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Bioactive Steroids from *Heeria insignis* Leaf: Isolation, NMR-Based Structural Elucidation, Antimicrobial Profiling, and Molecular Docking against *Plasmodium falciparum*

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Abstract

Despite continued researches, long-term use of antimalarial drugs has decreased their effectiveness because of increasing parasite resistance. This study accesses the efficacy of steroidal alkaloids from the leaf of *Heeria insignis* against *Plasmodium falciparum* parasite. Cold maceration was used for extraction. Chromatographic purification facilitated the isolation of four pure compounds, and their structural elucidation was performed using ^{1}H and ^{13}C NMR spectroscopy. Molecular docking investigations were conducted on these isolated ligands using the PDB structure 1ME6. Antimicrobial studies were performed using the well diffusion method, and the minimum inhibitory concentration (MIC) was determined. The dichloromethane (DCM) extract exhibited a significant antimicrobial effect, with inhibition zones ranging from 22 to 32 mm and an MIC of 0.625 mg/mL against *MRSA*, *Bacillus subtilis*, and *Candida albicans*. Molecular docking studies showed that Mefloquinine had a binding energy of -7.9 kcal/mol, while compound A3 demonstrated the lowest binding energy of -8.2 kcal/mol, indicating superior receptor interactions. Key target residues involved in these interactions included SER, ILE, VAL, TYR, and GLY, which play a crucial role in inhibiting *Plasmodium falciparum*-associated infections. The study highlights the potential of isolated compounds, particularly A3, as promising antimalarial agents due to their strong molecular interactions with key target residues. The potent antimicrobial activity of the DCM extract further supports its potential in combating infectious diseases.

Keywords: Molecular docking; chromatographic purification; structural elucidation*; P. falciparum*

Introduction

Approximately 500,000 people are killed and over 228 million are afflicted annually by malaria, a common infectious disease spread by the Anopheles mosquito [10, 11]. The well-known medicinal herb *Heeria insignis*, which has been used for millennia, is a member of the Anacardiaceae family. It is native to tropical regions and has been used to cure a variety of ailments, such as fever, inflammatory diseases, and infections [1, 2, 3]. Recent studies have shown its potential as an antimalarial medication due to its varied phytochemical makeup, which includes flavonoids, alkaloids, and terpenoids with bioactive properties against Plasmodium species [4].

The search for novel natural drug candidates is necessary due to the increased drug-resistant forms of malaria. In vitro, phytochemicals derived from *H. insignis* have demonstrated strong antiplasmodial action, indicating that they can stop Plasmodium falciparum from growing and interact with important molecular targets throughout the parasite's life cycle [5, 6]. Furthermore, it may work in concert with its antioxidant and antibacterial qualities to effectively combat malaria [7, 8]. Given these results, H. insignis is positioned as a viable target for additional research into the development of antimalarial treatments.

[9] reported the antimicrobial action of *H. insignis, a*ccording to the findings, H. insignis extract demonstrated strong antibacterial effects against the studied microbes. It has been stated that the consumption of H. insignis roots in Guinea-Bissau induces lactation [10].

The current study is focused on identifying, describing and evaluating the biologically active compounds from *Heeria insignis* (Cork Bush) leaves as potential antimalarial drugs against *Plasmodium falciparum's* plasm epsin II, an aspartyl protease.

Materials and Methods

Collection and identification of leaves sample

Leaves of *Heeria insignis* were collected fresh from their natural habitat in Zaria, Nigeria (Latitude 11.0667^0 N, Longitude 7.716^{0}E) during the rainy season in July 2023. To reduce exposure to heat and direct sunshine, the plant components were collected early in the morning. Standard plant collection procedures were followed during the collection process, ensuring that the environment would sustain little harm. The plant sample was verified and a voucher specimen (No. 014) was deposited. At room temperature, the freshly collected leaves sample were dried for a period of fourteen (14) days weeks to remove excess moisture and to prevent degradation from direct sunlight, ensuring that active compounds are preserved. The dried leaves were then pulverized and grounded into powder, which was stored until further used.

Extraction and isolation

Extraction was achieved by adopting a macceration procedure as described by [12, 13, 14]. The dichloromethane extract (6 g) was chromatographed, 100% n-hexane was used to continuously elute a 75 by 3.5 cm silica gel packed column. This was followed by solvent systems consisting of 100 % ethyl acetate, n-hexane:ethyl acetate and 100 % methanol. Fifty 100 mL portions were collected. Nine sub-fractions were obtained by pooling the 50 fractions together depending on how identical their thin layer chromatopgraphy (TLC) patterns were. Sub-fractions 4 underwent repeated column chromatography, yielding 71 sub-fractions. Four spots were found in sub-fractions 17–47, which were combined and given the designation HF417–47. To obtain the pure isolate, this fraction underwent preparative TLC; they were then analyzed using nuclear magnetic resonance (NMR) spectroscopy.

NMR Analysis

To confirm the chemical structures of the isolated compounds, NMR studies conducted using Bruker Advance III400 MHz Spectrometer at the Multi user Science Research Laboratory, Department of Chemistry, Ahmadu Bello University, Zaria.

Antimicrobial Evaluation

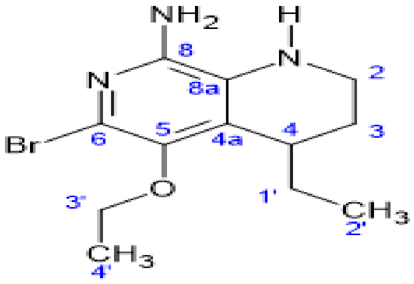
Antimicrobial potentials of the isolated compounds and the standard antibiotics (Ciprofloxacin, Sparfloxacin, and Fluconazole), were investigated against some microorganisms including; *Staphylococcus aureus*, *Streptococcus faecalis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Proteus rettgeri*, Vancomycin-resistant enterococci, *Bacillus subtilis*, *Candida stellatoidea*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Candida pseudotropicalis*, and *Candida albicans,* by adopting standard methods as described by [15, 16, 17].

Molecular Docking Investigations

In order to determine the optimum contact and the orientation that optimizes interactions and minimizes energy with the target receptor (PDB: 1ME6), molecular dockinginvestigations of the four isolated ligands were conducted. This was achieved by; prepataration of the receptor, preparation of the ligand, and the molecular docking analysis as described by [18, 19].

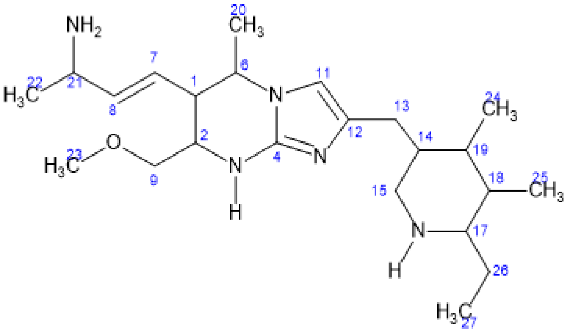
Results and Discussion

***bromo-5-ethoxy-4-ethyl-1,2,3,4-tetrahydro-1,7-naphthyridin-8-amine* (A1)** ^{13}C NMR: δ 131.13 (s), 128.73 (s), 67.38 (s), 38.74 (s), 30.26 (s), 29.67 – 28.27 (m), 23.58 (s), 22.75 (s), 13.44 (s), 10.42 (s). ^{1}H NMR: δ 7.77 (s, 14H), 7.59 (ddd, J = 314.1, 159.6, 156.1 Hz, 100H), 6.85 – 6.37 (m, 27H), 5.23 – 5.18 (m, 4H), 4.23 (qd, J = 16.2, 8.8 Hz, 34H), 3.60 (s, 24H), 3.29 (d, J = 9.3 Hz, 5H), 3.24 (s, 5H), 2.71 (d, J = 110.3 Hz, 67H), 2.28 (s, 32H), 2.20 (s, 7H), 2.11 (d, J = 9.2 Hz, 6H), 2.05 (dd, J = 3.9, 2.1 Hz, 100H), 1.96 (s, 9H), 1.90 (s, 9H), 1.80 (s, 9H), 1.69 (dd, J = 11.5, 5.8 Hz, 30H), 1.61 – 1.33 (m, 176H), 1.33 – 1.01 (m, 199H), 1.04 (d, J = 7.1 Hz, 22H), 0.99 – -0.75 (m, 301H), 0.61 (s, 118H), 0.61 (s, 6H), 1.01 – -6.80 (m, 389H), 1.07 – -6.80 (m, 410H).



