Antibacterial activity and chemical profile of the bioactive compounds from Aloe polyphylla Schönland

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Abstract

Background

The availability of knowledge regarding the ethnomedicinal value and phytochemistry of *Aloe polyphylla* Schönland has neither been documented nor fully explored. However, this aloe might possess phytocompounds of some medicinal importance.

Objective

This study aimed to evaluate the antibacterial activity and phytochemical profile of *A. polyphylla* Schönland gel extract.

Methods

The gel extract from *A. polyphylla* Schönland was evaluated for its antibacterial activity and chemical composition using the microdilution method and gas chromatography-mass spectrophotometry (GC-MS), respectively. The molecular interactions of the identified ligands with penicillin-binding protein 2x (PDP2) were studied by AutoDock Vina to predict the antibacterial mechanisms.

Results

The extract exhibited antibacterial activity against the selected bacteria with the minimum inhibitory concentration (MIC) values ranging from 0.05 to 0.781 mg/mL. The GC-MS analysis revealed 244 phytoconstituents with 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (15.66%), 5hydroxymethylfurfural (9.63%), tetradecanoic acid (3.93%), 3-butyl-4-nitro-pent-4-enoic acid, methyl ester (3.51%) and 2',4'-dihydroxy-3'-methylacetophenone (3.26%) as main compounds. The molecular structures of the ligands showed moderate interactions with PBP2, with the docking scores of -5.00, -4.8, -4.3, -5.0 and − 5.1 kcal/mol against 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 5-hydroxymethylfurfural, 2',4'-dihydroxy-3'-methylacetophenone, 3-butyl-4-nitro-pent-4-enoic acid, methyl ester and tetradecanoic acid, respectively.

Conclusion

The gel extract was revealed to have potential pharmacological applicability.

Introduction

Antimicrobial resistance has become a public health concern resulting in high morbidity and mortality rates, worldwide. More than 24 million people are hospitalized while over 700,000 die annually due to resistant pathogenic microorganisms [1]. The problem is worsened by antimicrobial resistance as a result of inadequate dosing, poor quality drugs, and genetic plasticity of the microorganisms [2]. Microorganisms developed different resistance mechanisms such as efflux pumps, secreted proteases, and alterations of the bacterial cell surface composition against antimicrobial agents. Resistance may cause an economic meltdown and weaken the success of health programs, globally [3]. Thus, the antimicrobial resistance coupled with limited knowledge of the mode of action and the undesired side effects of allopathic medicine has necessitated a search for alternative sources of novel therapeutic compounds. In recent years, researchers are considering discovering and developing new bioactive compounds of plant origin to control and overcome the burden imposed by multidrug-resistant pathogens [4].

Medicinal plants have been the predominant sources of bioactive compounds for centuries and are used in modern-day drug discoveries and developments [5]. Over 80% of drugs on the market shelves currently have their origin in medicinal plants [6]. Moreover, in developing countries, a major populace still relies on traditional herbal medicine [7]. Aloes are among the most used medicinal plants worldwide. The genus comprises over 600 known species that are widely distributed globally, with the highest diversity established in southern Africa, where over 144 species are regarded endemic [8]. Of recent, aloes are an attractive avenue of research conducted aiming to obtain novel phytochemicals for their ethnopharmacological effects against various diseases. Recent reviews have revealed that Aloe based compounds have profound biological activities such as antimicrobial, anti-inflammatory, antioxidant, anticancer, antidiabetic, antiglycation, anti-arthritic, anti-rheumatoid, antiulcer, laxative, and purgative properties and are used for detoxification, constipation treatment and promotion of proper digestion [9–12]. These biological activities are attributed to a various plethora of phytochemicals that include terpenoids, tannins, phenolic compounds, alkaloids, flavonoids, saponins, glycosides, and steroids, which tend to cumulatively work together effectively [13–15].

The most therapeutically used aloe parts are: (1) the outer green epidermis, which consists of the plant's structural components; (2) the outer pulp region just below the epidermis, consisting of aloe latex and (3) the inner leaf pulp, consisting of the gel [10]. The gel, the part that is proposed to be used in this study, is abundant in polysaccharides, vitamins, minerals, and a variety of therapeutic phytochemicals [16, 17]. Based on the literature, the most commonly used and researched Aloe species are *Aloe vera, Aloe secundiflora*, and *Aloe ferox.* At present, they account for the most economically valuable medicinal aloes and are often used in primary health treatment. Thus, only a marginal fraction of the ethnomedicinal uses and chemical profiles of other aloes such as *Aloe polyphylla* Schönland have been reported [18].

