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GC-MS Analysis, Antibiogram and Computational Studies of Kakamout (*Acacia polyacantha* L.) Leaves Hydro-Ethanolic Extract

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Abstract:

Medicinal plants represent the main source of therapeutic agents. The WHO stated that most of the world population use herbal drugs because they belief that, they are safe, cheap and affordable. Many modern drugs show resistance to pathogenic bacteria as one of the forms of antimicrobial resistance which is considered from the top problematic issues in the world. Fortunately, medicinal plants can be the hero for fighting this problem by providing new antibacterial agents. Sudan is a virgin area for phytomedicine research due to its diversity of traditions besides the diversity of climates that leads to variety of plant species. *Acacia polyacantha* tree is widely available in Sudan and is used traditionally for treating many bacterial diseases. This study aimed to analyze the hydro-ethanol extract of *Acacia polyacantha*leaves using GC-MS analysis and to determine its antibiogram against two standard bacterial strains by well diffusion method, besides molecular docking and ADMET studies.*A. Polyacantha* leaves

was extracted by cold maceration using Ethanol 70%. GC-MS analysis was carried out for the first time and proved to contain 23 compounds. The major compounds were4-O-Methylmannose (60.28%), Phytol (8.2%) and Adenosine N6-phenylaceticacid (4.74%). The extract was tested for antibacterial activity against standard bacterial strains of Gram +ve bacteria (*Staphylococcus aureus*) and Gram -ve bacteria (*Pseudomonas aeroginosa*) by well diffusion method which was active against *S.aureus* and inactive against *P.aeruginosa*. Moreover, binding mode and pharmacokinetics properties of the compounds present in the extract were further studied and reported. In conclusion, *A. Polyacantha* leaves extract is rich in phytochemical compounds having antibacterial activity.

Keywords: *Acacia polyacantha*, ADMET, Antibiogram, GC-MS, molecular docking.

Introduction:

Medicinal plants have been used since ancient times as a rich source of therapeutic agents for the treatment and prevention of diseases. Also, they deliver the main source of useful structures for the development of novel therapeutic agents[1]. WHO has reported that most of people (80 % of the world's population) use herbal medicine for primary health care, Because they trust that, herbal drugs are cheap, safe and affordable [2]. Many modern drugs have been stated to show resistance in bacterial infections. Also, these drugs are more expensive. At the same time most of African population survive under poverty line and cannot find the money for the expensive modern drugs. These challenges call for improved strategies on treatment, particularly in the development of antimicrobials. According to WHO, medicinal plants can offer the best alternative sources to obtain variety of drugs, so they can give new choices for solving antimicrobial resistance[3]. Sudan is a promising area for phytomedicine research because it contains a mixture of Islamic African and Arabic traditions, moreover the diversity of climates in Sudan results in a rich variety of plant species [4].

Acacia polyacantha Willd. (*Mimosaceae*) is widely available in tropical Africa. it is called Kakamout in Sudan and represents one of the main sources of gum Arabic there [5]. The leaves are twice compound with 14-35 sets of pinnae and 20-60 leaflets each pinna. Leaves are fairly large and arranged singly along shoots. The upper surface is

darker than the lower surface of the leaves, and mostly with hairs on the margins and on the stalk[6].The plant has been used in traditional medicine for the treatment of snakebite, livestock diseases such as Salmonellosis and gastrointestinal diseases, for venereal diseases and stomach disorders [3]. Also used as a remedy for snakebite and as an infusion to bath children who are restless at night [7]. Furthermore it is used as antimalarial [8]. Pharmacological studies of extracts and compounds from the plant included antibacterial activity, larvicidal activity [3], radical-scavenging activity [7] and anti-leishmaniasis [11]. Previous Phytochemical screening of Kakamout leavesmethanolic extract led to isolation of polyacanthoside A, stigma sterol, oleanolic acid, epicatechin, Chiroinositol, quercetin, and oleanolic acid [7].*Acacia polyacantha* plant is used traditionally in Sudan for treating many bacterial diseases. Thus, this study aimed to verify its traditional use as antibacterial and to specify its components which are essential for the antibacterial activity also the binding mode of the compounds found in the extract were analyzed via molecular docking analysis. Finally, *in silico* ADMET properties of the compound were evaluated using Schrodinger software.