(A1)

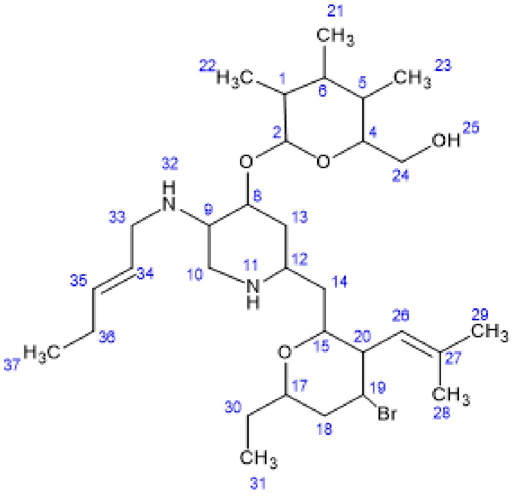
(E) ***-4-(2-((6-ethyl-4,5-dimethylpiperidin-3-yl)methyl)-7-(methoxymethyl)-5-methyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-6-yl)but-3-en-2-amine*** **(A2):** ^{13}C NMR: δ 131.11 (d, J = 5.4 Hz), 128.72 (s), 67.41 (s), 64.99 (s), 38.74 (s), 37.12 (s), 33.34 (s), 31.77 (d, J = 6.0 Hz), 30.35 (d, J = 16.6 Hz), 29.88 – 28.24 (m), 24.99 (d, J = 43.1 Hz), 24.75 – 24.07 (m), 23.59 (s), 22.75 (s), 20.58 (s), 18.96 (s), 18.48 (s), 13.46 (s), 13.11 (s), 11.64 (d, J = 18.8 Hz), 10.44 (s). ^{1}H NMR: δ 7.70 (dd, J = 35.9, 2.3 Hz, 25H), 5.26 – 5.15 (m, 5H), 5.13 – 5.02 (m, 4H), 4.48 (s, 3H), 4.57 – 4.18 (m, 29H), 4.08 (d, J = 8.2 Hz, 3H), 3.60 (s, 5H), 3.50 (s, 4H), 3.33 (s, 5H), 3.29 (d, J = 13.5 Hz, 10H), 3.27 (d, J = 24.8 Hz, 29H), 3.03 (dd, J = 192.1, 96.8 Hz, 52H), 3.39 – 2.45 (m, 59H), 3.39 – 2.31 (m, 66H), 2.64 (ddd, J = 138.9, 102.0, 51.2 Hz, 85H), 3.39 – 1.97 (m, 96H), 3.39 – 1.88 (m, 102H), 3.39 – 1.75 (m, 115H), 2.19 (dddd, J = 77.5, 47.7, 41.2, 21.4 Hz, 145H), 3.39 – 1.49 (m, 165H), 3.39 – 1.07 (m, 293H), 1.07 (s, 11H), 1.08 – 0.58 (m, 96H), 0.59 (s, 30H), 0.58 – -0.28 (m, 24H), 0.61 – -18.85 (m, 70H).



(A2)

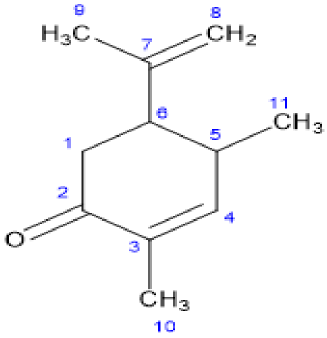
(F) ***-(6-((2-((4-bromo-6-ethyl-3-(2-methylprop-1-en-1-yl)tetrahydro-2H-pyran-2-yl)methyl)-5-(pent-2-en-1-ylamino)piperidin-4-yl)oxy)-3,4,5-trimethyltetrahydro-2H-pyran-2-yl)methanol*** **(A3):** ^{13}C NMR: δ 131.10 (d, J = 5.4 Hz), 128.72 (s), 64.99 (s), 37.12 (s), 33.34 (s), 31.78 (d, J = 5.7 Hz), 30.35 (d, J = 16.7 Hz), 29.81 – 28.30 (m), 25.21 (s), 24.74 (d, J = 6.9 Hz), 23.59 (s), 22.78 (d, J = 3.8 Hz), 22.44 (s), 20.96 (s), 20.59 (s), 18.96 (s), 18.49 (s), 13.50 (s), 13.13 (s), 11.65 (d, J = 19.0 Hz).^{1}H NMR: δ 7.86 – 7.48 (m, 5H), 6.04 (dd, J = 34.1, 15.4 Hz, 1H), 5.20 (dd, J = 15.3, 8.8 Hz, 1H), 5.07 (dd, J = 15.1, 8.7 Hz, 1H), 4.49

– 4.35 (m, 1H), 4.35 – 4.14 (m, 5H), 4.07 (dd, *J* = 17.0, 10.0 Hz, 1H), 3.60 (s, 1H), 3.50 (dd, *J* = 13.0, 8.4 Hz, 1H), 3.33 – 3.22 (m, 1H), 3.06 – 2.96 (m, 1H), 2.84 (d, *J* = 18.2 Hz, 1H), 2.31 – 2.22 (m, 3H), 2.14 – 2.02 (m, 2H), 1.92 (d, *J* = 28.7 Hz, 2H), 1.85 (dd, *J* = 45.7, 29.7 Hz, 4H), 2.00 – 1.60 (m, 12H), 2.00 – 1.55 (m, 15H), 2.00 – -0.55 (m, 73H), 0.51 (d, *J* = 62.8 Hz, 4H), 0.51 (d, *J* = 62.8 Hz, 8H), 0.40 – 0.10 (m, 2H).



(A3)

2,4-dimethyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one **(A4):** ^{1}H NMR: δ 6.49 (d, *J* = 12.9 Hz, 12H), 5.52 (s, 9H), 5.45 (s, 9H), 3.24 (s, 13H), 3.16 (s, 11H), 3.08 (s, 8H), 2.85 (s, 118H), 2.55 (s, 15H), 2.21 (s, 19H), 2.05 (s, 179H), 1.96 (s, 21H), 1.89 (s, 23H), 1.75 – 1.66 (m, 51H), 1.59 – 1.48 (m, 116H), 1.11 (s, 77H).



