

ARTICLE

Open Access



Exploring the medicinal potential of *Senna siamea* roots: an integrated study of antibacterial and antioxidant activities, phytochemical analysis, ADMET profiling, and molecular docking insights

Hadush Gebrehiwot¹, Urgessa Ensermu², Aman Dekebo^{1,3*} , Milkyas Endale⁴ and Mo Hunsen⁵

Abstract

Nowadays, infectious diseases pose an alarming global threat to human health. The genus *Senna* is among the most well-known taxonomic categories commonly used in folk medicine to confront these challenges. Motivated by its traditional uses, a comprehensive study was conducted on the roots extract of *Senna siamea*, aiming to address the in vitro antibacterial and antioxidant efficacy of phytochemicals from the dichloromethane: methanol (1:1) roots extract of the plant, along with in silico computational studies. The separation of compounds was achieved using silica gel column chromatography. Whereas, the antibacterial and antioxidant activities were examined using paper disc diffusion and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays, respectively. Silica gel column chromatography of the dichloromethane: methanol (1:1) roots extract afforded lupeol (**1**), β -sitosterol (**2a**) and stigmasterol (**2b**), chrysophanol (**3**), betulinic acid (**4**), and glyceryl-1-hexacosanoate (**5**). Although these compounds have been previously reported from the plant, proof of their medicinal applications via in vitro and in silico studies is still lacking. Notably, our findings showed remarkable inhibition zones by the extract (18.00 ± 0.00 mm and 17.17 ± 0.24 mm) against *E. coli* and *S. aureus*, respectively, at 50 mg/mL compared to ciprofloxacin (23.33 ± 0.47 mm and 22.00 ± 0.00 mm, respectively), showcasing its potential antibacterial efficiency. Considerable inhibition zones were also recorded by chrysophanol (**3**) against *E. coli* (16.33 ± 0.24 mm) and *S. pyogenes* (16.00 ± 0.00 mm) at 2 mg/mL, compared to ciprofloxacin which showed 23.33 ± 0.47 mm and 21.67 ± 0.47 mm, respectively, signifying its potent antibacterial activities. In addition, the crude extract and chrysophanol (**3**) exhibited substantial IC_{50} values (1.24 and 1.71 μ g/mL, respectively), suggesting their significant antioxidant potential compared to that of ascorbic acid (IC_{50} : 0.53 μ g/mL). Chrysophanol (**3**) fulfilled Lipinski's rule with no violation and lupeol (**1**), β -sitosterol (**2a**), stigmasterol (**2b**), betulinic acid (**4**), and glyceryl-1-hexacosanoate (**5**) displayed one violation each which were in favor of the drug-likeness predictions. All the compounds exhibited no cytotoxicity and except betulinic acid (**4**), all the compounds also showed no carcinogenicity properties which

*Correspondence:
Aman Dekebo
amandekeb@gmail.com

Full list of author information is available at the end of the article

were consistent with the prediction results of ciprofloxacin. The molecular docking computations revealed that all the compound isolates displayed strong and nearly strong binding affinities against all protein targets, ranging from -6.6 kcal/mol to -9.2 kcal/mol (lupeol (**1**) against *E. coli* DNA gyrase B and topoisomerase II α , respectively). Thus, the present findings suggest the roots of *Senna siamea* for potential medicinal applications against multi-drug resistant pathogens hence validating its ethno-medicinal uses.

Keywords Antibacterial, Antioxidant, Drug-likeness, Molecular docking, *Senna siamea*, Toxicity

Introduction

The genus *Senna*, also known as *Cassia*, belonging to the Fabaceae family, comprises more than 260 species found in Africa, Latin America, Southeast Asia, and Northern Australia. These species have attracted keen interest in phytochemical, biological, and pharmacological studies due to their vast therapeutic values [1]. The genus includes a range of plants, from giant trees to small seasonal herbs, with the majority being herbaceous perennials [2, 3]. Species of the genus are well known in folk medicine to cure a variety of internal and external disorders, including cancer [4], malaria [2], diabetes [5], bacterial and fungal infections, and mutagens [6, 7]. In Brazilian traditional medicine, *Senna* species have been used as purgatives, laxatives, and treatment of flu and colds [8]. In Ayurveda, these plants were employed against headache and fever and in Thai traditional medicine, the species have been used in the treatment of abdominal and skin infections [9].

Senna siamea Lam (synonym, *Cassia siamea*, commonly called the Kassod tree, Fig. 1) is widely grown in Africa, Oceania, and Latin America [10], with a wide spectrum of traditional uses. In Ethiopia, it is locally called *Yefereng digita* (Amharic name) and is used by traditional healers to treat dandruff, wounds, common warts, pyoderma, gonorrhea, anthrax, and snake bites [11]. Infusion of the pods is suggested to heal fever and as a laxative for pregnant women. Decoction of the pods is recommended against breathing difficulties and to treat intestinal worms. In addition, an infusion of extracts from the leaves is drunk to cure nosebleeds, convulsions, and flatulence [12]. More traditional claims have also been cited for *Senna siamea* to be used for the treatment of jaundice, abdominal pain, menstrual pain, typhoid fever, and ease of sugar levels in the blood [13]. The ethno-medicinal applications of the plant also revealed that it is used to treat microbial infections and asthma [14], sleeplessness, hypertension, malaria, liver disorder, constipation, toothache, diabetes [15, 16], digestive problems, herpes, genitourinary disorders, rhinitis, and as a blood cleaning agent [17].

Regardless of the subspecies, various parts of *S. siamea* have been evaluated for their antimalarial properties, a tropical endemic disease with high morbimortality and have displayed auspicious results [18, 19]. The compound chrobisiamone A isolated from the leaves extract

displayed attractive in vitro antiplasmodial activity against *Plasmodium falciparum* 3D7, and lupeol and emodin identified from the stem bark of the plant exhibited the antimalarial properties [20]. The stem bark extracts have been reported to possess anti-inflammatory and analgesic properties [21]. The leaves show antilipemic and antidiabetic effects [22], antibacterial activities [23], and antiproliferative effects [24]. Moreover, the roots and flowers exhibit antioxidant activities [25]. Hu and co-workers reported antiviral chromones from the stem of *S. siamea* with anti-HIV-1 and antitobacco mosaic virus (anti-TMV) activities [26]. Besides, cassiamin B reported from the plant exhibited chemo-preventing and antitumor-promoting effects [27].

The phytochemistry of the genus (*Senna*) is diverse. Thereby, the qualitative phytochemical screening of *S. siamea* revealed the presence of polyphenols (flavonoids, anthraquinones, tannins, anthrone, bisanthraquinones, isoflavonoids, and phenolics), chromones and derivatives (chromones glycosides, chromone alkaloids, bischromone, and dihydronaphthalenone compounds), steroids, alkaloids, carotenoids, saponins, minerals, reducing sugars, vitamins, and enzymes [28–32]. The phytochemical study of the plant also revealed various bioactive compounds, including barakol [33], anhydrobarakol, and 5-acetonyl-7-hydroxy-2-methylchromone [34] which have been reported from the chloroform extract of the leaves of *S. siamea*. Cassiarin A and B [35], chrysophanol, emodin, rhein, physicon, and sennosides [36], lupeol [37], and β -sitosterol [13] were reported from the methanol and ethanol extracts of *S. siamea* (leaves). Coumarin and betulinic acid [38], siamchromones A–G [26], kaempferol [39], emodin, and chrysophanol [40] have also been isolated from the stem and root barks of the plant. In addition, the *n*-hexane and aqueous extracts of the seeds of the plant afforded stigmasterol, palmitic, oleic, linoleic, and stearic acids [41], aloe emodin [42], vitamin B1, B2, B3, C, and E and various amino acids (valine, lysine, leucine, threonine, methionine, cysteine, histidine, glycine, and arginine) [43].

Despite the abundance of *S. siamea* in Ethiopia, scientific reports on the ethno-medicinal benefits of compounds isolated from its roots are still lacking. Although traditional healers use the plant in folk and livestock remedies, the medications are outdated and delivered without scientific support. Most traditional practices of the



Fig. 1 *Senna siamea* (picture by Hadush G., Adama Science and Technology University, Ethiopia, 2022)

plant in Ethiopia are related to wound healing and antibacterial, and antioxidant activities [11, 15]. In addition, *in silico* investigations such as pharmacokinetics, drug-likeness properties, and molecular docking studies of the compound isolates should be addressed. Thus, considering its promising multiple biological activities, this study aimed to evaluate the antibacterial, antioxidant, *in silico* cytotoxicity, and pharmacokinetic properties of compounds isolated from the roots of *S. siamea*, along with their *in silico* molecular modeling studies.

Materials and methods

Collection and identification of the plant material

Roots of *S. siamea* were harvested from Adama Science and Technology University campus, Adama, Ethiopia, and its surroundings in July 2022. Mr. Reta Regassa (chief botanist, Hawassa University) in collaboration with Mr. Melaku Wendafrash (chief botanist, Addis Ababa University) identified the plant. After identification, a voucher specimen (HSS006) was deposited at the National Herbarium, Addis Ababa University, Ethiopia. The fresh plant materials were freed from impurities with continuous washing using tap and distilled water consecutively, followed by air drying for three weeks at room temperature without direct exposure to sunlight. Thenceforward, the plant samples were crushed into a fine powder and kept in a polyethylene bag until extraction.

General experimental procedures

The plant materials were powdered using a grinder (Shanghai Jingke, JK-HSG-100 A, China). Solvents (analytical grade), reagents, spraying chemicals, and positive controls were purchased from local markets, in Addis

Ababa, Ethiopia. The powdered samples were macerated and concentrated on an orbital shaker (Gemmy Industries, VRN-200, Taiwan) and rotary evaporator (DW-RE-3000, China), respectively. The concentrates were chromatographed on silica gel (200 g, 60–200 mesh) in a column (size; 725 mm) and eluted with an increasing gradient of solvents. The purity of fractions was analyzed using TLC (thickness; 0.25 mm, size; 20×20 cm coated with high-grade silica gel, 230–400 mesh, pore size 60 Å, Merck Grade 64271, Darmstadt, Germany) and UV lamp (UV4AC6/2, CBIO Bioscience and Technologies, China, at 254 and 365 nm). Conversely, UV inactive compounds were detected using a spray (1% vanillin in ethanol and 2 mL sulphuric acid reagent) followed by direct heating (110 °C) over a stove for 5 min. Melting points were measured using a Japson-type apparatus (JA90161, India) and antioxidant activities were determined using a UV-Vis spectrophotometer (CE4001, UK) equipped with tungsten and deuterium lamps. NMR spectra were generated with the help of 400 and 600 MHz Bruker AVANCE type NMR instruments with deuterated chloroform and TMS as a solvent and reference, respectively. For antibacterial activity, an autoclave, incubator, micropipettes (1000 µL), Petri dishes (90 mm), and a hood equipped with laminar airflow and UV radiation were utilized.

Extraction and isolation

The pulverized roots of *S. siamea* (700 g) were extracted with 3.5 L of CH_2Cl_2 : CH_3OH (1:1) over an orbital shaker for 72 h by maceration technique. Whatman No. 1 and a vacuum rotary evaporator were used for the filtration and concentration techniques, respectively. The crude extract (24.0 g) was adsorbed on silica gel (25 g, 60–200 mesh), subsequently subjected to silica gel column chromatography (200 g, 60–200 mesh), and eluted with an increasing gradient of EtOAc in *n*-hexane followed by methanol in CHCl_3 ratio. The fractionation process generated a total of 75 fractions (100 mL each) and their purity was monitored using TLC, a UV lamp, and a vanillin-sulfuric acid reagent. Similar fractions with identical R_f values in the same solvent system were mixed and structural information of the purified compounds was established using 1D and 2D NMR spectroscopic techniques. Fractions 8–10 collected with 25% EtOAc in *n*-hexane were mixed and purified with a gradient elution of 100% *n*-hexane up to 50% EtOAc in *n*-hexane to afford lupeol (**1**, 12.6 mg) and a mixture of β -sitosterol (**2a**) and stigmasterol (**2b**) (23.8 mg). Fractions 15, 16, and 17 obtained with 40%, 45%, and 50% EtOAc in *n*-hexane, respectively, were combined and purified with a gradient elution of 20–70% EtOAc in *n*-hexane to yield chrysophanol (**3**, 10.3 mg) and betulinic acid (**4**, 13.8 mg). Furthermore, fraction 18, isolated with 50% EtOAc in *n*-hexane was purified with preparative TLC using 25% EtOAc in *n*-hexane as

a mobile phase to afford glyceryl-1-hexacosanoate (5, 9.5 mg).

Antibacterial activity

The CH₂Cl₂: CH₃OH (1:1) extract and the compound isolates were studied for in vitro antibacterial activities against four human bacterial pathogens (*Escherichia coli*, ATCC-25922; *Staphylococcus aureus*, ATCC-25923; *Pseudomonas aeruginosa*, ATCC-27853; and *Streptococcus pyogenes*, ATCC-19615) using paper disc diffusion assay. The activities were performed following the standard protocols adopted by Akhtar et al. (2017), Balouiri et al. (2016), and Habeeb et al. (2007) [44–46] using a commercial antibiotic (ciprofloxacin) and DMSO as reference drug and solvent, respectively. Different concentrations of the crude extract (25, 12.5, and 6.25 mg/mL) and isolated compounds (1, 0.5, and 0.25 mg/mL) were prepared in 4% DMSO from the corresponding stock solutions (50 mg/mL for the extract and 2 mg/mL for the compounds). The sterilized paper discs (6 mm) transferred to the bacterial culture-inoculated MHA were impregnated with 100 µL of each solution using a micropipette. Afterwards, the Petri dishes were left for 30 min for complete diffusion and incubated at 37 °C for 18–24 h. The clear zones of the paper discs revealed inhibition zones (mm) which were measured by a caliper (mm). The experiment was performed in triplicate and the results are expressed as mean ± standard error of the mean (SEM) using a Microsoft Excel 2016 spreadsheet.

Radical scavenging activity

The antioxidant activities that are related to the ability of the extract and compound isolates to transfer electrons or hydrogen atoms by bleaching the purple color of the DPPH solution in methanol were screened via 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method, following previous experimental protocol [47]. Various concentrations of the extract, compound isolates, and ascorbic acid standard were prepared in methanol by serial dilution and fresh DPPH solution (2 mL, 0.04% w/v in CH₃OH) was added to each solution. The sample solutions were left to stand in the dark at room temperature for half an hour and their absorbance was measured at λ = 517 nm using a UV-Vis spectrophotometer in triplicate. Finally, the scavenging activities were calculated as mean ± SEM using Eq. 1 and the IC₅₀ values were determined from the relationship curves.

$$\text{DPPH radical scavenging power (\%)} = \left(1 - \frac{A}{A_0}\right) * 100 \quad (1)$$

Where; A and A₀ imply the absorbance of sample solution in DPPH and DPPH solution, respectively.