Materials and methods:

Chemicals and reagents:

The antibiotics Vancomycin 30MCG, Item No SD045-5CT, Cat HIMEDIA and Ceftriaxone 30MCG, Item No SD065-5CT, Cat HIMEDIA, all experimental solvents such as Methanol (Purity 99.9%), Ethanol (Purity99.9%), were obtained from (SDFCL India). The purified distilled water was prepared at the faculty of Pharmacy University of Gezira laboratory and all other chemicals were of analytical grade. The filter papers were from what Mann No 1.

Equipment and Instruments:

The glassware such as beakers, conical flasks, round bottom flasks, cylinders, test tubes etc., were from (SanaiLab BORO 3.3 KSA). Electrical blender (Moulinex Blender the genuine 400 W, France), GC-MS (GC/MS-QP2010SE, Shimadzu Japan) and freeze dryer (LYO GT2, SRK-System technik GmbH, Germany).

Microorganisms:

The standard strains of Gram positive Bacteria *Staph. Aureus* ATCC 25923, Gram negative *Pseudomonas aeruginosa* ATCC27853 were used for the present study.

Plant materials and extraction:

The leaves of the plant Kakamout (*Acacia polyacantha* L.) were collected from Bazoora area - South of Algadaref State in Sudan. Then it has been identified and authenticated in the Herbarium of Medicinal & Aromatic Plants and Traditional Medicine Research Institute, National Center for Research. Khartoum, Sudan on Feb. 2022. The leaves were air dried and milled to give coarse powder using electrical blender. After that, 100g of powdered material was macerated for 72 hours with 1L of ethanol 70%. Then it was filtered using Buchner apparatus. Next, the solvent was evaporated and the extract was freeze dried and kept in a refrigerator till use.

GC-MS Analysis:

GC-MS analysis was performed in Medicinal & Aromatic Plants and Traditional Medicine Research Institute, Department of Pharmaceutics, Central Lab using a (GC/MS-QP2010SE, Shimadzu Japan) Serial number (O20535400496SA), equipped with capillary column (Rtx-5MS-30m×0.25 mmI. D×0.25µm). Analytical conditions: Injector temperature was 300°C. The injector was operated in the split mode. The oven temperature was programmed from 60°C to 300 °C at 10°C/min; The Carrier gas was helium at flow rate (1.6 mL/min), Volume of injection was 1µL. The MS conditions: Ion source temperature 200 °C and the Interface temperature was 250°C. The mass scan range (m/z) was 40–500 m/z, the total of run time is 34 minutes. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library (NIST) [12].

Antibacterial activity test:

Well diffusion method was used to screen the antibacterial activity of Kakamout leaves extract and performed by using Mueller Hinton agar (MHA).(HIMEDIA)[13].Colonies from sub-cultured bacteria were diluted with sterile normal saline (0.9%) to give 10^8 cfu/ ml (turbidity equivalent to McFarland's standard solution 0.5). McFarland was prepared by adding 0.6 ml of 1.17% w/v solution of barium chloride to 99.4ml of 1% v/v solution of sulfuric acid. About 20ml of melted MHA medium was poured into sterile plates and 200µl of bacterial suspension was added to the agar plates then was mixed gently to achieve equal distribution. After complete solidification of the media at room temperature, three circular wells, 8 millimeter in diameter,

were punched with the back of the sterile blue tips of graduated pipette[14]. Two wells were filled with 100 µl of the extracts using different concentrations of extract (500, 250, 100, 50, 25, 12.5, 6.25 and 3.125) mg/ml. while the third well was filled with 100 µl of methanol 50% as negative control. The positive control was Ceftriaxone disk 30 µgfor *P.aeroginosa* ATCC27853 and Vancomicin 30 µg disk for *S.aureus*ATCC25923 were placed on the surface of the media. The plates were left at room temperature for one hour and then incubated at 37°C for an overnight. The test was done in duplicate for each extract concentration. Finally, the mean diameters of the inhibition zones were obtained in (mm)using the following standard: < 9 mm zone was considered as inactive; 9-12mm as partially active while 13-18mm as active and > 18mm as very active [15].

