |
| ID 6453554                 | 0               | 0     | 1     | 0    | 2      | 0.55                     | 0     | 0     | 1                 |            |

indicates no violation or alert; positive integers indicate the number of violations or alerts. Lower synthetic accessibility score indicates easier predicted synthesis.



### 3.9 Pharmacokinetic and ADMET Profile

Predicted pharmacokinetic properties differentiated the selected ligands according to intestinal absorption, blood brain barrier permeability, P glycoprotein substrate status, and skin permeability (Table 15). Seven ligands were predicted to have high human intestinal absorption and seven were predicted to cross the blood brain barrier, while CID 6432585, CID 12315492, and CID 5281516 showed lower predicted intestinal absorption and no BBB permeation. All ten were predicted to be non substrates of P glycoprotein. Log Kp values ranged from  $-6.18$  to  $-3.00$ , indicating a broad range of predicted skin permeability. The correspondence between high HIA and BBB permeability for several ligands is consistent with their low TPSA and relatively lipophilic physicochemical profiles. The BOILED Egg framework predicts gastrointestinal and brain permeation from polarity and lipophilicity and provides a useful early stage comparison of small molecule distribution behaviour (Daina and Zoete, 2016). In the integrated workflow, the pharmacokinetic results were considered together with medicinal chemistry and docking. CID 5281515 and CID 3084954 were notable because they combined high predicted HIA, predicted BBB permeability, absence of P glycoprotein substrate liability, and favourable GyrB docking scores. The pharmacokinetic analysis therefore narrowed the computational prioritization by adding predicted exposure properties to the target interaction ranking.

Table 15. Predicted pharmacokinetic properties of the selected computational ligands

| S/No | Computational ligand ID | BBB | HIA  | P-gp substrate | Log Kp (cm/s) |
|------|-------------------------|-----|------|----------------|---------------|
| 1    | CID 5281515             | +   | High | No             | $-4.91$       |
| 2    | CID 6432585             | –   | Low  | No             | $-4.44$       |
| 3    | CID 91457               | +   | High | No             | $-5.15$       |
| 4    | CID 12315492            | –   | Low  | No             | $-4.00$       |
| 5    | CID 5281516             | –   | Low  | No             | $-3.00$       |
| 6    | CID 5318042             | +   | High | No             | $-4.83$       |
| 7    | CID 119838              | +   | High | No             | $-5.47$       |
| 8    | CID 3084954             | +   | High | No             | $-4.65$       |
| 9    | CID 244                 | +   | High | No             | $-6.18$       |
| 10   | CID 6453554             | +   | High | No             | $-5.93$       |

### 3.10 Molecular Docking against DNA Gyrase B

Molecular docking ranked the computational ligands within the ATP binding region of *S. aureus* GyrB. Novobiocin, the reference inhibitor, produced the most favourable score at  $-9.4$  kcal/mol. Among the selected ligands, CID 5281515 ranked first at  $-7.3$  kcal/mol, followed by CID 91457 and CID 3084954 at  $-7.1$  kcal/mol and CID 6432585 at  $-7.0$  kcal/mol. The remaining ligands showed scores from  $-6.7$  to  $-5.6$  kcal/mol. The interaction patterns included hydrophobic contacts,  $\pi$  mediated interactions, hydrogen bonds, and van der Waals contacts. CID 5281515 interacted with PHE204, TYR229, and ARG200, while CID 91457 contacted PHE204 and CID 3084954 interacted with VAL174, TYR141, and ILE148. GLU201 and ARG198 also appeared in the interaction networks of selected ligands and the novobiocin reference. These repeated contacts identify regions of the docking pocket that contribute to recognition of chemically distinct ligands. The stronger score of novobiocin was expected for an established GyrB inhibitor, while the leading natural product associated candidates provided a focused set for electronic and dynamic analysis.

Docking was used as a comparative ranking tool within a single receptor preparation and search protocol. Under these conditions, the difference between CID 5281515 and the other leading ligands was modest, which supported progression of several candidates rather than selection from docking score alone. Interaction geometry also contributed to prioritization, particularly where hydrogen bonding or recurrent pocket contacts complemented hydrophobic accommodation. This interpretation is consistent with the established use of docking to generate structural hypotheses and prioritize ligands for higher level calculations (Bissantz et al., 2010; Pantzar and Poso, 2018). The four ligands advanced to DFT and MD therefore represented a combination of favourable docking, medicinal chemistry, and pharmacokinetic properties.

