Molecular Docking Analysis and Evaluation for Antibacterial Activity of Chemical Constituents from the Stem Barks of *Podocarpus falcatus*

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Abstract

Chromatographic separation of an equal ratio of CH_2Cl_2-MeOH extract of the stem barks of *Podocarpus falcatus* led to the isolation of three compounds 1-3. The structures of the compounds were established based on the analysis of 1D and 2D NMR spectroscopic data. Compound 3 was reported from this plant for the first time. The crude extract and isolated compounds were tested for their antibacterial activity using the disk diffusion assay method against five bacteria strains. The crude extract showed strong activity against *S. aureus*. Compounds 1 and 2 showed relatively moderate activity against *S. flexineri* and *S. typhimurium* respectively, whereas, compound 3 demonstrated strong activity against *S. aureus*. Docking studies were performed against *S. aureus* Gyrase and human DNA topoisomerase IIβ by using AutoDock Vina. ADMET was predicted by SwissADME, PreADMET. In-silico molecular docking studies of compounds 1-3 showed strong interaction with *S. aureus* Gyrase with binding energy values ranging from −6.1 kcal/mol to −8.6 kcal/mol, concerning ciprofloxacin and Doxycycline-8.4 kcal/mol and -13.0 kcal/mol respectively, whereas, analysis against human topoisomerase IIβ with binding energy value ranging from -6.4 kcal/mol to -10.1 kcal/mol, for Vosaroxin and Abiraterone -10.2 kcal/mol and -11.8 kcal/mol respectively. The results obtained suggest that compounds 1-3 are potential *S. aureus* Gyrase inhibitors and might be used as antibacterial agents and compounds 1 and 2 are potential topoisomerase IIβ inhibitors and might be used as anticancer agents. The ADMET studies showed the highest drug-likeness properties for studied compounds 1-3. Compounds 1-3 have no acute toxicity. The studied compounds were predicted to be non- hepatotoxic, non-cytotoxic, non-mutagenic, and non-irritant. However, compound 2 is immunotoxic. The results of antibacterial activity and molecular docking suggested that this plant has potentially lead compounds for drug discovery.

Keywords: ADMET • Antibacterial activity • Molecular docking • *Podocarpus falcatus*

Introduction

Medicinal plants have been used in the treatment of various diseases sinThe *Podocarpus* (family, *Podocarpaceae*) is one of the largest genera of all conifers of the family containing about 94 species distributed from south temperate zones through the tropical highlands, West India, and Japan. Species from this genus have been reported to produce cytotoxic nor- and bisnor-diterpenoid dilactones generally known as nagilactones or podolactones [1]. In addition, totarane-type diterpenes such as totarol and their dimers (macrophyllic acid) have also been reported from many species of *Podocarpus*. These compounds are considered to be chemical markers of the genus [2]. *Podocarpus falcatus* (Thunb.) belongs to the vernacular name zigiba. It is an evergreen, dioecious, medium to large-sized tree up to 60 m tall and widely distributed in Ethiopia, Kenya, Tanzania, Mozambique, South Africa, and Madagascar [1, 2]. Besides its commercial and ecological importance, it has also known for its ethnobotanical values in Ethiopia. The roots of this plant are used as anticancer remedies [3,4]. Crushed and juice of the leaves taken for vomiting, whereas the root dried powdered, mixed with water taken for febrile illness. Decoction of the fruit serves as a tonic for cleaning the kidneys, lungs, and stomach

[5]. Despite the wider use of this plant by the communities for medicinal purposes, the phytochemical and bioactivity information about the stem bark of this plant is limited. Therefore, as part of the search for new bioactive molecules from Ethiopian medicinal plants, the isolation of five compounds and antibacterial activities of the extract and the compounds were reported here.

Materials and Methods

General method

Solvents and reagents used for extraction and purification of the compounds are of analytical and HPLC grade. Analytical TLC pre-coated sheets ALUGRAM®Xtra SIL G/UV_{254} (layer: 0.20 mm silica gel 60 with fluorescent indicator $UV_{F254/365}$) were used for purity analysis. For column chromatography, silica gel 100 mesh-200 mesh was used. Chromatograms were visualized on TLC by spraying with 10% H_2SO_4 and heating on a hot plate. NMR spectra data were recorded on an Avance 600 MHz spectrometer (Bruker, Billerica, MA, USA, at 600MHz (¹H) and 150MHz (¹³C). Chemical shifts are expressed in ppm, and J values are given in Hz and referenced to the central peak of the appropriate deuterated solvent's resonances (residual $CDCl_3$, $(CD_3)_2CO$, MeOD, and $(CD_3)_2SO$ at δ_H 7.26, 2.20, 3.35, 2.52 for protons and δ_C 79.16, 205.87, 49.77, 40.76 for carbons respectively). Whatman filter paper No.3, DMSO, Petri dishes, and gentamycin were used in the antibacterial analysis.

Plant materials

The stem bark of *P. falcatus* was collected from Horro Buluk, Horro Guduru Wollega zone, Oromia regional state, Ethiopia in September 2020. The plant material was identified by botanist (Dr. Fekadu Gurmessa) and the voucher specimen (DAD002Pf) has been deposited in Wollega University Herbarium. The collected plant part was washed thoroughly with tap water and cut into smaller pieces and dried under shade.

