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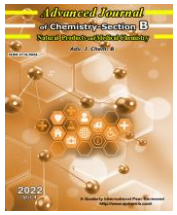

Original Research Article

Isolation and Characterization of Two Phytosterols from *Strychnos Innocua* (Delile) Root Bark, and In Silico Molecular Docking Studies as Antibacterial Agents

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ABSTRACT

Phytosterols derived from medicinal plants are well-known for their therapeutic effects in the treatment of diabetes, cardiovascular disease, cancer, and microbial infections. *Strychnos innocua* (a *Loganiaceae* family member) grows in numerous African countries and is widely used for medicinal purposes. This plant's (root bark) ethyl acetate extract was subjected to chromatographic separation, resulting in the isolation of Campesterol (**1**) and β-Sitosterol (**2**). Their structures were verified using mass spectrometry, nuclear magnetic resonance (1D and 2D NMR), and in comparison to published data. This is a novel report of phytosterol compounds which were isolated from *S. innocua* root bark. The in silico investigation found that the binding affinities of Campesterol (**1**) with binding sites of *Staphylococcus aureus* pyruvate carboxylase (PDB: 3HO8) and *Pseudomonas aeruginosa* virulence factor regulator (PDB: 2OZ6) were -7.8 and -7.9 kcal/mol, respectively. Furthermore, the binding affinities of β-Sitosterol (**2**) with binding sites of *S. aureus* and *P. aeruginosa* are -7.6 and -7.7 kcal/mol, respectively, while ciprofloxacin (standard drugs) exhibited binding affinities of -6.6 and -8.7 kcal/mol. This study concluded that the *S. innocua* root bark has a rich presence of Campesterol and β-Sitosterol, while their molecular docking studies revealed that they have excellent interactions with *S. aureus* and *P. aeruginosa.*

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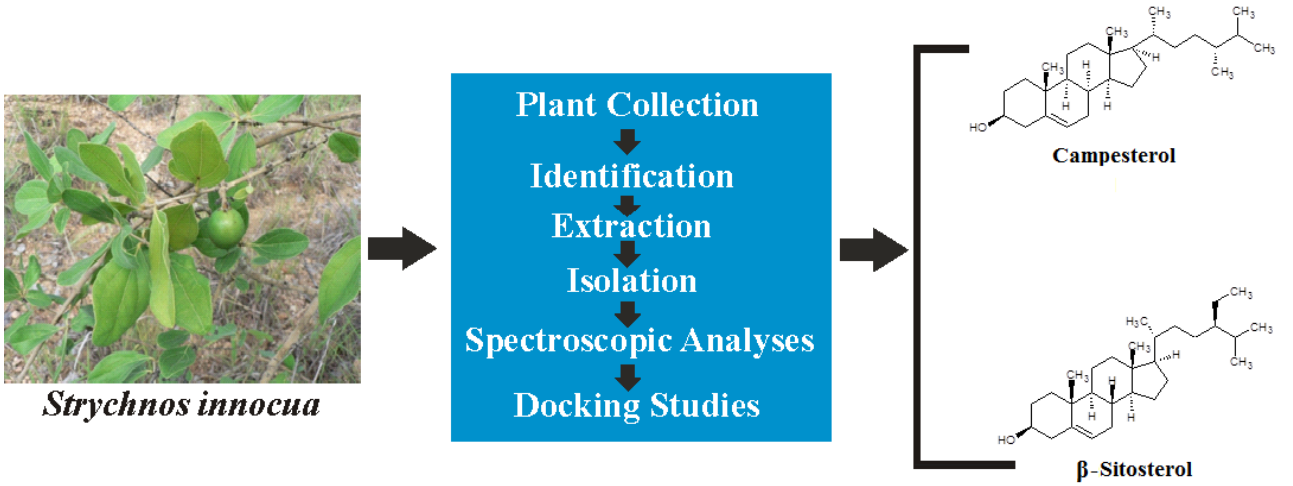
GRAPHICAL ABSTRACT



1. Introduction

Compounds isolated from plants' origins offer immeasurable prospects in the discovery of novel drugs [1]. 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 [2].

Plant phytochemical composition and secondary metabolites like phytosterols include active medicinal components and are linked to their therapeutic potential [3,4]. Campesterol is a naturally occurring plant sterol that has been shown to decrease cholesterol and fight cancer [5]. The presence of β-Sitosterol in *Plumbago zaylanica* is attributed to medicinal applications as antimalarial, antimicrobial, anti-inflammatory, antifertility, wound healing, blood coagulation, and anticancer activities [6].

Natural compounds derived from plants have been tested for antimicrobial activities utilizing a variety of methods (in vitro, in vivo, and in silico). Docking is one of these approaches that have received a lot of applications in the development of microbial medications [7,8].

Strychnos innocua (Figure 1) is a *Loganiaceae* plant that grows up to 18 meters tall and has a straight stem. It has a trunk diameter varying from 7 to 40 cm and many branches. Its leaves are usually plain, with a rounded base on rare occasions. *S. innocua* can be found in Malawi, Cameroon, and Nigeria. The root is reported to treat gonorrhoea, while an infusion of the plant's root (fresh) is used for the treatment of snake bites [9,10]. The plant can be harvested in Kaduna State, Nigeria.

Figure 1: *Strychnos innocua* showing branches fruit, and leaves

S. innocua root bark extracts have been studied for their chemical compositions and antimicrobial properties [11–14]. However,

there is a dearth of information in the literature about the isolation of phytosterols from *S. innocua* root bark. However, the phytosterols, Campesterol (**1**) and β-Sitosterol (**2**) were isolated from *S. innocua* root bark, elucidated, and docked in this study. This is a novel report of phytosterol compounds isolated from root bark of *S. innocua*.

