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Research Paper

Root-Derived Phytochemicals from *Inula confertiflora* for Antioxidant and Antibacterial Activities

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Abstract
Inula confertiflora, a medicinal herb indigenous to Ethiopia, often used in traditional treatments for inflammatory and pain-related conditions. The root of *I. confertiflora* was soaked with n-hexane, ethyl ether, and acetone solvents. The proportion of crude extracts derived from ethyl ether extracts was 1.4 times higher than that of n-hexane and 1.2 times higher than that of acetone. Analytical detection results of crude extracts confirmed that *I. confertiflora* contained a variety of different preliminary phytochemicals. The higher concentrations of total flavonoids and other polar phytoconstituents present in the ethyl ether extracts were associated with greater radical-scavenging effectiveness in the DPPH solution. More than half of the bacterial growth efficiency was restricted by phytochemicals derived from *I. confertiflora* root extracts using non-polar, medium-polar, and high-polar solvents. It is crucial to note that medium-polar extracts of *I. confertiflora* root decreased the inhibitory effect on the development of both gram-positive and gram-negative bacterial strains at a concentration of 100 μg/mL using acetone as the solvent. Additionally, they exhibited improved radical scavenging against a DPPH solution. Moreover, molecular docking simulation clearly revealed that *I. confertiflora* extracts have a strong binding affinity toward four key bacterial target proteins (LasB (-7.9kcal/mol), PBP2a (-8.0kcal/mol), FabH (-11.3kcal/mol), and MurA1 (-4.7kcal/mol)). Collectively, these findings suggest that *I. confertiflora* extracts exhibit substantial potential as antibacterial agents by targeting diverse and functionally important bacterial proteins.

Keywords
Inula confertiflora, Phytochemicals, Antioxidant, Antibacterial Activities

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1. INTRODUCTION

Antibacterial resistance has become one of the most serious risks to the global public health because it reduces the effectiveness of existing antibiotics and increases the risk of treatment failure, prolonged infection, and mortality. Various adaptive mechanisms are responsible for the emergence of antibacterial resistance. The identification of antibacterials should not just emphasise growth suppression but also assess whether candidate compounds might disrupt resistance-related mechanisms, such as efflux activity, membrane integrity, virulence regulation, and biofilm formation. Besides the treatment and prevention of infectious illnesses, people have used plants for millennia for many biological, economic, and cultural advantages (Ajeethan et al., 2026; Eshete and Molla, 2021; Langwick, 2015; Moges and Moges, 2019). This association implies that the world has benefited tremendously from the symbiotic relationship between humans and plants in a variety of applications (Faccio, 2020; Nesbitt et al., 2010; Zuluaga, 2024). From ancient times, empirical observations of individuals have been employed to create a variety of flavours, cosmetics, and drugs from plants. This has resulted in the creation of contemporary pharmaceuticals, cosmetics, and flavours that are currently in use in the developed world (Krief et al., 2005).

Traditional medical systems in several regions worldwide, particularly in sub-Saharan Africa, continue to depend on plant-based chemicals for therapeutic preparations. This technique has been bolstered by the progress of fundamental sciences, like natural product chemistry and pharmaceutical chemistry (Plaatjie et al., 2024). A World Health Organization (WHO) assessment indicates that roughly 65% to 80% of individuals

in underdeveloped nations consistently use and trust the efficacy of medicinal plant-based treatments (Feyisa et al., 2024; Gedlu et al., 2024). In the Eighth General Program of Work (1990–1995), the World Health Organization (WHO) broadened the meaning of traditional medicine to encompass therapeutic practices that had been employed for centuries before the development and dissemination of modern medicine (Brown et al., 2006). Medicinal plants contain a variety of active compounds that are critical to facilitate the growth and development of the plants, as well as to their natural defenses and capacity to address environmental issues (Agidew, 2022). These diverse bioactive compounds, referred to as secondary metabolites, are essential to medicine and are utilized by patients either directly or indirectly under the guidance of traditional healers. Ethiopia is home to numerous common and endemic medicinal plants that are widely utilized by many people to treat a variety of illnesses (Ali et al., 2018). In this context, plant-derived phytochemicals provide a promising source of antibacterial compounds because plants naturally produce structurally diverse secondary metabolites as defense molecules against microbial invasion. Phenolics, flavonoids, terpenoids, alkaloids, tannins, and saponins have been reported to induce antibacterial effects via multiple mechanisms, such as the inhibition of critical enzymes and proteins and the disruption of bacterial membranes, the interference with nucleic acid synthesis, the suppression of quorum sensing, and the inhibition of biofilm formationPhytochemicals has the capacity to provide multi-target actions, hence diminishing the probability of fast resistance development, unlike several conventional antibiotics that operate on a single molecular target. The many mechanisms of action of plant metabolites render them particularly relevant for combating illnesses produced by resistant bacterial strains.

There are more than 100 species of Inula in the Compositae family, which includes the Asteraceae. These species are distributed across the continents of Africa, Asia, and Europe (Seca et al., 2014; Tavares et al., 2022). There are thought to be about 400 different chemicals in the Inula genus, mostly flavonoids, alkaloids, and terpenoids (Talebi et al., 2023). Numerous of these compounds exhibit encouraging pharmacological properties and are crucial to scientific and medical research (Seca et al., 2014; Sun et al., 2021). *I. confertiflora* is a medicinal plant that is indigenous to Ethiopia and is widely used to treat a variety of skin and ophthalmic conditions, such as fungal infections, wound infections, and eczematous lesions (Gebre-Mariam et al., 2006; Habtamu et al., 2024). The desiccated root segments of *I. confertiflora* have historically been used as a conventional treatment for leprosy and as a fumigant for ailments associated with delivery (Ayele et al., 2024). However, there is no scientific data regarding the preliminary phytochemicals present in *I. confertiflora* root parts and their bioassay activity results against bacterial strains. Consequently, this investigation presents the preliminary compounds that were identified in the root parts of *I. confertiflora* as a result of extraction through successive maceration with various solvents. Furthermore, it assesses the crude extract's antibacterial activity by assessing its ability to impede the proliferation of specific gram-positive and gram-negative bacterial strains. In addition, molecular docking simulations were also confirmed that *I. confertiflora* extracts have a strong binding affinity toward four key bacterial target proteins which provide potential as antibacterial agents targeting diverse and essential bacterial proteins. This research development was carried out considering that root-derived phytochemicals from *Inula confertiflora* contain bioactive secondary metabolites, particularly phenolics, flavonoids, terpenoids, and other antimicrobial-associated compounds, that contribute to measurable antioxidants and antibacterial activities. Integrating phytochemical profiling, antibacterial assays, and molecular docking into a unified analytical framework allows for a more comprehensive elucidation of the biological activity of the root extract by correlating the presence of specific phytochemicals with their inhibitory effects on bacterial pathogens and their predicted molecular-level interactions involving essential bacterial target proteins. The novelty of this study is the development of an integrated phytochemical, biological, computational framework to evaluate root-derived phytochemicals from *Inula confertiflora*. Unlike previous studies that mainly reported phytochemical constituents or in vitro antioxidant and antibacterial activities separately. This approach provides a stronger scientific basis for identifying *I. confertiflora* roots as a promising source of phytogenic antibacterial agents.

2. EXPERIMENTAL SECTION

2.1 Materials

Some of the organic solvents that were employed to produce the many reagents used in this inquiry and to cleanse and immerse the plant components include n-hexane, ethyl ether, acetone, and deionised water. The following chemicals are employed to prepare reagents for the investigation of bioactive secondary compounds, including flavonoids, alkaloids, tannins, terpenoids, saponins, carotenoids, phenols, and steroids: vanillin, potassium hydroxide, sulphuric acid, and hydrochloric acid, (85% Mumbai, India), acetic anhydrous, and magnesium rubber (British Drug House Ltd., UK), and ferric chloride (British Drug House Ltd., England), potassium iodide, and iodine. DPPH, FRAP, Quercetin, hydrogen peroxide, and Gallic acid were acquired from Sigma-Aldrich (Mumbai, India). The study directly employed all of these analytical-grade chemicals without the need for any purification procedures. The plant of *I. confertiflora* were obtained from the Yebokilla district, Amhara region, Ethiopia, in August 2024. The identified voucher specimen of *I. confertiflora* was labelled AB-002.

