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Coumarin and fatty alcohol from root bark of *strychnos innocua* (delile): isolation, characterization and in silico molecular docking studies

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Abstract

Background: Coumarin and fatty alcohol are abundant in nature, particularly in plants, and have been reported to have therapeutic uses. *Strychnos innocua* (*Loganiaceae* family) is commonly utilized for medicinal purposes in several African countries. Ethyl acetate extract of the plant (root bark) was subjected to chromatography separation, leading to the isolation of Umbelliferone (**1**) and 2,13-octadecadien-1-ol (**2**).

Results: Their structures were verified using mass spectrometry (MS) and nuclear magnetic resonance (NMR) and then compared with published data. This is the first time these compounds (**1** and **2**) have been isolated from *S. innocua* root bark. In the molecular docking analysis, the binding scores of the compounds (**1** and **2**) with the binding sites of *Staphylococcus aureus* pyruvate carboxylase (PDB: 3HO8) and *Pseudomonas aeruginosa* virulence factor regulator (PDB: 2OZ6) were − 5.6 and − 4.7 kcal/mol, and − 6.9 and − 5.7 kcal/mol, respectively. These were compared with ciprofloxacin (standard drug), which had docking scores of -6.6 and -8.7 kcal/mol, respectively.

Conclusions: In conclusion, this study established the rich presence of Umbelliferone and 2,13-octadecadien-1-ol in the plant root bark, and their docking studies revealed moderate binding potential with the binding sites of *S. aureus* and *P. aeruginosa*.

Keywords: Isolation, *Strychnos innocua*, Umbelliferone, 2,13-octadecadien-1-ol, Docking

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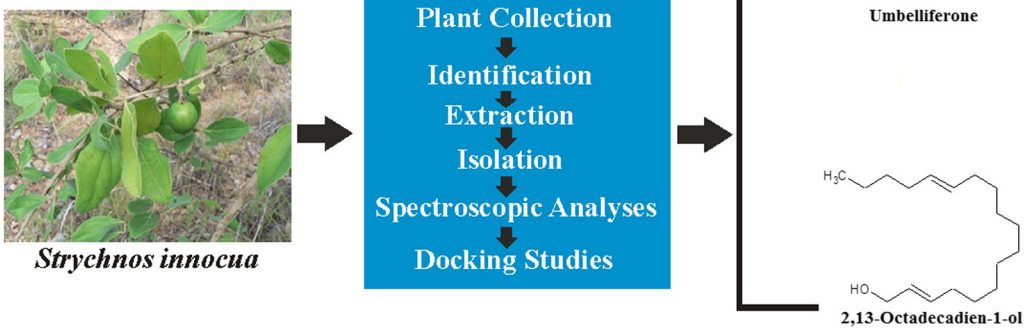
Graphical Abstract



Background

Compounds isolated from plants have enormous potential in the development of new drugs (Sasidharan et al. 2011). In general, plants can be found all over the world, and their parts (leaves, stems, roots, fruit, and flowers) are employed in a variety of uses, including medicine (Umaru et al. 2019).

The phytochemical composition and secondary metabolites of plants such as coumarin and fatty alcohols contain active therapeutic components and are associated

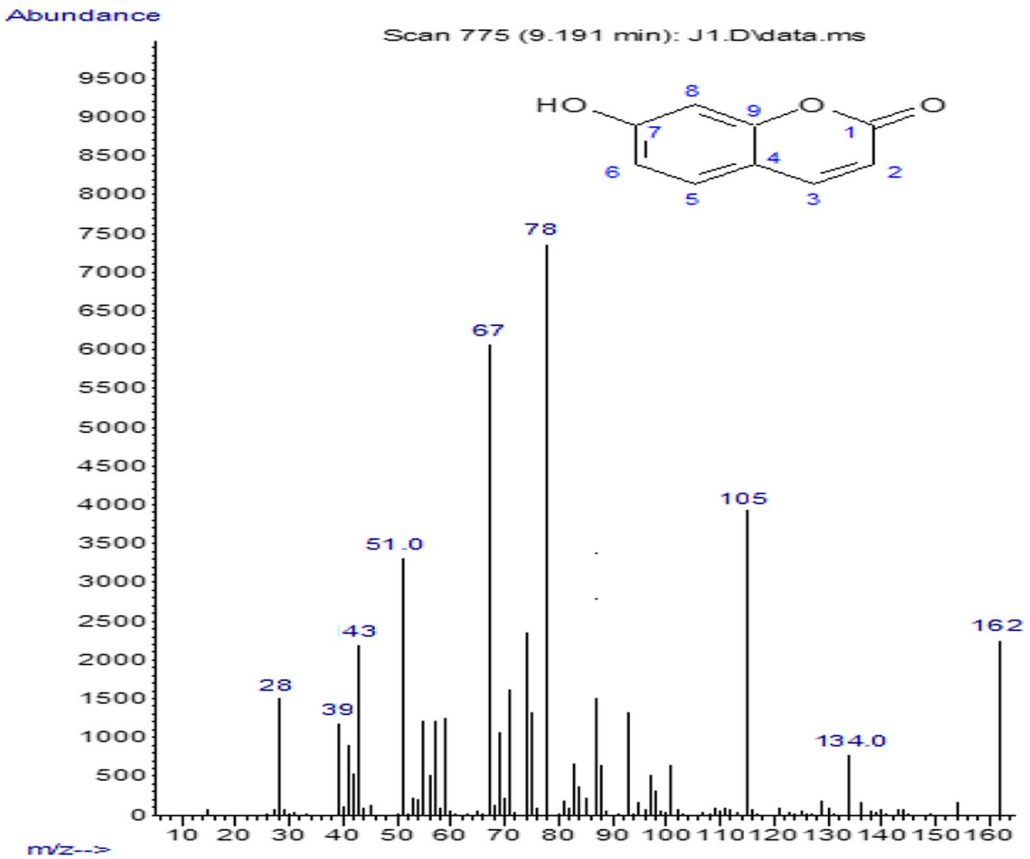


Fig. 1 Mass spectrum of compound (1)

Table 1 The NMR (400 MHz) data of the compound (1)

