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Phylogenetic analysis and antibacterial potency of indigenous mushrooms in Arabuko Sokoke forest, Kenya

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ABSTRACT: Indigenous mushrooms are a rich source of bioactive compounds with potential pharmacological application. This study investigated the diversity, molecular identity and antibacterial activity of indigenous mushroom collected from Arabuko Sokoke forest, Kenya. Fifteen mushroom accessions were purposively sampled and subjected to DNA extraction using an optimized CTAB protocol. Molecular identification was carried out through ITS1 and ITS4 amplification followed by sequencing and phylogenetic analysis. Crude extracts of the mushrooms were screened for antibacterial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) using the disc diffusion method. Active extracts were further profiled using Gas Chromatography-Mass Spectrometry to identify bioactive metabolites. Molecular docking was performed to predict compound-protein interactions with bacterial targets. Sequencing and phylogenetic analysis identified 12 distinct taxa, including *Ganoderma mbrekobenum*, *Leiotrametes lactinea*, *Trametes polyzona* and *Auricularia polytricha*. Among the 15 extracts, only *G. mbrekobenum* (MU005) and *L. lactinea* (MU012) exhibited antibacterial activity. The two mushrooms produced zones of inhibition of 24.1-34.3mm, which were comparable to or greater than ampicillin controls. Gas Chromatography- Mass Spectrometry profiling of these two mushrooms revealed four bioactive compounds: 2,4-di-tert-butylphenol, 3-deoxy-D-mannoic lactone, 4-thiazolecarboxylic acid derivative, and 2-formylamino-3-methylbut-2-enoic acid (unique to *G. mbrekobenum*). Molecular docking indicated strong binding affinities of these compounds for key bacterial enzymes, including dihydrofolate reductase, enolpyruvyl transferase, phospholipase A2, and penicillin-binding protein 1, which supports the observed antibacterial activity. The findings highlight *G. mbrekobenum* and *L. lactinea* as promising sources of antibacterial agents, with activity comparable to standard antibiotics. The integration of molecular identification, chemical profiling, and in silico docking provides a robust framework for further discovery of natural products. Further studies should expand antibacterial screening to more pathogens, determine minimum inhibitory concentration and minimum bactericidal concentration values, and validate compound activity through bioassay-guided fractionation.

KEYWORDS: Antibacterial activity, *Ganoderma mbrekobenum*, GC-MS, *Leiotrametes lactinea*, Molecular docking.

INTRODUCTION

Fungi, especially mushrooms, are essential components of land ecosystems, acting as saprotrophs, mutualistic partners, and pathogens, thereby impacting all ecosystems. Globally, about 3000 mushroom species are considered edible, but only approximately 100 of them have been economically cultivated. Out of the 100, just over 10 have been produced on an industrial scale [1]. In most developing countries, poor identification and improper recording of medicinal mushroom species have caused discrepancies in their traditional medicinal use and mythological beliefs surrounding them [2]. Additionally, in these countries, the diversity and market value of mushroom species are not well understood; they rarely appear in global markets. While cultivated mushrooms are widely accepted by consumers worldwide, wild mushrooms are traditionally consumed due to their nutritional and medicinal qualities,

without further exploitation. Traditionally, these mushrooms are used in managing infectious diseases in certain ethnic groups [3]. The slow global response towards their impact in the pharmaceutical industry therefore affects their exploitation to full capacity.

Infectious diseases are among the main causes of death globally. This is driven by the emergence of new pathogens, the re-emergence of deadly infections, and the rise of antimicrobial-resistant strains. The result is a significant threat to public health. The rapid decline in antibiotic efficacy, coupled with increasing drug-resistant bacteria and the stagnation in new drug development, depicts an impending global public health crisis. Mushrooms, long recognized in the food industry for their nutritional benefits, need to get attention in the pharmaceutical industry. This can be in the form of dietary supplements, nutraceuticals, and as mycotherapy products. Medicinal mushrooms, since ancient times, have always been used as traditional medicine. They are always prepared as extracts, infusions, decoctions, and other therapeutic formulations [4].

Lately, scientific advancements have increasingly illuminated the medicinal potential of mushrooms. This establishes them as valuable reservoirs of bioactive compounds for drug discovery and development. *Ganoderma lucidium* (Reishi), known for its abundance of polysaccharides and triterpenoids, has been widely researched. The research focuses on the mushroom's antioxidant, antimicrobial, anticancer, immunomodulatory, anti-inflammatory, and antidiabetic properties [5]. Furthermore, an advanced review on *Fomitopsis* species emphasizes its wide range of secondary metabolites. The metabolites include: phenolic compounds, polysaccharides, and terpenoids. The metabolites have been discovered to possess immunomodulatory, anti-inflammatory, antimicrobial and antioxidant properties. This depicts their potential use in cancer treatment, cardiovascular wellness, and in the regulation of the immune system [6]. Likewise, a 2025 review on mushroom-derived bioactive compounds highlights their significant anticancer, antimicrobial, antidiabetic, and antioxidant properties. It further emphasizes the therapeutic relevance of the key compounds including: terpenoids, steroids, polyphenols, flavonoids, β-glucans, proteins, and peptides [7].

The de novo drug discovery and development process is currently the predominant method for creating new medications. The process requires significant time and financial investment, and it is experiencing high failure rates [8]. The overall process of getting a drug through initial discovery to market availability can take up to 20 years. In the United States of America, as a first-world country, this timeline captures the period after the U.S. Food and Drug Administration (FDA) approval, meaning it may actually take longer to get to the approval stages. The whole process incurs costs up to $2 billion, with only a 5% likelihood of completing clinical trials and achieving market entry. In contrast, drug repurposing has emerged as a faster and more efficient strategy for identifying new uses for already established drugs [9]. The process involves finding new applications for existing medications of compounds previously shelved. This study employs an experimental and analytical design that integrates a tripartite relationship between phylogenetic analysis, antibacterial screening, and pharmacological assays aimed at profiling the medicinal potential of mushroom crude extracts.

Traditionally, the World Health Organization (WHO) has placed limited emphasis on medicinal mushrooms. However, recent worldwide initiatives aimed at advancing Universal Health Coverage (UHC) have sparked a reevaluation. This is happening amidst healthcare expenses and constrained budgets in nations. With time it is globally realized that traditional pharmaceuticals are prohibitively expensive and difficult to access in most regions. Mushroom-based medicines on the other hand are more accessible, affordable and culturally accepted. This has therefore encouraged the WHO to incorporate traditional and complementary medicine into healthcare frameworks [10].

The process of isolating pure compounds from crude extracts demands a lot of input. Typically, the compounds or extracts go through bioassays informed by their documented traditional and ethnobotanical applications. The process focuses on how the naturally derived compounds influence biological systems. This clarifies their underlying mechanisms, mapping their interactions with particular molecular or cellular targets described through structure-activity relationships. In present-day drug discovery, researchers generate extensive libraries of purified candidate molecules. The libraries are then subjected to rapid evaluation, mainly against enzymes or cell-based models using high-throughput screening (HTS) approaches [11]. Contemporary drug discovery initiatives focus on developing drug candidates or hit compounds that can effectively engage with specific molecular targets [12]. These targets may include enzymes, proteins, various components of the human genome, DNA segments, and complex/simple units, all of which ensure the modulation of the body's functions to diseases.

According to Nawaz et al. [13], crude extracts are obtained using a variety of solvents ranging from polar to non-polar with relatively consistent methods of production. The chemical composition of the extracted constituents is anticipated based on solvent polarity as used during extraction or fractionation [14]. Non-polar solvents, such as n-hexane, are effectively used in isolating lipids, fatty acids, and terpenoids with low polarity. In contrast, solvents of

intermediate polarity, chloroform and ethyl acetate, are commonly used to extract alkaloids, phenolic compounds, and related metabolites. On the other hand, polar solvents, like water and methanol, are particularly effective in obtaining sugars, glycosidic alkaloids, flavonoids, and various highly oxygenated biomolecules [14].

Every organism is defined by its unique genetic makeup, but the observable traits, or phenotypes, arise from the expression of these genes, as in the case of secondary metabolites' synthesis [15]. Early identification of bioactive compounds of interest aids in their isolation. Therefore, analyzing metabolite profiles is critical in functional genomics as it facilitates the identification of new bioactive compounds with potential pharmacological applications [15]. The majority of clinically effective drugs derived from fungi exhibit some level of antibacterial activity, for instance, penicillins and cephalosporins. Their therapeutic uses, however, primarily lie outside medicinal applications. This study, therefore, is an exploratory screening investigation aimed at identifying indigenous mushroom species from the Arabuko Sokoke Forest that exhibit antibacterial activity. It precisely integrates molecular phylogenetic identification, in vitro antibacterial screening, GC-MS-based chemical profiling, and in silico molecular docking to give preliminary insights into the bioactive potential of selected mushroom extracts. By doing so, their therapeutic applications in the management of bacterial-caused diseases can be validated further.

MATERIALS AND METHODS

Study site

Samples were carefully collected from the natural Arabuko Sokoke forest in the coastal region of Kenya. The forest is inland between Malindi and Watamu at 3º 20' S and 39º 50'E, covering an area of 420 km^2. It makes part of the coastal lowlands inclusive of mangroves, tropical forests and savannas. This forest hosts diverse native vegetation that creates an ideal environment for mushroom growth. It therefore harbors a documented total of 32 indigenous mushrooms [16].

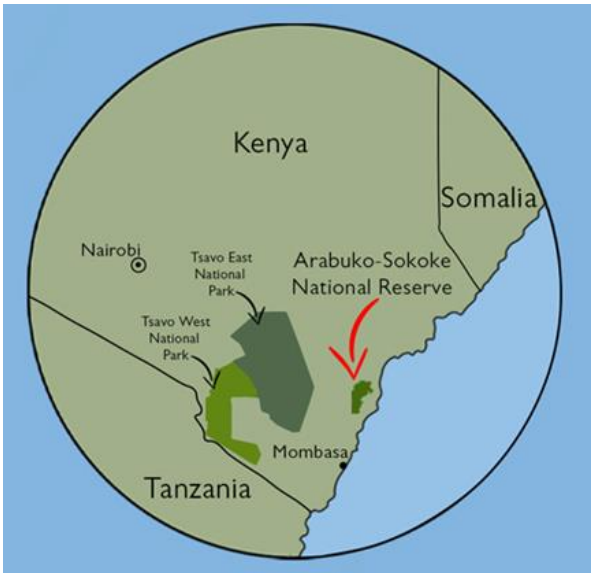


Figure 1. A Map showing the location of the Arabuko Sokoke forest in the coastal region of Kenya.

Sampling

Due to the dwindling diversity of mushrooms indigenous to the Arabuko Sokoke forest, Purposive sampling was employed, and 15 mushroom accessions were collected. This was done in replicates based on visible morphological diversity, availability during the sampling period, and ethnomycological relevance reported in the Arabuko Sokoke Forest. While the forest hosts a wider diversity of macrofungi, the selected accessions represent commonly encountered and ecologically distinct taxa. For each accession, replicate specimens were collected and processed independently to enhance reproducibility and reduce sampling bias [17; 18]. The samples were obtained during the rainy season, specifically between April and May 2024. The fresh fruit bodies were examined immediately, photographed in situ, and preserved in silica gel, awaiting molecular analysis [18].

DNA extraction

Using an optimized cetyltrimethylammonium bromide (CTAB) extraction protocol adapted from the method described by Carter-house et al. [19] and Riverside and Stajich [20], genomic DNA was isolated from the dried fruiting body of replicates of 15 samples representative of phenotypically unique clusters of mushrooms collected during a sampling exercise. This method has proven highly efficient for isolating DNA from diverse sample types. This is inclusive of herbarium-preserved materials, freshly collected field samples, and woody basidiomata rich in secondary metabolites and polysaccharides. The mushroom specimens, previously stored in silica gel, were freeze-dried and subsequently ground into a fine powder using a laboratory blender. A lysis buffer stock solution was freshly prepared by mixing 14.0 ml of SDS, 2.5 ml of NaCl, 10ml of EDTA, and 5ml of NaCl, then adjusting the final volume to 100ml using PCR-grade water. Approximately 0.5 g of the powdered sample was transferred into 1.5 ml Eppendorf tubes, after which 500µl of the lysis buffer was added, followed by 1 µl of proteinase K. The contents were gently mixed and incubated in a water bath at 65ºC for 35 minutes with occasional inversion to facilitate lysis. Following incubation, the mixture was homogenized and centrifuged at 10,000 x g for 10 minutes. The resulting

supernatant was carefully transferred to fresh tubes, and 625µl of a 4M potassium acetate (KAc) solution, equivalent to two-thirds of the final volume, was introduced. The mixture was gently agitated and placed on ice (0ºC) for 30 minutes to enhance protein precipitation. A subsequent centrifugation step at 10,000 x g for 10 minutes at 4ºC was performed to remove residual proteins. The clarified supernatant was transferred to new tubes, and two-thirds of the volume of pre-chilled isopropanol was added to facilitate DNA precipitation. Samples were incubated at -20ºC for a minimum of 16 hours, followed by centrifugation to pellet the genomic DNA. The DNA pellet was washed with 500µl of 70% ethanol, after which the tubes were inverted on absorbent paper and air-dried for approximately one hour. Finally, the DNA was dissolved in 100µl of PCR-grade water, gently tapped to ensure complete resuspension, and stored at -20ºC.

