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RESEARCH ARTICLE

In-vitro and *In-silico* Study of Bioactive Compounds from *Coccocarpia erythroxyli* as Inhibitors Targeting PBP3 (6ILE) for Antibacterial Applications

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Abstract

Lichens are recognized as sources of diverse bioactive metabolites with potential antibacterial properties. This study evaluated the antibacterial activity of *Coccocarpia erythroxyli* (Spreng.) Swinscow & Krog and its interaction with Penicillin-Binding Protein 3 (PBP3, PDB ID: 6I1E). Methanol extracts of *C. erythroxyli* were tested against six bacterial strains using the disc diffusion method, revealing moderate antibacterial activity, with the highest inhibition zone observed against *Escherichia coli* (18.6 ± 0.44 mm), compared to chloramphenicol (29.13 mm). GC-MS analysis identified 33 bioactive compounds, which were further evaluated through *in-silico* drug-likeness screening and molecular docking. Among these, Cembrene showed the highest binding affinity (−5.9 kcal/mol), interacting with hydrophobic residues PHE182 and TRP185, while the control compound displayed a slightly stronger affinity (−6.3 kcal/mol). These results highlight the moderate antibacterial potential of *C. erythroxyli* and suggest that its metabolites warrant further investigation as lead compounds in antibacterial drug development, particularly in the context of antibiotic resistance.

Keywords: Antibacterial, Bioactive compounds, *Coccocarpia erythroxyli,* Penicillin-binding protein

Introduction

Lichens are symbiotic systems composed of fungi, algae, and / or cyanobacteria, and they represent promising sources of low molecular weight secondary metabolites. Over 1000 distinct secondary chemicals have been identified in lichens and their cultured mycobionts [1]. Lichens produce two types of metabolites: Primary and secondary. Primary metabolites are intracellular in origin and produced independently by both symbionts. These primary compounds include chitin (found in hyphal walls), lichenin, isolichenin, hemicellulose, pectins, disaccharides, polyalcohols, amino acids, and pigments such as chlorophyll, β-carotenes, and xanthophylls [2]. In contrast, secondary metabolites are generated by the fungal partner and are exported beyond the hyphae, often crystallizing in various regions of the thallus, including the upper cortex and fruiting bodies [3]. Lichens have been utilized as ingredients in folk medicine for centuries, with many cultures employing them to treat various ailments as part of their traditional healing practices [4]. Some lichens possess therapeutic properties recognized in Ayurvedic and Unani systems, and they are used to address a wide range of health issues, including blood and heart problems, bronchitis, scabies, leprosy, asthma, and stomach disorders [5].

Coccocarpia is a genus consisting of approximately 25 lichen species, primarily found in humid tropical climates, with a few species extending into both the northern and southern hemispheres. This genus belongs to the family Coccocarpiaceae and is distributed worldwide in both tropical and temperate regions. Coccocarpia species are characterized by a foliose thallus with a prominent cortex and white medulla, and most exhibit a distinct lobate shape. Key identifying features include the presence of isidia,

apothecia, and unique morphological variations in the isidia [6,7].

While antimicrobial properties have been observed in several *Coccocarpia* species, such as *C. palmicola* [8], the antibacterial activity of *C. erythroxyli* has also been reported by Vinayaka [9]. The antibacterial activity of *C. Erythroxyli* is concentration-dependent, with the highest activity observed against *Bacillus cereus*. However, the specific bioactive compounds responsible for its antibacterial effects remain largely unexplored. Furthermore, the chemical composition and molecular mechanisms underlying its antimicrobial activity have not been thoroughly investigated, leaving a significant gap in current knowledge.

Addressing this gap is crucial, as further exploration of *C. erythroxyli*'s bioactive metabolites could lead to the discovery of novel antibacterial agents with valuable therapeutic potential. The present study aims to explicitly test the hypothesis that bioactive compounds from *C. erythroxyli* inhibit PBP3, a key protein involved in bacterial cell wall synthesis, and thus offer a potential alternative to traditional antibiotics. By employing *in vitro* assays, GC-MS analysis, PASS (Prediction of Activity Spectra for Substances), and molecular docking studies, this research seeks to characterize the bioactive compounds of *C. erythroxyli* and investigate their molecular interactions with PBP3. This investigation will help establish a deeper understanding of its medicinal potential and support its development for pharmaceutical applications.

Bacterial cell wall production is a complex and tightly regulated process essential for cellular survival and morphogenesis. Peptidoglycan (PG), a key component of the cell wall, surrounds the bacterial membrane, protecting it from osmotic lysis. It also serves as a platform for binding virulence factors and adhesins [10]. Penicillin-binding proteins (PBPs) are classified into high-molecular-weight (HMW) and low-molecular-weight (LMW) groups based on sequence homology and molecular weight. They belong to the acyl-serine transferase family [11]. Among PBPs, PBP3 plays a critical role in septal peptidoglycan synthesis during bacterial cell division. It is a key component of the divisome complex [12]. Inhibition of PBP3 disrupts bacterial division, leading to cell death. Thus, PBP3 is an important target for antibiotics [12,13]. However, the rise in resistance to β-lactam antibiotics targeting PBPs, including PBP3, is a growing health concern. Resistance mechanisms such as reduced membrane permeability, PBP mutations, and β-lactamase production have reduced the efficacy of these antibiotics.

New agents, such as cefiderocol and tricyclic β-lactams, offer enhanced strategies by improving membrane penetration and overcoming resistance [14]. PBP3 binds noncovalently to (5S)-penicilloic acid, a hydrolysis product of piperacillin. This interaction provides valuable insights into PBP3 and inhibitor design. In Gram-negative bacteria, reduced membrane permeability and β-lactamase production further complicate antibiotic development. However, compounds like cefiderocol and the novel tricyclic β-lactam address these challenges by enhancing membrane penetration and combating resistance [15].

The PDB structure 6I1E represents PBP3 bound to an inhibitor, offering insights into the binding interactions and the mechanism of these drugs. This structural information is crucial for understanding how inhibitors affect PBP3 function. Such knowledge aids in the development of new antibacterial agents and strategies to combat bacterial resistance. Remarkably, metabolites derived from lichens, such as those from *C. erythroxyli*, have shown the ability to inhibit PBP3 in a manner similar to synthetic antibiotics. This highlights the potential of lichen-derived compounds as natural alternatives to combat the growing challenge of bacterial resistance. These findings suggest new approaches to improving the efficacy of treatments against resistant bacterial strains.

Materials and methods

Preparation of the lichen extracts

C. erythroxyli (Spreng.) Swinscow & Krog collected from Bukit Barisan Grand Forest Park, North Sumatra, Indonesia, were first cleaned and dried at room temperature. The dried lichens were ground into powder using a blender. Approximately 10 g of lichen powder was subjected to maceration extraction with 100 mL of 70% methanol (v/v) at a ratio of 1:10 (lichen powder:solvent). The extraction was carried out at room temperature for 24 h per cycle, using a shaker set at 150 rpm for three consecutive cycles. The mixture was then filtered using Whatman No.1 filter paper to separate the

filtrate. The combined filtrates were concentrated under reduced pressure at the boiling point of methanol (about 65 °C) using a rotary evaporator until a thick extract was obtained. The concentrated extract was subsequently diluted to 50%(v/v) using Dimethyl Sulfoxide (DMSO) to obtain the final extract for further analysis. The concentrated extract was stored at −20 °C until further use.

Antibacterial assays of lichens

Pathogens used for antibacterial activity

The antibacterial activity was evaluated using pathogenic microbes consisting of three Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Propionibacterium acnes* ATCC 6919, and *Streptococcus mutans* ATCC 35668), three Gram-negative bacteria (*Salmonella enterica* serovar Typhii IPBCC b 11 669, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 15442), and *Candida albicans* ATCC 10231.