Computational studies:

The crystal structure of *Staphylococcus aureus* enoyl-acyl carrier protein reductase (fabI) (PDB ID: 3GR6) was downloaded from protein data bank and prepared using Protein Preparations Wizard of Schrodinger. The structures of the compounds were retrieved from PubChem database and minimized using Macro Model tool. Molecular docking studies were carried out using XP (extra precision) mode of Glide of Schrodinger and binding site was determined using Receptor Grid Generation tool. MM-GBSA free energy calculations were predicted using Prime module of Schrodinger. The QikProp module of Schrodinger suite was utilized to predict the drug gable properties of the compounds. The compounds were assessed for pharmacokinetic properties and toxicity. Lipinski's rule of five was also examined for the compounds[16].

Results and Discussion:

GC-MS analysis: The GC-MS analysis of *A. polyacantha* leaves extract was proved to contain 23 compounds (Table 1). The most abundant of them were 4-O-Methylmannose (Peak area 60.28%),Phytol (Peak area 8.2%) and Adenosine, N6-phenylaceticacid (Peak area 4.74%).Previously, 4-O-Methylmannose was found as the major compound (45.2%) in a GC-MS investigation on Ethanolic extract of *Acacia ehrenbergiana* aerial parts together with Phytol and fatty acid ethyl esters of various types [17]. Hexadecanoic acid methyl ester and Octadecanoic acid ethyl ester were also detected in

Table 1: Results of GC-MS analysis of A. *polyacantha* leaves extract

Peak#	Name	R.Time	Area	Area%
1	Benzoicacid	6.303	359927	0.67
2	1-Tridecene	6.739	428013	0.80
3	Benzofuran,2,3-dihydro-	7.140	743007	1.39
4	2-[4-(3,4-Dimethoxyphenyl)-3-methyl-1,2-oxazol-5-yl]-	8.528	252483	0.47
5	3-Octadecene,(E)-	9.449	819749	1.53
6	2,4-Di-tert-butylphenol	11.022	235821	0.44
7	1-Heptadecene	11.922	657837	1.23
8	Adenosine,N6-phenylaceticacid	12.554	2537554	4.74
9	4-O-Methylmannose	12.894	32292532	60.28
10	1-Nonadecene	14.157	432161	0.81
11	Hexadecanoicacid,methylester	15.539	464051	0.87
12	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dion	15.609	1674839	3.13
13	n-Hexadecanoicacid	15.865	807030	1.51
14	Dibutylphthalate	16.000	570001	1.06
15	1,4-Naphthalenediol,decahydro-,(1.alpha.,4.beta.,4a.alp	16.142	354851	0.66
16	Hexadecanoicacid,ethylester	16.201	1083814	2.02
17	9,12-Octadecadienoicacid(Z,Z)-,methylester	17.189	2342683	4.37
18	Phytol	17.354	4391472	8.20
19	9,12,15-Octadecatrienoicacid,ethylester,(Z,Z,Z)-	17.864	738215	1.38
20	Octadecanoicacid,ethylester	18.055	396140	0.74
21	Squalene	23.077	891255	1.66
22	.beta.-Amyrin	27.985	515717	0.96
23	Germanicol	28.083	581676	1.09
Total		-	53570828	100.00

A. polyacantha seed oil extract [12]. However, β-amyrin triterpenoid which was detected in trace amount was Previously reported in Phytochemical studies of *A. polyacantha* leaves methanolic extract [18].

Antibacterial activity:

The antibacterial activity of *A. polyacantha* Leaves extract was tested using agar well diffusion method against the selected clinically important microorganisms; Gram

positive *Staphylococcus aureus* (ATCC 25923) and Gram negative *Pseudomonas aeroginosa* (ATCC27853). The extract was found to be active against *S. aureus* to exhibit a remarkable inhibition of bacterial

growth in a dose dependent manner (Figures 1 and 2), a result agreed to what Ashu *et al*., had reported[19]. On the other hand, the leaves extract was not active against *P. aeroginosa* ATCC27853 in a concentration up to 500mg/ml in contrast to a study conducted by Mambe *et al*.,[7] who found that *A. polyacantha* leaves methanolic extract was active against *P. aeroginosa* PA01 and PA124. In this case, the variability of results mostly due to susceptibility difference of *Pseudomonas* strains. Moreover, seasonal and maturity differences, geographical location, genetic variations, growth stages, post-harvest drying, storage and extraction techniques can affect the quality and quantity of metabolites[20]