DNA quality evaluation

The evaluation of the quality of the extracted DNA was conducted following a modified approach outlined by the protocols described by Lucena-Aguilar et al. [21] and Revathy et al. [22]. Five primary parameters were considered in determining the quality of the extracted DNA: "Overall DNA integrity, visual coloration of the solution, purity ratio derived from spectral absorbance (A 260/280), final DNA concentration expressed as ng/µl of purified DNA, and efficiency of PCR amplification for the ITS region". Assessment of amplified DNA quality was carried out using 0.8% agarose gel electrophoresis prepared in 0.5x tris-borate-EDTA(TBE) buffer. The gel was stained with 2.5 µl of GelRed®, and a 1Kb lambda DNA ladder was used as a molecular size marker. A total of 3 µl of the DNA sample was combined with an equal volume of loading dye and carefully loaded into the wells. Electrophoresis was performed at 100V for 45 minutes to visualize DNA fragments.

Assessment of amplified DNA quality was carried out using "0.8% agarose gel electrophoresis prepared in 0.5X Tris-Borate-EDTA (TBE) buffer. The gel was stained with 2.5 µl of GelRed, and a 1 Kb Lambda DNA ladder was used as a molecular size marker. A total of 3 µl of the DNA sample was combined with an equal volume of loading dye and carefully loaded into the wells. Electrophoresis was performed at 100 V for 45 minutes to visualize the DNA fragments". The extracted DNA was categorized based on its molecular condition as:

Category 1: High-quality DNA with intact, high molecular weight bands and no visible degradation.

Category 2: Moderately degraded DNA with partial smearing but retaining a distinct high molecular weight band.

Category 3: Highly degraded DNA showing significant smearing and/or low molecular weight bands.

Additionally, the color of the DNA solution was classified into three groups: 1) clear, transparent, or whitish, 2) slightly yellowish yet still transparent, and 3) dark opaque, indicating possible contamination. DNA concentration and purity were determined using a Thermo Scientific NanoDrop 2000 spectrophotometer, measuring absorbance at A 260/280 to confirm its quality and suitability for downstream applications.

PCR amplification of extracted DNA

According to the outlined guidelines and as directed by (23)"Polymerase Chain Reaction (PCR) amplifications were carried out using a Techne TC plus thermocycler in combination with the Accupower® Taq PCR premix Kit. For each reaction, a total volume of 20µl was prepared, consisting of 2µl of the diluted DNA template (20 ng/µl), 0.8 µl of each primer, and 16.4 µl of Milli-Q water. The thermal cycling conditions were adapted from the manufacturer's protocol with minor modifications. An initial denaturation at 95ºC for 5 minutes, followed by 35 amplification cycles comprising denaturation at 95ºC for 30 seconds, annealing at 60ºC for 30 seconds, and extension at 72ºC for 1 minute. A final elongation step was performed at 72ºC for 10 minutes, after which the samples were cooled to 10ºC to terminate the reaction". The primers used for amplification were [24]:

ITS1(Forward): 5'-TCCGTAGGTGAACCTGCGG-3'

ITS4 (Reverse): 5'-TCCTCCGCTTATTGATATGC-3'

PCR amplicons were analyzed through electrophoresis on a 1.8% agarose gel prepared in a 0.5x tris-borate-EDTA (TBE) buffer, containing 2.5µl of GelRed® nucleic acid stain. A Thermos Scientific GeneRuler 100bp DNA ladder was used as a size reference. Amplicons were subsequently purified using the GeneJET PCR purification Kit, following the manufacturer's guidelines [25]. The concentration and purity of the purified PCR products were quantified using a Nanodrop 2000 spectrophotometer by measuring absorbance ratios at A 260/280 [26]. Finally, the purified DNA samples were sent to Macrogen Asia Pacific Pte. Ltd. (Singapore) for sequencing.

Data analyses

The sequences generated from this study, together with the obtained sequences that were retrieved from GenBank, were assembled. The sequences were then thoroughly validated for potential errors using the CLC

Workbench platform [27]. Multiple sequence alignments of the amplified fragments were performed using CLUSTAL W [28] integrate within MEGA version 11 [29]. This was done using the software's default alignment parameters. To infer evolutionary relationships, phylogenetic trees were constructed using both the Maximum Likelihood (ML) and Neighbour-Joining (NJ) approaches, adopting the Kimura2 parameter model [30]. The results in robust topologies were evaluated by bootstrap analysis based on 1,000 replicates. Preliminary tree structures were automatically generated using neighbour-joining and BioNJ algorithms [31]. This was derived from a distance matrix computed via the maximum composite likelihood (MCL) method [32]. Finally, a tree exhibiting the highest log-likelihood score was selected, and sequence identity percentages were calculated using Clustal Omega [26].

Determination of antibacterial efficacy

The evaluation of the antibacterial potential of the medicinal extracts followed the general procedure described by Erbiai et al. [33]. The standard bacterial strains used as test organisms are: "Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATCC 25922)". The antibacterial properties of the mushroom extracts were tested by disc-diffusion, adapted with minor modifications to align with the specific experimental conditions as outlined by Ansari et al. [34]. Sterile paper discs were impregnated with mushroom extracts at a concentration of 20 µl/disc. Those dipped in distilled water served as the negative control, while ampicillin 10µg/disc was used as the positive control.

Data management and analysis

Quantitative data inform of inhibition zones from the antibacterial assays were documented in Microsoft Excel. This was done for all treatment triplicates included in the experimental setup. Statistical analyses were carried out by SAS software version 9.1 using the General Linear Model (PROC-GLM). This was used to perform the analysis of Variance (ANOVA) on the collected data. Graphs representing mean values along with their corresponding standard errors were generated using GraphPad Prism. Tukey's Honest Significant Difference (HSD) test was used to evaluate the differences among the means. This was achieved by comparing and distinguishing the diameters of the inhibition zones [35].

Extracts preparation

50 mg of freeze-dried and crushed mushroom samples were placed in a thimble, and Soxhlet extraction tools were set in place. Soxhlet extraction was done with 200 ml of solvent (DCM: Methanol (1:1) per sample). The extracts were concentrated with a rotary evaporator and passed through Whatman Number 1 filter papers for purification, and subsequently preserved at 4ºC for future use [36].

Metabolite analysis

Chemical profiling of the mushroom extracts was done using a Gas Chromatography- Mass Spectrometry (GC-MS) system named Shimadzu GCMS-QP2010 SE. The equipment was interfaced with a computer workstation. Before injection, the extracts were prepared in a 1:1 mixture of dichloromethane and methanol. This ensured an efficient extraction of both lipid-soluble and water-soluble analytes, including volatile and semi-volatile components. An SGE BPX5 capillary column of size 30m x 0.25mm x 0.25µm was used to facilitate compound separation 99.999% pure helium gas was used as a carrier, flowing at 10ml/minute under a constant pressure of 14.5 psi. 1µl aliquots of each sample were added to the injector operating at 280ºC with a 10:1 split mode. The oven was programmed for a 40-minute analytical run. It started at 100ºC, held for 2 minutes, then a 10ºC per minute ramp to 200ºC, then 7ºC per minute to 249ºC, and finally 3ºC per minute to 300ºC, a 2-minute hold at the maximum temperature. The interface temperature was set at 290ºC. The mass spectrometer was set in positive electron ionization mode at 70eV. The ion source temperature, on the other hand, ranged between 200ºC and 250ºC, and the system was configured to scan mass-to-charge (m/z) fragments [35 and 37]. The resulting spectral data were interpreted by comparing the obtained mass profiles with entries from the National Institute of Standards and Technology (NIST) 2014 spectral database, thus enabling the proper identification of phytoconstituents present in the samples [38].

In silico and network pharmacology

Advanced computer-based simulations were employed to predict potential molecular targets for the tested compounds by evaluating parameters like binding site compatibility and ADME characteristics (adsorption, distribution, metabolism, and excretion). This approach being supportive evidence rather than proof of mechanism, enabled the identification of a lead compound possibly responsible for the observed bioactivity, while subsequent

simulations highlighted possible derivative molecules tailored for specific targets. To support this, databases relevant to computational herbal medicine research were explored to locate structurally related analogs of the bioactive compounds detected in the mushrooms [39].

For protein and target identification, the Protein Data Bank (PDB) served as the primary repository since it offers comprehensive access to publicly available three-dimensional protein structures essential for in-silico analyses [39]. In addition, specialized resources such as the Therapeutic Target Database (TTP) and the Potential Drug Target Database (PDTD) were used as well. Both provided curated information on proteins relevant to drug discovery and the elucidation of phytochemical mechanisms of action [39]. A range of bioinformatics tools facilitated these analyses, including ligand-based virtual screen software, pharmacophore modelling platforms, and molecular docking programs like Biovia, Chimera, and PyRx. Furthermore, pattern recognition algorithms and proteomics/ genomics visualization tools were integrated to enhance data interpretation and ensure accurate computational predictions [40]. Nonetheless, the docking results obtained would require biochemical or enzymatic for validation.

RESULTS

Sampling

Purposive sampling was done, and 15 distinct samples were collected from the Arabuko Sokoke forest with the aid of a Research Assistant. They were labelled and preserved in silica gel and transported to the PAUSTI lab and stored at -80°C.

Table 1. Table showing 15 collected mushroom accessions indigenous to Arabuko Sokoke Forest, Kenya.

Sample Image	Substrate	Latitude S3°	Longitude E39°	Altitude
MU001 	wood	18'6.27400"	59'44.02860"	21m
MU002 	wood	18'7.23840"	59'39.95740"	21m
MU003 	wood	18'7.73400"	59'40.16960"	16m
MU004 	wood	18'7.55670"	59'46.0100"	16m

MU005		wood	18'7.89400"	59"37.95170"	16m
MU006		wood	18'8.05440"	59'43.41210"	16m
MU007		wood	18' 8.06320"	59'43.52560"	16m
MU008		soil	17'54.86590"	58'56.17400	29m
MU009		Soil	17'55.45280	58'4985780	25m
MU010		Soil	19,20.51600"	56'20.40910"	18m
MU011		Soil	17'56.19430"	58'57.40460"	29m

Citation: Audrey MS, Kamau PK, and Neondo J. Phylogenetic analysis and antibacterial potency of indigenous mushrooms in Arabuko Sokoke forest, Kenya. *J Life Sci Biomed*, 2025; 15(4): 78-102. DOI: https://dx.doi.org/10.54203/jlsb.2025.10

MU012		Soil	15'50.67810"	58'29.60840"	25m
MU013		wood	15'50.43140"	58'29.59000"	25m
MU014		wood	15' 50.3832"	58'29.62970"	25m
MU015		Soil	14' 49.6379"	56' 28.82521"	25m

DNA extraction, PCR and Sequencing

High-quality translucent genomic mushroom DNA was extracted from the 15 mushroom samples using an optimized cetyltrimethylammonium bromide (CTAB) extraction protocol (20). PCR was then done using the universal ITS1 and ITS4 as forward and reverse primers, respectively, as initially described, and a confirmatory gel electrophoresis followed.

Table 2- Table presenting the evaluation of DNA quality by examining its structural integrity, color characteristics, absorbance ratio at 260/280 nm, and the resulting concentration of purified DNA expressed in ng/µL.