Prediction of drug-likeness and ADMET properties

The pharmacokinetics (ADME: absorption, distribution, metabolism, and excretion) properties of the compound isolates along with the standard ciprofloxacin were computed by SwissADME (<http://www.swissadme.ch/>) and ADMETSAR (<http://lmmd.ecust.edu.cn/admet sar1>) online web tools [48]. The drug-likeness predictions were also established based on Lipinski's rule [49]. Furthermore, the organ toxicity predictions of the compound isolates were generated using Pro Tox II web tool (<https://tox-new.charite.de/>) [50].

Molecular docking studies

The modes of binding of the compound isolates to protein targets of the bacterial strains and human enzymes were studied using molecular docking experiments and compared to the reference drugs. Accordingly, DNA gyrase B of *E. coli* (PDB ID: 6F86), PK of *S. aureus* (PDB ID: 3T07), 10782 streptopain of *S. pyogenes* (PDB ID: 6UKD), human myeloperoxidase (PDB ID: 1DNU), and topoisomerase II α (PDB ID: 4FM9) were obtained from the Protein Data Bank (PDB) (<http://www.rcsb.org>) and saved in the PDB format. The appropriate 3D structures of the compounds were retrieved from PubChem and/or Zinc docking databases, downloaded in SDF format, converted to PDB format via the Open Babel GUI application, and saved in PDB format. Proteins and ligands were prepared as per previous protocols [51, 52] and molecular docking was computed by studying the binding interaction of the ligand and the receptor on the active sites of the protein targets. The 3D sizes of the structure-based design (SBD) site sphere of each protein were retrieved as follows; PDB ID: 1DNU (36.727, 9.922, and 10.989), PDB ID: 6F86 (61.680, 28.330, and 64.290), PDB ID: 6UKD (-25.515, -13.743, and 13.171), PDB ID: 3T07 (0.013, 0.147, and -0.003), and PDB ID: 4FM9 (17.245, 39.350, and 25.275) Å for x, y and z dimensions, respectively. The docking simulation was performed using the MGL AuthoDock Tools 1.5.6 program and all the files dropped to the appropriate working directory were computed via the Command Prompt application. Nine different conformations were generated and only a single conformation with the most stable binding affinity and root mean square deviation (RMSD) was chosen. Finally, the ligand interactions, hydrogen bonds, residual amino acid interactions, 2D and 3D structural orientations, and image preparations were run using the Biovia Discovery Studio Visualizer 2021 [53].

Results and discussion

Extraction yield

The maceration of the CH₂Cl₂: CH₃OH (1:1) roots extract of *S. siamea* afforded 25.0 g (3.57%) of crude extract.

Structure elucidations

In the present work, lupeol (1), β -sitosterol (2a) and stigmasterol (2b), chrysophanol (3), betulinic acid (4), and glyceryl-1-hexacosanoate (5) were isolated from the CH_2Cl_2 : CH_3OH (1:1) roots extract of *S. siamea* and their structures were confirmed with 1D and 2D NMR spectroscopic techniques.

Compound 1 (12.6 mg, R_f : 0.45 in 10% EtOAc in *n*-hexane) was obtained as white sparkles with a melting point of 212–214 °C which was in good agreement with the reported values for lupeol [37, 54]. The ^1H NMR (400 MHz, CDCl_3) spectrum showed a pair of doublets at δ 4.67 (1H, *d*, $J=2.5$, H-29a) and 4.55 (1H, *d*, $J=1.2$, H-29b) suggesting the presence of terminal isopropenyl group in the lupane series of triterpenoids. A broad singlet and doublet of doublets observed at δ 3.63 (1H, *brs*, OH) and 3.18 (1H, *dd*, $J=11.2$, 5.0, H-3) attributed to hydroxyl and oxymethine protons at C-3, respectively. The multiplet signals at δ 2.36 (1H, *m*, H-19) were characteristic of a pentacyclic methine proton where the isopropenyl group is attached. Signals of seven singlets displayed at δ 1.66, 1.01, 0.95, 0.93, 0.81, 0.77, and 0.74 (21 H, *s*) belong to the methyl groups of the structure assignable to H-30, H-26, H-27, H-23, H-25, H-28, and H-24, respectively. The remaining protons overlapped within the range of δ 1.65–1.15 (24 H, *m*) (Additional file 1: Table S1; Fig. S1). The ^1H - ^1H coupling of adjacent protons was also supported by the COSY correlations. Accordingly, important ^3J correlations were observed between H-2 (δ 1.54) and H-3 (δ 3.18), H-19 (δ 2.36) and H-21 (δ 1.33), and H-29a

(δ 4.67) and H-29b (δ 4.55) (Additional file 1: Table S1; Fig. S4).

The ^{13}C NMR (400 MHz, CDCl_3) spectrum revealed thirty well-resolved signals attributed to the lupane series of a triterpenoid skeleton. Characteristics of sp^2 hybridized carbon signals were observed at δ 151.1 and 109.4 assignable to C-20 and C-29, respectively, of which the former belongs to an sp^2 quaternary carbon, whereas, the latter belongs to a terminal sp^2 methine carbon (from the DEPT-135 spectrum). The signal at δ 79.1 (C-3) indicated an oxymethine carbon and groups of methine signals were observed at δ 55.3 (C-5), 50.5 (C-9), 48.3 (C-18), 48.0 (C-19), and 38.1 (C-13). Seven methyl signals were also clearly evident at δ 28.0 (C-23), 19.4 (C-30), 18.0 (C-28), 16.2 (C-25), 16.0 (C-26), 15.5 (C-24), and 14.6 (C-27) in good agreement with the triterpenoid skeleton. Furthermore, the DEPT-135 spectrum exhibited an sp^2 olefinic methylene signal at δ 109.4, an sp^3 oxymethine signal at δ 79.1, five methine, ten methylene, and seven methyl signals consistent with the ^{13}C NMR spectrum (Additional file 1: Table S1; Fig. S2 and S3). Overall, the spectral data obtained were in close agreement with the reported values for lupeol (1) [37, 55] (Fig. 2). The compound was previously reported from the stem barks of *S. siamea* [56].

Compound 2 (23.8 mg, R_f : 0.82 in 20% EtOAc in *n*-hexane) was isolated as white needles. Based on the integration patterns and intensity of signals, its ^1H NMR (600 MHz, CDCl_3) spectrum revealed resonances of a mixture of two compounds (approximately 3:1 of 2a and 2b). The

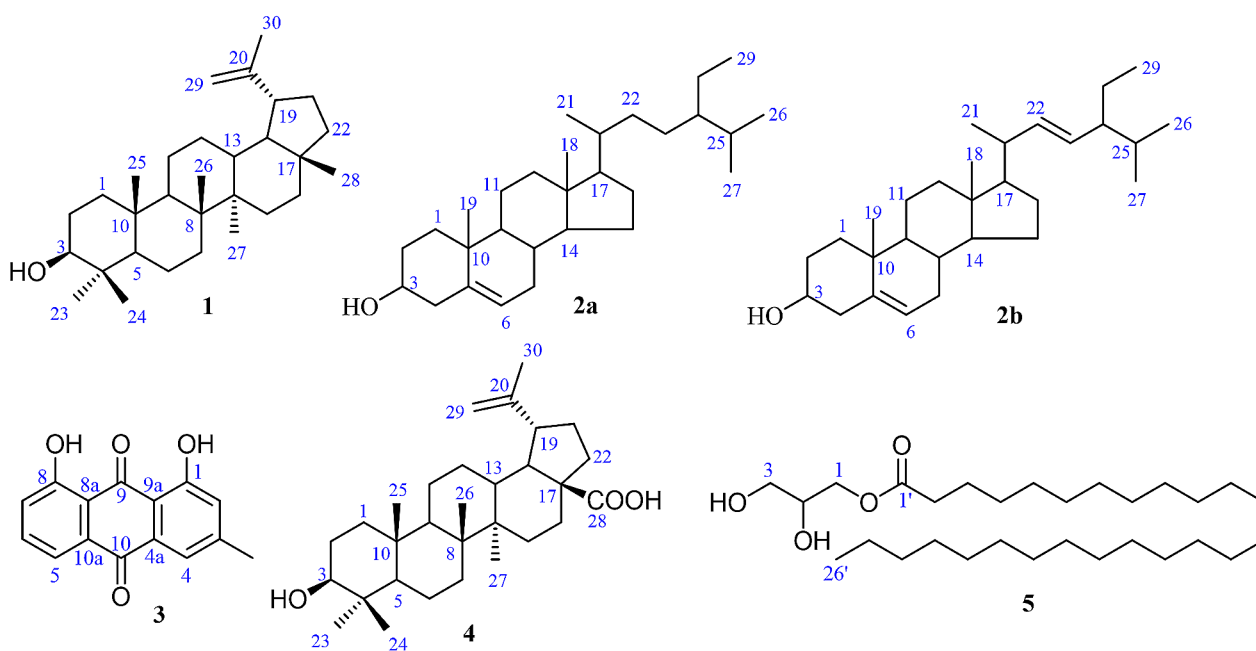


Fig. 2 Chemical structures of the isolated compounds from the roots of *S. siamea*

spectrum displayed four sp^2 methine signals (two overlapped) at δ 5.35 (2H, *m*, H-6a, 6b), 5.15 (1H, *dd*, J =15.1, 8.7, H-22b), and 5.02 (1H, *dd*, J =15.2, 8.7, H-23b), sp^3 oxymethine signals at δ 3.53 (2H, *m*, H-3a, 3b), and ten groups of methyl protons at δ 1.02 (3H, *d*, J =6.7, H-21b), 1.01 (6 H, *s*, H-19), 0.92 (3H, *d*, J =6.5, H-21a), 0.84 (3H, *t*, H-29a), 0.83 (6 H, *d*, J =6.1, H-27), 0.82 (3H, *d*, J =6.3, H-26b), 0.81 (3H, *d*, J =6.2, H-26a), 0.79 (3H, *t*, J =6.0, H-29b), 0.69 (3H, *s*, H-18b), and 0.68 (3H, *s*, H-18a) which were characteristics of a mixture of phytosterols (Additional file 1: Table S2; Fig. S5). The ^{13}C NMR (600 MHz, $CDCl_3$) spectrum associated with the DEPT-135 spectrum revealed resonances of fifty-eight carbon atoms including the signals overlapped in the same chemical environment with the under-cited functionalities. Six olefinic carbons at δ 140.7 (2x, C-5a, 5b), 138.3 (C-22b), 129.3 (C-23b), and 121.7 (2x, C-6a, 6b), two oxymethine carbons at δ 71.8 (2x, C-3a, 3b), fourteen methine carbons at δ 56.9 (C-14b), 56.8 (C-14a), 56.0 (C-17b), 55.9 (C-17a), 51.2 (C-24b), 50.2 (C-9b), 50.1 (C-9a), 45.8 (C-24a), 40.5 (C-20b), 36.1 (C-20a), 31.9 (3x, C-8a, 8b, 25b), and 29.1 (C-25a), four sp^3 quaternary carbons (from the DEPT-135 spectrum) at δ 42.3 (C-13b), 42.2 (C-13a), and 36.5 (2x, C-10a, 10b), twenty methylene carbons at δ 42.3 (2x, C-4a, 4b), 39.8 (C-12b), 39.7 (C-12a), 37.2 (2x, C-1a, 1b), 33.9 (C-22a), 31.9 (2x, C-7a, 7b), 31.6 (2x, C-2a, 2b), 29.4 (C-16b), 28.2 (C-16a), 26.0 (C-23a), 25.4 (C-28b), 24.7 (C-15a), 24.3 (C-15b), 23.0 (C-28a), and 21.1 (2x, C-11a, 11b), and twelve methyl carbons at δ 21.2 (C-21b), 21.1 (C-26b), 19.8 (C-19b), 19.4 (2x, C-19a, 26a), 19.0 (C-21a), 18.9 (C-27b), 18.8 (C-27a), 12.2 (C-29b), 12.0 (2x, C-18b, 29a), and 11.8 (C-18a) (Additional file 1: Table S2; Fig. S6 and S7). Many of the chemical shifts in the ^1H and ^{13}C NMR spectra of compounds **2a** and **2b** were overlapped, designating resemblances in chemical structures. The only difference noted was the presence of a double bond at C-22 and C-23 in compound **2b** which were converted to methylene carbons at δ 33.9 (C-22a) and 26.0 (C-23a) in the case of **2a** and the methine carbon at δ 51.2 (C-24b) in **2b** were shielded to δ 45.8 (C-24a) in **2a**.

The ^1H - ^1H and ^1H - ^{13}C NMR assignments of the compounds were verified by 2D NMR experiments (COSY and HSQC, respectively). Thereby, the COSY spectrum exhibited ^3J correlations between H-6 (δ 5.35) and H-7 (δ 1.98), H-3 (δ 3.53) and H-4 (δ 2.28), H-3 (δ 3.53) and H-2 (δ 1.51), H-7 (δ 1.98) and H-8 (δ 1.51), and many more overlapped correlations in the up filled region (Additional file 1: Table S2; Fig. S8). Furthermore, the HSQC spectrum exhibited connectivity between H-6 (δ 5.35) and C-6 (δ 121.7), H-22b (δ 5.15) and C-22b (δ_C 138.3), H-23b (δ 5.02) and C-23b (δ 129.3), H-3 (δ 3.53) and C-3 (δ 71.8), H-4 (δ 2.28, 2.23) and C-4 (δ 42.3), H-12 (δ 2.01, 1.16) and C-12 (δ 39.7), H-2 (δ 1.82, 1.51) and C-2 (δ

31.6), H-1 (δ 1.83, 1.08) and C-1 (δ 37.2), H-18a (δ 0.68) and C-18a (δ 11.8), H-18b (δ 0.69) and C-18b (δ 12.0), H-21a (δ 0.92) and C-21a (δ 19.0), H-21b (δ 1.02) and C-21b (δ 21.2), H-26a (δ 0.81) and C-26a (δ 19.4), H-26b (δ 0.82) and C-26b (δ 21.1), H-27a (δ 0.83) and C-27a (δ 18.8), H-27b (δ 0.83) and C-27b (δ 18.9), H-29a (δ 0.84) and C-29a (δ 12.0), and H-29b (δ 0.79) and C-29b (δ 12.2) (Additional file 1: Table S2; Fig. S9). Overall, the spectral analyses were consistent with the reported values [57] of β -sitosterol (**2a**) and stigmasterol (**2b**) (Fig. 2).