A. polyphylla Schönland, also known as *lekhala-la-kharatsa* in Sesotho and *spiral aloe* in English, is one of the most sought-after aloes that are endemic to Lesotho and is highly concentrated in the Thaba Putsoa Range and Drakensberg mountains [19, 20]. It is well-adapted to the cold (subzero) temperatures that are mostly experienced in these regions. *A. polyphylla* Schönland is a succulent plant that belongs to

the *Xanthorrhoeaceae* family. It is rare and the least known aloe [21]. Its medicinal potential has neither been documented nor explored. Moreover, the lack of information on its life cycle and ecology does limit the availability of the knowledge regarding its ethnomedicinal value [20, 22, 23]. Nevertheless, *A. polyphylla* is used traditionally to treat diabetes and sexually-transmitted diseases such as HIV/ AIDS, although there is a lack of scientific proof to validate its efficacy.

The present study aimed at evaluating the antibacterial activity and chemical constituents of the gel extract from *A. polyphylla* Schönland. Solvent extraction was used to obtain the plant secondary metabolites, which were then identified by gas chromatography-mass spectrometry. The molecular interactions of the GC-MS-identified ligands with the target proteins were assessed using AutoDock Vina to investigate the mode of action involved during antibacterial activity.

Material And Methods

Media and chemicals

The media and chemicals used in this study were procured from Sigma-Aldrich and Merck (Pty) Ltd. The water that was used was distilled and autoclaved at the University of Zululand, KwaZulu-Natal, South Africa.

Plant collection, preparation, and extraction

The aloe leaves were collected from Drakensberg Mountains (latitude 29.4667° S, longitude 29.2667° E) in KwaZulu-Natal, April 2021 and were transported to the University of Zululand, KwaZulu-Natal, South Africa. The leaves were authenticated by Prof. Ntuli, Department of Botany, University of Zululand, KwaDlangezwa at the University Herbarium and the specimen number is AK01 and was deposited at the University Herbarium [ZULU], which is available mainly to researchers. Ethical approval to collect the plant was obtained from the research ethical committee at the University of Zululand (UZREC 171110-030 PGM 2021/56). The leaves were washed with autoclaved distilled water to get rid of dirt. The leaves were dissected longitudinally into small pieces of 5 cm and the spikes and epidermidis were removed. *A. polyphylla* Schönland gel was then scooped out from the leaves and dried in shade at 32 °C. Thereafter, the dried gel was ground to powder and about 10 g was extracted with 100 mL of a mixture of ethanol (70%) and methanol (80%) at 1:10 at room temperature for 72 hrs. The extractant was centrifuged at 5000 rpm for 25 minutes, filtered with Whatman No.1 filter paper, and subsequently evaporated to dryness by using a rotary evaporator at 40 ^{o}C. Thereafter, the obtained extract was stored at 4 °C for further experiments [24].

Minimal inhibitory concentrations (MIC) of the extract

The antibacterial activity was determined against *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 by the assessing minimal inhibitory concentration of the extract using broth micro-dilution method with p-

iodonitrotetrazolium violet (INT) serving as the growth indicator [25]. Briefly, the bacterial cells were grown overnight at 37 °C in a nutrient broth medium and diluted in the same medium to give 1×10^6 CFU/ mL. The extract was serially diluted to vary its concentrations in a range of 2.5 to 0.02 mg/mL, followed by the addition of 50 µL of the adjusted bacteria. The 96 well microplates were then placed in an incubator (IncoTherm LABOTEC) set at 37 °C for 24 hours. Thereafter, 40 µL of INT (0.2 mg/mL) was poured and the plate was re-incubated at 37 °C for 30 minutes. The MIC was considered the lowest concentration of the extract at which no bacterial growth was observed.

Minimum bactericidal concentration (MBC) of the extract

The MBC was evaluated by withdrawing 20 µL of bacterial suspensions from the wells that showed no growth during the evaluation of MIC. The suspensions were pipetted into 50 µL of NB in a sterile 96 well plate. The plate was incubated at 37 °C, overnight. Thereafter, 40 µL of INT was poured and the plate was re-incubated at 37 °C for 30 minutes. The lowest concentration that displayed no bacterial growth was identified as the MBC of the extract [26].

Preliminary compounds identification

The standard methods were used to screen different classes of compounds within the crude extract. The compounds that were screened included; phenols, flavonoids, quinones, coumarins, anthraquinone, and saponins [27].