Species Name	Concentration (µg/mL)	Absorbance at 260/280 Ratio
Kretzschmaria zonata	78.447	1.875
Marasmius sp	78.667	1.875
MU003	135.72	1.873
Gymnopus brunneigracilis	134.42	1.840
Ganoderma mbrekobenum	77.843	1.752
Geotrichum candidum	134.96	1.781
Auricularia polytricha	140.04	1.798
Agaricus sp	150.71	1.876
Neonothopanus hygrophanus	131.51	1.845
MU010	87.75	1.865
MU011	147.65	1.870
Leiotrametes lactinea	134.35	1.853
Pycnoporus sanguineus	134.45	1.851
Trametes polyzona	101.87	1.842
Chlorophyllum globosum	123.34	1.856

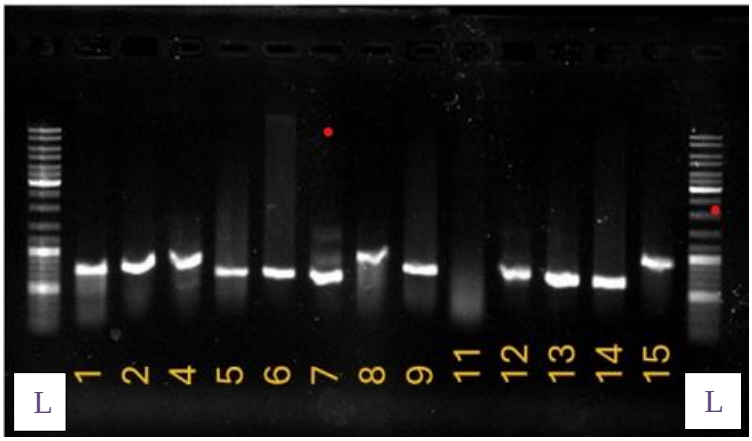


Figure 2: Visualization of the amplification products obtained from the selected isolates. Well labels: L=100bp DNA Ladder, 1=MU001, 2=MU002, 4=MU004, 5=MU005, 6=MU006, 7=MU007, 8=MU008, 9=MU009, 11=MU011, 12=MU012, 13=MU013, 14=MU014, and 15=MU015

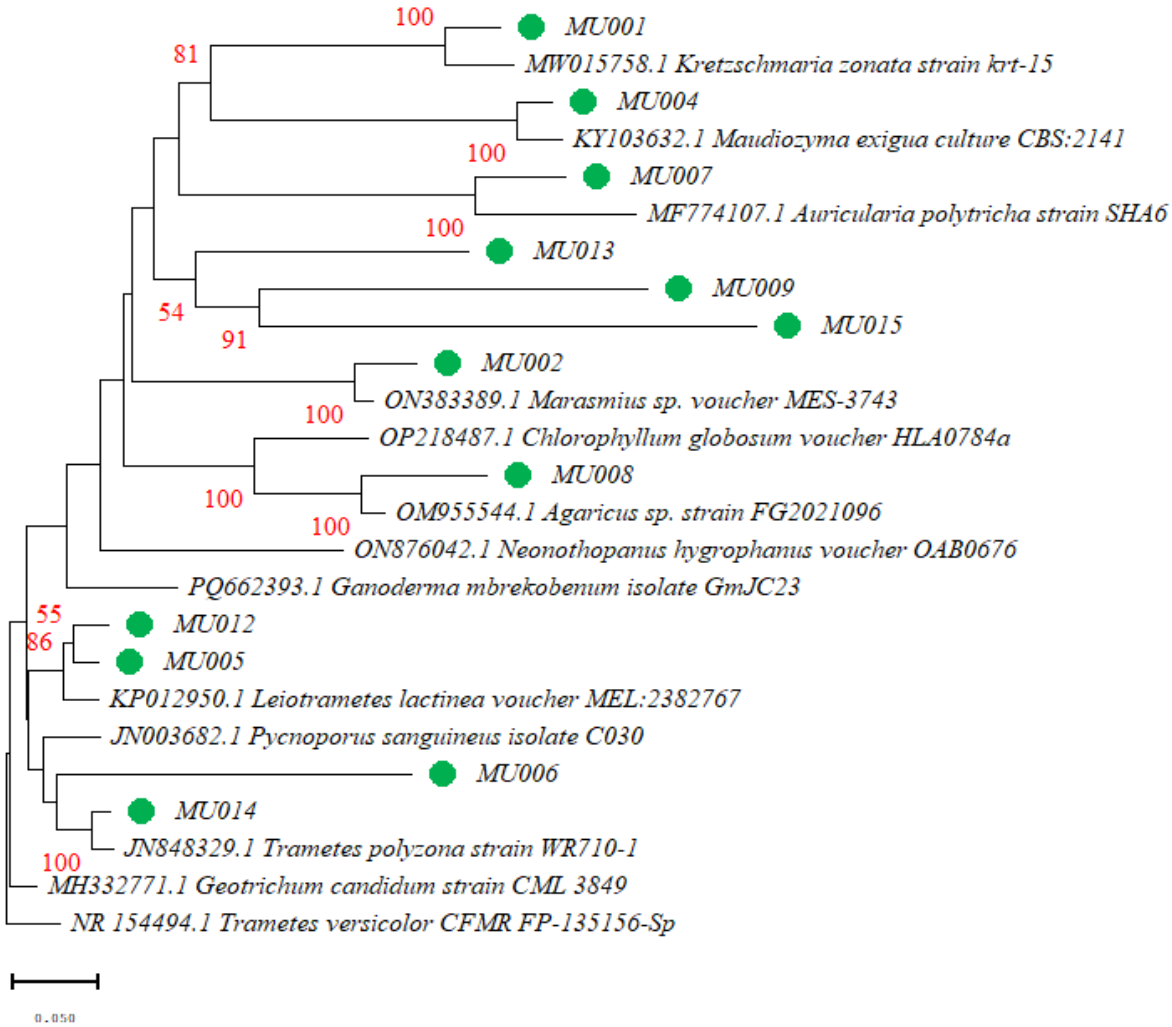


Figure 3. Image of an unrooted phylogenetic tree illustrating the relationship between the indigenous mushroom accessions acquired from the Arabuko Sokoke forest, Kenya.

The phylogenetic relationships were reconstructed using neighbor-joining (NJ) algorithm as described by Saitou and Ne [30]. The resulting best-fit tree was presented with bootstrap values based on 1000 replicates, as initially stated alongside their respective branches, as outlined by Felsenstein [41]. The tree was proportionally scaled, and the lengths of branches represent equal measurement units as evolutionary distances used when building the topology. The distances were approximated using the Kimura 2-parameter model [42]. This is denoted as the number of nucleotide substitutions in each site. To enable accurate analysis, 25 nucleotide sequences and

the positions containing gaps or ambiguous data were excluded using a complete deletion strategy. The final dataset comprised 169 conserved positions. All phylogenetic computations and visualizations were performed with MEGA version 11 [29].

This unrooted tree illustrates the phylogenetic relationship between 12 out of 15 mushroom accessions acquired from Arabuko Skoke forest Kenya with *Trametes versicolor* as the out-group. The Neighbor-Joining phylogenetic analysis based on ITS DNA sequences revealed that 12 of the 15 mushroom accessions clustered reliably with reference sequences of known fungal taxa retrieved from GenBank. This is evident from the high bootstrap support values (≥81-100%) across most nodes that indicated strong phylogenetic confidence at the species or genus level and confirm that ITS sequencing successfully resolved the majority of the sampled macrofungi. Several accessions grouped within the Polyporaceae, consistent with morphological observations of wood-decaying bracket fungi, where MU001 clustered with *Kretzschmaria zonata* with 100% bootstrap support, confirming its taxonomic identity with high confidence, while MU004 and MU007 grouped with Marasmius exiguus related taxa, also supported by strong bootstrap values (100%). Additionally, MU013 and MU009 formed a closely related subclade, suggesting either the presence of closely related species within the same genus or intraspecific variation within a single taxon, with the tight clustering of high bootstrap values supporting accurate molecular identification. Within the agaricoid fungi, MU002 clustered with *Marasmius* species with 100% boostrap support, consistent with its morphological traits such as pileus form and stipe characteristics, thus supporting the reliability of ITS as a barcode marker for agaricoid fungi. In the Ganodermataceae clade, MU005 and MU012 clustered with Gernodema mbrekobenum and Leiotrametes reference sequences, with moderate-to high bootstrap values (85-91%), suggesting correct genus-level placement and possible strain-level divergence or geographic variation. This is a pattern commonly observed among wild tropical Ganoderma species due to inherent ITS variability.

Furthermore, MU006 clustered near Pycnoporus sanguineus and Trametes species, supporting its classification among medicinal polypores. Trametes versicolor served as an appropriate fungal outgroup, effectively rooting the tree, validating the evolutionary direction of branching. This confirmed that all ingroup taxa share a common fungal ancestry, making its use methodologically sound for ITS-based fungal phylogenetics. The reduced clustering of only 12 accessions is explained by technical limitations affecting three samples. MU011 failed to yield usable sequence data due to sample spillage during transportation to the sequencing facility, and repeat sequencing was not possible due to financial constraints. MU003 and MU010 failed to amplify with ITS primers despite protocol optimization, likely due to primer mismatch or the presence of PCR inhibitors common in wild macrofungi. Consequently, these three accessions were excluded from the final phylogenetic reconstruction, highlighting practical challenges associated with molecular characterization of macrofungal species. Nodes with bootstrap values bigger than 70, were considered reliable. Samples were therefore identified from the point of divergence as follows:

MU001- *Kretzschmaria zonata*
MU002- *Marasmius sp*
MU004- *Maudiozyma exigua*
MU005- *Ganoderma mbrekobenum*
MU006- *Geotrichum candidum*
MU007- *Auricularia polytricha*
MU008- *Agaricus sp*
MU009- *Neonothopanus hygrophanus*
MU012- *Leiotrametes lactinea*
MU013- *Pycnoporus sanguineus*
MU014- *Trametes polyzona*
MU015- *Chlorophyllum globosum*

Antibacterial assay

The study investigated the antibacterial potency of indigenous mushroom extracts from the Arabuko Sokoke forest against two study organisms, "*Escherichia coli* and *Staphylococcus aureus*". The findings revealed that out of the fifteen mushroom extracts tested, only *Ganoderma mbrekobenum* and *leiotrametes lactinea* demonstrated significant antibacterial activity. The two showed measurable zones of inhibition comparable to ampicillin, the positive control. All antibacterial assays were conducted in triplicate, and results are presented as mean values with corresponding standard deviations. However, observed activity of crude extracts should not be interpreted as directly comparable to purified antibiotics such as ampicillin, but rather as indicative of the presence of potentially bioactive compounds.

Table 3. Table representing results in the form of means from the zone of inhibition readings, represented in millimetres after three replications on all 15 mushroom samples. n(Replications)=3, R=Resistant, S=susceptible.

TEST ORGANISM	SAMPLE NUMBER															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	+VE CTRL
E. coli	0.0	0.0	0.0	0.0	24.167	0.0	0.0	0.0	0.0	0.0	0.0	27.0	0.0	0.0	0.0	25.333
S. aureus	0.0	0.0	0.0	0.0	34.333	0.0	0.0	0.0	0.0	0.0	0.0	30.83	0.0	0.0	0.0	26.00
Interpretation	R	R	R	R	S	R	R	R	R	R	R	S	R	R	R	S

Because the standardized CLSI or EUCAST breakpoint criteria do not apply to crude natural extracts, antibacterial activity was interpreted using zone-of- inhibition- based activity categories adapted from previously published antimicrobial screening studies. Extracts producing inhibition zones ≥ 15mm were considered to exhibit strong activity, zones of 10-14mm moderate activity, zones of 7-9 mm weak activity, and zones <7mm were considered inactive. These categorizations are meant for comparative screening purposes and do not represent clinical susceptibility classification [43; 44].

The results from the disc diffusion showed that *Ganoderma mbrekobenum* exhibited 24.167mm as a zone of inhibition against *Escherichia coli* and 34.333mm against *Staphylococcus aureus*. *Leiotrametes lactinea* showed 27.0mm and 30.83mm, respectively. These values are comparable to the positive control (ampicillin), which recorded inhibition zones of 25.333mm for *Escherichia coli* and 26.00mm for *S. aureus*. This significant inhibition observed suggests that bioactive compounds present in these mushrooms possess potent antibacterial properties.

On the other hand, the remaining thirteen mushroom extracts did not exhibit any inhibitory effect with disc diffusion against either bacterial strain. This indicates either the absence of antimicrobial compounds or insufficient concentrations in the tested extracts. The resistance observed in these samples is consistent with previous studies where certain fungal species lack antibacterial activity despite their bioactive compound diversity, as shown from *Trametes polyzona* below in Figure 6.

Figure 4. Illustration depicting the zones of inhibition produced by *Ganoderma mbrekobenum* against *E. coli* and *S. aureus* using 10 µg ampicillin discs in a disc diffusion assay.