Compound **3** (10.3 mg, R_f : 0.55 in 30% EtOAc in *n*-hexane, mp: 193–194 °C) was isolated as orange crystals. The melting point range of the compound matches the reported values of chrysophanol (194–195 °C) in the literature [58]. Its ^1H NMR (400 MHz, $CDCl_3$) spectrum displayed two deshielded protons at δ 12.12 (1H, *s*) and 12.01 (1H, *s*) with peri-effect properties assignable to the hydroxyl protons at C-1 and C-8, respectively. Five aromatic signals were also observed at δ 7.81 (1H, *dd*, J =7.5, 1.2, H-5), 7.67 (1H, *dd*, J =8.2, 1.2, H-6), 7.64 (1H, *s*, H-4), 7.28 (1H, *dd*, J =8.4, 1.2, H-7) and 7.09 (1H, *s*, H-2) attributed to ABX and AB type spin patterns in rings A and C of an anthraquinone skeleton, respectively. The singlet signal observed at δ 2.46 (3H, *s*) revealed a characteristic of methyl substituent attached to C-3 of ring C (Additional file 1: Table S3; Fig. S10). The ^{13}C NMR (400 MHz, $CDCl_3$) spectral data exhibited fifteen well-resolved carbon signals including two carbonyl carbons at δ 192.6 and 182.1 assignable to C-9 and C-10, respectively. The chemical shift difference between the two carbonyl carbons is more than 10 ppm, suggesting that C-9 is deshielded due to the peri-effect of hydroxyl groups at C-1 and C-8 [59]. In addition, the spectrum revealed two oxygenated aromatic carbons at δ 162.8 (C-1) and 162.5 (C-8), five quaternary aromatic carbons (from DEPT-135 spectrum) at δ 149.4 (C-3), 133.7 (C-4a), 133.3 (C-10a), 115.9 (C-8a), and 113.8 (C-9a), five sp^2 aromatic methine signals at δ 137.0 (C-6), 124.7 (C-7), 124.5 (C-2), 121.5 (C-4) and 120.0 (C-5), and a sp^3 methyl signal at δ 22.4 (CH_3) (Additional file 1: Table S3; Fig. S11). Furthermore, the DEPT-135 spectrum revealed five aromatic methines and a single sp^3 methyl signal consistent with the ^{13}C NMR spectral analyses of the compound (Additional file 1: Table S3; Fig. S12). Notably, the assignments of adjacent protons were validated by COSY correlations. Accordingly, the spectrum revealed important ^3J correlations between H-5 (δ 7.81) and H-6 (δ 7.67), and H-6 (δ 7.67) and H-7 (δ 7.28) (Additional file 1: Table S3; Fig. S13). After all, the spectral data agreed with the literature values of chrysophanol (**3**) [58, 60] (Fig. 2). Previous reports also support the isolation of chrysophanol from the stem barks of *S. siamea* [56, 61].

Compound **4** (13.8 mg, R_f : 0.55 in 30% EtOAc in *n*-hexane, mp: 296–298 °C) was collected as a white crystalline

solid and exhibited a very close melting point range (295–298 °C) to the reported values of betulinic acid [62, 63]. Its ^1H NMR (400 MHz, CDCl_3) spectrum revealed two proton signals corresponding to geminal sp^2 methylene protons at δ 4.73 (1H, s, H-29a) and 4.60 (1H, s, H-29b). The broad singlet and doublet of doublet signals observed at δ 12.05 (1H, brs, acidic OH) and 3.18 (1H, dd, $J=11.1$, 4.9, H-3) suggest the presence of carboxylic acid and carbinol methine protons, respectively. The attachment of the β -hydroxyl group at C-3 was verified by the coupling constant ($J=11.1$) of the biaxial protons (H-3 α , δ 3.18 and H-2 α δ 1.60). The spectrum also showed six methyl signals at δ 1.67 (3H, s, H-30), 0.96 (3H, s, H-23), 0.95 (3H, s, H-27), 0.92 (3H, s, H-26), 0.81 (3H, s, H-25), and 0.74 (3H, s, H-24) suggesting a characteristic pattern of a lupane type skeleton (Additional file 1: Table S4; Fig. S14). The ^{13}C NMR (400 MHz, CDCl_3) spectrum exhibited a resonance of thirty well-resolved carbon signals including a carbonyl signal at δ 176.7 (C-28), vinylic signals at δ 150.4 (C-20) and 109.8 (C-29), and carbinol methine signal at δ 79.1 (C-3) associated with the structure of lupane type triterpenoid. In agreement with the DEPT-135 spectrum, the ^{13}C NMR spectrum also revealed five sp^3 quaternary carbons at δ 56.3 (C-17), 42.5 (C-14), 40.7 (C-8), 38.9 (C-4), and 37.3 (C-10), five sp^3 methine signals at δ 55.4 (C-5), 50.6 (C-9), 49.3 (C-18), 46.9 (C-19), and 38.4 (C-13), ten sp^3 methylene signals at δ 38.8 (C-1), 37.1 (C-22), 34.4 (C-7), 32.2 (C-16), 30.6 (C-21), 29.8 (C-15), 27.4 (C-2), 25.5 (C-12), 20.9 (C-11), and 18.3 (C-6), and six methyl signals at δ 28.0 (C-23), 19.4 (C-30), 16.2 (C-26), 16.1 (C-25), 15.4 (C-24), and 14.7 (C-27) (Additional file 1: Table S4; Figs. S15 and S16). In addition, the orientations of adjacent protons were confirmed by COSY correlations. Accordingly, the spectrum showed ^3J correlations between H-2 (δ 1.60) and H-3 (δ 3.18), H-5 (δ 0.67) and H-6 (δ 1.50, 1.36), and ^2J correlations between H-29a (δ 4.73) and H-29b (δ 4.60) (Additional file 1: Table S4; Fig. S17). Overall, the spectral data agreed with the reported values of betulinic acid (4) [62] (Fig. 2).

Compound 5 (9.5 mg, R_f : 0.50 in 30% EtOAc in *n*-hexane, mp: 92–94°C) was isolated as a white solid. The melting point range was very close to the reported values of glyceryl-1-hexacosanoate (91–93°C) in the literature [64]. Its ^1H NMR (400 MHz, CDCl_3) spectrum revealed signals of geminal oxymethylenes at δ 4.20 (1H, dd, $J=11.7$, 4.6, H-1a), 4.14 (1H, dd, $J=11.6$, 6.1, H-1b), 3.69 (1H, dd, $J=11.4$, 4.0, H-3a), and 3.59 (1H, dd, $J=11.4$, 5.8, H-3b), and an oxymethine proton at δ 3.92 (1H, m, H-2) which are characteristic of a glycerol protons. In addition, four methylene protons were observed at δ 2.34 (2H, t, $J=14.6$, H-2') and 1.62 (2H, m, H-3'). Three methyl protons were displayed at δ 0.86 (3H, t, $J=12.8$, H-26') attributed to fatty acid/ester protons nearest to

the carboxylic/ate group and terminal methyl protons, respectively. The remaining protons overlapped in the region δ 1.31–1.23 (44H, brs) (Additional file 1: Table S5; Fig. S18). The ^{13}C NMR (400 MHz, CDCl_3) spectral data exhibited a carbonyl (ester) signal at δ 174.5 (C-1'), three sp^3 carbinol signals at δ 70.3 (C-2), 65.2 (C-1), and 63.4 (C-3), four methylene signals at δ 34.2 (C-2'), 32.0 (C-24'), 25.0 (C-3'), and 22.8 (C-25'), and a terminal methyl signal at δ 14.2 (C-26'). Twenty sp^3 methylene signals observed in the range δ 29.8–29.2 (C-4'–C-23') suggest some long-chain fatty acid-containing glycerol (Additional file 1: Table S5; Fig. S19). Moreover, the proton-proton coupling assignments of the compound were supported by COSY correlations, thereby the ^2J correlations between H-1a (δ 4.20) and H-1b (δ 4.14), H-3a (δ 3.69) and H-3b (δ 3.59), and ^3J correlations between H-2' (δ 2.34) and H-3' (δ 1.62) revealed confirmatory information of the structure (Additional file 1: Table S5; Fig. S20). Finally, the spectral analyses agreed with the reported values of glyceryl-1-hexacosanoate (5) [64, 65] (Fig. 2).

Antibacterial activity

In the present work, the antibacterial efficacy of the CH_2Cl_2 : CH_3OH (1:1) extract and compound isolates were tested in vitro against four bacterial strains. Results showed that the extract exhibited potential activity against *E. coli*, *S. aureus*, and *S. pyogenes* and the highest activity was recorded against *E. coli* (18.00 ± 0.00 mm) and *S. aureus* (17.17 ± 0.24 mm) at 50 mg/mL compared to ciprofloxacin (23.33 ± 0.47 mm and 22.00 ± 0.00 mm against *E. coli* and *S. aureus*, respectively) (Table 1). At the smallest concentration (6.25 mg/mL), the extract also displayed a better inhibition zone against *E. coli* (12.00 ± 0.41 mm) followed by *S. aureus* (10.33 ± 0.47 mm) and *S. pyogenes* (10.00 ± 0.00 mm). Of the compound isolates, chrysophanol (3) exhibited the highest activity against *E. coli* (16.33 ± 0.24 mm) and *S. pyogenes* (16.00 ± 0.00 mm) at 2 mg/mL compared to ciprofloxacin (23.33 ± 0.47 and 21.67 ± 0.47 mm, respectively). At 0.25 mg/mL, chrysophanol (3) displayed the highest inhibition diameter against *E. coli* (11.17 ± 0.24 mm) followed by lupeol (1) against *S. pyogenes* (9.83 ± 0.24 mm), chrysophanol (3) against *S. aureus* and *S. pyogenes* (9.83 ± 0.24 mm), and betulinic acid (4) against *E. coli* and *S. pyogenes* (9.83 ± 0.24 mm) (Table 1). Our findings corroborate previous research reports wherein the leaves ethanol extract of the plant exhibited comparable activities against *E. coli* which were 14.00 ± 0.00 , 10.00 ± 0.00 , and 9.00 ± 0.00 mm at 50, 30, and 10 mg/mL, respectively. Whereas, against *S. aureus* it displayed 15.00 ± 0.00 , 13.00 ± 0.00 , and 11.00 ± 0.00 mm at 40, 30, and 10 mg/mL, respectively [66]. The antibacterial effects of acetone, ethanol, and aqueous extracts of *S. siamea* have been reported previously. According to Vasait, (2016),

Table 1 Inhibition zones (mean ± SD) of CH₂Cl₂: CH₃OH (1:1) extract and the compound isolates (1–5) from the roots of *S. siamea*

Bacterial strain	Conc. (mg/mL)	Inhibition zone (mm) of the CH ₂ Cl ₂ : CH ₃ OH (1:1) extract and compounds (3 trials)						Ciprofloxacin (30 µg/disc)
		CH ₂ Cl ₂ : CH ₃ OH (1:1) extract	1	2	3	4	5	
<i>E. coli</i>	2	18.00±0.00	14.17±0.24	13.50±0.41	16.33±0.24	14.33±0.47	10.00±0.00	23.33±0.47
	1	15.67±0.47	12.17±0.24	12.00±0.00	14.33±0.24	12.33±0.47	8.67±0.47	
	0.5	13.50±0.41	10.67±0.47	10.67±0.47	12.50±0.41	10.83±0.24	7.00±0.00	
	0.25	12.00±0.41	9.50±0.41	9.33±0.47	11.17±0.24	9.83±0.24	NA	
<i>S. aureus</i>	2	17.17±0.24	14.00±0.00	12.50±0.41	14.67±0.47	14.33±0.47	10.67±0.47	22.00±0.00
	1	14.83±0.24	11.83±0.24	11.33±0.47	13.17±0.24	12.00±0.00	9.00±0.00	
	0.5	12.50±0.41	10.50±0.41	10.00±0.00	11.33±0.47	11.00±0.41	7.25±0.20	
	0.25	10.33±0.47	9.33±0.47	9.00±0.00	9.83±0.24	9.67±0.47	NA	
<i>P. aeruginosa</i>	2	11.67±0.47	10.00±0.00	10.50±0.41	10.33±0.47	10.17±0.24	8.33±0.47	20.00±0.41
	1	9.67±0.47	8.33±0.47	8.67±0.47	8.33±0.47	8.33±0.47	7.00±0.00	
	0.5	8.50±0.41	7.00±0.00	7.67±0.47	7.00±0.00	7.00±0.00	NA	
	0.25	7.17±0.24	NA	7.00±0.00	7.00±0.00	NA	NA	
<i>S. pyogenes</i>	2	16.50±0.41	14.33±0.47	13.83±0.24	16.00±0.00	14.67±0.47	9.67±0.47	21.67±0.47
	1	13.67±0.47	12.33±0.47	12.00±0.00	13.00±0.00	12.67±0.47	8.50±0.41	
	0.5	11.83±0.24	11.00±0.00	10.50±0.41	11.33±0.47	11.17±0.24	7.00±0.00	
	0.25	10.00±0.00	9.83±0.24	9.33±0.47	9.83±0.24	9.83±0.24	NA	

Conc.; concentration, NA; No Activity. For CH₂Cl₂: CH₃OH (1: 1) extract, 2; 50 mg/mL, 1; 25 mg/mL, 0.5; 12.5 mg/mL, and 0.25; 6.25 mg/mL

Table 2 DPPH radical scavenging activity (%) of the CH₂Cl₂: CH₃OH (1:1) extract and isolated compounds (1–5) of *S. siamea*

Conc. (µg/mL)	% of scavenging activity (mean ± SD)						
	CH ₂ Cl ₂ : CH ₃ OH (1:1) extract	1	2	3	4	5	Ascorbic acid
62.5	73.50±0.00	65.67±0.41	67.23±0.21	70.07±0.33	69.02±0.23	66.26±0.42	77.38±0.35
125	76.79±0.35	68.50±0.35	70.37±0.00	74.40±0.25	71.64±0.27	69.70±0.35	80.44±0.41
250	81.19±0.15	72.46±0.12	74.77±0.18	78.50±0.35	76.19±0.18	73.28±0.15	84.62±0.27
500	86.34±0.21	77.08±0.18	78.80±0.30	82.61±0.18	79.92±0.31	77.68±0.30	88.95±0.21
1000	89.25±0.00	80.44±0.21	81.19±0.00	85.59±0.15	83.73±0.25	80.82±0.27	92.76±0.28
IC ₅₀ (µg/mL)	1.24	3.93	2.35	1.71	2.06	3.06	0.53

the acetone extract of *S. siamea* exhibited remarkable bactericidal activity against *E. coli* (22.00±00 mm) and *S. aureus* (20.00±00 mm) at 0.1 mg/mL with more efficient activity than the present findings. The ethanol and aqueous extracts were also susceptible to *E. coli* (20.00±00 and 18.00±00 mm, respectively), and *S. aureus* (17.50.00±00 mm and 13.00±00 mm, respectively), at 0.1 mg/mL [67]. Melaku et al. [68], reported the antibacterial activity of chrysophanol (3) which is positively correlated with the present work. According to the report, the compound exhibited an inhibition diameter of 13.00±0.20 mm at 1 mg/mL against *S. aureus* which was comparable to the inhibition zone of this study (13.17±0.24 mm). Thus, the findings of this study support the traditional practices of *S. siamea* against infectious diseases caused by the four bacterial pathogens.