Position	Compound (1)			Literature Data (Mazimba 2017)		
	^{1}H (ppm)	^{13}C (ppm)	DEPT	^{1}H (ppm)	^{13}C (ppm)	DEPT
C-1		161.19	C		160.5	C
C-2	6.00 (d, 1H)	111.59	CH	6.16 (d, 1H)	112.0	CH
C-3	7.75 (d, 1H)	144.15	CH	7.87 (d, 1H)	144.2	CH
C-4		110.00	C		111.9	C
C-5	7.31 (d, 1H)	129.32	CH	7.50 (d, 1H)	129.7	CH
C-6	6.75 (dd, 1H)	109.43	CH	6.83 (dd, 1H)	113.5	CH
C-7		160.90	C		161.6	C
C-8	6.51 (s, 1H)	102.58	CH	6.76 (d, 1H)	102.5	CH
C-9		156.34	C		156.2	C
OH	9.63 (s, 1H)			N.L		

N.L Not Listed

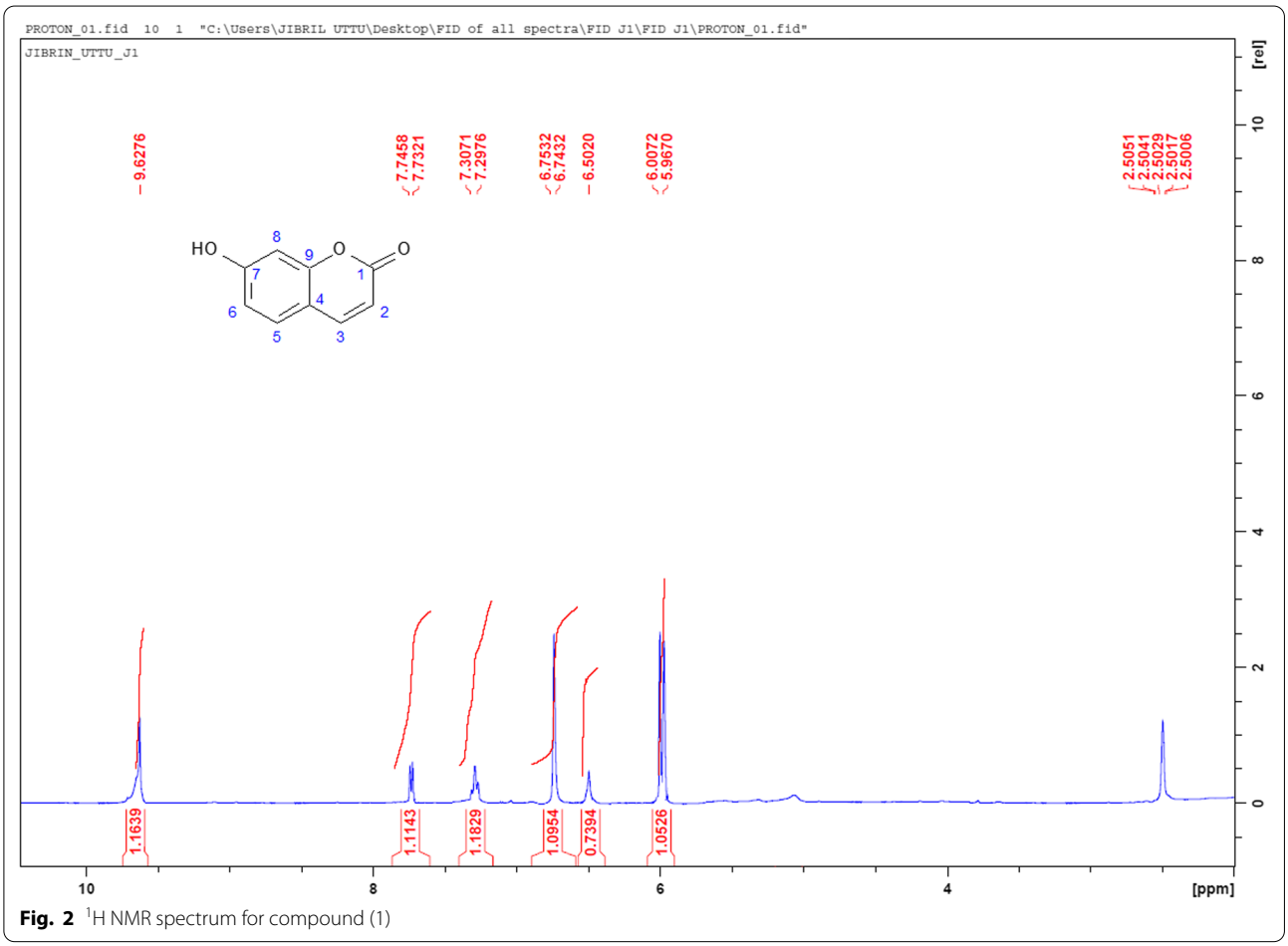


Fig. 2 ^{1}H NMR spectrum for compound (1)