Extraction and isolation

The powdered stem bark of *P. falcatus* (850 gm) was extracted with an equal ratio of CH_2Cl_2-MeOH (3 Lx3 L) at room temperature for 48 hours each with occasional shaking. The crude extract was filtered from marc using Whatman filter paper. The solvent was evaporated under reduced pressure using a rotary evaporator at 40⁰C to yield (20 gm, 2.7%) of dark brown crude extract. About 18 g of the extract was adsorbed on 25 gm of silica gel and subjected to column chromatography, packed with silica gel (320 g). The column was eluted with hexane with an increasing gradient of ethyl acetate to afford 30 major fractions *ca*. 100 mL each. Fractions with similar TLC profiles were combined for further purification. Fractions 6−10 (2% EtOAc in hexane) were combined 20 mg and refined by Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) afforded (1 mg, 15 mg) while fractions 15-20 (3% EtOAc in hexane) showed similar TLC profiles were combined gave 18 mg and purified by washing with excess petroleum ether gave compound (2 mg, 12 mg). Fractions 26-30 (6% EtOAc in hexane) showed similar spots combined afforded 15 mg and purified further by Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) afforded compound (3 mg, 10 mg) and compound (4 mg, 8 mg).

Antibacterial activity assay

Five pathogenic bacterial strains, one gram-positive (*Staphylococcus aureus* (ATCC25923)) and four gram-negative (*Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhimurium* (ATCC13311), and *Shigella flexneri* (ATCC29903) were obtained from the Department of Biology, Wollega University and used for evaluation of antibacterial activities. Antibacterial activities of the methanol extract and isolated compounds were tested against five bacterial strains using the disc diffusion method [6] with slight modification. The test solutions were prepared with a known weight of crude extract (0.01 mg) and isolated compounds were dissolved in 1 mL of DMSO. A 0.6 mm diameter sterile Whatman test disk was placed on the surface of the

inoculated Mueller Hinton Agar in a 90 mm Petri dish and then 0.01 mg/mL of the crude extract and the isolated compound 1-3 were applied onto the disks. Gentamicin (10 µg) and DMSO were used as the positive and negative controls respectively. The test samples were allowed to diffuse for 30 min and the plates were then kept in an incubator at 37°C for 24 hours [7]. The experiments were carried out in triplicate and the mean diameter of the inhibition zones was calculated. The antibacterial activity was determined by measuring the zone of growth inhibition surrounding the disks Antibacterial inhibition activities were measured against the standard.

In silico molecular docking methodology

Preparation of ligands

The 2D structures (.mol) of all isolated compounds 1-3 were drawn and each structure was analyzed by using ChemDraw 16.0. The selected molecules were treated quantum mechanically by applying the DFT method using the Gaussian 09 program suite at the Becke-3-Lee-YangPar (B3LYP) level combined with the standard 6-31G (d,p) basis set. During the optimization procedure, all the parameters were set to obtain a stable structure with minimum energy. The global minimum energy of the title compound was determined from the structure optimization procedure. The 3D coordinates (PDB) of each molecule were obtained through the optimized structure.

Preparation of macromolecules

The crystal structure of receptor molecules *S. aureus* Gyrase (PDB ID 2XCT) and human topoisomerase IIβ with DNA (PDB ID 3QX3) were downloaded from the protein data bank. The protein preparation was done using the reported standard protocol by removing the co-crystallized ligand, selected water molecules and cofactors, the target protein file was prepared by leaving the associated residue with protein by using Auto Preparation of target protein file Auto Dock 4.2.6 (MGL tools 1.5.6).

Autodock Vina analysis

The graphical user interface program Auto Dock 4.2.6 was used to set the grid box for docking simulations. The grid was set so that it surrounds the region of interest in the macromolecule. The docking algorithm provided with Auto Dock Vina was used to search for the best-docked conformation between ligand and protein. During the docking process, a maximum of nine conformers were considered for each ligand. The conformations with the most favorable (least) free binding energy were selected for analyzing the interactions between the target receptor and ligands by the Discovery studio visualizer. AutoDock Vina with the standard protocol was used to dock the protein S. aureus Gyrase (PDB ID 2XCT) and human topoisomerase IIβ with DNA (PDB ID 3QX3) and isolated ligands 1-3 into the active site of proteins. The molecular docking studies were carried out using Auto Dock Tools (ADT) [8], which is a free Graphical User Interface (GUI) for the AutoDock Vina program. The grid box was constructed using 20x20x20, pointing in x, y, and z directions, respectively, with a grid point spacing of 0.375 Å. The center grid box is 62 Åx30 Åx62 Å for 2XCT and of 65 Åx40 Åx65Å for 3QX3. Nine different conformations were generated for each ligand scored using AutoDock Vina

functions and were ranked according to their binding energies. The ligands are represented in a different color, H-bonds, and the interacting residues are represented in stick model representation.

Result and Discussion

The stem bark of *P. falcatus* was exhaustively extracted with an equal ratio of CH_2Cl_2-MeOH solvent combination. The extract was subjected to column chromatography on silica gel followed by purification on Sephadex LH-20 and afforded three compounds 1-3 (Figure 1).