Identification of volatile secondary metabolites

The volatile secondary metabolites profile was determined by gas chromatography coupled with mass spectrometry. Briefly, the analysis conditions were as follows: split ratio: 1:20, and the flow rate of the carrier gas-Helium was 1 mL/minute. The injector volume of 1 µL of the gel extract was used. The injector temperature was programmed to 250 °C and the column temperature was set at 150 °C. The initial and column temperatures were set to increase to 220 °C at the rate of 15 °C per minute. After 1 min, the temperature was pre-set to slowly increase to 280 °C by 5 °C and kept at this temperature for 12 minutes. The ion source temperature was set at 200 °C and the ionization energy was 70 eV. The mass scans were done within the range of m/z 22 - 390 at a rate of 0.15 scan/second with an intermediate time of 0.03 seconds between the scans. The constituents were characterized by comparing their mass spectra with those found in the NIST 5 mass spectra library. The components were expressed as the peak area percentage of the total compound [28].

Molecular docking simulation

Computer-based molecular docking method was employed to understand the molecular interactions involved during the inhibition of bacterial growth based on the binding scores and types of bonds formed by the two most predominant GC-MS identified ligand and ciprofloxacin with the receptor protein, penicillin-binding protein 2x (PDP2). PDP2 is a membrane-associated enzyme essential for cell growth as

it is associated with peptidoglycan biosynthesis [29]. The three-dimensional structures of PDP2 were retrieved from Protein Data Bank (PDB ID: 1QMF). The protein was optimized by deleting water molecules, heteroatoms, and other ligands before the addition of polar hydrogens using the Discovery Studio tool [30]. Thereafter, the chemical structures of the GC-MS identified ligands were procured from PubChem online database, and energy was minimized using UCSF Chimera [31]. AutoDock Vina was then used to execute a molecular docking study of the prepared ligands and the target protein. The best-docked conformations were selected based on their binding scores [32]. Discovery Studio visualizer was employed in the visualization and analysis of the docked complexes.

Statistical analysis

All extractions, chromatographic analyses, and other experiments were done in triplicate. The obtained data were expressed as mean ± standard deviation. The significant differences between the obtained results were analysed with a one-way analysis of variance and considered to be significantly different at $p < 0.05$.

Result

Antimicrobial activity of the gel extract

In the present study, the antibacterial activity of the gel extract was evaluated against four bacterial strains by the micro-dilution technique and the results are summarized in Table 3. The inhibitory effect of the gel extract was more pronounced against *S. aureus* (ATCC 25925) and *B. cereus* (ATCC 10102) with the MIC values of 0.05 and 0.1 mg/mL. The Gram-negative strains- *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922) had the MIC values of 0.39 and 0.78 mg/mL, respectively.

Table 1 MIC and MBC of the bacterial extract against the selected strains

Bacteria	Gel extract		Ciprofloxacin	
	MIC (mg/mL)	MBC (mg/mL)	MIC (µg/mL)	MBC (µg/mL)
S. aureus (ATCC 25925)	0.05	1.56	0.02	0.03
B. cereus (ATCC 10102)	0.10	12.5	0.02	0.06
P. aeruginosa (ATCC 27853)	0.39	25.0	0.06	0.24
E. coli (ATCC 25922)	0.78	25.0	0.02	0.03

MBC of the gel extract

The MBC assay was assessed against the indicator strains. The most potent activity was displayed against *S. aureus* (ATCC 25925) and *B. cereus* (ATCC 10102) with the MBC values of 1.56 and 12.5 mg/mL, respectively. The MBC value of the Gram-negative bacteria (*P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922)) was the highest concentration of 25 mg/mL that was used in this study (Table 1).

Preliminary compounds identification

A qualitative phytochemical evaluation of different classes of compounds within the gel extract was done. The compounds; phenols, flavonoids, quinones, and coumorins were found present whereas saponins and anthraquiones were not detected (Table 2).

Table 2 The qualitative chemical constituents of the bacterial extract

Compounds	Presence of compounds
Phenols	+
Flavonoids	+
Quinones	+
Coumarins	+
Anthraquinones	-
Saponins	-

Key: + indicates presence and – denotes absence.

Identification and quantitative analyses of different compounds in the extract were performed using GC-MS. GC-MS spectrum revealed about 244 compounds with 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-m being the main active ingredient (15.66%) based on percentage peak area followed by 5-hydroxymethylfurfural (9.63 %), tetradecanoic acid (3.93%), 3-butyl-4-nitro-pent-4-enoic acid, methyl ester (3.51%) and 2',4'-dihydroxy-3'-methylacetophenone (3.26%). The other minor compounds were present in the gel extract in the amount of less than 2% (see Table 3, Table S1, and Figure 1).