2. Materials and Methods

2.1. Plant collection

The plant of *S. innocua* was collected from the wild in Kaduna State, Nigeria, identified, and authenticated in the Department of Biological Sciences at ABU, Zaria by Mr. Namadi Sunusi, where V/N – 01884 is the herbarium voucher number.

2.2. 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 by using the maceration technique with solvents (n-hexane solvent, ethyl acetate solvent, and methanol solvent) in increasing polarity, as reported by [11].

2.3. General experimental procedure

A GC-MS analysis of the isolated compounds was done on GC 7890B, MSD 5977A, Agilent Tech. The NMR (1D and 2D) spectra were obtained on a Varian–Vnmrs 400 MHz spectrometer with Chloroform ($CdCl_3$), while chemical shift (δ) were reported in ppm.

2.4. Reagents and chemicals used

All of the chemicals and reagents utilized in the study are of analytical grade.

2.5. Isolation and purification

Thin layer chromatography of ethyl acetate extract revealed many spots using several solvent systems. The extract (30 g) was combined with 60-120 mesh silica gel and allowed to dry. After parking (using silica gel and HEX), the dried extract was placed into a column (size, 5 cm × 60 cm) and eluted with a suitable solvent (HEX:EA) at gradually increased polarity (HEX 100%, 9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 3:7, 2:8, 1:9, and 100% EA) at a flow rate of 1 drop/sec, resulting in 261 collections of 50 mL. A pre-coated TLC with spraying reagent (CH_3OH:CH_3COOH:H_2SO_4:$CH_3OC_6H_4CHO$ at a ratio of 85:10:5:0.l mL) were used to monitor these collections, yielding 24 fractions (F1 – F24). The fractions 8 and 9 were combined and separated on column chromatography eluting with HEX:EA in increasing concentration (HEX 100 %, HEX:EA, 9:1) to obtain 60 collections of 5 mL each. The collections were also monitored using a pre-coated TLC plate to give eight subfractions (FF1 – FF8). The subfractions, FF4 and FF5 were further merged and chromatographed on a small column and eluted with HEX:EA (9.1), to give three smaller fractions (SF1, SF2, and SF3). SF2 revealed one spot on TLC to represent compound **1** (Rf = 0.41), the yield was 46 mg. While the subfraction FF2 and FF3 were also merged and eluted with HEX:EA (9:1) to give four smaller fractions (SF1, SF2, SF3, and SF4). SF3 revealed one spot on TLC to give compound **2** (Rf = 0.18), the yield value was 37 mg.

2.6. Molecular docking analyses

Compounds (**1** and **2**), as well as Ciprofloxacin (standard drug), were docked in silico with target receptors (PDB: 3HO8 and 2OZ6) downloaded from (www.rcsb.org). ChemDraw professional 16.0 was used to create their two-dimensional (2D) structure, which was then converted into three-dimensional (3D) geometrical optimization using Spartan 20v.1.1/2020. The target receptors were created in three dimensions using Discovery Studio Visualizer, stored in a file format (PDB), and then they were uploaded for docking using Pyrx software. The docking output was shown in Discovery Studio with the binding score to examine the protein-ligand interactions [15,16].

3. Results and Discussion

Compound **1** (46 mg), in the form of white powder, has a melting point of 162 °C. The mass spectrum (Figure 2) of **1** showed peaks of m/z 400 as molecular ion and fragment ions m/z 367, 316, 289, 255, 213, 173, 145, 109, 81, and 43, suggesting its molecular formula to be $C_{28}H_{48}O$. The NMR spectra data (Table 1) of **1** was very similar to literature for Campesterol with ^{1}H NMR (Figure 3) displaying δ_H for ten methylene protons (δ_H 1.99 H-1, 1.82 H-2, 1.60 H-4, 1.13 H-7, 1.12 H-11, 1.20 H-12, 2.12 H-15, 1.92 H-16, 2.20 H-22, and 1.08 H-23), six methyl protons (δ_H 0.83 H-18, 0.67 H-19, 0.81 H-21, 0.77 C-26, 0.80 H-27, and 0.66 C-28), eight methine protons (δ_H 3.53 H-3, 1.80 H-8, 0.98 H-9, 1.46 H-14, 1.80 H-17, 2.27 H-20, 0.90 H-24, and 1.27 H-25), one olefinic methine proton (δ_H 5.51 H-6), and one hydroxyl proton (δ_H 4.53 OH). The ^{13}C NMR (Figure 4) and DEPT revealed 28 carbon signals for ten methylene carbons (δ_C 37.44 C-1, 31.85 C-2, 42.49 C-4, 32.12 C-7, 23.25 C-11, 39.97 C-12, 24.51 C-15, 26.22 C-16, 34.31 C-22, and 34.13 C-23), six methyl carbons (δ_C 15.58 C-18, 12.19 C-19, 14.35 C-21, 21.28 C-26, 20.01 C-27, and 15.64 C-28), eight methine carbons (δ_C 72.03 C-3, 32.09 C-8, 51.44 C-9, 56.96 C-14, 56.23 C-17, 36.35 C-20, 39.25 C-24, and 33.90 C-25), three quaternary carbons (δ_C 145.43 C-5, 36.71 C-10, and 46.01 C-13), and one olefinic methine carbon (δ_C 121.94 C-6).