(A4)

Table 1: Binding Scores of Isolated compounds and Mefloquinine against the active site of (PDB: 1ME6)

Ligands	Binding Score (Kcal/mol)	Protein Interaction	Types of Interaction	Bond Distance Å
6-bromo-5-ethoxy-4-ethyl-1,2,3,4-tetrahydro-1,7-naphthyridin-8-amine (A1)	-6.9	SER B:218	Donor-Donor	1.29
		SER B:218	Conventional	3.03
		SER B:79	Conventional	2.95
		LEU B:292	Alkyl	5.46
		ILE B:300	Alkyl	4.89
		VAL B:78	Alkyl	3.90
		VAL B:78	Alkyl	4.21
		TYR B:77	Pi-Alkyl	4.74
		TYR B:77	Pi-Sigma	2.81
*(E)-4-(2-((6-ethyl-4,5-dimethylpiperidin-3-yl)methyl)-7-(methoxymethyl)-5-methyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrimidin-6-yl)but-3-en-2-amin*e (A2)	-7.2	GLY B:216	Conventional	2.98
		ASP B:214	Conventional	2.44
		VAL B:78	Alkyl	4.86
		GLY B:216	Conventional	2.98
		GLY B:216	Conventional	2.18
		TYR B:77	Pi-Alkyl	5.21
		SER B:218	Carbon	2.86
		TYR B:17	Carbon	2.58
		PHE B:11	Pi-Alkyl	4.84
		ALA B:117	Carbon	2.91
		ALA B:117	Alkyl	3.77
(E)-(6-((2-((4-bromo-6-ethyl-3-(2-methylprop-1-en-1-yl)tetrahydro-2H-pyran-2-yl)methyl)-5-(pent-2-en-1-ylamino)piperidin-4-yl)oxy)-3,4,5-trimethyltetrahydro-2H-pyran-2-yl)methanol (A3)	-8.2	ILE B:123	Alkyl	4.16
		ILE B:32	Alkyl	4.38
		PHE B:11	Pi-Alkyl	5.07
		PHE B:11	Pi-Alkyl	5.28
		ILE B:14	Alkyl	3.97
		VAL B:78	Alkyl	3.98
		ILE B:290	Alkyl	5.15
		ILE B:290	Alkyl	4.43
		ALA B:219	Alkyl	3.71
		ALA B:219	Alkyl	4.62
		PHE B:244	Pi-Alkyl	4.25
		SER B:218	Carbon	2.96
		SER B:218	Carbon	2.12
		GLY B:216	Carbon	2.25
	-6.2	SER B:79	Conventional	2.31

*2,4-dimethyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-on*e (A4)		ILE B:123	Alkyl	4.03
		ILE B:123	Alkyl	4.97
		ILE B:123	Alkyl	5.11
		ILE B:32	Alkyl	4.26
		ILE B:32	Alkyl	4.29
		ILE B:32	Alkyl	5.44
		PHE B:111	Pi-Alkyl	4.55
		PHE B:120	Pi-Alkyl	4.69
		TYR B:77	Pi-Alkyl	4.31
Mefloquinine	-.7.9	GLY B:216	Conventional	2.08
		ILE B:300	Alkyl	4.86
		LEU B:292	Alkyl	5.38
		THR B:221	Fluorine	2.61
		ASP B:214	Fluorine	3.02
		TYR B:77	Pi-Alkyl	5.28
		VAL B:78	Conventional	2.11
		VAL B:78	Alkyl	5.14
		VAL B:78	Pi-Alkyl	4.00
		GLY B:36	Fluorine	3.65
		ASP B:34	Fluorine	3.36

Table 2: Antimicrobial activity of Isolated Compounds at 500 µg/mL

Test organisms	A1	A2	CFX	SFX	FCZ
MRSA	24(125/250)	22(125/500)	35	35	-
VRE	-	-	0	35	-
S. aureus	27(62.5/250)	29(62.5/500)	37	41	-
S.feacalis	24(125/250)	27(62.5/250)	34	37	-
B. subtilis	30(62.5/125	31(62.5/125)	31	34	-
P.aeruginosa	-	-	0	32	-
Enterobacter sp	25(125/250)	24(125/250)	34	35	-
P. retgeris	-	-	35	37	-
S. dysenteriae	32(62.5/125)	29(62.5/125)	39	40	-
C. stellatoidea	24(125/500)	25(125/250)	-	-	37
C. pseudotropicalis	-	-	-	-	32
C.albicans	22(125/500)	24(125/250)	-	-	36

Key: SFX = Sparfloxacine, VRE = Vancomycine resistant enterococi, FCZ = Fluconazole, (-) = no activity, CFX = Ciprofloxacine,

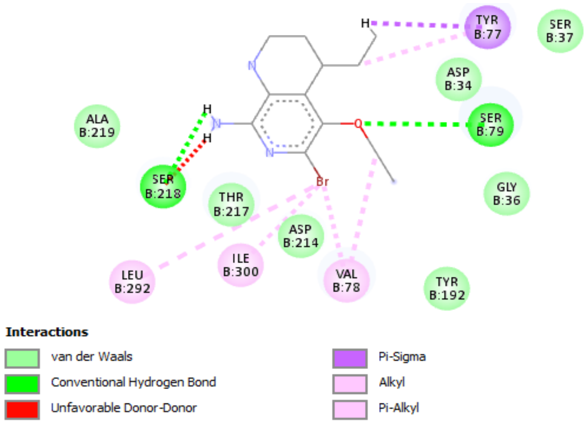


Figure 1: A1-Receptor complex in 2D

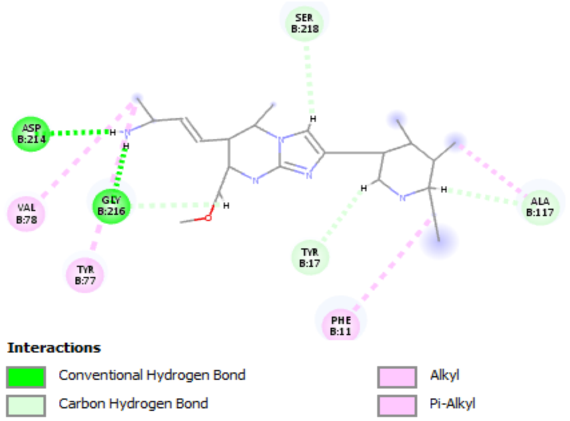


Figure 2: A2-Receptor complex in 2D

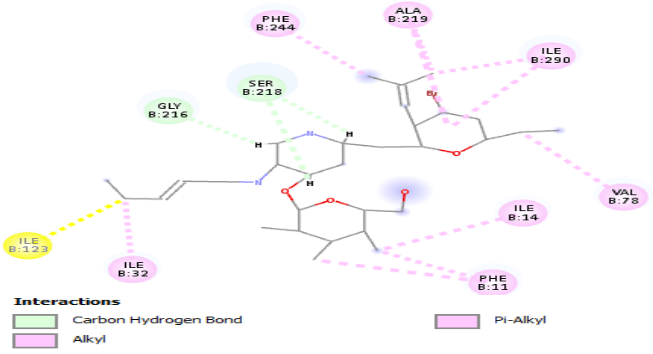


Figure 3: A3-Receptor complex in 2D

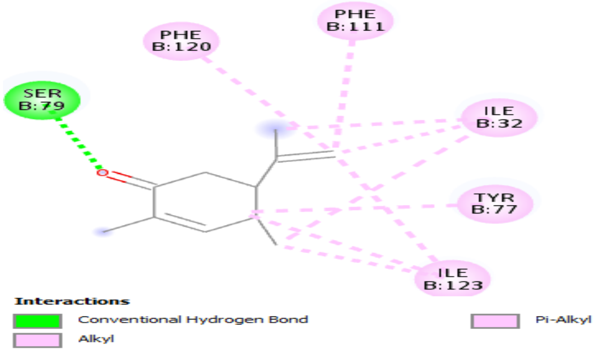


Figure 4: A4-Receptor complex in 2D

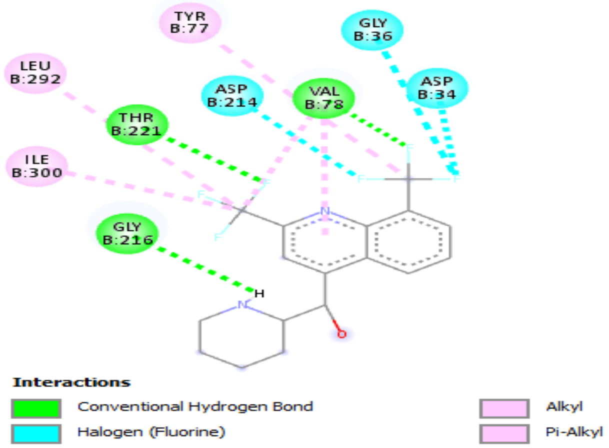


Figure 5: Mefloquine-Receptor in 2D

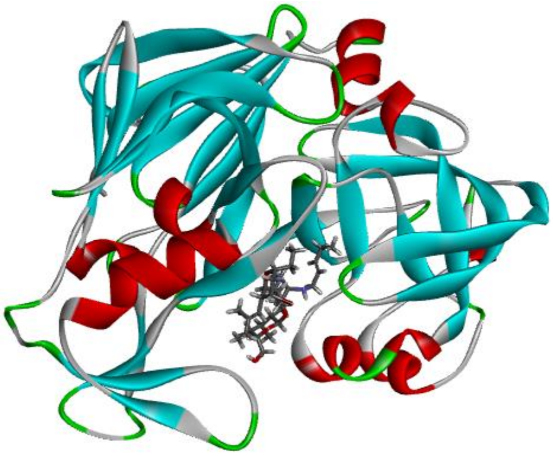


Figure 6: Expanded 3D Interaction of Compound A**3** with (PDB: 1ME6).