Antibacterial activity

The antibacterial activity was tested using the disc diffusion method as a preliminary screening. Twenty milliliters of Mueller-Hinton Agar (MHA) medium were poured into sterile petri dishes and allowed to solidify. A bacterial suspension was prepared to a turbidity equivalent to 0.5 McFarland standard and evenly spread on the agar surface using a sterile cotton swab. Discs infused with 100 µL of the lichen methanol extract were placed on the agar surface. Chloramphenicol was used as a positive control, while DMSO served as the negative control. The plates were incubated at room temperature for 24 h. The diameter of the inhibition zones surrounding each disc was measured to assess bacterial growth inhibition. Although the Minimum Inhibitory Concentration (MIC) was not determined in this study, the disc diffusion method provided an initial indication of antibacterial potential.

GC-MS analysis

The filtered sample was injected into a GC-MS instrument with a volume of 1 µl. Volatile component composition analysis was conducted using a Shimadzu GCMS-QP2010 Plus, equipped with a split-splitless injector set at 250 °C. The sample was injected using the split method, and the MS detector temperature was set to 280 °C. The column used was a Restek Rtx®-50 (Crossbond® 5% phenyl-50% methyl polysiloxane) with an internal diameter of 0.25 mm, a length of 30 m, and a thickness of 0.25 µm. Helium was used as the carrier gas at a pressure of 64.1 kPa. The oven temperature program started at 80 °C with a 2-minute hold and ended at 280 °C with an 8-minute hold. The total flow was 4.9 mL/min, with a column flow of 0.99 mL/min, linear velocity of 36.6 cm/s, and purge flow of 3.0 mL/min. The mass spectrum of each detected compound peak in the chromatogram was compared against known compounds in the Wiley9.LIB database. Compounds with a *match factor* score of ≥ 85% were considered as valid identifications. However, compounds with lower match factor scores will be further validated using pure standards or additional confirmation methods such as LC-MS/MS or NMR in future analyses. These additional validation techniques are recommended to enhance the reliability of the compound identification and ensure the accuracy of the results.

Protein-Ligan preparation

The bioactive compounds from *C. erythroxyli* (Spreng.) Swinscow & Krog were used in this study. The compounds and their respective PubChem IDs are listed as follows: 9-octadecenal, (z)- (5364492), Propiolic acid (10110), 2-Propanamine, 1-methoxy- (123458), Ammonium acetate (517165), Borine, ethylisopropylpropyl- (535106), Pyrazine, methyl- (7976), 3-dimethylsilyloxytridecane (6328885), 1H-Tetrazaborole, 4,5-dihydro-1,4-dimethyl- (6329220), Benzene, (2-methoxyethoxy)- (96375), Cyclopentane, (1-methylethyl)- (19751), 2-pyrrolidinone (12025), 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on (5371506), Isosorbide (12597), (7ar,8r,13as,15s)-11-ethoxy-1,1,7a,8,13a-pentamethyloctadecahydro-2h-cyclopropa[8a',1']naphtho[2',1'] (88003), Phenol, 2-(1,1-dimethylethyl)- (6923), Piperidin-4-one, 3-methyl-1-(2,2,6,6-tetramethyl-4-piperidyl)- (547348), 2-hydroxy-3,5,5-trimethyl-2-cyclohexenone (551084), Piperidine, 1-(1-cyclohexen-1-yl)- (18118), Ethanimine, N-t-butyl- (138901), Heptadecanoic acid, 10-methyl-, methyl ester (554139), P-Methoxyphenyl

glycidyl ether (16646), Pentadecanoic acid, methyl ester (23518), Docosane (12405), Hexadecanoic acid, methyl ester (8181), Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester (78435), Tridecanoic acid (12530), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (5284421), Octadecanoic acid, ethyl ester (8122), Cembrene (6430770), Tetratetracontane (23494), 9,12-Octadecadienoic acid (Z,Z)- (5280450), 8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)- (5363092), methyl .gamma.-linolenoate (-). Furthermore, the compound 2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid (Control) (PubChem CID: 5287717) was employed as a reference control chemical to evaluate the inhibitory properties of the bioactive compounds obtained from *C. erythroxyli*. The 3D structures of the active ingredients found in *C. erythroxyli* and the control were acquired from the appropriate chemical databases on PubChem (https://pubchem.ncbi.nlm.nih.gov). Subsequently, these structures were optimized for energy and transformed into the .pdb file format using Open Babel in PyRx software.

Biological activities prediction using the PASS online

The prediction of biological activity of the compound *C. erythroxyli* (Spreng.) Swinscow & Krog was conducted using the Prediction of Activity Spectra for Substances (PASS) server via the Way2drug server (http://way2drug.com/PassOnline/). The prediction process involved obtaining the SMILES structure from PubChem (http://pubchem.ncbi.nlm.nih.gov) and submitting it to the Way2drug server. The following were the criteria for the PASS Online test's result (Pa value): A Pa value > 0.7 indicates a high probability of biological activity, while a Pa value $0.5 \leq Pa \leq 0.7$ indicates low biological activity, and a Pa value < 0.5 indicates deficient activity.

Prediction drug-likeness

The compounds' drug-likeness was assessed using Lipinski's rule of five. SwissADME (http://www.swissadme.ch/) was employed to evaluate the drug-likeness of all compounds. This resource comprises parameters such as the molecule's number of hydrogen bond acceptors and donors, molecular weight, and bioavailability. Lipinski's rule of five facilitates the determination of whether a compound can be orally assimilated.

Molecular docking analysis

The study employed Autodock Vina within PyRx 8.0.0 to perform docking analysis, treating the protein (6I1E) as the macromolecule and examining bioactive compounds alongside 2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid as ligands. Docking utilized specific grid parameters centered at (1.3057×1.4901×0.3121) with dimensions (36.7569×19.5379×18.1727 $Å^3$). To validate the docking method, redocking of the original ligand (GDP) into the protein's binding site was carried out to ensure the accuracy of binding predictions. Visualization of the docking outcomes was accomplished using Discovery Studio 2024 software.

Results and discussion

Lichen morphology

C. erythroxyli (Spreng.) Swinscow & Krog, belonging to the *Coccocarpiaceae* family, is a corticolous lichen characterized by a foliose thallus that is rounded in shape. The thallus has a smooth texture with uneven edges and does not fully adhere to the substrate. Its underside features rhizines, with thallus lengths ranging from 10 to 35 cm and lobe widths between 0.5 to 1 cm. The upper surface of the thallus is gray, while the underside is black. Reproductive structures consist of rare black apothecia (**Figure 1**). Chemical tests reveal that the upper cortex reacts K+ (yellow), while it is C- and KC-. This species has been found in Indonesia, specifically in the Batang Toru Forest [16] and Taman Hutan Raya Bukit Barisan in North Sumatra [17]. It thrives in fully lit areas at altitudes of 874 to 903 m above sea level.