2.2 Methods

2.2.1 Plant Material Extraction

The mature root parts of *I. confertiflora* that were gathered were cleansed with distilled water to remove impurities, detritus, and sediment under the flow of potable water. The plant material was first dried under shade conditions and subsequently pulverized into fine fragments using a grinder. In in line with their polarity, n-hexane, ethyl ether, and acetone were used to

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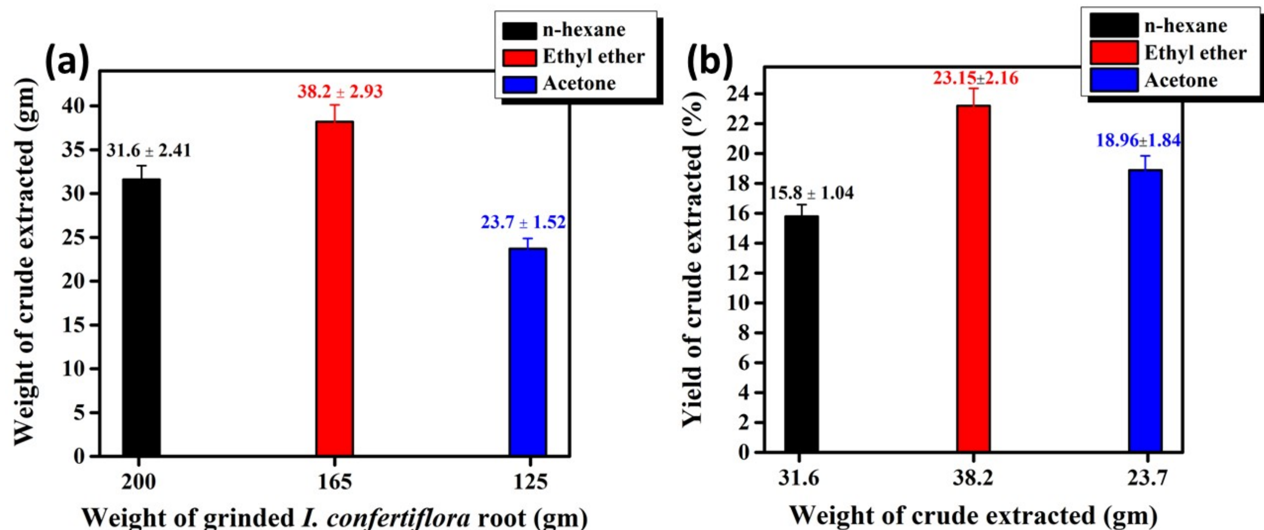

Figure 1. (a) Net Weight in Grams and (b) Yield in Percentage of Crude Extracts *I. confertiflora* Root in n-hexane, Ethyl Ether, and Acetone Solvent

Table 1. Compounds of *Inula confertiflora*

Compound	Source	Reference	Pubchem ID
Isotelekin	Leaf	(Ahmed et al., 2024)	12304585
asperilin	Leaf	(Ahmed et al., 2024)	257274
Carabrone	Leaf	(Ahmed et al., 2024)	164879
Carpesioline	Leaf	(Ahmed et al., 2024)	10015663
graveolide	Leaf	(Ahmed et al., 2024)	11043090
inuviscolide	Leaf	(Ahmed et al., 2024)	176489
Epifriedelanol	Root	(Gashu, 2022)	119242
Dammara-20,24-dien-3-yl acetate	Root	(Gashu, 2022)	14137680

macerate the powdered root parts of the plant for 3 consecutive days. Following a 72-h room temperature maceration with the corresponding solvent in a crude-to-solvent ratio of 1:5, in accordance with the solvent's boiling point, the mixture was filtered and concentrated using a rotating vacuum evaporator. The well-evaporated and dried crude extracted yield from the root *I. confertiflora* was then refrigerated for further experimental study. The extract yields were ascertained using the suitable solvents by computing the dried and centrifuged extracts of the plant according to the following formula (1):

$$\text{Percentage (\%) yield of the extracted crudes} = \frac{\text{Weight of centrifuged and dried extracts}}{\text{Weight of the washed and dried root of } \textit{I. confertiflora}} \times 100 \quad (1)$$

2.2.2 In Vitro Qualitative Phytochemicals Screening Test

The presence of secondary metabolites, such as phenols, carotenoids, alkaloids, terpenoids, saponins, steroids, flavonoids, and tannins, was determined in the crudes obtained from *I. confertiflora* root extracts using various solvents, as per the standard procedures detailed by Kokate et al. (2001).

2.2.3 In-Vitro Quantitative Phytochemical Assessment

2.2..3.1 Estimation of Total Phenolic Contents

The Folin-Ciocalteu method using Gallic acid as a standard, as described by Samatha et al. (2012) with slight modification. In summary, the standard Gallic acid solution (0.1 to 3.2 μg/mL), the solvent of the dissolution, and the five independent concentrations of root extracts (10 and 60 mg/mL) were pipetted into distinct test tubes. The reaction was vortexed after the addition of approximately 2.0 mL of Folin-Ciocalteu reagent, and it was allowed to stand at ambient temperature for approximately 5 minutes. Consequently, the extracts' absorbance was determined at 765 nm using an ultraviolet-visible spectrophotometer after the mixture was vortexed with approximately 1 mL of 7.5% anhydrous sodium carbonate solution and allowed to remain at 37 °C for 30 to 40 minutes. Triplicates of this experiment were implemented. Following the calculation of the concentration of Gallic acid in the root extract from the calibration curve using the absorbance of Gallic acid, the total phenolic content of the extracts was represented as milligrams of Gallic acid equivalent per gram of dry extract (mg GAE/g) using the following formula (2):

$$\text{TPC} = C\frac{V}{M} \quad (2)$$

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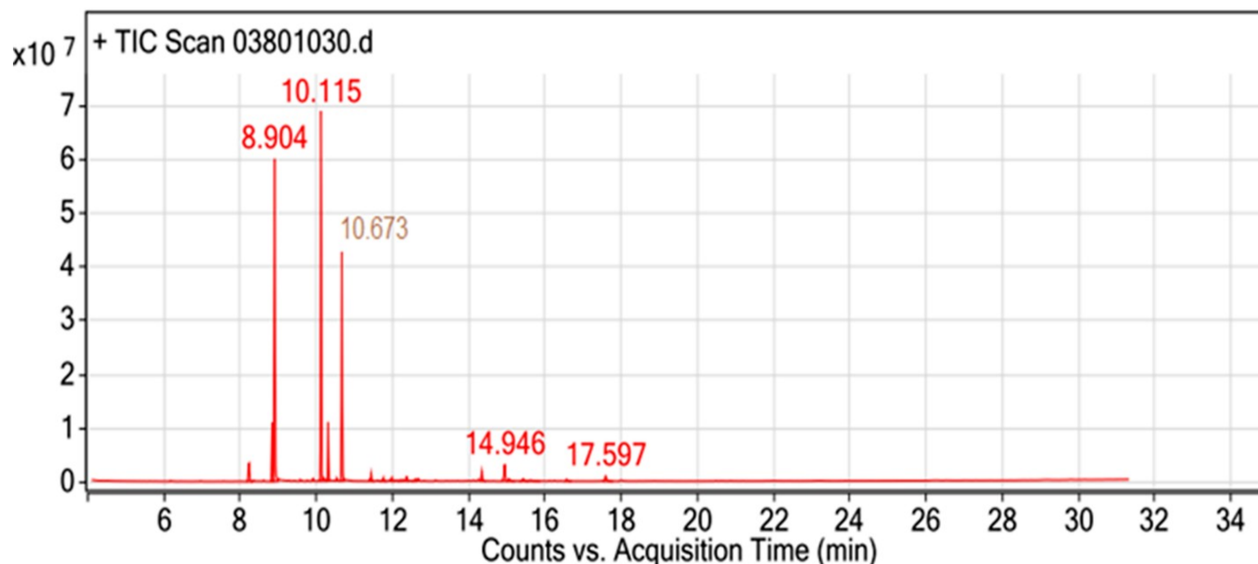

Figure 2. GC-MS Results of Root Powder of *I. confertiflora*

TPC : total phenolic content in mg/g expressed as GAE (Gallic acid equivalent)
C : concentration of the Gallic acid determined from the calibration curve in mg/mL
V : volume of the root extract in mL
M : mass of the dry root of *I. confertiflora* extract in gm.

2.2.3.2 Calculation of the Total Concentration of Flavonoids

The colorimetric approach using aluminium trichloride as delineated by Kamtekar et al. (2014) was used with little alteration. In brief, the standard quercetin solution (0.1 to 3.2 μg/mL), the dissolving solvent, and five independent root extracts (10 and 60 mg/mL) were dispensed into separate test tubes. After the incorporation of about 2.0 mL of distilled water, the mixture was subjected to vortexing. Subsequently, about 0.5 mL of 5% sodium nitrate was incorporated into each combination, which was then allowed to equilibrate at ambient temperature for around 10 minutes. 1mL of 1M sodium hydroxide was added to the mixture after 10 mins, and after that, 0.2 mL of aluminum chloride was added to the mixture and let to stand for an additional 20 mins. The solution was diluted with 4 mL of distilled water and properly mixed before measuring the absorbance at 510 nm using an ultraviolet-visible spectrophotometer. This experiment was conducted in triplicate. The total flavonoid content of the extracts was quantified as milligrams of quercetin equivalent per gram of dried extract (mg QE/g) by determining the extract concentration in the root extract from the calibration curve based on their absorbance (3):

$$TFC = C\frac{V}{M} \tag{3}$$

TFC : total flavonoid content in mg/g expressed as QE (quercetin equivalent)
C : concentration of the quercetin determined from the calibration curve in mg/mL
V : volume of the root extract in mL
M : mass of the dry root of *I. confertiflora* extract in gm.

2.2.4 Extracts' Chemical Composition

The possible volatile components in the essential oil of about 50 grams of *I. confertiflora* root powder were investigated using the hydro-distillation technique, and the outcomes were analyzed using GC-MS.