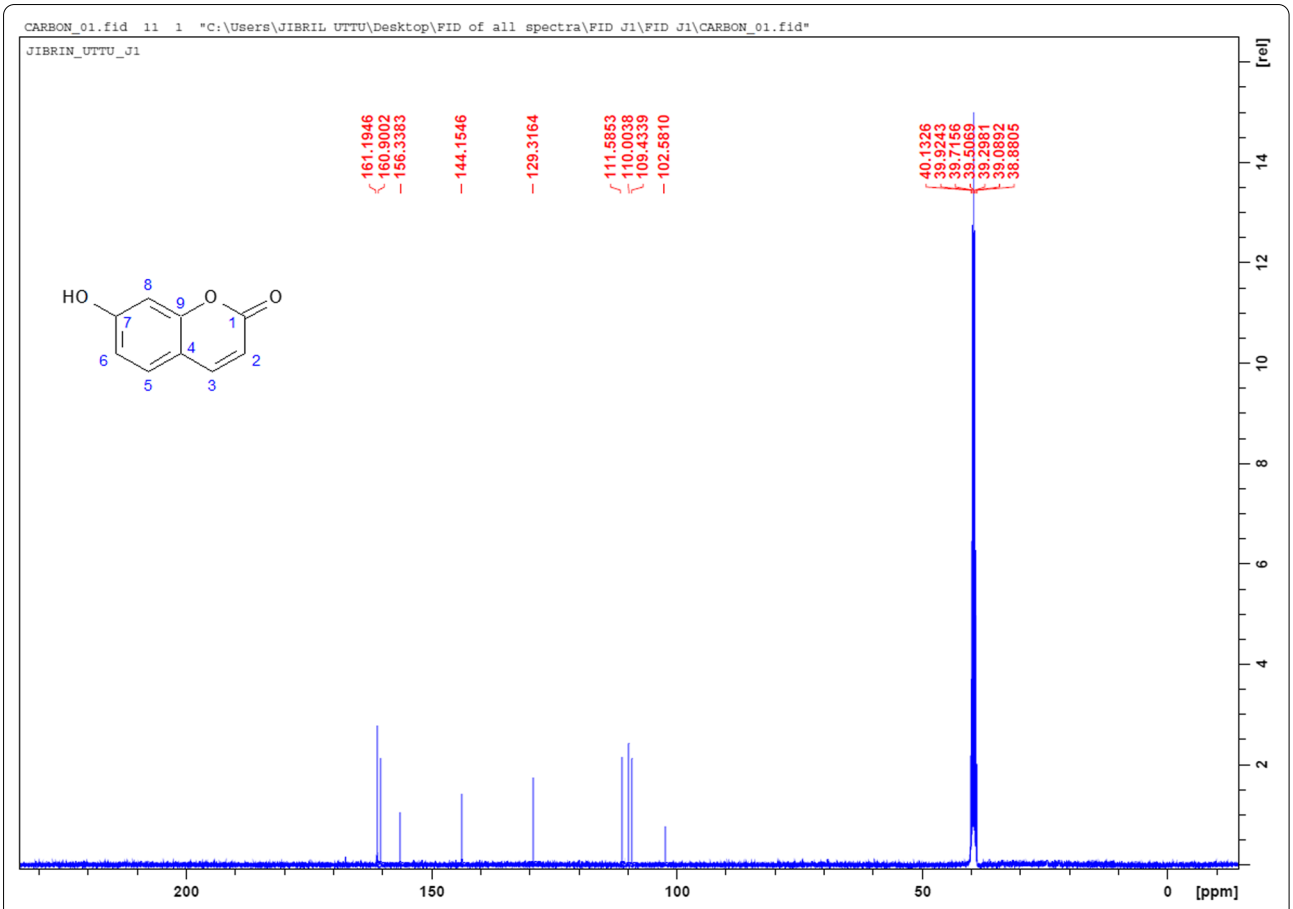


Fig. 3 ^{13}C NMR spectrum for compound (1)

with their pharmacological potential (Mondal et al. 2019; Uttu et al. 2022). Umbelliferone (7-hydroxycoumarin) is sometimes utilized as a fluorophore. It has a large π–π conjugated system that allows it to be used as a fluorescent sensor for a variety of biological applications (Raunio et al. 2020), such as antidiarrheal, antiulcerogenic, antibacterial, mutagenic, and antioxidant activities (Cruz and de-Figueriredo G. F., Pedro L. P., Amorin Y. M., Andrade J. T., Passos T. F. and Araujo M. G. D. 2020), whereas 2,13-octadecadien-1-ol was identified as one of the major phytochemical compounds in *Epipremnum aureum* and possesses diuretic, antimicrobial, antiulcer, anti-inflammatory, antioxidant, and pesticide activities (Meshram et al. 2016).

The antimicrobial potential of natural compounds from plant origins has been assessed using a variety of in vitro, in vivo, and in silico computational approaches. Docking is one of the techniques that have seen a lot of application in microbial medication development (Nour et al. 2021).

Strychnos innocua is a *Loganiaceae* plant with a straight-stem that can reach up to 18 m in height. It has multiple branches, with a trunk diameter ranging from 7 to 40 cm. Its leaves are normally simple, with a rarely rounded base. The plant is found in Sudan, Malawi, Cameroon, Uganda, and Nigeria, to name a few countries. Its root is claimed to help with gonorrhoea, while root decoction of the plant in fresh form is reported to help in the treatment of snake bites [9.10]. In Kaduna State, Nigeria, the plant can be obtained.

The root bark of this plant has been previously found to contain important chemical compositions (Ibrahim et al. 2021; Iyun et al. 2022; Sallau et al. 2022). However, there is a dearth of information regarding in silico molecular docking studies of compounds isolated from *S. innocua* root bark. In this research, 7-hydroxycoumarin (**1**) and 2,13-octadecadien-1-ol (**2**) were isolated from *S. innocua* root bark, characterized, and subjected to docking analysis. This is the first time these compounds have been isolated from this plant.

Methods

Plant collection

The plant of *S. innocua* was collected from the wild in Kaduna State of Nigeria. It was then identified and