Using molecular docking, the compounds' interactions with the target receptor's active site (PDB: 1ME6) were examined, and the results were compared to those of the antimalarial medication mefloquinine. Table 1 revealed the binding energies of the isolated compounds against the binding sites of plasmepsin II (an aspartyl protease from Plasmodium falciparum) (PDB: 1ME6) and all showed a remarkable binding affinities compared to the standard drug.

Plasmepsins II, a parasite of malaria, is among the four aspartic proteases in *P. falciparum* which is essential enzyme for degradation of haemoglobin (Hb) which is also significant for the growth of the parasite in human [20, 21, 22]. Researchers have now show interest to develop antimalarial drugs that will targets plamepsins.

Generally, The molecular docking studies revealed that Mefloquinine's binding energy is -7.9 kcal/mol, whereas (E)-(6-((2-((4-bromo-6-ethyl-3-(2-methylprop-1-en-1-yl)tetrahydro-2H-pyran-2-yl)methyl)-5-(pent-2-en-1-ylamino)piperidin-4-yl)oxy)-3,4,5-trimethyltetrahydro-2H-pyran-2-yl)methanol (A3) has the lowest binding energy (-8.2 kcal/mol), indicating superior intermolecular interactions with the receptor. All of the compounds' interactions with the receptor's binding site demonstrated that SER, ILE, VAL, TYR, and GLY are the target residues that are involved in different types of interactions with isolated compounds. Figures 1, 2, 3, 4, and 5 showed the 2D interactions of the compounds with (PDB: 1ME6) [23, 24],

The antimicrobial activity results of compounds A1, A2, A3 and A3 reveal moderate effectiveness against tested microbes. Among the pathogens tested, Shigella dysenteriae exhibited the highest susceptibility to compounds A1 and A2, with a zone of inhibition of 32 mm. This finding is consistent with previous reports on the antimicrobial activity of similar compounds against Shigella species [30]. In contrast, Candida albicans and Methicillin-resistant Staphylococcus aureus (MRSA) showed reduced susceptibility, with zones of inhibition of 22 mm. This is not surprising, considering the notorious resistance of MRSA to various antimicrobial agents [25, 26, 27].

Compound A1 (MIC) of 62.5 µg/ml against Staphylococcus aureus and Shigella dysenteriae and inhibited the growth of MRSA, *Streptococcus feacalis, Enterobacter sp., C. stellatoidea, and C. albicans* at a MIC of 125 µg/ml, (MBC/MFC) for A1 was recorded at: 125 µg/ml against Bacillus subtilis and Shigella dysenteriae, 250 µg/ml against MRSA, S. aureus, S. feacalis, and Enterobacter sp., 500 µg/ml for C. stellatoidea and C. albicans. Compound A2 demonstrated MIC of 62.5 µg/ml against S. aureus, S. feacalis, B. subtilis, and S. dysenteriae and sowed an MBC of 125 µg/ml against B. subtilis and S. dysenteriae [28, 29].

These results suggest that Compound A1 and Compound A3 can potentially become lead for the production of antimicrobial candidates to treat diseases caused by S. aureus, B. subtilis, S. dysenteriae, and other pathogens. Remarkably, these findings align with earlier data on the antibacterial efficacy of identical molecules [30, 31, 32, 33].

Conclusion

This study's result emphasizes the ongoing difficulty of treating malaria and the pressing need for novel treatment approaches, with an (MIC) of 0.625 mg/mL, the study revealed that the dichloromethane (DCM) extract showed strong antibacterial activity and could successfully target resistant bacterial and fungal species, such as MRSA and Candida albicans. Together with molecular docking investigations, the structural elucidation of four isolated compounds revealed (E)-(6-((4-bromo-6-ethyl-3-(2-methylprop-1-en-1-yl)With the lowest binding energy (-8.2 kcal/mol), tetrahydro-2H-pyran-2-yl)methyl)-5-(pent-2-en-1-ylamino)piperidin-4-yl)oxy)-3,4,5-trimethyltetrahydro-2H-pyran-2-yl)methanol (A3) is the most promising ligand. Inhibiting disorders linked to P. falciparum required important interactions with residues including SER, ILE, VAL, TYR, and GLY in the receptor binding site. This research opens the door for the creation of powerful antimalarial drugs while also advancing our knowledge of bioactive chemicals.

References

[1] AbdulRazaq T, Nasiru M M, Maryam I, Murtala Y, Tajuddeen J A. Phytochemical Screening and Evaluation of the Antioxidant Potentials of the Stem Bark Extracts of *Erythrophleum Africanum* (African Blackwood) *Advanced Journal of Chemistry, Section B,* 2022, 4(3), 202-208. DOI: 10.22034/ajcb.2022.345261.1123, URL: http://www.ajchem-b.com/article_158150.html.

[2] Abreu, P.M., Martins, E. S., Kayser, O., Bindseil, K.U., Siems, K., Seemann, A., Frevert, J., (1999). *In vitro* topoisomerase inhibition of roots of *O. insignis. Journal of Phytomedicine*, 6: 187-90.

[3] Agunu, A., Ahmadu,A. A. , Afolabi, S. O., Yaro, A.U., Ehinmidu, J.O., Mohammed,Z. (2011). Evaluation of the Antibacterial and Antidiarrhoeal Activities of **Heeria Insignis** O. Ktze. *Indian Journal of Pharmaceutical Sciences,*73(3): 328– 332.

[4] Ayedoun, M.A., Moudachirou, M., Garneau, F.X., Gagnon, H., Jean, F.I., Tomi, F., Casanova, J. (1998). Constituents of the Leaf and Flower Oils of *Heeria insignis* Del. from Benin. *Journal of Essential Oil Research,* 10 (5): 11-20.

[5] Burkill, H. M.(1985). The Useful Plants of West Tropical Africa, 2th ed., *Royal Botanic Gardens: Kew :*1

[6] Centers for Disease Control and Prevention.(2020). Tuberculosis(TB) Treatment.Centers for Disease Control and Prevention. (2020). Antibiotic Resistance Threats in the United States.\

[7] Dalziel, J.M., (1955). The useful plants of west tropical Africa London: *Crown Agents for Overseas Government and Administration,* : 738.

[8] Himanshu, J., Gyanendra, K. S., Vikas, S., Ekta, A., Rahul, P. S.(2013). Phytochemical Investigation, Isolation and Characterization

of Betulin from Bark of Betula Utilis. *Journal of Pharmacognosy and Phytochemistry,*2 (1): 145-151.

[9] Kumar et al. Kumar, S, Kumar, V, & Mahapatra, S. (2018). Antimicrobial activity of compounds isolated from *Heeria insignis*. Journal of Ethnopharmacology, 211, 145-153.

[10] Liu, J., Wang, Y., Li, Z., & Chen, L. (2019) Lupeol-type triterpenes from the leaves of *Heeria insignis* exhibit anti-tuberculosis activity. Journal of Ethnopharmacology, 231, 112-118.

[11] World Health Organization (2020). Global Tuberculosis Report 2020. World Health Organization.