Figure 5. Illustration of the zones of inhibition produced by *Leiotrametes lactinea* against *E. coli* and *S. aureus* in a disc diffusion assay using 10 µg ampicillin discs.

Figure 6. Illustration of the absence of inhibition zones when *E. coli* and *S. aureus* were exposed to *Trametes polyzona* using 10 µg ampicillin discs, representing the twelve mushroom extracts that exhibited no antimicrobial activity in the disc diffusion assay.

To statistically evaluate the differences in zones of inhibition between the active mushroom extracts and the standard antibiotic, ampicillin, an analysis on variance (ANOVA) was done [45]. Data were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare zones of inhibition among isolates for *E. coli* and *Staphylococcus aureus*. Assumptions of normality and homogeneity of variance were evaluated and found acceptable; therefore, equal variances were assumed. Where ANOVA indicated significant differences, Tukey's post hoc test was applied. Statistical significance was set at $\alpha = 0.05$. All analyses were performed with a sample size of n = 3 per isolate. For *E. coli*, the one-way ANOVA showed no statistically significant differences among isolates, with results being ($F(3,8)=2.08$ and $p=0.182$. In contrast, for *Staphylococcus aureus*, significant differences were observed as $F(3,8)=11.25$ and $p=0.003$. The one-way ANOVA indicated no statistically significant differences in the zones of inhibition produced by *Ganoderma mbrekobenum* and the positive control (ampicillin) against *Escherichia coli* ($F(3,8)=2.08$, $p=0.182$), suggesting comparable antibacterial activity. In contrast, a significant difference was observed among treatments against *Staphylococcus aureus* ($F(3,8)=11.25$, $p=0.003$), with *G. mbrekobenum* producing larger zones of inhibition than ampicillin, indicating enhanced antibacterial efficacy. *Leiotrametes lactinea* also exhibited notable inhibitory activity against both bacterial strains, further supporting its antibacterial potential. This highlights its promise as a notable inhibitory activity against both bacterial strains, thus a natural source of antibacterial compounds. Upon further analysis, with GraphPad Prism software, ANOVA-based mean comparisons (mean± standard error) were visualized. Tukey's Honest Significant Difference test (HSD), a post-hoc practice, was used to determine the pairwise significance [46] generating the graphs in Figures 7 and 8.

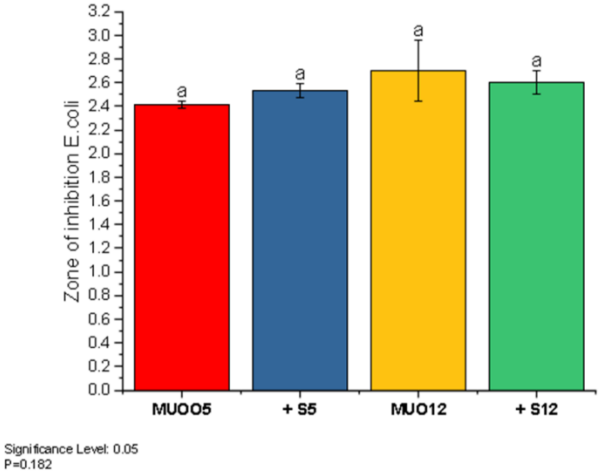


Figure 7. Graphical representation of the analysis of variance (ANOVA) results with the values representing mean±SD (n=3), generated using GraphPad Prism software. Tukey's Honest Significant Difference (HSD) test was applied to evaluate and distinguish variations in the mean inhibition zone diameters of *E. coli*. MU005 = *Ganoderma mbrekobenum*, MU012= *Leiotrametes lactinea*

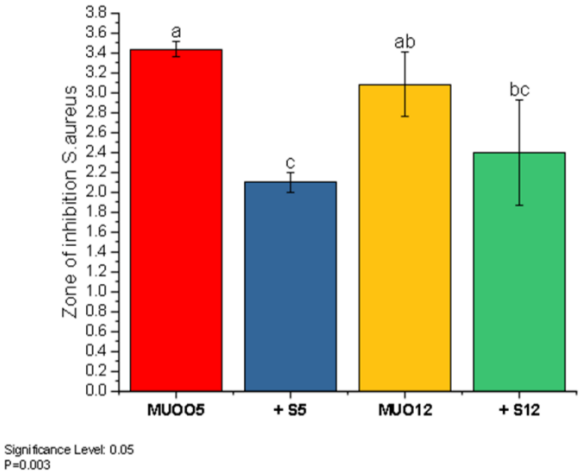
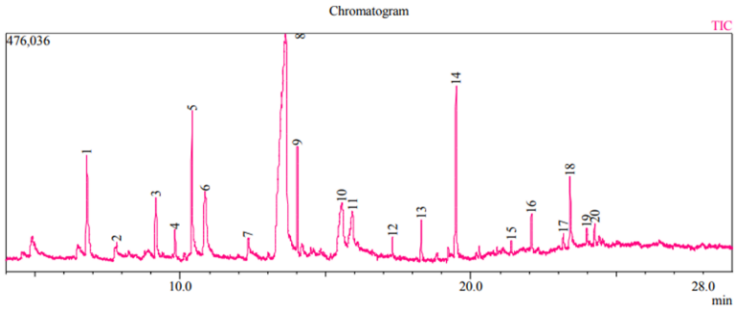


Figure 8. Graphical representation of the ANOVA results with the values representing mean±SD (n=3), generated using GraphPad Prism software. Tukey's Honest Significant Difference (HSD) test was applied to evaluate and distinguish the differences in the mean diameters of the inhibition zones against *S. aureus*. MU005= *Ganoderma mbrekobenum*, MU012= *Leiotrametes lactinea*.

GC-MS

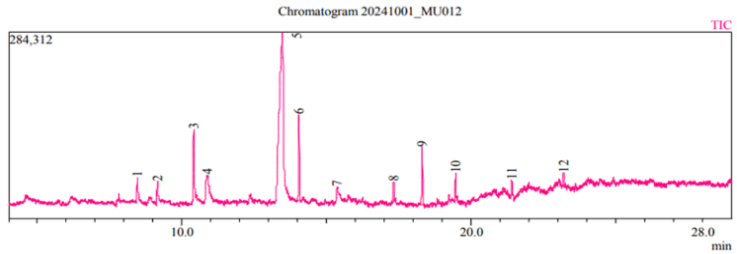
To identify the potential bioactive constituents, GC-MS analysis was conducted on the two mushroom accessions that demonstrated antibacterial properties with disc-diffusion. One representative from the inactive group was also included. The analysis resulted in the profile represented by the chromatograms in Figures 9, 10 and 11 and the table 4 below. The GC-MS analysis identified key secondary metabolites that are bioactive compounds in the two active mushroom extracts [47]. *Leiotrametes lactinea* had 2,4-Di-tert-butylphenol, 3-Deoxymannoic lactone, and 4-Thiazole carboxylic acid. *Ganoderma mbrekobenum* contained 2-formylamino-3-methylbut-2-enoic acid in addition to the compound present in *Leiotrametes lactinea*. These four compounds have been previously reported for their antibacterial properties, likely contributing to the observed inhibition zones.



Peak Report TIC

Peak#	R.Time	I.Time	Area	Area%	Name
1	6.781	6.740	626478	6.16	2-Formylamino-3-methylbut-2-enoic acid
2	7.809	7.800	10498	0.10	Nonane, 2-methyl-
3	9.164	9.115	282624	2.78	Benzoic acid
4	9.812	9.785	93873	0.92	4-Penten-2-ol, trifluoroacetate
5	10.401	10.360	513599	5.05	4-Thiazolecarboxylic acid, 4,5-dihydro-5,5-d
6	10.845	10.785	486626	4.79	2-Butenoic acid, 2-methyl-, hexyl ester, (Z)-
7	12.327	12.290	88976	0.88	Sulfurous acid, cyclohexylmethyl hexadecyl
8	13.620	13.250	5533046	54.45	Butoxyacetic acid
9	14.028	13.995	339936	3.35	2,4-Di-tert-butylphenol
10	15.556	15.385	687001	6.76	3-Deoxy-d-mannoic lactone
11	15.924	15.775	284000	2.79	3-Deoxy-d-mannoic lactone
12	17.303	17.280	55786	0.55	1-Octadecene
13	18.290	18.255	138421	1.36	1,2-Benzenedicarboxylic acid, bis(2-methylp
14	19.488	19.460	537453	5.29	Phosphine, 1,2-ethanediylbis[bis(1-methyleth
15	21.389	21.365	28624	0.28	n-Tetracosanol-1
16	22.080	22.060	77100	0.76	Phenol, 4,4',4"-ethylidynetris-
17	23.180	23.155	33213	0.33	13-Docosenamide, (Z)-
18	23.407	23.380	263247	2.59	Oxantrene-2,3-dicarbonitrile
19	23.982	23.950	36256	0.36	Fcimic chloride
20	24.259	24.225	45212	0.44	Fcimic chloride
			10161969	100.00	

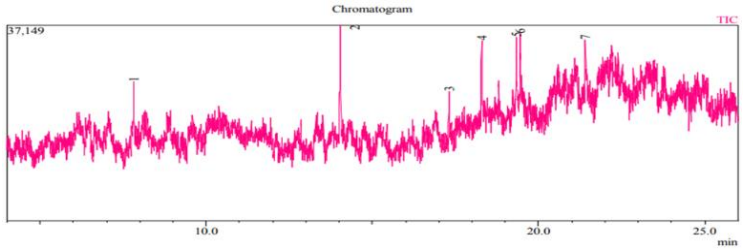
Figure 9. Chromatogram representing compounds present in *Ganoderma mbrekobenum* extracted in DCM: Methanol (1:1) after GC: MS analysis.



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	8.450	62556	2.01	Ethyl(hydroxymethyl)malonic acid
2	9.149	33564	1.08	Benzoic acid
3	10.398	175425	5.64	4-Thiazolecarboxylic acid, 4,5-dihydro-5,5-d
4	10.865	195291	6.28	2-Butenoic acid, 2-methyl-, hexyl ester, (Z)-
5	13.464	2093559	67.37	Butoxyacetic acid
6	14.033	216722	6.97	2,4-Di-tert-butylphenol
7	15.350	40815	1.31	3-Deoxy-d-mannoic lactone
8	17.307	45113	1.45	9-Eicosene, (E)-
9	18.292	160012	5.15	1,2-Benzenedicarboxylic acid, bis(2-methylp
10	19.450	44222	1.42	Trifluoroacetic acid, pentadecyl ester
11	21.392	24507	0.79	3-Chloropropionic acid, heptadecyl ester
12	23.177	15926	0.51	1-Tetracosene
		3107712	100.00	

Figure 10. Chromatogram representing compounds present in *Leiotrametes lactinea* extracted in DCM: Methanol (1:1) after GC: MS analysis.



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	7.818	5888	6.96	Octane, 2,7-dimethyl-
2	14.032	37977	44.86	2,4-Di-tert-butylphenol
3	17.310	12310	14.54	E-15-Heptadecenal
4	18.291	13598	16.06	1,2-Benzenedicarboxylic acid, bis(2-methylp
5	19.333	4610	5.45	Phthalic acid, butyl 3-fluorophenyl ester
6	19.449	7669	9.06	1-Tricosene
7	21.409	2603	3.07	Oxalic acid, hexyl tetradecyl ester
		84655	100.00	

Figure 11. Chromatogram representing compounds present in *Trametes polyzona* extracted in DCM: Methanol (1:1) after GC: MS analysis.

Table 4. Table illustrating the structural and molecular characteristics of the profiled phytochemicals. G.M. *Ganoderma mbrekobenum* L.L. *Leiotrametes lactinea*.