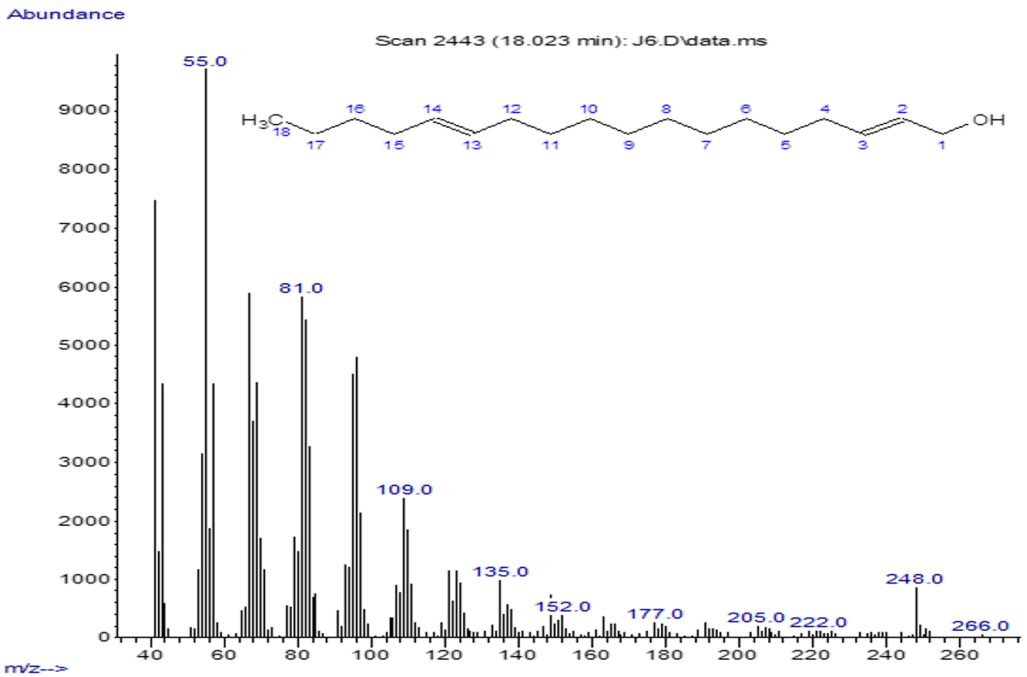


Fig. 4 Mass spectrum of compound (2)

Table 2 The NMR (400 MHz) data of the compound (2)

Position	Compound (2)			Literature Data (Hoskovec et al. 1990)		
	^{1}H (ppm)	^{13}C (ppm)	DEPT	^{1}H (ppm)	^{13}C (ppm)	DEPT
C-1	4.57 (d, 2H)	66.28	CH_2	4.20 (d, 2H)	58.61	CH_2
C-2	5.90 (m, 1H)	129.86	CH	5.54 (m, 1H)	128.26	CH
C-3	5.80 (m, 1H)	132.41	CH	5.67 (m, 1H)	133.28	CH
C-4	2.28 (m, 2H)	27.35	CH_2	2.02 (m, 2H)	27.43	CH_2
C-5	1.21 (m, 2H)	29.94	CH_2	1.22 (m, 2H)	29.75	CH_2
C-6	1.35 (m, 2H)	29.71	CH_2	1.39 (m, 2H)	29.60	CH_2
C-7	1.29 (m, 2H)	29.62	CH_2	N.L	29.55	CH_2
C-8	1.16 (m, 2H)	29.55	CH_2	N.L	29.52	CH_2
C-9	1.27 (m, 2H)	29.43	CH_2	N.L	29.47	CH_2
C-10	1.37 (m, 2H)	29.32	CH_2	N.L	29.44	CH_2
C-11	1.19 (m, 2H)	29.21	CH_2	1.21 (m, 2H)	29.28	CH_2
C-12	2.17 (m, 2H)	24.81	CH_2	2.02 (m, 2H)	26.91	CH_2
C-13	5.53 (m, 1H)	130.16	CH	5.28 (m, 1H)	129.83	CH
C-14	5.55 (m, 1H)	130.16	CH	5.43 (m, 1H)	129.83	CH
C-15	2.17 (m, 2H)	27.29	CH_2	2.02 (m, 2H)	27.19	CH_2
C-16	1.21 (m, 2H)	32.06	CH_2	1.22(m, 2H)	31.96	CH_2
C-17	1.43 (m, 2H)	22.88	CH_2	1.39 (m, 2H)	22.34	CH_2
C-18	0.83 (t, 3H)	14.23	CH_3	0.90 (t, 3H)	13.99	CH_3
OH	5.21 (s, 1H)			5.21 (s, 1H)		