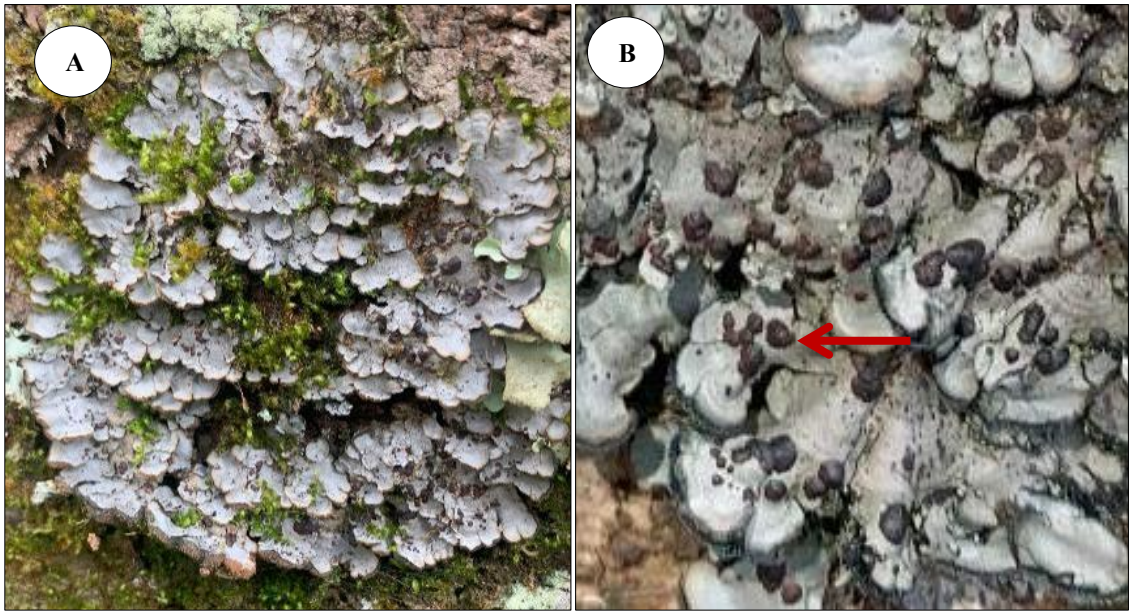

Figure 1 *C. erythroxyli* (Spreng.) Swinscow & Krog. (A) Talus (B) *Apotheciav*.

Table 1 Antibacterial activity of of methanol extract from *C. Erythroxyli.*

Extract	Microorganism		Inhibition Zone (mm)	
			Methanol Extract (10 µl/disk)	Chloramphenikol
Coccocarpia erythroxyli	Gram-Negative Bacteria	*Salmonella enterica* serovar Typhii IPBCC b 11 669	15.8 ± 0.25	31.76
		Escherichia coli ATCC 8739	18.6 ± 0.44	29.13
		Pseudomonas aeruginosa ATCC 15442	11.53 ± 0.11	26.53
	Gram-Positive Bacteria	*Staphylococcus aureus* ATCC 6538	15.16 ± 0.24	31.16
		Propionibacterium acnes ATCC 6919	15.36 ± 0.34	37.43
		Streptococcus mutans ATCC 35668	16.13 ± 0.24	22.8

Table 1 presents the antibacterial activity of the methanol extract from *C. erythroxyli*, measured in terms of inhibition zones (in mm) against various microorganisms. For Gram-negative bacteria, the extract demonstrated varying degrees of inhibitory effects. The highest inhibition zone was observed against *E. coli* ATCC 8739, measuring 18.6 ± 0.44 mm, followed by *S. enterica* serovar Typhii IPBCC b 11 669 at 15.8 ± 0.25 mm, and *P. aeruginosa* ATCC 15442 with an inhibition zone of 11.53 ± 0.11 mm. In comparison, chloramphenicol showed significantly larger inhibition zones ranging from 26.53 mm to 31.76 mm for these bacteria. In terms of Gram-positive bacteria, the extract's inhibition zones ranged from 15.16 ± 0.24 mm for *S. aureus* ATCC 6538 to 16.13 ± 0.24 mm for *S. mutans* ATCC 35668. Chloramphenicol, in contrast, exhibited inhibition zones between 22.8 and 37.43 mm for the tested Gram-positive strains. Although some inhibition zones produced by the methanol extract exceeded 15 mm, they were consistently smaller than those of chloramphenicol. Therefore, the antibacterial activity of the extract can be categorized as moderate, rather than strong.

The relatively larger inhibition zone observed for *E. coli* may be attributed to several factors, including the susceptibility of this strain to the bioactive

compounds present in the methanol extract. *E. coli* is known to have a relatively permeable outer membrane, which may facilitate the uptake of antimicrobial compounds, making it more vulnerable to inhibition. Additionally, the extract may contain specific bioactive constituents that are particularly effective against the cellular processes of *E. coli*, such as interfering with metabolic pathways or disrupting cell wall synthesis [18,19]. Moreover, while efflux pumps in Gram-negative bacteria like *E. coli* often contribute to antibiotic resistance, certain compounds in the extract might inhibit these pumps, thereby enhancing antimicrobial efficacy [20]. The composition of the methanol extract, including its phytochemical constituents, likely plays a significant role in its antibacterial properties, meriting further investigation to isolate and characterize the active compounds and their mechanisms of action. Overall, the methanol extract from *C. erythroxyli* demonstrated moderate antibacterial activity, with a particularly notable effect against *E. coli* ATCC 8739. These findings suggest potential applications for *C. erythroxyli* in the development of natural antibacterial agents, particularly for managing infections caused by Gram-negative pathogens.

However, it should be noted that the disc diffusion method used in this study provides only semi-quantitative results based on the inhibition zone and does not directly measure the antibacterial strength, such as MIC or MBC. A more precise evaluation of antibacterial potency would require MIC/MBC testing, which was not performed in this study, representing a limitation in the accuracy of the antibacterial evaluation.

Gas Chromatograpy-Mass spectrometry analysis

Raw data from GC-MS and individual chromatograms were processed using Origin software. The GC-MS analysis revealed 33 bioactive compounds in *C. erythroxyli*. These compounds include: 9-octadecenal, (z)-, Propiolic acid, 2-Propanamine, 1-methoxy-, Ammonium acetate, Borine, ethylisopropylpropyl-, Pyrazine, methyl-, 3-dimethylsilyloxytridecane, 1H-Tetrazaborole, 4,5-dihydro-1,4-dimethyl-, Benzene, (2-methoxyethoxy)-, Cyclopentane, (1-methylethyl)-, 2-pyrrolidinone, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on, Isosorbide, (7ar,8r,13as,15s)-11-ethoxy-1,1,7a,8,13a-pentamethyloctadecahydro-2h-cyclopropa[8a',1']naphtho[2',1'], Phenol, 2-(1,1-dimethylethyl)-, Piperidin-4-one, 3-methyl-1-(2,2,6,6-tetramethyl-4-piperidyl)-, 2-hydroxy-3,5,5-trimethyl-2-cyclohexenone, Piperidine, 1-(1-cyclohexen-1-yl)-, Ethanimine, N-t-butyl-, Heptadecanoic acid, 10-methyl-, methyl ester, P-Methoxyphenyl glycidyl ether, Pentadecanoic acid, methyl ester, Docosane, Hexadecanoic acid, methyl ester, Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester, Tridecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Octadecanoic acid, ethyl ester, Cembrene, Tetratetracontane, 9,12-Octadecadienoic acid (Z,Z)-, 8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-, methyl .gamma.-linolenoate. Their retention times are listed in **Table 2**, and the chromatogram is shown in **Figure 2**. According to a screening in PubChem, some compounds lack existing 3D structures, so for docking analysis, 23 of these compounds listed in the database will be utilized.