Characterization

Compound 1 was isolated as a white powder with melting points of 175⁰C-177⁰C. The[1]HNMR spectrum showed 12 signals, with a highly downfield shifted proton signal at 12.07 assigned to carboxylic acidic proton whereas, proton signals at 6.96 (1H, d, 8.6 Hz) and 6.41 (1H,d, 8.6 Hz) assigned to *ortho*-coupled aromatic protons (H-11, H-12), two overlapped doublets at 1.33 (6H, d, 7.1 Hz) were an isopropyl moiety and the rest proton signals correspond to non-aromatic protons. ^{13}C NMR spectrum showed six aromatic carbon signals resonating at $δ_c$ 149.5, 142.1, 134.6, 133.4, 126.6 and 113.4 assigned to C-13, C-9, C-14, C-8, C-11, and C-12 carbons, respectively. The remaining protons and carbons were assigned based on 2D-NMR data, notably, HSQC and HMBC. The COSY spectrum showed coupling between H-2/H-3 and H-5/H-6. The HSQC spectrum showed the presence of twelve signals suggesting that compound 1 possesses four saturated methylene groups, although their respective proton signals could not be fully determined due to their significant overlapping. The HMBC correlations between methyl protons H-16 and H-17 with C-14 indicated that the isopropyl group is attached to the aromatic ring at C-14. Moreover, a cross peak in the HMBC spectrum between H-18 and $δ_c$ at 178.9 ppm, confirmed the presence of a carboxylic acid group attached at C-4. Similarly, C-4 at $δ_c$ 43.4 was assigned based on an HMBC cross peak to H-18 (Figure 2). The above evidence was in agreement with a totarane-type diterpenes skeleton and corresponded to the known compound 4*β*-carboxy-19-nortotarol, which matched with the reported data for this compound [9].

Compound 2 was isolated as a white powder with melting points of 134⁰C−136⁰C. The structure of this compound was identified to be β-sitosterol using ¹H and ¹³C spectra data. The[1]H NMR spectrum showed an olefinic proton at $δ_H$ 3.54 (1H, tdd, 11.2, 6.5, 4.6 Hz) corresponding to H-6 and oxymethine proton at $δ_H$ 3.54 (1H, tdd, 11.2, 6.5, 4.6 Hz) for H-3. It also showed proton signals at $δ_H$ 0.69 (3H, s), 1.02 (3H, s), 0.94 (3H,d, 6.5 Hz), 0.84 (3H, d, 6.8 Hz), 0.81(3H, d, 6.8 Hz), 0.85 (3H, t, 7.2 Hz) for six methyl groups and were assigned to H-18, H-19, H-21, H-26, H-27 and H-29, respectively. The ¹³C NMR spectrum showed signals for 29 carbon atoms including signals for six methyl (19.8, 19.4, 19.1, 18.8, 11.9 and 11.8), eleven methylene ($δ_c$ 42.2, 39.8, 37.3, 33.9, 31.9, 31.6, 28.3, 26.1, 24.3, 23.1 and 21.1), nine methine ($δ_c$ 121.7, 71.8, 56.8, 56.1, 50.1, 45.8, 36.2, 31.9 and 29.2) and three quaternary ($δ_c$ 140.7, 42.3 and 36.5) carbon atoms. The recognizable signals at 140.9 (C-5) and 121.9 (C-6) are typical alkenes double bonds. The signals at δ 19.2 and 12.1 correspond to angular methyl carbon atoms (C-19) and (C-18) respectively. Signal at 71.9 is assignable to the β-hydroxyl group attached to the carbon
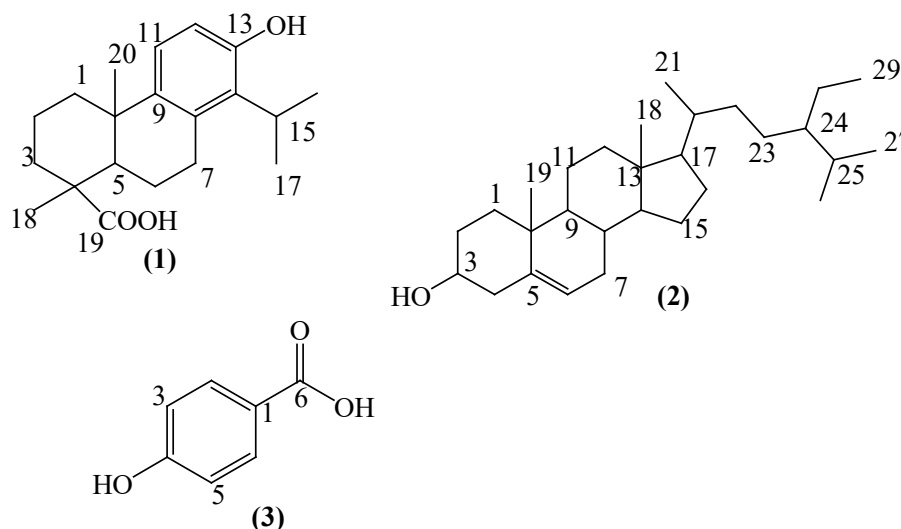

Figure 1. Structure of isolated compounds from stem bark of *P. falcatus*.

at (C-3). Therefore, based on these spectral data agrees with existing literature reported on β-sitosterol [10].