Compound **2** (37 mg), in the form of a clear crystal, has 147 °C as the melting point. The mass spectrum (Figure 5) of **2** indicated fragment ion peaks at m/z 396, representing a H_2O was loss from the molecular ion peak (m/z 414). Other fragmentation ions included *m/z* 381, 342, 303, 255, 213, 173, 145, 109, 81, and 43, suggesting its molecular formula to be $C_{29}H_{50}O$. The NMR spectra data (Table 1) of **2** were very similar to the literature for β-Sitosterol, with ^{1}H NMR (Figure 6) displaying δ_H for eleven methylene protons (δ_H 1.72 H-1, 1.93 C-2, 2.32 H-4, 1.95 H-7, 1.19 H-11, 1.22 H-12, 1.53 H-15, 1.20 H-16, 1.43 H-22, 1.22 H-23, and 1.39 H-28), six methyl protons (δ_H 0.87 H-18, 0.78 C-19, 0.97 H-21, 0.82 H-26, 0.85 H-27, and 0.88 H-29), nine methine protons (δ_H 3.57 H-3, 5.46 H-6, 2.19 H-8, 1.10 H-9, 1.15 H-14, 1.21 H-17, 1.51 H-20, 1.14 C-24, and 1.57 H-25), and one hydroxyl proton (δ_H 4.82 OH). The ^{13}C NMR (Figure 7) and DEPT displayed 29 carbon signals for eleven methylene carbons (δ_C 38.89 C-1, 30.27 C-2, 41.25 C-4, 32.14 C-7, 22.91 C-11, 40.09 C-12, 28.95 C-15, 28.88 C-16, 33.20 C-22, 28.98 C-23, and 24.85 C-28), six methyl carbons (δ_C 14.31 C-18, 19.52 C-19, 20.70 C-21, 22.86 C-26, 22.81 C-27, and 14.35 C-29), eight methine carbons (δ_C 72.06 C-3, 31.88 C-8, 51.46 C-9, 56.52 C-14, 55.00 C-17, 33.77 C-20, 45.02 C-24, and 29.91 C-25), three quaternary carbons (δ_C 143.71 C-5, 37.34 C-10, and 43.51 C-13), one olefinic methine carbon (δ_C 122.21 C-6).

In the phytochemical study, the ethyl acetate extract showed the presence of steroids, and the extract had potent antibacterial activity against *S. aureus*, *P. aeruginosa*, and *B. subtilis* [13]. After subjecting the extract to chromatography separation, Campesterol and β-Sitosterol were isolated (Figure 8 and 9), and their structures were determined using spectroscopic analyses and in comparison with data from the literature [17-22]. They compounds are found in a wide range of plant species; their biological activities have been extensively examined, and pharmaceutical effects have been demonstrated. [23] investigated the antifungal activity of Campesterol and β-Sitosterol obtained from *D. asper* against some fungi pathogens and discovered that they have outstanding antifungal properties.

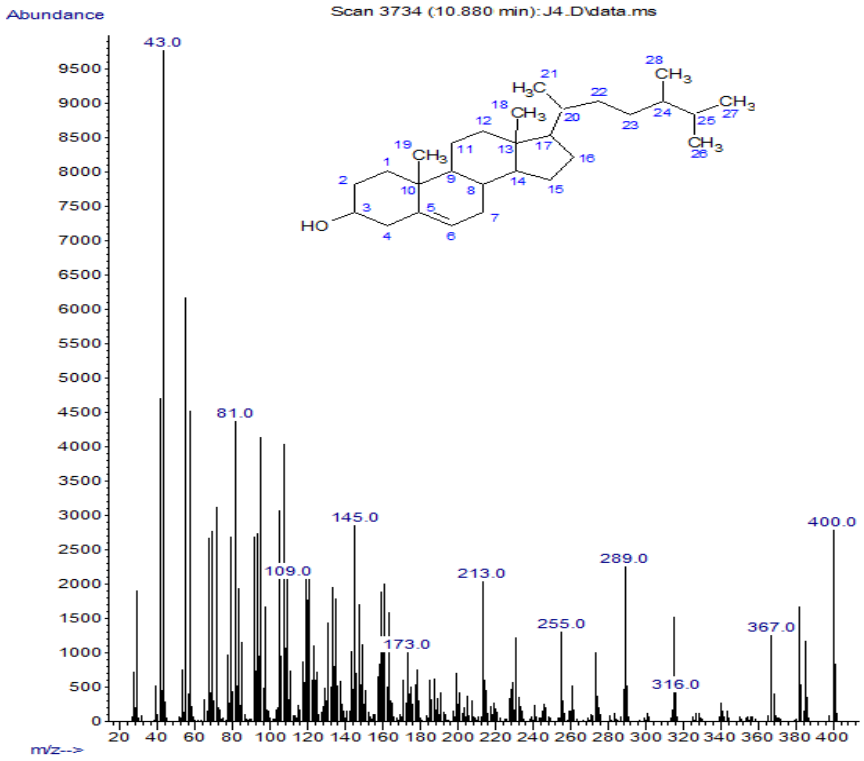


Figure 2: Mass Spectrum of Campesterol (**1**)