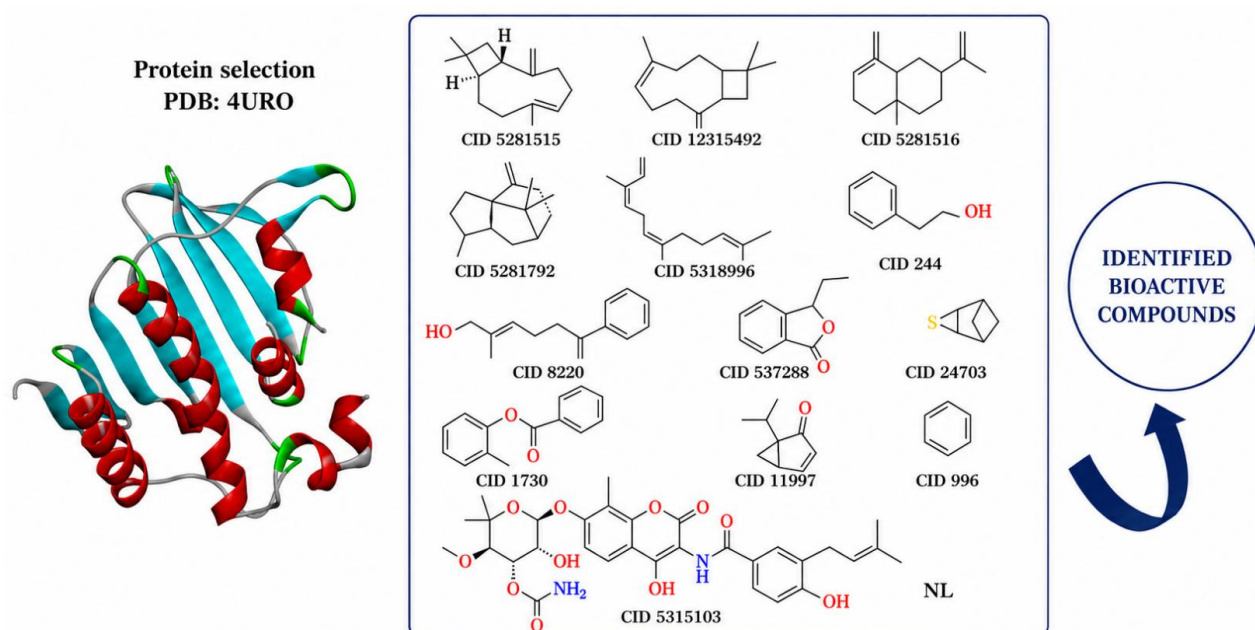


Figure 7A. Overview of the GyrB docking target and the computational ligand set used for molecular docking.