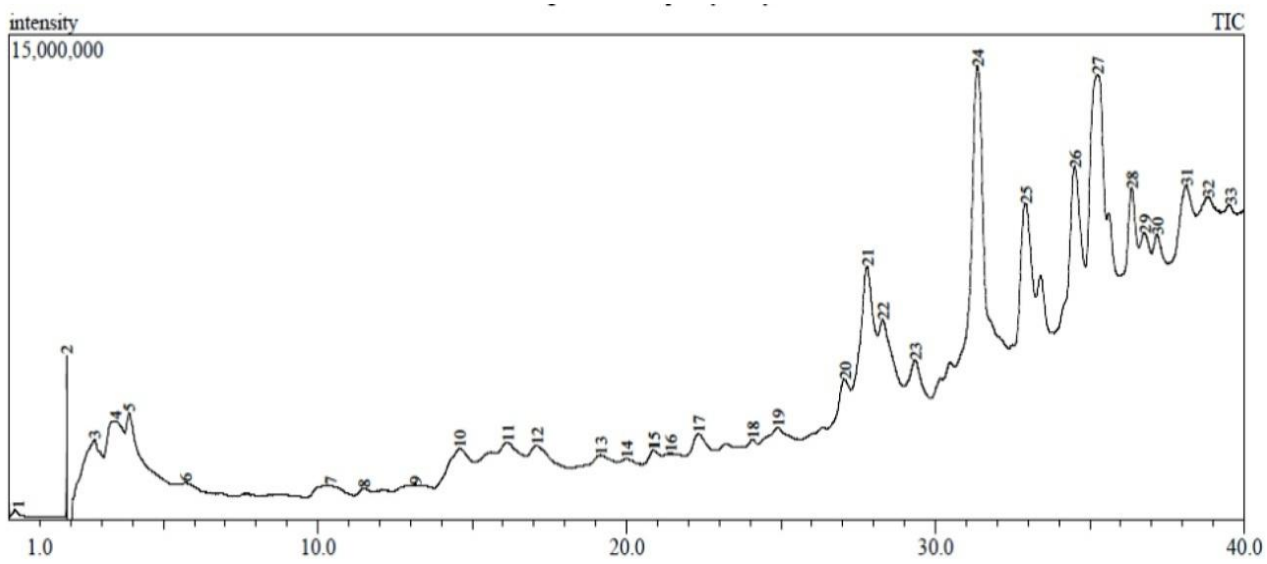

Figure 2 GC-MS chromatogram of *C. erythroxyli*.

Table 2 Compounds of *C. erythroxyli*.

No	Compound name	Mol. Formula	Mass (g/mol)	R. time (min)	Smiles	PubChem ID
1	9-octadecenal, (z)-	C18H34O	266	0.198	CCCCCCCCC=CCCCCCCC=O	5364492
2	Propiolic acid	C3H2O2	70	1.865	C#CC(=O)O	10110
3	2-Propanamine, 1-methoxy-	C4H11NO	89	2.777	CC(COC)N	123458
4	Ammonium acetate	C2H7NO2	77	3.462	CC(=O)[O-].[NH4+]	517165
5	Borine, ethylisopropylpropyl-	C8H19B	126	3.891	B(CC)(CCC)C(C)C	535106
6	Pyrazine, methyl-	C5H6N2	94	5.725	CC1=NC=CN=C1	7976
7	3-dimethylsilyloxytridecane	C15H34OSi	258	10.417	CCCCCCCCCCC(CC)O[Si](C)C	6328885
8	1H-Tetrazaborole, 4,5-dihydro-1,4-dimethyl-	C2H7BN4	98	11.507	[B]1N(N=NN1C)C	6329220
9	Benzene, (2-methoxyethoxy)-	C9H12O2	152	13.165	COCCOC1=CC=CC=C1	96375
10	Cyclopentane, (1-methylethyl)-	C8H16	112	14.605	CC(C)C1CCCC1	19751
11	2-pyrrolidinone	C4H7NO	85	16.159	C1CC(=O)NC1	12025
12	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on	C6H8O4	144	17.111	CC(=C(C)C(=O)O)C(=O)O	5371506
13	Isosorbide	C6H10O4	146	19.178	C1C(C2C(O1)C(CO2)O)O	12597
14	(7ar,8r,13as,15s)-11-ethoxy-1,1,7a,8,13a-pentamethyloctadecahydro-2h-cyclopropa[8a',1']naphtho[2',1'	C29H48O4	460	20.017	CCCCCCCCCCCOC(=O)C1=CC=CC=C1C(=O)OCCCCCCCCCC	88003

No	Compound name	Mol. Formula	Mass (g/mol)	R. time (min)	Smiles	PubChem ID
15	Phenol, 2-(1,1-dimethylethyl)-	C10H14O	150	20.897	CC(C)(C)C1=CC=CC=C1O	6923
16	Piperidin-4-one, 3-methyl-1-(2,2,6,6-tetramethyl-4-piperidyl)-	C15H28N2O	252	21.434	CC1CN(CCC1=O)C2CC(NC(C2)(C)C)(C)C	547348
17	2-hydroxy-3,5,5-trimethyl-2-cyclohexenone	C9H14O2	154	22.344	CC1=C(C(=O)CC(C1)(C)C)O	551084
18	Piperidine, 1-(1-cyclohexen-1-yl)-	C11H19N	165	24.103	C1CCN(CC1)C2=CCCCC2	18118
19	Ethanimine, N-t-butyl-	C6H13N	99	24.916	CC=NC(C)(C)C	138901
20	Heptadecanoic acid, 10-methyl-, methyl ester	C19H38O2	298	27.082	CCCCCCCC(C)CCCCCCCCC(=O)OC	554139
21	P-Methoxyphenyl glycidyl ether	C10H12O3	180	27.805	COC1=CC=C(C=C1)OCC2CO2	16646
22	Pentadecanoic acid, methyl ester	C16H32O2	256	28.313	CCCCCCCCCCCCCCC(=O)OC	23518
23	Docosane	C22H46	310	29.362	CCCCCCCCCCCCCCCCCCCCCC	12405
24	Hexadecanoic acid, methyl ester	C17H34O2	270	31.386	CCCCCCCCCCCCCCCC(=O)OC	8181
25	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	C10H12O4	196	32.937	CC1=CC(=C(C(=C1C(=O)OC)O)C)O	78435
26	Tridecanoic acid	C13H26O2	214	34.534	CCCCCCCCCCCCC(=O)O	12530
27	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294	35.27	CCCCCC=CCC=CCCCCCCCC(=O)OC	5284421
28	Octadecanoic acid, ethyl ester	C20H40O2	312	36.382	CCCCCCCCCCCCCCCCCC(=O)OCC	8122
29	Cembrene	C20H32	272	36.792	CC1=CCCC(=CCC=C(C=CC(CC1)C(C)C)C)C	6430770
30	Tetratetracontane	C44H90	618	37.199	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	23494
31	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	38.145	CCCCCC=CCC=CCCCCCCCC(=O)O	5280450
32	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	C21H36O2	320	38.838	CCCCCC=CCC=CCC=CCCCCCCCC(=O)OC	5363092
33	methyl .gamma.-linolenoate	-	-	-	-	-

Prediction of compound biological activities

Numerous bioactive compounds derived from *Coccocarpia erythroxyli* show significant potential for antibacterial applications, as highlighted by the PASS online analysis (**Table 3**). This comprehensive assessment identifies various compounds from *C. erythroxyli* that exhibit notable antibacterial potential, aligning with promising antibacterial activity observed in previous studies. Compounds such as 9-octadecenal, propiolic acid, and pyrazine, methyl-, demonstrate high predicted activity against bacterial infections, suggesting their potential as anti-infective agents. The presence of lysostaphin inhibitors and other antibacterial compounds further supports mechanisms that could disrupt bacterial growth or viability. For example, the high score of propiolic acid as both an antieczeumatic agent and lysostaphin inhibitor indicates its potential effectiveness against infections caused by *Staphylococcus* species, which are often resistant to many antibiotics [21,22].