Antioxidant activity

The antioxidant potentials of the CH₂Cl₂: CH₃OH (1:1) roots extract and compounds were determined via DPPH radical scavenging assay. The extract showed a scavenging activity of 89.25±0.00 and 73.50±0.00% at 1000 and

62.5 µg/mL, respectively, with IC₅₀ value of 1.24 µg/mL compared to ascorbic acid (92.76±0.28 and 77.38±0.35% at the 1000 and 62.5 µg/mL, respectively, with IC₅₀ value of 0.53 µg/mL. Of the compounds, chrysophanol (3) exhibited the highest scavenging potential at 1000 µg/mL (85.59±0.15%) and 62.5 µg/mL (70.07±0.33%) with an IC₅₀ value of 1.71 µg/mL. Whereas, the smallest scavenging activity was displayed by lupeol (1) with an IC₅₀ value of 3.93 µg/mL (Table 2; Fig. 3). Antioxidants have been investigated to show cell protection from free radicals causing oxidative cleavage which avoids aging and cancer. They serve as oxygen scavengers by interacting with free radicals, thereby disrupting the oxidation process [69]. Previous studies revealed that synthetic antioxidants loom to cause cancer diseases. Consequently, antioxidants of natural origin have become the target of modern research [70]. This study suggests that the extract and compound isolates of *S. siamea* exhibit promising antioxidant activities which align with previous reports. In a study by Oyebade et al. (2021) [71], the antioxidant properties of the leaf extracts of *S. siamea* were tested in vitro and the hexane, ethyl acetate, ethanol, and aqueous

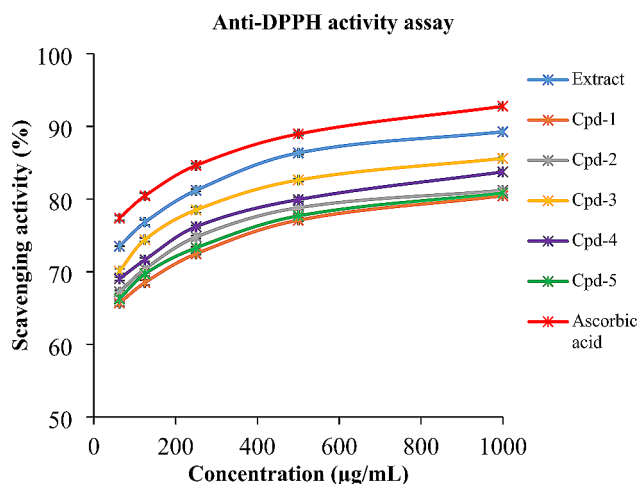


Fig. 3 DPPH radical scavenging activity (%) of the CH₂Cl₂: CH₃OH (1:1) extract, compounds (1–5), and ascorbic acid against different concentrations (graphical portrayal)

extracts showed potential DPPH scavenging results with IC₅₀ values of 35.41, 32.77, 25.73, and 12.89 µg/mL, respectively, and the activities were promising compared to ascorbic acid (IC₅₀: 11.12 µg/mL). The antioxidant activity of chrysophanol (3) was also the subject of previous researches. Accordingly, Melaku et al. (2022) [68] reported the DPPH radical scavenging activity of the compound with an IC₅₀ value of 6.2 µg/mL compared to ascorbic acid (IC₅₀: 3.38) accounting comparable scavenging values with the present finding.

The IC₅₀ values were calculated from the relationship curves on a Microsoft Excel 2016 spreadsheet. The trend line options were displayed and relationships that revealed the highest coefficient of determination (R²) values were selected for the calculations. Accordingly, the logarithmic relationship curves of each sample showed the highest R² values and the following equations were used for the determination of the IC₅₀ values. CH₂Cl₂: CH₃OH (1:1) extract; $Y = 5.92 \ln(x) + 48.71$, R²=0.99, lupeol (1); $Y = 5.49 \ln(x) + 42.46$, R²=0.99, β-sitosterol (2a) and stigmasterol (2b); $Y = 5.24 \ln(x) + 45.51$, R²=0.99, chrysophanol (3); $Y = 5.66 \ln(x) + 46.96$, R²=0.99, betulinic acid (4); $Y = 5.43 \ln(x) + 46.07$, R²=0.99, glyceryl-1-hexacosanoate (5); $Y = 5.35 \ln(x) + 43.99$, R²=0.99, and ascorbic acid; $Y = 5.66 \ln(x) + 53.54$, R²=0.99 and the Y values were labeled as 50 (Fig. 3).

Drug likeness and ADMET properties

Many drug candidates fail to afford the clinical trials due to their poor ADMET properties. Thereby, the study of toxicity and pharmacokinetic properties are crucial parameters in drug discovery [72]. In the present study, the drug-likeness predictions of the compound isolates were anticipated by adopting Lipinski's rule of five [49] and Veber's rule [73]. Compounds that afford MW < 500

Daltons, LogP (iLogP) < 5, NHD < 5, and NHA < 10 favor Lipinski's rule of 5, and their drug candidacy is likely. In addition, compounds with TPSA < 140 and NRB < 10 favor Veber's rule and possess good oral bioavailability. Compounds with zero or one Lipinski's violation afford drug candidacy. Thus, chrysophanol (3) fulfilled Lipinski's rule with zero violation, while lupeol (1), β-sitosterol (2a) and stigmasterol (2b), betulinic acid (4), and glyceryl-1-hexacosanoate (5) displayed one violation each and were in favor of the drug-likeness predictions. Except for glyceryl-1-hexacosanoate (5), all the compounds satisfy Veber's rule with TPSA < 140 and NRB < 10 and therefore exhibit safe absorption in the gut. The affinity of compounds to lipophilic properties is related to their LogP values [74]. Hence, all the compounds except stigmasterol (2b) and glyceryl-1-hexacosanoate (5) showed < 5 values suggesting their optimum lipophilicity. Chrysophanol (3) exhibited better LogKp (cm/s) values than the others compared to ciprofloxacin indicating lesser skin permeability [48]. Lupeol (1), β-sitosterol (2a), and glyceryl-1-hexacosanoate (5) were computed as non-inhibitors of all the cytochromes and agreed with the predictions of ciprofloxacin (Table 3).

In this work, the toxicity predictions of all the compound isolates were computed. The results revealed that all the compounds exhibited lethal dose values between $800 < LD_{50} \leq 5000$ mg/kg with toxicity classes 4 and 5 suggesting their slight toxicity and no compound favored acute toxicity [75]. The prediction tool also showed toxicological (hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity) endpoints. Accordingly, all the computed compounds revealed no hepatotoxicity results, thereby no problem with liver functions. All the compounds exhibited no cytotoxicity and except betulinic acid (4), all the compounds also revealed no carcinogenicity properties which were consistent with the prediction results of ciprofloxacin. Only chrysophanol (3) displayed mutagenicity properties and except glyceryl-1-hexacosanoate (5), all the compounds exhibited immunotoxicity properties (Table 4). Thus, the majority of the ADMET properties revealed that the investigated compounds possess drug-like properties and we suggest further experimental and computational studies.

The drug-likeness properties of lupeol (1), β-sitosterol (2a), chrysophanol (3), and betulinic acid (4) were supported by bioavailability radar predictions. The insolubility (INSOLU), lipophilicity (LIPO), polar surface area (POLAR), rotatable bonds (FLEX), molecular weight (SIZE), and optimal area for unsaturation (INSATU) properties were displayed in the bioavailability radar (Fig. 4). Drug-like molecules are suggested to exhibit SIZE (MW) < 500 g/mol, -6 < INSOLU (Log S) < 0, 0 < FLEX (NRB) < 9, -0.7 < LIPO (Log P) < 5, 20 Å² <

Table 3 *In silico* drug-likeness and ADME properties of the isolated compounds (**1–5**) computed by the SwissADME online web tool

Predicted parameters	Compounds						
	1	2a	2b	3	4	5	Ciprofloxacin
Drug likeness							
MF	C ₃₀ H ₅₀ O	C ₂₉ H ₅₀ O	C ₂₉ H ₄₈ O	C ₁₅ H ₁₀ O ₄	C ₃₀ H ₄₈ O ₃	C ₂₉ H ₅₈ O ₄	C ₁₇ H ₁₈ FN ₃ O ₃
MW (g/mol)	426.71	414.71	412.69	254.24	456.70	470.77	331.34
NRB	1	6	5	0	2	28	3
NHD	1	1	1	2	2	2	2
NHA	1	1	1	4	3	4	5
LogP (iLogP)	4.72	4.79	5.01	2.22	3.83	6.64	2.24
TPSA (Å ²)	20.23	20.23	20.23	74.60	57.53	66.76	74.57
Lipinski's Ro5	1	1	1	0	1	1	0
Veber's rule	Yes	Yes	Yes	Yes	Yes	No (1)	Yes
ADME predictions							
LogKp (cm/s)	-1.90	-2.20	-2.74	-5.34	-3.26	-0.83	-9.09
GIA	Low	Low	Low	High	Low	Low	High
BBB	No	No	No	Yes	No	No	No
Inhibitor interaction							
P-gp substrate	No	No	No	No	No	No	Yes
CYP1A2	No	No	No	Yes	No	No	No
CYP2C19	No	No	No	No	No	No	No
CYP2C9	No	No	Yes	No	Yes	No	No
CYP2D6	No	No	No	No	No	No	No
CYP3A4	No	No	No	Yes	No	No	No

Abbreviations BBB; blood-brain barrier, CYP; cytochrome-P, GIA; gastrointestinal absorption, LogKp; skin permeation value, MF; molecular formula, MW; molecular weight, NHA; number of hydrogen acceptors, NHD; number of hydrogen donors, NRB; number of rotatable bonds, P-gp; p glycoprotein, Ro5; rule of five, TPSA; total polar surface area

Table 4 Toxicity prediction of the compound isolates (**1–5**) computed by ProTox-II

Compound No.	LD ₅₀ (mg/kg)	Toxicity class	Toxicity				
			Immuno	Hepato	Cyto	Carcino	Mutagen
1	2000	4	Yes	No	No	No	No
2a	890	4	Yes	No	No	No	No
2b	890	4	Yes	No	No	No	No
3	5000	5	Yes	No	No	No	Yes
4	2610	5	Yes	No	No	Yes	No
5	5000	5	No	No	No	No	No
Ciprofloxacin	2000	4	No	No	No	No	Yes

Abbreviations Carcino; carcinogenicity, Cyto; cytotoxicity, Hepato; hepatotoxicity, Immuno; immunotoxicity, LD; lethal dose, Mutagen; mutagenicity

POLAR (TPSA) < 140 Å², and 0.25 < INSATU (fraction Csp3) < 1 prediction [76]. Except for the Log S (INSOLU) value of lupeol (**1**) (-6.74) and β -sitosterol (**2a**) (-6.19), and INSATU value of chrysophanol (**3**) (0.07), the radar plots showed drug-like properties for all the compounds. In addition, the pharmacokinetic properties of the compounds were established using the BOILED-Egg model to explain the human intestinal absorption (HIA) and blood-brain barrier (BBB) characteristics. Compounds with high BBB penetration and HIA are located in the yolk (yellow) and albumin (white) regions, respectively [76]. Accordingly, chrysophanol (**3**) was located inside the yolk region explaining its high penetration in the brain and good absorption in the gastrointestinal. Whereas, betulinic acid (**4**) was located inside the

albumin (white) region showcasing its passive absorption by the gastrointestinal tract. In addition, lupeol (**1**) and β -sitosterol (**2a**) located outside the BOILED-Egg explain their poor penetration and absorption. Chrysophanol (**3**) and betulinic acid (**4**) were also designated along with a red circle describing their non-substrate (PGP-) properties (Fig. 4). Thus, the bioavailability radar and BOILED-Egg model predictions of chrysophanol (**3**) and betulinic acid (**4**) suggest their drug-like potentials.