2.2.5 In Vitro Antioxidant Activity of the Root Extracts

2.2.5.1 2, 2-Diphenyl-1-picrylhydrazyl Radical Scavenging Activity Assay

The in vitro antioxidant activity experiment was performed using the DPPH technique established by Kibiti and Afolayan (2015) to evaluate the free radical scavenging activity of *I. confertiflora* root extracts, with minor changes added. In brief, a 0.4mM DPPH solution in methanol was made, and 2 mL of the DPPH solution was combined with 2 mL of each plant fraction (5 μg/mL to 320 μg/mL) for every standard ascorbic acid concentration. Additionally, a control solution was created, including DPPH and standard methanol. Following the vertexing of the solution, it was let to remain at ambient temperature for 30 minutes in the absence of light. The absorbance of the solution at 517 nm was measured using a UV-Vis spectrophotometer. Methanol served as the blank solution, whilst ascorbic acid functioned as the positive control. Finally, the overall free radical scavenging efficacy, expressed as a percentage, of crude extracts used with various solvents was determined using the following formula (4):

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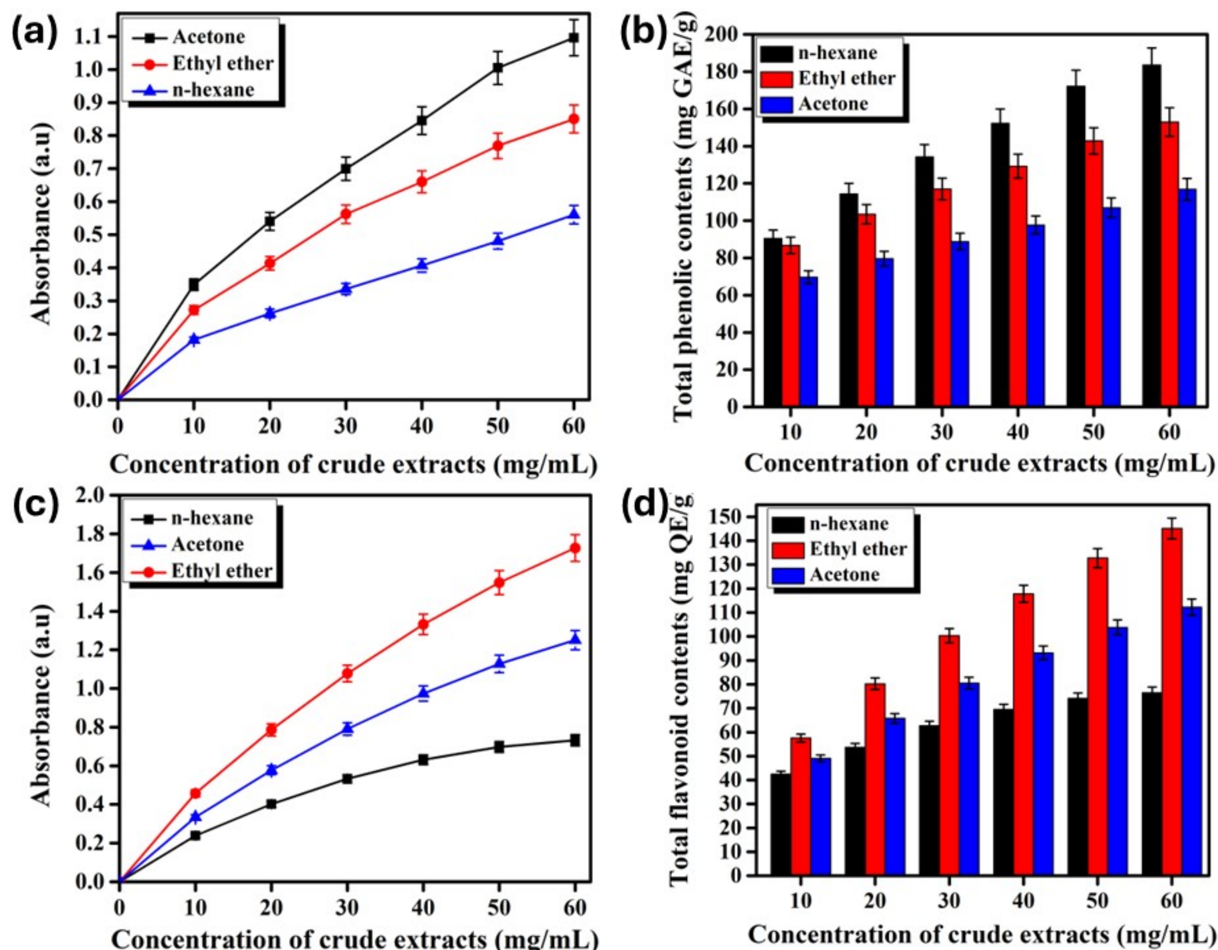

Figure 3. (a) The UV-Vis Absorbance Intensity (a and c), and (b), and Total Content of Flavonoids (d) in Crude Extracts at Corresponding Solvents at Various Concentrations

$$\text{DPPH scavenging efficiency in percentage} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (4)$$

Where Asample represents the absorbance of the solution containing the DPPH solution mixed with extracts, while Acontrol represents the absorbance of the control, which consists of the DPPH solution and methanol. Three separate runs of the experiment were carried out.

2.2.5.2 Ferric Reducing Antioxidant Power of the Plant Root Extracts

The ferric reducing capacity of root extracts obtained from n-hexane, ethyl ether, and acetone solvents was assessed using the procedures outlined by Aiyegoro and Okoh (2010) with minor modification. A mixture comprising approximately 1.5 mL of 0.2 M phosphate buffer solution at pH 6.6 and 1.5 mL of 1% potassium hexacyanoferrate solution was combined with 2.0 mL of root extracts of *I. confertiflora*, which were obtained using n-hexane, ethyl ether, and acetone solvents, along with a standard solution ranging from 5 μg/mL to 320 μg/mL concentration. The solution was incubated at 50 °C for around 20 minutes. Following incubation of the mixture, the reaction was terminated by the addition of 1.5 mL of 10% trichloroacetic acid. Subsequently, the mixture was vortexed for 5 minutes. Subsequently, roughly 1.5 mL of the supernatant was extracted, and the residual mixture was amalgamated with an additional 1.5 mL of distilled water. The mixture was then mixed with 0.5 mL of freshly prepared $FeCl_2$ and $FeCl_3$, each at a concentration of 0.1%, and allowed to stand for approximately 10 mins. The absorbance of the compounds was measured at 593 nm using an ultraviolet-visible spectrophotometer. The buffer solution served as a control, whilst potassium hexacyanoferrate acted as a positive control. The proportion of FRAP in crude extracts obtained from various solvents was determined using the following formula (5):

$$\text{FRAP value in percentage} = \left[\frac{(A_c - A_b)x^2}{(A_s - A_b)}\right] \times 2 \qquad (5)$$

Where Ac represents absorbance of control with FRAP reagents, As represents absorbance of sample with frap reagent, and Ab represents absorbance of blank, reacted with solvent

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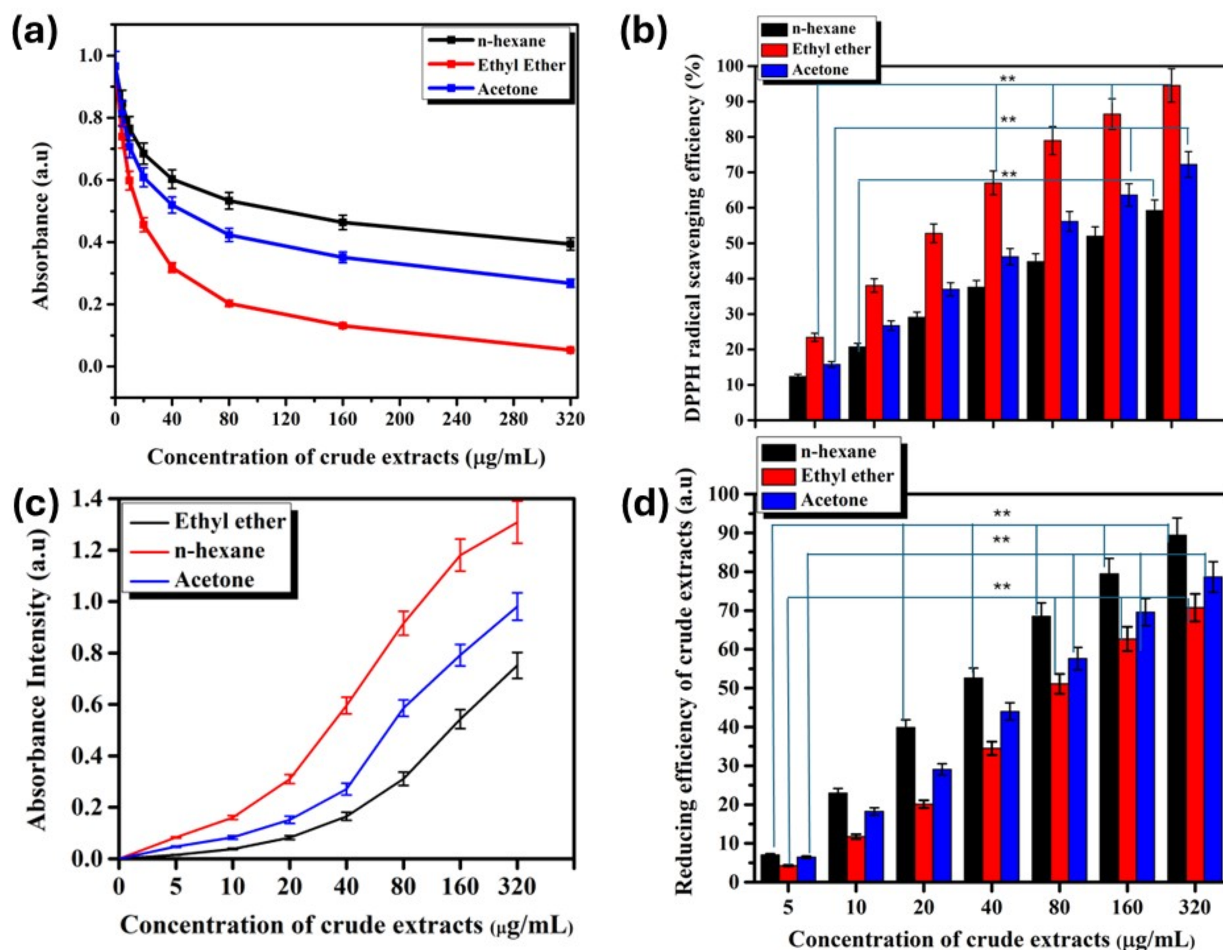