ID	Retention time		Name	Area (%)		MW	MF	Structure Type
	G.M	L.L		G.M	L.L			
7311	14.028	14.033	2,4-Di-tert-butylphenol	3.35	6.97	206.32 g/mol	$C_{14}H_{22}O$	Phenol
575144	10.401	10.398	4-Thiazolecarboxylic acid, 4,5-dihydro-5,5-dimethylester	5.05	5.64	173.24 g/mol	$C_7H_{11}NO_2S$	Heterocyclic compound with; thiazoles Carboxylic acids and their derivatives and Esters
549345	6.781	———	2-Formylamino-3-methylbut-2-enoic acid	6.16	———	143.14 g/mol	$C_6H_9NO_3$	Amino acid
541561	31.48	15.350	3-Deoxy-D-mannoic lactone	9.55	1.31	162.14 g/mol	$C_6H_{10}O_5$	lactone

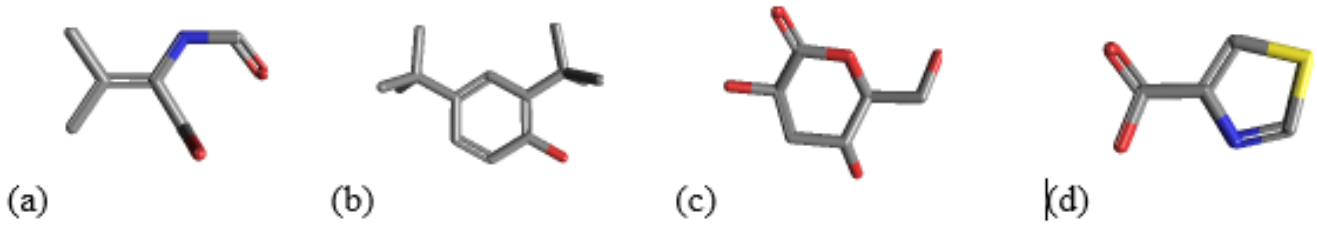


Figure 12. Images showing 3D structures of (a) 2-Formylamino-3-methylbut-2-enoic acid, (b) 2,4-Di-tert-butylphenol, (c) 3-Deoxy-D-mannoic lactone, and (d) 4-Thiazolecarboxylic acid

Table 5. Table showing the target binding affinity between the profiled phytochemicals and bacterial target enzymes.

Mushroom species	phytochemicals	Target binding affinity			
Ganoderma mbrekobenum *Leiotrametes lactinea*	2-Formylamino-3-methylbut-2-enoic acid	Phospholipase A2 1OXL	UDP-N-acetylglucosamine Enolpyruvyl Transferase (MurA) 3KR6:	Dihydrofolate Reductase (3GHW)	Penicillin-Binding Protein 1 (704B)
		-5.3	-4.1	-4.0	-4.6
	3-Deoxy-D-mannoic lactone	-5.6	-5.0	-4.2	-4.9
	2,4-Di-tert-butylphenol	-5.9	-4.3	-5.4	-3.8
	4-Thiazolecarboxylic acid, 4,5-dihydro-5,5-dimethylester	-4.8	-3.2	-3.7	-4.4

With the aid of docking programs (Chimera, PyRx and Biovia), the activity of four bioactive compounds was docked against target enzymes (from PDB) to determine their affinity and efficiency thereof. Molecular docking analyses showed string binding affinities of the identified compounds to bacterial target enzymes. The enzymes include phospholipase A2, UDP-N-acetylglucosamine, enolpyruvyl transferase (MurA), and dihydrofolate reductase (DHFR). These interactions suggest that the bioactive compounds interfere with essential bacterial metabolic pathways, leading to growth inhibition. Notably, 2,4-di-tertbutylphenol exhibited the highest binding affinity of -5.9 kcal/mol against DHFR, and enzyme crucial for bacterial DNA synthesis.

Molecular docking results

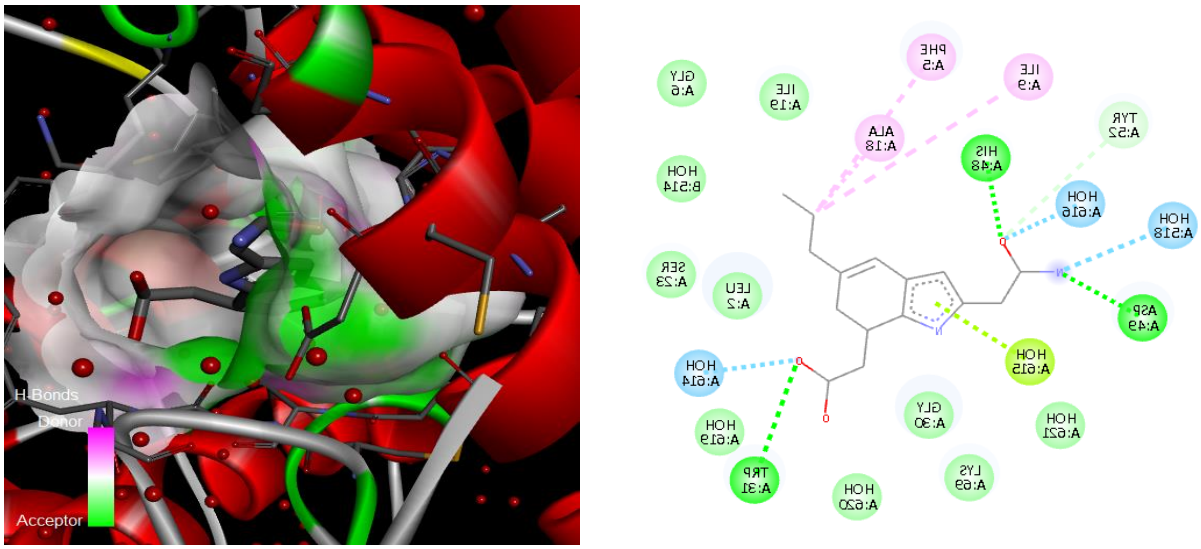


Figure 13. Image showing Hydrogen bond interactions between 2-Formylamino-3-methylbut-2-enoic acid and phospholipase A2 at the active site.

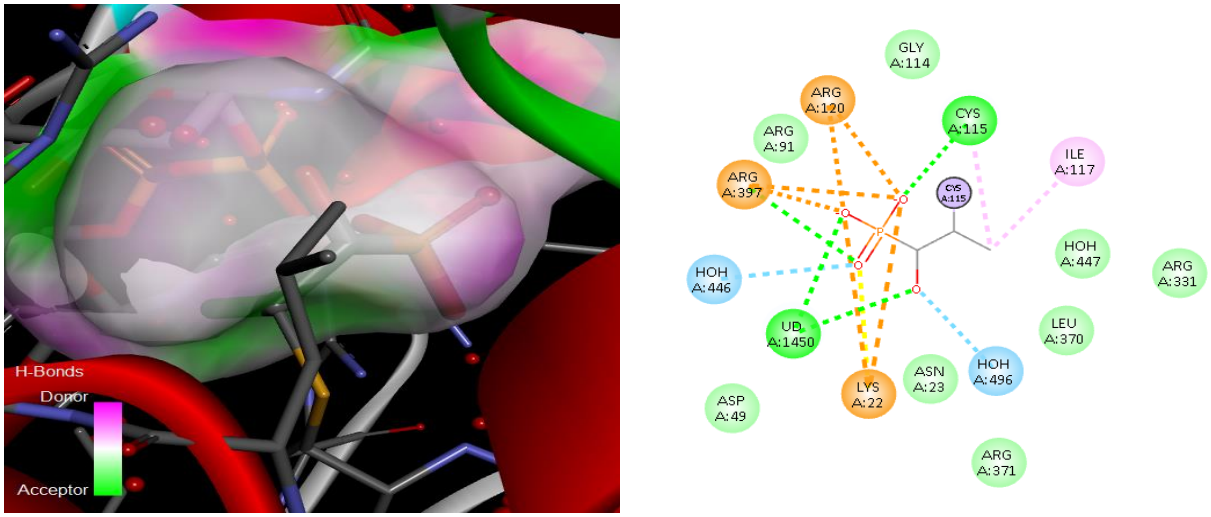


Figure 14. Image showing Hydrogen bond interactions between 2-Formylamino-3-methylbut-2-enoic acid and MurA at the active site.

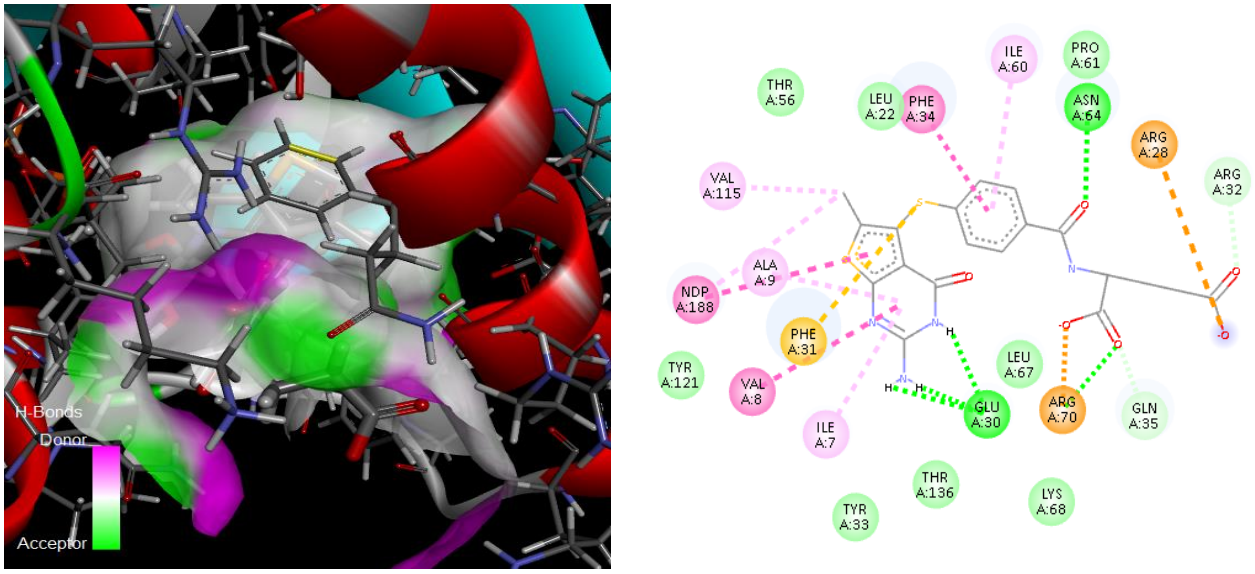


Figure 15. Image showing Hydrogen bond interactions between 2-Formylamino-3 hylbut2-enoic acid and dihydrofolate reductase at the active site.

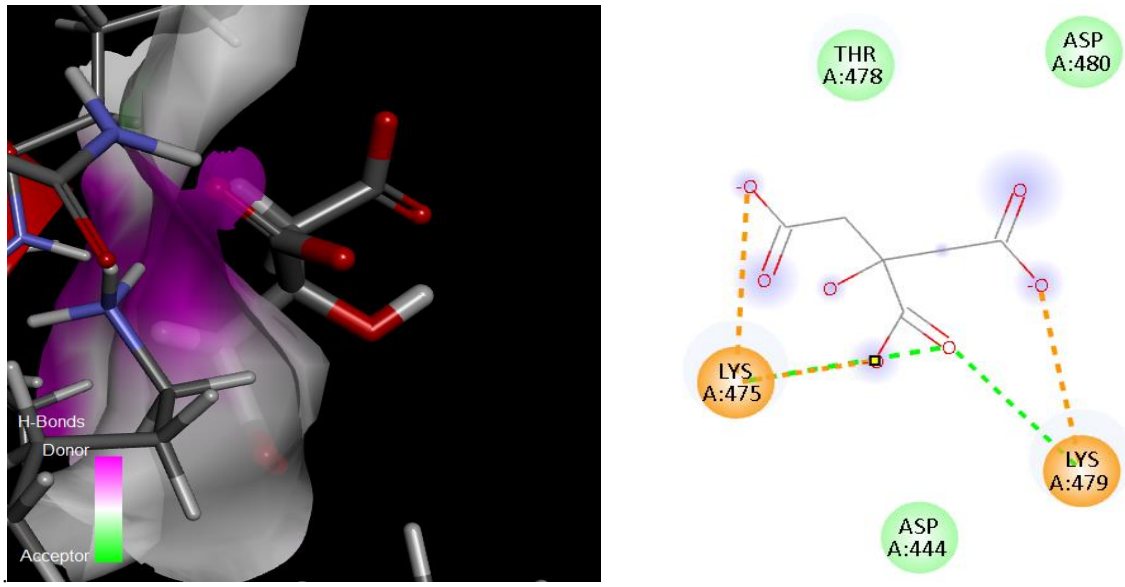


Figure 16. Image showing Hydrogen bond interactions between 2-Formylamino-3-methylbut-2-enoic acid and Penicillin Binding Protein 1 at the active site.