[12] Hwang, B. Y, Lee, J, Kim, H.S, & Hong, Y.S. (2012). Antimicrobial activity of betulin and its derivatives. Journal of Microbiology, Biotechnology and Food Sciences, 2(2),631-638.

[13] Kumar, S., Kumar, V., & Mahapatra, S. K. (2013). Lanostene derivatives as potential inhibitors of Mycobacterium tuberculosis. European Journal of Medicinal Chemistry, 66,253-263.

[14] Santos, F. A., et al. Santos, F. A., Silva, R.M., & Bento, E. S. (2017). Betulin and its derivatives: A review of their pharmacological activities. European Journal of Pharmacology, 794, 187-198.

[15] Liu, Y, Liu, J, Zhang, L, & Chen, Ling (2019). Methicillin-resistant Staphylococcus aureus: a review of the current situation. Journal of Medical Microbiology, 68(10),1511-1522.

[16] Khan, I, Khan, S, Ali, S, & Ahmed, S. (2020). Synthesis and antimicrobial evaluation of novel compounds. Journal of Pharmaceutical Research, 19(2), 1-9.

[17] Momoh H., Dambata M.B., B. Ibrahim and Oladosu P.O. (2017c). Lannea Humilis (Oliv) Leave Extracts Inhibits Bacteria, Fungi And Mycobacterium Bovis. *World Journal of Pharmaceutical and medical research,* Vol.3 No. 6: 58-63.

[18] Momoh, H., Habila, J.D., Ayo, R.G., Ndukwe, G.I. and Tajuddeen,N. (2015c). Antitubercular And Antimicrobial Triterpenes From The Leaves Of Clerodendrum Capitatum (Wlld). *Nigerian Journal of Pharmaceutical Sciences,* Vol. 14 No.2: 71-78.

[19] Momoh, S., Oladipo, A. O., & Akpan, I.(2015). Antimicrobial activity of some medicinal plants against clinical isolates of bacteria and fungi. Journal of Medicinal Plants Research, 9(15), 537-545.

[20] Moyo, M., et al. Moyo, M, Ndlovu, B, & Van Staden, J. (2013). Antimycobacterial activity of some plant extracts and compounds. Journal of Ethnopharmacology,147(2), 546-553.

[21] Clinical and Laboratory Standards Institute (CLSI).(2015). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Tenth Edition. CLSI document M07-A10.

[22] Nyaberi, M. O., Onyango, C. A., Mathooko, F. M., Maina, J. M., Makobe, M. and Mwaura, F. (2010). Bioactive fractions in the stem charcoal of Ozoroa insignis used by the pastoral communities in West Pokot to preserve milk. *Journal of Applied Biosciences,* 26: 1653 – 1658.

[23] Oladosu, P.O., Isu, N.R., Ibrahim, K., Orishade, A.T., Oladepo, D. and Lovett, L.(2013). Antituberculosis activity of bioactive compounds from fruits extracts of Acacia nilotica. *Journal of microbiology Research,* 3: 247-254.

[24] Pedro, J.M.A., and Yonghong, L. (2007). Ozoroalide, a new macrolide from *Ozoroa insignis Fitoterapia,* 78: 388-389.

[25] Rea, A.I., Schmidt, J.M., Setzer, W.N., Sibanda, S., Taylor, C., Gwebu, E.T.(2003).

Cytotoxic activity of *Ozoroa insignis* from Zimbabwe. ¸ Dec;74(7-8): 732-5.

[26] Silva, G.L., Lee, I. and Douglas, K.A. (1998). Special problems with extraction of plants.In: Cannel, J.P.R. (ed.). *Natural Products Isolation.* Humana press publishers, New Jersey (USA): 356-358.

[27] Srinivasan, S., Rajan, M., & Kumar, R. (2018). Antimicrobial activity of novel compounds against Shigella species. Journal of Antibiotics, 71(10), 831-838.

[28] Tijjani, .A, Ndukwe, I.G., and Ayo, R.G., (2012). Isolation and Characterization of Lup- 20(29)- ene-3, 28-diol (Betulin) from the Stem-Bark of *Adenium obesum (Apocynaceae). Tropical Journal of Pharmaceutical Research,* 11 (2): 259-262.

[29] Von, T., Van, I., Wyk, A.E. (1996). Taxonomic significance of pericarp and seed structure in *Heeria argentea* (Thunb.) Meisn. (Anacardiaceae), including reference to pachychalazy and recalcitrance. *Botanical Journal of the Linnean Society,* 122(4): 335-352.

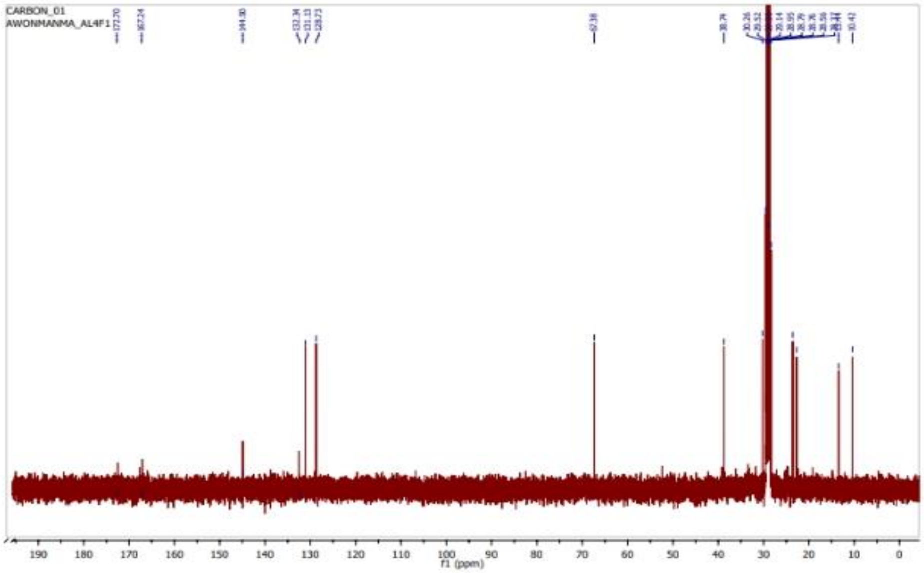
[30] Wilson, K.W., Rhea, M.S., Josua, J.W, Peter, S.R., Benfang, R., George, J.S J. (1996). Analysis of C27 sterols by nuclear magnetic resonance spectroscopy. *Journal of lipid research,* 37: 1529-1554.

[31] World Health Organization (2019). Treatment of tuberculosis: guidelines for national programmes.

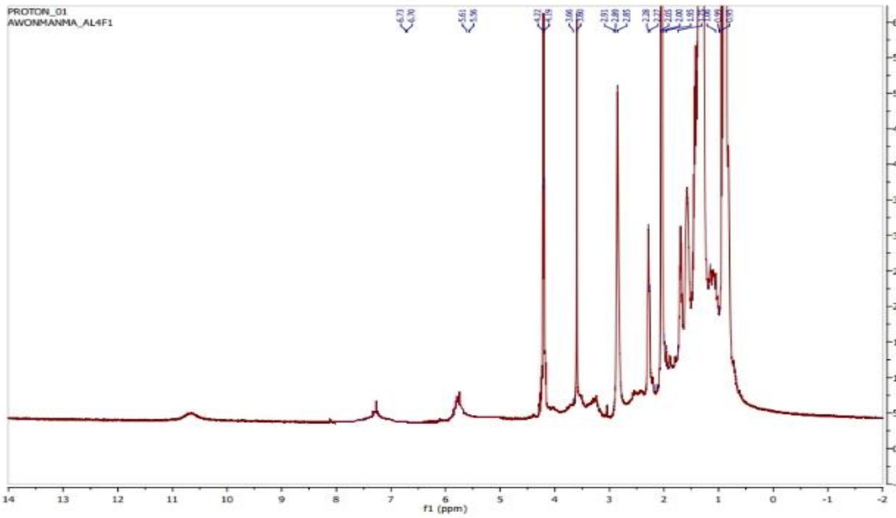
[32] World Health Organization. (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics.