Figure 4. The UV-Vis Absorbance (a and d), Radical Scavenging (b) and Reducing (d) Efficency of Crude Extracts of Root *I. confertiflora* from Corresponding Solvent at Various Concentrations. Statistical Analysis; * = $p > 0.05$, ** = $p \leq 0.05$, (n = 3)

and FRAP reagent. Three separate runs of the experiment were carried out.

2.3 Bacterial Strains

The antibacterial effectiveness of extracts from *I. confertiflora* root, obtained using n-hexane, ethyl acetate, and acetone, was investigated against two gram-positive pathogens (*S. aureus* and *S. pneumonia*) and two gram-negative strains (*E. coli* and *Pseudomonas aeruginosa*). The Department of Biology of Bahir-Dar University, College of Natural Sciences, cultivated all the bacterial strains.

2.3.1 In Vitro Antibacterial Activity of Root Extracts

The antibacterial activity test for crude root *I. confertiflora* employed at several solvents was assessed against gram-positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains using the Hudzicki (2009) disc-diffusion method with certain modifications. In particular, the isolated bacterial strains were cultivated in nutrient broth and cultured for 24 hours at 37 °C. After that, 20 milliliters of nutrient agar were added to sterile plates and left to settle. Continually, using a sterile swab spreader, 100 μL of each test organism was streaked uniformly across the plates. The sterile paper discs were made by impregnating them with distinct crude extracts that were obtained in various solvents at a 10% w/v concentration in DMSO. The plates were incubated at 37 °C for 24 hours after each disc was loaded with 100 μg/mL of 20 μL of the separate crude-extracted solution. The zone of inhibition that formed around the discs was measured using a calliper. The DMSO solvent was employed as a negative control, and chloramphenicol, an antibiotic, was employed as a standard.

2.3.2 Molecular Docking Simulation

Molecular docking analysis was performed to further confirm the antibacterial activity observed in the experimental assays by evaluating the interaction of selected ligands with four key bacterial target proteins, including LasB from *Pseudomonas aeruginosa*, PBP2a from *Staphylococcus aureus*, FabH from *Escherichia coli*, and MurA1 from *Streptococcus pneumoniae*. The compounds of *I. confertiflora* were used as ligands which first identified through a study literature, then their three-dimensional struc-

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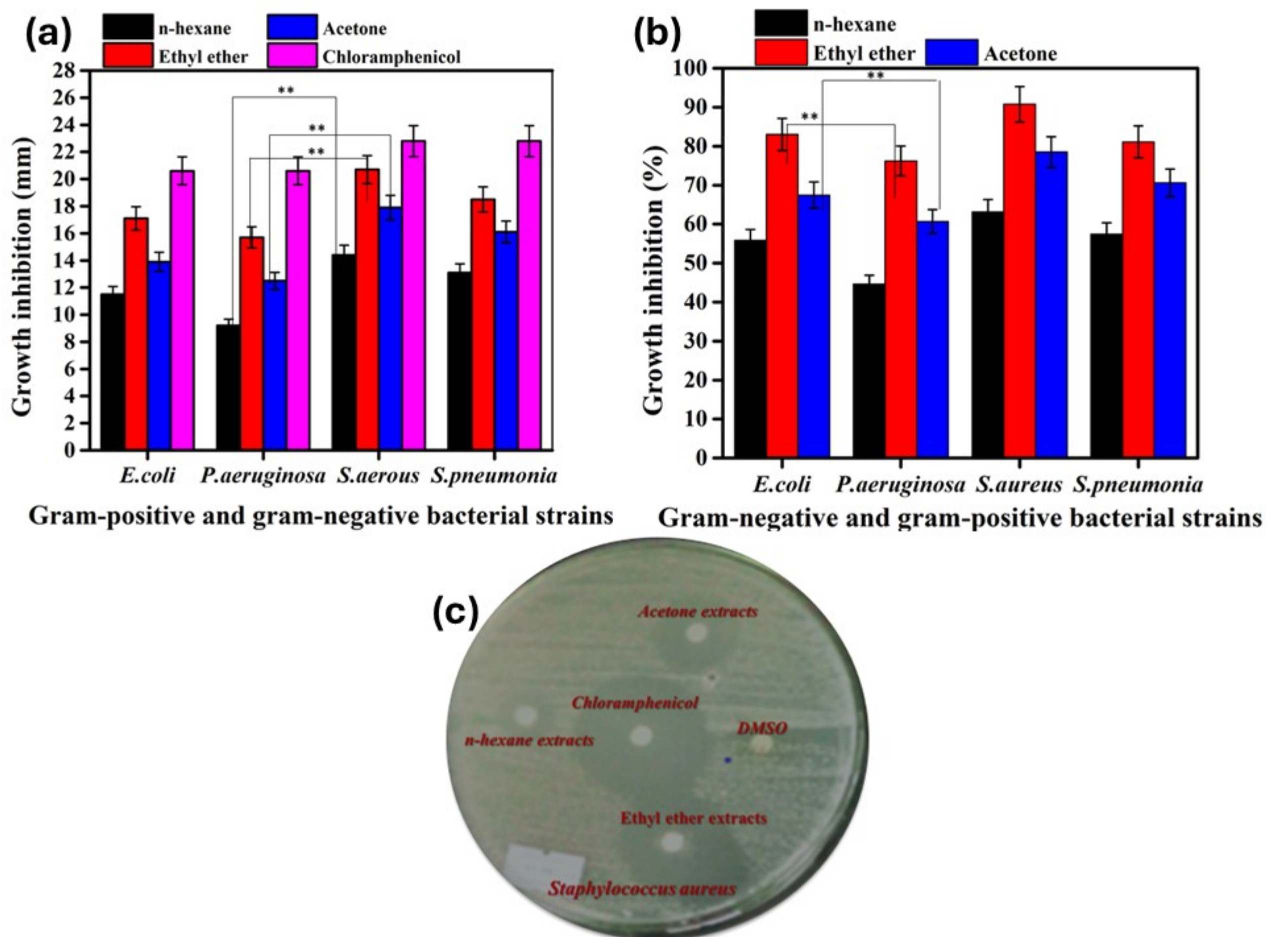

Figure 5. Growth Inhibition in Millimeter (a) and in Percent Efficiency (b) of Crude Extracts Against Gram-Negative and Gram-Positive Bacterial Strains as Well as Inhibition Disc (c) Against *Staphylococcus aureus* Bacterial Strains. Statistical Analysis; * = $p > 0.05$, ** = $p \leq 0.05$, (n = 3)

Table 2. Preliminary Phytochemicals Result of *I. confertiflora* Root Found in Corresponding Solvent Crude Extracts

Phytochemicals	n-Hexane Extracts	Ethyl Ether Extracts	Acetone Extracts
Phenols	+	+	+
Carotenoids	+	−	−
Alkaloids	−	+	+
Terepenoids	−	+	+
Saponins	+	+	+
Steroids	+	+	−
Flavonoids	+	+	+
Tannins	−	+	+

ture was retrieved from PubChem database (Table 1). The crystal structures of the target proteins and their native ligands were downloaded from the Protein Data Bank database with ID number 5bns for FabH; 8cc4 for LasB; 1mwt for PBP2a; and 3zh3 for MurA1. The proteins and native/control ligand were prepared using BIOVIA Discovery Studio. Molecular docking simulations were conducted using PyRx integrated with AutoDock Vina, with the active sites defined based on conserved catalytic residues and the coordinates of native ligands. The interaction patterns and binding affinities from the docking results were evaluated to assess their potential as antibacterial candidates using BIOVIA Discovery Studio.