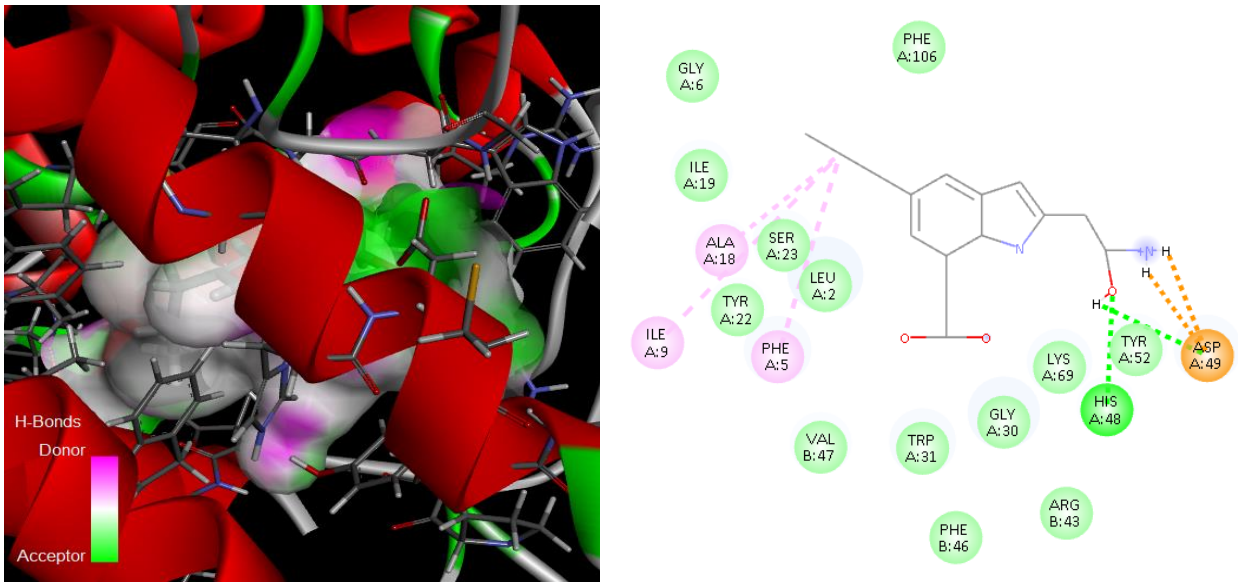


Figure 17. Image showing Hydrogen bond interactions between 3-Deoxy-D-mannoic lactone and phospholipase A2 at the active site.

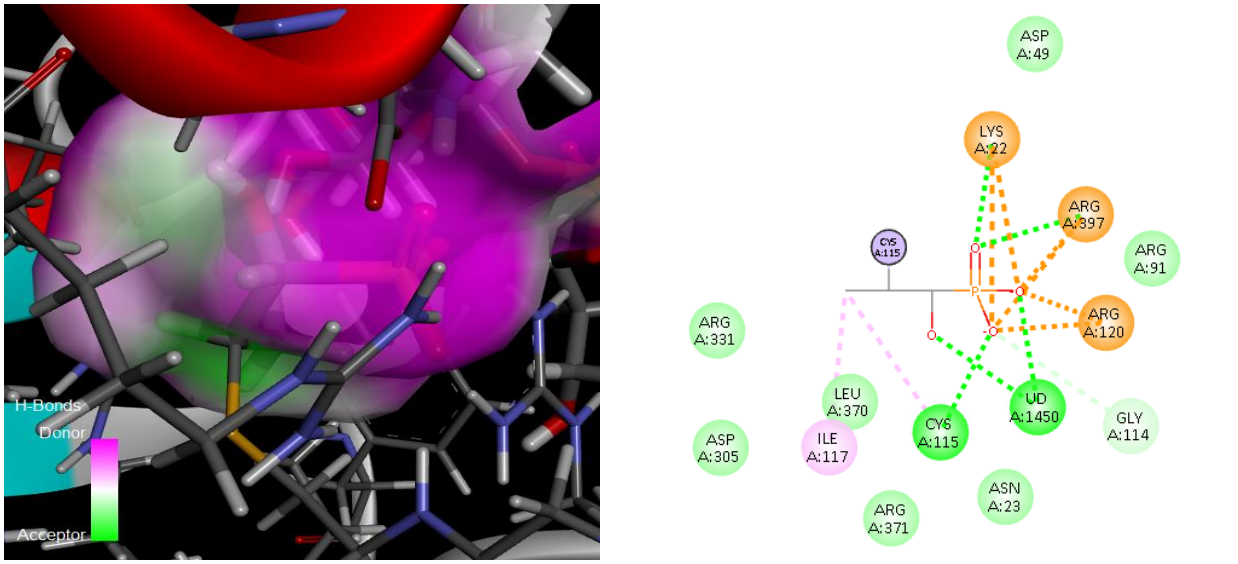


Figure 18. Image showing Hydrogen bond interactions between 3-Deoxy-D-mannoic lactone and MurA at the active site.

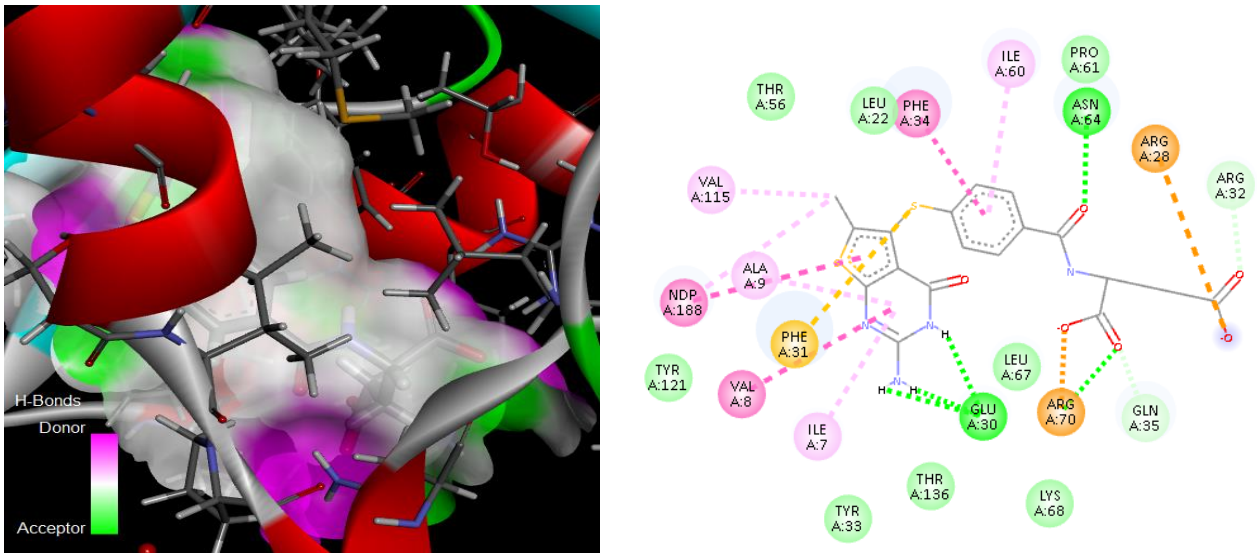


Figure 19. Image showing Hydrogen bond interactions between 3-Deoxy-D-mannoic lactone and dihydrofolate reductase at the active site.

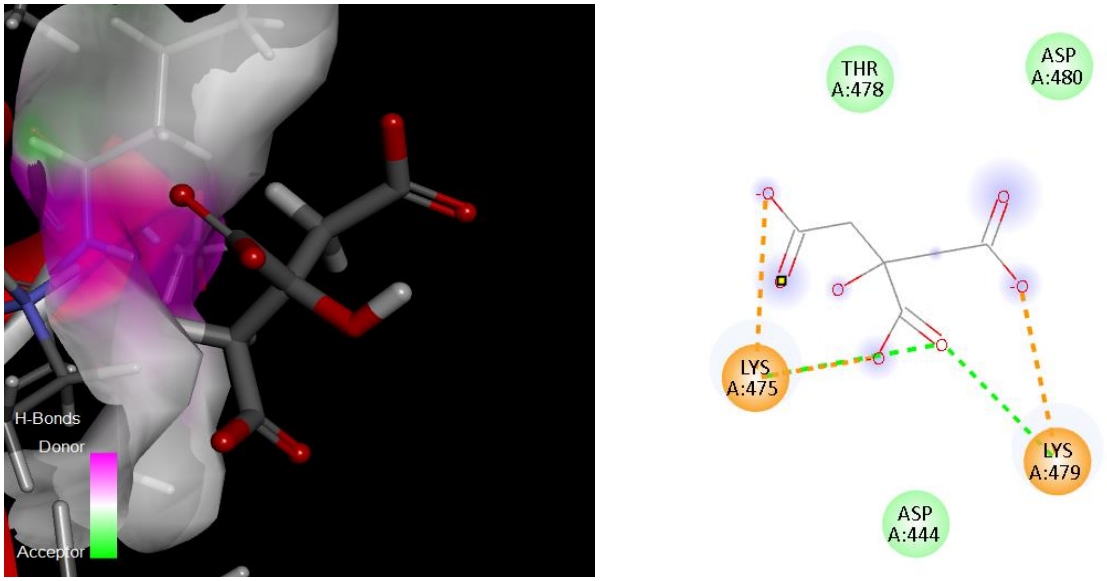


Figure 20. Image showing Hydrogen bond interactions between 3-Deoxy-D-mannoic lactone and Penicillin Binding protein 1 at the active site.

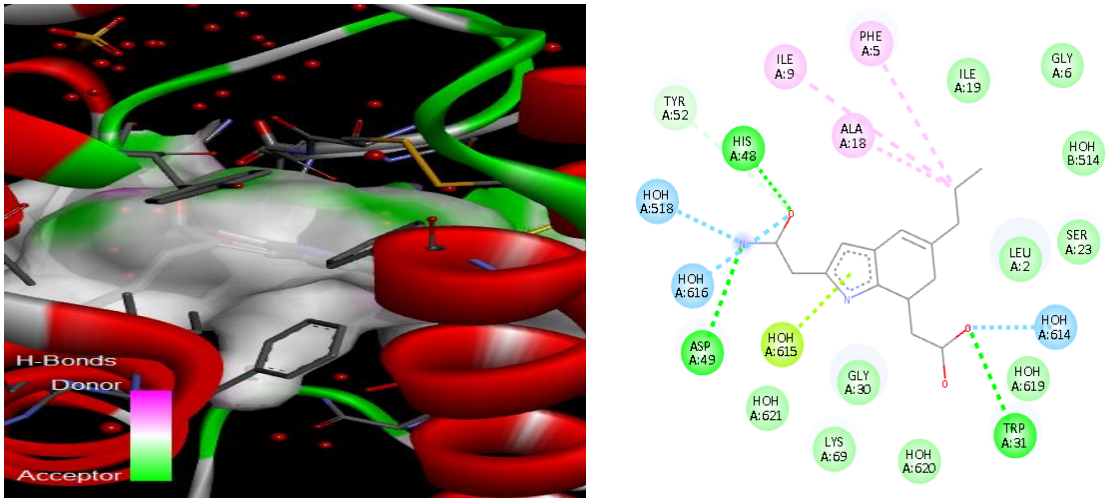


Figure 21. Image showing Hydrogen bond interactions between 2, 4-Di-tert-butylphenol and Phospholipase A2 at the active site.

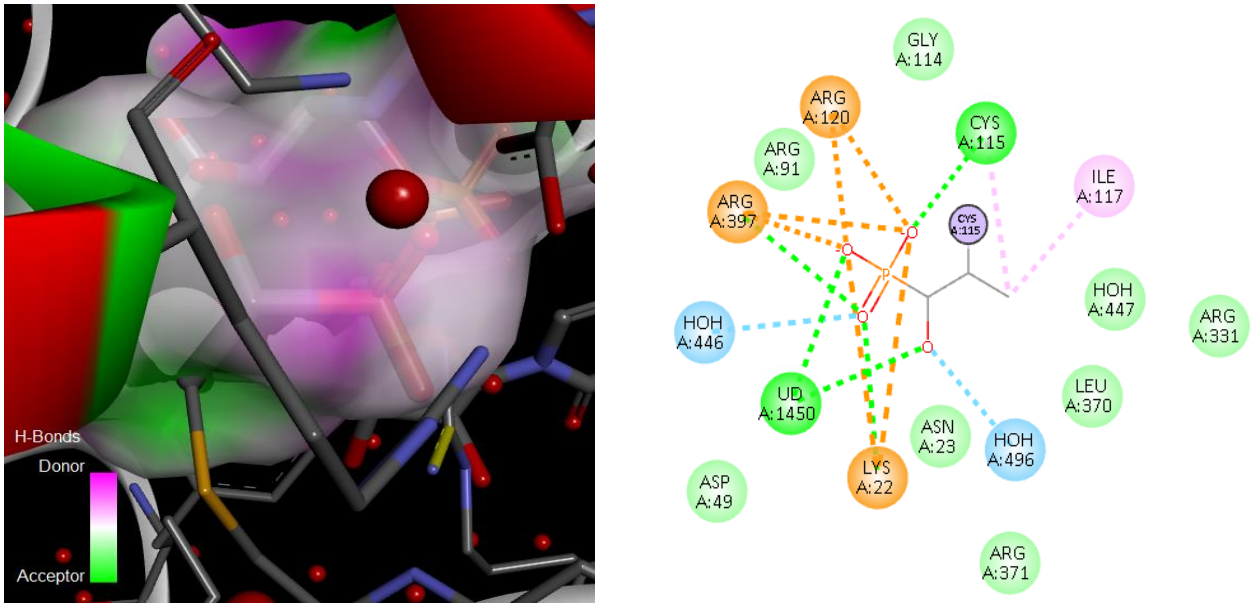


Figure 22. Image showing Hydrogen bond interactions between 2, 4-Di-tert-butylphenol and MurA at the active site.