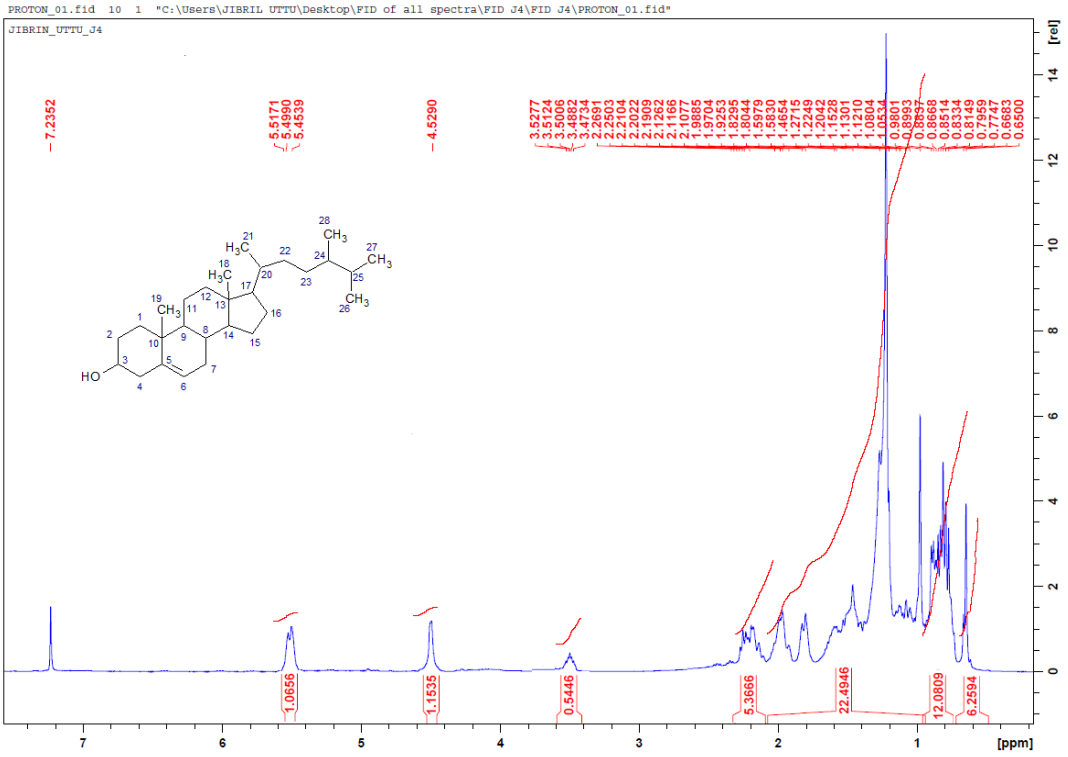


Figure 3: ^{1}H NMR Spectrum of Campesterol (**1**)

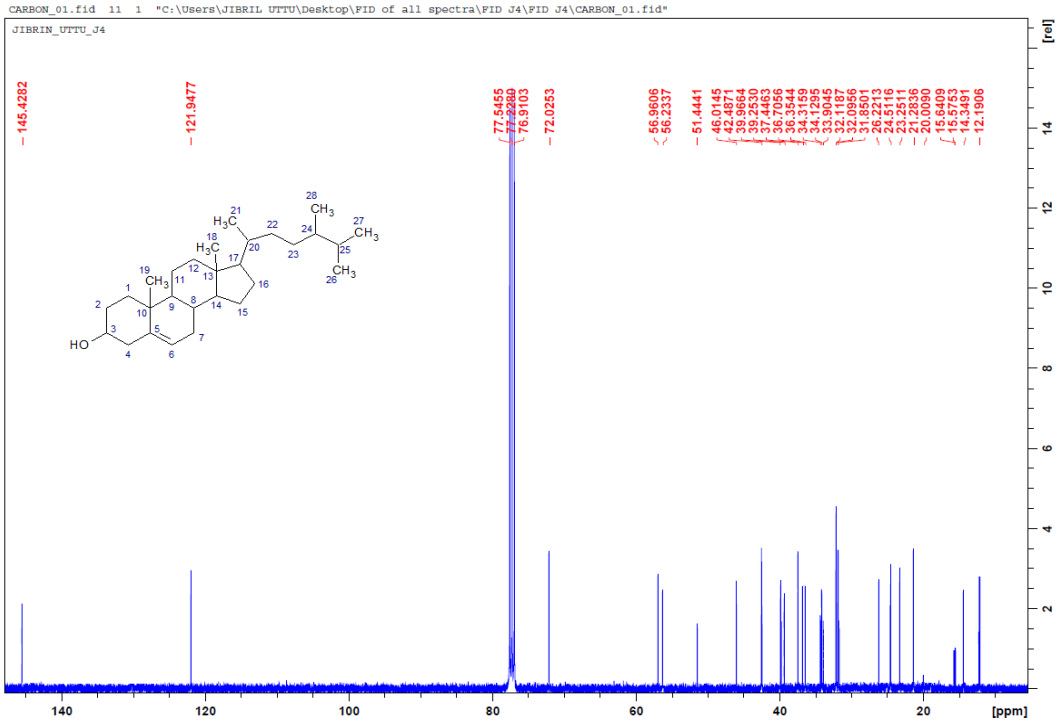


Figure 4: ^{13}C NMR Spectrum of Campesterol (**1**)

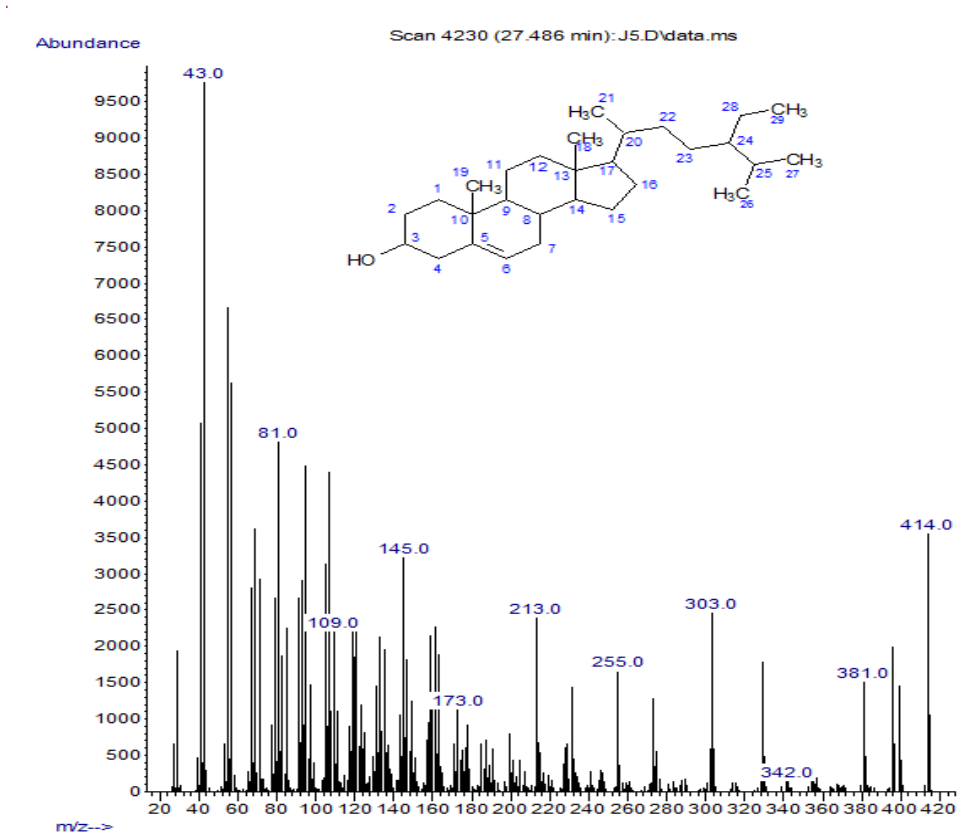


Figure 5: Mass Spectrum of β-Sitosterol (**2**)