Additionally, compounds like 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on and phenol, 2-(1,1-dimethylethyl)-, exhibit promising inhibitory potential against specific bacterial enzymes, supporting their role in inhibiting bacterial cell wall synthesis or other critical metabolic processes. The high predicted activity of these compounds suggests they warrant further exploration for their antibacterial properties. However, while PASS predictions indicate promising antibacterial activity, experimental validation through in vitro testing and molecular docking studies remains essential to confirm these predictions. Some compounds, such as piperidin-4-one, 3-methyl-1-(2,2,6,6-tetramethyl-4-piperidyl)-, exhibit low predicted antibacterial activity. Nonetheless, the overall profile of *C. erythroxyli* suggests a rich source of bioactive compounds that could effectively combat bacterial infections, especially in the context of rising antibiotic resistance.

It is important to note that although PASS predictions offer valuable insights into the potential antibacterial activity of these compounds, actual binding affinities and inhibitory effects observed in molecular docking studies may not always align with these predictions. In cases where a discrepancy exists, future research should focus on the compounds with both high PASS scores and favorable docking affinities. Experimental validation and further in vitro and in vivo studies are necessary to confirm the real-world efficacy of these compounds. Future research should focus on isolating these active compounds, investigating their specific antibacterial mechanisms, optimizing their efficacy, and potentially developing them into natural antibacterial agents. The findings from this analysis underscore the potential of *C. erythroxyli* as a valuable resource in the search for new antibiotics.

Table 2 Compounds of C. erythroxyli.

No	Compound name	Biological activity	Pa	Pi	Cri
1	9-Octadecenal, (Z)- (CAS)	Antiinfective	0.781	0.005	high
		Antifungal	0.566	0.022	low
2	Propiolic acid	Antieczematic	0.783	0.022	high
		Lysostaphin inhibitor	0.858	0.003	high
3	2-Propanamine, 1-methoxy-	Mucositis treatment	0.757	0.018	high
		Venombin AB inhibitor	0.781	0.007	high
4	Ammonium acetate				
5	Pyrazine, methyl- (CAS)	IgA-specific serine endopeptidase inhibitor	0.788	0.007	high
		Chloride peroxidase inhibitor	0.782	0.004	high

No	Compound name	Biological activity	Pa	Pi	Cri
6	Benzene, (2-Methoxyethoxy)-	Chlordecone reductase inhibitor	0.864	0.009	high
		Nicotinic alpha6beta3beta4alpha5 receptor antagonist	0.777	0.015	high
7	Cyclopentane, (1-methylethyl)- (CAS)	Polyporopepsin inhibitor	0.747	0.029	high
		Acrocylindropepsin inhibitor	0.823	0.016	high
8	2-Pyrrolidinone (CAS)	Creatininase inhibitor	0.804	0.005	high
		Pterin deaminase inhibitor	0.855	0.003	high
9	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on	Fragilysin inhibitor	0.912	0.003	high
		Allyl-alcohol dehydrogenase inhibitor	0.921	0.002	high
10	Isosorbide	Pectate Lyase Inhibitor	0.700	0.005	high
		Polyporopepsin inhibitor	0.756	0.028	high
11	Phenol, 2-(1,1-dimethylethyl)- (CAS)	Glucan Endo-1,6-beta-Glucosidase Inhibitor	0.873	0.004	high
		Dextranase Inhibitor	0.836	0.004	high
12	Piperidin-4-one, 3-methyl-1-(2,2,6,6-tetramethyl-4-piperidyl)-	Antibacterial	0.236	0.091	extremely low
		Antimycobacterial	0.257	0.113	extremely low
13	2-Hydroxy-3,5,5-Trimethyl-2-Cyclohexenone	Peptidoglycan glycosyltransferase inhibitor	0.538	0.015	low
		Membrane integrity agonist	0.819	0.032	high
14	Piperidine, 1-(1-cyclohexen-1-yl)- (CAS)	Chymosin inhibitor	0.682	0.049	low
		Nootropic	0.596	0.078	low
15	Ethanimine, N-t-butyl-	Chymosin inhibitor	0.874	0.008	high
		Saccharopepsin inhibitor	0.874	0.008	high
16	p-Methoxyphenyl glycidyl ether	Acrocylindropepsin inhibitor	0.973	0.001	high
		Chymosin inhibitor	0.973	0.001	high
17	Pentadecanoic Acid, Methyl Ester	Saccharopepsin inhibitor	0.962	0.002	high
		Polyporopepsin inhibitor	0.942	0.003	high
18	Hexadecanoic acid, methyl ester (CAS)	Acrocylindropepsin inhibitor	0.942	0.003	high
		Acylcarnitine hydrolase inhibitor	0.942	0.003	high
19	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester (CAS)	Antiseptic	0.819	0.004	high
		Membrane integrity agonist	0.909	0.009	high
20	Tridecanoic acid (CAS)	Glycosylphosphatidylinositol phospholipase D inhibitor	0.860	0.005	high
		Lipoprotein lipase inhibitor	0.910	0.003	high

No	Compound name	Biological activity	Pa	Pi	Cri
21	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)	Antieczematic	0.953	0.002	high
		Pediculicide	0.717	0.003	high
22	Cembrene	Antieczematic	0.869	0.008	high
		Antifungal	0.510	0.029	low
23	9,12-Octadecadienoic acid (Z,Z)-(CAS)	Fusarinine-C ornithinesterase inhibitor	0.785	0.009	high
		Pseudolysin inhibitor	0.752	0.012	high

Prediction of Drug-likeness

The analysis reveals varying adherence to drug-likeness criteria among the compounds derived from *C. erythroxyli*, as presented in **Table 4**. These physicochemical properties were evaluated according to Lipinski's rule of five, which serves as a guideline for assessing drug-likeness and potential oral bioavailability. Each compound is analyzed based on four key criteria: Molecular weight (MW), ideally less than 500 Da; MlogP (logarithm of the partition coefficient), with an optimal value of ≤ 4.15 to indicate hydrophobicity; the number of oxygen atoms (NorO), ideally ≤ 10 to enhance solubility; and the number of hydrogen bond donors (NHorOH), which should not exceed 5 for better membrane permeability. The "Violation" column indicates whether a compound fails to meet any of these criteria, with "Yes" denoting violations. For instance, compounds like 9-Octadecenal (Z)- and Cembrene violate multiple criteria, potentially limiting their suitability as drug candidates. Overall, while some compounds exhibit favorable drug-like properties, others may require further optimization to enhance their pharmacological profiles. Future research should focus on modifying these compounds to improve their therapeutic potential.

The "Rule of Five" is a guideline for evaluating whether a biologically active compound is likely to be an orally active drug in humans, requiring it to meet five criteria related to molecular weight, hydrogen bond acceptors and donors, and lipophilicity. Poor absorption or permeation is likely if it exceeds specific thresholds [23]. It suggests that a potential orally active drug should have no more than one violation of its criteria for optimal drug-likeness yet acknowledges that many effective drugs may not conform to these rules. Despite its widespread application, the rule has limitations, including equal weighting of criteria, rigid violation boundaries, and the exclusion of natural and biological compounds, as well as metabolic considerations. While the "Rule of Five" outlines important criteria for assessing drug bioavailability, some effective drugs, particularly antibiotics and antifungals, may not meet these criteria due to their structural features that allow interaction with natural transporters in the body, enabling their efficacy despite falling outside the rule's parameters [24]. Moreover, compounds derived from natural sources, such as those from *C. erythroxyli*, may not always conform to these criteria yet still exhibit potent biological activity. This highlights the need to consider alternative mechanisms of drug action, particularly for compounds targeting extracellular bacterial structures, where factors like molecular interaction with bacterial surfaces can play a key role in efficacy. Therefore, while the 'Rule of Five' provides a valuable framework for initial drug-likeness evaluation, it should not be applied rigidly, particularly when dealing with natural compounds that may operate through non-traditional mechanisms.