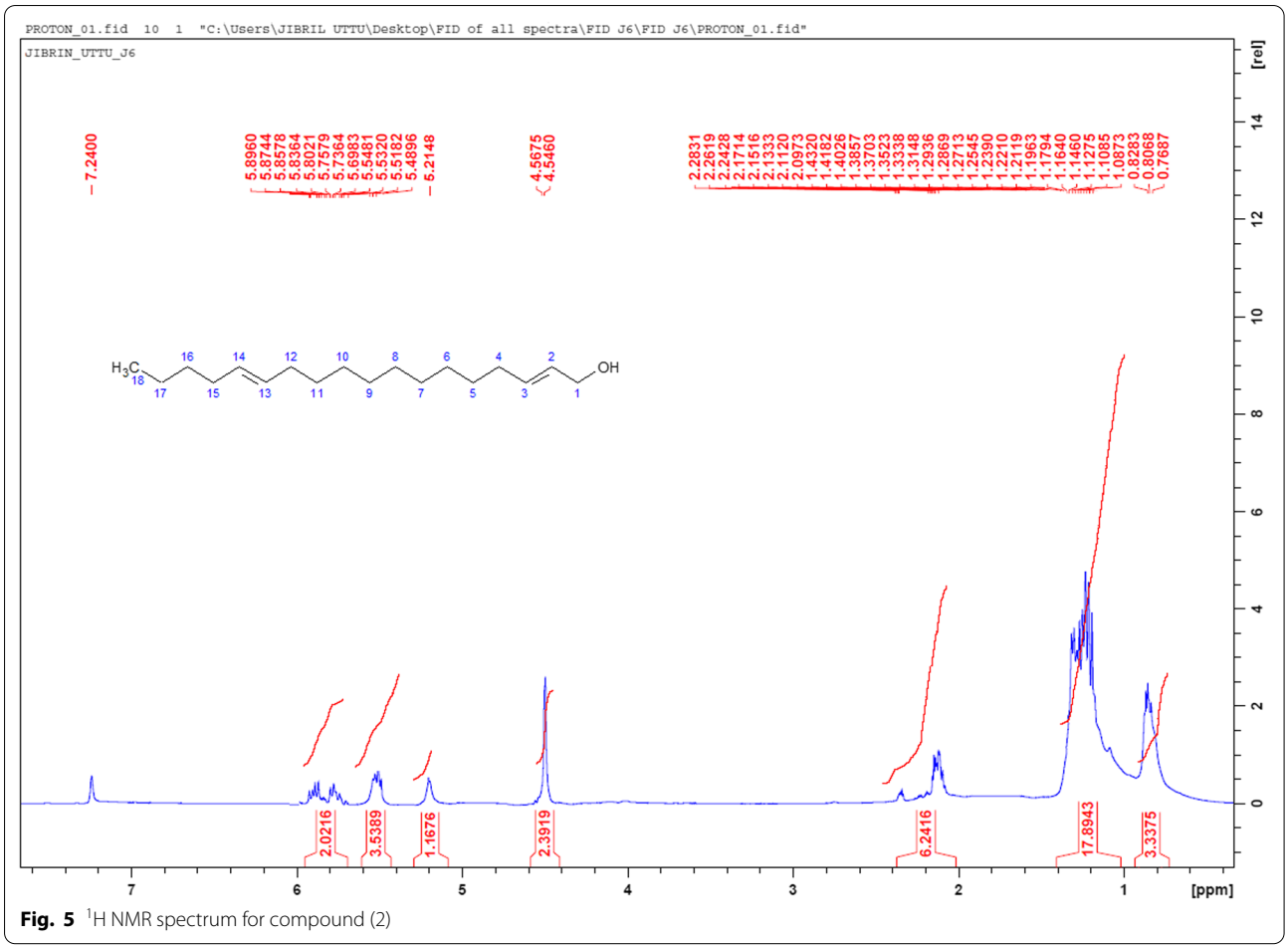


Fig. 5 ^{1}H NMR spectrum for compound (2)

authenticated in the Department of Biology at ABU, Zaria, by Mr. Namadi Sunusi, where V/N–01884 is the herbarium voucher number.

Extraction

The root bark of *S. innocua* was dried under shade. Subsequently, it was then crushed to a fine powder. The powder (i.e., pulverized sample, 2 kg) was subjected to extraction using the maceration technique with solvents *n*-hexane solvent, ethyl acetate solvent, and methanol solvents, respectively, in increasing polarity as reported by Ibrahim et al. (2021); Iyun et al. 2022; Sallau et al. 2022).

General experimental procedure

GC–MS of the isolated compounds was done on GC 7890B, MSD 5977A, Agilent Tech. The NMR (1D and 2D) spectra were measured on Varian–VNMRS 400 MHz spectrometer using dimethyl sulfoxide (DMSO) for Compound (**1**) and chloroform ($CdCl_3$) for Compound (**2**) as the solvent, while ppm is the unit of the chemical shift (δ).

Reagents and chemicals used

All the reagents and chemicals used in this research (n-hexane, ethyl acetate, chloroform, and methanol) are of analytical grade.

Isolation of compounds and purification

The ethyl acetate extract was checked on a pre-coated TLC, which revealed many spots using various solvent systems. The extract (30 g) was mixed with silica gel (60–120 mesh) and left to dry. The dried extract was then transferred into a column (size: 5 cm × 60 cm) after packing with silica gel and n-hexane and eluting with a suitable solvent (n-hexane: ethyl acetate) with increasing polarity (n-hexane 100%, 9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 3:7,

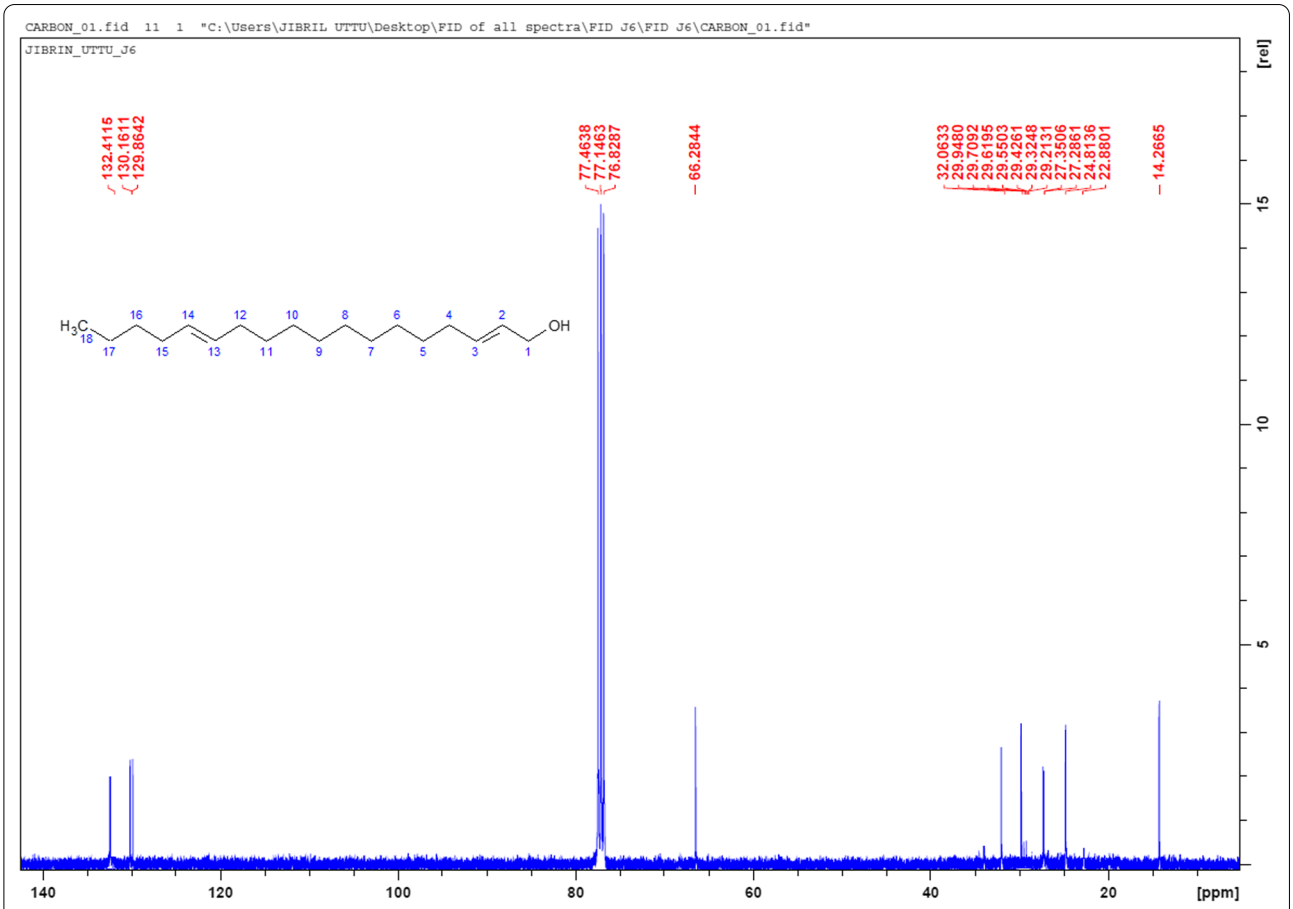


Fig. 6 ^{13}C NMR spectrum for compound (2)