Table 1: The NMR (400 MHz, $CDCl_3$) Data of Campesterol (1)

	Campesterol			Literature Data [17,18]		
Position	**^{1}H (ppm)**	**^{13}C (ppm)**	**DEPT**	**^{1}H (ppm)**	**^{13}C (ppm)**	**DEPT**
C-1	1.99 (m, 2H)	37.44	CH_2	1.55 (m, 2H)	37.30	CH_2
C-2	1.82 (m, 2H)	31.85	CH_2	1.52 (m, 2H)	28.90	CH_2
C-3	3.53 (m, 1H)	72.03	CH	3.40 (m, 1H)	71.90	CH
C-4	1.60 (m, 2H)	42.49	CH_2	1.40 (m, 2H)	42.30	CH_2
C-5		145.43	C		142.40	C
C-6	5.51 (m, 1H)	121.94	CH	5.31 (m, 1H)	121.90	CH
C-7	1.13 (m, 2H)	32.12	CH_2	1.33 (m, 2H)	31.80	CH_2
C-8	1.80 (m, 1H)	32.09	CH	1.73 (m, 1H)	31.00	CH
C-9	0.98 (m, 1H)	51.44	CH		51.20	CH
C-10		36.71	C		36.50	C
C-11	1.12 (m, 2H)	23.25	CH_2	1.13 (m, 2H)	21.10	CH_2
C-12	1.20 (m, 2H)	39.97	CH_2	1.21 (m, 2H)	39.80	CH_2
C-13		46.01	C		43.10	C
C-14	1.46 (m, 1H)	56.96	CH	1.83 (m, 1H)	56.90	CH
C-15	2.12 (m, 2H)	24.51	CH_2		21.80	CH_2
C-16	1.92 (m, 1H)	26.22	CH_2	1.92 (m, 1H)	25.00	CH_2
C-17	1.80 (m, 1H)	56.23	CH	1.73 (m, 1H)	56.10	CH
C-18	0.83 (s, 3H)	15.58	CH_3	1.10 (s, 3H)	19.80	CH_3
C-19	0.67 (s, 3H)	12.19	CH_3	0.73 (s, 3H)	12.20	CH_3
C-20	2.27 (m, 2H)	36.35	CH	2.17 (m, 1H)	32.50	CH
C-21	0.81 (d, 3H)	14.35	CH_3	0.81 (d, 3H)	19.10	CH_3
C-22	2.20 (m, 2H)	34.31	CH_2		34.50	CH_2
C-23	1.08 (m, 2H)	34.13	CH_2	1.20 (m, 2H)	30.30	CH_2
C-24	0.90 (m, 1H)	39.25	CH	1.08 (m, 2H)	42.40	CH
C-25	1.27 (m, 1H)	33.90	CH	1.77 (m, 1H)	36.10	CH
C-26	0.77 (d, 3H)	21.28	CH_3	0.83 (d, 3H)	21.20	CH_3
C-27	0.80 (d, 3H)	20.01	CH_3	0.79 (d, 3H)	19.10	CH_3
C-28	0.66 (d, 3H)	15.64	CH_3	0.70 (d, 3H)	15.39	CH_3
OH	4.53 (s, 1H)					

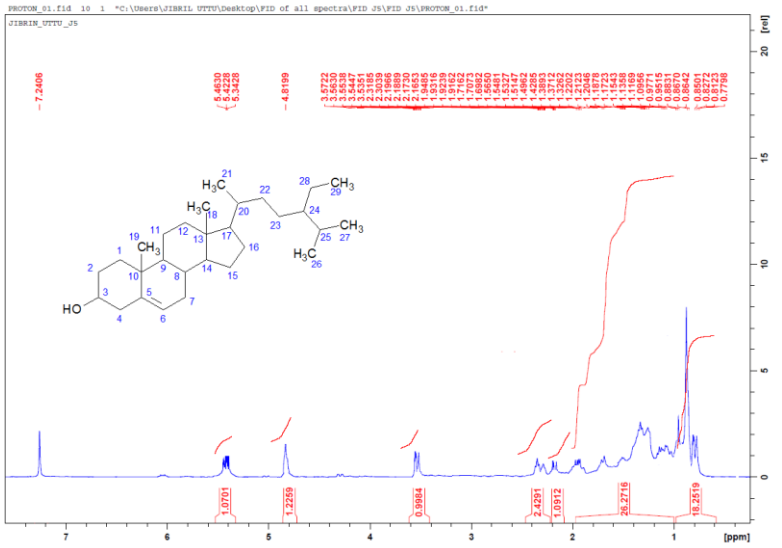


Figure 6: ^{1}H NMR Spectrum of β-Sitosterol (**2**)