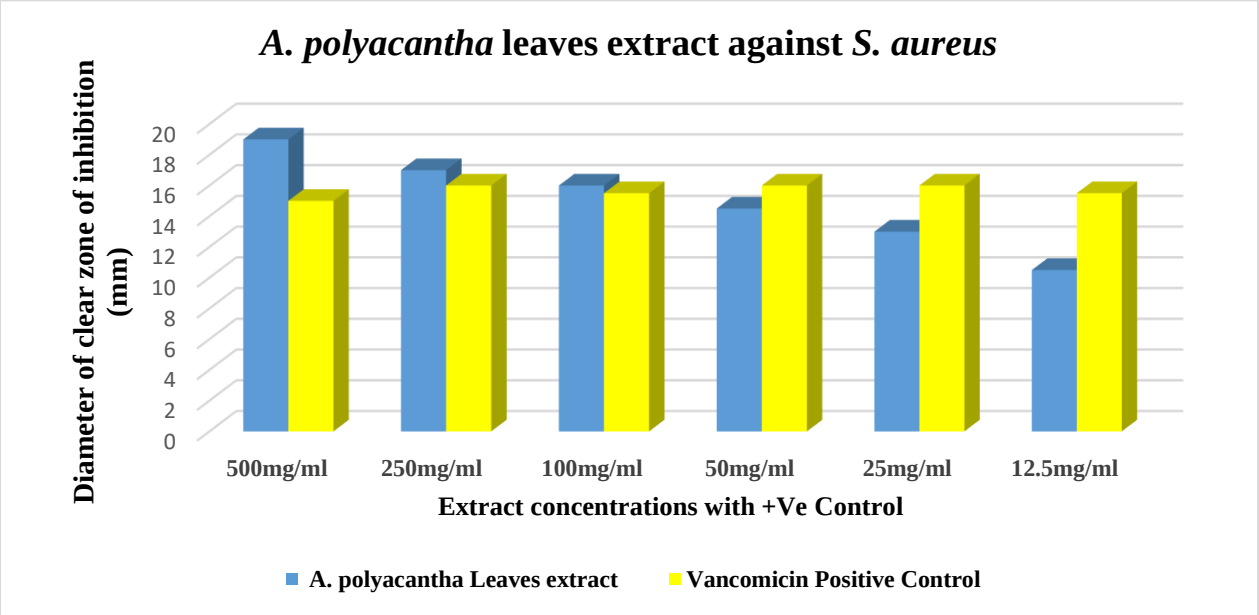

Figure 1: The Susceptability of *Staphylococcus aureus* (ATCC 25923) to *A. polyacantha* leaves extract at different concentrations**.**