Compound 3 was isolated as white amorphous with melting points of 214^0C-215^0C. The ^{1}H-NMR spectrum showed four proton signals. Signals at$δ_H$ 7.78 (2H, d, 8.5Hz) were assigned to two overlapping aromatic protons (H-2, H-6), and signals at 6.82 (2H, d, 8.6Hz) were allocated to two overlapping protons (H-3, H-5). Whereas proton signals at 12.41(1H; s) correspond to a carboxylic acid proton, and signal at 10.22 (1H, s) is assigned to an OH proton. The ^{13}C NMR spectrum showed seven carbons signals corresponding to four aromatic methine at $δ_C$ 131.9 assigned to two overlapping carbons (C-2, C-6) and at 115.6 assigned to two overlapping carbons (C-3, C-5), three quaternary, one for carboxylic acid at 167.6 (C-7),121.8 (C-1) and 162.1(C-4). The 2D experiment COSY and HSQC spectra of compound 3 allowed, respectively, the detection of the scalar couplings of the protons and connectivity of each proton to a directly linked carbon atom. The COSY spectrum shows the coupling between H-2/ H-3 and H-5/H-6.The HSQC shows two protonated carbons at 7.79 (H-2 and H-6) linked with 131.9 (C-2 and C-6) and 6.82 (H-3 and H-5) linked with 115.6 (C-3 and C-5). The HMBC spectrum reveals the correlation between the cross peak $δ_H$ 7.79 (H-2and H-6) correlated with 115.6 (C-3and C-5), 131.9 (C-4) and 167.6 (C-7), the proton at 6.82 (H-3 and H-5) correlated with 121.8 (C-1), 115.6 (C-3), 162.1 (C-4) and proton at 10.22 (OH proton) correlated with 115.6 (C-3 and C-5) and 162.1(C-4) that reveals the position of OH at C-4. Based on spectral analysis of 1D and 2D NMR compound 3 was identified as 4-hydroxybenzoic acid which is in agreement with reported data for 4-hydroxybenzoic acid [11].

Antibacterial activity result

The antibacterial activity of the crude extract and the isolated compounds result (Table 1) showed varying degree of inhibition of the growth of bacterial strains. The crude extract showed considerable activity on both Gram-positive and Gram-negative bacterial strains with a zone of inhibition ranging from 18.23 mm ± 0.21 mm-23.03 mm ± 0.05 mm with the highest activity (23.03 mm ± 0.05 mm) was observed against S. aureus, which is even greater than that of the reference drug (gentamycin, 19.5 mm ± 0.04 mm) against the same strain Whereas, the isolated compounds showed moderate activities against all the test strains. Compound 3 displayed strong activity against S. aureus (18.13 ± 0.12 mm) and compound 2 showed better inhibition zone against S. typhimurium (18.01 mm ± 0.47 mm). This variation in inhibition of the bacterial growth by the crude extract and isolated compounds could be related to the synergetic effects of the various kinds of compounds present in the crude extracts or the minor compounds in the extract that could showed this activity have not been isolated. Therefore, the antibacterial activity displayed by the stem extracts of P. falcatus reveals the presence of compound 1-3. In general, the remarkable activities of the crude extract from this medicinal plant (P. falcatus) support the traditional use of the plant and could be used as a potential candidate in the development of novel antibacterial agents.

Molecular docking analysis

The results obtained from the molecular docking study revealed that the isolated compounds 1-3 showed a strong binding affinity towards S. aureus Gyrase (PDB ID 2XCT) with binding energy values ranging from −6.1 kcal/mol to −8.6 kcal/mol, concerning ciprofloxacin and Doxycycline −8.4 kcal/mol and 13.0 kcal/ mol respectively (Table 2 and Figure 2). Compound 1 showed strong binding energy (−8.6 kcal/mol) compared to ciprofloxacin −8.4 kcal/mol and compounds 2 and 3 had good binding energy (-7.5 kcal/ mol, -6.1 kcal/mol) respectively compared to ciprofloxacin (−8.4 kcal/ mol). Compounds 1 and 2 showed one hydrogen bond each, compound 1 via an acid functional group with Arg-458 and compound 2 through its

hydroxyl functional group with Met-1121 protein residues. Compound 3 displayed 5- Hydrogen bonds including 3 via its acid functional group with (DA-13, DG-9and DT-8) and 2 through its hydroxyl functional group with Arg-1122 and Gly-1082 protein residues. Hydrophobic interactions were observed for all isolated compounds 1-3 suggesting the compounds act as inhibitors of S. aureus Gyrase. On the other hand, the isolated compounds 1 and 2 showed a strong binding affinity towards Human topoisomerase IIβ with binding energy values ranging from −9.2 kcal/mol to −10.1 kcal/ mol, for Vosaroxin −10.2 kcal/ mol (Table 3, Figure 3). Compound 3 showed weak binding energy ranging from −6.4 kcal/mol compared to Vosaroxin (−10.2 kcal/mol). Compound 1 displayed 3-Hydrogen bonds 2 via its acid group with DC-11 and DG-10 and one trough hydroxyl group with Arg-503 protein residues. Compound 2 showed 2- Hydrogen bonds through its hydroxyl group with Glu-477 and Asp-557 protein residues and compound 3 displayed 5-Hydrogen bonds including 3 via its acid functional group with Arg-503, DG-13, DC-8, and 2 through its hydroxyl group with Asp-479 and Gly-478 protein residues. The result obtained suggests that compounds 1-3 are potential topoisomerase IIβ inhibitors and might be used as anticancer agents.