Molecular docking studies

In this study, the bacterial protein targets were selected based on their metabolic importance to the microorganisms, the in vitro antibacterial and antioxidant profiles of the pathogens hosting them, and the likeness of the

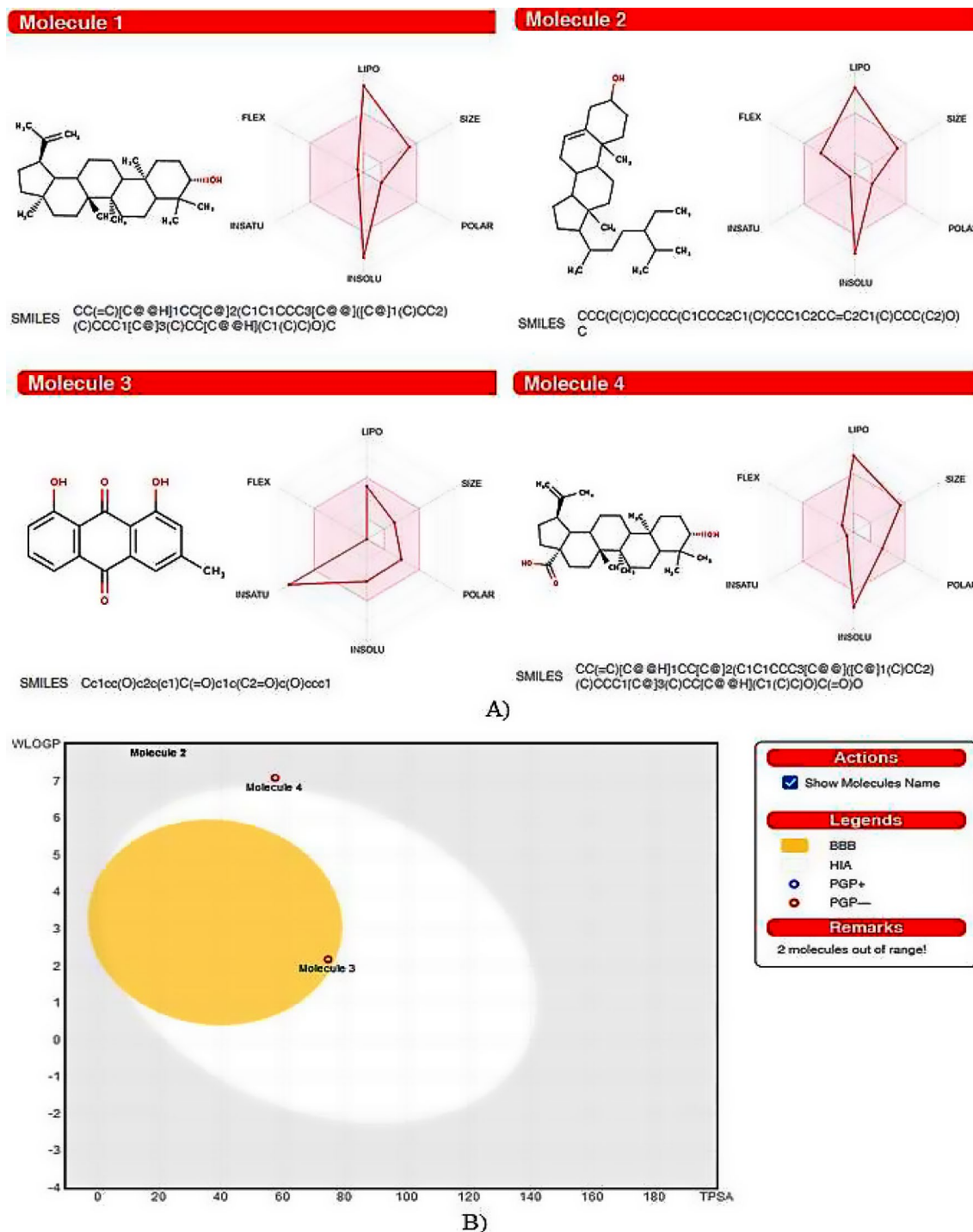


Fig. 4 Bioavailability radar (A) and BOILED-Egg model (B) of lupeol (1), β -sitosterol (2a), chrysophanol (3), and betulinic acid (4). Molecule 1; lupeol (1), molecule 2; β -sitosterol (2a), molecule 3; chrysophanol (3), and molecule 4; betulinic acid (4)

compounds to the co-crystallized ligands in the protein complex before preparation. DNA gyrase plays a vital role in the survival of bacterial cells and is mostly used as a drug target for *in silico* computational studies [77]. Reports on the DNA gyrase revealed that its enzymatic activities are critical in transcription, initiation, and

elongation during DNA replication, DNA superhelicity regulation, and chromosome decatenation [78]. Pyruvate kinase (PK), the enzyme at the last stage of glycolysis catalyzes the formation of adenosine triphosphate (ATP) and pyruvate by transferring a phosphoryl group from phosphoenolpyruvate (PEP) to adenosine diphosphate

(ADP) [79]. PK plays a crucial role in metabolic flux distribution and energy generation. Inhibition of PK disrupts the metabolic stability and energy production of bacteria [80]. Streptococcal streptopain is also an important virulence factor leading to different streptococcal diseases including glomerulonephritis, fever, shock-like syndrome, and pharyngitis [81]. Thus, considering the aforementioned factors, we showed a desire to select the bacterial protein targets for this study.

The *in silico* molecular docking analyses of compounds exhibiting promising *in vitro* activities were performed and results showed that lupeol (1), β -sitosterol (2a), chrysophanol (3), and betulinic acid (4) displayed promising binding affinities against all the protein targets. The binding scores, hydrogen bonds, and residual amino acid interactions of the compound isolates along with reference drugs are depicted in Tables 5 and 6, and their 2D and 3D representations are presented in Figs. 5, 6, 7, 8 and 9. Chrysophanol (3) exhibited the highest binding affinity against all bacterial protein targets which were in line with the *in vitro* activities of the compound. During molecular docking studies, hydrogen bonds are among the most fundamental evidence that have to be considered while identifying active sites and they are essential bonds in determining the stability of the protein structure. This is because proteins are comprised of OH and NH groups which can donate electrons and hydrogen bonds to other groups. Therefore, hydrogen bonds help to specify protein-ligand interactions, thereby stabilizing the ligand in the binding site [82]. Accordingly, chrysophanol (3) showed ≥ 2 H-bonds with all protein targets and thus exhibited better stability than the other compound isolates.

DNA gyrase B belongs to the bacterial type II A topoisomerase enzymes and regulates the topology of DNA during replication, recombination, and transcription by importing transient breaks to DNA strands and providing the necessary energy for catalytic functions [77]. Lupeol (1), chrysophanol (3), and betulinic acid (4) showed minimum binding affinities of -6.6, -7.2, and -7.0 kcal/mol, respectively, suggesting their inhibitory effects against *E. coli* DNA gyrase B. The binding scores of chrysophanol (3) and betulinic acid (4) were also comparable to the ciprofloxacin standard (-7.6 kcal/mol) and the scores were consistent with the *in vitro* activities of the compounds. The molecular docking interactions revealed a set of one H-bond (Glu-50) for lupeol (1) and three H-bonds each for chrysophanol (3) (Asn-46, Gly-77, and Pro-79) and betulinic acid (4) (Asn-46, Val-120, and Glu-50) of which the latter two compounds showed one common H-bond interaction (Asn-46) with ciprofloxacin (Arg-76, Asn-46, Asp-73). In addition, considerable residual amino acid interactions were observed for the compounds (Table 5; Fig. 5).

Pyruvate Kinase (PK) catalyzes the last step of glycolysis including the unalterable transformation of phosphoenolpyruvate (PEP) into pyruvate followed by phosphorylation of ADP into ATP [83]. Lupeol (1), chrysophanol (3), and betulinic acid (4) were investigated for their molecular docking performances against *S. aureus* PK and the results were auspicious with binding affinities of -7.7, -8.1, and -8.0 kcal/mol, respectively. The scores were also very close to the binding affinity of ciprofloxacin (-8.2 kcal/mol), signifying their potential antibacterial effects. Lupeol (1) displayed no H-bond, while chrysophanol (3) and betulinic acid (4) showed two (Ser-362 and Ala-358) and one (Asp-339) H-bonds, respectively, of which the former shared similar H-bond interaction (Ser-362) with ciprofloxacin (Ser-362 and Asn-369) (Table 5; Fig. 6). Streptopain proteins are cysteine proteases articulated by *S. pyogenes* and are key virulence factors in streptococcal exposures triggering different human diseases, including glomerulonephritis, fever, pharyngitis, and shock-like syndromes. In this work, lupeol (1), β -sitosterol (2a), chrysophanol (3), and betulinic acid (4) were performed for their molecular docking potential against 10,782 streptopain and revealed possible antibacterial interactions against *S. pyogenes* with binding affinities of -7.0, -6.8, -7.3, and -7.1 kcal/mol, respectively. The close binding scores of the compounds to the ciprofloxacin standard (-7.5 kcal/mol) also support their potential activities against *S. pyogenes*. Results also showed that lupeol (1), β -sitosterol (2a), chrysophanol (3), and betulinic acid (4) exhibited H-bond interactions with Gly-163 and Asn-161, Gly-339, Asn-161, and Trp-359, and Asn-167 and Gly-163, respectively (Table 5; Fig. 7).

In the present study, the antioxidant potency of β -sitosterol (2a), chrysophanol (3), and betulinic acid (4) exhibiting promising IC_{50} values were supported with *in silico* molecular docking computations and the results were consistent with the *in vitro* experimental data. The compounds revealed minimum binding affinities of -7.2, -7.5, and -7.4 kcal/mol for β -sitosterol (2a), chrysophanol (3), and betulinic acid (4), respectively, and the values were comparable with the binding score of ascorbic acid (-7.7 kcal/mol). Chrysophanol (3) and betulinic acid (4) formed three H-bonds each with (Ile-160, Asn-162, and Arg-323) and (Arg-31, Asn-162, and Phe-29), respectively, of which Ile-160, Arg-323, and Arg-31 were also displayed by ascorbic acid (Arg-31, Arg-161, Ala-35, Ile-160 and Arg-323) (Table 6; Fig. 8). The molecular docking study was extended to *in silico* cytotoxicity investigations. Accordingly, lupeol (1), β -sitosterol (2a), chrysophanol (3), and betulinic acid (4) were analyzed against topoisomerase II α . The results showed that lupeol (1) exhibited the most stable binding affinity (-9.2 kcal/mol) followed by β -sitosterol (2a) (-9.0 kcal/mol) with

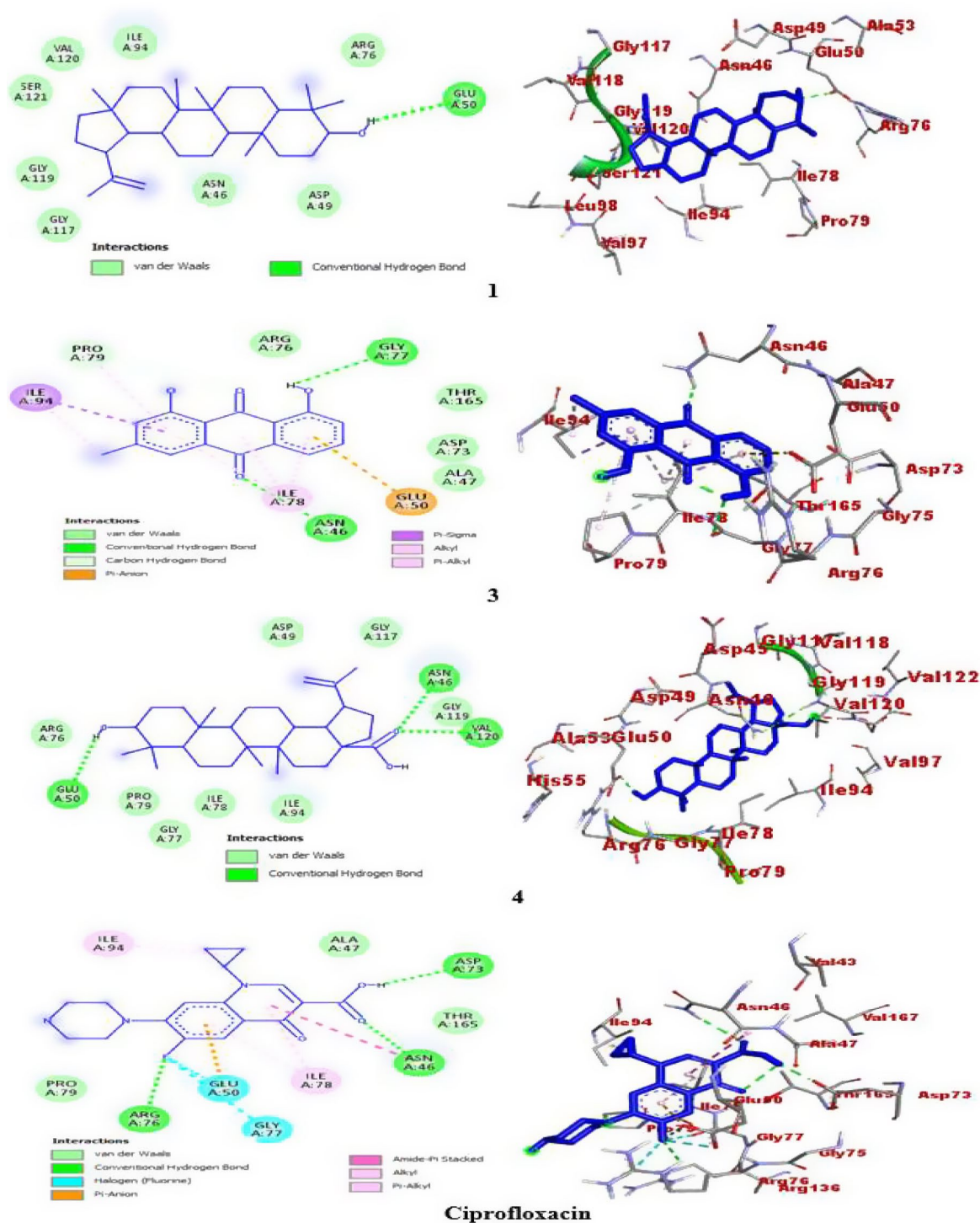


Fig. 5 2D (left) and 3D (right) binding interactions of compounds **1**, **3**, **4**, and ciprofloxacin against *E. coli* DNA gyrase B

comparable binding scores with etoposide standard (-9.4 kcal/mol). Furthermore, all the investigated compounds formed at least one H-bond with some amino acids of the target protein showcasing their stability (Table 6; Fig. 9). Thus, we suggest additional *in vitro* and

in vivo cytotoxicity studies on the compound isolates to support the *in silico* findings presented in this study.