Table 4 Physicochemical properties of compounds from *C. erythroxyli* based on Lipinski's rule of five test.

No	Compound Name	Lipinski				
		MW	**MlogP ≤ 4.15**	**NorO ≤ 10**	**NHorOH ≤ 5**	**Violation**
1	9-octadecenal, (z)-	266	4.68	1	0	Yes (1)
2	Propiolic acid	70	−0.08	2	1	Yes (0)
3	2-Propanamine, 1-methoxy-	89	−0.18	2	1	Yes (0)
4	Ammonium acetate	77	−5.05	2	1	Yes (0)
5	Pyrazine, methyl-	94	−0.51	2	0	Yes (0)
6	Benzene, (2-methoxyethoxy)-	152	1.53	2	0	Yes (0)
7	Cyclopentane, (1-methylethyl)-	112	3.81	0	0	Yes (0)
8	2-pyrrolidinone	85	−0.31	1	1	Yes (0)
9	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on	144	0.15	4	2	Yes (0)
10	Isosorbide	146	−1.52	4	2	Yes (0)
11	Phenol, 2-(1,1-dimethylethyl)-	150	2.76	1	1	Yes (0)
12	Piperidin-4-one, 3-methyl-1-(2,2,6,6-tetramethyl-4-piperidyl)-	252	1.86	3	1	Yes (0)
13	2-hydroxy-3,5,5-trimethyl-2-cyclohexenone	154	0.97	2	1	Yes (0)
14	Piperidine, 1-(1-cyclohexen-1-yl)-	165	2.59	0	0	Yes (0)
15	Ethanimine, N-t-butyl-	99	1.39	1	0	Yes (0)
16	P-Methoxyphenyl glycidyl ether	180	0.83	3	0	Yes (0)
17	Pentadecanoic acid, methyl ester	256	4.19	2	0	Yes (1)
18	Hexadecanoic acid, methyl ester	270	4.44	2	0	Yes (1)
19	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	196	1.36	4	2	Yes (0)
20	Tridecanoic acid	214	3.42	2	1	Yes (0)
21	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	4.7	2	0	Yes (1)
22	Cembrene	272	5.63	0	0	Yes (1)
23	9,12-Octadecadienoic acid (Z,Z)-	280	4.47	2	1	Yes (1)

Molecular interactions of bioactive compounds *C. erythroxyli* with 6IE1 protein

Based on the molecular docking modeling results, a comparison of the control compound,2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid, and several bioactive compounds from *C. erythroxyli* revealed substantial differences in the types of interactions and binding energies with protein targets. The control compound exhibited the highest binding energy at −6.3 kcal/mol. Significant interactions include hydrogen bonds with residues ALA 220, SER 435, THR 437, and ARG 438; hydrophobic interactions with residues LEU 219 and VAL 439; and an electrostatic interaction with residue TRP 185. These interactions demonstrate its strong affinity for the target protein, suggesting that 2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid effectively inhibits the target protein (**Table 4** and **Figures 3** and **4**).

Table 5 Residue and biding energy of ligand and 6IE1 Protein interaction.

No	Ligands	Interaction type					Binding Afinity
		Hydrogen Bond		Hydrophobic	Electrostatis	Unfavorable	
		Hydrogen Bond	Carbon Hydrogen Bond				
Control							
1	2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid (Control)	ALA 220, SER 435, THR 437, ARG 438	-	LEU 219, VAL 439	TRP 185	LEU 434	– 6.3
Bioactive components of the *C. erythroxyli*							
2	9-octadecenal, (z)-	ALA 220	-	PHE 182, TRP 185, LEU 219	-	-	–3.9
3	Propiolic acid	SER 435	-	PHE 182, LEU 381, PHE 383	-	MET 436, THR437	–3.2
4	2-Propanamine, 1-methoxy-	SER 435, THR 437, ARG 438	-	VAL 439	-		–2.8
5	Ammonium acetate	SER 435, THR 437, ARG 438	-	-	-	-	–2.8
6	Pyrazine, methyl-	ASP 378	-	HIS 420	-	-	–3.2
7	Benzene, (2-methoxyethoxy)-	SER 435, THR 437	-	VAL 439, PRO 443	-	-	–4.3
8	Cyclopentane, (1-methylethyl)-		-	TRP 185	-	-	–3.5
9	2-pyrrolidinone	LEU 434, SER 435, THR 437, ARG 438	-	LEU 381	-	-	–3.6
10	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on	ASN 69, ASP 224, ARG 226	-	LEU 260, MET 553	-	-	–4.1
11	Isosorbide	SER 435, THR 437, ARG 438	-		-	-	–4
12	Phenol, 2-(1,1-dimethylethyl)-	ARG 438	-	LEU 219, ALA 220	-	-	–4.2
13	Piperidin-4-one, 3-methyl-1-(2,2,6,6-tetramethyl-4-piperidyl)-	SER 435, THR 437, ARG 438	LEU 381	PHE 182, PHE 383	-	-	–5.2

No	Ligands	Interaction type					Binding Afinity
		Hydrogen Bond		Hydrophobic	Electrostatis	Unfavorable	
		Hydrogen Bond	Carbon Hydrogen Bond				
14	2-hydroxy-3,5,5-trimethyl-2-cyclohexenone	SER 435, THR 437	-	PHE 182, LEU 381, PHE 383, MET 436	-	-	–5.1
15	Piperidine, 1-(1-cyclohexen-1-yl)-	-	-	TRP 185, LEU 219, ALA 220, ARG 438	-	-	–4.5
16	Ethanimine, N-t-butyl-	ASP 378	-	-	-	-	–3.1
17	P-Methoxyphenyl glycidyl ether	SER 435, MET 436, THR 437	LEU 434	VAL 439	-	-	–4.3
18	Pentadecanoic acid, methyl ester	SER 435, MET 436, THR 437	-	PHE 182	-	-	–4.1
19	Hexadecanoic acid, methyl ester	SER 435, MET 436, THR 437	LEU 434	PHE 182, TRP 185	-	-	–4.3
20	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	SER 435, THR 437	-	PHE 182, LEU 381, VAL 439, PRO 443	-	-	–4.8
21	Tridecanoic acid	ALA 220	-	PHE 182, TRP 185	-	ARG 438	–4.4
22	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	SER 435, MET 436, THR 437	-	PHE 182, TRP 185, LEU 219, ALA 220	-	-	–4.7
23	Cembrene		-	PHE 182, TRP 185	-	-	–5.9
24	9,12-Octadecadienoic acid (Z,Z)-	ALA 220	-	PHE 182, TRP 185	-	ARG 438	–4.8

The analysis of molecular interactions between bioactive compounds from *Coccocarpia erythroxyli* and the 6IE1 protein provides valuable insights into their potential as antibacterial agents. The 6IE1 protein plays a crucial role in bacterial cell wall synthesis, making it a key target for new antibiotic development. Docking studies reveal varying binding affinities and interaction types among the tested compounds. The control compound, 2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid, is the original ligand for 6IE1. It has a binding energy of –6.3 kcal/mol, which represents the reference value for comparison. This value suggests a relatively strong interaction with protein 6IE1, as more

negative binding energies typically indicate stronger binding. Major interactions observed include hydrogen bonds with ALA 220, SER 435, THR 437, and ARG 438, hydrophobic interactions with LEU 219 and VAL 439, and an electrostatic interaction with TRP 185. These interactions suggest that this compound could be an effective inhibitor of the target protein.