In silico pharmacokinetics (drug likeness) and toxicity analysis

The structures of isolated compounds 1-3 were converted to their canonical Simplified Molecular-Input Lineentry System (SMILE) and submitted to the Swiss ADME tool to estimate in silico pharmacokinetic parameters (drug-likeness properties) according to 'Lipinski's rule of five [12]. Lipinski's rule of five implies that the drugs and/or candidates should obey the five-parameter rule, which states that Hydrogen-Bond Donors (HBDs) should be less than 5, Hydrogen-Bond Acceptors (HBAs) should be less than 10, molecular mass should be less than 500 Da, log P should not be less than 5, and Total Polar Surface Area (TPSA) should not be greater than 140Å. Drug-likeness is a prediction that screens whether a particular organic molecule has properties consistent with being an orally active drug [12]. In the present study, the Swiss ADME prediction revealed that compounds 1-3 obeyed Lipinski's rule of five and they are likely to be orally active (Table 4). The TPSA value of the compounds 1-3 was noticed in the range from 20.23 Å to 57.53 Å and is well below the limit of 140 Å. The calculated numbers of rotatable bonds NRB values for the isolated compound 1-3 are less than 10 (Table 4), which indicated the compounds are conformationally stable.

ADMET properties

The Absorption, Distribution, Metabolism, Excretion, And Toxicity (ADMET) studies of isolated compounds 1-3 were predicted using Swiss ADMET. The skin permeability value (Kp) in cm/s indicates the skin absorption of molecules. In silico, the skin permeability, Kp, values of all compounds ranged from −2.20 cm/s to −6.02 cm/s suggesting low skin permeability and are within the range of broad-spectrum antibiotic Doxycycline (−9.03 cm/s) and under the clinical trial anticancer agent Vosaroxin (−8.98 cm/s). Additionally, Gastrointestinal (GI) and Blood−Brain Barrier (BBB) permeation indicate the absorption and distribution of drug molecules. The in-silico prediction results of Absorption, Distribution, Metabolism and Excretion (ADME) of the compounds 1-3 studied are presented in (Table 5). The Swiss ADME prediction parameters indicated that compound 1 and 3 showed high gastrointestinal (GI) absorption, whereas compound 2 displayed low absorption and do not show Blood−Brain Barrier (BBB) permeation. Moreover, a range of cytochromes (CYP's) regulates drug metabolism, in which CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 are vital for the biotransformation of drug molecules [13]. Thus, in silico SwissADME prediction, only compound 1 inhibited cytochrome CYP2C9. However, the compounds 2 and 3 are neither cytochromes inhibitor nor a substrate of permeability glycoprotein (P-gp).

Table 1. Antibacterial activity test for crude and isolated compounds from P. falcatus.

Crude extract/ compound	Bacteria inhibition zone (mm)				
	E.coli	S. aureus	S. flexineri	S. typhimurium	P. aeruginosa
PfME	21.03 ± 0.03	23.03 ± 0.05	19.07 ± 0.02	18.23 ± 0.21	20.13 ± 0.04
1	11.16 ± 0.24	11.33 ± 0.47	10.16 ± 0.24	12.33 ± 0.47	9.66 ± 0.24
2	16.00 ± 0.41	12.16 ± 0.24	14.16 ± 0.24	18.01 ± 0.47	10.33 ± 0.24
3	18.06 ± 0.09	18.13 ± 0.12	16.23 ± 0.33	17.22 ± 0.47	13.10 ± 0.08
Gentamycin	22.13 ± 0.05	19.5 ± 0.04	20.03 ± 0.05	20.10 ± 0.03	14.06 ± 0.06
DMSO	-	-	-	-	-

*PfME- Podocarpus falcatus Methanol Extract

(1)

(2)

(3)