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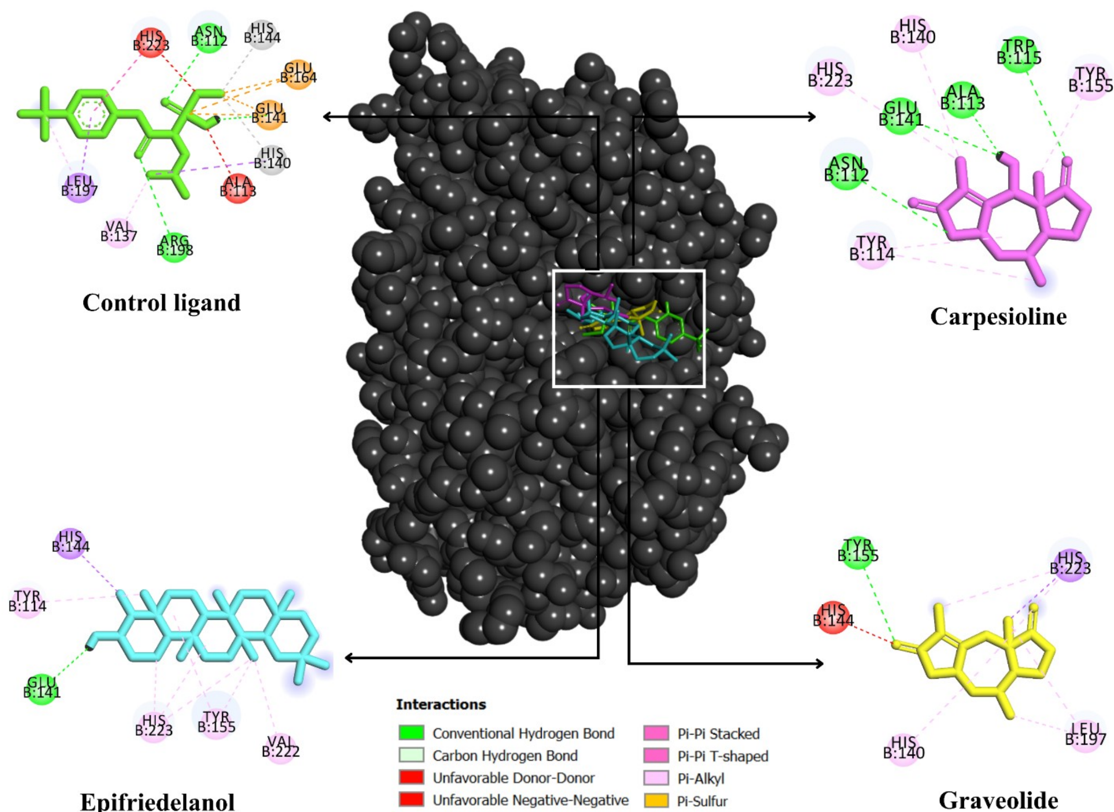

Figure 6. Molecular Docking Analysis of Control Ligand and Phytochemical Ligands (Graveolide, Carpesioline, and Epifriedelanol) with the LasB Elastase Protein of *Pseudomonas aeruginosa*

Table 3. Chemical Composition of Essential Oil from *I. confertiflora* Root 50 mg Oil, 0.1% Yield

Peak No	Compound Name	Chemical Structure	Retention Time (min)	% Comp	Quality
1	2-Methyl-3-phenyl-2-propenal	477	8.231	02.50	70%
2	3-Methyl-4-isopropylphenol	478	8.841	04.93	93%
3	Thymol	233	8.904	30.92	70%
5	2,5-Dimethoxy-4-isopropyltoluene	479	10.115	31.86	91%
6	1,4-Dimethoxy-2-methyl-5(prop-1-en-2-yl) benzene	480	10.311	04.70	94%
8	1-Methoxy-4-(1-methylethyl) benzene	481	10.673	18.10	76%

3. RESULTS AND DISCUSSIONS

3.1 Extraction Yield

The 200-gram powdered root sections of *I. confertiflora* were effectively soaked in n-hexane, ethyl ether, and acetone, respectively for 72 h. The residue from the crude extract was soaked three times for a few mins each with a little amount of the corresponding solvent in order to extract the residual components that might have a tendency to extract with the corresponding solvents. The polarity nature of the organic solvent is a crucial factor in determining the extraction yield and the type of components present in the *I. confertiflora* root portions that are extracted from the associated solvent. The yield of crude extracts obtained from *I. confertiflora* roots in n-hexane shown in Figure 1a was noticeably higher than that of crude extracts derived in acetone solvent. The increased yield from the plant's root sections in n-hexane solvents may indicate that the *I. confertiflora* root contains a large number of non-polar chemicals. This finding suggests that a high concentration of lipophilic compounds, such as certain terpenoids and components based on long-chain fatty acids, were extracted from the plant using n-hexane (Braca et al., 2001; Youn et al., 2019). Furthermore, the higher yield of extracts in n-hexane compared to acetone extracts implies that more coarse-natured

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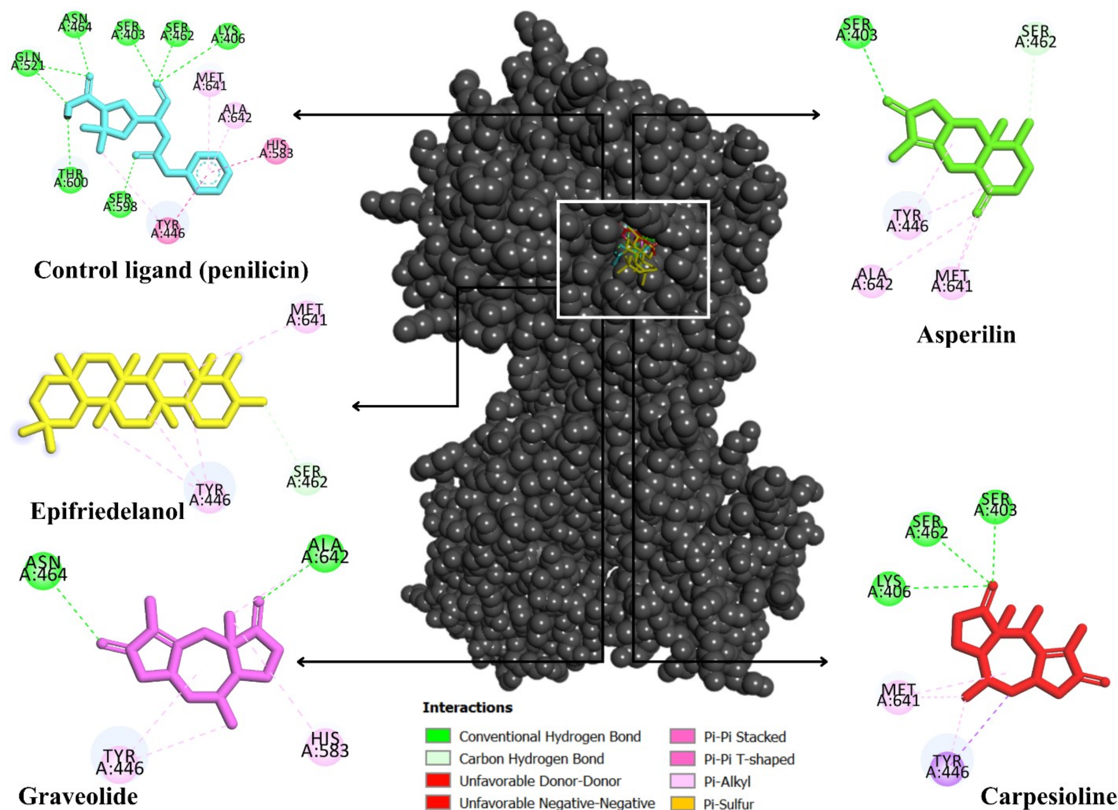

Figure 7. Binding Poses and Interaction Maps of Control Ligand (Penicillin) and Phytochemical Ligands (Epifriedelanol, Asperilin, Graveolide, and Carpesioline) Docked to the PBP2a Protein of *Staphylococcus aureus*

Table 4. Binding Affinity Between *Inula confertiflora*'s Compound with Bacterial's Proteins

Ligand	Binding Affinity (kcal/mol)			
	LasB Protein	PBP2a Protein	Fabh Protein	MurA1 Protein
LasB's Control	-7.9	-	-	-
PBP2a's Control	-	-8.0	-	-
Fabh's Control	-	-	-11.3	-
MurA1's Control	-	-	-	-4.7
Dammara-20,24-dien-3-yl Acetate	-	-	-7.8	-7.8
Graveolide	-7.1	-8.2	-7.0	-
Epifriedelanol	-7.4	-8.9	-7.1	-8.6
Carpesioline	-7.2	-8.3	-	-7.0
Asperilin	-	-8.2	-	-

phytochemicals from *I. confertiflora* root were recovered with non-polar characteristics in line with the "like dissolves like" concept (Carreno-Quintero et al., 2024). These compounds could play a significant role in the plant's overall medicinal properties, warranting further investigation into their potential applications in pharmaceuticals. On the other hand, the choice of solvent plays a significant role in the extraction efficiency of bioactive compounds from the plant (Nabi et al., 2025). Moreover, the yield of crude extraction delivered using the ethyl ether solvent was much higher than those in the acetone and n-hexane solvents. Ethyl ether, which is more polar than n-hexane and less polar than acetone, was used to extract fine-natured phytochemicals with intermediate polarity found in the root sections of *I. confertiflora* (Rathod and Sihare, 2024). These findings suggest that the remaining plant root components were medium- to high-polar, with more active functional groups, such as amine, carbonyl, and hydroxyl moieties. These functional groups may enhance the solubility of certain com-