Table 3 The five major chemical constituents of the gel extract with their different retention time intervals and peak areas

Peak	Compounds	Retention time (min)	Area%
1	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	8.789	15.66
2	5-Hydroxymethylfurfural	9.830	9.63
3	2',4'-Dihydroxy-3'-methylacetophenone	14.767	3.26
4	3-Butyl-4-nitro-pent-4-enoic acid, methyl ester	14.948	3.51
5	Tetradecanoic acid	15.288	3.93

Molecular docking studies

AutoDock Vina was used to simulating the interaction of the four most dominant GC-MS identified antimicrobial ligands and ciprofloxacin against the antibacterial target protein, PBP2 (PDB: 1QMF). The molecular structures of the ligands revealed moderate interactions with PBP2 with the docking scores of -5.00, -4.8, -4.3, -5.0, and -5.1 kcal/mol against 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 5-hydroxymethylfurfural, 2',4'-dihydroxy-3'- methylacetophenone, 3-butyl-4-nitro-pent-4-enoic acid, methyl ester, and tetradecanoic acid, respectively (Table 4). The positive control-ciproflaxaxin demonstrated a free energy score of -7.7 kcal/mol.

Table 4 Molecular docking study results of the ligands with binding energy and H-bond interactions with amino acids inside the active site of the PBP2 (PDB: 1QMF)

Compounds	Binding scores (kcal/mol)	H-bonds interaction residues
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	-5.00	ARG A:151, THR A:165, ARG A:241, and HIS A:293
5-Hydroxymethylfurfural	-4.8	ASP A:288, LYS A:289, LYS A:290, GLU A:268 and TYR A:269
2',4'-Dihydroxy-3'-methylacetophenone	-4.3	A:ARG151:HN and HIS A:293
3-Butyl-4-nitro-pent-4-enoic acid, methyl ester	-5.0	ARG A:151, THR A:165, and ARG A:241
Tetradecanoic acid	-5.1	VAL A:277 and ASP A:275
Ciprofloxacin	-7.7	THR A:165, THR A:216, and HIS A:293

The amino acids ARG A:151, THR A:165, ARG A:241, and HIS A:293 of the active site of PBP2 interacted with 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl through H-bond (Table 4, Figure 2 and 3). The other formed types of interactions were alkyl and carbon-hydrogen interactions. ASP A:288, LYS A:289, GLU A:268, LYS A:290, and TYR A:269 interacted with 5-hydroxymethylfurfural by H-bond (Table 3 and

Fig). The other bond that was revealed by 5-hydroxymethylfurfural with PBP2 was pi-Alkyl with A:LYS A:289. 2',4'-Dihydroxy-3'-methylacetophenone revealed only two hydrogen bonds with ARG A:151 and HIS A:293 of PBP2. It also displayed van der Waals, alkyl, and carbon-hydrogen bonds. 3-Butyl-4-nitro-pent-4-enoic acid, methyl ester had three hydrogen bonds with ARG A:151, THR A:165, and ARG A:241, carbon-hydrogen bond (ARG A:151, SER A:149, and GLU A:239) and pi-Alkyl (HIS A:293). Tetradecanoic acid revealed two hydrogen bonds (VAL A:277 and ASP A:275), alkyl and pi-alkyl. Ciproflaxaxin demonstrated three hydrogen bonds (THR A:165, THR A:216, and HIS A:293), carbon-hydrogen bond, pi-alkyl, and pi-donor hydrogen bond.

Discussion

The antibacterial profile of the gel extract was assessed against four microorganisms. All bacterial strains were found to be sensitive to the gel extract at MIC < 1 mg/mL. The low MIC values displayed by the gel extract translated the efficacy of the extracted antimicrobial phytochemicals. It was also observed that the activity of the gel extract was generally profound against the Gram-positive strains with a noteworthy activity (MIC value less than 1 mg/mL) than the Gram-negative strains. Thus, the differences in the bioactivity of the extract against different classes of bacteria were the indication that the antibacterial effect was cell-wall related. Gram-negative in comparison to Gram-positive bacteria have phospholipid membranes with structural lipopolysaccharide components that enable their cell wall impermeability to some antimicrobial agents [33]. Arbab *et al.* [34] observed similar results whereby the extract`s antibacterial activity was more profound against the Gram-positive than Gram-negative strains. The observed antibacterial activity of the extract was ascribed to the synergistic effects of the different antibacterial compounds within the gel extract. In addition, the extract exhibited a bactericidal effect on both Gram positive and Gram negative bacteria within the tested concentrations (Table 2). This implies that the gel extract has the potential not only to inhibit but also to kill bacteria.