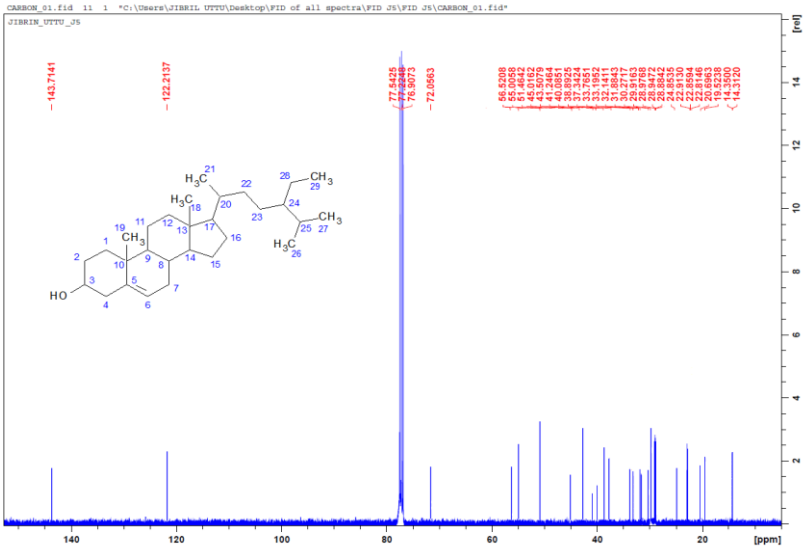


Figure 7: ^{13}C NMR Spectrum of β-Sitosterol (**2**)

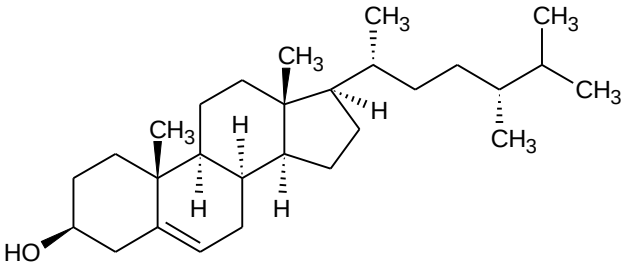


Figure 8: Structure of Campesterol (**1**)

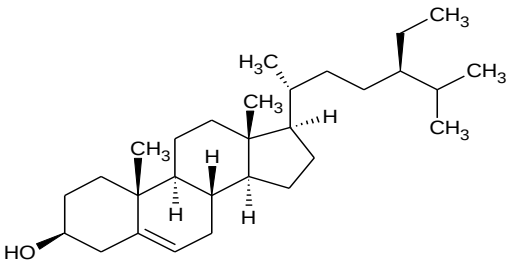


Figure 9: Structure of β-Sitosterol (**2**)

The compounds' interactions with the target receptors (PDB: 3HO8 and 2OZ6) were investigated using molecular docking and compared with ciprofloxacin (standard drug). In comparison to ciprofloxacin, all of the compounds have significantly higher binding scores (Table 3) with *S. aureus* pyruvate carboxylase 3HO8 (receptor). Although the binding energy of Campesterol (-7.8 kcal/mol) is higher than that of β-Sitosterol (-7.6 kcal/mol), their interactions with the receptor are displayed in Figures 10 and 11, respectively. Ciprofloxacin's binding energy was -6.6 kcal/mol, and its interaction with the receptor is depicted in Figure 12. Campesterol identified from *Fiscus religiosa* demonstrated strong interactions with binding sites of crystal structure of the Kelch-Neh2 complex (PDB: 2FLU), suggesting that it is a suitable competitive agent to counteract keapl, and so it offers cancer chemoprevention (24).

Furthermore, the compounds have significantly moderate binding scores (Table 4) with the *P. aeruginosa* virulence factor regulator 2OZ6 (receptor) than ciprofloxacin. Though, the Campesterol has a higher binding energy (-7.9 kcal/mol) than β-Sitosterol (-7.7 kcal/mol), their interactions with the receptor are illustrated in Figures 13 and 14, respectively. The binding energy of ciprofloxacin was -8.7 kcal/mol, and Figure 15 depicts its interaction with the receptor. β-Sitosterol was also identified in *Fiscus religiosa*, exhibited significant interactions with binding sites in the crystal structure of the Kelch-Neh2 complex (PDB: 2FLU), suggesting that it is a potential competitive drug to counteract keapl, and hence offers cancer chemoprevention (24).

Table 2: The NMR (400 MHz, $CDCl_3$) Data of β-Sitosterol (**2**)

	β-Sitosterol			Literature Data [19,20]		
Position	**^{1}H (ppm)**	**^{13}C (ppm)**	**DEPT**	**^{1}H (ppm)**	**^{13}C (ppm)**	**DEPT**
C-1	1.72 (m, 2H)	38.89	CH_2	1.85 (m, 2H)	37.39	CH_2
C-2	1.93 (m, 2H)	30.27	CH_2	1.95 (m, 2H)	31.76	CH_2
C-3	3.57 (m, 1H)	72.06	CH	3.55 (m, 1H)	71.95	CH
C-4	2.32 (m, 2H)	41.25	CH_2	2.38 (m, 2H)	42.39	CH_2
C-5		143.71	C		140.85	C
C-6	5.46 (m, 1H)	122.21	CH	5.37 (m, 1H)	121.85	CH_2
C-7	1.95 (m, 2H)	32.14	CH_2	1.99 (m, 2H)	32.06	CH_2
C-8	2.19 (m, 1H)	31.88	CH	2.00 (m, 1H)	31.93	CH
C-9	1.10 (m, 1H)	51.46	CH	0.94 (m, 1H)	50.28	CH
C-10		37.34	C		36.64	C
C-11	1.19 (m, 2H)	22.91	CH_2	1.02 (m, 2H)	21.22	CH_2
C-12	1.22 (m, 2H)	40.09	CH_2	1.16 (m, 2H)	39.92	CH_2
C-13		43.51	C		42.46	C
C-14	1.15 (m, 1H)	56.52	CH	1.00 (m, 1H)	56.90	CH
C-15	1.53 (m, 2H)	28.95	CH_2	1.58 (m, 2H)	28.39	CH_2
C-16	1.20 (m, 2H)	28.88	CH_2	1.09 (m, 2H)	28.35	CH_2
C-17	1.21 (m, 1H)	55.00	CH	1.12 (m, 1H)	56.18	CH
C-18	0.87 (s, 3H)	14.31	CH_3	0.85 (s, 3H)	12.12	CH_3
C-19	0.78 (s, 3H)	19.52	CH_3	0.82 (s, 3H)	19.40	CH_3
C-20	1.51 (m, 1H)	33.77	CH	1.35 (m, 1H)	36.29	CH
C-21	0.97 (d, 3H)	20.70	CH_3	0.95 (d, 3H)	18.92	CH_3
C-22	1.43 (m, 2H)	33.20	CH_2	1.33 (m, 2H)	34.07	CH_2
C-23	1.22 (m, 2H)	28.98	CH_2	1.16 (m, 2H)	26.14	CH_2
C-24	1.14 (m, 1H)	45.02	CH	0.94 (m, 1H)	45.99	CH
C-25	1.57 (m, 1H)	29.91	CH	1.66 (m, 1H)	28.91	CH
C-26	0.82 (d, 3H)	22.86	CH_3	0.83 (d, 3H)	21.38	CH_3
C-27	0.85 (d, 3H)	22.81	CH_3	0.84 (d, 3H)	19.18	CH_3
C-28	1.39 (m, 2H)	24.85	CH_2	1.25 (m, 2H)	23.20	CH_2
C-29	0.88 (m, 3H)	14.35	CH_3	0.85 (m, 3H)	12.19	CH_3
OH	4.82 (s, 1H)					