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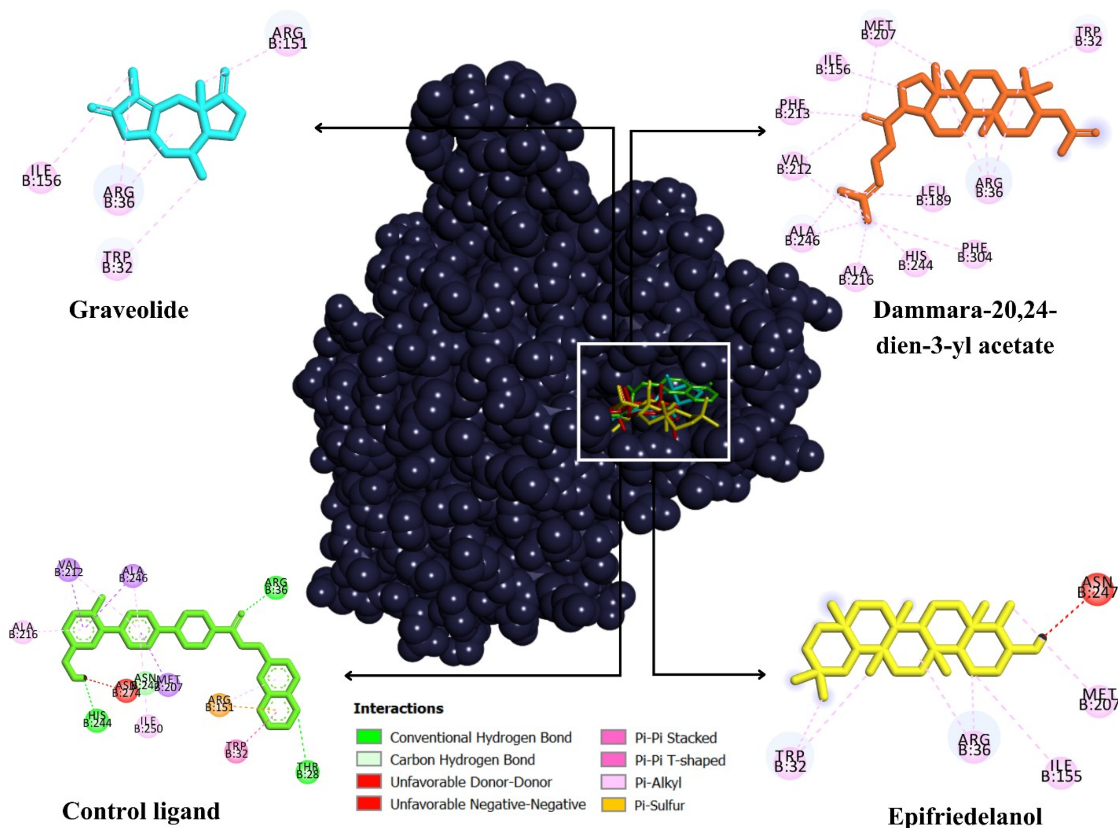

Figure 8. Docking Visualization of the Control Ligand and Phytochemical Ligands (Dammara-20,24-Dien-3-yl Acetate, Epifriedelanol, and Graveolide) within the Active Site of FabH Protein from *Escherichia coli*

pounds in ethyl ether, thereby improving extraction efficiency. The crude extracts obtained with ethyl ether had a somewhat higher percentage yield than those obtained with n-hexane, as illustrated in Figure 1b. A popular solvent for the extraction of specific, mid-polarity plant components, ethyl ether's polarity index position makes it ideal for isolating substances that need a mild environment to dissolve (Kamtekar et al., 2014; Kibiti and Afolayan, 2015). This could suggest that the ethyl ether solvent extracts somewhat more polar components. These polar components may include a variety of phytochemicals that could have different biological activities compared to the non-polar compounds extracted using n-hexane. The percentage yield of *I. confertiflora* root crude extracts from acetone solvent was much higher than that of n-hexane extracts. This could suggest that stronger polar solvents were used to extract most of the polar components present in the root of *I. confertiflora*. Additionally, this finding suggests that the bioactive compounds in *I. confertiflora* may be more soluble in acetone, facilitating a more efficient extraction process. This efficiency in extraction could lead to a more potent formulation for potential therapeutic applications, as the bioactive compounds are likely to play a crucial role in the plant's medicinal properties. Taking into consideration the yield of the crude extracts produced at different solvents, the quality and quantity of phytochemicals delivered at different solvents are further examined. This analysis may help identify the most effective solvent for extracting specific compounds, thereby enhancing the overall efficacy of the extracts. A preliminary phytochemical screening test was conducted to determine the kind and nature of components present in the crude extracts obtained from the root of *I. confertiflora* in corresponding solvent.

3.1.1 Qualitative Phytochemical Studies of *I. confertiflora* Root

The medicinal effectiveness of plants is determined by the internal secondary phytochemical constituents produced by different parts of the plant at various stages. These phytochemicals can vary significantly in type and concentration depending on several factors, including the type of plant and its environment. Preliminary phytochemicals, or secondary metabolites, are diverse substances produced by natural organisms with differing capacities for surviving in various environments. These compounds often play crucial roles in plant defense mechanisms, helping to deter herbivores and inhibit the growth of pathogens. The majority of secondary phytochemicals were detected in the ethyl ether extracts from the *I. confertiflora* root, as shown in Table 2. These results shows that polar and semi-polar-based compounds percolated in the ethyl ether solvents. These interactions may lead to variations in solubility and extraction

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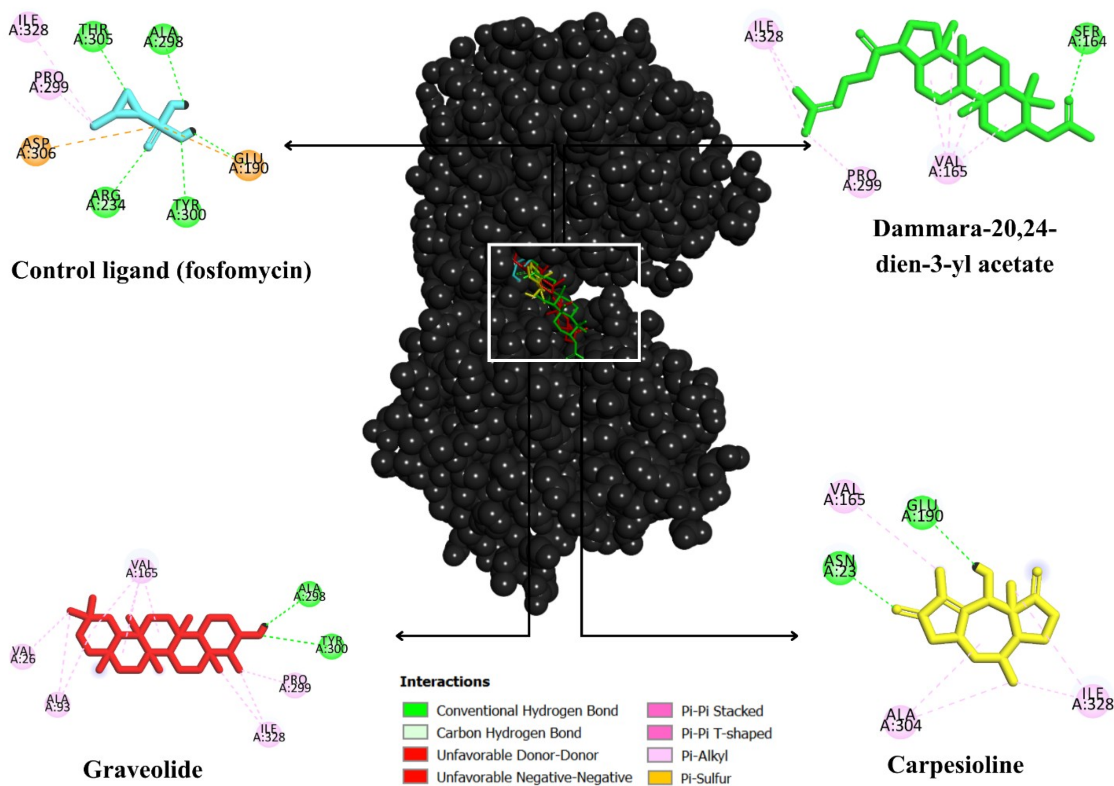

Figure 9. Molecular Docking Interactions of Control Ligand (Fosfomycin) and Selected Phytochemical Ligands (Dammara-20,24-dien-3-yl Acetate, Graveolide, and Carpesioline) with the MurA1 Protein of *Streptococcus pneumoniae*

efficiency, affecting the overall yield of desired compounds. Furthermore, these findings also suggest that ethyl ether may be utilized to extract potential bioactive constituents from *I. confertiflora* roots. The fact that the common and extensive polar-based constituents were delivered in the acetone extracts of *I. confertiflora* root suggests that acetone extracts may contain more polar molecules than those discovered in ethyl ether extracts. This finding indicates that acetone could be a more effective solvent for isolating a broader range of polar compounds from this plant species.