The pharmaceutical importance of aloes lies in the activity of their metabolites. The detected compounds: phenols, flavonoids, quinonis, and coumorins were perceived to have contributed to the inhibitory activity of the gel extract as they are mostly reported in literature to possess antibacterial activity. Phenols and flavonoids are well known for their antimicrobial, anti-inflammatory, anesthetic, antioxidant, anticancer, and analgesic activities [35, 36]. Quinones and coumorins are also reported to exhibit antimicrobial action [37, 38]. The presence of these antimicrobial compounds is an indication of the antibacterial potential of the *A. polyphylla* gel extract.

The major constituents of the gel extract according to GC-MS analysis were identified as 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 5hydroxymethylfurfural, 2',4'-dihydroxy-3'-methylacetophenone, 3-butyl-4-nitro-pent-4-enoic acid, methyl ester and tetradecanoic acid. 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl is known for its antimicrobial and anti-inflammatory properties [39] whereas 5hydroxymethylfurfural and 2',4'-dihydroxy-3'-methylacetophenone are reported to have antimicrobial activities [40, 41]. 3-Butyl-4-nitro-pent-4-enoic acid, methyl ester has an anticarcinogenic effect [42]. Tetradecanoic acid is reported to possess antimicrobial, antispasmodic, and anti-inflammatory activities

[43]. Some minor constituents of the gel extract (see Table S1) have also been reported in literature to possess potential antimicrobial activity. Among this compounds are: pentadecanoic acid [44], hexadecanoic acid, methyl ester [45], phytol [46], 8-nonenoic acid [47], tetradecane [48], n-decanoic acid [49], stevioside [50], dodecane [51], eicosane [52], 2-furanmethanol [53], Z-5-nonadecene [54], 3deoxydmannoic lactone [55] and n-hexadecanoic acid [56]. Therefore, the synergistic effects and the variety of the major and minor components present within the gel extract should be taken into consideration for the observed antibacterial activity in this study. Moreover, the results illustrated the potential of the extract to serve as a renewable resource for antimicrobial compounds [57].

Molecular docking studies were carried out to understand the binding energies (kcal/mol) and amino acid residue interactions of the most predominant ligands (Table 4). According to the findings, the interactions revealed the negative values of the binding energy (Table 4). The low negative values indicated very decent fitness of the ligands in the binding pocket of the receptor, implying that ligands have established good interaction with the receptor [58]. Moreover, the free energy scores suggested that tetradecanoic acid has more binding affinity than the other test compounds as it displayed the lowest energy value. However, the positive control-ciproflaxaxin demonstrated better binding affinity as it displayed a much lower free energy score than the all test ligands (Table 4). Nevertheless, it can be concluded that the test ligands have the potential to inhibit cell wall synthesis as they revealed moderate binding capacities with PBP2, which is involved in peptidoglycan biosynthesis of the cell wall in bacterial strains [59].

The binding of ligands to receptors is governed by the concept of chemical bonding. Intermolecular interactions play a vital role in the binding chemistry between the ligands and the receptors [60]. To analyse the types of interactions formed between the ligands and PBP2, the desirable poses were visualized by the discovery studio. H-bonds were found to be present in all ligand-receptor interactions (Table 4 and Fig. 2). H-bonds are important in drug-target interactions as they are responsible for the stabilization of ligand-receptor protein complexes, consequently leading to the inhibition of bacterial growth [61]. The other types of bonds that were revealed by the ligands with PBP2 are carbon-hydrogen, alkyl, pi-alkyl, and van der Waals bonds. Alkyl bond is a covalent bond. Covalent bonds are strong and hence ligands forming them often bind permanently to their target. Thus it is expected that the ligands have the potential to strongly bind to PBP2 and result in cell death.

Conclusions

A. polyphylla gel extract showed noteworthy antibacterial activity against the tested strains. The antibacterial activity was more profound against the Gram-positive bacteria than the Gram-negative strains. The GC-MS analysis revealed 244 phyto-constituents of which the five main compounds were; 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 5hydroxymethylfurfural, 2',4'-dihydroxy-3'-methylacetophenone, 3-butyl-4-nitro-pent-4-enoic acid, methyl ester, and tetradecanoic acid. The phytochemicals are considered to have bioactivity including antimicrobial activity. Thus, the profound antibacterial activity observed in this study was attributed to the synergistic effects of these compounds. Moreover, the identified compounds displayed the binding scores which correlated to the moderate

binding affinities, implying that they have the potential to inhibit PBP2, consequently leading to the observed antibacterial activities. For future studies, extraction and characterisation of individual compounds and *in vivo* studies are recommended.