Doxycycline
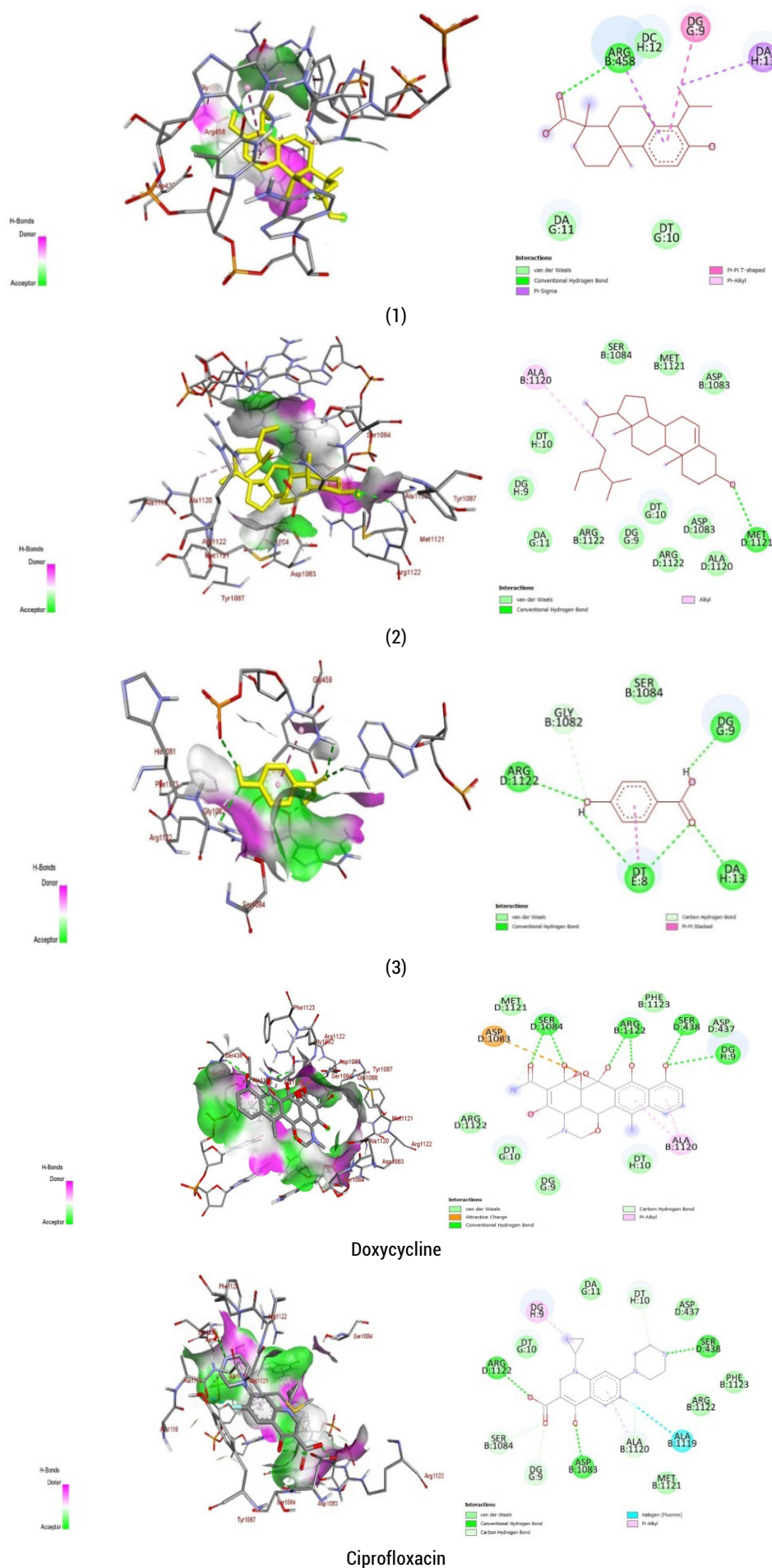
Ciprofloxacin

Figure 2. The 2D and 3D binding interactions of compounds (1-3) and Reference drugs Doxycycline and Ciprofloxacin agains*t S. aureus Gyrase* (PDB ID: 2XCT).

(1)

(2)

(3)