Moreover, hydrodistillation of powdered roots (50 g) produced 50 mg of essential oil, giving an extraction yield of 0.1%. Subsequent GC–MS characterization identified six major volatile constituents that collectively accounted for 93.01% of the oil composition, while several other compounds were detected at lower concentrations (Table 3 and Figure 2). Among the identified constituents, 2,5-dimethoxy-4-isopropyltoluene (31.86%) (479) was the most abundant, followed by thymol (30.92%) (233) and 1-methoxy-4-(1-methylethyl)benzene (18.10%) (481).

3.1.2 Quantitative Phytochemical Studies of *I. confertiflora* Root

The crude extracts of *I. confertiflora* root contain an estimated total amount of phenols and flavonoids. The standard procedures were employed to analyse the confertiflora that was obtained using n-hexane, ethyl ether, and acetone solvents. The calibration curve for gallic acid, with concentrations from 10 to 120 ppm, was measured at a wavelength of 764 nm, yielding a calibration equation of $Y = 124.59X + 47$, used to ascertain the total phenolic content of the extracts.

As shown in Figure 3a, the absorbance intensity of n-hexane crude extracts was lower than the ethyl ether and acetone extracts, which implies that phenolic and phenolic-based compounds were extracted using polar solvents. However, trace amounts of semi-polar phenolics compounds can be recovered and exhibit positive colorimetric values, like blue, in non-polar solvents. The absorbance intensity of crude extracts from root *I. confertiflora* using acetone solvent was higher than that of ethyl ether extracts, suggesting that more concentrated and interacting phenolic and phenolic-based compounds were recovered in acetone solvents. Importantly, as the concentration of the extracts increased from 10 to 60 mg/mL, the absorbance intensity increased significantly, which may imply that the aggregation in each solvent increased significantly. This suggests that higher concentrations of phenolic-based compounds within the crude extracts could enhance the interaction between molecules, leading to greater aggregation. The estimated total amount of the phenols presents in the root *I. confertiflora* extracts using n-

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hexane, ethyl ether, and acetone solvents was determined using the gallic acid calibration curve and the precise absorbance of the crude extracts with the corresponding concentration at 764 nm. According to Figure 3b, the estimated total phenol content in crudes extracted from ethyl ether and acetone is less than 100 mg GAE gm-1 at the lowest concentration. This could indicate that the electron donation efficiency needed to diminish the complex of phosphotungstic and phosphomolybdic acids was insufficient. The estimated total amount of phenols found in the crude extracts was increased by the concentration, which may indicate a direct proportionality between the concentration and total phenolic-based phytochemicals. More importantly, crude extracts of *I. confertiflora* roots obtained with acetone solvent had greater total phenolic content than those delivered with ethyl ether solvents. These findings describe that the highest phenolic and phenolic-based contents were dissolved in more polar acetone solvents. phenolic-based compounds within each extract have the potential to interact internally and show higher absorbance intensity at higher concentration. This finding indicates that ethyl ether is a more effective solvent for isolating phenolic compounds from the root of *I. confertilora*. As shown in Figure 3c, the total estimated flavonoid contents extracted using acetone were marginally lower than those extracted using ethyl ether. These confirmed that medium polar solvents were used to extract the lower and medium flavonoid-based constituents. Flavonoid and flavonoid-based compounds play a significant role in treating various diseases; as a result, the total flavonoid content found in root *I. confertiflora* extracted in n-hexane, ethyl ether, and acetone was investigated using a UV-Vis spectrophotometer at a maximum wavelength of 510 nm.

As shown in Figure 3d, the crude extracts obtained in n-hexane exhibited a lower absorbance intensity compared to the ethyl ether and acetone extracts. This could indicate that the extraction efficiency of flavonoid-based polar constituents from *I. confertiflora* root using non-polar solvents was low. The absorbance intensity of ethyl ether extracts was significantly higher than that of n-hexane and acetone, which may suggest that high- and medium-polarity phenolic compounds were soluble and extracted in ethyl ether solvents. Moreover, the estimated total flavonoids extracted with more polar acetone solvents were lower than the total flavonoids obtained in ethyl ether solvents, suggesting that the higher polar solvents extracted higher molecular flavonoid-based compounds from *I. confertiflora* root in three days of soaking. The total estimated content of flavonoids was lower than the total phenols derived from *I. confertiflora* root in all solvents at the same concentration. This implies that lower, medium, and higher flavonoid-based compounds are a subclass of phenols. This finding highlights the intricate relationship between flavonoids and phenolic compounds, suggesting that while they may exhibit distinct biological activities, they are interconnected within the broader category of polyphenols. The presence of preliminary phytochemicals in plants plays a significant role in maintaining health through scavenging radicals from toxic chemical excretion in the body. These compounds can help reduce oxidative stress and inflammation, ultimately contributing to overall wellness.

The antioxidant properties of plants are significantly improved by the presence of preliminary phytochemicals, including saponins, flavonoids, and alkaloids, in their roots (Aiyegoro and Okoh, 2010). The UV-Vis absorbance intensity was measured at 517 nm for each concentration made from crude extracts of *I. confertiflora* roots obtained from n-hexane, ethyl ether, and acetone solvents. As shown in Figure 4a, the absorbance intensity features of crude extracts in DPPH from *I. confertiflora* root exhibited variation at various concentrations in n-hexane, ethyl ether, and acetone solvents at a consistent wavelength. Importantly, the change of color from purple at low concentration to intensive yellowish at higher concentration of the crude extracts was inversely proportional to the absorbance intensity characteristics in DPPH solution, implying that there was a possible change in antioxidant qualities of the extracts at different concentrations. The absorbance intensity of the ethyl ether crude extracts was lower than acetone and n-hexane crude extracts with increasing concentration, which may suggest that the presence of different phytochemical constituents enhances the reduction of free radicals from DPPH. The lower absorbance intensity at 517 nm for the ethyl ether extract indicates a higher degree of free radical scavenging compared to the other extracts. This suggests that ethyl ether effectively extracted the compounds with antioxidant properties from the *I. confertiflora* root, which then reacted with and neutralized the DPPH free radicals, causing the purple color to fade (and thus reducing the absorbance at 517 nm). Moreover, using a standard formula, the radical scavenging efficacy of crude extracts in percentage for *I. confertiflora* root extracts in comparable solvents was assessed independently, and the outcomes showed substantial variation across the solvents. As shown in Figure 4b, the overall percentage radical scavenging efficiency of n-hexane extracts was lower than the acetone crude extracts of root *I. confertiflora*. The efficiency of DPPH radical scavenging in percentage against n-hexane crude extracts was 59.22 ± 3.1 at 320 μg/mL and 12.31 ± 0.8 at the lowest concentration of 5 μg/mL. This suggests that the capacity of constituents found in the low polar extracts to donate hydrogen atoms increased gradually at higher concentrations. Similarly, at a greater concentration of 320 μg/mL, the electron donation efficiency of acetone crude extracts was $72.25 \pm 3.6\%$, suggesting that the crudes obtained at higher polar solvents had the propensity to donate electrons to diminish the free radicals present in DPPH. Compared to n-hexane and acetone extracts, ethyl ether crude extracts of *I. confertiflora* demonstrated a higher reduction efficiency of free radicals detected in DPPH. This could suggest that the inclusion of medium- and low-polar components extracted with medium-polar ethyl ether solvents may enhance the hydrogen donation to reduce free radicals found in DPPH and produce diphenyl picrylhydrazine's deep yellowish color. More significantly, the hydrogen donation efficiency increased as the concentration of acetone crude extracts increased. This may suggest that the intermolecular interaction between low-

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and semi-polar constituents in methanol solution accelerates the migration of electrons or hydrogen to form stable DPPH-H. Furthermore, the antioxidant efficiency of root *I. confertiflora* was assessed using the FRAP assay.

The antioxidant efficiency of crude extracts was measured by the reducing power of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to intensify the blue ferrous-tripyridyltriazine complex (Fe^{2+}-TPTZ) with an absorption maximum at 593nm. As shown in Figure 4c, the absorbance intensity of crude extracts delivered in nonpolar n-hexane solvents from *I. confertiflora* root was increased significantly, implying that the ferric reducing efficiency of constituents found in crude extracts of nonpolar was lower. The absorbance intensity of ethyl ether extracts was increased slightly with increasing concentration, which may suggest that the presence and interaction of various constituents enhance ionization potential. As demonstrated in Figure 4d, the percentage of reduced ferrous-tripyridyltriazine by crude extracts increased with increasing concentration, indicating that potential internal intermolecular interactions among constituents may improve the transfer of hydrogen and reduction potential in a particular solution. This suggests that optimizing the concentration of these extracts could enhance their effectiveness in reducing ferrous ions. Based on the result, the free radical scavenging and ferric-reducing capacity exhibited by the root extracts of *I. confertiflora*, provided a basis for subsequent evaluation of their in vitro antibacterial activity against a range of Gram-positive and Gram-negative bacterial species.