Abbreviations

A. polyphylla Schönland
Aloe polyphylla Schönland
S. aureus
Staphylococcus aureus
B. cereus
Bacillus cereus
E. coli
Escherichia coli
P. aeruginosa
Pseudomonas aeruginosa
ATCC
American Type Culture Collection
MIC
minimum inhibitory concentration
MBC
minimum bactericidal concentration
DMSO
dimethyl sulfoxide
INT
piodonitrotetrazodium violet
PDP2
penicillin-binding protein 2x
PDB
Protein Data Bank
GC-MS
Gas chromatography-mass spectrometry.

Declarations

a) Ethics approval and consent to participate

The collection of the plant was compiled and approved by the Research Ethical Committee of the University of Zululand (UZREC171110-030 PGM 2021/56). The experimental research and the collection of plant material comply with relevant institutional, national and international guidelines, and legislation.

b) Consent for publication

No consent for publication was required.

c) Availability of data and materials

All data available on the manuscript.

d) Competing interests

The authors declare that they have no competing interests.

e) Funding

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f) Authors' contributions

The study was conceived and designed by TSM and JSES. The experiments were conducted by OJP. LSN analysed the data. TNS and LSN contributed to the study by revising the manuscript. All authors read and approved the final manuscript.

g) Acknowledgements

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Figures

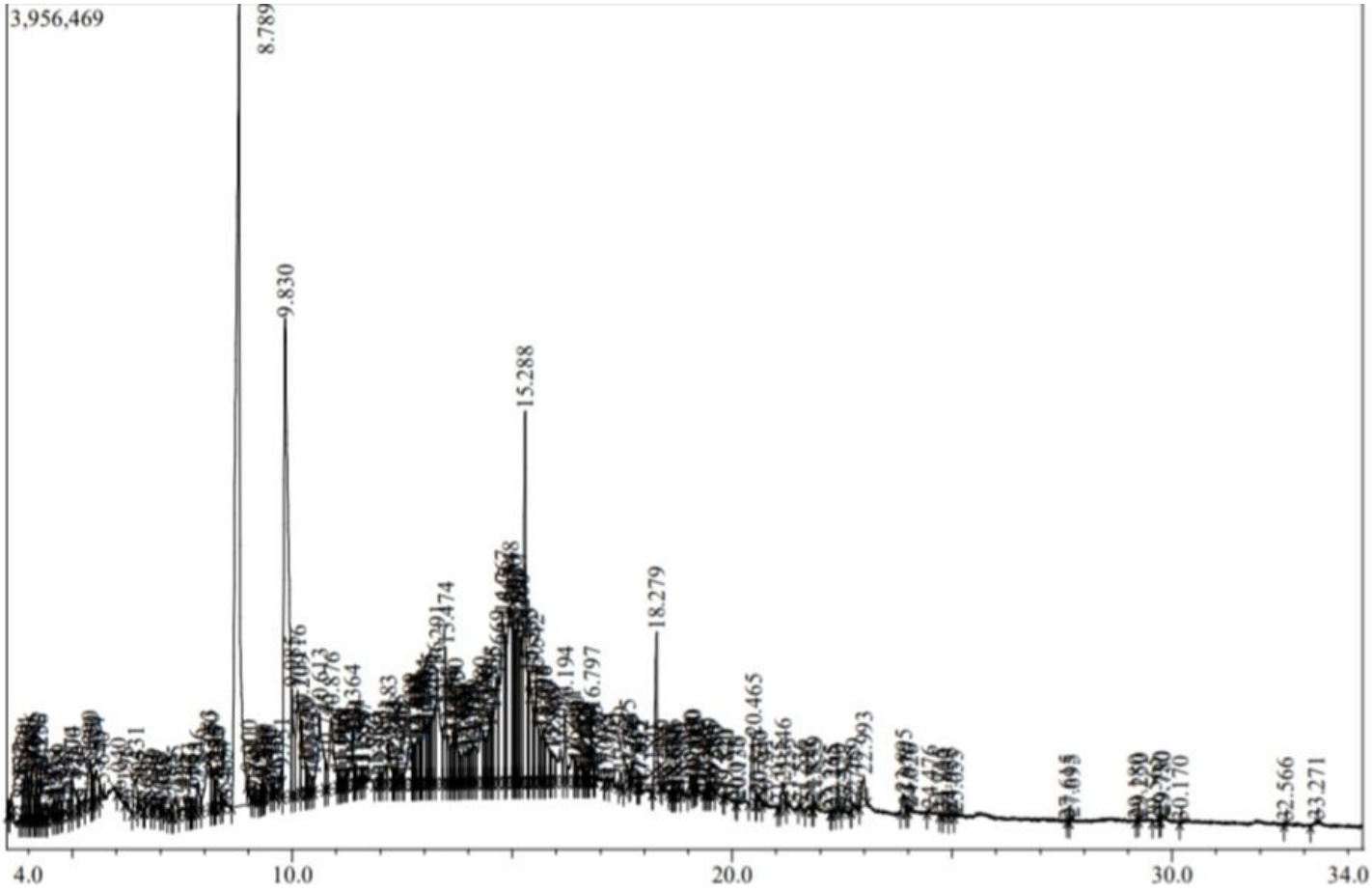

Figure 1

GC-MS chromatogram of the gel extract of *A. polyphylla* Schönland

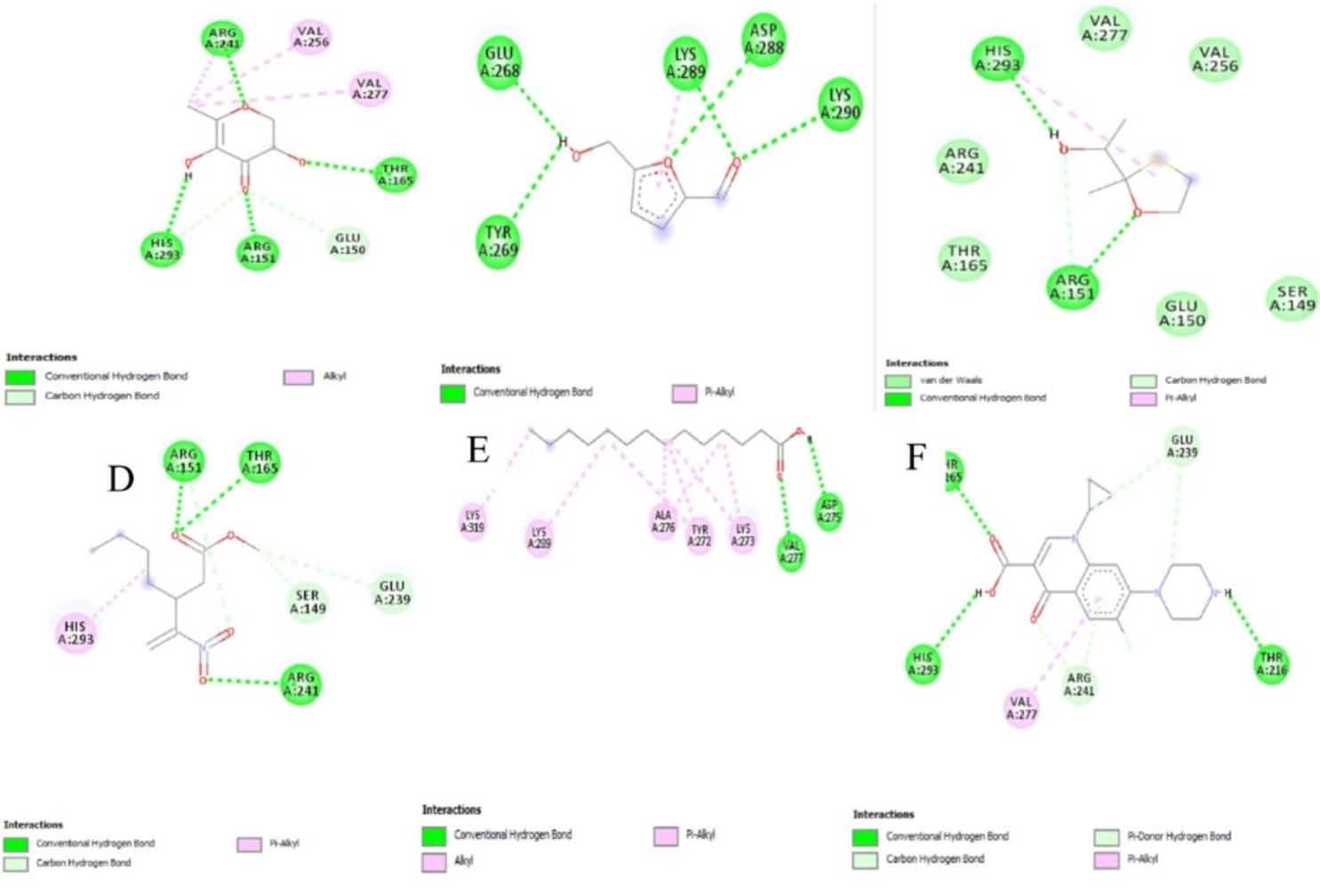

Figure 2

The 2D structures of the interactions between the ligands and PBP2; A 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-PBP2, B 5-hydroxymethylfurfural-PBP2, C2',4'-dihydroxy-3'-methylacetophenone-PBP2, D 3-butyl-4-nitro-pent-4-enoic acid, methyl ester-PBP2, E tetradecanoic acid-PBP2 and F ciprofloxacin-PBP2.