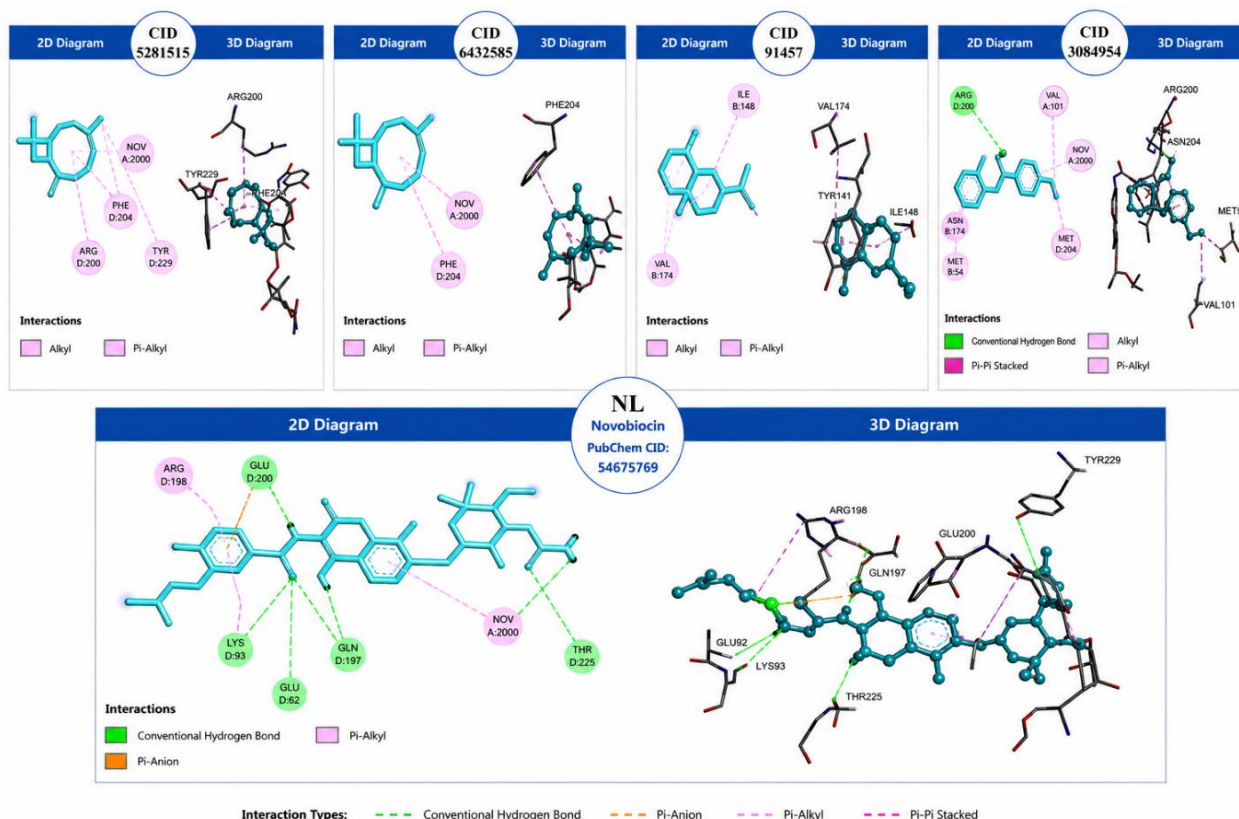


Figure 7B. Representative two dimensional and three dimensional binding interactions of selected computational ligands within the GyrB binding region.

### 3.11 Density Functional Theory and Quantum Chemical Analysis

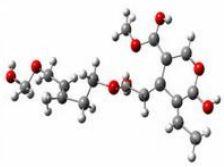
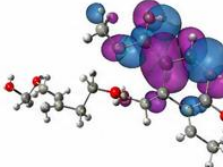
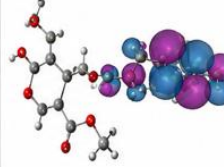
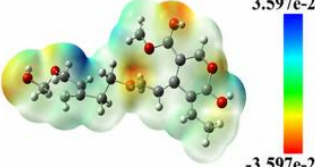
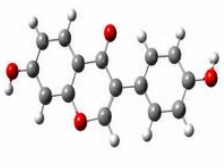
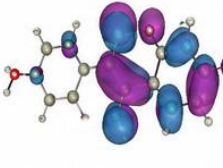
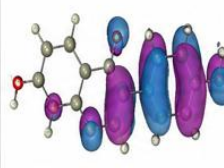
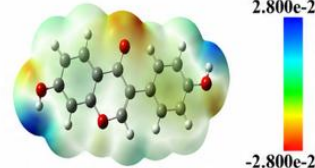
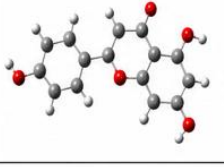
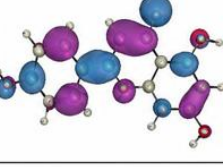
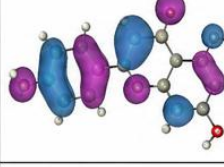
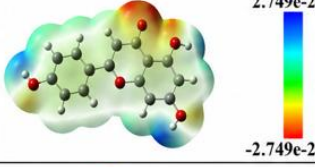
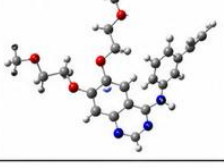
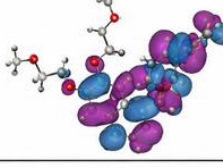
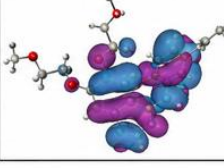
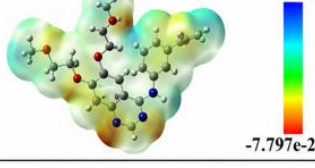
DFT analysis showed clear electronic differentiation among CID 5281515, CID 6432585, CID 91457, and CID 3084954 (Table 16; Figure 8). HOMO energies ranged from  $-0.3248$  to  $-0.2966$  au and LUMO energies from  $-0.1860$  to  $-0.0815$  au. The corresponding HOMO LUMO gaps ranged from 3.322 to 6.479 eV. CID 6432585 had the smallest gap, lowest hardness, and highest softness, identifying it as the most electronically responsive molecule in the selected set. CID 3084954 had the largest gap, highest hardness, lowest softness, and the highest dipole moment, indicating a more electronically stable but more strongly polarized structure. CID 91457 showed the highest electrophilicity, while CID 5281515 occupied an intermediate position across several descriptors. These differences provide an electronic context for the chemically distinct docking poses.