Crude extracts delivered from various plant components can have antibacterial properties through different mechanisms, including increasing intracellular osmotic pressure, blocking nucleic acid, and rupturing cell membranes (Kozhantayeva et al., 2024; Krakowska-Sieprawska et al., 2022; Mallhi et al., 2018). Importantly, the root of *I. confertiflora* includes a variety of compounds listed in Table 3 that may enhance the antibacterial properties against both gram-positive and gram-negative bacterial strains. Thus, using the agar well diffusion method, the antibacterial activity of crude extracts from the plant, administered in non-polar, mid-polar, and polar solvents, was evaluated against both gram-positive and gram-negative bacterial strains. In this investigation, chloramphenicol, a widely used antibiotic medication, was employed as a positive control, and methanol served as a negative control. As shown in Figure 5a, the inhibitory zone, measured in millimeters, of crude extracts delivered from *I. confertiflora* roots against gram-negative microorganisms of *Escherichia coli* and *Pseudomonas aeruginosa* was marginally smaller than that of crude extracts at a concentration of 100 μg/mL obtained in acetone solvent. The maximum millimeters of growth on the disk were recorded for the crude extracts present in the ethyl ether extracts against specific gram-negative bacterial strains of *Staphylococcus aureus*, as confirmed in Figure 5c. This suggests that the presence of various phytochemicals effectively combats bacterial strains and reduces the likelihood of bacterial growth. Overall, the bacterial growth restriction efficiency of crude extracts against *E. coli* was slightly higher than that of *S. aeruginosa*. This could suggest that *E. coli* was more susceptible to *I. confertiflora* crude extracts due to its defensive mechanism and bacterial nature (Okoduwa et al., 2024). Similarly, under comparable experimental conditions, ethyl ether crude extracts were more effective than n-hexane and acetone extracts in inhibiting the growth of Gram-positive bacterial strains of *S. aureus* and *S. pneumoniae*. Furthermore, the effectiveness of *I. confertiflora* root n-hexane crude extracts against all specific bacterial strains was lower than that of the other crude extracts. This suggests that the presence of low and nonpolar elements was insufficient to stop the growth of bacterial strains in the disk. On the other hand, medium- and higher-polarity-based phytochemicals found in medium- and higher-polarity solvents are effective in restricting the growth of bacterial strains. The inhibition efficiency, expressed as a percentage of crude extracts against selected bacteria, was further assessed. Figure 5b shows that n-hexane crude extracts had a decreased penetration efficacy against *P. aeruginosa*. This suggests that the nonpolar components of *I. confertiflora* root are compatible with *P. aeruginosa* bacteria. Acetone extracts were found to inhibit the growth of bacterial strains by over 50% in both gram-positive and gram-negative bacteria. This suggests that the crude extracts contain more polar-based components that may be able to penetrate the bacterial cell wall and inhibit growth (Abeysinghe et al., 2021; Atina et al., 2025; Manayia et al., 2025; Masfria et al., 2023). Furthermore, having more medium-polar and polar components in the crude extracts of medium-polar solvents improves their interaction with specific bacterial strains and inhibits growth on the disk. This enhanced interaction can lead to more effective antimicrobial properties, making medium-polar solvents a valuable tool in the development of natural antibacterial agents. Overall, several phytochemical components with functional groups were present in the extracts derived from the medium-polar elements using ethyl acetate. These preliminary phytochemicals may enhance compatibility with both gram-positive and gram-negative bacterial strains, thereby limiting the growth tendency of the selected bacteria. This offers convincing evidence for those who rely on *I. confertiflora* to treat a range of ailments.

3.2 Molecular Docking Simulations

The molecular docking analysis was further interpreted through a quantitative comparison between phytochemical ligands and control ligands. The molecular docking results demonstrated that several phytochemical ligands exhibited strong binding affinities toward the four key bacterial target proteins, including LasB (*Pseudomonas aeruginosa*), PBP2a (*Staphylococcus aureus*), FabH (*Escherichia coli*), and MurA1 (*Streptococcus pneumoniae*) (Table 4). The binding affinities of the phytochemicals ranged from moderate to strong (-7.0 to -8.9 kcal/mol), indicating their potential antibacterial mechanisms at the molecular level. Based the resulte, epifriedelanol consistently exhibited strong and competitive binding across multiple targets (LasB: -7.4; PBP2a: -8.9; FabH: -7.1; MurA1: -8.6 kcal/mol), highlighting its potential as a multi-target antibacterial agent. This multi-target profile is particularly advantageous, as it may reduce

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the likelihood of resistance development by simultaneously disrupting multiple essential bacterial pathways.

For the LasB protein, the control ligand exhibited a binding affinity of -7.9 kcal/mol. Among the tested phytochemicals, epifriedelanol (-7.4 kcal/mol), carpesioline (-7.2 kcal/mol), and graveolide (-7.1 kcal/mol) showed slightly lower binding affinities as shown at Figure 6. The interaction showed contacts with residues such as His223, His140, Glu141, and His144, which are critical for catalytic zinc dependent proteolytic activity (Kawamoto et al., 1993; Waheeb et al., 2026) (Figure 5). The LasB produced by *Pseudomonas aeruginosa* is a crucial virulence factor and is known to play a central role in the establishment and progression of pseudomonal infections (Cathcart et al., 2011). Although these values are weaker than the control, suggesting that these ligands may still effectively interact with the LasB protein.

In the case of PBP2a, the control ligand showed a binding affinity of -8.0 kcal/mol. Notably, epifriedelanol demonstrated a stronger binding affinity (-8.9 kcal/mol), followed by carpesioline (-8.3 kcal/mol), graveolide and asperilin (-8.2 kcal/mol), all of which surpassed the control as shown at Figure 7. This suggests that these phytochemicals may have a higher binding stability within PBP2a. Phytochemicals showed engagement with Tyr446, the active site gatekeeper residue in PBP2a (Aribisala and Sabiu, 2022). PBPs are key enzymes that catalyze the final stages of peptidoglycan synthesis in bacterial cell walls. In *Staphylococcus aureus*, the enzyme PBP2 plays a crucial role in completing the final steps of peptidoglycan formation and is therefore the primary target of penicillin (Manayia et al., 2025; Pinho and Errington, 2005).

As shown at Figure 8 For the FabH protein, the control ligand displayed a markedly strong binding affinity (-11.3 kcal/-mol), which was significantly higher than all tested phytochemicals (-7.0 to -7.8 kcal/mol). This result indicated that the phytochemicals are less competitive toward FabH compared to the native ligand. Ligands showed interactions with key catalytic residues such as Trp32 and Arg36 (López-López et al., 2022). These results supported the potential of the tested compounds to inhibit fatty acid biosynthesis pathways in Gram-negative bacteria.

For the MurA1 protein, the control ligand exhibited a relatively weak binding affinity (-4.7 kcal/mol) as shown at Figure 9. Several phytochemicals demonstrated significantly stronger binding, including epifriedelanol (-8.6 kcal/mol), dammara-20,24-dien-3-yl acetate (-7.8 kcal/mol), and carpesioline (-7.0 kcal/mol). These compounds might act as effective MurA1 inhibitors. MurA1 catalyzes the first step committed in peptidoglycan biosynthesis, stronger ligand binding at this site could directly impair bacterial cell wall formation, leading to bactericidal effects. MurA1 protein a bacterial enzyme essential for the synthesis of the cell wall's peptidoglycan (Bensen et al., 2012; Fathalla et al., 2022). This protein might be an ideal target for developing new antibacterial drugs because it's essential for bacteria but has no counterpart in human cells (Ahmed et al., 2024; Gashu, 2022).

4. CONCLUSIONS

This study demonstrated that root-derived phytochemicals from *Inula confertiflora* possess promising biological potential, as indicated by their antioxidant capacity, antibacterial activity, and predicted molecular interactions with selected bacterial target proteins. The antioxidant results suggest that the root extract may contain compounds capable of reducing oxidative stress through radical-scavenging mechanisms, while the antibacterial assays indicate that these phytochemicals may contribute to the inhibition of bacterial growth. The presence of various phytochemicals was confirmed in each crude extract using analytical detection methods. The ethyl ether crude extracts of *I. confertiflora* contained high levels of total flavonoids and a variety of phytochemicals, and they demonstrated superior restriction efficacy against the development of both gram-positive and gram-negative bacterial strains. Similarly, molecular docking results provide the potential of *I. confertiflora* roots as antibacterial activities against several bacterial target proteins. These further investigations are essential before *I. confertiflora* root-derived compounds can be considered strong candidates for antibacterial drug discovery or natural therapeutic development.

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