Abiraterone
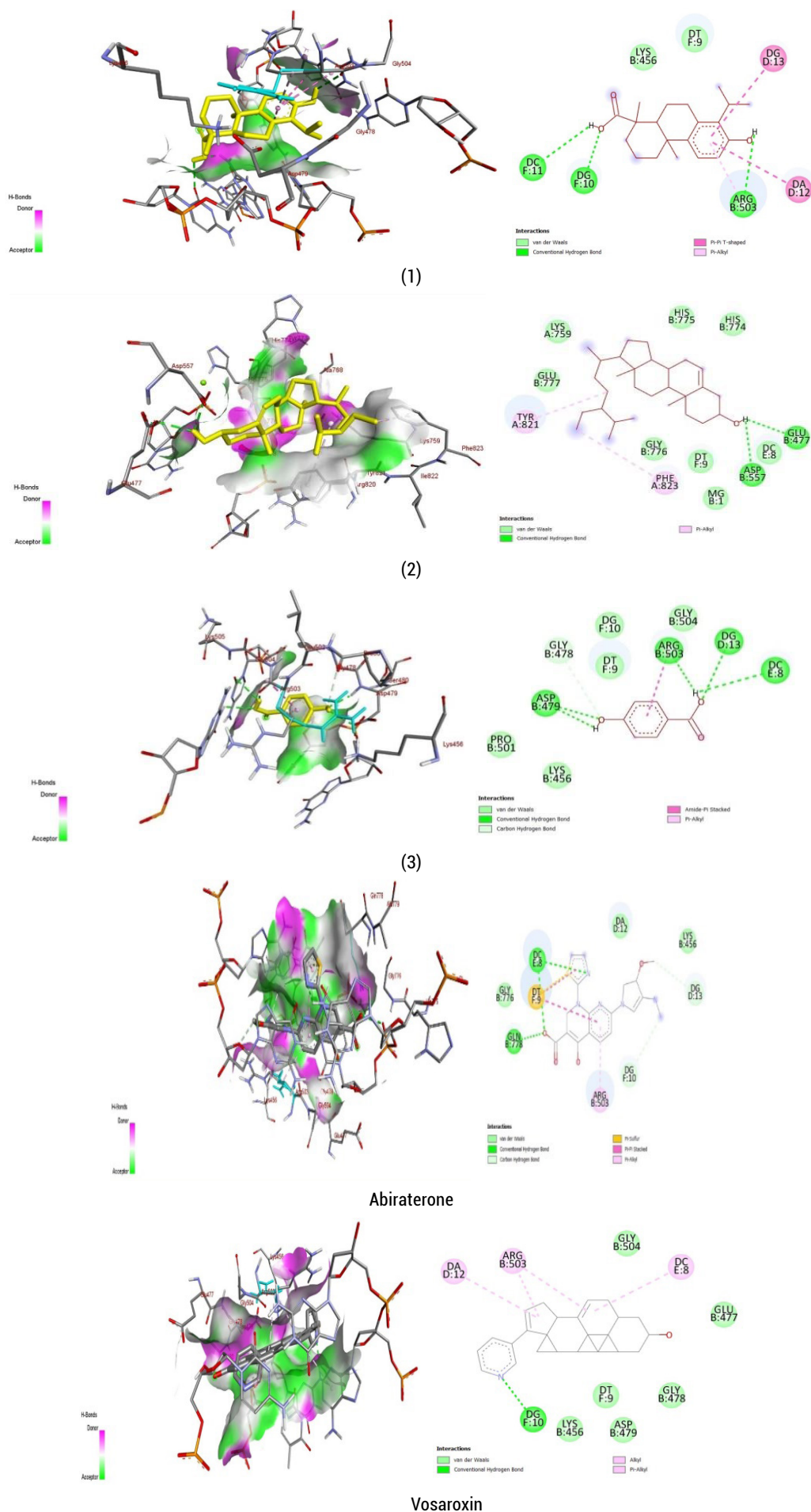
Vosaroxin

Figure 3. The 2D and 3D binding interactions of compounds (1-3) and reference drugs Abiraterone and Vosaroxin against human topoisomerase IIβ (PDB ID: 3QX3).

Table 2. Molecular docking results of isolated compounds against *S. aureus* Gyrase (PDB ID 2XCT).

Compound	Binding Affinity (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic, Electrostatic & others	Van der Waals
1	− 8.6	Arg-458	Arg-458, DA-13 DG-9, Arg-458	DC-12, DA-11, DT-10
2	− 7.5	Met-1121	Ala-1120	Ser-1084, Met-1121, Asp-1083, DT-10, DG-9, DA-11, Arg-1122, Ala-1120
3	− 6.1	Arg-1122, DA-13, DG-9, Gly-1082, DT-8	DT-8	Ser-1084
Doxycycline	−13.0	Ser-438, Ser-1084, Ser-1084: HG, Ser-1084: Arg-1122: HH12, DG-9	Asp-1083, Ala-1120	Arg-1122, Met-1121, Phe-1123, Asp-437, DG-9, DT: G-10, DT: H-10
Ciprofloxacin	− 8.4	Ser-438, Arg-1122, Asp-1083, Ser-1084, Ala-1120, DG-9, DT-10	Ala-1119, Ala-1120, DG-9	DA-11, Asp-437, Phe-1123, Arg-1122, Met-1121, DT-10

DA= Deoxyadenosine; DG=Deoxyguanosine; DT=Deoxythymidine; DC=Deoxycytidine

Table 3. Molecular docking results of isolated compounds against Human topoisomerase II β (PDB ID 3QX3).

Compound	Binding Affinity (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic, Electrostatic and others	Van der Waals
1	− 9.2	DG-10, DC-11, Arg-503	DA-12, DG-13 Arg-503	DT-9, Lys-456
2	− 10.1	Glu-477, Asp-557	Tyr-821, Phe-823	Glu-477, Lys-759, His-775, His-774, Gly-776, DT-9, DC-8, Mg-1
3	− 6.4	DG-13, Arg-503, DC-8, Gly-478, Asp-479	Arg-503, Arg-503	DG-10, DT-9, Gly-504, Pro-501, Lys-456
Abiraterone	− 11.8	DG-10	Arg-503, DA-12, DC-8	Gly-504, Glu-477, Gly-478, Lys-456, Asp-479, DT-9
Vosaroxin	− 10.2	Gln-778, DG-10, DG-13, DC-8	DT-9, Arg-503	DA-12, Lys-456, Gly-776

DA=Deoxyadenosine; DG = Deoxyguanosine; DT=Deoxythymidine; DC=Deoxycytidine

Table 4. Drug-likeness predictions of compounds, computed by Swiss ADME.

Compound	Mol. Wt. (g/mol)	NHD	NHA	NRB	TPSA (A²)	Log P (iLOGP) Lipophilicity	Log P (MLOGP) Lipophilicity	Log S (ESOL) Water Solubility	Lipinski's rule of five with zero violations
1	316.43	2	3	2	57.53	2.57	3.84	−4.66	0
2	414.71	1	1	6	20.23	4.79	6.73	−7.90	1
3	138.12	2	3	1	57.53	0.85	0.99	−2.07	0
Abiraterone	349.51	1	2	1	33.12	3.42	4.42	−5.03	1
Doxycycline	444.43	6	9	2	181.62	1.11	−2.08	−2.59	1
Vosaroxin	401.45	2	7	5	137.82	2.18	0.19	−2.19	0

NHD=Number of Hydrogen Donors, NHA=Number of Hydrogen Acceptors, NRB=Number of Rotatable Bonds, and TPSA=Total Polar Surface Area

Table 5. ADME predictions of compounds, computed by SwissADME and PreADMET.