Nowadays, antibiotic resistance has become a key problem in various virulent pathogens due to the widespread usage of antimicrobial drugs [69]. Many antibiotics now

Table 5 Molecular docking study of compounds **1**, **3**, **4**, and ciprofloxacin against *E. coli* DNA gyrase B, and *S. aureus* PK, and compounds **1**, **2a**, **3**, **4**, and ciprofloxacin against *S. pyogenes* 10782 streptopain

Compound/ligand	Binding affinity (kcal/mol)	H-bond	Residual amino acid interactions	
			Hydrophobic/Electrostatic	Van der Waals
Against <i>E. coli</i> DNA gyrase B				
1	-6.6	Glu-50		Ile-94, Arg-76, Asp-49, Asn-46, Gly-117, Val-120, Gly-119, and Ser-121
3	-7.2	Asn-46, Gly-77, and Pro-79	Pro-79, Glu-50, Ile-78, and Ile-94	Arg-76, Thr-165, Asp-73, and Ala-47
4	-7.0	Asn-46, Val-120, and Glu-50		Asp-49, Gly-117, Gly-119, Ile-94, Ile-78, Gly-77, Pro-79, and Arg-76
Ciprofloxacin	-7.6	Arg-76, Asn-46, and Asp-73	Ile-78, Ile-94, Glu-50, Gly-77, Asn-46, and Arg-76	Ala-47, Pro-79, and Thr-165
Against <i>S. aureus</i> PK				
1	-7.7		Lys-260 and Tyr-302	Arg-264, Lys-342, Asp-303, Asp-339, Gln-338, Tyr-302, and Lys-260
3	-8.1	Ser-362 and Ala-358	His-365 and Ile-361	Thr-353, Asn-369, Ile-361, Thr-366, and His-365
4	-8.0	Asp-339	Lys-342	Gln-338, Ala-337, Asp-339, Tyr-302, Lys-342, Arg-264, Leu-343, Asp-346, Asp-303, and Lys-260
Ciprofloxacin	-8.2	Ser-362 and Asn-369	Ile-361 and His-365	Thr-353, Thr-348, Asn-369, Ile-361, His-365, Ser-362, Met-467, Ala-358, and Thr-366
Against <i>S. pyogenes</i> 10782 streptopain				
1	-7.0	Gly-163 and Asn-161		Gln-162, Ala-189, Trp-359, Ser-279, Cys-192, Gly-339, Gly-338, Val-337, and Leu-377
2a	-6.8	Gly-339	Ala-189, His-340, and Trp-359	Gln-186, Phe-183, Gly-163, Leu-377, Gly-338, Val-337, Phe-386, Ser-279, Gln-162, Pro-278, Ala-188, and His-187
3	-7.3	Asn-161 and Trp-359	Trp-359, Ala-376, Leu-377, and Ala-189	Gln-162, Gly-163, and Val-362
4	-7.1	Asn-167 and Gly-163 (C-H bond)		Gln-162, Asn-161, Asn-164, His-187, Gln-186, Phe-183, Pro-278, Ala-189, Ser-279, Trp-359, and Leu-377
Ciprofloxacin	-7.5	Gln-332, His-340, Ser-279, and Gly-281 (C-H bond)	Ser-280, Cys-192, Gly-339, and Val-334	Val-193, Ser-282, Tyr-389, Ala-341, and Gly-333

in application have negative side effects such as immunosuppression, hypersensitivity, and toxicity creating public health problems. Thus, the search for alternative therapies from plant-based natural products has become the concern of many research activities. Our research findings correlated the in vitro and in silico techniques to identify potential antibacterial and antioxidant compounds from the roots of *S. siamea* and the results showed notable progress compared to previous findings. Though the in vitro antibacterial and antioxidant investigations of the extract and isolated compounds from *S. siamea* aren't novel, we noticed that the previous studies lack further experimental and computational validations. In our case, the in vitro activities of the compound isolates were supported by *in silico* pharmacokinetic and molecular docking computations to verify possible therapeutic properties. Thus, we believe that the contributions of this work to the scientific world are vital and we suggest further investigations to study their efficacy in vivo.

In the present study, six known compounds were reported along with in vitro antibacterial, antioxidant, *in silico* ADMET properties, and molecular docking studies. The in vitro antibacterial evaluations revealed that the roots extract (18.00 ± 0.00 mm) and chrysophanol (**3**) (16.33 ± 0.24 mm) exhibited the highest inhibition diameters against *E. coli*, at 50 mg/mL and 2 mg/mL, respectively. The comparable IC_{50} values of chrysophanol (**3**), betulinic acid (**4**), and β -sitosterol (**2a**) (IC_{50} : 1.71, 2.06, and 2.35 μ g/mL, respectively), to ascorbic acid (IC_{50} : 0.53 μ g/mL) were also evidence for the potential antioxidant potency of the compounds. The *in silico* pharmacokinetics, ADMET, and drug-likeness properties in combination with the molecular docking results of lupeol (**1**), β -sitosterol (**2a**), chrysophanol (**3**), and betulinic acid (**4**) suggest potential uses of these compounds as prospective antibacterial and antioxidants. Thus, the present study supports the traditional relevance of *S. siamea*, and further works are recommended on other biological activities of the plant.

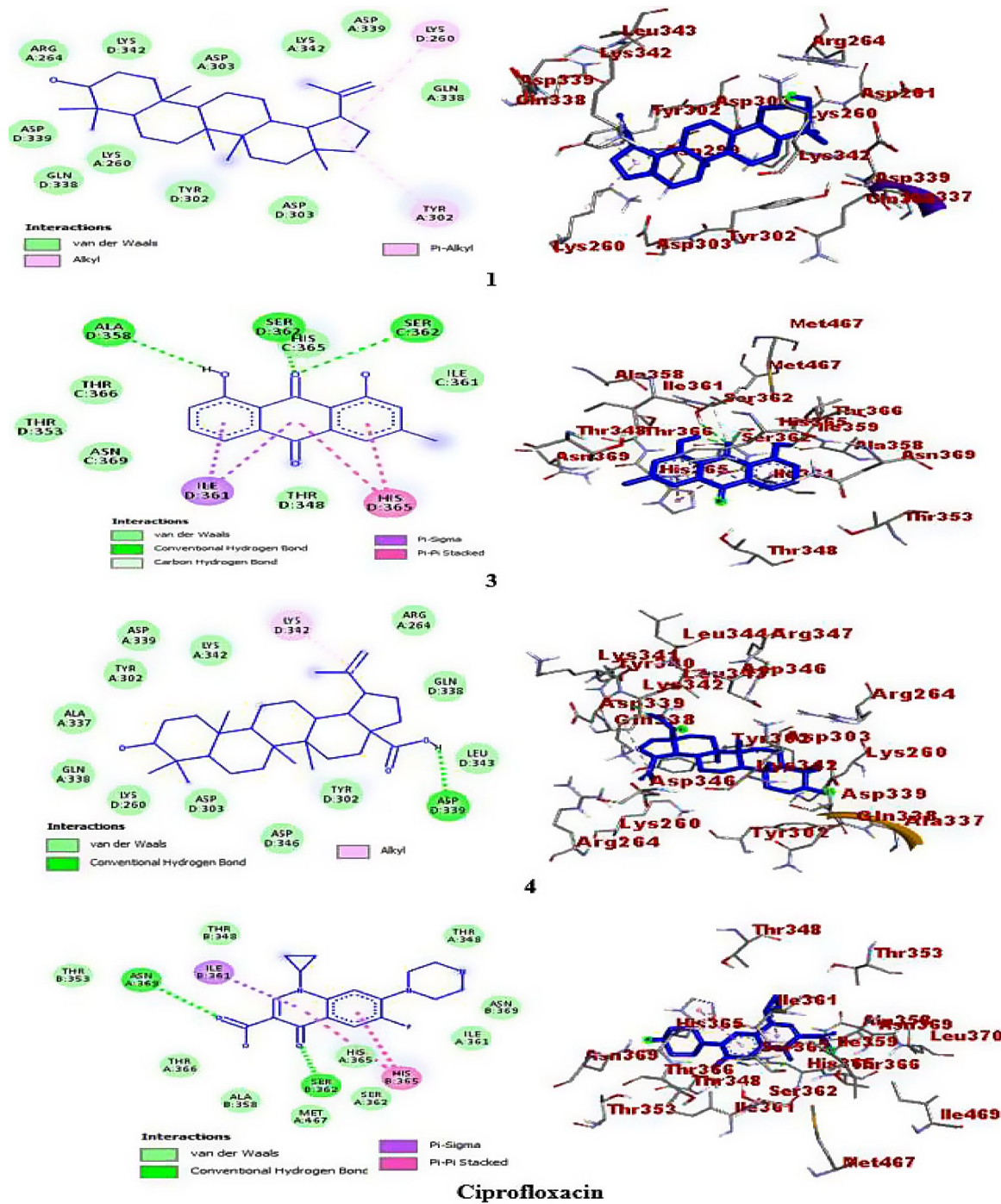


Fig. 6 2D (left) and 3D (right) binding interactions of compounds **1**, **3**, **4**, and ciprofloxacin against *S. aureus* PK

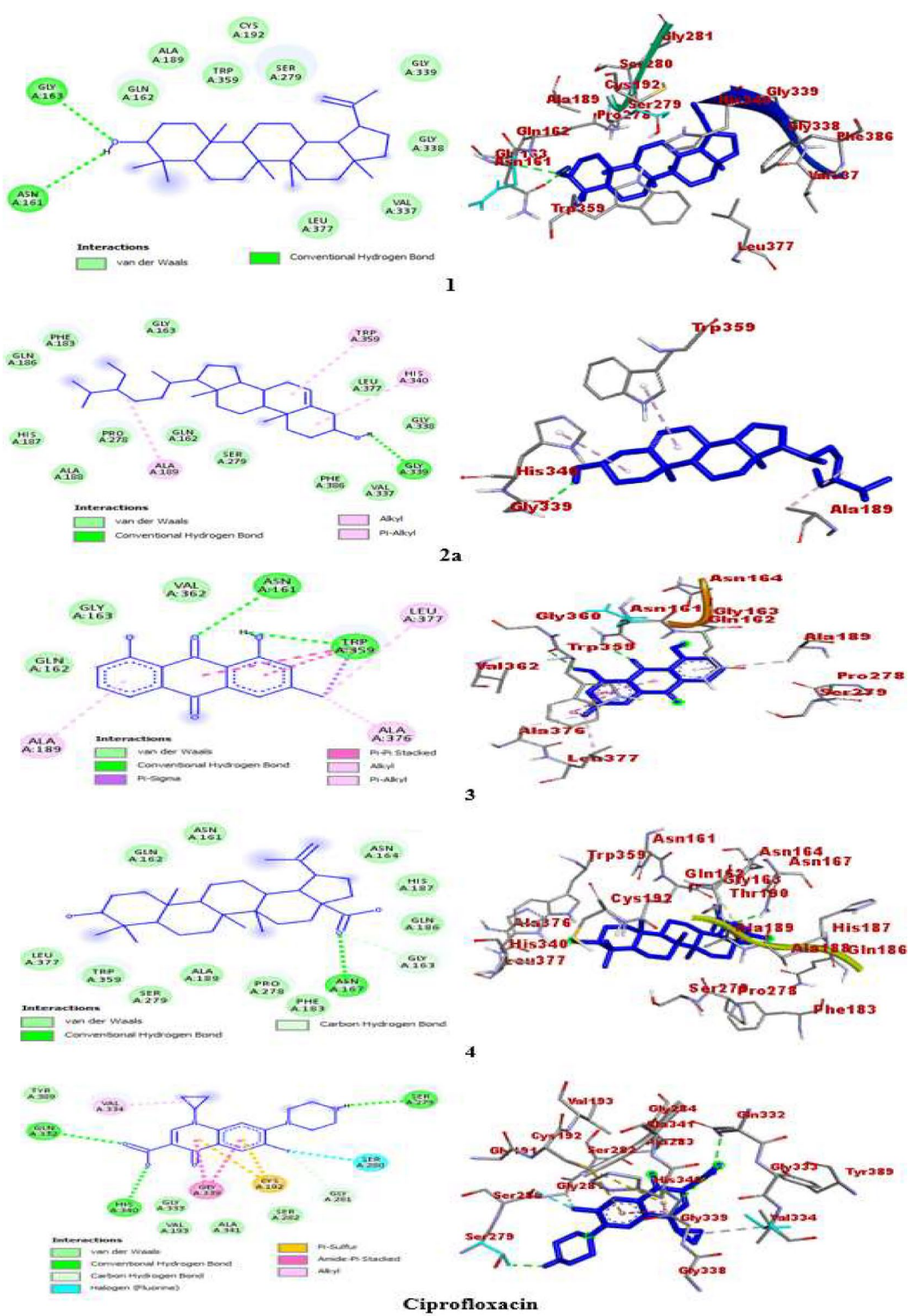


Fig. 7 2D (left) and 3D (right) binding interactions of compounds **1**, **2a**, **3**, **4**, and ciprofloxacin against 10,782 streptopain

Table 6 Molecular docking study of compounds **2a**, **3**, **4**, and ascorbic acid against human myeloperoxidase and compounds **1**, **2a**, **3**, **4**, and etoposide against topoisomerase II α

Compound/ligand	Binding affinity (kcal/mol)	H-bond	Residual amino acid interactions	
			Hydrophobic/Electrostatic	Van der Waals
Against myeloperoxidase				
2a	-7.2		Ala-152, Arg-31, Ile-160, and Cys-153	Ile-160, Thr-159, Asn-162, Arg-323, Val-30, Arg-31, and Ala-28
3	-7.5	Ile-160, Asn-162, and Arg-323	Cys-153 and Arg-31	Pro-34, Val-30, Ala-28, Thr-159, Ala-152, and Phe-29
4	-7.4	Arg-31, Asn-162, and Phe-29		Lys-505, Asp-321, Pro-34, Arg-323, Trp-32, Ile-160, Ala-35, Ala-28, Val-30, and Arg-31
Ascorbic acid	-7.7	Arg-31, Arg-161, Ala-35, Ile-160, and Arg-323		Ala-28, Asn-162, Phe-29, Pro-34, Val-30, and Thr-159
Against topoisomerase IIa				
1	-9.2	Ser-717	Phe-1003	Asp-1004, Gly-1007, Arg-727, Glu-712, Pro-724, Ser-709, Arg-713, Lys-728, Asp-831, Leu-829, Glu-839, and Trp-840
2a	-9.0	Asp-1004	Pro-724, Lys-728, Tyr-757, His-758, His-759, and Phe-1003	Glu-837, Asp-831, Glu-712, Gly-1007, His-1005, Ser-717, Trp-840, Glu-839, Arg-727, Leu-829, Gly-725, and Arg-713
3	-8.3	Gln-544 and Leu-685	Pro-593 and Leu-705	Tyr-684, Asp-683, Glu-682, Arg-672, Leu-592, Gln-542, Tyr-590, Tyr-686, and Ser-591
4	-7.9	Glu-839 and His-758 (C-H bond)	Leu-829	Lys-728, Asp-831, Pro-838, Trp-840, Arg-727, Glu-837, Pro-724, Glu-712, Tyr-757, and His-759
Etoposide	-9.4	Gln-544, Arg-727, Ser-756, Pro-838, Glu-837, and Glu-712 (C-H bond)	Lys-728, Lys-614, His-758	Asp-832, Val-836, Asp-831, Pro-724, Leu-829, Glu-839, Trp-840, Gly-1007, Glu-712, Arg-713, Ser-709, and His-759