[33] Pedro A. M., John B. D., DavidA., Fidock (2009).Role of *Plamodium falciparum* digestive vacuole plasmepsins in the specificity and antimalarial mode of action of cysteine and aspartic protease inhibitors. Antimicrobial agents and chemostherapy 53:, DOI: https://doi.org/10.1128/aac.00882.09.

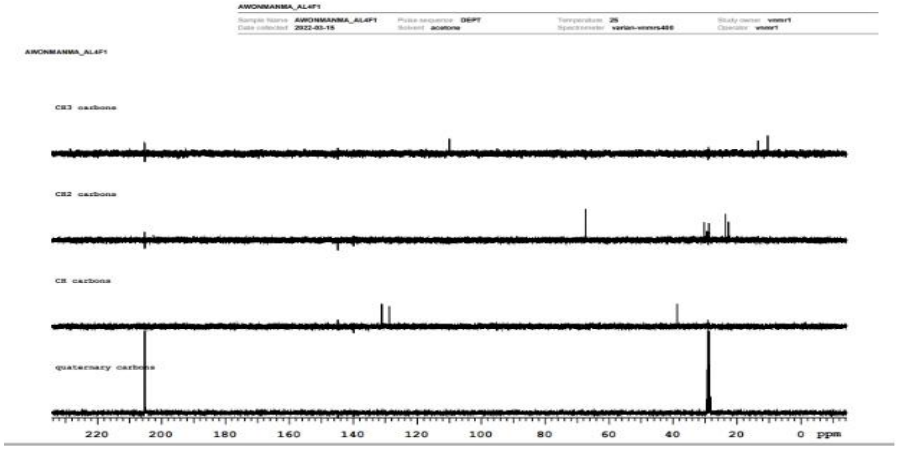
Appendices



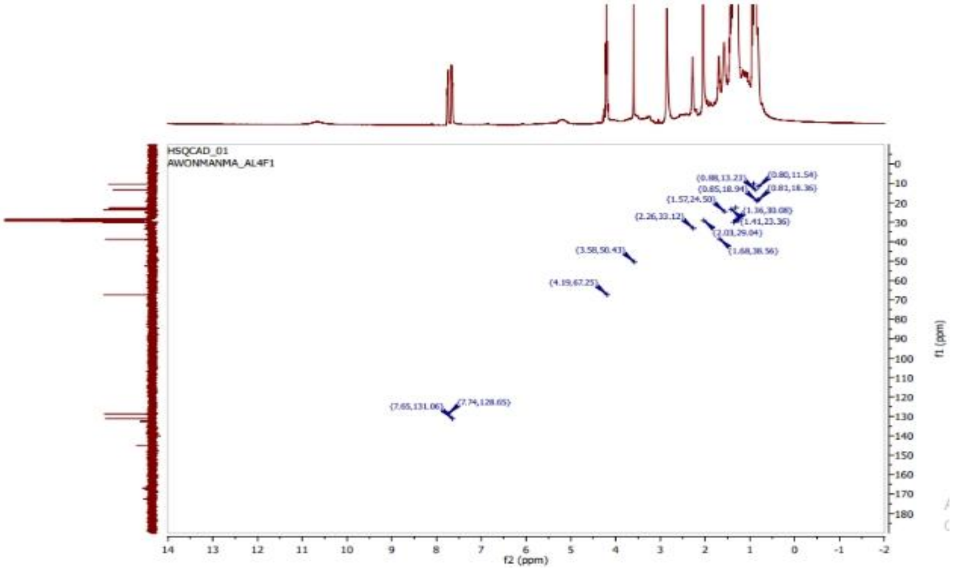
Appendix 1: ^{13}C-NMR Spectrum of A1



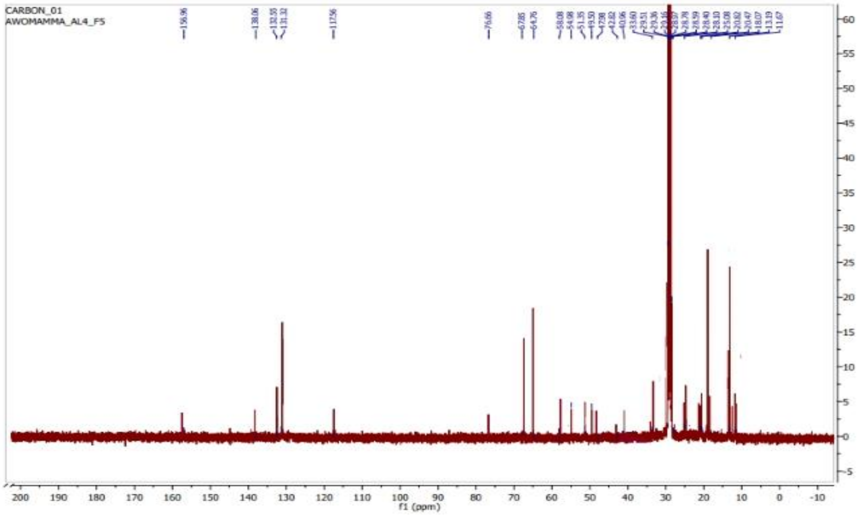