Compound	Skin Permeation Value (Log Kp) cm/s	GI Absorption	BBB Permeability	Inhibitor Interaction					
				Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
1	−5.08	High	Yes	Yes	No	No	Yes	No	No
2	−2.20	Low	No	No	No	No	No	No	No
3	−6.02	High	Yes	No	No	No	No	No	No
Abiraterone	−5.14	High	Yes	No	Yes	No	No	No	No
Doxycycline	−9.03	Low	No	Yes	No	No	No	No	No
Vosaroxin	−8.98	High	No	Yes	Yes	No	No	No	No

GI=Gastrointestinal, BBB=Blood Brain Barrier, P-gp=P-Glycoprotein, and CYP=Cytochrome-P

Table 6. Toxicity prediction of compounds, computed by ProTox-II and OSIRIS property explorer.

Compound	LD50 (mg/kg)	Toxicity Class	Organ Toxicity					
			Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Irritant
1	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive	No
2	890	4	Inactive	Inactive	Active	Inactive	Inactive	No
3	2200	5	Inactive	Inactive	Inactive	Inactive	Inactive	No
Abiraterone	830	4	Inactive	Inactive	Active	Inactive	Inactive	No
Doxycycline	1007	4	Active	Inactive	Active	Inactive	Inactive	No
Vosaroxin	500	4	Active	Inactive	Inactive	Inactive	Inactive	No

Toxicity

Acute toxicity prediction results, such as toxicity class classification and LD_{50} values, predict that all of the isolated compounds 1-3 have no acute toxicity. The toxicological prediction gives results of endpoints such as hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. The studied compounds were predicted to be non- hepatotoxic, non- cytotoxic, non-mutagenic and non-irritant. However, compound 2 is immunotoxic as shown in (Table 6).

Conclusion

Phytochemical investigation of DCM-MeOH extract of stem bark. *P. falcatus* led to the isolation of four compounds 4β-carboxy-19-nor-totarol (1), β-sitosterol (2) and 4-hydroxybenzoic acid (3), and their structures were established on the basis of their ^{1}H and ^{13}C NMR spectral data and comparing with existing literature. Compound 3 is reported for the first time from the genus *Podocarpus*. The crude extract showed strong activity against *S. aureus.* Whereas, the isolated compounds showed moderate activity against all test strains. In silico molecular docking and ADMET analysis of isolated compounds 1-3 revealed that these compounds could be considered as antibacterial and anticancer agents. The results obtained from molecular docking, drug-likeness properties, ADMET analysis, in good agreement with those obtained from experimental studies suggesting the potential use of the isolated compounds as potential drug leads, which corroborate with the traditional uses of the roots of *P. falcatus.* Further comprehensive evaluations including *in vivo* activity tests could be done for conclusive decision on potential candidacy of the plant for formulation and medicinal uses.

Conflict of interests

The authors declare that they have no conflict of interest.

Availability of data and material

NMR data of compounds are available.

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References

1. Barrero, A. F., et al. "Podolactones: A group of biologically active norditerpenoids. Studies in Natural Products Chemistry" 28 (2003): 453-516.

2. Ito, S. & Kodama, M. "Norditerpene dilactones from Podocarpus species." *Heterocycles*, 4 (1976): 595–624.

3. Aerts, R. Afrocarpus falcatus (Thunb) 2008. http://database.prota.org/PROTAhtml/Afrocarpus%20falcatus En.htm.

4. Lulekal, E., et al. (2008). "An ethnobotanical study of medicinal plants in Mana Angetu District, southeastern Ethiopia." *J Ethnobiol Ethnomedicine*, 4.1 (2008): 1-10.

5. Abdillahi, H. S., et al. "Anti-inflammatory, antioxidant, anti-tyrosinase and phenolic contents of four Podocarpus species used in traditional medicine in South Africa." *J Ethnopharmacol*, 136.3 (2011): 496-503.

6. Wayne, P. A. "Performance Standards for Antimicrobial Disc Susceptibility Test, Approved Standard: M02-A11" 11th edition. National Committee for Clinical Laboratory Standards (NCCLS), New York, USA, 32.1 (2012): 1-76.

7. Nascimento, G., et al. "Antibacterial activities of plant extracts and phytochemicals on antibiotic resistant Bacteria." *Braz J Microbiol*. 31(2000): 247-256.

8. Trott, O., & Olson, A. J.,(2010). "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading." *J. Comput. Chem*. 31 (2009): 455–461.

9. Park, H. S., et al."New cytotoxic norditerpene dilactones from leaves of Podocarpus macrophyllus var. maki" *Heterocycles*, 63.2 (2004): 347-357. [Google Scholar] [Cross Ref]

10. Arjun, P., et al. (2010). "Isolation and characterization of stigmast-5-en-3β-ol (βsitosterol) from the leaves of Hygrophila spinosa T.Anders" *Int J Pharm Sci. Res.* 1.2 (2010): 95-100.

11. Chen, J., et al. Isolation and characterization of the chemical constituents from Gynura bicolor and G. divaricata. *Planta Medica*, *78*.11(2012):PF6.

12. Lipinski, C.A., et al. "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings". *Adv Drug Deliv Rev*, 23 (1997) 3– 25

13. Das, P., et al. "In-silico approach for identification of effective and stable inhibitors for COVID-19 main protease (Mpro) from flavonoid based phytochemical constituents of Calendula officinalis". *J Biomol Struct Dyn*.; 38.16 (2021): 6265-6280.