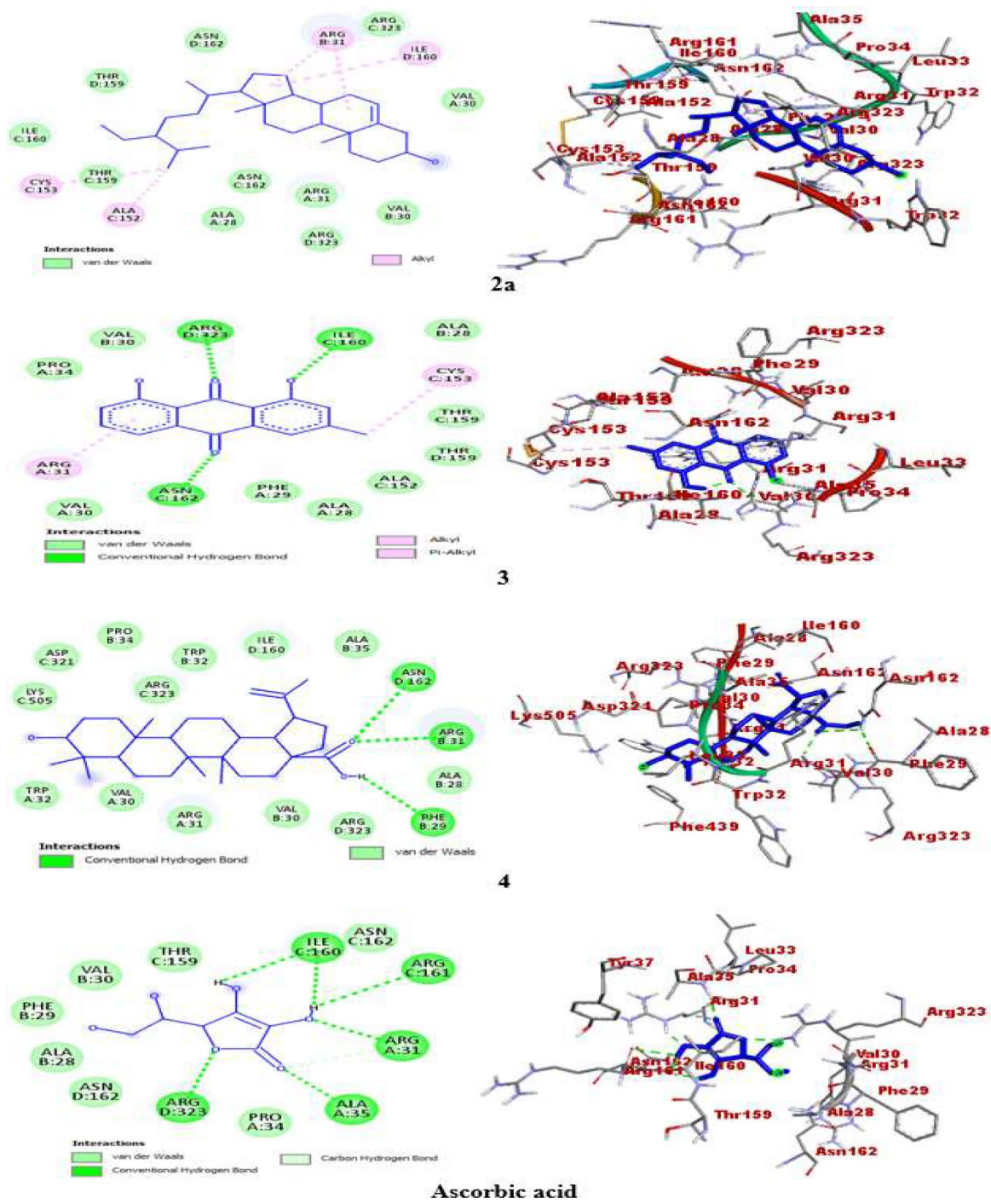


Fig. 8 2D (left) and 3D (right) binding interactions of compounds **2a**, **3**, **4**, and ascorbic acid against human myeloperoxidase

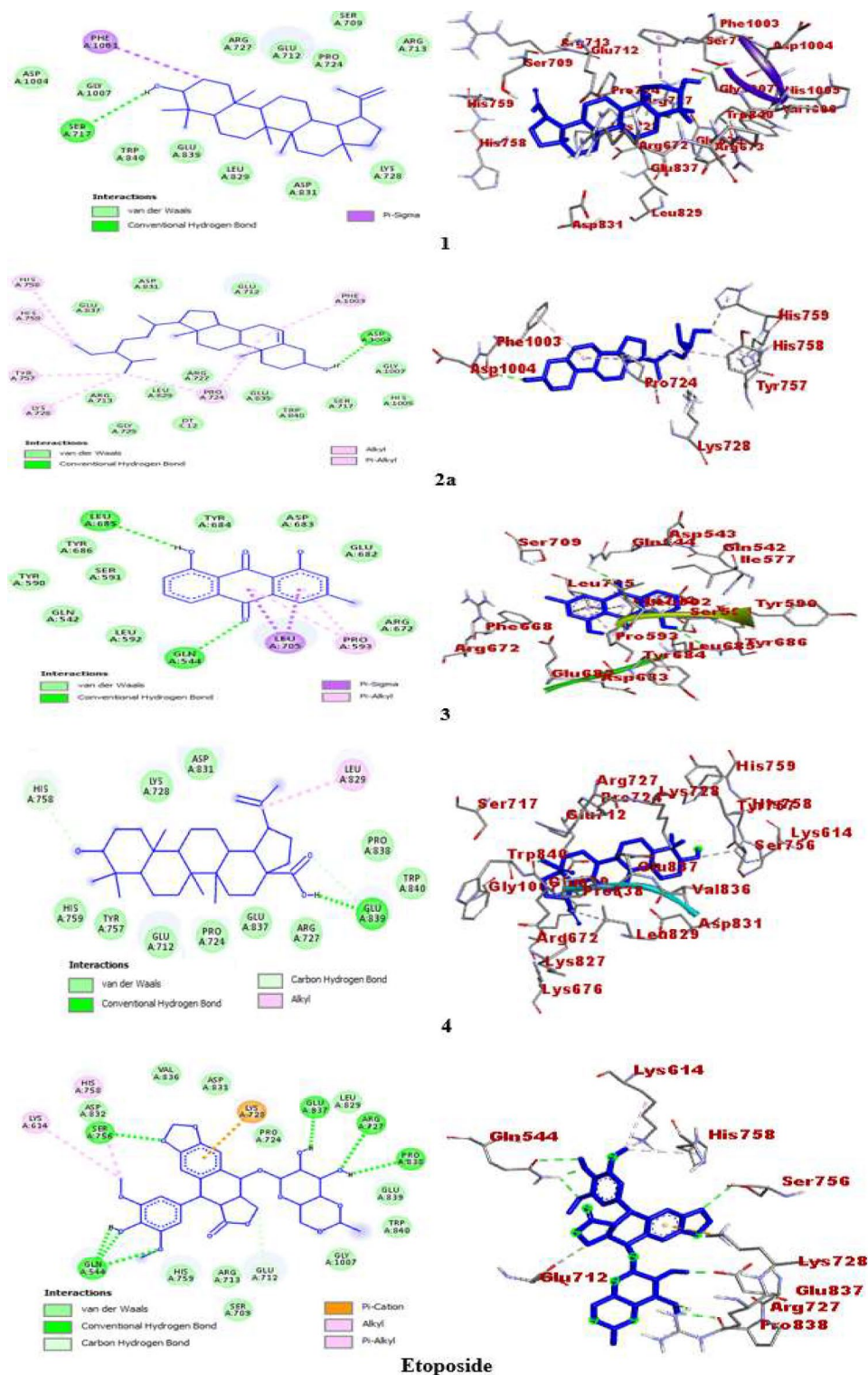


Fig. 9 2D (left) and 3D (right) binding interactions of compounds **1**, **2a**, **3**, **4**, and etoposide against topoisomerase II α

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-024-00899-2>.

Supplementary Material 1

Acknowledgements

The authors are grateful to Adama Science and Technology University, Ethiopia, for funding, with grant number: ASTU/AS-R/003/2020. We also thank the Department of Chemistry, Kenyon College, Gambier, USA, for NMR facilities.

Author contributions

All authors made substantial contributions to the conception, drafting, and analysis of the research work. Aman Dekebo and Milkyas Endale designed and supervised the overall work, and edited the manuscript. Hadush Gebrehiwot collected the sample materials, implemented the experimental works, computed the molecular docking results, operated antioxidant activities, conducted structure elucidations, and drafted the manuscript. Urgessa Ensermu performed the antibacterial activities and Mo Hunsen generated the NMR spectra and edited the manuscript. All the authors read and approved the manuscript for publication.

Funding

This research was supported by grants from Adama Science and Technology University, Ethiopia, with grant number: ASTU/AS-R/003/2020.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Applied Chemistry, Adama Science and Technology University, P.O. Box 1888, Adama, Ethiopia

²Department of Applied Biology, Adama Science and Technology University, P.O. Box 1888, Adama, Ethiopia

³Institute of Pharmaceutical Sciences, Adama Science and Technology University, P.O. Box 1888, Adama, Ethiopia

⁴Traditional and Modern Medicine Research and Development Directorate, Armauer Hansen Research Institute, P.O. Box 1242, Addis Ababa, Ethiopia

⁵Department of Chemistry, Kenyon College, Gambier, OH 43022, USA

Received: 19 January 2024 / Accepted: 9 April 2024

Published online: 22 May 2024

References

- Phongpaichit S, Puenjob N, Rukachaisirikul V, Ongsakul M (2004) Antifungal activity from leaf extracts of *Cassia alata* L., *Cassia fistula* L. and *Cassia tora* L. Songklanakarin J Sci Technol 26(5):741–748
- Gupta VK, Singh A, Pathak A, Verma R, Dayal S, Jain A, Gahlot M (2015) *In vitro* antimicrobial potential of *Cassia* Genus, an overview. Br J Pharm Res Int 7:236–246
- Tripathi V, Goswami S (2011) Generic relationship among *Cassia* L., *Senna* Mill. and *Chamaecrista* Moench using RAPD markers. Int J Biodivers Conserv 3(3):92–100
- Prasanna R, Harish C, Pichai R, Sakthisekaran D, Gunasekaran P (2009) Anti-cancer effect of *Cassia auriculata* leaf extract *in vitro* through cell cycle arrest and induction of apoptosis in human breast and larynx cancer cell lines. Cell Biol Int 33(2):127–134
- Jalalpure S, Patil M, Pai A, Shah B, Salahuddin M (2004) Antidiabetic activity of *Cassia auriculata* seeds in alloxan-induced diabetic rats. Niger J Nat Prod Med 8:22–23
- Sule W, Okonko I, Joseph T, Ojezele M, Nwanze J, Alli J, Adewale O (2010) *In vitro* antifungal activity of *Senna alata* Linn. crude leaf extract. Res J Biol Sci 5(3):275–284
- Yadav J, Arya V, Yadav S, Panghal M, Kumar S, Dhankhar S (2010) *Cassia occidentalis* L.: a review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia 81(4):223–230
- Jothy SL, Torey A, Darah I, Choong YS, Saravanan D, Chen Y, Latha LY, Deivanai S, Sasidharan S (2012) *Cassia spectabilis* (DC) Irwin Et Barn: a promising traditional herb in health improvement. Molecules 17(9):10292–10305
- Mazumder PM, Percha V, Farswan M, Upaganlawar A (2008) *Cassia*: a wonder gift to medical sciences. Int J Clin Pharm 1(2):16–38
- Thongsaard W, Chainakul S, Bennett G, Marsden C (2001) Determination of barakol extracted from *Cassia siamea* by HPLC with electrochemical detection. J Pharm Biomed Anal 25(5–6):853–859
- Teka A, Asfaw Z, Demissew S, Van Damme P (2020) Medicinal plant use practice in four ethnic communities (Gurage, Mareqo, Qebena, and Silti), South Central Ethiopia. J Ethnobiol Ethnomed 16:1–12
- Abdo BM (2017) Sennosides determination of Ethiopian *Senna alexandrina* Mill accessions. Nat Prod Chem Res 5(7):1–4
- Bukar A, Mukhtar M, Hassan A (2009) Phytochemical screening and antibacterial activity of leaf extracts of *Senna siamea* (Lam) on *Pseudomonas aeruginosa*. Bayero J Pure Appl Sci 2(1):139–142
- Osunga S, Amuka O, Machocho AK, Getabu A (2023) Ethnobotany of some members of the genus *Cassia* (Senna). Int J Novel Res Life Sci 10(5):1–14
- Kamagaté M, Koffi C, Kouamé NM, Akoubet A, Alain N, Yao R, Die H (2014) Ethnobotany, phytochemistry, pharmacology and toxicology profiles of *Cassia siamea* Lam. J Phytopharm 3(1):57–76
- Ratnampally SK, Venkateshwar C (2017) Quantitative analysis of phytochemicals in the bark extracts of medicinally important plant *Cassia fistula* Linn. Int J Curr Microbiol Appl Sci ISSN, 6(4):2319–7706
- Aliyu B (2006) Some ethno medicinal plants of the Savannah regions of West Africa: description and phytochemicals. Triumph Pub Comp 1:135–152
- Koudouvo K, Karou DS, Kokou K, Essien K, Aklikokou K, Glitho I, Simpore J, Sanogo R, De Souza C, Gbeassor M (2011) An ethnobotanical study of anti-malarial plants in Togo Maritime Region. J Ethnopharmacol 134(1):183–190
- Otimenyin S, Kolawole J, Nwosu M (2010) Pharmacological basis for the continual use of the root of *Senna siamea* in traditional medicine. Int J Pharma Bio Sci 1(3):PS67
- Ajaiyeoba E, Ashidi J, Okpako LC, Houghton P, Wright CW (2008) Antiplasmodial compounds from *Cassia siamea* stem bark extract. Phytother Res 22(2):254–255
- Ntandou GN, Banzouzi J, Mbatchi B, Elion-Itou R, Etou-Ossibi A, Ramos S, Benoit-Vical F, Abena A, Ouamba J (2010) Analgesic and anti-inflammatory effects of *Cassia siamea* Lam. Stem bark extracts. J Ethnopharmacol 127(1):108–111
- Kumar S, Kumar V, Prakash O (2010) Antidiabetic and anti-lipemic effects of *Cassia siamea* leaves extract in streptozotocin-induced diabetic rats. Asian Pac J Trop Med 3(11):871–873
- Majji LN, Battu GR, Jangiti RK, Talluri MR (2013) Evaluation of *in vitro* antibacterial activity of *Cassia siamea* leaves. Int J Pharm Pharm Sci 5(3):263–265
- Esakkirajan M, Prabhu N, Arulvasu C, Beulaja M, Manikandan R, Thiagarajan R, Govindaraju K, Prabhu D, Dinesh D, Babu G (2014) Anti-proliferative effect of a compound isolated from *Cassia auriculata* against human colon cancer cell line HCT 15. Spectrochim Acta Mol Biomol Spectrosc 120:462–466
- Deshpande S, Kewatkar SM, Paithankar VV (2013) *In vitro* antioxidant activity of different fractions of roots of *Cassia auriculata* Linn. Drug Invent Today 5(2):164–168
- Hu Q-F, Zhou B, Gao X-M, Yang L-Y, Shu L-D, Shen Y, Li G-P, Che C-T, Yang G-Y (2012) Antiviral chromones from the stem of *Cassia siamea*. J Nat Prod 75(11):1909–1914
- Sastry B, Sreelatha T, Suresh Babu K, Madhusudhana Rao J (2003) Phytochemical investigation of *Cassia siamea* Lam. National seminar on biodiversity conservation and commercial exploitation of medicinal plants, Department of Botany. Osmania University, Hyderabad
- Kaur P, Arora S (2010) Polyphenols of Caselpiniaceae. J Chin Clin Med 5(5):282
- Mohammed A, Liman M, Atiku M (2013) Chemical composition of the methanolic leaf and stem bark extracts of *Senna siamea* Lam. J Pharmacogn Phytotherapy 5(5):98–100
- Smith YA (2009) Determination of the chemical composition of *Senna siamea* (Cassia leaves). Pak J Nutr 8(2):119–121
- Subramanian D, Venugopal S (2011) Comparative study of antioxidant activities of *Cassia auriculata* and *Cassia siamea* flowers. Int Res J Pharm 2(12):208–212
- Veerachari U, Bopaiah A (2011) Preliminary phytochemical evaluation of the leaf extract of five *Cassia* species. J Chem Pharm Res 3(5):574–583