Among the bioactive compounds analyzed, Cembrene shows a binding energy of –5.9 kcal/mol, which is still in the weak to moderate range for antibacterial activity, though it is relatively close to the control compound. Cembrene interacts with PHE 182 and TRP 185 through hydrophobic interactions, suggesting it could bind effectively to the protein target. However, its binding affinity is somewhat lower than that of the control compound, indicating it may require further optimization for therapeutic use. The hydrophobic contacts made by Cembrene could contribute to its stability and efficacy in biological systems, but its potential as an antibacterial agent needs further investigation.

In comparison, other bioactive compounds show varying binding affinities. For instance, 9-octadecenal (Z) has a binding energy of –3.9 kcal/mol, with hydrogen bonding to ALA 220. Propiolic acid shows a lower affinity of –3.2 kcal/mol, interacting through SER 435 and several hydrophobic contacts. Other compounds, such as Piperidin-4-one and 2-hydroxy-3,5,5-trimethyl-2-cyclohexenone, have higher binding affinities of –5.2 and –5.1 kcal/mol, respectively, with significant hydrogen bonding interactions. These findings indicate that these compounds may also inhibit the target protein, though their binding strengths are not as high as Cembrene.

Cembrene is a bicyclic sesquiterpene known for its unique structure and potential biological activities, including antimicrobial and anti-inflammatory effects. In medicinal chemistry, Cembrene and its derivatives have garnered interest due to their pharmacological properties. It has shown the ability to inhibit bacterial growth by disrupting cell walls or interfering with metabolic processes, positioning it as a promising candidate for new antimicrobial agents. Its structural features may improve its binding affinity to protein targets involved in bacterial resistance, offering a potential solution for treating antibiotic-resistant infections. Further research is necessary to explore Cembrene's mechanisms of action and optimize its therapeutic efficacy [25-27].

Protein 6IE1, also known as Penicillin-Binding Protein 3 (PBP3), plays a critical role in bacterial cell wall synthesis, especially during the transpeptidation stage of peptidoglycan formation. This process is vital for maintaining the structural integrity of the bacterial cell wall. PBP3 is, therefore, an essential target for antibacterial therapies. Amoxicillin, a β-lactam antibiotic, inhibits PBP3 by covalently binding to its active site, disrupting cell wall synthesis and leading to bacterial cell death [28].

Similarly, Cembrene binds to hydrophobic residues on PBP3, such as PHE 182 and TRP 185, with a binding energy of approximately –5.9 kcal/mol. This binding resembles that of β-lactam antibiotics, highlighting its potential as an antibacterial agent. However, Cembrene relies more on hydrophobic and electrostatic interactions, which may offer an advantage in overcoming antibiotic resistance. Other compounds tested, such as 9-octadecenal (Z), also show antibacterial activity, though with a lower affinity compared to Cembrene. This finding is consistent with the results of Atni *et al.* [29] on *Parmotrema xanthinum*, where 9-octadecenal (Z) also exhibited antibacterial effects. These results suggest that bioactive compounds from *C. erythroxyli* could be promising candidates for developing novel antibacterial agents with distinct mechanisms that may combat antibiotic-resistant bacteria [30,31].

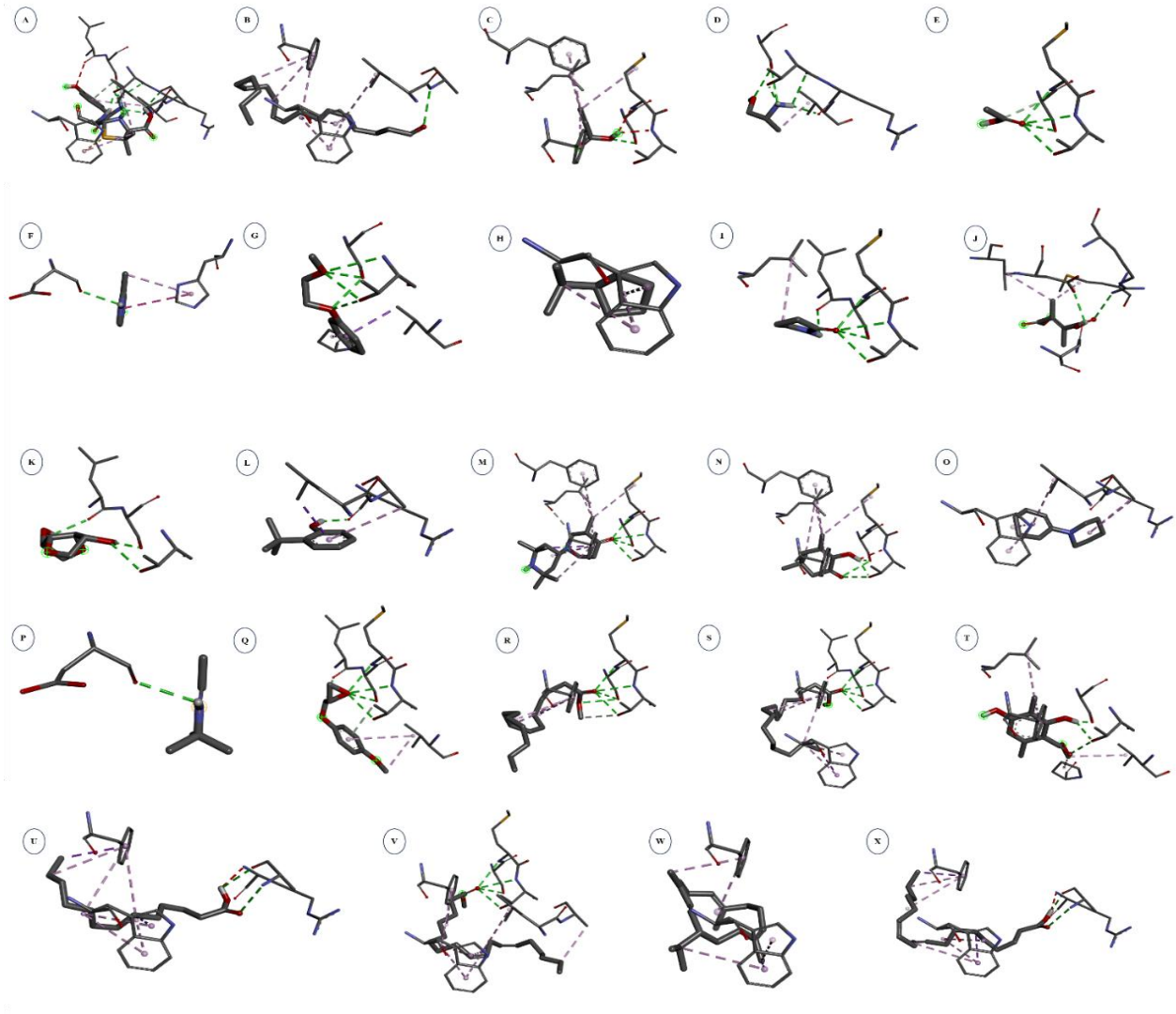

Figure 3 3D interactions of 2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid (Control) and bioactive compounds from Coccocarpia erythroxyli (Spreng.) Swinscow & Krog against the 6IE1 protein. (A) 2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid; (B) 9-octadecenal, (z)-; (C) Propiolic acid; (D) 2-Propanamine, 1-methoxy-; (E) Ammonium acetate; (F) Pyrazine, methyl-; (G) Benzene, (2-methoxyethoxy)-; (H) Cyclopentane, (1-methylethyl)-; (I) 2-pyrrolidinone; (J) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on; (K) Isosorbide; (L) Phenol, 2-(1,1-dimethylethyl)-; (M) Piperidin-4-one, 3-methyl-1-(2,2,6,6-tetramethyl-4-piperidyl)-; (N) 2-hydroxy-3,5,5-trimethyl-2-cyclohexenone; (O) Piperidine, 1-(1-cyclohexen-1-yl)-; (P) Ethanimine, N-t-butyl-; (Q) P-Methoxyphenyl glycidyl ether; (R) Pentadecanoic acid, methyl ester; (S) Hexadecanoic acid, methyl ester; (T) Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester; (U) Tridecanoic acid; (V) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; (W) Cembrene; dan (X) 9,12-Octadecadienoic acid (Z,Z)-.