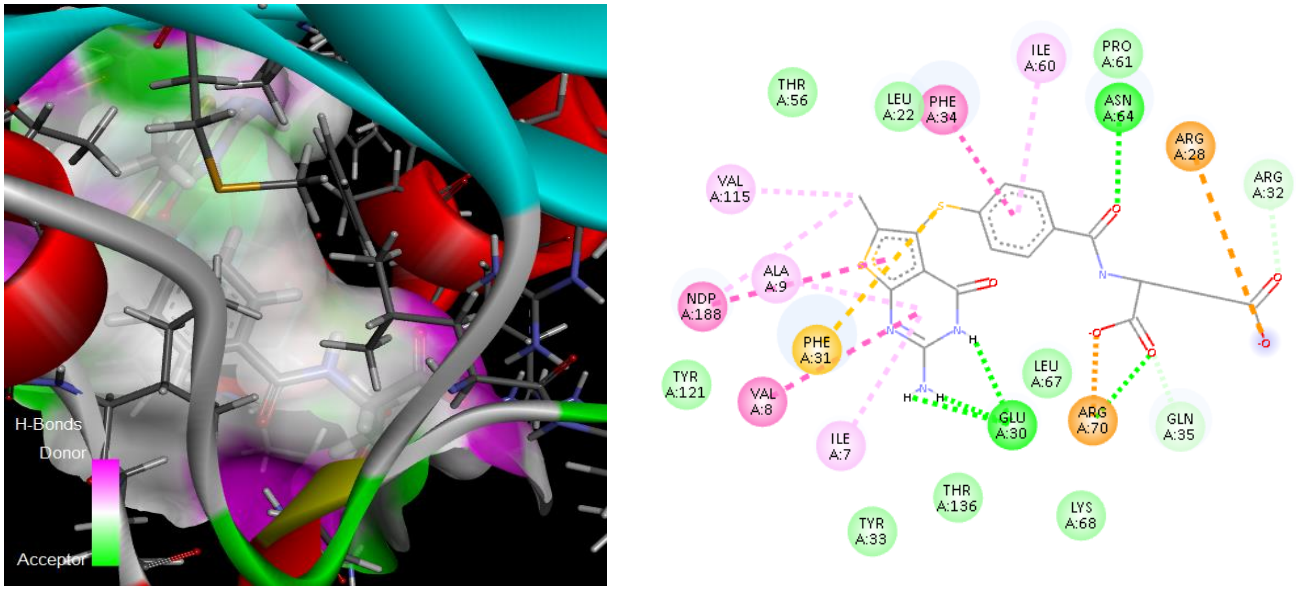


Figure 23. Image showing Hydrogen bond interactions between 2, 4-Di-tert-butylphenol dihydrofolate reductase at the active site.

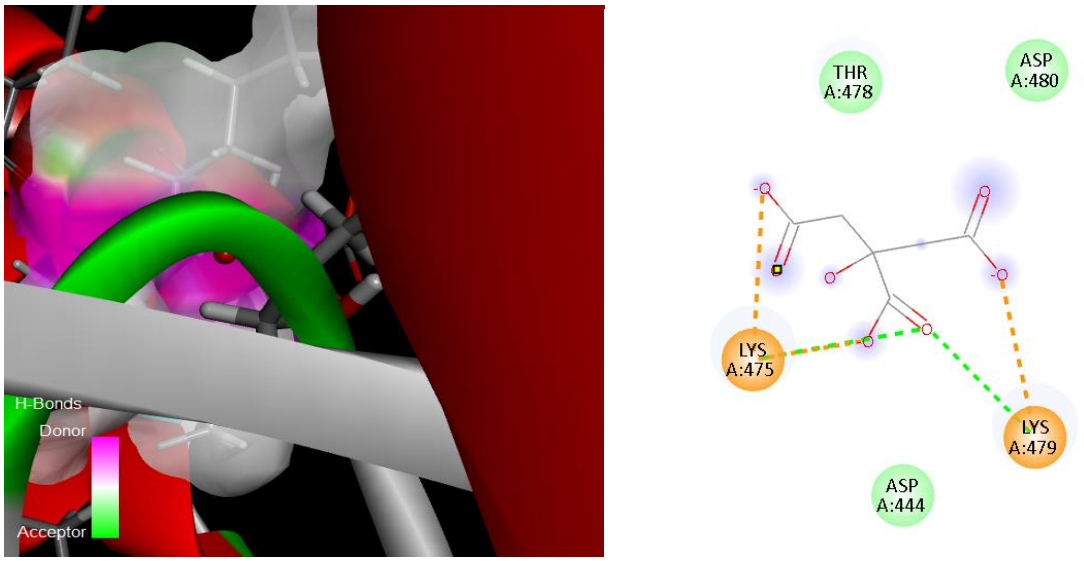


Figure 24. Image showing Hydrogen bond interactions between 2, 4-Di-tert-butylphenol and penicillin binding protein 1 at the active site.

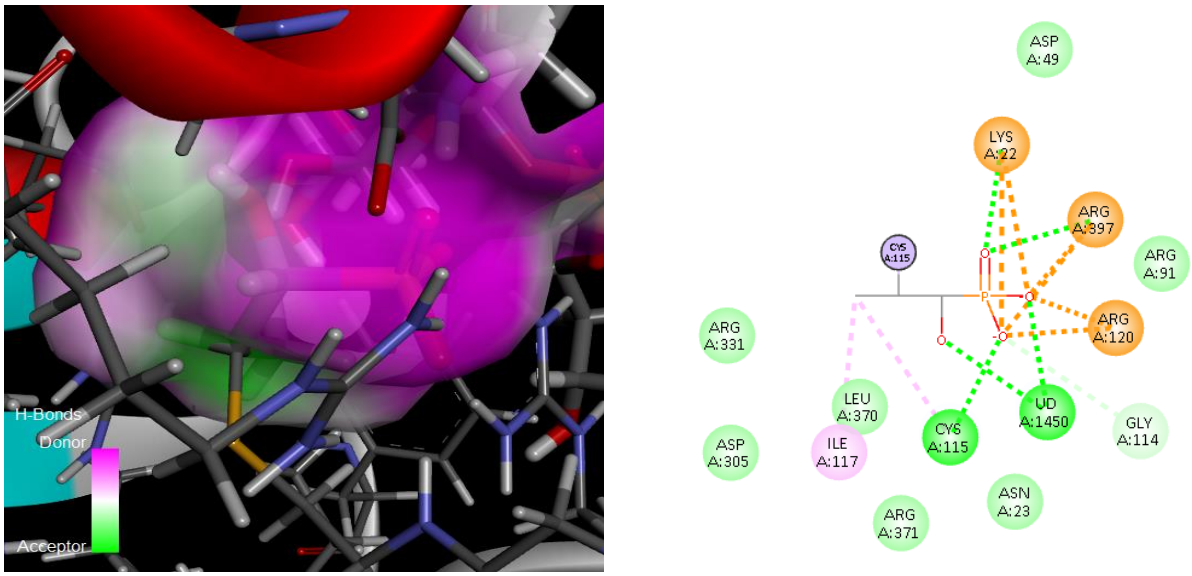


Figure 25. Image showing Hydrogen bond interactions between 4-Thiazolecarboxylic acid, 4, 5-dihydro-5,5-dimethyl-, methyl ester and MurA at the active site.

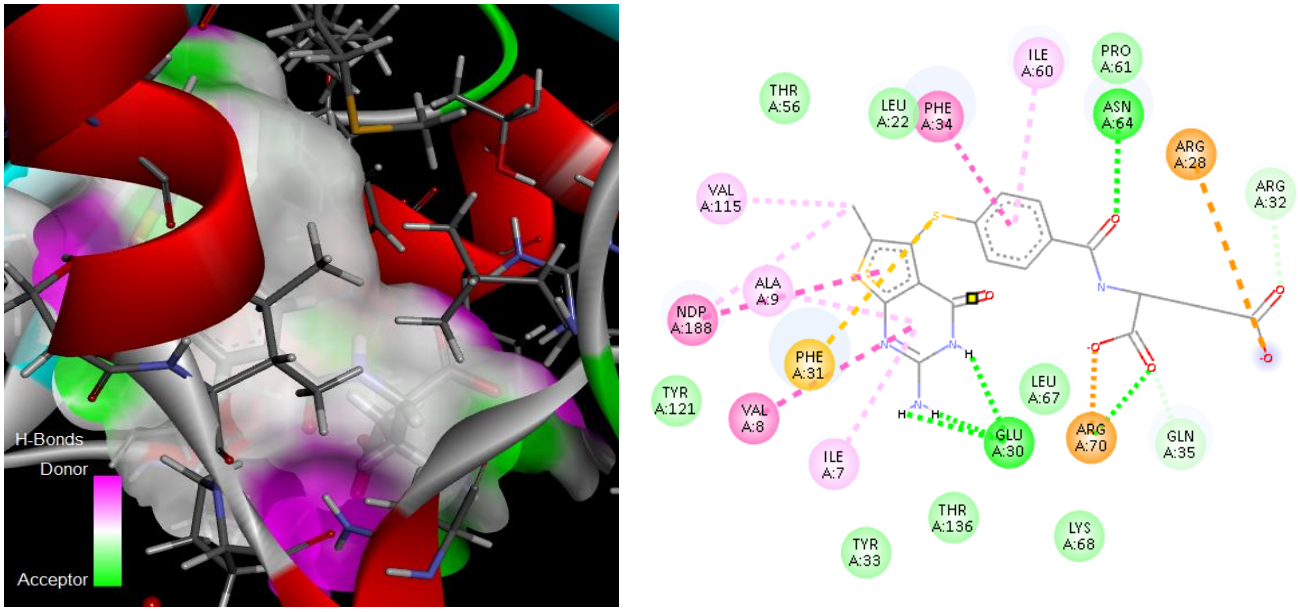


Figure 26. Image showing Hydrogen bond interactions between 4-Thiazolecarboxylic acid, 4, 5-dihydro-5,5-dimethyl-, methyl ester and dihydrofolate reductase at the active site.

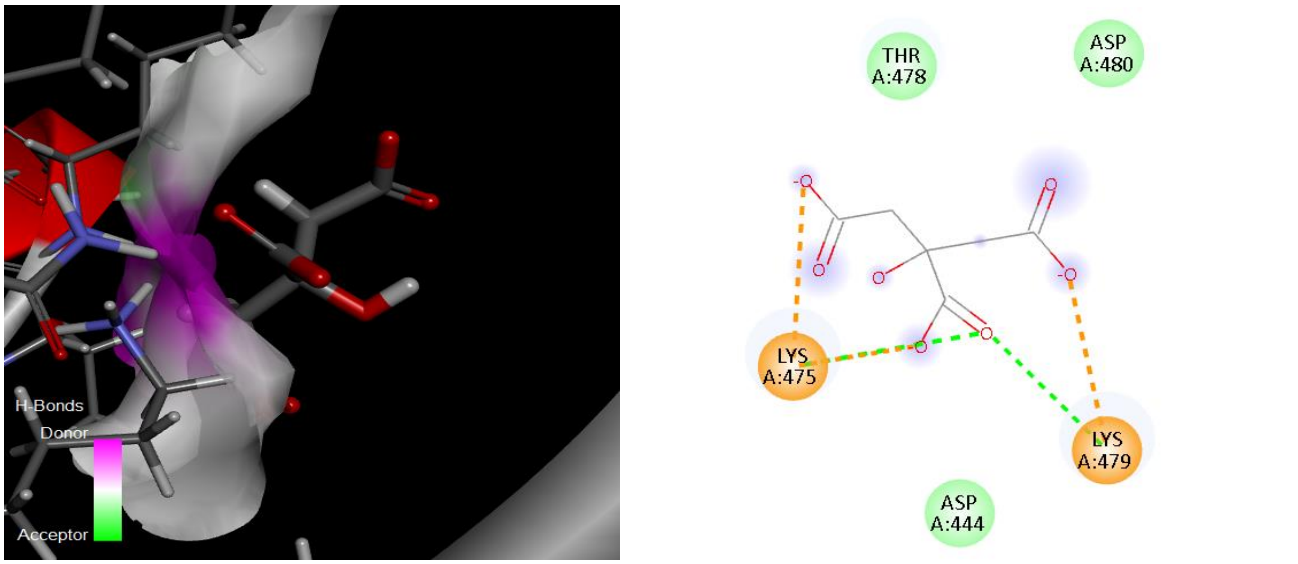


Figure 27. Image showing Hydrogen bond interactions between 4-Thiazolecarboxylic acid, 4, 5-dihydro-5,5-dimethyl-, methyl ester and Penicillin Binding Protein at the active site.