The DFT ranking did not mirror the docking ranking directly. CID 6432585 had the smallest energy gap but did not produce the most favourable docking score, whereas CID 5281515 achieved the best natural ligand docking score despite intermediate hardness, softness, and electrophilicity. CID 3084954 combined the largest energy gap with a docking score comparable to CID 91457. The dipole moment followed another pattern, with CID 3084954

reaching 3.1296 D while the other three ligands remained below 0.60 D. These contrasts show that GyrB recognition depended on the combined influence of electronic distribution, three dimensional shape, hydrophobic complementarity, and the specific orientation of interaction sites within the ATP binding pocket rather than on a single electronic descriptor.

Electrophilicity further distinguished the ligands, with CID 91457 showing the highest value at 13.01 eV, followed by CID 6432585 and CID 5281515, while CID 3084954 had a markedly lower value. The combined DFT and docking results therefore identified different electronic routes to favourable receptor accommodation. CID 5281515 combined the strongest docking score with an intermediate electronic profile; CID 91457 combined favourable docking with relatively high electrophilicity and softness; CID 6432585 showed the greatest electronic responsiveness; and CID 3084954 combined high polarity and electronic hardness with favourable docking. This diversity justified dynamic evaluation of the four complexes rather than assuming that one quantum chemical descriptor would predict complex behaviour.

The DFT stage refined the computational prioritization by adding electronic structure to the physicochemical, ADMET, and docking results. CID 5281515 remained the leading candidate because its favourable docking was supported by a balanced electronic profile, while CID 91457, CID 6432585, and CID 3084954 represented contrasting electronic chemotypes. These four ligands were therefore taken forward to 200 ns MD simulations to determine how the static docking poses behaved in a fully dynamic solvated protein environment.

| Name of ligands | Optimized Structure                                                                 | HOMO                                                                                | LUMO                                                                                 | MEP                                                                                   |
|-----------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| CID 5281515     |  |  |  |  |
| CID 6432585     |  |  |  |  |
| CID 91457       |  |  |  |  |
| CID 3084954     |  |  |  |  |

Note: HOMO = Highest Occupied Molecular Orbital; LUMO = Lowest Unoccupied Molecular Orbital; MEP = Molecular Electrostatic Potential.

Figure 8. Optimized structures, HOMO, LUMO, and molecular electrostatic potential maps of the four ligands selected for DFT analysis.

Table 16. DFT derived quantum chemical parameters of the four selected computational ligands.

| Computational<br>ligand ID | Dipole<br>Moment<br>(Debye) | HOMO<br>(au) | LUMO<br>(au) | Energy<br>Gap<br>$\Delta E$<br>(eV) | Energy<br>Gap<br>(au) | Hardness<br>$\eta$ (eV) | Softness<br>S (eV <sup>-1</sup> ) | Electrophilicity<br>$\omega$ (eV) |
|----------------------------|-----------------------------|--------------|--------------|-------------------------------------|-----------------------|-------------------------|-----------------------------------|-----------------------------------|
| CID 5281515                | 0.5919                      | -0.3248      | -0.1809      | 3.916                               | 0.1439                | 1.96                    | 0.255                             | 12.06                             |
| CID 6432585                | 0.4242                      | -0.2966      | -0.1745      | 3.322                               | 0.1221                | 1.66                    | 0.301                             | 12.38                             |
| CID 91457                  | 0.3850                      | -0.3192      | -0.1860      | 3.625                               | 0.1332                | 1.82                    | 0.275                             | 13.01                             |
| CID 3084954                | 3.1296                      | -0.3196      | -0.0815      | 6.479                               | 0.2381                | 3.24                    | 0.154                             | 4.60                              |

Key: HOMO = highest occupied molecular orbital; LUMO = lowest unoccupied molecular orbital;  $\Delta E$  = HOMO LUMO energy gap;  $\eta$  = hardness; S = softness;  $\omega$  = electrophilicity.