33. Padumanonda T, Suntornsuk L, Gritsanapan W (2006) Quantitative analysis of barakol content in *Senna siamea* leaves and flowers by TLC-densitometry. *Med Princ Prac* 16(1):47–52
34. Oshimi S, Tomizawa Y, Hirasawa Y, Honda T, Ekasari W, Widyawaruyanti A, Rudyanto M, Indrayanto G, Zaini NC, Morita H (2008) Chrobisiamone A, a new bischromone from *Cassia siamea* and a biomimetic transformation of 5-acetyl-7-hydroxy-2-methylchromone into cassiarin A. *Bioorg Med Chem Lett* 18(13):3761–3763
35. Morita H, Oshimi S, Hirasawa Y, Koyama K, Honda T, Ekasari W, Indrayanto G, Zaini NC (2007) Cassiarins A and B, novel antiparasitic alkaloids from *Cassia siamea*. *Org Lett* 9(18):3691–3693
36. Koyama J, Morita I, Tagahara K, Nobukuni Y, Mukainaka T, Kuchide M, Tokuda H, Nishino H (2002) Chemopreventive effects of emodin and cassiamin B in mouse skin carcinogenesis. *Cancer Lett* 182(2):135–139
37. Adedoyin BA, Adeniran OI, Muhammed AB, Dangoggo SM, Nahar L, Sharples GP, Sarker SD (2020) Isolation and characterization of propitious bioactive compounds from *Cassia singueana* L. *Adv Med Plant Res* 8(4):89–100
38. Nsonde-Ntandoua G, Lucantoni L, Banzouzi J, Ndonga M, Yerbanga S, Ouambaf J, Habluetzel A, Esposito F, Abena A (2010) Mosquitocidal and antifecundity effects of coumarin and betulinic acid isolated from *Cassia siamea* (Fabaceae) stem bark chloroform extract on female *Anopheles stephensi* (Diptera: ciliidae). Abstract, actes du Symposium International, Bénin
39. Calderon-Montano M, Burgos-Morón J, Pérez-Guerrero E, López-Lázaro C M (2011) A review on the dietary flavonoid kaempferol. *Mini Rev Med Chem* 11(4):298–344
40. Dave H, Ledwani L (2012) A review on anthraquinones isolated from *Cassia* species and their applications. *Indian J Nat Prod Resour* 3(3):291–319
41. Teangpook C, Paosangtong U, Titatarn Y, Onhem S, Puminat W (2011) Production and nutrition of khi lek (*siamese cassia*) curry from Central Thailand. *Agric Nat Resour* 45(3):510–520
42. Ahmed R, Nagori K, Kumar T, Singh M, Dewangan D (2011) Phytochemical estimation of anthraquinones from *Cassia* species. *Int J Res Ayurveda Pharm* 2(4):1320–1323
43. Ingweye J, Kalio G, Ubua J, Effiong G (2010) The potentials of a lesser known Nigerian legume, *Senna siamea* seeds as a plant protein source. *Aust J Basic Appl Sci* 4(8):2222–2231
44. Akhtar MS, Hossain MA, Said SA (2017) Isolation and characterization of antimicrobial compound from the stem-bark of the traditionally used medicinal plant *Adenium obesum*. *J Tradit Complement Med* 7(3):296–300
45. Balouiiri M, Sadiki M, Ibnsouda SK (2016) Methods for *in vitro* evaluating antimicrobial activity: a review. *J Pharm Anal* 6(2):71–79
46. Habeeb F, Shakir E, Bradbury F, Cameron P, Taravati MR, Drummond AJ, Gray AI, Ferro VA (2007) Screening methods used to determine the anti-microbial properties of *Aloe vera* inner gel. *Methods* 42(4):315–320
47. Khorasani Esmaili A, Mat Taha R, Mohajer S, Banisalam B (2015) Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L. (Red Clover). *BioMed Res Int* 2015:1–12
48. Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 7(1):42717
49. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 23(1–3):3–25
50. Banerjee P, Eckert AO, Schrey AK, Preissner R (2018) ProTox-II: a web server for the prediction of toxicity of chemicals. *Nucleic Acids Res* 46(W1):W257–W263
51. Baby ST, Sharma S, Enaganti S, Cherian PR (2016) Molecular docking and pharmacophore studies of heterocyclic compounds as Heat shock protein 90 (Hsp90) inhibitors. *Bioinformation* 12(3):149–155
52. Seeliger D, de Groot BL (2010) Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *J J Comput Aided Mol Des* 24(5):417–422
53. Biovia DS (2021) Discovery Studio Visualizer v21. 1.0. 20298. Dassault Systèmes, San Diego
54. Musa NM, Sallau MS, Oyewale AO, Ali T (2024) Antimicrobial activity of lupeol and β -amyrin (triterpenoids) isolated from the rhizome of *Dolichos pachyrhizus* harm. *Adv J Chem A* 7(1):1–14
55. Shwe HH, Win KK, Moe TT, Myint AA, Win T (2019) Isolation and structural characterization of lupeol from the stem bark of *Diospyros ehereioides* Wall. *IEEE-SEM* 7(8):140–144
56. Omonike O, Johnson A, Edith A, Ramsay K, Mohammed C (2014) Anthraquinones and triterpenoids from *Senna siamea* (Fabaceae) Lam inhibit poliovirus activity. *Afr J Microbiol Res* 8(31):2955–2963
57. Cayme J-MC, Ragasa CY (2004) Structure elucidation of β -stigmaterol and β -sitosterol from *Sesbania grandiflora* [Linn.] Pers. and β -carotene from *Heliotropium indicum* Linn. By NMR spectroscopy. *Kimika* 20(1):5–12
58. Mining J, Lagat Z, Akenga T, Tarus P, Imbuga M, Tsanuo M (2014) Bioactive metabolites of *Senna didymobotrya* used as a biopesticide against *Acanthoscelides obtectus* in Bungoma, Kenya. *J Appl Pharm Sci* 4(9):56–60
59. Schripsema J, Dagnino D (1996) Elucidation of the substitution pattern of 9, 10-anthraquinones through the chemical shifts of peri-hydroxyl protons. *Phytochemistry* 42(1):177–184
60. Kuwabara N, Matsuo Y, Ueki T, Nakamura Y, Kase N, Okayasu T, Mimaki Y, Tachikawa E, Sato S, Yamada H (2022) Inhibitory effect of *Rhei rhizoma* constituents on the cortisol production in acth-stimulated bovine adrenal fasciculata cells. *Trad Med* 3(2):1–7
61. Ledwani L, Singh M (2004) Isolation and characterization of anthraquinones from the stem bark of *Cassia siamea*. *Indian J Chem* 43B:2257–2258
62. Aung EE, Kristanti AN, Aminah NS, Takaya Y, Ramadhan R (2023) Phytochemicals constituents in medicinal plant *Syzygium aqueum* (Burm.) Alston (Myrtaceae). *Egypt J Chem* 66(3):301–308
63. Santos R, Conceição A, Lula I, Oliveira R (2018) Triterpenes esterified with fatty acid isolated from *Pouteria macahensis* TD Penn (Sapotaceae) leaves. *Rev Virtual Quim* 10(5):1–8
64. Mbouangouere R, Tane P, Ngamga D, Djemgou P, Choudhary M, Ngadjui B (2007) Piptaderol from *Piptadenia Africana*. *Afr J Tradit Complement Altern Med* 4(3):294–298
65. Chimi Fotso S, Tcho Tadjong A, Tsopgni WDT, Lenta BN, Nkenfou CN, Wansi JD, Toze FAA (2021) Chemical constituents and antimicrobial activities of some isolated compounds from the Cameroonian species of *Senna alata* (*Cassia alata* L. Roxb synonym, the plant list 2013). (Leguminosae). *Trends Phytochem Res* 5(1):37–43
66. Abdallah M, Idriss A, Yahaya S, Aliyu I (2019) Antibacterial activity of ethanolic leaves extracts of *Cassia siamea* against some bacterial isolates from infantile diarrhoeal attending General Hospital Damaturu. *Glob Acad J Pharm Drug Res* 1(1):5–9
67. Vasait RD (2016) Screening of antibacterial properties of crude leaf extracts of plant *Cassia siamea* collected from Baglan Region. *Indian J Appl Res* 6(11):42–43
68. Melaku Y, Getahun T, Addisu M, Tesse H, Eswaramoorthy R, Ankita G (2022) Molecular docking, antibacterial and antioxidant activities of compounds isolated from Ethiopian plants. *Int J Second Metab* 9(2):208–328
69. Islam MA, Mondal SK, Islam S, Shorna A, Nourin M, Biswas S, Uddin MS, Zaman S, Saleh MA (2023) Antioxidant, cytotoxicity, antimicrobial activity, and *in silico* analysis of the methanolic leaf and flower extracts of *Clitoria ternatea*. *Biochem Res Int* 2023:1–12
70. Jacob L, Latha M (2013) *In vitro* antioxidant activity of *Clitoria ternatea* Linn. *Int J Res Phytochem Pharmacol* 3(1):35–39
71. Oyeade K, Daspan A, Denkok Y, Alemika T, Ojerinde O (2021) Antimicrobial, antioxidant and antiproliferative properties of the leaves of *Senna siamea*. *J Complement Altern Med Res* 14(1):22–29
72. Abishad P, Niveditha P, Unni V, Vergis J, Kurkure NV, Chaudhari S, Rawool DB, Barbudhe SB (2021) *In silico* molecular docking and *in vitro* antimicrobial efficacy of phytochemicals against multi-drug-resistant enteroaggregative *Escherichia coli* and non-typhoidal *Salmonella* spp. *Gut Pathog* 13(1):1–11
73. Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD (2002) Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem* 45(12):2615–2623
74. Daina A, Zoete V (2016) A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem* 11(11):1117–1121
75. Gadaleta D, Vuković K, Toma C, Lavado GJ, Karmaus AL, Mansouri K, Klein-streuer NC, Benfenati E, Roncaglioni A (2019) SAR and QSAR modeling of a large collection of LD₅₀ rat acute oral toxicity data. *J Cheminformatics* 11(1):1–16
76. Islam MA, Pillay TS (2019) Identification of promising anti-DNA gyrase antibacterial compounds using de novo design, molecular docking, and molecular dynamics studies. *J Biomol Struct Dyn* 2019:1798–1809
77. Collin F, Karkare S, Maxwell A (2011) Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Appl Microbiol Biotechnol* 92:479–497
78. Levine C, Hiasa H, Mariani KJ (1998) DNA gyrase and topoisomerase IV: biochemical activities, physiological roles during chromosome replication, and drug sensitivities. *Biochim Biophys Acta Gene Struct Expression* 1400(1–3):29–43

79. Valentini G, Chiarelli L, Fortin R, Speranza ML, Galizzi A, Mattevi A (2000) The allosteric regulation of pyruvate kinase: a site-directed mutagenesis study. *J Biol Chem* 275(24):18145–18152
80. Zhai Z, An H, Wang G, Luo Y, Hao Y (2015) Functional role of pyruvate kinase from *Lactobacillus bulgaricus* in acid tolerance and identification of its transcription factor by bacterial one-hybrid. *Scie Rep* 5(1):17024
81. Chen C-Y, Luo S-C, Kuo C-F, Lin Y-S, Wu J-J, Lin MT, Liu C-C, Jeng W-Y, Chuang W-J (2003) Maturation processing and characterization of Streptopain. *J Biol Chem* 278(19):17336–17343
82. Dhorajiwala TM, Halder ST, Samant L (2019) Comparative in silico molecular docking analysis of l-threonine-3-dehydrogenase, a protein target against African trypanosomiasis using selected phytochemicals. *J Appl Biotechnol Rep* 6(3):101–108
83. Kumar NS, Dullaghan EM, Finlay BB, Gong H, Reiner NE, Selvam JJP, Thorson LM, Campbell S, Vitko N, Richardson AR (2014) Discovery and optimization of a new class of pyruvate kinase inhibitors as potential therapeutics for the treatment of methicillin-resistant *Staphylococcus aureus* infections. *Bioorg Med Chem* 22(5):1708–1725

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.