Fig. 7 *Strychnos innocua* showing branches fruit and leaves

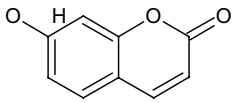


Fig. 8 Structure of Umbelliferone (1)

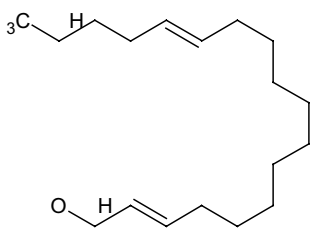


Fig. 9 Structure of 2,3-Octadecadien-1-ol (2)

2:8, 1:9, and 100% ethyl acetate) at a flow rate of 1 drop/sec, resulting in 261 collections of 50 mL. These collections were monitored using a pre-coated TLC with spraying reagent which is a mixture of CH_3OH: CH_3COOH:

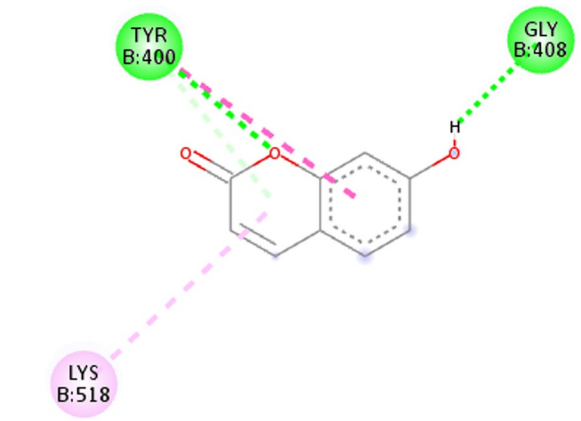


Fig. 10 2D interaction of compound **1** with crystal structure of *S. aureus* (PDB: 3HO8)

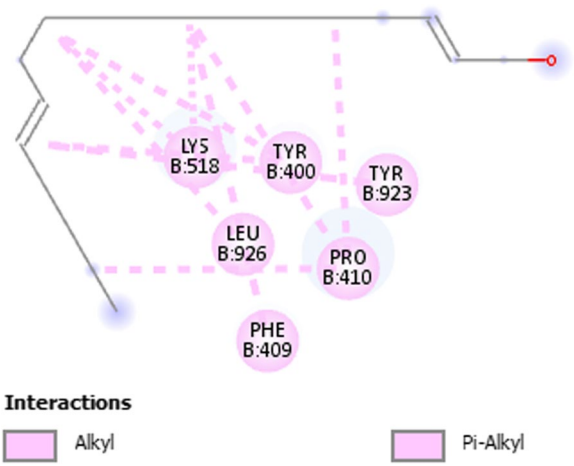


Fig. 11 2D interaction of compound **2** with crystal structure of *S. aureus* (PDB: 3HO8)

H_2SO_4: $CH_3OC_6H_4CHO$ at a ratio of 85:10:5:0.l, respectively, resulting in 24 fractions (F1–F24). The fractions 8 and 9 were combined and separated on column chromatography eluting with HEX: EA in increasing concentrations (HEX: 100%, HEX: EA, 9:1) to obtain 60 collections of 5 mL each. The collections were monitored using a pre-coated TLC plate to give eight subfractions (FF1–FF8). The FF8 was further chromatographed on a small column and eluted with HEX: EA (9.1) to give two

Table 3 Result of the binding scores of isolated compounds/ciprofloxacin with active site of receptor (PDB: 3HO8)

Ligands	Binding Score (Kcal/mol)	Protein interaction	Types of interaction	Bond distance Å
Umbelliferone	− 5.6	TYR400	Conventional Hydrogen bond Pi-Alkyl	2.43
		GLY408		3.25
		LYS518		5.21
2,13-octadecadien-1-ol	− 4.7	PRO410	Alkyl	4.25
		PRO410		4.97
		PRO410		4.06
		LYS519		4.50
		LYS519		4.30
		LEU926		5.27
		TYR400	Pi-Alkyl	4.98
		TYR923		5.46
		PHE409		4.90
		TYR400		4.93
Ciprofloxacin	− 6.6	PRO410	Pi-Sigma	3.70
		PHE934	Pi-Alkyl	5.28
		PHE409		5.12
		PRO410		5.06
		LYS518	Alkyl	4.14
		PRO410		5.15
		ASN403	Conventional Hydrogen bond	2.73

Table 4 Result of the binding scores of the isolated compounds/ciprofloxacin with target receptor (PDB: 2OZ6)

Ligands	Binding score (Kcal/mol)	Protein interaction	Types of interaction	Bond distance Å
Umbelliferone	− 6.9	ARG75	Conventional Hydrogen bond	2.45
		GLY69		2.52
		GLU67		2.87
		LEU68		2.21
		SER76	Pi-Donor Hydrogen Bond	2.88
		ILE25	Pi-Alkyl	5.48
		ARG75		5.32
		LEU68		4.41
		ALA77		4.91
		ILE25		5.26
2,13-octadecadien-1-ol	− 5.7	ALA77	Conventional Hydrogen bond	2.59
		SER76		2.69
		LEU68	Alkyl	5.27
		ALA77		4.25
		ILE44		4.14
		LEU68		5.25
		ARG116		5.10
		ARG116		4.78
		ARG116		4.88
		ILE56		4.88
		ILE44		4.34
		ILE44		4.77
		ILE44		4.47
		LEU59		4.04
Ciprofloxacin	− 8.7	GLU57	Pi-Anion	4.48
		ILE44	Pi-Sigma	3.99
		ALA77	Carbon Hydrogen Bond	2.52
		LEU68	Alkyl	4.76
		ALA77		5.14
		ALA77		4.72
		ILE56		4.42
		ALA77		4.72
		ARG116	Pi-Alkyl	4.84
		LEU68		5.43
		ILE44		4.16
		THR120	Conventional Hydrogen bond	2.37
		GLY66		2.37

smaller fractions (SF1 and SF2). SF2 revealed one spot on TLC to represent compound **1** (Rf = 0.27). The yield was 51 mg. Subfractions FF2 and FF3 were merged and eluted with HEX: EA (9:1) to give four smaller fractions (SF1, SF2, SF3 and SF4). The SF2 revealed one spot on TLC to give compound **2** (Rf = 0.64), the yield value of 24 mg.