### 3.12 Molecular Dynamics Simulation and Complex Dynamics

The 200 ns MD simulations provided the final computational stage of the study by testing the time dependent behaviour of the four selected GyrB ligand complexes against the novobiocin reference (Figures 9 to 12). RMSD, RMSF, radius of gyration, and SASA were interpreted together because they describe different aspects of conformational deviation, local flexibility, global compactness, and solvent exposure. This multidescriptor analysis allowed the docking ranking to be examined under dynamic conditions rather than relying on the static lowest energy pose alone (Case et al., 2023; Roe and Cheatham, 2013).

The RMSD trajectories separated the complexes into distinct dynamic profiles. The novobiocin system generally remained within approximately 2.5 to 3.2 Å after early equilibration. CID 5281515 and CID 91457 showed moderate deviations with recurrent fluctuations, whereas CID 6432585 moved rapidly into a higher range of approximately 4.5 to 5.5 Å. CID 3084954 increased progressively and approached 5 to 6 Å during the later part of the trajectory. CID 5281515 therefore showed the clearest convergence between a favourable docking score and comparatively restrained global conformational deviation. CID 91457 displayed a second

relatively consistent profile, while CID 3084954 and CID 6432585 underwent larger conformational rearrangements.

RMSF analysis showed that novobiocin, CID 5281515, and CID 91457 generally produced lower residue level fluctuations than CID 6432585. Several regions of the CID 6432585 trajectory reached substantially larger fluctuations, indicating increased local mobility in that complex. The location of high RMSF regions is important because flexible loops and termini can move extensively without disrupting the core fold, whereas persistent motion near the binding region can alter ligand recognition. The overall RMSF pattern therefore complemented the RMSD result by identifying CID 6432585 as the most locally mobile system among the simulated complexes.

Radius of gyration remained broadly within 26 to 27.5 Å for all systems. The relatively narrow Rg range indicates that the global compactness of GyrB was maintained throughout the simulations despite the larger RMSD and RMSF values observed in some complexes. This distinction was especially informative for CID 6432585 and CID 3084954: both showed greater positional deviation, but neither produced a major expansion of the protein structure. The MD results therefore support conformational rearrangement within a preserved global fold rather than large scale loss of compactness.

SASA profiles provided a complementary measure of solvent exposure. CID 6432585 showed the highest solvent accessible surface values at several points, reaching approximately 28,000 to 29,000 Å<sup>2</sup>, while CID 91457 also showed elevated exposure during parts of the trajectory. CID 5281515 and CID 3084954 occupied intermediate ranges. The combination of elevated SASA, RMSD, and RMSF for CID 6432585 is consistent with a more mobile and solvent exposed conformational ensemble. In contrast, CID 5281515 combined comparatively moderate RMSD, lower residue fluctuations, stable Rg, and intermediate SASA, giving the most internally consistent dynamic profile among the selected ligands.

Comparison of docking and MD showed that static affinity ranking and dynamic behaviour were related but not identical. CID 5281515 retained the strongest combined profile because it ranked first among the selected ligands in docking and also showed comparatively restrained dynamics. CID 91457 combined favourable docking with moderate RMSD and RMSF. CID 3084954 showed a favourable docking score but greater late stage conformational deviation, while CID 6432585 showed the largest residue level mobility despite a relatively strong docking score. The MD stage therefore added discriminatory information that was not available from docking alone.

Integration of physicochemical screening, ADMET prediction, docking, DFT, and MD identified CID 5281515 as the leading computational candidate in this dataset, with CID 91457 as a second candidate of interest. CID 3084954 and CID 6432585 remained informative because their favourable docking was accompanied by distinct electronic and dynamic behaviour. The combined computational evidence provides a rational basis for subsequent biochemical testing of the prioritized ligands against GyrB and for direct antimicrobial testing of structurally confirmed isolated constituents from the active *T. versicolor* extract.