Table 3: Results of Binding Scores of Isolated Compounds/Ciprofloxacin with Receptor (PDB: 3HO8)

Ligands	Binding Score (Kcal/mol)	Protein Interaction	Types of Interaction	Bond Distance Å
Campesterol	-7.8	PHE516	Alkyl	4.74
		PRO410	Alkyl	5.03
		PRO410	Alkyl	5.46
		PRO410	Alkyl	5.16
		LYS518	Alkyl	4.92
		LYS518	Alkyl	5.46
		LYS518	Alkyl	4.23
		VAL404	Alkyl	4.37
		PHE516	Pi-Alkyl	5.42
		TYR400	Pi-Alkyl	5.37
		TRY400	Pi-Alkyl	5.27
		GLY408	Carbon Hydrogen Bond	2.81
β-Sitosterol	-7.6	PRO410	Alkyl	4.97
		PRO410	Alkyl	4.00
		PRO410	Alkyl	4.70
		LEU926	Alkyl	5.03
		LYS518	Alkyl	5.25
		LYS518	Alkyl	4.77
		LYS518	Alkyl	4.14
		VAL404	Alkyl	4.79
		PHE516	Pi-Alkyl	4.81
		PHE409	Pi-Alkyl	4.68
		PHE934	Pi-Alkyl	5.13
		TYR400	Pi-Alkyl	5.06
		TYR400	Pi-Alkyl	4.61
		TRY923	Pi-Alkyl	4.77
		GLY408	Carbon Hydrogen Bond	2.99
Ciprofloxacin	-6.6	PRO410	Pi-Sigma	3.70
		PHE934	Pi-Alkyl	5.28
		PHE409	Pi-Alkyl	5.12
		PRO410	Pi-Alkyl	5.06
		LYS518	Alkyl	4.14
		PRO410	Pi-Alkyl	5.15
		ASN403	Conventional Hydrogen bond	2.73

Table 4: Results of Binding Scores of Isolated Compounds/Ciprofloxacin with Receptor (PDB: 2OZ6)

Ligands	Binding Score (Kcal/mol)	Protein Interaction	Types of Interaction	Bond Distance Å
Campesterol	-7.9	ALA77	Alkyl	3.79
		LEU59	Alkyl	4.43
		ILE44	Alkyl	4.22
		VAL79	Alkyl	5.46
		ARG116	Alkyl	5.26
		ARG116	Alkyl	4.05
		ARG116	Alkyl	5.57
		LEU117	Alkyl	4.92
		LEU68	Alkyl	3.82
		LEU68	Alkyl	5.42
		LEU68	Alkyl	4.48
		LEU68	Alkyl	4.48
		LEU68	Alkyl	4.42
β-Sitosterol	-7.7	ILE56	Alkyl	4.16
		ILE44	Alkyl	4.44
		ILE44	Alkyl	3.95
		LEU59	Alkyl	4.25
		ARG116	Alkyl	4.20
		ARG116	Alkyl	4.50
		LEU68	Alkyl	4.70
		LEU68	Alkyl	4.84
		MET113	Alkyl	4.74
		MET113	Alkyl	4.32
		LEU117	Alkyl	4.16
		LEU117	Alkyl	5.03
		LEU117	Alkyl	4.25
		LEU117	Alkyl	4.19
Ciprofloxacin	-8.7	GLU57	Pi-Anion	4.48
		ILE44	Pi-Sigma	3.99
		ALA77	Carbon Hydrogen Bond	2.52
		LEU68	Alkyl	4.76
		ALA77	Alkyl	5.14
		ALA77	Alkyl	4.72
		ILE56	Alkyl	4.42
		ALA77	Alkyl	4.72
		ARG116	Pi-Alkyl	4.84
		LEU68	Pi-Alkyl	5.43
		ILE44	Pi-Alkyl	4.16
		THR120	Conventional	2.37
		GLY66	Hydrogen bond	2.37