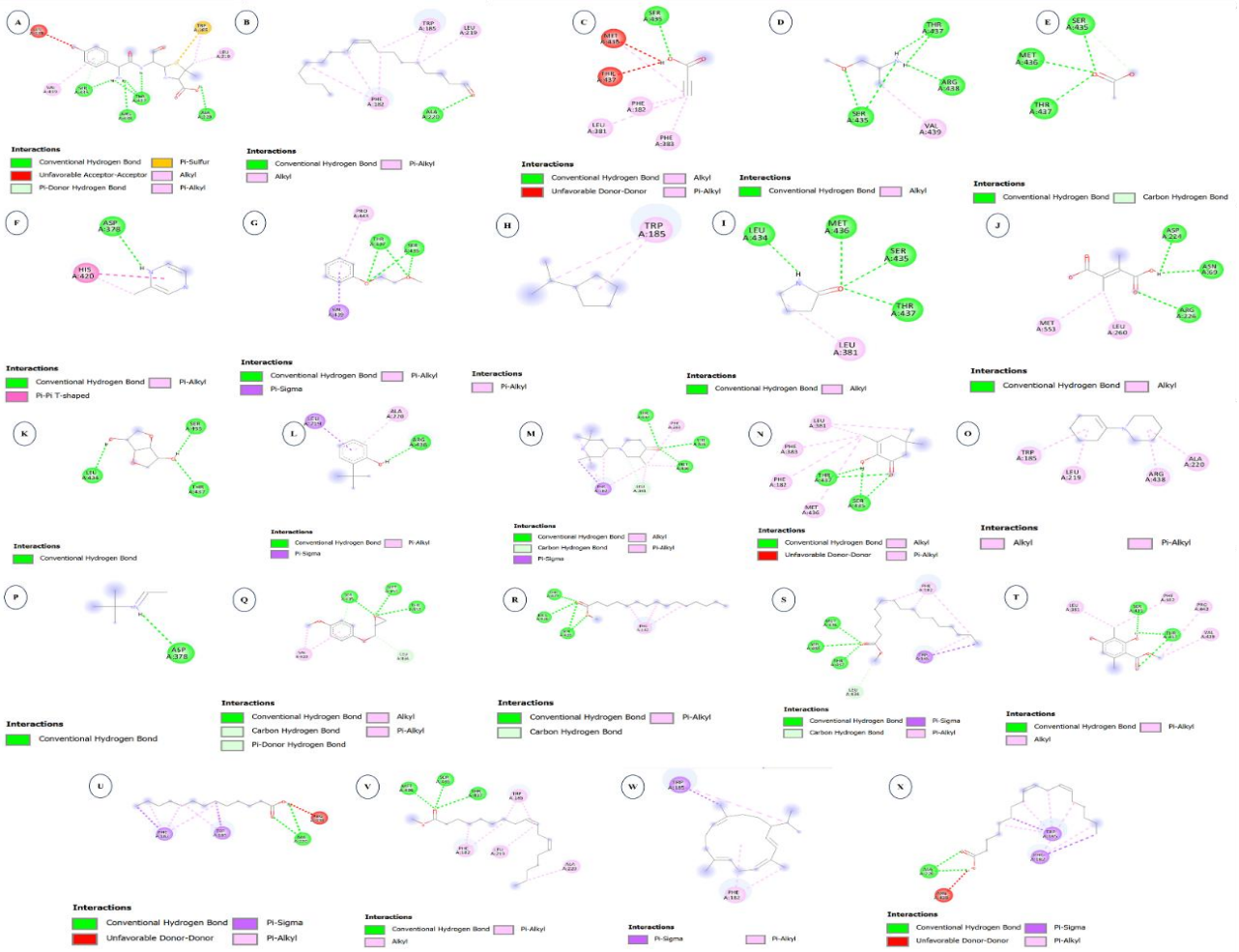

Figure 4 2D interactions of 2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid (Control) and bioactive compounds from Coccocarpia erythroxyli (Spreng.) Swinscow & Krog against the 6IE1 protein. (A) 2-{1-[2-Amino-2-(4-hydroxy-phenyl)-acetylamino]-2-oxo-ethyl}-5,5-dimethyl-thiazolidine-4-carboxylic acid; (B) 9-octadecenal, (z)-; (C) Propiolic acid; (D) 2-Propanamine, 1-methoxy-; (E) Ammonium acetate; (F) Pyrazine, methyl-; (G) Benzene, (2-methoxyethoxy)-; (H) Cyclopentane, (1-methylethyl)-; (I) 2-pyrrolidinone; (J) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-on; (K) Isosorbide; (L) Phenol, 2-(1,1-dimethylethyl)-; (M) Piperidin-4-one, 3-methyl-1-(2,2,6,6-tetramethyl-4-piperidyl)-; (N) 2-hydroxy-3,5,5-trimethyl-2-cyclohexenone; (O) Piperidine, 1-(1-cyclohexen-1-yl)-; (P) Ethanimine, N-t-butyl-; (Q) P-Methoxyphenyl glycidyl ether; (R) Pentadecanoic acid, methyl ester; (S) Hexadecanoic acid, methyl ester; (T) Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester; (U) Tridecanoic acid; (V) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; (W) Cembrene; dan (X) 9,12-Octadecadienoic acid (Z,Z)-.

Conclusions

This study presents promising preliminary evidence of the antibacterial potential of *Coccocarpia erythroxyli* (Spreng.) Swinscow & Krog, a foliose lichen from the Coccocarpiaceae family. The methanol extract showed notable antibacterial activity, particularly against Gram-negative bacteria, with the highest inhibition zone of 18.6 ± 0.44 mm against *Escherichia coli* ATCC 8739. GC-MS analysis identified 33 bioactive compounds, among which Cembrene displayed a binding energy of –5.9 kcal/mol in molecular docking studies. It exhibited key hydrophobic interactions with residues PHE182 and TRP185, similar to the control compound, when docked with the 6IE1 protein, a target involved in bacterial cell wall synthesis. These molecular interactions suggest a potential inhibitory effect on PBP3. Although some compounds may require further optimization to improve

their drug-likeness, these findings provide a preliminary foundation for exploring the antibacterial properties of *C. erythroxyli* and its metabolites in the development of future therapeutic agents. These results support the need for *in-vivo* studies and SAR optimization to enhance the antibacterial efficacy of *C. erythroxyli* compounds.

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Declaration of Generative AI in Scientific Writing

No generative AI tools were used in the writing of this manuscript.

CRediT author statement

E.M.: Conceptualization, investigation, reviewing and editing, corresponding author; O.K.A.: Investigation, methodology, research data collection, data analysis, writing original draft; and N.P.: Research design, project administration, writing reviewing, data curation and editing. All authors have read and accepted the published version of the work.

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