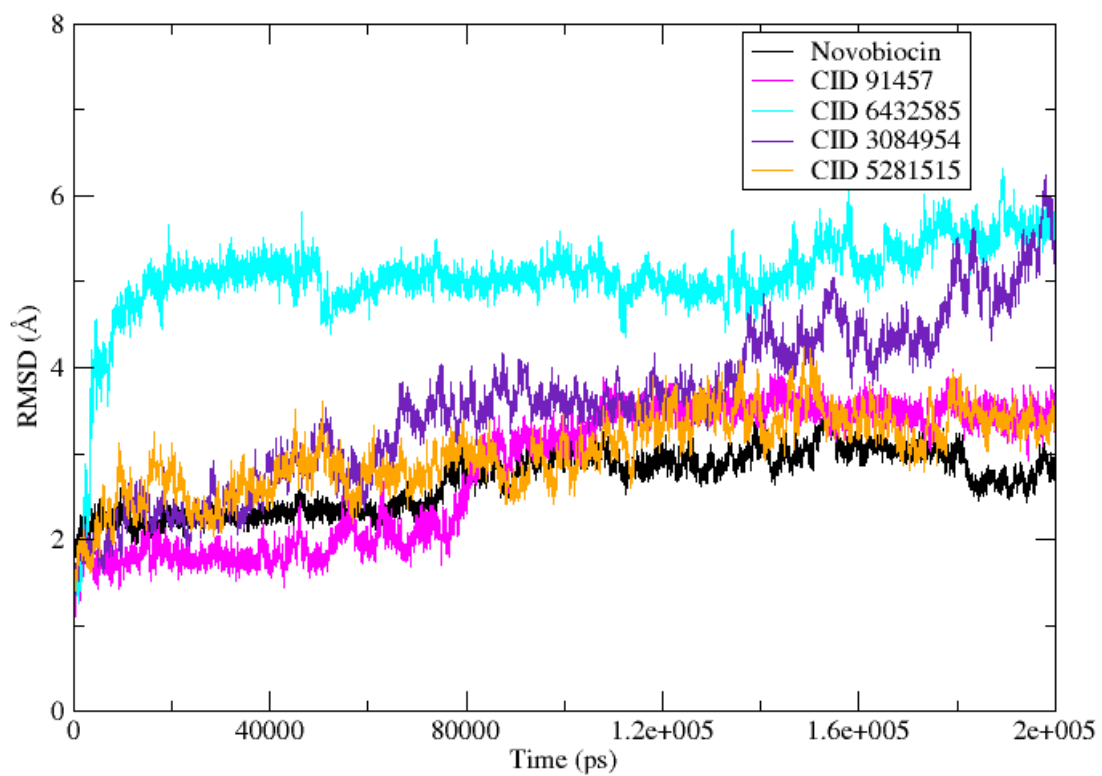


*Figure 9. Root mean square deviation profiles of the GyrB ligand complexes during the 200 ns molecular dynamics simulations.*

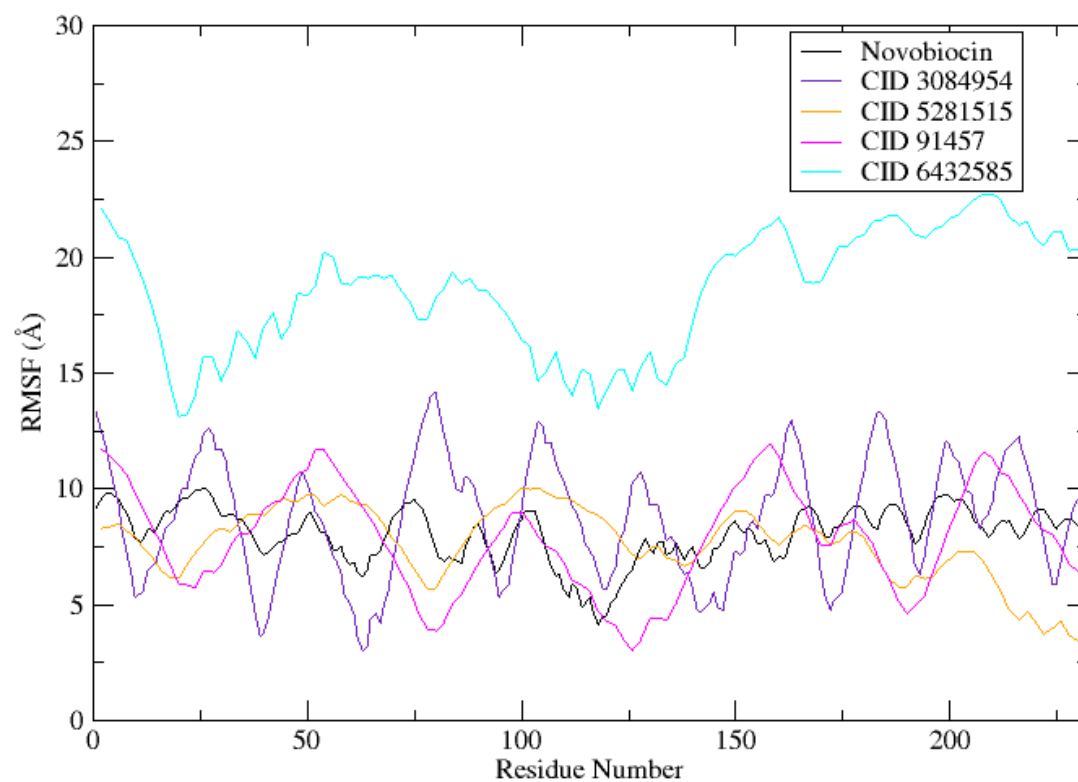


*Figure 10. Root mean square fluctuation profiles of the GyrB ligand complexes during the 200 ns molecular dynamics simulations.*