Appendix 2: ^{1}H-NMR Spectrum of A1



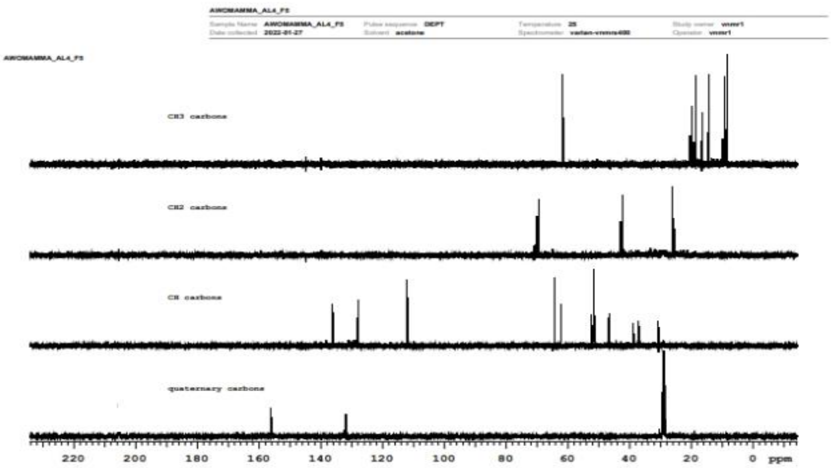
Appendix 3: Dept Spectrum of A1



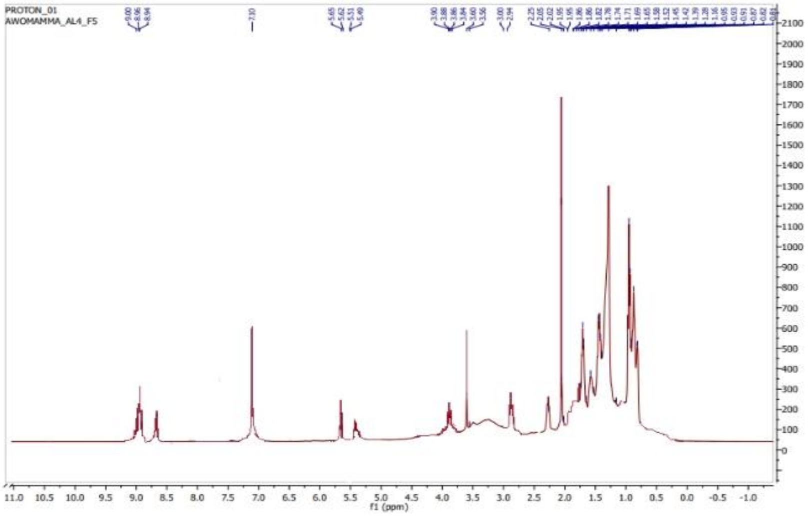
Appendix 4: HSQC Spectrum of A1



Appendix 5: ^{13}C-NMR Spectrum of A2



Appendix 6 : ^{13}C-NMR Spectrum of A2



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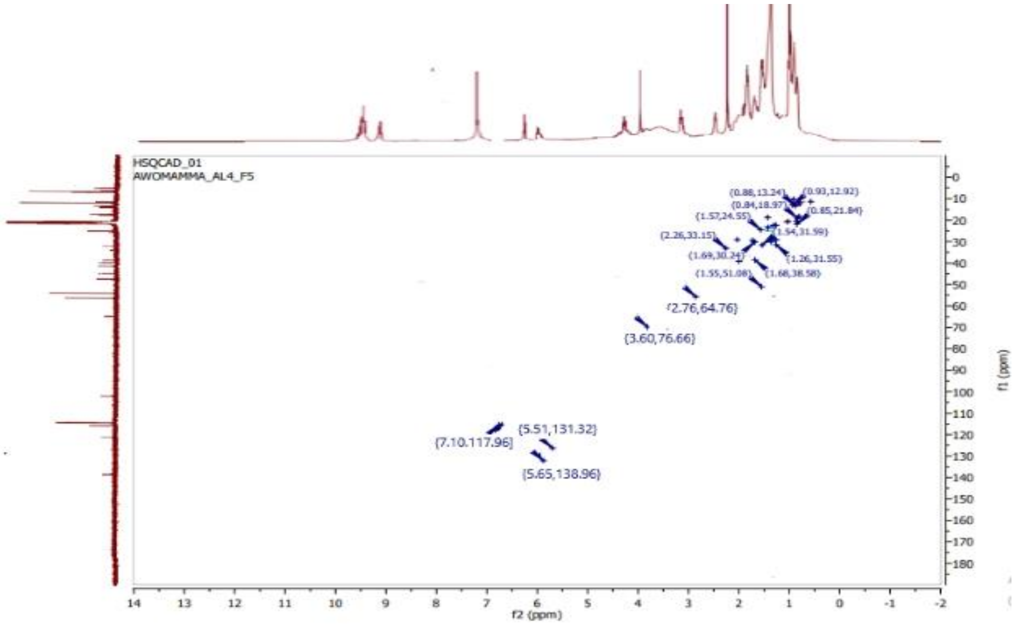
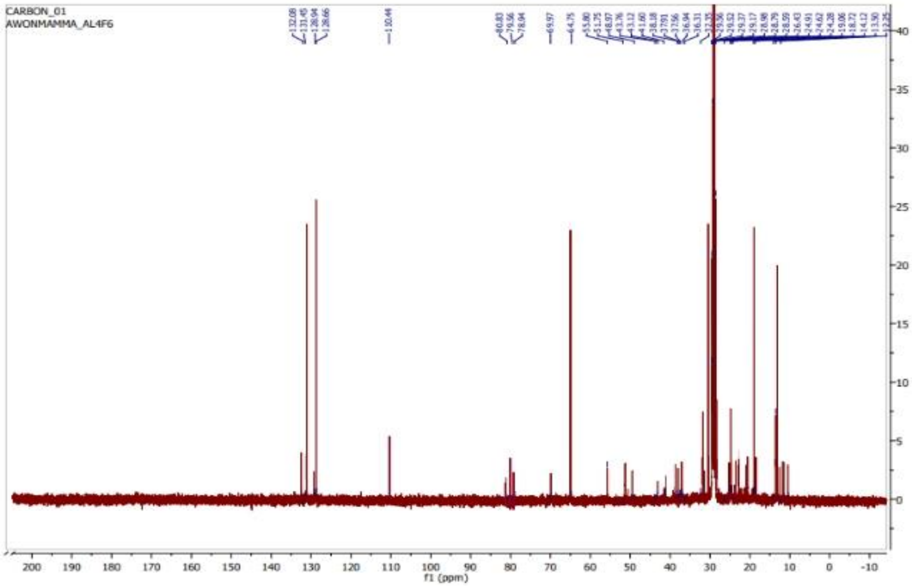
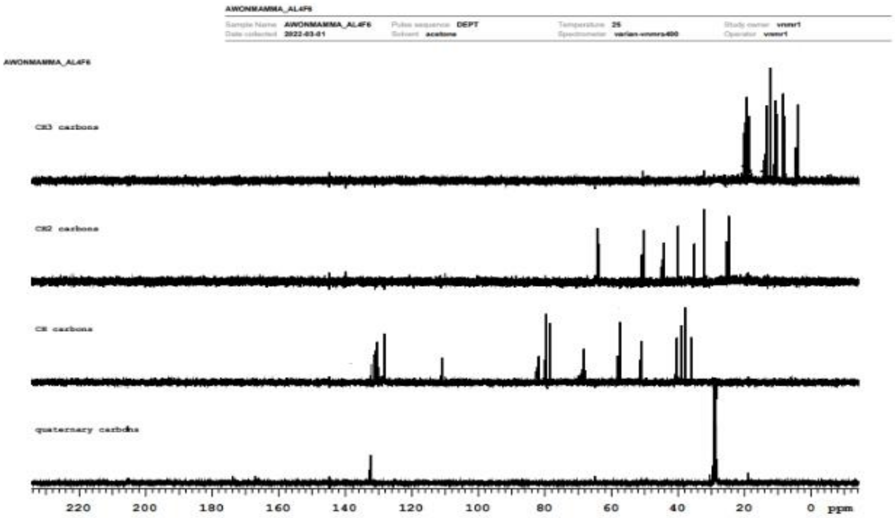