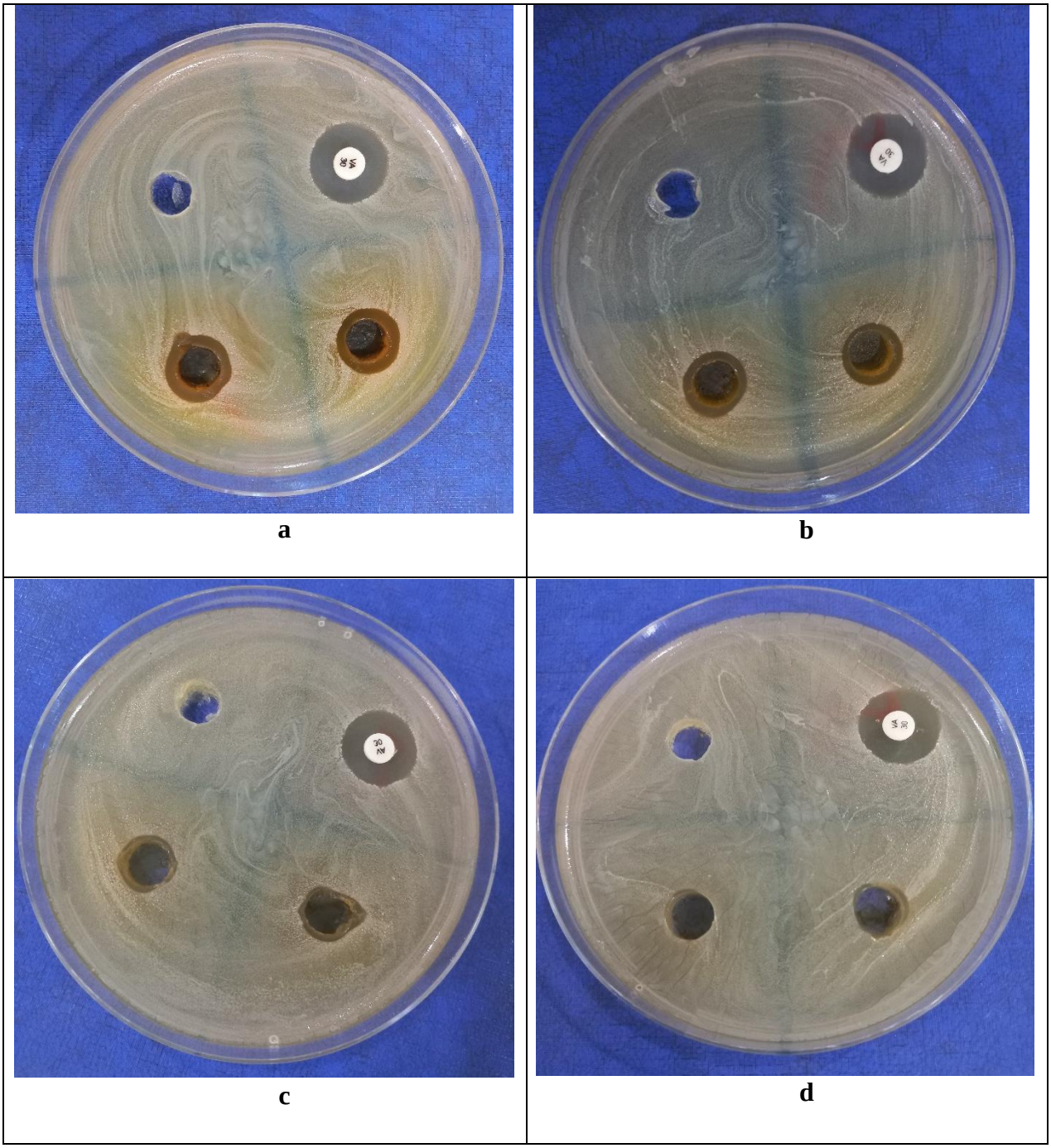

Figure 2: Agar well diffusion test for *A. polyacantha* leaves extracts against *S. aureus* ATCC 25923; (a for 50mg/ml, b for 25mg/ml, c for 12.5mg/ml and d for 6.25mg/ml).

Computational studies:

In computational drug discovery methods, molecular docking determines the binding mode of the ligand-protein complex. Yet, the binding affinity and stability of certain complexes cannot be measured via docking only. So, post docking investigation was employed to avoid any false-positive results. The binding affinity of the top ligand-protein complexes was calculated to confirm the accuracy of docking results by using post-docking free binding energy calculation by Molecular Mechanics Generalized Born and surface area (MM-GBSA); an important technique that determines the binding affinity, where the higher negative energy values indicate greater complex affinity. Additionally MM-GBSA takes into account the influence of the solvent in the binding energy calculation. [16].