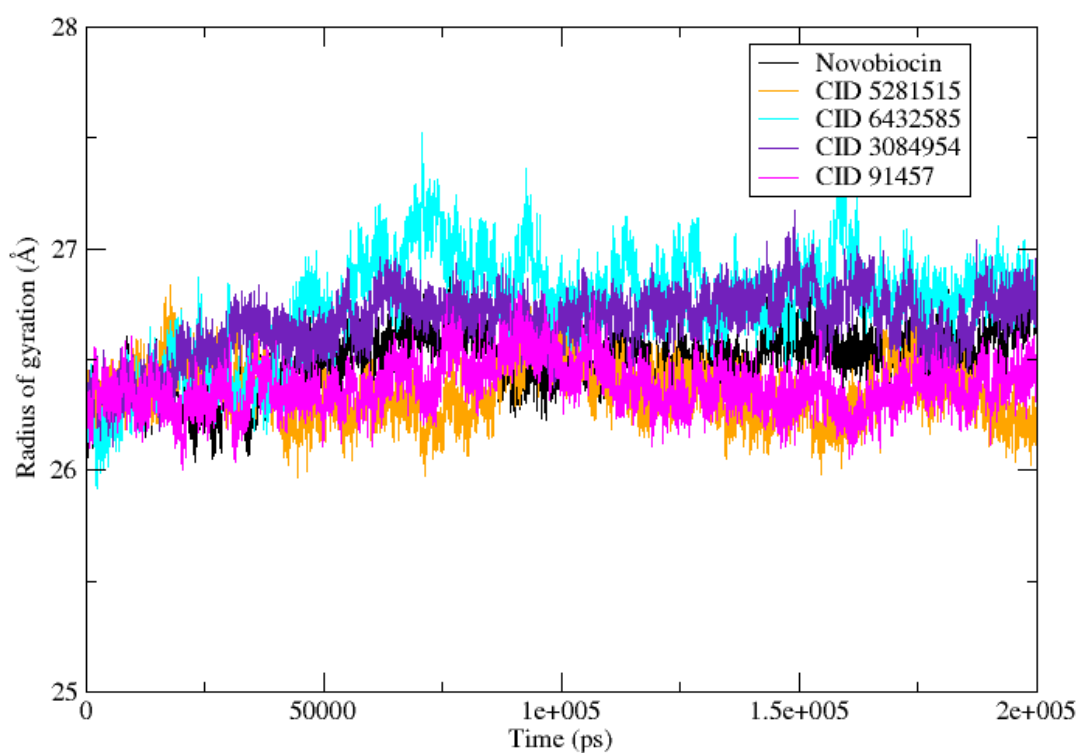


Figure 11. Radius of gyration profiles of the GyrB ligand complexes during the 200 ns molecular dynamics simulations.