In Silico docking studies

In silico docking studies were performed on compounds (**1** and **2**) and ciprofloxacin (standard drug) with target receptors (PDB: 3HO8 and 2OZ6) downloaded from www.rcsb.org. Their two-dimensional structure (2D) was generated from ChemDraw professional 16.0 and

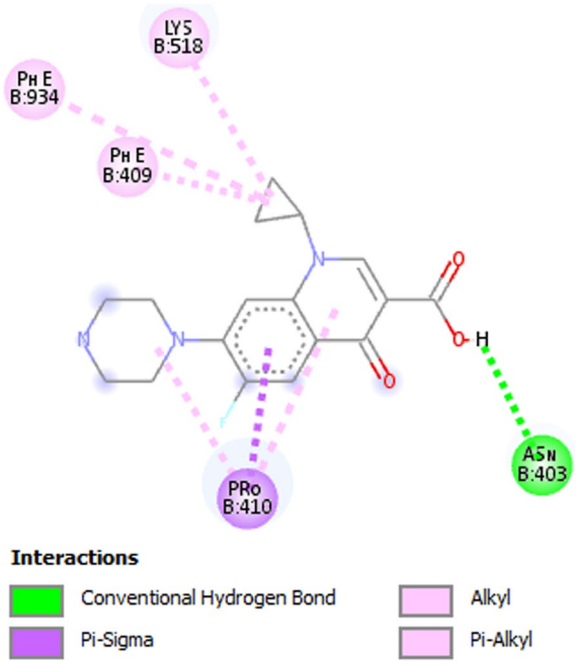


Fig. 12 2D interaction of ciprofloxacin with crystal structure of *S. aureus* (PDB: 3HO8)

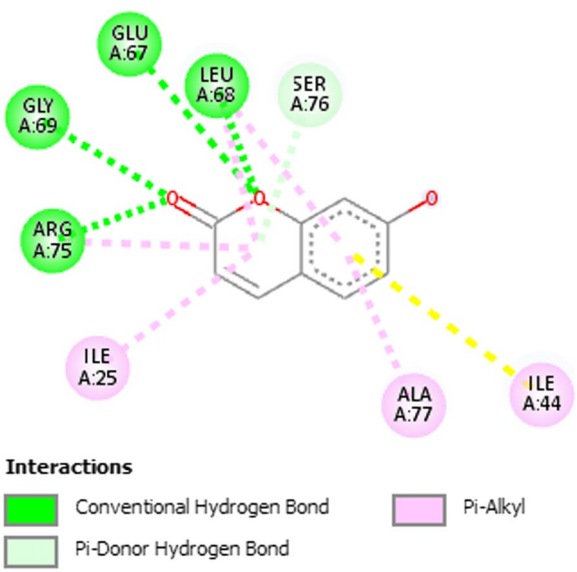


Fig. 13 2D interaction of compound **1** with crystal structure of *P. aeruginosa* (PDB: 2OZ6)

converted to three-dimensional (3D) geometrical optimization using Spartan 20v.1.1 /2020. The target receptors in three-dimensional form were prepared using Discovery Studio Visualizer and saved in PDB and then uploaded for docking studies using Pyrx. The docking output with binding score was visualized in Discovery

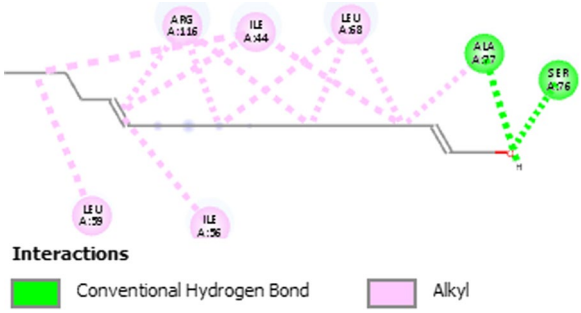


Fig. 14 2D interaction of compound **2** with crystal structure of *P. aeruginosa* (PDB: 2OZ6)

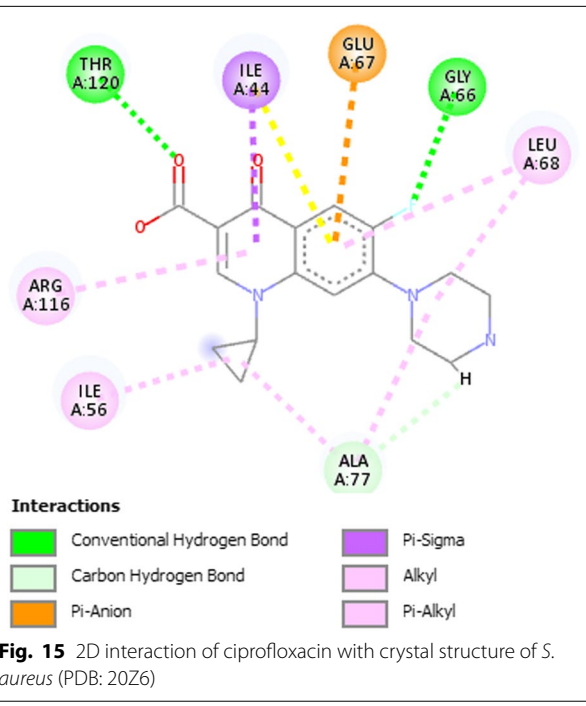


Fig. 15 2D interaction of ciprofloxacin with crystal structure of *S. aureus* (PDB: 20Z6)

Studio to analyze the protein–ligand interactions (Ejeh et al. 2021; Tukur et al. 2022).