In an attempt to identify the hypothetical binding modes of the compounds present in the leaves extract, the compounds were docked into the binding cavity of *Staphylococcus aureus* enoyl-acyl carrier protein reductase (fabI) (PDB ID: 3GR6) which is essential for fatty acid synthesis in *S. aureus*. Using the co-crystalized ligand with the protein, the binding cavity was determined using Receptor Grid Generation tool of Schrodinger. The docking scores of the top3 compounds Adenosine, N6-phenylacetic acid (PubChem ID:13821354), Benzoic acid(PubChem ID:243) and 4-O-Methylmannose(PubChem ID:345716)were-7.599, -7.219, -6.615 Kcal/mol, respective and the binding free energies(MM-GBSA calculations) were -40.49,-30.74 and -20.8 Kcal/mol in succession which had been in harmonization with the docking scores as shown in (Table 2). These indicate good binding affinity of the three compounds with the Target. The three compounds formed hydrophobic contacts with the Target protein. CID13821354 and CID243 interacted with TYR157 through pi-pi interactions. Also, CID243 formed H-bond with TYR157. CID345716 showed two hydrogen bonds with TYR157 and SER197 (Figure 3). The three compounds displayed acceptable ADME properties and no cardiac toxicity as depicted in Table 2. The antibacterial activity presented by the leaves extract mostly could be due to 4-O-Methylmannose being the major compound (60.28%) as evident in previous studies [17] which supported by Kawsar *et al* report [21]. Phenyl acetic acid as antibacterial compound[22]could possibly in a synergism act as antibacterial in the extract [23].

Table 2: Docking scores, MM-GBSA binding free energy and ADMET properties of top 3 scoring compounds with 3GR6

PubChem ID	**docking score**	**MMGBSA dG Bind**	**QPlogPo/w[a]**	**QPlogS[b]**	**QPlogHERG[c]**	**QPPCaco[d]**	**QPlogBB[e]**	**QPPMDCK[f]**	**RuleOfFiv[g]**
13821354	-7.599	-40.49	-0.213	-2.91	-3.634	3.611	-2.868	1.442	1
243	-7.219	-30.74	1.866	-1.474	-1.695	245.912	-0.304	138.132	0
345716	-6.615	-20.8	-1.692	-0.86	-2.78	205.377	-1.188	89.392	0

[a]Predicted octanol/water partition coefficient log P (acceptable range - 2.0–6.5).

[b]Predicted aqueous solubility in mol/L (acceptable range - 6.5–0.5).

[c]Predicted IC50 value for blockage of HERG K+ channels (concern below -5.0).

[d]Predictedcaco cell permeability in nm/s (acceptable range: <25 is poor and >500 is great).

[e]Predicted blood brain barrier permeability (acceptable range -3–1.2).

[f]Predicted apparent MDCK cell permeability in nm/s (acceptable range in nm/s (acceptable range: <25 is poor and >500 is great).

[g]Lipinski rule of five (Up to 4)