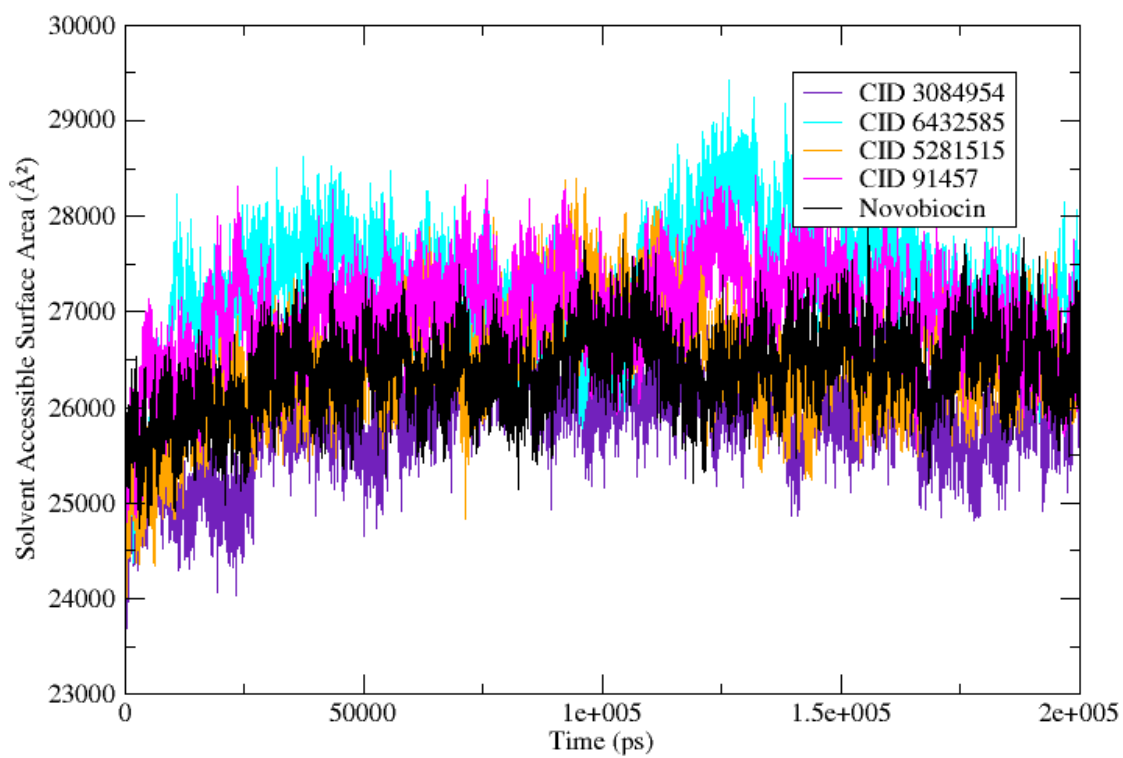


Figure 12. Solvent accessible surface area profiles of the GyrB ligand complexes during the 200 ns molecular dynamics simulations.

#### **4. Conclusions**

This study integrated cultivation, extraction, antimicrobial testing, chemical profiling, medicinal chemistry, ADMET prediction, molecular docking, DFT, and 200 ns molecular dynamics simulation to evaluate the antimicrobial potential of *T. versicolor*. Supplemented sawdust shortened the cultivation cycle and increased fresh yield and biological efficiency, providing a substantially more productive source of fungal biomass. Water recovered the greatest crude extract mass, while methanol produced the highest overall disc diffusion activity, demonstrating that extraction yield and antimicrobial potency were not directly proportional. The antimicrobial response depended strongly on extract, concentration, microorganism, and their interactions, and the broth dilution experiment further showed organism specific growth responses across the concentration series. Qualitative phytochemical screening demonstrated a broader metabolite profile in the methanolic and ethanolic extracts, and GC MS profiling of the methanolic extract revealed a terpenoid rich mixture of volatile and semi volatile constituents. The computational workflow differentiated the selected ligands by drug likeness, pharmacokinetic behaviour, predicted GyrB binding, electronic properties, and complex dynamics. CID 5281515 produced the most favourable docking score among the selected ligands and also showed the most consistent overall MD profile, while CID 91457 remained a strong secondary candidate. The convergence of extract level antimicrobial activity, chemical profiling, and target based computational prioritization identifies *T. versicolor* as a useful source for continued antimicrobial lead discovery and supports experimental evaluation of structurally confirmed constituents against bacterial GyrB.

Future work should prioritise isolation and structural confirmation of the major constituents of the active methanolic extract, followed by direct antimicrobial testing and purified GyrB assays for the leading candidates, including CID 5281515 and CID 91457. Extending the molecular dynamics analysis with independent replicates and binding free energy calculations, together with cytotoxicity, metabolic stability, and experimental pharmacokinetic evaluation, would further strengthen the case for these compounds as antimicrobial leads.

#### **Author Contributions**

Fanna Abba Ali Garra: Writing- Original Draft, Investigation, performed both experimental and computational phases, Adam Mustapha and Abubakar Shettima: Supervised the experimental phase, Mustafa Alhaji Isa: Visualization, software, and Review of Manuscript, Ahmed Abbator: Review and Design methodology, and Statistics.

#### **Acknowledgments**

I am thankful to Allah Almighty, who blessed me with a cooperative crew of people that proves helpful to polish my abilities and provide me with moral support as well. Special thanks to all the team who assist me to conduct a smooth research protocol.

## Consent

The explicit consent for publication was also obtained from participants. The researchers sought informed consent from all participants before recruitment for data collection.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

Data will be made available on request.

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