Discussion

Compound **1** (41 mg) was gotten as a pale yellow crystal having a 231 °C (melting point). The mass spectrum (Fig. 1) of **1** indicates *m/z* 162 as the molecular ion peak, while *m/z* 134, 105, 78, 67, 51, 43, 39, and 28 are the fragment ions, suggesting its molecular formula to be $C_9H_6O_3$. The NMR spectra data (Table 1) of **1** were very similar to literature for Umbelliferone with ^{1}H NMR (Fig. 2) displaying δ_H for five olefinic methine protons (δ_H 6.00 H-2, 6.51 H-8, 6.75 H-6, 7.31 H-5, and 7.75 H-3). The ^{13}C NMR (Fig. 3) and DEPT displayed 9 carbon

signals for five olefinic methine (δ_C 102.58 C-8, 109.43 C-6, 111.59 C-2, 129.32 C-5, and 144.15 C-3), and four quaternary carbon (δ_C 110.00 C-4, 156.34 C-9, 160.90 C-7, and 161.19 C-1).

Compound **2** (24 mg) was obtained as a clear crystal. The mass spectrum (Fig. 4) of the **2** indicates *m/z* 266 as a fragmentation ion, which represents H_2O was lost from *m/z* 248. Other fragment ions are *m/z* 222, 205, 177, 152, 135, 109, 81, and 55 suggesting its molecular formula to be $C_{18}H_{34}O$. The NMR spectra data (Table 2) were very similar to literature for 2,13-octadecadien-1-ol with ^{1}H NMR (Fig. 5) displaying δ_H for thirteen methylene protons (δ_H 1.16 H-8, 1.19 H-11, 1.21 H-5/16, 1.27 H-9, 1.29 H-7, 1.35 H-6, 1.37 H-10, 1.43 H-17, 2.17 H-12/15, 2.28 H-4, and 4.57 H-1), Four signals for olefinic methine protons (δ_H 5.53 H-13, 5.55 H-14, 5.80 H-2, and 5.90 H-3), one signals for methyl protons (δ_H 0.83 H-18), and one hydroxyl proton (δ_H 5.21). The ^{13}C NMR (Fig. 6) and DEPT displayed 18 carbon signals for thirteen methylene carbons (δ_C 22.88 C-17, 29.55 C-8, 29.71 C-6, 29.62 C-7, 29.43 C-9, 29.32 C-10, 29.94 C-5, 29.21 C-11, 32.06 C-16, 27.29 C-15, 27.35 C-4, 24.81 C-12, and 66.28 C-1), one methyl carbon (δ_C 14.23 C-18), and four olefinic methine carbons (δ_C 129.86 C-2, 130.16 C-13/14, and 132.41 C-3).

The ethyl acetate extract of *S. innocua* (Fig. 7) revealed the presence of coumarin in the phytochemical study, and the extract showed antibacterial activity against *B. subtilis*, *S. aureus,* and *P. aeruginosa* (Sallau et al. 2022). When the extract was subjected to chromatography separation, Umbelliferone and 2,3-Octadecadien-1-ol (Figs. 8 and 9) were isolated, and their structures were determined by spectroscopic analysis and compared to published data (Naka et al. 2006; Singh et al. 2010; Mazimba 2017; Hoskovec et al. 1990). Umbelliferone is found in nature in a variety of plant families and has been shown to have pharmacological effects such as antioxidants and antimicrobials (Mazimba 2017), while 2,13-octadecadien-1-ol was discovered among the major chemical constituents of *Cedrela sinensis* seed, and the plant's antibacterial activity in vitro was found to be significant (Lin et al. 2012).

The interactions of the compounds with the target receptors (PDB: 3HO8 and 2OZ6) were investigated using molecular docking (Tables 3 and 4) and in comparison with ciprofloxacin (standard drug). The binding scores of the compounds with *S. aureus* pyruvate carboxylase 3HO8 (receptor) are all moderately lower than ciprofloxacin, as shown in Table 3. Though Umbelliferone binding energy (-5.6 kcal/mol) is higher than 2,13-octadecadien-1-ol (− 4.7 kcal/mol), their interactions with the receptor are demonstrated in Figs. 10 and 11, while the binding energy of ciprofloxacin was − 6.6 kcal/mol and its interaction is presented in Fig. 12. In addition, Table 4 also demonstrates that the compounds bind to the active site of ***P. aeruginosa virulence factor regulator 2OZ6 (receptor)*** with lower binding scores than ciprofloxacin. Also, Umbelliferone has a higher binding energy (− 6.9 kcal/mol) than 2,13-octadecadien-1-ol (− 5.6 kcal/mol), their interactions with the receptor are depicted in Figs. 13 and 14, respectively. The binding energy of ciprofloxacin was − 8.7 kcal/mol, and its interactions are depicted in Fig. 15.

Conclusions

Isolation of two compounds (Umbelliferone and 2,13-octadecadien-1-ol) from *S. innocua* root bark was carried out through column chromatography, and their structures were characterized using MS and NMR spectroscopic analyses. Docking study results revealed that Umbelliferone showed moderate binding scores of − 5.6 and − 6.9 kcal/mol, while 2,13-octadecadien-1-ol showed − 4.7 and − 5.7 kcal/mol slightly lower than Umbelliferone. These results were all relatively lower than ciprofloxacin (− 6.6 and − 8.7 kcal/mol), hence the compounds might be a potential antibacterial agents against ***P. aeruginosa and*** *S. aureus*.

Abbreviations

NMR: Nuclear magnetic resonance; PDB: Protein data bank; ABU: Ahmadu Bello University; V/N: Voucher number; TLC: Thin-layer chromatography; HEX: Hexane; EA: Ethyl acetate; Rf: Retention factor; 2D: Two dimensional; 3D: Three dimensional; ppm: Parts per millions; N.L: Not listed.

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Author contributions

M.S.S. gave the procedure for isolation, A.J.U carried out the experiments/write the manuscript, O.R.I. assisted in supervising the experiment, and H.I. contributed in NMR elucidation. All authors have read and approved the final Manuscript.

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