DISCUSSION

Traditional mushrooms have been valued for centuries in natural medicine. This is because they are known for their potential therapeutic effects, including boosting the immune system and improving brain function [48]. However, the increasing rates of bush burning and deforestation pose significant threats to their natural habitats. This leads to a decline in wild mushroom populations. Moreover, the limited understanding of the genetic diversity and medicinal potential of these indigenous species hampers their integration into the pharmaceutical and food industries, particularly in Kenya. This gap in knowledge restricts the sustainable utilization and conservation of these valuable fungal resources [49].

This study highlights four bioactive compounds, all of which have potentially remarkable antibacterial properties. This suggests their potential as promising leads for pharmaceutical development upon further experimental validation**.** Their characteristics are summarized in Table 4. Among them is 2-formylamino-3-methylbut-2-enoic acid, which is a structural derivative of 3-methylbut-2-enoic acid and a chemical block in medicinal chemistry. The compound's derivatives, like 4,4-trisulfanediylbis(but-2-enoic acid) (TSDB), exhibit antibacterial activity, and others, like sulfonylpiperidine derivatives, inhibit cell membrane integrity(50). This is an enzyme responsible for bacterial DNA synthesis by blocking the phosphorylation of thymidine monophosphate, in

turn disrupting nucleotide metabolism [51]. To add to that, such metalloprotease inhibitors also suppress the organism's growth by inhibiting peptide deformylase (PDF) [52]. This derivative's formyl amino group suggests possible interactions with bacterial enzymes that are analogous to PDF or TMPK [53; 54].

2,4-Di-tert-butylphenol, found in both *Ganoderma mbrekobenum* and *Leiotrametes lactinea*, has been associated with potent broad-spectrum inhibitory effects. It has potentially exhibited antibacterial activity through mechanisms involving the disruption of bacterial cell membrane stability [55]. 2,4- DTBP is a phenolic compound recognized for its significant therapeutic potential and wide range of pharmacological activities [55]. The compound has exhibited efficacy by interfering with quorum-sensing mechanisms that reduce virulence factor production and the formation of biofilm [56]. In *Pseudomonas aeruginosa*, 2,4- DTBP inhibits quorum sensing by binding to LasR and Rh1R receptors. This, in turn, reduces the expression of elastases and proteases. The compound induces oxidative stress in *Ralstonia solanacearum*. This is the pathogen responsible for bacterial wilt. It manifests by stimulating the production of reactive oxygen species (ROS), which activate lipid peroxidation and ultimately compromise the integrity of cell membranes [57]. Additionally, 2,4-DTBP exhibits antibacterial effects on *Cutibacterium acnes*, suggesting potential applications in bacterial acne treatment [58]. The compound also exhibits strong antifungal activity, posing as a potential biopreservative. Studies indicate that this compound effectively suppresses mycelial development of "*Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium chrysogenum*". In fungal cells, it appears to disrupt cytoskeletal structures by binding to β-tubulin. This impairs microtubule dynamics, thus inhibiting spore germination and mycelial growth [55].

3-Deoxy-D-mannoic lactone inhibits bacterial quorum sensing. This is a crucial mechanism for pathogenicity in several bacterial species. The compound reduces their ability to form biofilms [59]. The characteristic is especially important in addressing antibiotic-resistant infections, where the development of biofilms significantly strengthens bacterial survival and persistence. The sugar molecule 3-deoxy-D-manno-octulosonic acid, Kdo, is a vital component of the lipopolysaccharides in Gram-negative bacteria. It therefore plays a major role in maintaining the integrity and stability of their outer membrane [60]. 3-Deoxy-D-mannoic lactone targets the viability of this sugar as an additive to inhibitors of KDO hydrolase. These derivatives target the enzymes responsible for LPS biosynthesis. For instance, Kdo transferase (WaaA) catalyzes the addition of Kdo residues to lipid A. 3-deoxy-D-mannoic lactone, as a natural derivative in fungi, prevents proper LPS formation, thereby weakening the outer membrane and increasing susceptibility to other agents [61]. Additionally, studies show that 3-Deoxy-D-mannoic lactone may also have α-glucosidase inhibitor activities [62; 63]. α-glucosidase catalyzes the breakdown of glucose to simple sugars. In bacteria, this is a crucial step for carbohydrate metabolism, cell-wall biosynthesis and energy production. Since bacteria live in diverse environments, they must break down plant-derived polysaccharides (starch to glucose) for survival. Pathogenic bacteria use this enzyme to rummage for sugars from host tissues. This leads to colonization, biofilm formation, infection, and ultimately resistance to antibiotics and immune defenses. Inhibiting this enzyme starves, reduces bacteria's ability to grow or form biofilms and cause infections, making the inhibitor a potential antibacterial agent upon further studies.

4-thiazolecarboxylic acid, detected in both mushroom species, belongs to the thiazole derivative group. The group is well recognized for exhibiting significant antibacterial potential [64]. Its mode of action involves chelating zinc ions within the active sites of class B metallo-β-lactamases (MBLs). This action prevents these enzymes from functioning. Such enzymes, including NDM-1 and VI -2, are commonly produced by gram-negative bacteria, and they break down β-lactam antibiotics, ultimately rendering the drugs ineffective [65]. Derivatives of the compound exhibit a broad-spectrum activity against pathogens like *E. coli* and *S. aureus* [66]. As a potent enzyme inhibitor, the compound precisely targets the cell wall synthesis process, causing cell lysis and death in the end [65]. The inhibitory effects observed in this study support its potential role in the formation of new antibacterial drugs if further experiments prove so, particularly against resistant strains such as *S. aureus*.

Ganoderma mbrekobenum is a medicinal mushroom proven to have antimicrobial and antifungal properties previously. The mushroom has recently shown mild antimalarial activity, as eleven undescribed lanostanes were isolated from its extracts [67]. From previous studies, the bioactive compounds present in the Arabuko Sokoke isolate include polysaccharides that exhibit extensive antibacterial properties [68]. Present alkaloids and ganoderic acid are used in medicines and dietary supplements, and they offer leads in drug discovery, as the genus *Ganoderm*a is known for its medicinal properties [69].

Leiotrametes lactinea, also known as *Trametes lactinea,* is a tropical white-rot fungus that is often found on broadleaf tree felling piles [70]. The fungus predominantly produces polysaccharides that function as complex macromolecules that exhibit diverse biological activities [71]. Research findings indicate that this fungus exhibits notable immunosuppressive capabilities. Extracts of *Trametes lactinea* have been reported to inhibit enzymes like hyaluronidase, lipoxygenase, and xanthine oxidase. These are enzymes linked to oxidative stress and inflammation

pathways [72]. In a study investigating fungal-fungal interactions, *Leiotrametes lactinea* showed elevated superoxide dismutase activity in response to interspecific stress [73]. Additionally, this fungus has demonstrated significant analgesic effects and offers protective benefits according to Dong et al. (74) and Liu et al. (75). The massive antibacterial effect from this fungus is acquainted to the sesquiterpenoids it synthesizes [76].

Given the rising global challenge of antibiotic resistance, these bioactive compounds could serve as the foundation for novel antibacterial therapies if further experimental studies prove so. Natural compounds like these offer a vast, underutilized resource for drug discovery. Further research into their pharmacokinetics, toxicity, and clinical efficacy is essential [77; 21]. Incorporating these bioactive compounds into pharmaceutical formulations holds significant promise for improving the performance of the current antibiotics. This also paves way for entirely new classes of antibacterials. To fully harness their therapeutic potential, future research should focus on investigating their synergistic interactions with existing antibiotics. Additionally, comprehensive mechanistic studies need to be conducted to improve the understanding of the molecular basis of their antibacterial activity. The scalability of extraction and synthesis methods must therefore be evaluated to ensure their feasibility for large-scale pharmaceutical production [78].

Implications and future research

The findings of this study suggest that *Ganoderma mbrekobenum* and *Leiotrametes lactinea* are promising sources of novel antibacterial compounds. Given the increasing prevalence of antibiotic resistance, natural products with potent antimicrobial activity are crucial for developing alternative therapeutic strategies. Future studies should focus on fractionation and purification of the active compounds, followed by in vivo validation to assess their clinical applicability.

CONCLUSIONS AND RECOMMENDATIONS

The study successfully extracted high-quality genomic DNA from 15 distinct mushroom samples, allowing for effective genetic characterization. The identification of species highlights the rich biodiversity present in the region. The antibacterial assays demonstrated varying levels of efficacy among the mushroom extracts against common pathogenic bacteria. Notably, extracts from *Ganoderma mbrekobenum* exhibited significant inhibition against *Staphylococcus aureus*, suggesting its potential as a natural antibacterial that is to be further validated by extensive studies. The study identified a range of bioactive metabolites exhibiting significant antibacterial potential, notably 2,4-di-tert-butylphenol and 4-thiazolecarboxylic acid. These molecules appear to target and interfere with essential bacterial enzymes, suggesting promising prospects for the development of novel antibiotics. Moreover, the results highlight the critical need to preserve native mushroom species, given their dual importance in maintaining ecological balance and serving as valuable resources for future nutraceutical and pharmaceutical innovations. The genetic erosion due to habitat destruction and overharvesting calls for immediate conservation efforts. The study, therefore, gives antibacterial evidence that can form a basis for a broader antimicrobial screening in future work.

Although molecular docking provides valuable insights into the potential binding modes and relative affinities of bioactive compounds with target proteins, it remains a predictive and in silico-based approach with inherent limitations. Docking simulations rely on static protein structures and simplified scoring functions, which may not fully capture protein flexibility, solvent effects, or the dynamic nature of ligand-protein interactions under physiological conditions. Consequently, the binding affinities and interaction profiles reported in this study should be interpreted as indicative rather than definitive. To substantiate the biological relevance of the predicted interactions, further biochemical and enzymatic validation assays, such as enzyme inhibition kinetics, binding affinity measurements, or cell-based functional studies, are necessary. Such experimental validation would confirm target engagement, mechanism of action, and the therapeutic potential of the identified compound.

GC-MS used in this study only detected volatile/semi-volatile metabolites; non-volatile bioactive compounds like polysaccharides and peptides were not captured. Further studies to comprehensively evaluate the antimicrobial potential and additional techniques (LC-MS) that can capture hydrophobic compounds should be considered. Additionally, docking predictions require further validation through enzyme inhibition assays.

DECLARATIONS

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Data availability

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

Authors' contributions

MA conceived and designed the study, coordinated fieldwork, and collected mushroom samples from Arabuko Sokoke forest. She performed DNA extraction, PCR amplification, sent samples to Macrogen Asia for sequencing and did phylogenetic analysis. Conducted antibacterial assay against E. coli and S. aureus, analyzed inhibition zone data, and prepared the initial draft of the manuscript. Managed correspondence with the journal and is responsible for all inquiries regarding the research.

JN, jointly with MA, conceived and designed the study, supervised the overall research process and provided technical guidance in molecular biology, microbiology, and bioinformatics. Verified DNA sequence quality, assisted in phylogenetic tree construction, and ensured adherence to scientific and ethical standards. Contributed substantially to data interpretation, manuscript structuring, and critical revision for intellectual content.

PK performed GC-MS alongside MA and identified bioactive compounds. Offered guidance on molecular docking simulations to evaluate compound-protein interactions analyzed the docking scores. Correlated the findings with antibacterial assay results together with MA. Assisted in literature review, manuscript editing, and preparation of figures and tables.

All authors read, reviewed, and approved the final version of the manuscript before submission.

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Competing interests

The authors declare that they have no competing interests

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