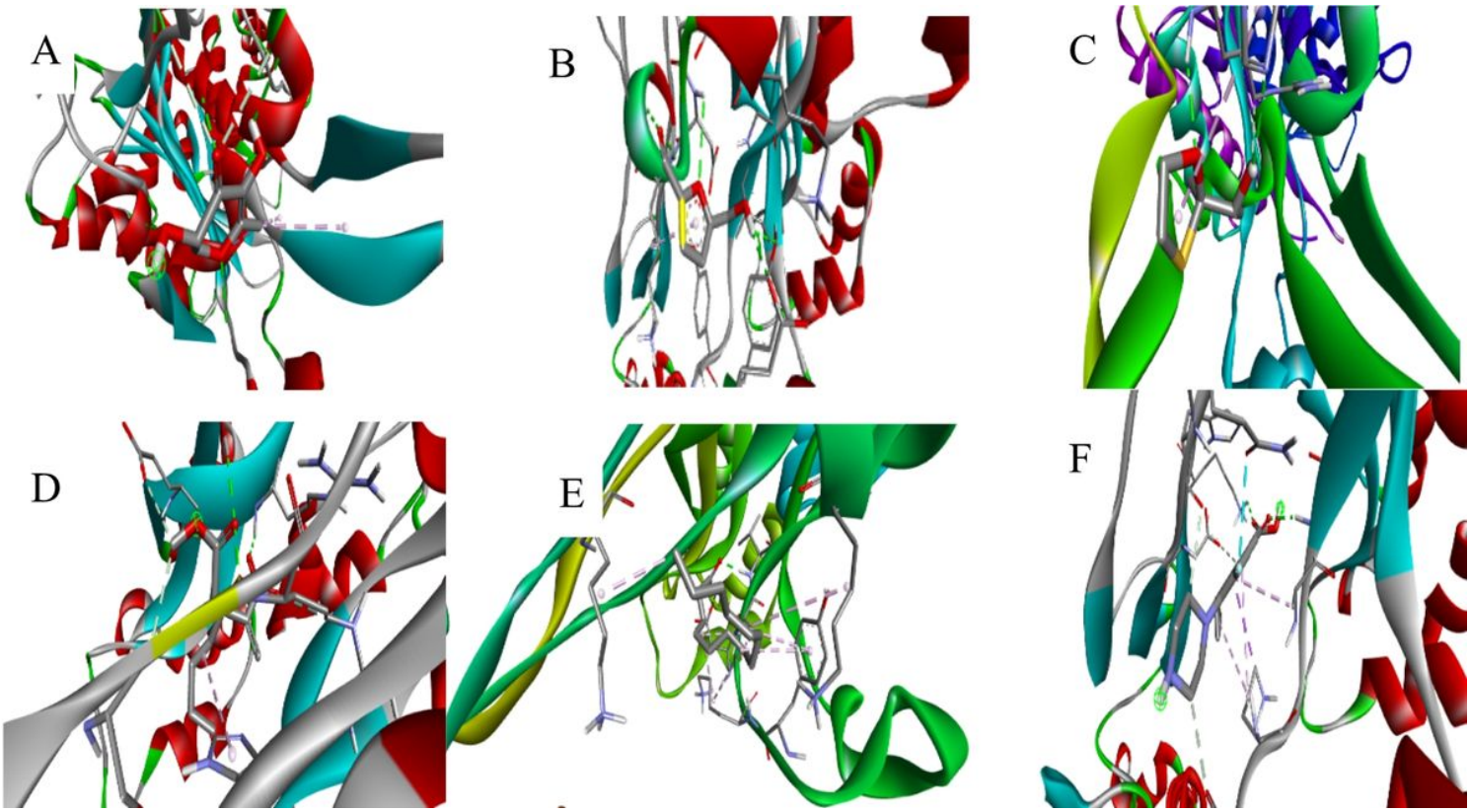

Figure 3

The 3D structures of the ligands docked with PBP2; A 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-PBP2, B 5-hydroxymethylfurfural-PBP2, C 2',4'-dihydroxy-3'-methylacetophenone-PBP2, D 3-butyl-4-nitro-pent-4-enoic acid, methyl ester-PBP2, E tetradecanoic acid-PBP2 and F ciprofloxacin-PBP2.

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