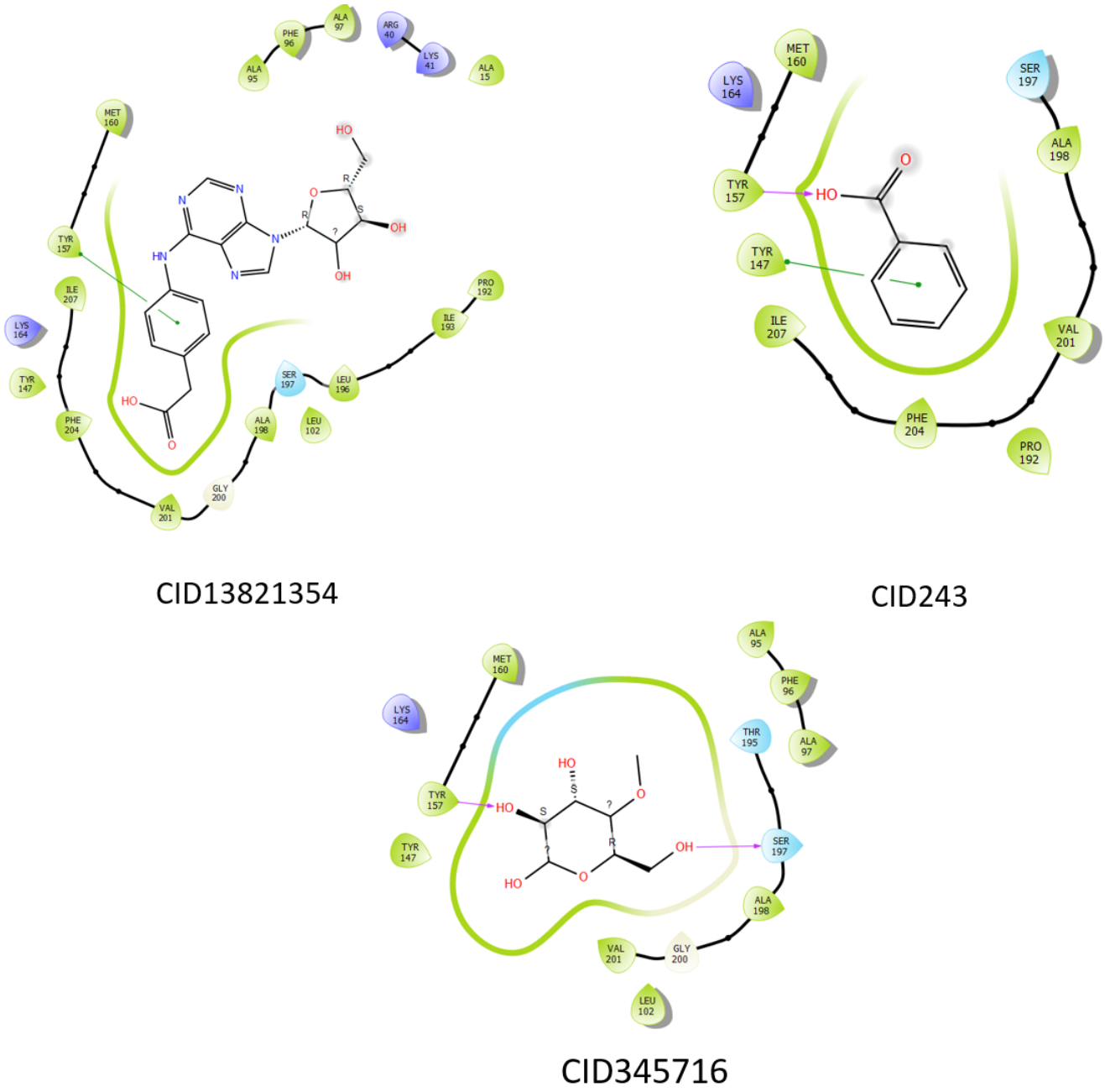

Figure 3: 2D interaction diagrams of top 3 scoring compounds with 3GR6.

The Pharmacokinetic profile of drug-like compounds is very important in drug discovery. The QikProp module of Maestro had been used to predict the ADMET properties of the compounds

present in the leaves extract. Various physicochemical and pharmacokinetic characteristics were calculated as listed in Table 2; QPlogPo/w predicts the lipophilicity profile of molecules, QPlogS indicates possible aqueous solubility, QPlogHERG predicts the cardiac toxicity, QPPCaco assumes the permeability of the molecule to cell membranes, QPlogBB which indicates the permeability of the molecule to the brain, QPPMDCK indicates kidney cell permeability and Lipinski's rule of five which is a crucial measure of drug-likeness, it refers to number of violations of Lipinski's rule of five. The rulesare: molecular weight < 500, QPlogPo/w < 5, H-bond donors ≤ 5, H-bond acceptors≤ 10. Compounds that satisfy these rules are considered drug like (The "five" refers to the limits, which are multiples of 5).

Conclusion:

The first report on GC-MS analysis of *Acacia polyacantha* leaves extract revealed the presence of 23 components the major of them were 4-O-Methylmannose (60.28%), Phytol (8.2%) and Adenosine, N6-phenylaceticacid (4.74%). The extract showed good antibacterial activity against the Gram+ve *Staphylococcus aureus* (ATCC 25923) and found to be devoid of activity against Gram-ve *Pseudomonas aeroginosa* (ATCC27853). As evidenced by the good docking scores and MM-GBSA calculations. The top scoring compounds showed good binding affinities to the enzyme enoyl-acyl carrier protein reductase which is essential for formation of fatty acid synthesis in *S. aureus*. The top scoring compounds also displayed acceptable ADME properties. Finally, this study showed that *A. polyacantha* leaves extract as rich in Phytochemical constituents which could have possible antibiogram potential especially Gram positive strains. Further studies should be encouraged by using different types of Bacterial strains and clinical isolates in addition to the fungal pathogens after that, isolation and clinical studies for the active constituents could be evaluated.

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