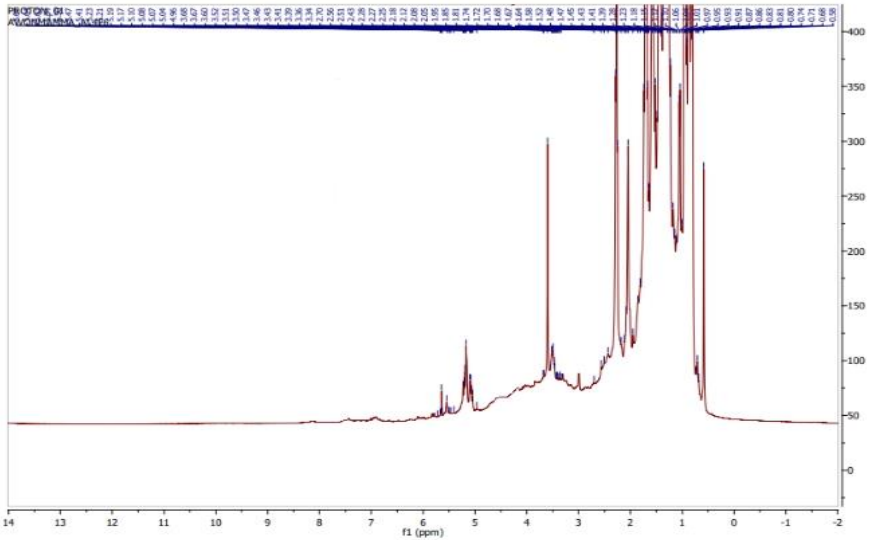
Appendix 8: HSQC Spectrum of A2



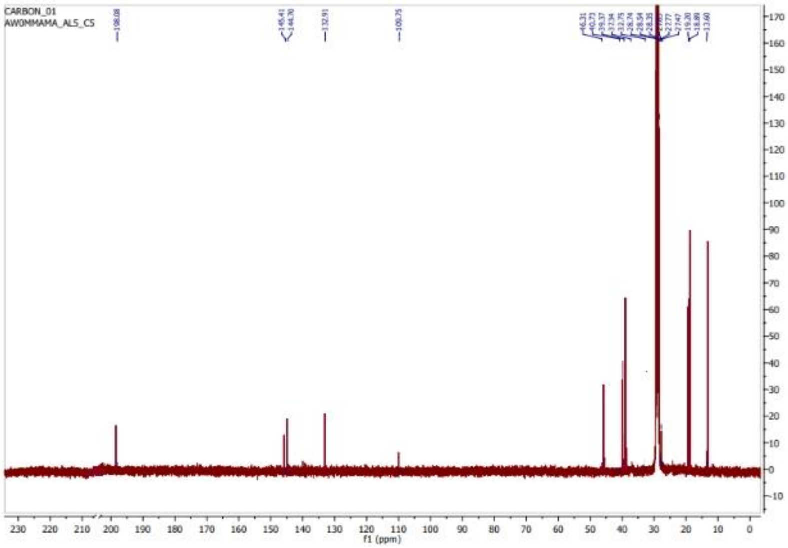
Appendix 9: ^{13}C-NMR Spectrum of A3



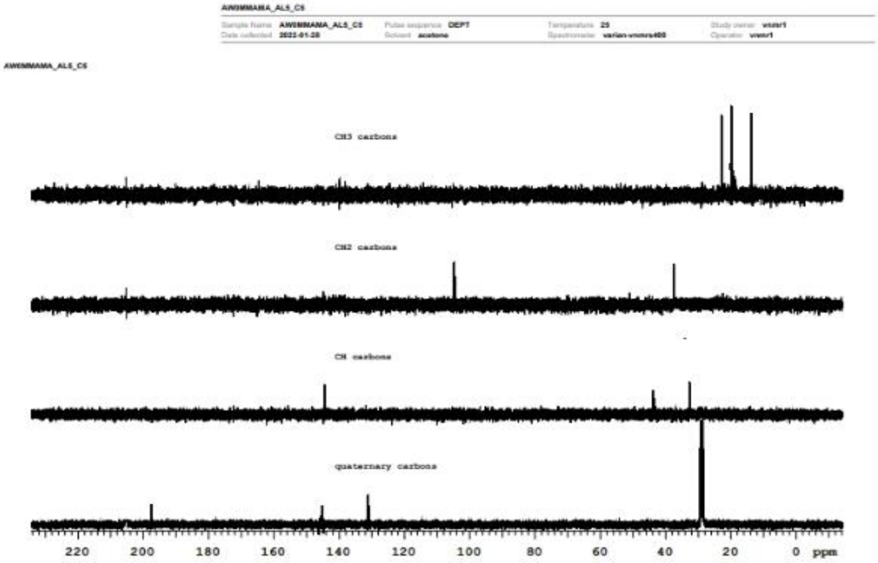
Appendix 10: DEPT Spectrum of A3



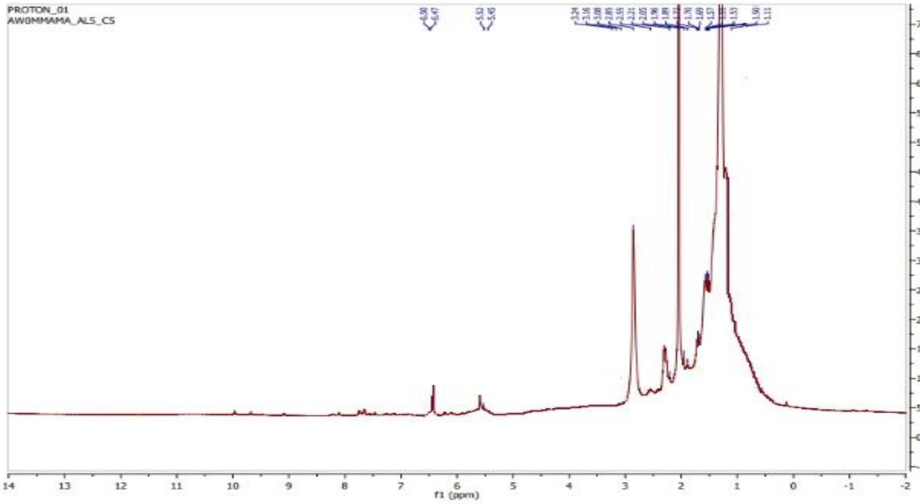
Appendix 11: ^{1}H-NMR Spectrum of A3



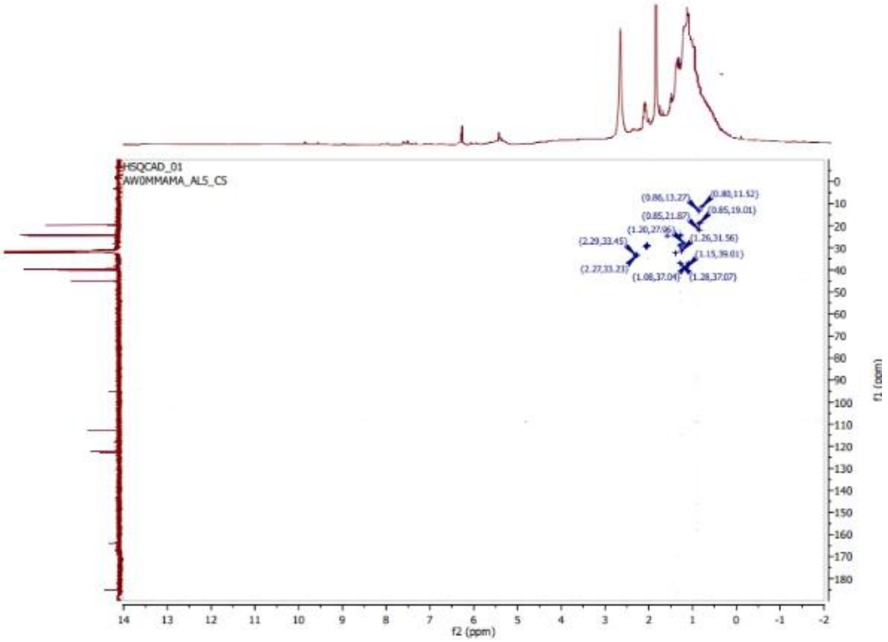
Appendix 13: ^{13}C-NMR Spectrum of A4



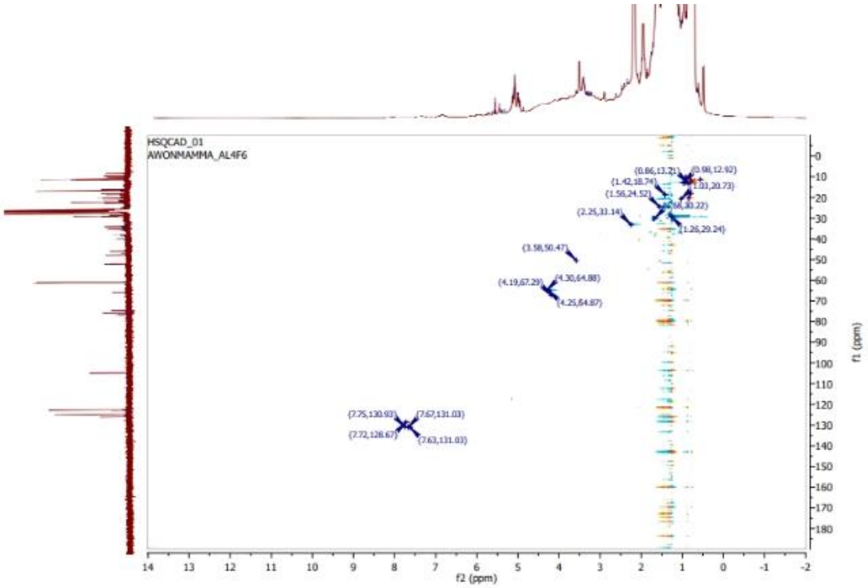
Appendix 14: DEPT Spectrum of A4



Appendix 14: ^{1}H-NMR Spectrum of A4



Appendix 16: HSQC Spectrum of A4



Appendix 12: HSQC Spectrum of A3