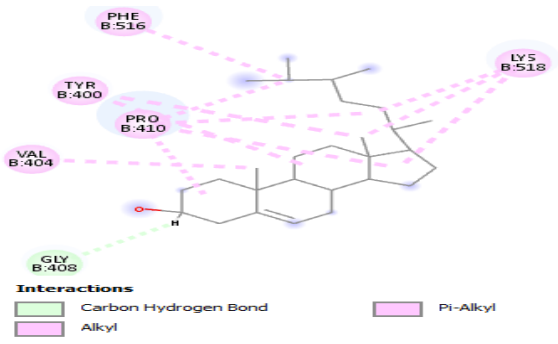


Figure 10: 2D Interaction of Campesterol (**1**) with crystal structure of *S. aureus* (PDB: 3HO8)

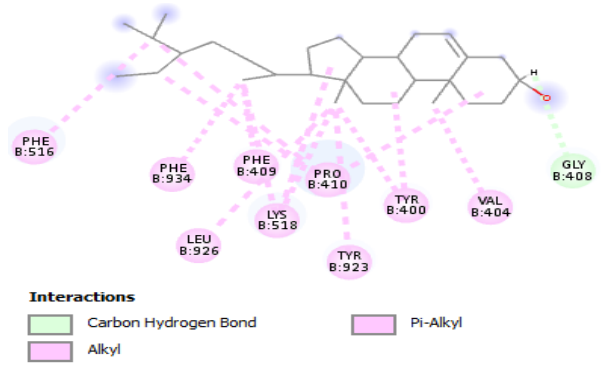


Figure 11: 2D Interaction of β-Sitosterol (**2**) with crystal structure of *S. aureus* (PDB: 3HO8)

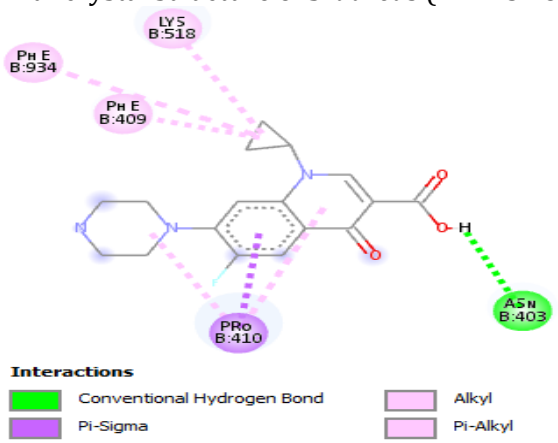


Figure 12: 2D Interaction of Ciprofloxacin with crystal structure of *S. aureus* (PDB: 3HO8).

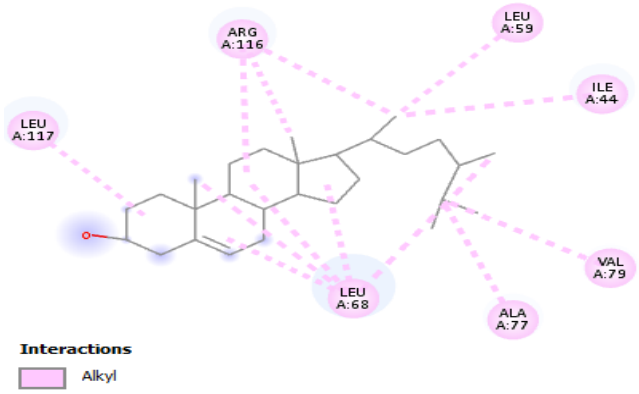


Figure 13: 2D Interaction of Campesterol (**1**) with crystal structure of *P. aeruginosa* (PDB: 2OZ6)

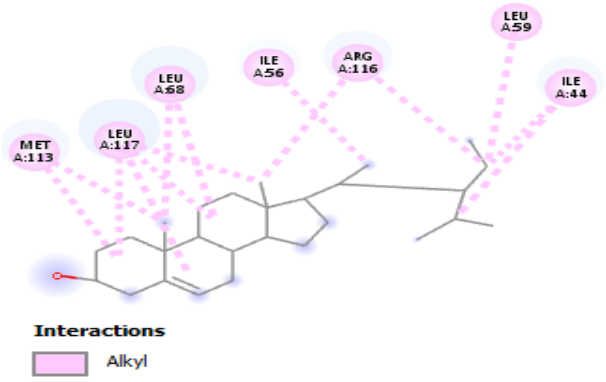


Figure 14: 2D Interaction of β-Sitosterol (**2**) with crystal structure of *P. aeruginosa* (PDB: 2OZ6)

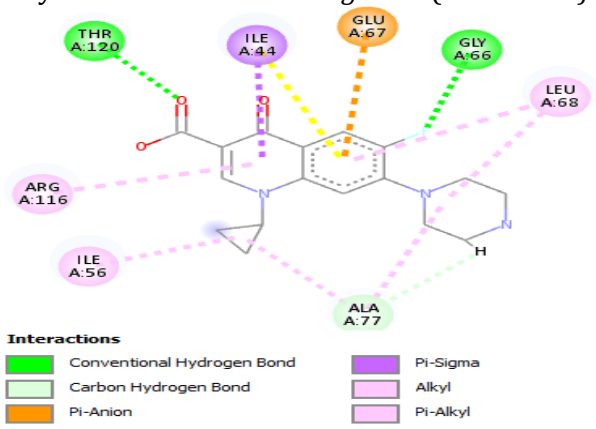


Figure 15: 2D Interaction of Ciprofloxacin with crystal structure of *P. aeruginosa* (PDB: 2OZ6).

4. Conclusion

The structures of two compounds (Campesterol and β-sitosterol) isolated from *S. innocua* root bark were determined using MS, and NMR spectroscopy. Campesterol and β-Sitosterol had a binding score of -7.8 and -7.7 kcal/mol with the binding site of *S. aureus* (PDB: 3HO8) in the docking investigation, which is higher than Ciprofloxacin (drug). Furthermore, the compounds also demonstrated binding affinity of -7.9 and 7.7 kcal/mol with the binding site of *P. aeruginosa* (PDB: 2OZ6), which is comparable with ciprofloxacin (-8.7 kcal/mol). This suggests

that the compounds might be possible agents for antibacterial activity.

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